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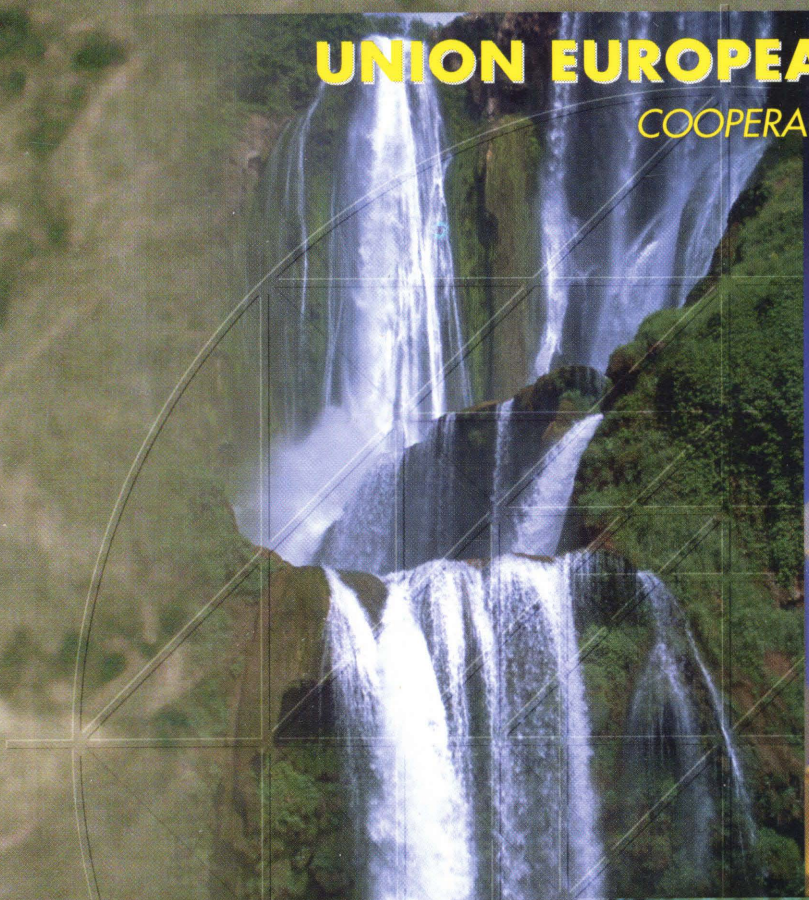

EUROPEAN UNION - LATIN AMERICA

SCIENTIFIC COOPERATION IN THE 90's

Vol I: Life Sciences and Technologies for Developing Countries (STD III)

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Research DG/E - INCO-DEV Programme

Contact: Mr Jaak Sinnaeve - rue de la Loi, 200, B-1049 Brussels
Tel: (32-2) 295 40 45 - Fax (32-2) 296 62 52

EUROPEAN UNION - LATIN AMERICA

SCIENTIFIC COOPERATION IN THE 90' s

UNION EUROPEA - AMERICA LATINA

COOPERACION CIENTIFICA EN LOS AÑOS 90

Vol I: Life Sciences and Technologies
for Developing Countries (STD III)

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Preface

European Union - Latin America Scientific Cooperation in the 90's

It gives me great satisfaction to present this overview of the results of almost a decade of continuous support from the European Community to cooperation between our scientists and their Latin American counterparts. In addition, this publication provides researchers with a valuable source of information on the projects supported, their scope, objectives, and results, and gives full details of the teams involved and how to contact them.

The reader will find in the pages that follow the practical results of the Community's policy on scientific cooperation with the Latin American region. As in the case of other developing regions, Community policy has sought to harmonise a contribution to the region's socio-economic progress with our own scientific interests.

Implementation of this policy has allowed Community scientists to gain access to localities displaying particular environmental, agricultural, ecological and public health characteristics, and to undertake their research in these areas. As a counterbalance, we believe that Latin American researchers have derived great benefit from interaction with their European peers. Given their own scientific quality, this sharing of experience places local teams in a privileged position from which to contribute to finding science-based solutions to problems faced by their communities.

It is precisely with the aim of tackling these problems effectively that, after extensive dialogue with the scientific authorities and communities of the region, the Commission selected areas on which to target cooperation. Agriculture and agroindustry, health and environmental issues were considered the most important priorities, as the reader will be able to see in the body of this publication. However, in order to capitalize on the human potential available, research in other relevant fields such as earth sciences, materials and different branches of engineering was also supported when resources permitted.

We firmly believe that our cooperation has led to the creation of a permanent network of scientific interaction, embracing a vast number of Latin American and European scientists, and which is even broader and more far-reaching than the sum of the results of the projects presented here.

The importance of Latin America for the European Community has recently been brought to the forefront by the Summit of Heads of State of Latin America and the Caribbean, and the European Union, which took place last June in Rio de Janeiro. The dialogue that has taken place over the years in different fora has been reinforced by the Heads of State of the two regions with their decision to establish a Working Group of Representatives. This institutionalised Working Group should provide a renewed impetus to our cooperation: whether this will be achieved through the enlargement of the specific programme for cooperation, by further facilitating access to the specific thematic programmes of the framework programmes, by the conclusion of cooperation agreements, or by the combination of some of these options, is still an open question.

The Working Group of Representatives will be the forum for reflection and advice on the most appropriate way to develop the full potential of our cooperation in the future. The Rio Summit underscored the will of both regions to deepen that cooperation, and the European Commission will apply its best efforts and full capacity to the successful achievement of that aim.

Brussels, October 1999

J. Gabolde
Director

Introduction

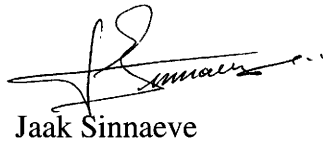
During the 1990s, the European Community pursued scientific cooperation with Latin America through a series of different programmes.

For the period 1990-1994 two complementary schemes were in operation. First, the Life Sciences and Technologies for Developing Countries (STDIII) programme, which formed part of the EC's Third Framework Programme for Research and Technological Development aimed at mobilizing EC and Developing Country scientists to work on pressing problems of all developing countries, including Latin American countries, in the areas of human health and agriculture. Second, the International Scientific Cooperation (ISC) scheme, which aimed at developing long-lasting working relationships between EC and Latin American scientists, covered a wider range of subjects and set priorities by mutual agreement with the national authorities of individual countries. Through these two schemes a wide-ranging development effort was complemented by a country-specific initiative. The ISC scheme also granted fellowships for Latin American scientists to do research in European laboratories and develop contacts with the European scientific community.

In 1994, a new scheme combining these ideas was introduced. This was the INCO-DC programme (Scientific and Technological Cooperation with Developing Countries), which formed part of the EC's Fourth RTD Framework Programme and which ran until 1998. It focussed specifically on three sectors of widespread importance (sustainable management of renewable natural resources, sustainable improvement of agricultural and agroindustrial production, and health) and used a regional basis, in this case the region being Latin America, on which to set research priorities and build projects.

The newest programme, which started in 1999 and runs for a further four years, is the Research for Development (INCO-DEV) component of the Fifth RTD Framework Programme. This programme targets research of a problem-orientated nature, maintains the regional approach and subject-matter coverage of the earlier INCO-DC programme but adds to it a section on policy research for sustainable development.

This volume contains summaries of joint research projects involving partners in Latin America. It covers all STDIII and INCO-DC projects, and ISC projects which started in the 1992-1994 period. A table summarizing the number of activities carried out and EC financial contribution is given below.



Jaak Sinnaeve
Head of Unit XII-E-4
Research for Development

EC-Latin America S + T cooperation activities			
	Number of activities	Number of institutional partners	EC financial contribution (million ECU)
Joint Research projects			
STD III (1990-1994)	96	388*	31.76
ISC (1990-1994)	363	933	57.88
INCO-DC (1994-1998)	121	818*	58.50
Fellowships (1990-1994)	319	638	10.44
TOTAL	**899**	**2777**	**158.58**

** Includes some partners from non-Latin American developing countries*

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STD III

Agriculture

Contract number: TS3*CT910003

Period: October 1991 to July 1995

INFLUENCE OF CULTIVATION ON ORGANIC NITROGEN STATUS IN TROPICAL SOILS. ADJUSTMENT OF A MATHEMATICAL MODEL TO NITROGEN FERTILITY

Co-ordinator: Consejo Superior de Investigaciones Científicas, Santiago de Compostela, Spain
(Tarsy Carballas)

Objectives

- Evaluating the effects of usual agricultural practices (nitrogen fertilisation and rotation) on the state and evolution of N reserves in soils in the Venezuelan Llanos (savanna system).
- Modelling the N cycle in tropical areas to achieve both improvements in soil productivity and conservation.
- Performing a study of social representations of soil fertility and N fertilisation.
- Using the results of the research to draft recommendations aimed at changing, if necessary, current fertilisation and cultivation habits.

Activities

- To annually cultivate a local variety of maize (two crops in two years) and a permanent pasture (*Digitaria decumbens*) fertilised with ^{15}N-urea in an Alfisol located on the Western Venezuelan Llanos.
- To follow the evolution of N derived from the soil (NDDS) and derived from the fertiliser (NDDF) in plants to evaluate N uptake.
- To follow the evolution of soil N to determine the N status and to evaluate soil N reserves.
- To evaluate acidification induced by urea fertilisation.
- To investigate, during the cropping period, the distribution of soil N and the N derived from ^{15}N labelled urea in reservoirs with different turnover rates: granulometric fractions, microbial biomass and biochemical fractions.
- To adapt existing mathematical models to tropical environments and to improve them by taking into account new N reservoirs.
- To apply sociology techniques to the field of soil fertility and N fertilisation to improve the oral and written discourse of industries and institutions to farmers.

Results

In spite of an N equilibrium, important losses of N derived from fertiliser and from the soil N were detected. N volatilization, which takes place early the crop cycle when there is a high concentration of N derived from urea in the soil and in the plant, was the process that most contributed to N losses: losses by leaching and run-off were of less importance. Denitrification and N losses directly from the plant were not discarded. N losses were higher in the soil under maize than under pasture. A high percentage of the N derived from the fertiliser was immobilised

in the soil in organic N forms at the end of the crop cycle. The immobilising activity of the microbiota in the soil under pasture was higher than in the soil under maize. At the end of the crop, only 15% of the fertiliser that remained in the soil was used by the next crop. The N incorporated into the plants over the whole of the crop cycle contained only 2% of the fertiliser added the preceding year; this value could characterise the composition of the available N fraction. The acidifying effect induced by urea fertilisation only affected the surface layer; this effect, which was lower in the soils under pasture than in the soils under maize, was of minor importance, temporary, and easily controlled by liming. In general, mineral N levels varied during the crop cycle but initial levels were restored at the end of the crop. Ammoniacal N predominated over nitrate N in the pasture soil but the reverse occurred in the maize soil. In both crops, nitrates were scarce and were mainly found at the last stages of the crops, which seems to be the pattern in the area. A hybrid model adapted to tropical conditions, from the CERES MAIZE and NCESWAP models, was designed and the basis for its complete development was established. Simulation of plant phenology, foliage surface calculation, plant weight and root development was modified in the CERES plant model. For the N model, four organic N reservoirs were defined and their evolution was followed in the crops. Microbial N, bicarbonate N, permanganate N and easily hydrolyzable N (H1) represented about 5, 5, 20-25 and 50% of total N respectively, and each of them received about 3% of N derived from urea. The use of H1 as active organic matter reservoir gave satisfactory results for the prediction of plant dry-matter production, N exportation by the plant, and evolution of microbial biomass N. According to this, 50% of total N could be involved in N mineralization-immobilization processes in the short term. The social representations of fertility were much less precise than those of fertilisation. The discourse on fertility and fertilisation varied according to the category of interviewee. The social representations on fertility and fertilisation were mainly determined by political aspects, where the institutional variable had the higher weight, and by the economic aspect, where profitability was the most important variable. These facts minimised the social aspect and ecological problems related to soil conservation and fertility. There is an imposition of knowledge on farmers, reinforced by political decisions concerning agricultural credits, and no integration of such knowledge by the institution responsible for fertiliser distribution.

Partners

CONSEJO SUPERIOR DE INVESTIGACCIONES CIENTIFICAS
Bioquímica del Suelo
Avda. De Vigo S/N, Apartado 122
E-15080 Santiago de Compostela
Spain

Tarsy Carballas
Tel.: +34-981-59 09 58
Fax: +34-981-59 25 04
E-mail: tcf@cesga.es

UNIVERSIDAD DE LOS ANDES
Facultad de Ingeniería
Apartado postal 30 – La Hechicera
Merida
Venezuela

J.M. Hetier
Tel.: +58-74-40 16 06
Fax: +58-74-40 12 86

Now as follows:

IRD (ex-**ORSTOM**)
URFPV CIRAD CA
BP 5036
F-34032 Montpellier
France

Tel.: +33-67-61 65 98
Fax: + 33-67-61 71 73
E-mail:Hetier@cirad.fr

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
Université Claude Bernard – Lyon 1
Ecologie Microbienne du Sol
43, boulevard du 11 November 1918
F-69622 Villeurbanne cedex
France

L. Jocteur-Monrozier
Tel.: +33-72-44 80 00
Fax: +33-72-43 12 23

KATHOLIEKE UNIVERSITEIT LEUVEN
Fac. of Agricultural Sciences
Lab. of Soil Fertility and Soil Biology
Kardinaal Mercierlaan 92
B-3001 Leuven
Belgium

R. Merckx
Tel.: +32-16-22 01 31
Fax: +32-16-29 38 05

Contract number: TS3*CT910004

Period: January 1992 to December 1994

EXTRACTIVISM IN CENTRAL AMAZONIA: VIABILITY AND OPTIMIZATION

Co-ordinator: IRD (ex-ORSTOM), Paris, France (Lescure)

Objectives

- Collect biological and ecological data on the different non-timber forest products exploited in the region.
- Describe production practices and evaluate their impacts on the vegetation.
- Document the importance of extractivism in different kinds of production systems practised by forest dwellers and how extractivism is linked to other subsistence and/or commercial activities.
- Find ways for improving production by conversion to agroforestry.

Activities

Field activities (Brazil and Ecuador)

- Analysis of extraction activities in different regions with a wide range of ecological and socio-economic conditions. Observations at different biological, ecological and sociological levels.
- Nutrients cycling in brazil nuts under natural and plantation conditions.

Laboratory activities

For converting production practices from extractivism to agroforestry a better understanding of the problems of germination is needed. Therefore, different conditions of seed conservation and germination were tested at INPA.

Results

⇒ Extractive activities do not provide significantly more income than traditional farming activities such as growing manioc. Agroforestry systems appear to be superior from this standpoint. The interest of integrating extractive activities in development policies therefore remains related to their conservationist character.

⇒ Extractive activities are not in themselves activities aimed at conservation. Since, by definition, they can only be practised in forest ecosystems, with perhaps some degree of management, they may in some cases have a strong impact on plant populations and should be limited by management rules which should be discussed for each case.

⇒ The factors limiting extractive activities are basically socio-economic: lack of access to the resources and land, markets and distribution channels, lack of tax incentives and failure to include them in development policies.

⇒ The major assets of extractive activities are their flexibility and ability of being integrated into complex production systems. This flexibility is basically due to the diversity of the products exploited and the exploitation practices. Such activitiesalso have the advantage of being well integrated in local cultures. Last but not least, they are capable of evolving towards agroforestry practices.

Recommendations

- Development policies should take seriously extractive activities as a component of the production systems to increase the value of forest products.
- The gradual transition from extractive activities towards agroforestry practices should be strongly encouraged.
- The development of extractivism should also be considered outside the framework of extractive reserves.
- A tax policy supporting extractive activities should be set up.
- Strong support should be given to integrating environmental value in product prices.
- The desirable transition to agroforestry production requires a re-examination of land-tenure.
- Agricultural assistance services need to evolve and gain new expertise in ecosystem management if these proposals are to be successfully implemented.
- Product exploitation legislation, based on the results of research and on potential impacts, which should be considered in space-time frameworks specific to each product, need to evolve. The data on such impacts are missing and research is required in this area.

Selected publications

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Partners

IRD (ex-ORSTOM)
Département Milieux et Activité Agricole
273 rue Lafayette
F-75480 Paris cedex 10
France
Lescure
Tel.: +33-1-48 03 77 77
Fax: +33-1-40 35 17 13

INSTITUTO NACIONAL DE PESQUISAS DA AMAZONIA
Alameda Cosme Ferreira 1756
BR 69011 Manaus
Brazil
Ferraz
Tel.: +55-92-642 21 18
Fax: +55-92-642 21 18

UNIVERSITY OF AARHUS
Aarhus
Denmark
Baslev
Tel.: +45-86-12 51 77
Fax: +45-86 13 99 19

Contract number: TS3*CT910010

Period: February 1992 to January 1996

COAT PROTEIN MEDIATED RESISTANCE OF SOLANUM TUBEROSUM AND NICOTIANA TABACUM TOWARDS ANDEAN POTATO MOTTLE VIRUS

Co-ordinator: Rijksuniversiteit Gent, Ghent, Belgium (Marc Van Montagu)

Objectives

- Determination of the level of resistance obtained through the expression of the APMV coat proteins (CPs) in tobacco and potato.
- Comparison of the effectiveness of developing resistance by expressing one or both coat proteins in the host plant.
- Outline a general approach towards engineering resistance against viruses with multiple coat proteins.
- Determination of the protection of the transgenic plants against other viruses.
- Development of a detection kit for APMV presence by applying "Polymerase Chain Reaction" technology.
- Development of double transgenic plants through crossing of CP plants with "defence-related genes" expressing transgenic lines.

Activities

* Cloning the coding sequence of both CPs in plant expression vectors, separately and combined.
* Developing a regeneration protocol for the potato cultivar Delta, and transformation of the above mentioned constructs.
* Characterization of the transgenic plants (with one or both CPs) and scoring their resistance towards APMV infection.
* Scoring resistance of transgenic plants co-expressing viral CPs and common defense genes (such as ß-1,3-glucanases, a proline-rich cell wall protein HRGP, a pathogen-related protein - PRms of the PR1 family), towards infection with APMV and other viruses.
* Development of a detection kit for APMV using PCR technology.

Expected outcome

⇒ The project will lead to the understanding of the role of the amount of the CPs in the resistance mechanism against viruses, and consequently to the outlining of a general approach towards engineering resistance against viruses with multiple coat proteins.

⇒ Developing resistance towards APMV will substantially increase the yield of important crops such as potato, tomato and eggplant (up to 20% loss due to APMV infection) and reduce the need for insecticides which are detrimental for the environment. The combination with common defence-related genes might improve the expected yield increases. The development of a detection kit for APMV is a test case to be used to work out kits for other viruses.

Selected publications

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Brioso P.S.T. 1995. Caracterização Viral, detecção de virus vegetais e identificação de fonte de resistencia. PhD thesis presented to the Department of Genetics, Federal University of Rio de Janeiro, Brazil, March 1995. Promotor: Prof. D. E. de Oliveira.

Partners

RIJKSUNIVERSITEIT GENT
Laboratorium voor Genetica
K.L. Ledeganckstraat 35
B-9000 Gent
Belgium

Marc Van Montagu
Tel.: +32-91-64.51.70
Fax: +32-91-64.53.4

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
Departamento de Genetica
Instituto de Biología
Caixa Postal 68011
21941 Rio de Janeiro
Brazil

Dulce Eleonora de Oliveira
Tel.: +55-21-590.01.11
Fax: +55-21-590.01.11

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Centro de Investigación y Desarrollo
Jordi Girona 18
E-8034 Barcelona
Spain

Pedro Puigdomenech
Tel.: +34-93-204.06.00
Fax: +34-93-204.59.04

Contract number: TS3*CT910014

Period: March 1992 to August 1996

GENETIC IMPROVEMENT OF BANANA FOR LOCAL CONSUMPTION AND FOR EXPORT, WITH REFERENCE TO CERCOSPORIOSIS RESISTANCE

Co-ordinator: CIRAD-FLHOR, Montpellier, France (Hugues Tezenas du Montcel))

Objectives

- Select sweet-banana and plantain cultivars resistant to *Mycosphaerella fijiensis*, causal agent of black-sigatoka disease which is a very serious threat. Assess these cultivars vis-à-vis other diseases and pests.
- Better understand banana genetics and resistance mechanisms.
- Develop traditional breeding based on this understanding and integrate non-conventional techniques.

Results

⇒ This project has enriched the *in vivo* collections at CRBP and CIRAD. The majority of the diploid germplasm introduced came from Papua New Guinea and Vietnam. This was characterised using isozymatic, molecular markers.

⇒ Two successive maps were constructed; based on two different segregating populations. Using these two maps a composite map including 157 loci was drawn up. This map will be used to locate any QTL of interest to breeders.

⇒ CIRAD's strategy to create triploid hybrid varieties was confirmed.

⇒ Three AAB hybrids of the sweet type, resistant to black sigatoka disease, are undergoing commercialization trialsin various countries.

⇒ At CRBP, tetrapoloid hybrids of plantain resistant to black sigatoka disease- were created using the M53 male parent.

⇒ Systems for cell establishment and regeneration were developed to be used in transgenesis.

⇒ Conformity in the field of "Grande Naine" (Big Dwarf) banana from somatic embryogenesis was confirmed.

⇒ Stable transformants of 'Grande Naine' and dark French were obtained.

⇒ Somatic fusion (but without plant regeneration) was obtained.

⇒ *Mycosphaerella fijiensis* populations were characterized, and the variability of these populations was noted.

⇒ In addition, the project also has led to:

- –14 doctoral theses, 15 university degrees and training opportunities for several students
- –4 meetings among the project participants for discussing results
- – presentation of the results by members of the project team in more than 10 international seminars
- – publication of 34 articles and 62 communications.

Selected publications

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Partners

CIRAD-FLHOR
B.P. 5035
F-34032 Montpellier cedex
France
Hughues Tezenas du Montcel
Tel.: +33-4-67 61 58 60
Fax: +33-4-67 61 58 71

FAC. UNIV. DES SC. AGRONOMIQUES DE LA COMMUNAUTE FRANÇAISE DE BELGIQUE
Avenue du Maréchal Juin 13
B-5030 Gembloux
Belgium
Philippe Lepoivre
Tel.: +32-81-62 24 34
Fax: +32-81-61 01 26

CIRAD GERDAT
Unité de recherche BIOTROP
B.P. 5035
F-34032 Montpellier cedex 1
France
Jacques Schwendiman
Tel.: 33-67-61 58 29
Fax: 33-67-61 57 92

UNIVERSITE DE PARIS SUD
Bât. 360
F-91405 Orsay cedex
France
Line Rossignol
Tel.: +33-1-69 41 70 49
Fax: +33-1-64 46 19 92

CENTRO AGRONOMICO TROPICAL DE INVESTICAGION Y ENSEÑANZA
CR-7170 Turrialba
Costa Rica
Jean-Vincent Escalant
Tel.: +506-56 01 69
Fax: +506-56 15 33

CENTRE REGIONAL BANANIERS ET PLANTAINS
Boîte postale 832
Douala
Cameroon
Christophe Jenny
Tel.: +237-42-71 29
Fax: +237-42-57 86

KATHOLIEKE UNIVERSITEIT LEUVEN
Oude Markt 13
B-3000 Leuven
Belgium
Rony Swennen
Tel.: +32-16-22 09 31
Fax: +32-16-22 00 98

RESEAU INTERNATIONAL POUR L'AMELIORATION DE LA BANANE ET LA BANANE PLANTAIN
Parc Scientifique Agropolis, Bât. 7
Boulevard de la Lironde
F-34980 Montferriez-sur-Lez
France
David Jones
Tel.: +33-67-61 13 02
Fax: +33-67 61 03 34

Contract number: TS3*CT910021

Period: April 1992 to August 1994

NUTRIENT CYCLING AND SUSTAINABILITY IN ALLEY-CROPPING SYSTEMS IN THE HUMID TROPICS: II : PHOSPHORUS, LABILE SOIL ORGANIC PHOSPHORUS AND BASE-CATIONS

Co-ordinator: University of Cambridge, Cambridge, United Kingdom
(Timothy Bayliss-Smith)

Objectives

- Maintain the experimental alley-cropping (AC) and open field (control) plots in lowland tropical-rain-forest sites in Costa Rica that were established under the original STD2 funding (1988-92).
- In both AC and control plots, to analyse the cycling of phosphorus, by means of ecosystem budgets and isotopic root-uptake studies.
- Investigate the potential role of nutrient supplements in AC systems, in particular base cations, so as to provide guidelines for sustainable land use on acid, leached soils.
- Experiment further on the role of permanent mulch cover in the context of mycorrhizal and free-living microbiota and their phosphatase activity.

Activities

We originally worked on two sites, La Conquista, Sarapiqui (LaC), and Co-ope San Juan, San Carlos (CSJ), but from 1992 onwards the research focused on CSJ. Here the AC trials have been monitored continuously for 5 years, following an initial slash-and-burn operation in 1989. The whole CSJ site covered 2 ha and corresponded to the size and scale of a family holding. It comprised 8 AC and 8 control plots, each with or without additions of rock phosphate, plus supplementary plots and experiments. We intentionally maintained an intense and stressful regime of two crops per year, with maize and beans alternating (a simple analogue of indigenous practice), together with 4 prunings per year in the alley plots.

01/02. Cambridge : project co-ordinator T. Bayliss-Smith was based in U.K. while Research Associate M.R. Hands was stationed in Costa Rica organising the field trials, managing the local labour force and overseeing the lab work at UCR.

03. ITE : A.F. Harrison and J. Dighton conducted fieldwork at CSJ on isotopic root uptake in mid-1993.

04. Granada : R. Azcon visited Costa Rica in mid-1993 to liaise with UCR project researcher M. Bermudez on the on-going experiments on VAM mycorrhizae, using soils from the experimental sites at CSJ.

05. UCR : A. Alvarado, Director of Centro de Investigaciones Agronomicas, liaised with M.R. Hands on the facilities needed for the ITE work on isotopes and for analysis of soils and biomass at University of Costa Rica.

Expected outcome

The environmental context in Costa Rica is typical of rain forests in the lowland humid tropics : acid, leached latasols and a high rainfall. The project is about the sustainability of alley cropping for small farmers in this environment : to what extent is this from of agroforestry, using minimal inputs, a viable alternative to shifting cultivation ? We aim to gain an understanding of the cycling of phosphorus, with or without the benefit of tree mulch from the AC hedgerows, in sustaining the productivity of agricultural systems in the tropical rain forest zone. There are anticipated benefits to development planning as well as to our scientific knowledge of this important agro-ecosystem

Partners

UNIVERSITY OF CAMBRIDGE
Department of Geography
Downing Place
UK-CB2 3EN Cambridge
United Kingdom

Timothy Bayliss-Smith
Tel.: +44-1223-33.33.78
Fax: +44-1223-33.33.92

UNIVERSIDAD DE COSTA RICA
Centro de Investigaciones Agronómicas
Ciudad Universitaria
San José
Costa Rica

Freddy Sancho
Tel.: +506-24.37.12
Fax: +506-34.16.27

INSTITUTE OF TERRESTRIAL ECOLOGY
Merlewood
Grange Over Sands
Cumbria
United Kingdom

Anthony Harrison
Tel.: +44-15395-322.64

ESTACION EXPERIMENTAL DEL ZAIDIN
Profesor Alvarado 1
E-18008 Granada
Spain

José Miguel Barea
Tel.: +34-58-12.10.11
Fax: +34-58-12.96.00

Contract number: TS3*CT920017

Period: April 1992 to March 1996

FARMER STRATEGIES AND PRODUCTION SYSTEMS IN FRAGILE ENVIRONMENTS IN MOUNTAINOUS AREAS OF LATIN AMERICA

Co-ordinator: University of Leeds, Leeds, United Kingdom (David Preston)

Objectives

- Identify and measure physical components of environmental deterioration at different geographical levels - from field to physiographic region.
- Identify the present and historical links between production systems, social structures and environmental deterioration in relation to agricultural production and food provision in selected areas in Honduras and Bolivia.
- Identify and explain reasons for differences in land uses and levels of production in similar physical environments at a household, community and regional level.
- Identify and evaluate, according to locally and scientifically-acceptable criteria, those existing and novel farming practices most capable of facilitating sustainable food production.

Activities

* Collection of basic socio-economic data on production systems in the study areas to place land use decisions in a broader context. The collection of basic information about locally-perceived environmental problems and ways of overcoming them.
* The establishment of a sample of farmers to be studied in more depth and through whom a close monitoring of activity over three years will provide data on land use decisions and evidence of environmental problems as well as a record of changes in physical soil characteristics.
* The study of evidence of vegetation change and association with particular land use strategies in order to develop a model of vegetation degradation.
* The identification of key issues to be examined in detail to reveal structural pressures on land users in the recent past.

Throughout the period of work, there was a continuous dialogue with rural people in the communities being investigated to identify a set of practices, appropriate to each ecological situation, that facilitate sustainable food production and the provision of an acceptable level of living.

Expected outcome

Our research will enable a much deeper understanding than has hitherto been possible of the links between physical environmental change and household strategies. In particular it should document the extent to which apparent environmental deterioration is a consequence of current land use management. It will also enable to recording of the range of land use practices that most contribute to sustainable production systems.

Selected publications

David Preston and Lorraine Clewer. 1993. Wine production in a marginal area: Tarija wine in the 1990s. Journal of Wine Research. **4(3)**: 227-232.

Kees Jansen. 1995. Ecological degradation in the production of food and export crops in north-west Honduras. In : M. Mörner & M. Rosendal (eds.) Threatened peoples and environments in the Americas (University of Stockholm, Institute of Latin American Studies) 161-189.

Partners

UNIVERSITY OF LEEDS
School of Geography
Woodhouse Lane
UK-LS2 9JT Leeds
United Kingdom

David Preston
Tel.: +44-113-233.33.43
Fax: +44-113-233.33.08
E-mail: d.a.preston@leeds.ac.uk

WAGENINGEN AGRICULTURAL UNIVERSITY
Dept. of Sociology of Rural Development
Hollandseweg 1
NL-6707 KN Wageningen
Netherlands

Norman Long
Tel.: +31-317-48.20.75
Fax: +31-317-48.40.37

UNIVERSIDAD DE VALENCIA
Departamento de Geografía
Apartado 22060
Valencia
Spain

Adolfo Calvo
Tel.: +34-6-386.42.30
Fax: +34-6-386.42.49

UNIVERSIDAD NACIONAL AUTONOMA DE HONDURAS
Postgrado Centroamericano en Economía y Planificaciòn del Desarrollo
Apartado 1748
Tegucigalpa, D.C.
Honduras

Rafael del Cid
Tel.: +504-32.21.10
Fax: +504-31.32.89

UNIVERSIDAD MAYOR DE SAN ANDRES LA PAZ
Instituto de Ecología
Casilla 10077 Correo Central
La Paz
Bolivia

Maximo Liebermann
Tel.: +591-2-79.25.82
Fax: +591-2-39.11.76

Contract number: TS3*CT920061

Period: January 1993 to October 1996

IRRIGATION WATER MANAGEMENT AND SALINIZATION: INTERCOMPARISON OF SIMULATION MODELS IN ARGENTINA AND EGYPT

Co-ordinator: DLO Winand Staring Centre for Integrated Land, Soil and Water Research, Wageningen, The Netherlands (Massimo Menenti)

Objectives

- Compare two models (SIWARE and TUNIN) that describe the regional aspects of salt and water balance in an entire irrigation scheme, and two models (BIWASA and SWASALT) that describe coupled salt and water flow in soil profiles, considering accuracy and sensitivity to input data and to the schematization of the irrigation and drainage systems.
- Assess the practical applicability of these models to the context of water administration practices in Argentina and Egypt.

Activities

- ***Comparison of the models BIWASA and SWAP.*** Two field tests were carried out in Argentina, near Lavalle (Mendoza) and in Egypt near Zagazig. At both sites, the data needed to apply and validate the models were collected. Soil hydrological properties were determined on undisturbed soil cores at the laboratories of the participating institutes in Argentina, Egypt and The Netherlands. The solute concentration in soil water was determined by extracting samples of soil water with tensiometers and vacuum pumps. The intercomparison study was based on four simulation studies, since each model was applied at both field sites.
- ***Comparison tests of the models SIWARE and TUNIN.*** The data needed to apply these models were collected for the irrigation system of the Rio Tunuyan Medio (Mendoza, Argentina) and for the eastern Nile delta. The model SIWARE was applied to both irrigation systems. The model TUNIN required the determination of irrigation efficiencies. Because of the limited scope of the investigation, volumes of irrigation water calculated with SIWARE were used instead to determine the required path efficiencies for the eastern delta case study.
- ***Analysis of water management practices in Spain, Argentina and Egypt.*** The physical, legal and administrative settings of irrigation in Spain, Argentina and Egypt were reviewed. Ongoing efforts to improve the effectiveness of water laws in these countries were addressed in the review.

Results

⇒ ***SWAP and BIWASA***: agreement of simulated soil water content with field measurements was good in all cases, while the vertical profile of solute concentration at the Lavalle test site could not be reproduced satisfactorily. A better parameterization of adsorption is necessary. Accurate determination of soil hydrological properties was also difficult, and part of the field observations were used for model calibration. Two-dimensional

(BIWASA) modelling was necessary to describe soil and water flow at the Lavalle test site, where lateral variability is significant due to furrow irrigation. Numerical models are useful for detailed comparison of irrigation strategies using indicators or irrigation performance.

⇒ ***TUNIN and SIWARE***: monthly irrigation volumes within the eastern delta irrigation system were reproduced with rather good accuracy, using the relatively simple TUNIN algorithm based on time-independent path efficiencies. The latter implies that irrigation water is allocated proportionally to the irrigated area. The detailed description of agrohydrological processes included in SIWARE is necessary in a limited range of situations where water volumes are not proportional to the irrigated area. The SIWARE case study in Argentina underscored the difficulty of determining all required input variables with sufficient accuracy at the level of spatial aggregation, on which this model was built

Partners

DLO WINAND STARING CENTRE FOR INTEGRATED LAND, SOIL AND WATER RESEARCH
Marijkeweg 11/22
P.O. Box 125
NL-6700 AC Wageningen
The Netherlands

Massimo Menenti
Tel.: +31-317-47 42 00
Fax: +31-317-42 48 12
E-mail: postkamer@sc.dlo.nl

INSTITUTO NACIONAL DE CIENCIA Y TECNICA HIDRICAS
Centro Regional Andino
Cas. Correo 6
Belgrano 210
RA-5500 Mendoza
Argentina

Jorge Luís Chambouleyrón
Tel.: +54-61-24 19 93
Fax: +54-61-38 02 51
E-mail: postmaster@inccra.edu.ar

INSTITUTO NACIONAL DE REFORMA Y DESARROLLO AGRARIO
Paseo de la Castellana 112
E-28071 Madrid
Spain

Julián Martínez Beltrán
Tel.: +34-91-347 18 39
Fax: +34-91-564 52 35
411 37 70

UNIVERSITY OF CAIRO
Faculty of Engineering
Dept. of Irrigation and Hydraulics
Gizeh, Cairo
Egypt

Mohamed El Sherbiny M. Kiwan
M.H. Salem
Tel.: +20-2-72 85 32 ext. 3125
Fax: +20-2-72 70 09
E-mail: kiwan@cairo9.eg

DRAINAGE RESEARCH INSTITUTE
Qanater 1362/5
Cairo
Egypt

Shaden Abdel Ghawad
Tel.: +20-2-95 93 83
Fax: +20-2-95 91 53

Contract number: TS3*CT920069

Period: October 1992 to September 1996

GENETIC IMPROVEMENT OF PHASEOLUS FOOD LEGUMES FOR THE LOWLAND AND HIGHLAND TROPICS OF COLOMBIA AND PERU

Co-ordinator: Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium (Jean-Pierre Baudoin)

Objectives

- Exploit the genetic potential of the food legumes *Phaseolus lunatus* and *P. polyanthus* in the lowland and highland areas of the two countries.
- Develop improved cultivars of *P. vulgaris* from hybridization with *P. polyanthus* resistant to *Phoma exigua* var. *diversispora* (causing *Ascochyta* blight).
- Refine the *in vitro* culture techniques of immature *Phaseolus* embryos, and create new interspecific hybrids.
- Search for DNA molecular markers linked with the genes of resistance to *Phoma* in *Phaseolus*.

Activities

- Study of the genomes of *P. Polyanthus* and other related taxa, using DNA restricton polymorphism and allozyme markers in Gembloux (Belgium).
- Refinement of *in vitro* culture of early heart-shaped embryos and development of new interspecific hybrids with *P. vulgaris* in Gembloux (Belgium), Rio Negro (Colombia), Lima (Peru).
- Screening for field resistance to *Phoma* in Colombia and Peru, and DNA fingerprinting of *Phoma* and *Phaseolus* in Bonn (Germany).
- Breeding of interspecific hybrids of *P. vulgaris* in highland stations of Colombia and Peru, using recurrent selection schemes and multiple cropping systems.
- Breeding of *P. lunatus* to overcome the major agronomical constraints in Colombia and Peru.

Results

⇒ The biochemical and molecular characterization of *Phaseolus* allowed the determination of the phyletic relationship between taxa, to orient interspecific hybridization and to define in *P. lunatus*, a secondary gene pool made of Andean wild *Phaseolus* species.

⇒ Field inheritance studies revealed a monogenic resistance, with complete and partial dominance, to *Ascochyta* blight. *P. polyanthus* is characterized by a high level of field resistance to this disease.

⇒ A new technique to rescue early heart-shaped embryos was developed, using two successive media: one for maturation-germination, and one for rooting. Application of this technique allowed to obtain more than 20 new interspecific combinations and several hybrids in the recombination nursery of the recurrent selection scheme adopted for interspecific crosses (*P. vulgaris* x *P polyanthus* and *P. vulgaris* x *P. coccineus*).

⇒ Improved varieties of P. polyanthus and P. lunatus were created for the traditional cropping systems in highland and lowland areas of the Andes.

Follow up

- Total and chloroplastic genome analyses were carried out to specify the genetic organization at intra- and interspecific levels of *Phaseolus*.
- The new improved *Phaseolus* varieties are being tested in traditional cropping systems of Andean regions, with a view to determining appropriate crop husbandry, particularly in intercropping systems (such as maize or bean).

Selected publications

Schmit V., du Jardin P., Baudoin J.P., Debouck D.G.D. 1993. Phylogenetic study of seven *Phaseolus* taxa, namely *P. vulgaris* and *P. coccineus*, using chloroplast DNA. Theor. Appl. Genet. **87**:506-516.

Baudoin J.P., Camarena F., Lobo M. 1995. Amélioration de quatre espèces de légumineuses alimentaires tropicales: *Phaseolus vulgaris*, *P. coccineus*, *P. polyanthus*, et *P. lunatus*. Sélection intra- et interspécifique. *In*: Quel avenir pour l'amélioration des plantes? J. Dubois, Y. Demarly (eds.) AUPELF-UREF. John Libbey. Eurotext, paris. pp. 31-39.

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Maquet A., Zoro Bi.I., Delvaux M., Wathelet B., Baudoin J.P. 1997. Genetic structure of a Lima-bean base collection, using allozyme markers. Theor. Appl. Genet. **95**: 980-991.

Mergeai G., Schmit V., Lecomte B., Baudoin J.P. 1997. Mise au point d'une technique de culture *in vitro* d'embryons immatures de *Phaseolus*. Biotechnol. Agron. Soc. Environ. **1**: 49-58.

Partners

FAC. UNIV. DES SCIENCES AGRONOMIQUES DE GEMBLOUX
Unité de Phytotechnie des régions intertropicales
Passage des Déportés 2
B-5030 Gembloux
Belgium

Jean-Pierre Baudoin
Tel.: +32-81-62 21 12
Fax: +32-81-61 45 44
E-mail: baudoinjp@fsagx.ac.be

CORPORACION COLOMBIANA DE INVESTIGACION AGROPECUARIA
C.I. La Selva
A.A. 470
Rionegro (Antioquia)
Colombia

Mario Lobo
Tel.: +57-4-537 00 04
Fax: +57-4-461 20 82

UNIVERSIDAD NACIONAL AGRARIA LA MOLINA
Programa Leguminosas y Oleaginosas
Apartado 456
1 Lima
Peru

Felix Camarena Mayta
Tel.: +51-1-435 20 35
Fax: +51-1-435 20 73
E-mail: camafe@lamolina01.lamolina.edu.pe

RHEINISCHE FRIEDRICH-WILHELMS UNIVERSITAET BONN
Institut für Genetik
Abt. Biochemische Genetik
Kirschallee 1
D-5300 Bonn
Germany

Milan Höfer
Tel.: +49-228-73 55 19
Fax: +49-228 73 55 45
E-mail: unb121@ibm.rhrz.uni-bonn.de

Contract number: TS3*CT920071

Period: January 1993 to June 1996

ADAPTATION OF MAIZE TO ACID SOILS OF THE TROPICS

Co-ordinator: Universität Hannover, Hanover, Germany (Walter Horst)

Objectives

The overall objective of the project was to improve and enhance the breeding of maize cultivars adapted to acid soils of the tropics through:

- identification of the soil factors most limiting maize growth in selected acid soils in Brazil, Cameroon and Guadeloupe
- better understanding of the physiological, morphological, biochemical mechanisms of Al toxicity, Al- and acid-soil resistance
- development of quick laboratory screening procedures reflecting the performance of maize cultivars on acid soils in the field.

Activities

- Morphological and physiological responses of maize plants to Al
- Physiology of Al resistance
- Screening for Al resistance
- Screening and breeding for adaptation to acid soils of the tropics

Results

⇒ Inhibition of root elongation by Al, induction of callose formation in root tips, and the hematoxylin staining of Al in root tips are parameters of Al sensitivity which can easily be assessed in hydroponically-grown maize seedlings. Which of these techniques is the most appropriate to be incorporated into a breeding programme for adaptation of maize to acid soils needs to be established. Studies with the objective to further characterize a physiological/biochemical marker of Al resistance have to be intensified.

⇒ Although Al resistance is a prerequisite for adaptation to acid soils at most locations, this study clearly showed that, at some locations, other factors of the acid soil complex may be equally or even more important. Interaction of Al resistance with factors such as Mn toxicity, P deficiency and H^+ toxicity need to be taken into consideration in the improvement of screening techniques for acid soil tolerance.

⇒ Acid-soil-resistance traits from exotic germplasm can be and need to be incorporated into locally otherwise adapted germplasm. This, however, requires quick and reliable screening techniques allowing the evaluation of large numbers of genotypes.

Follow-up

The activities are pursued with a modified focus in the INCO research project fitting maize into cropping systems on acid soils of the tropics (ERBIC 18CT960063)

Selected publications

Barcelo J., Poschenrieder Ch., Vásquez M. D., Gunsé B. 1996. Aluminium phytotoxicity. A challenge for plant scientists. Fertilizer Research **43**, 217-223.

Calba H., Jaillard B., Fallavier P., Arvieu J.-C. 1996. : Agarose is a suitable substrate for use in the study of Al dynamics in the rhizosphere. Plant and Soil, **178**, 67-74.

Horst W.J., Schmohl N., Püsche, A.K. 1997. Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. Plant and Soil. **192**, 23-30,

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Pintro J.C., Barloy P., Fallavier P. 1996. Aluminium effects on the growth and mineral composition of corn plants cultivated in nutrient solution at low aluminium activity. J. Plant Nutr. **19**, 729-741.

Partners

UNIVERSITAET HANNOVER
Institut für Pflanzenernährung
Herrenhaeuserstrasse 2
D-3000 Hannover 21
Germany

Walter Horst
Tel.: +49-511-762 26 26
Fax: +49-511-762 30 15

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
Laboratoire d'Agronomie
65 rue de Saint-Brieuc
F-35042 Rennes
France

Jean Barloy
Tel.: +33-99-28 54 71
Fax: +33-99-28 75 10

UNIVERSIDAD AUTONOMA DE BARCELONA
Facultad de Ciencias
Laboratorio de Fisiología Vegetal
E-08193 Bellaterra
Spain

Juán Barceló
Tel.: +34-93-581 12 67
Fax: +34-93-581 20 03

CIRAD CA
Département GERDAT
BP 503.5
F-34032 Montpellier cedex 1
France

Paul Fallavier
Tel.: +33-4-67 61 58 22
Fax: +33-4-6761 59 86

INSTITUT DE RECHERCHE AGRONOMIQUE DU CAMEROUN
Dept. of Plant Breeding and Agronomy
BP 2123
Yaoundé
Cameroon

Charles The
Tel.: +237-23 35 38
Fax: +237-22 18 73

CIRAD CA
Station d'Amélioration des Plantes
F-97184 Pointe-à-Pitre
Guadeloupe

Danièle Clavel
Tel.: +590-25 59 45
Fax: +590-25 59 24

UNIVERSIDADE ESTADUAL DE LONDRINA
Departamento do Agronomía
VR-BP 6001 Londrina – Parana
Brazil

Maria de Fatima Guimarães
Tel.: +55-43-221 20 00
Fax: +55-43-227 69 32

Contract number: TS3*CT920091

Period: December 1992 to November 1997

EVALUATION OF LOCAL POULTRY RESOURCES FOR CREATING GENETIC STOCK WITH IMPROVED ADAPTABILITY, PRODUCTIVITY AND DISEASE RESISTANCE FOR TROPICAL ENVIRONMENTS

Co-ordinator: Technische Universität Berlin, Berlin, Germany (Peter Horst)

Objectives

- Utilization of local genic resources and identification of valuable characteristics such as productive adaptability, disease tolerance and special qualities for market preferences.
- Genetic improvement by complementing special fitness characteristics of local genotypes with potential productivity with high-yield exotic gene material. The targets were:
  - Evaluation of local fowl strains in different continents with respect to major gene-related external characteristics, productive performance and disease resistance.
  - Identification at genomic level, genetic variation *within* and genomic differentiation *between* local strains.
  - Exploitation of these gene resources to develop genetic stock with high adaptability, productivity and disease resistance under the existing environmental conditions.
  - Test of crossbreeding effects on performance under unfavourable conditions by introducing a dual-purpose exotic strain and to search for the use of distinct tropical major genes in those crossbreeds as a basis for further decisions on autonomous breeding systems.

Activities

Since the purpose of the project was to incorporate widely distributed populations, most work was carried out by local partner institutions in Asia, Africa and South-America. Collection of material, multiplication of stock and performance testing was done overseas, and the main laboratory work and training was done in Europe. The activities were the following:

- Screening of local populations and evaluation of special characteristics: in each developing country a survey was conducted on the different ecotypes to investigate morphological features, special characteristics, beliefs about biological role, availability of major genes and special use by the local populations in the regions concerned.
- Investigation of the disease-resistance status of local populations through investigating humoral and cellular immunity responses in birds. An overall immunity-competence index was created to evaluate the different population tested.
- Estimation of genetic distances and genetic variability within populations. This was done by DNA fingerprint and microsatellite-marker systems. In combining those results, a dendrogram of all genotypes was established, displaying the actually existing genetic differentiation.

* Upgrading of local populations to improve productive adaptability and disease resistance. This was achieved by crossing the selected local populations with a high-yield promoter line. The local, exotic and F1 crosses were tested simultaneously for various performance traits. Selected F1s will be back-crossed with the exotic high-yield population. The best fitting genetic groups have been investigated further to verify the desirable characteristics of adaptability and disease resistance.

Selected publications

Agbede G., Demey F., Verhulst A. and Bell G.J. 1995. Divergent selection for specific immune reactions and resistance to Newcastle disease in native chickens of Cameroon. In: Advances in Avian Immunologie Research, Reading: 111-117.

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Rodríguez-Palacios Z. 1997. Caractérisation hématologique et immunologique des poules locales Boliviennes, des poules exotiques GDR et de la descendance de leur croisement. IMT M.Sc. thèse, pp. 52.

Singh,V.K. 1997. Studies on some economic parameters of various Indian native breeds of chickens and their crosses with Dahlem Red (Exotic Breed). M.V.Sc. Thesis, submitted to Rajendra Agricultrural University, PUSA (Samastipur), Bihar, India.

Partners

HUMBOLDT UNIVERSITY OF BERLIN
Institute of Animal Sciences
Lentzeallee 75
D-14195 Berlin
Germany

Peter Horst
Tel.: +49-30-31.47.11.20
Fax: +49-30-31.47.14.26
+49-30.31.47.13.01
E-mail: czarnetz@elster.iae.tu-berlin.de

PRINCE LEOPOLD INSTITUTE OF TROPICAL MEDICINE
Nationalestraat 155
B-2000 Antwerpen
Belgium

Felix Demey
Tel.: +32-3-247.63.92
Fax: +: 32-3-216.14.31
E-mail: animprod@vet.itg.be

CENTRAL AVIAN RESEARCH INSTITUTE
Avian Genetics and Breeding Division
Izatnagar (U.P.)
IND-243 122 Bareilly
India

Dinesh Pased Singh
Tel.: +91-581-44.64.20
Fax: +91-581-44.73.21

OBAFEMI AWOLOWO UNIVERSITY
Department of Animal Science
Ile-Ife
Nigeria

Emmanuel Sonaiya
Tel.: +234-36-23.02.90
Fax: +234-36-23.13.20
E-mail: fsonaiya@oauife.edu.ng

UNIVERSIDAD TECNICA DE ORURO
Casilla 49
Oruro
Bolivia

C. Fausto Choque and O. Iñíguez
Tel.: +591-52.616.45
Fax: +591-52.422.15

Contract number: TS3*CT920093

Period: February 1993 to July 1997

THERMOCHEMICAL UPGRADING OF BIOMASSES TO GASEOUS AND LIQUID FUELS AND FEEDSTOCKS

Co-ordinator: European Centre for Coal Specimens SBN, Eygelshoven, The Netherlands
(A.M.H. Van Der Veen)

Objectives

- The project aimed to investigate the development of technologies for the thermochemical conversion of biomass for energy production in developing countries with a positive effect on the environment.
- The conversion of biomass was investigated into two directions: (hydro)pyrolysis/ hydrocracking and gasification.

Activities

* Preparation of samples from feed materials, catalysts, and substrates and long term storage of the various samples for future research
* Characterization of feed materials, intermediates and final products
* Hydropyrolysis / hydrocracking experiments - Atmospheric fluidized bed gasification experiments
* Fluidized bed pyrolysis experiments.

The experiments were carried out with wood residues and residues from sugar cane. Several catalysts were tested during the course of the project.
The characterization of the solid materials included chemical composition, SEM analyses, and surface area determinations. Liquid products were analyzed by means of GC-MS, UV-fluorescence spectroscopy, and size exclusion chromatography.

Expected outcome

The project provided the scientific community with well-characterized samples of biomass feedstocks and catalysts for the upgrading of these feedstocks. The engineering experiments led to an improvement of existing techniques for the thermochemical upgrading of these feedstocks into different directions (gasification, pyrolysis, and hydropyrolysis). These improvements will lead to a more efficient use of biomass feedstocks for long-term power generation and for use as transport fuel. This kind of technology is of special interest to developing countries, since they do not have to import (expensive) fossil fuels for their energy consumption.

Partners

EUROPEAN CENTRE FOR COAL SPECIMENS SBN
Rimburgerweg 2
NL-6471 Eygelshoven
The Netherlands
A.M.H. Van Der Veen
Tel.: +31-455-35.20.11
Fax: +31-455-46.56.53

IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE
Dept. of Chemical Engineering and Chemical Technology
Prince Consort Road
UK-SW7 2BY London
United Kingdom
Rafael Kandiyoti
Tel.: +44-171-589.51.11
Fax: +44-171-584.11.70

UNIVERSIDAD DE CONCEPCION
Departamento de Ingeniería Química
Casilla 53-C, Correo 3
Concepción
Chile
Alfredo L. Gordon
Tel.: +56-41-23.49.85 - Ext. 2534
Fax: +56-41-24.02.80

UNIVERSITY OF ALEXANDRIA
Faculty of Science
Alexandria
Egypt
Mohamed Y Bakr
Tel.: +20-3-545.45.35
Fax: +20-3-595.05.20

Contract number: TS3*CT920094

Period: July 1992 to June 1995

DEVELOPMENT OF AN INTEGRATED SYSTEM TO CONTROL BEAN DISEASES IN TROPICAL AND SUBTROPICAL REGIONS

Co-ordinator: Universität Hannover, Hanover, Germany (Bernhard Hau)

Objectives

- Establish single and multiple disease-loss relationships for field situations and quantify the effects of control measures on disease progression.
- Design a system for integrated control of the following bean diseases: bean rust (*Uromyces appendiculatus*), angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), Fusarium yellows (*Fusarium oxysporum* f. sp. *phaseoli*), Rhizoctonia root rot (*Rhizoctonia solani*), and bean golden mosaic (GMV).

Activities

* Field experiments with leaf diseases in Piracicaba and Viçosa (Brazil), and in Hanover (Germany).
* Microplot experiments with soil-borne diseases in Córdoba (Spain), in order to determine inoculum density and disease incidence relationships.
* Growth chamber experiments for leaf diseases in Piracicaba in order to determine monocyclic disease parameters, and for soil-borne diseases in Córdoba, to investigate the effects of temperature on disease development for single and multiple diseases.
* Construction of computer models for single diseases and for disease complexes including their effects on the growth and yield of beans, carried out in Hanover (Germany).

Results

⇒ For reliable disease assessment, diagrammatic scales were developed for bean rust, angular leaf spot, anthracnose and bean golden mosaic. In all field experiments, disease parameters, like the area under disease progress curve, were not, or only weakly, correlated with yield parameters. The diseases did not only reduce the photosynthetic active leaf area by their lesions, but affected the growth of the bean plant by dropping diseased leaves (angular leaf spot) or reducing further plant growth (bean rust). Therefore, host parameters reflecting these effects, for instance the leaf area index duration, were more closely related to yield and yield loss.

⇒ Under controlled conditions, monocyclic parameters of the fungal diseases were determined and used, to model the disease dynamics. Monocyclic parameters of pathogens can be altered if other diseases interfere, as shown for the effects of a preinfection by the bean line-pattern mosaic virus on the parameters of *U. appendiculatus* and *P. griseola*. Interactions between diseases were also observed in microplot experiments in Spain, in which *F. oxysporum* and *R. solani* showed antagonistic effects.

⇒ Based on the experimental results, a coupled model was developed, that can describe host and disease development simultaneously. For bean rust and angular leaf spot, host area

and the initial part of the disease progress curves can be simulated satisfactorily, but the decline of diseases due to removals of leaves has to be improved.

⇒ For the application of an integrated system, methods were tested with a view to facilitating the assessment of host and disease parameters, for instance by estimating disease severity with a modified disease incidence or by assessing host area with a multiple linear equation based on the number of leaves and the mean disease severity.

Selected publications

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Partners

UNIVERSITAET HANNOVER
Institut für Pflanzenkrankheiten und Pflanzenschutz
Herrenhaeuserstrasse 2
D-30419 Hannover
Germany
Bernhard Hau
Tel.: +49-511-762.35.03
Fax: +49-511-762.30.15
E-mail: bernhard.hau@mbox.ipp.uni-hannover.de

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Instituto de Agricultura Sostenible
Apartado de Correos 4084
E-14080 Córdoba
Spain
Rafael M. Jímenez Díaz
Tel.: +34-57-499.222
Fax: +34-57-499.252
E-mail: ag1jidir@lucano.uco.es

UNIVERSIDADE DE SAO PAULO
Escola Superior „Luiz deQueiroz"
Departamento de Fitopatología
Avenida Padua Días 11
BR-13418-900 Piracicaba-SP
Brazil
Armanda Bergamín Filho
Tel.: +55-194-29.42.67
Fax: +55-194-34.48.39
E-mail: abergami@carpa.ciagri.usp.br

UNIVERSIDADE FEDERAL DE VICOSA
Departamento de Fitopatología
Campus UFV
BR-36571-000 VICOSA - MG
Brazil
Francisco X. R. Do Vale
Tel.: +55-31-899.26.20
Fax: +55-31-899.22.40
E-mail: dovale@mail.ufv.br

Contract number: TS3*CT920098

Period: March 1993 to August 1997

BIOCONTROL OF DAMAGING ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPP.) PESTS OF STAPLE FOOD AND CASH CROPS BY INDUCING SUPPRESSIVE SOILS WITH THE BACTERIAL PARASITE *PASTEURIA PENETRANS*

Co-ordinator: Scottish Crop Research Institute, Dundee, United Kingdom
(David Trudgill)

Objectives

- Determine the distribution/importance of root-knot nematodes (RKN) in selected tropical countries and levels of parasitism with *P. penetrans*.
- Test effects of crop rotation on levels of parasitism of RKN with *P. penetrans*.
- Examine methods of managing RKN and effects on yields of susceptible crops.
- Investigate factors involved in the specificity and effectiveness of *P.penetrans*.

Results (in Ecuador)

⇒ 205 of 207 vegetable crops surveyed (10 plants/crop) were RKN infested. Average gall indices (on 1 to 10 scale) were 6.0 (Coastal region), 5.4 (Highland), and 4.8 (Orient), indicating 30 to 40% yield losses. Isozyme analyses (ORSTOM) showed 85% were *M. incognita*. Infection with *P. penetrans*. ranged from 52% in the Coastal region to only 9% in the Highlands. *P. penetrans* occurred in all soil types.

⇒ In a micro-plot trial on RKN infested soil, an exotic *P. penetrans* (from NRI) was added to soil already containing a low indigenous population of *P. penetrans*. After six cropping cycles, gall indices had been significantly decreased from a mean of 8.5 to 5.1, parasitism of RKN juveniles had been increased from 22% to 97% and yields of tomato increased by 32%. In a rotational field trial, *P. penetrans* integrated with groundnuts gave higher tomato yields, in the fifth cropping cycle, than maize or beans.

⇒ The existence of a very serious and widespread RKN problem was demonstrated on vegetable crops in Ecuador: It is likely that the introduction of small amounts of an exotic isolate greatly increased the pathogenicity of the local isolate of *P. penetrans*, but this needs to be confirmed and developed as part of an integrated strategy involving appropriate resistant cultivars and non-host crops (e.g. ground nuts).

Selected publications

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Partners

SCOTTISH CROP RESEARCH INSTITUTE
Zoology Department
Invergowrie, Dundee DD2 5DA
United Kingdom

David Trudgill
Tel.: +44-1382-56 27 31
Fax: +44-1382-56 24 26

NATURAL RESOURCES INSTITUTE
Chatham Maritime, Central avenue
ME4 4TB Chatham
United Kingdom

Gowen Simon
Tel.: +44-1634-800 88
Fax: +44-1634-880 066

ORSTOM
Dept. MAA UR3C Nematologie
B.P. 5045
F-34032 Montpellier
France

Mireille Fargette
Tel.: +33-4-67 61 74 00
Fax: +33-4-67 54 78 00

NATIONAL AGRICULTURAL RES. FOUNDATION
Institute of Subtropical Plants and Olive Trees
Agrokipio, Chania
Greece

Effie Vouyoukalou
Tel.: +30-821-571 42
Fax: +30-821-439 63

IACR-ROTHAMSTED EXPERIMENTAL STATION
Lawes Agricultural Trust
AL5 AJQ Harpenden
United Kingdom

Brian Kerry
Tel.: ++44-1582-76 31 33
Fax: ++44-1582-76 09 81

CARIBBEAN AGRICULTURAL RES. AND DEV.
University Campus
St. Augustine
Trinidad

Gordon Muller
Tel.: +809-645 12 06
Fax: +809-645 12 08

INST. NAC. DE INVESTIGACIONES AGROPECUARIAS
P.O. Box 2600
Quito
Ecuador

Carmen Travino
Tel.: +593-2-52 86 50
Fax: +593-2-50 42 40

LAB. DE RECH. DE LA PROTECTION DES VEGETAUX
B.P. 403
Bobo-Dioulasso
Burkina Faso

Abdoussalam Sawadogo
Tel.: 226-98 03 73

MINISTERE DE L'AGRICULTURE
Bvumbwe Research Station
Bvumbwe
Malawi

A. Daudi

RESEARCH AND TRAINING INSTITUTE TUMBI
P.O. Box 306
Tabora
Tanzania

Joseph Madulu
Tel.: +255-61 24 31

Contract number: TS3*CT920106

Period: January 1993 to December 1995

DEFINITION AND CONDITIONS OF USE OF FIELD IMMUNODIAGNOSTICS FOR PARASITIC DISEASES PREVAILING IN EXTENSIVELY BRED CATTLE

Co-ordinator: Institut National de la Recherche Agronomique, Monnaie (Nouzilly), France
(Chantal Boulard)

Objectives

- Characterise antigens for an early diagnosis of five endoparasites of ruminants: *Fasciola hepatica*, *Hypoderma bovis*, *Gasterophilus sp.*, *Oestrus ovis* and *Dictyocaulus viviparus*.
- Produce this or these antigens as pure as possible to develop kits of immunodiagnosis.
- Set up a field immuno-test.
- Start an immuno-epidemiological survey of each of these parasites.

Activities

- Definition of the antigenic pattern of each parasite, using sequential sampling of sera, following experimental monoinfestation, by western blotting. Analysis of antigenic community between these parasites.
- Characterisation of the kinetic of the antibodies during a natural infestation and effect of a chimiotherapeutic treatment on this kinetic will be studied by ELISA.
- Liquid chromatography, monoclonal antibodies or molecular biology were used to produce the protein.
- Antigen concentration, serum dilution and condition of use of the other reagents were determined.
- In order to use these field tests in optimal conditions, monthly blood samplings were carried out during the first two years of the programme, to determine the ideal period to start an immuno epidemiological survey of each disease.
- In the last year of the programme, a large epidemiological survey was carried out, with the developed test, by each participating group.

Expected outcome

The results of this project are expected to lead to the knowledge of the prevalence of the different endoparasites studied in our countries. This will help in starting and evaluating the efficiency of control programme to improve animal production.

Selected publications

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Sampaio Silva M.L., Correia Da Costa J.M., Viana Da Costa A.M., Pires M.A., Lopes S.A. Castro A.M. and Monjour L. 1996. Antigenic components of excretory-secretory products of adult *Fasciola hepatica* recognized in human infections. Am. J. Trop. Med. Hyg. **54(2)**: 146-148.

Selected Partners

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
Station de Pathologie Aviaire et de Parasitologie
Centre de Recherches de Tours-Nouzilly
F-37380 Monnaie (Nouzilly)
France
Chantal Boulard
Tel.: +33-47.42.77.57
Fax: +33-47.42.77.74

ECOLE NATIONALE VETERINAIRE
Service de Parasitologie
Maladies Parasitaires
Case Postale 3013
F-44087 Nantes
France
Alain Chauvin
Tel.: +33-40.68.77.00
Fax: +33-40.68.77.78

ECOLE NATIONALE VETERINAIRE
Laboratoire de Parasitologie
Avenue Pasteur
BP 161
16200 El-Harrach (Alger)
Algérie
Fifi Oussaid
Tel.: +213-2-76.67.81

UNIVERSIDAD DE SANTIAGO DE COMPOSTELA
Facultad Veterinaria
Parasitología y Enfermedades Parasitarias
Avenida de Madrid 81
E-27002 Lugo
Spain
Pablo Diez-Banos
Tel.: +34-82-252361
Fax: +34-82-252195

INSTITUTO NACIONAL DE INVESTIGACIONES FORESTALES Y AGROPECUARIAS
División Trematodiasis CENID
Laabo. Parasitología Veterinaria
Carretera Federal Cuernavaca KM 11.5
Cuautla 62500
Cuernavaca, Estado de Morelos
Mexico
Froulan Ibarra Velarde
Tel.: +52-73-19.02.02 - Ext. 192860
Fax: +52-73-20.43.62

INSTITUTO NACIONAL DE SAUDE
Depto. de Parasitología
P-4000 Porto
Portugal
Maria Sampaio Silva
Tel.: +351-2-57.15.09
Fax: +351-2-200.53.23

Contract number: TS3*CT920109

Period: April 1993 to March 1996

ESTUDIOS BIOQUÍMICOS E HISTOLÓGICOS DE LOS CEFALÓPODOS RELACIONADOS CON LA APLICACIÓN DE TECNOLOGÍAS CONVENCIONALES Y NUEVAS Y CON EL CONTROL DE CALIDAD

Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
(Antonio Moral Rama)

Objetivos

- Contribuir al conocimiento bioquímico e histológico de los cefalópodos de interés comercial en sus distintas fases de desarrollo gonadal.
- En base a la caracterización de las especies estudiadas, conseguir la aplicación correcta del frío y de otras tecnologías a bordo y en tierra, y elaboración de productos convencionales de alta calidad y nuevos de alto valor añadido para el comercio interior o para la exportación.
- Estudiar nuevos índices de calidad basados en la evolución de los componentes bioquímicos y en las posibles modificaciones histológicas cuando se apliquen las diferentes tecnologías y durante la conservación.
- Disponer en puerto de una materia prima diversificada de alta calidad, mejorar la elaboración de productos convencionales e incrementar el desarrollo de nuevos productos en base a las tecnologías menos contaminantes.
- Optimizar el empleo de las tecnologías convencionales y desarrollar, si es posible, nuevos equipos para la manipulación, el tratamiento, la conservación y presentación comercial de los productos acabados.
- Disponer de un grupo amplio de investigadores para conseguir una metodología común de optimización de los distintos procedimientos de conservación y elaboración de productos.

Actividades

Las actividades de este proyecto están concebidas en base a conseguir una posible mejora del particular comportamiento de muchos cefalópodos de uso reciente en alimentación humana, frescos/ refrigerados o congelados, cuando se aplican los procedimientos de utilización convencionales. Sistemáticamente se aprecian fenómenos de retracción, dureza (elasticidad) y a veces sabores extraños del manto.

Para tratar de solucionar estos problemas se realizarán las siguientes tareas:

- Caracterización histológica, anatómica y fisiológica durante el ciclo biológico: tejido conectivo, muscular, edad, estado de madurez gonadal, índice de condición y alimentación.
- Caracterización de los componentes bioquímicos e histológicos de las especies objeto de estudio, durante el ciclo biológico. Especialmente de los compuestos nitrogenados. En la fracción nitrogenada no protéica se estudiarán los aminoácidos libres, oxido de trimetilamina, betaínas, nucleotidos, compuestos guanidinicos (octopina, argiminfosfato) y ácidos propioácetico y diprapionico. En la fracción protéica, las proteínas miofibriles, proteínas sarcoplásmicas y con especial atención las proteínas del tejido conectivo por la intervención en los fenómenos de retracción y dureza del músculo. En las tres fracciones protéicas se medirán propiedades funcionales (solubilidad, capacidad de emulsión, hidrofobicidad y módulo de elasticidad de los geles). También se aplicaran técnicas elecroforéticas y de calorimetría diferencial de barrido.
- En base a los resultados de los estudios de caracterización reseñados se procederá a la aplicación de las tecnologías convencionales y nuevas que se consideren más adecuadas.
- Se seleccionarán los componentes bioquímicos más idóneos en base a las modificaciones que sufran durante el tratamiento y conservacíon de las especies objeto de estudio para utilizarlas como índices

de calidad objetivos. Las especies seleccionadas para estos estudios han sido: *Illex coindeti*, y *Eledone cirrhosa* (España); *Illex argentinus* (Argentina) y *Dosidicus gigas* (Chile).

✶ Resultados esperados

⇒ En base a los estudios de caracterización, fisiológicos y de los componentes químicos y bioquímicos se espera poder, en cada caso, aplicar la tecnología más adecuada para obtener materia prima de alta calidad que permita el desarrollo de productos de alto valor añadido.

⇒ Disponer de técnicas objetivas específicas para el control de calidad de los cefalópodos.

⇒ En base a los estudios histológicos / anatómicos se espera disponer de datos para el diseño de equipos de manipulación y aplicación correcta de ciertas tecnologías. Establecer una colaboración permanente entre los distintos grupos investigadores que participan así como con otros que puedan estar interesados en el tema.

Partners

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Instituto del Frío
Ciudad Univeristaria
E-28040 Madrid
Spain

Antonio Moral-Rama
Tel.: +34-1-544.56.07
Fax: +34-1-549.36.27

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Instituto de Investigaciones Marinas
Eduardo Cabello 6
E-36208 Vigo
Spain

Guerra Sierra Angel
Tel.: +34-86-23.19.30
Fax: +34-86-29.27.62

CIRAD - SAR
Div. Génie & Technologie Alimentaires
Avenue du Val de Montferrand
B.P. 5035
F-34032 Montpellier
France

Stéphane Guilbert
Tel.: +33-67.61.55.36
Fax: +33-67.41.40.15

INSTITUTO NACIONAL DE TECNOLOGIA INDUSTRIAL (INTI)
Centro de Inivestigación de Teconología Pesquera
División Bioquímica Aplicada
Marcelo T. de Alvear 1168
7600 Mar del Plata
Argentina

Marcos Crupkin
Tel.: +54-23-80.37.94/28.01
Fax: +54-23-80.37.94

UNIVERSIDAD DE SANTIAGO DE CHILE CENTRO DE ESTUDIOS EN CIENCIA Y TECNOLOGIA DE LOS ALIMENTOS (CECTA)
Avenida Bernardo O'Higgins 3363
Santiago de Chile
Chile

Claudio Romo
Tel.: +56-2-681.13.81
Fax: +56-2-681.21.08

Contract number: TS3*CT920110

Period: November 1992 to December 1995

ADDING VALUE TO PRODUCTS, BY-PRODUCTS AND WASTE FROM SMALL- AND MEDIUM-SIZED CASSAVA-PROCESSING INDUSTRIES IN LATIN AMERICA

Co-ordinator: CIRAD-AMIS, Montpellier, France (Dany Griffon)

Objectives

Develop the small- and medium-sized cassava-processing sector by:

- increasing the added value of processing
- designing, developing and marketing high-quality products
- reducing the impact of processing industries on the environment.

Activities and results

Research focused on five areas:

Area 1

Raw materials, processing methods, and quality of cassava meal and starches:

Determining the influence of technological parameters on product quality; bread-making testing by including up to 20% cassava meal; testing for new expansion and panification functional properties of cassava starch by fermentation and sun-drying;, improvement of traditional processes and equipment; development of new equipment (hydrocyclone) ensuring reduced consumption and recycling of extraction water.

Area 2 - Processing of solid and liquid waste

Testing of several pilot anaerobic residual-water processes, and recommendations with regard to their use in the socio-economic environment of local small-size starch-extraction industries in Colombia; determining the advantages of the use of horizontal-flow land-reactors; use of methane fermentation for starch-effluent cleaning; ability to enhance cassava meal and fibrous residue by using them with *Tricosporon* yeast as substrates in the production of aromas; possible recovery of proteins and of A and C vitamins from cassava leaves by osmotic dehydration.

Area 3 - Bioconversion of cassava meals and starches

Adding proteins to meals by Fermentation in Solid Environment, through *Rhizopus* fungus action; using these meals for bread-making (up to 20% mixed with wheat flour); bioconversion of meals through amylolytic lactic bacteria (*Lactobacillus Plantarum* A6, alone or in association with *Lactobacillus lactis*), and formulation of new amylaceous lactic drinks like liquid yoghurt.

Area 4 - Improvement of the functional properties of cassava meals and starches

Producing starch hydrolysates on a pilot scale and obtaining concentrated maltose syrup (50-55%), with a 20 l/h/m2 flow; designing and locally implementing a mono-screw extruder, and elaborating new formulations for cassava-based pre-cooked extruded meals, using maltodextrines in the preparation of light processed-meat products with a calorie value reduced by 11-12 %; microwave processing of cassava starch, with a view to obtaining physical by-products analogous to the fats used in food industries.

Area 5 - Market studies for new cassava by-products.

Presentation, in the form of case studies, of the traditional "farinha", sweet and fermented starches, chips for animal feed, and flours for bread-making industries; highlighting the various constraints linked to supply, to production and processing costs, to the organization of marketing, to demand fluctuation, and to quality perception by consumers.

Selected publications

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Giraud E., Champailler A., Raimbault M. 1994. Degradation of raw starch by a wild amylolytic strain of *lactobacillus plantarum.* Appl. Microbiol.,**vol 60 (12)** 4319-4323.

García V. 1996. Transitions thermiques de l'amidon de manioc en milieu peu hydraté. Doctoral thesis, Institut National Agronomique Paris-Grignon, 169p.

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Partners

CIRAD-AMELIORATION DES METHODES POUR L'INNOVATION SCIENTIFIQUE
Av. Agropolis
B.P. 5035
F-34032 Montpellier Cedex 1
France

Dany Griffon and Nadine Zakhia
Tel.: +33-4-67.61.57.07
Fax : +33-4-67-61-44-55
E-mail: dany.griffon@cirad.fr

UNIVERSIDAD DE BUENOS AIRES - FCE Y N.
Laboratorio de Microbiología de Alimentos
Ciudad Universitaria - Pabellón II-Piso 3
1428 Buenos Aires
Argentina

Susana V. De Fabrizio
Tel.: +54-1-781-05-29
Fax : +54-1-331-32-72

NATURAL RESOURCES INSTITUTE
Chatham Maritime, Central Avenue
UK-ME4 4TB Chatham
Kent
United Kingdom

June Wenham
Tel.: +44-1634-88-00-88
Fax : +44-1634-88-00-66

ORSTOM
Laboratoire de Biotechnologie
Boîte Postale 5045
F-34032 Montpellier
France

Maurice Raimbault and D. Alazard
Tel.: +33-4-67-61-75-83
Fax: +33-4-67-54-78-00

CENTRO INTER. DE AGRICULTURA TROPICAL
Apartado Aereo 6713
Cali
Colombia

Guy Henry
Tel.: +57-2-330-72-85
Fax : +57-2-339-72-64

UNIVERSIDADE ESTADUAL PAULISTA .
Faculdade de Ciencias Agronómicas
Grupo de Trabalho em Tecnología, Fazenda Experim. Lageado
Caixa Postal 237 – Campus de Botucatu
18600 Lageado
Brazil

Marney Pascoli Cereda and G. Chuzel
Tel.: +55-149-22-33-88
Fax : +55-149-22-34-38

CIRAD - DEPARTEMENT CULTURES ANNUELLES
Programme Climat Plantes Production
Boîte postale 5035
F-34032 Montpellier Cedex 1
France

Francis Forest
Tel.: +33-4-67-61-56-41
Fax : +33-4-67-61-71-60
E-mail: forest@cirad.fr

Contract number: TS3*CT920111

Period: February 1993 to January 1997

INTEGRATION DE STRATEGIES D'AMELIORATION DE LA RESISTANCE DU RIZ À LA PYRICULARIOSE (*MAGNAPORTHE GRISEA*) DANS LES NOUVEAUX PROGRAMMES DE CREATION VARIETALE

Co-ordinator: CIRAD – CA, Montpellier 1, France (Jean-Loup Notteghem)

Objectifs

- Connaître la structure et la fonction de gènes de pathogénie de *M. grisea* agent de la pyriculariose du riz.
- Connaître l'expression de la résistance partielle chez des plants de riz hybrides Fl hétérozygotes.
- Dnner des stratégies d'obtention de cultivars de riz hybrides Fl pourvus d'une résistance stable à la pyriculariose;
- Obtenir des formules de riz hybrides pourvues d'une résistance durable à la pyriculariose.

Activités

* L'analyse de gènes de pathogénie de *M. grisea* sera conduite par le CIRAD-CA et l'UPS Orsay en plusieurs étapes. La première année permettra la caractérisation de 4 gènes d'avirulence identifiés dans le croisement GUY11 X 2/0/3. La cartographie moléculaire des gènes sera réalisée la première année et affinée la seconde. Le travail de clonage des gènes sera commencé la première année et poursuivi les années suivantes. L'analyse de leur fonction et la recherche de nouvelles stratégies de création de cultivars résistants sera conduite les troisième et quatrième années.

* L'analyse de l'expression des gènes contrôlant des composantes de la résistance partielle sera conduite par le CIRAD-CA, l'IDESSA et le CNPAF au cours des première et deuxième années. Puis la résistance de cultivars Fl expérimentaux sera testée en vraie grandeur. Dans le même temps la résistance des lignées candidates à l'élaboration d'hybrides Fl, sera analysée. Les meilleures formules de riz hybrides résistants seront définies au cours de la quatrième année.

Résultats attendus

⇒ On aura cloné des gènes d'avirulence de *M. grisea*, analysé leurs séquences et leurs fonctions et tenté de définir de nouvelles stratégies de création de cultivars résistants.

⇒ On connaîtra le niveau d'expression des composantes de la résistance à la pyriculariose, des lignées de riz modifiées par l'introduction de gènes de stérilité génocytoplasmiques et des lignées restauratrices. On connaîtra également le niveau d'expression des composantes de la résistance des cultivars de riz hybrides Fl. Des cultivars résistants F1 pourront être proposés.

⇒ Le CIRAD-CA, l'IDESSA et le CNPAF seront en mesure de proposer des hybrides F1 résistants à la pyriculariose. On évitera ainsi un emploi accru de fongicides pour les cultivars F1 destinés à une culture intensive.

Publications choisies

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Tharreau D., Lebrun M.H. and Notteghem J.L. 1997. Mutations affecting pertihecium development and asexual sporulation in *Magnaporthe grisea.* Fungal Genetica and Biology. **21**: 206-213.

Partners

CIRAD - CA
Unité de Recherche de Phytopathologie et Malherbologie
BP 5035
F-34032 Montpellier 1
France

Jean-Loup Notteghem
Tel.: +33-67.61.55.44
Fax: +33-67.61.56.03

INSTITUTO SUPERIOR DE AGRONOMIA SECCAO AUTONOMA DE SANIDADE E PATOLOGIA VEGETAL
Tapada da Ajuda
P-1399 Lisboa Codex
Portugal

Jorge Francisco Pinto Ganhao
Tel.: +351-1-363.81.61
Fax: +351-1-363.50.31

UNIVERSITE DE PARIS-SUD
Institut de Génétique & Microbiologie
Division Cryptogamie
Bâtiment 400
F-91405 Orsay
France

Marc-Henri Lebrun
Tel.: +33-1-69.41.70.06
Fax: +33-1-69.41.72.96

EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA
Caixa Postal 179
74000 Golania Goias
Brazil

Pedro Antonio Arraes Pereira
Tel.: +55-62-261.30.22
Fax: +55-62-261.38.80

INSTITUT DES SAVANES
Département Cultures Vivrières
Laboratoire de Phytopathologie
BP 633
Bouake
Côte d'Ivoire

Michel Vales
Tel.: +225-63.31.26
Fax: +225-63.20.45

Contract number: TS3*CT920115

Period: October 1992 to September 1996

BIOSYSTEMATIC INVESTIGATIONS OF THE (SUB)TROPICAL TUBER-BEARING LEGUME *GENUS PACHYRIZMUS* (YAM BEANS), WITH SPECIAL REFERENCE TO THE DEVELOPMENT OF HIGH PERFORMANCE VARIETIES

Co-ordinator: Royal Veterinary and Agricultural University, Frederiksberg C, Copenhagen, Denmark (Marten Sørensen)

Objectives

- To create interspecific hybrids and analyze their compatibility and performance.
- To evaluate the agronomic adaptability of cultivars, landraces, wild material and interspecific hybrids under different climatic conditions: tropical and Mediterranean.
- To study the environmental impact of yam bean production under different edafic and cultivation conditions.
- To examine the practical insecticidal uses of the rotenone content of the seeds.
- To clarify producer/consumer acceptability and the potential marketability.

Results

The project has succeeded in developing high yielding, early and photothermally neutral varieties of yam bean with good pest and disease resistance. Furthermore, the selected varieties have been demonstrated to have a high efficient biological nitrogen fixation, thus ensuring a high level of sustainability for farmers in developing countries. In addition, field trials in Portugal have led to the identification of a new and attractive tuber crop for cultivation in the Mediterranean region. Finally, the analyses of rotenone levels have led to the identification of multiple-purpose varieties.

Selected publications

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Partners

ROYAL VETERIN. AND AGRICULT. UNIVERSITY
Botanical Section, Dept. of Botany, Dendrology, and Forest Genetics
Rolighedsvej 21
DK-1958 Frederiksberg C (Copenhagen)
Denmark

Marten Sørensen
Tel.: +45-35-28.28.15
Fax: +45-35-28.28.21
E-mail: MS@kvl.dk

UNIVERSITE PARIS 7 'DENIS DIDEROT'
Centre de Recherche de Botanique
Lab. Biochimie et Physiologie de l'Adaptation Végétale
Case 7019
2 Place Jussieu
F-75251 Paris 05
France

Yasmine Zuily
Tel.: +33-1-44.27.60.69
Fax: +33-1-40.51.71.08
E-mail: zuily@ccr.jussieu.fr

UNIVERSITE NATIONALE DU BENIN,
Faculté des Sciences Agronomiques,
Laboratoire de Biologie Végétale Appliquée
BP 526 Cotonou
République du Benin

D.F. Adjahossou,
Fax: +229-35.06.32
E-mail: adjahoss@syfed-bj.bj.refer.org

CENTRE D'ETUDE REG. POUR L'AMELIOR. DE L'ADAPTATION A LA SECHERESSE (CERAAS)
B.P. 3320 Thiès Escale
Thiès
Senegal

H. Roy-Macauley
Fax: +221-51.50.03
E-mail: ceraas@syfed.refer.sn

MINISTRY OF AGRICULTURE AND FORESTRY
Research Division
Vaini Research Station
P.O. Box 14, Nuku'alofa
Tonga

V.T. Manu
Fax: +676-24.271

INSTITUTO NACIONAL DE INVESTIGACIONES FORESTALES Y AGROPECUARIAS (INIFAP)
Secr. de Agric. y Recursos Hidraulicos
Campo Experimental Bajío, CIRCE,
Apdo. Postal 112,
C.P. 38000 Celaya, Gto.,
Mexico

M.C. A. Heredia Z.
E-mail: cebaj@cirpac.inifap.conacyt.mx
Fax: +52-461-15.431

CENTRO AGRONOMICO TROPICAL DE INVESTIGACION Y ENSENANZA (CATIE)
Unidad Biodiversidad y Sistemas de Producción
Apdo. 7170 Turrialba
Costa Rica

A. Q. Mora
E-mail: amora@catie.ac.cr
Fax: +506-556.1533

INSTITUTO NACIONAL DE INVESTIGACIONES AGROPECUARIAS (INIAP)
Departamento Nacional de Recursos Fitogenéticos y Biotecnología (DENAREF)
Estación Experimental Santa Catalina
Casilla 17-01-340, Quito
Ecuador

R. Castillo
Fax: +593-2-690.991
E-mail: rcastil@ecnet.ec

UNIVERSIDAD TECNICA DE ESMERALDAS "LUIS VARGAS TORRES"
Jardín Tropical
Casilla 08-01-0173, Esmeraldas
Ecuador

A. Arévalo T.
Fax: +593-6-726.446

Contract number: TS3*CT920125

Period: October 1992 to September 1995

BIOLOGICAL MANAGEMENT OF IRRIGATION CHANNEL WEED PROBLEMS IN IRRIGATED SEMI-ARID AGRICULTURE

Co-ordinator: Institute for Infrastr. Hydraulic & Environmental Engineering, Delft, The Netherlands (M.J.M. Hootsmans)

Objectives

- Produce a model of the interactions between water quality (especially turbidity) and submerged weed growth in irrigation/drainage channels in Argentina, which will be of practical value in aiding management decisions relating to weed management.
- Develop innovative channel management regimes for the target irrigation systems, integrating biological control measures based on the use of fish, and other management options.
- Transfer and implement the new technology in the target area.

Activities

- Assessment of the present status of aquatic weed problems and fish populations in the two target irrigation areas. To this end, inventories were made on the distribution and abundance of fish and weeds, water chemistry, and light regime.
- Provision of input and test data permitting the adaptation, calibration and validation of an existing model for simulating macrophyte growth, and development in relation to environmental parameters (mainly light regime- in the target channels. The main aim was to predict the likely effects of changing light availability due to altered sediment loadings on macrophyte growth.
- Experimental trials using common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*). Assessment of the effectiveness of treatments both as knock-down impact and longer-term effects on weed propagule bank size.
- Technology transfer phase: staff training (MSc, PhD levels). Implementation of results in a demonstration set-up, using the assistance of local organisations for irrigation and agricultural extension. Production of a video document, and access to the modelling package to support management decisions.
- Research was carried out in Argentina, the UK and The Netherlands. All field experiments were performed in Argentina. Laboratory experiments were also performed in the Netherlands and in the UK. Results from field and laboratory experiments, together with data from field surveys were used to successfully adapt the present macrophyte growth model SAGA1, developed at IHE Delft (The Netherlands).

Results

⇒ On the basis of field and laboratory experiments, and guided by model predictions based on detailed knowledge of weed life cycles, it was concluded that manipulation of the already existing common carp populations appears very promising in combination with

less frequent mowing operations. In this way, the use of herbicides can be limited, if not excluded completely. Grass carp could be exploited also.

⇒ Four functional vegetation types were identified: two groups, differing in dominant species, were found to be well adapted for disturbance tolerance, while being more vulnerable to higher stress pressures. A third group was found in slightly less disturbed sites, with moderate stress due to salinity, turbidity, or drought. A last group consisted of species that are quite tolerant to high saline-stress conditions.

⇒ The nuisance vegetation types present in the systems targeted by this study are reasonably representative of those found in many such systems world-wide. The recommendations resulting from this study should therefore at least be suitable as the basis for suggesting appropriate fish-based sustainable management measures for controlling aquatic weeds in other semi-arid irrigated areas, both in Argentina and elsewhere in the world.

⇒ The proposed new approach in itself should lead to more environmental awareness at the level of local staff involved in the actual management. This can have an important effect on areas more remote from the actual irrigation scheme.

Selected publications

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Partners

INTERNATIONAL INSTITUTE FOR INFRASTRUCTURAL HYDRAULIC AND ENVIRONMENTAL ENGINEERING
P.O. Box 3015
NL-2601 DA Delft
The Netherlands

Michiel J.M. Hootsmans
Tel.: +31-15-215 17 89
Fax: +31-15-212 29 21
E-mail: mih@ihe.nl

CENTRO DE RECURSOS NATURALES RENOVABLES
CC 738
8000 Bahía Blanca
Argentina

Osvaldo A. Fernández
Tel.: +54-91-347 75
Fax: +54-91-278 76

UNIVERSITY OF READING
Aquatic Biology Research Unit
78 Elmhurst Road
GB-RG1 5HY Reading
England

Jeremy Domaniewsky
Tel.: +44-1734-69 27 85
Fax: +44-1734-31 01 80

Contract number: TS3*CT920128

Period: December 1992 to November 1995

CONSERVATION AND REGENERATION OF SOIL FERTILITY IN TROPICAL AGRICULTURAL SYSTEMS BY THE MANIPULATION OF EARTHWORM COMMUNITIES (MACROFAUNA PROJECT - SECOND PHASE)

Co-ordinator: IRD (ex-ORSTOM de Bondy, Bondy, France (Patrick Lavelle)

Objectives

- Continue the development and experimentation of techniques to improve plant production and preserve or restore soil fertility through the introduction of selected earthworm species.
- Extend the experiment at the real scale of a farmer's plot and check for social acceptability and economic feasibility.
- Consolidate the integration and formation of an international group of research able to face the increasing need for research in tropical soil biology.

Activities

* Development of databases of earthworm species and communities to allow a better knowledge of the existing fauna.
* Basic ecological studies of species with large environmental tolerance and/or extended distribution
* Effects of selected earthworm species on soil processes at different scales of time and space.
* Experimentation of earthworm introduction at the real scale of a farmer's plot at Yurimaguas (Peruvian Amazonia.
* Application to the regeneration of degraded soils in intensive tea plantations in India and degraded vertisols submitted to intensive market gardening in Martinique.
* Description and quantification of the socio-economic value of earthworms in low-input agricultural systems .

Results

⇒ The research will produce a catalogue of earthworm species that could be used to improve fertility of cultivated soils. Characteristics of soil and microclimate suitable for each species, their demographic parameters and short-term effects on mineralization of organic N and P from the soil will be indicated.

⇒ The effects of selected species on different plants, in different soil types will be assessed in standardized conditions to help choose the most efficient earthworm species in any condition.

⇒ Finally, large-scale manipulation of earthworm populations will be tested at a real scale and the economic validity of this technique will be assessed. At the end of the research period it will be possible to either propose management options to improve plant production and soil conservation through the direct or indirect manipulation of earthworm communities, or abandon these options if they prove inefficient or impractical.

Selected publications

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Partners

CENTRE IRD (EX-ORSTOM) DE BONDY
Laboratoire d'Ecologie des Sols Tropicaux
32 avenue Henri Varagnat
F-93143 Bondy
France

Patrick Lavelle
Tel.:+33-1-48- 02 55 01
Fax: +33-1-48- 47 30 88
e-mail: lavelle@bondy.Orstom.fr

ORSTOM
B.P. 8006
F-97259 Fort-de-France
France

Alain Albrecht
Tel.: +596-63 06 09
Fax: +596-71 73 16

UNIVERSIDAD COMPLUTENSE DE MADRID
Facultad de Biología
Departamento de Biología Animal
Isaac Peral
E-28040 Madrid
Spain

Ana Moreno
Tel.:+34-1-549 13 97
Fax: +34-1-543 83 45

INSTITUTO DE ECOLOGIA A.C.
Apartado Postal 63
MEX-9100 Xalapa, Ver.
Mexico

Isabelle Barois Boullard
Tel.: +52-28-18 60 00
Fax: +52-28-18 69 10

NORTH CAROLINA STATE UNIVERSITY
Misión Universidad Carolina del Norte
Javier Prado Este 1894
San Borja
P.O. Box 248
Lima
Perú

Beto Pashanasi
Tel.: +51-1-475 04 10
Fax: +51-1-475 90 16

UNIVERSITE NATIONALE DU RWANDA
Campus Universitaire de Butare
B.P. 117
Butare
Rwanda

Jean Kanyonyo Ka Kajondo
Tel.: +250-30 272
Fax: +250-30 858

Partners (cont'd)

SAMBALPUR UNVIERSITY
School of Life Sciences
Ecology Section
TH 715
IND-768019 Jyoti Vihar
India

Bikram Senapati
Tel.: +91-663-82 309
Fax: 91-663-20 000

WAGENINGEN AGRICULTURAL UNIVERSITY
Dept. of Soil Science and Geology
P.O. Box 37
NL-6700 AA Wagenigen
The Netherlands

Lijbert Brussaard
Tel.: +31-317-48 24 34
Fax: +31-317-48 24 19

Contract number: TS3*CT920131

Period: March 1993 to February 1996

ORGANISATION OF INFORMATION SYSTEMS ON PRODUCTION INPUTS, CATCHES AND CHARACTERISTICS OF SMALL-SCALE FISHERY IN ECUADOR

Co-ordinator: Scottish Office, Aberdeen, Scotland, United Kingdom (Robin Cook)

Objectives

- Establishment of an information system of data collection and management for the catch and fishing effort in the Artisanal fishery, with national coverage.
- Estimation of maximum sustainable yield for the fisheries of large demersal and pelagic fish exploited by the Artisanal fishing fleet.
- Identification of the Artisanal fishing community and analysis of its technical and socio-economic characteristics.

Activities

* A new questionnaire will be designed to be used by technical staff for interviews with fishermen at their landing sites along the Ecuadorian coast.
* The technical staff will visit the landing sites at the time of landing and ask the fishermen about their catch. Details of the catch will be recorded, or if this is not possible, estimates will be made.
* Simultaneously with compiling information about catch and fishing effort, covering the vessels and fishing gear used, there will be studies of the Artisanal fishing communities. These studies will collect additional data on:
  - demographic aspects - inhabitants, families, homes
  - services - schools, cultural facilities, health, transport, waste disposal
  - infrastructure - social organisations, commercial and cultural activities, cooperatives, quality of life

Expected outcome

The socio-economic data collected from community studies and interviews with fishermen will be analysed along with biological data on the catch, fishing effort and production inputs. When a sufficiently good database has been established, standard methods for assessing fish stocks will be applied to indicate trends in stock sizes and the optimum level of fishing effort. This kind of information is essential background for fishery management actions aimed at the development of the Artisanal fishery sector.

Selected publications

Declich F., King D., and Montano R. (eds.) 1996. Las comunidades pesqueras artesanales en la costa ecuadoriana : un enfoque multidisciplinario. Memorias del seminario de clausura del proyecto STD3, Gyauaquil.

Partners

AGRICULTURE & FISHERIES DEPARTMENT
Scottish Office
Marine Laboratory
Victoria Road
P.O. Box 101
UK-AB9 8DB Aberdeen
United Kingdom

Robin Cook
Tel.: +44-1224-87.65.44
Fax: +44-1224-29.55.11

INSTITUTO NACIONAL DE PESCA
Letamendi 102 y la Ría
Guyaquil
Ecuador

Segundo Miguel Coello Cisneros
Tel.: +593-4-40.17.76
Fax: +593-4-40.23.04

COMITATO INTERNAZIONALE PER LO SVILUPPO DEI POPULI
Marziale 47
I-00136 Roma
Italy

Carlo Tassara
Tel.: +39-06-374.14.90
Fax: +39-06-325.07.51

Contract number: TS3*CT920134

Period: February 1993 to January 1996

A PROJECT TO SIGNIFICANTLY IMPROVE THE HANDLING AND PROCESSING OF SMALL PELAGIC FISH FOR AQUACULTURE AND FOOD USE

Co-ordinator: Danish Institute for Fisheries Technology and Aquaculture, Hirtshals, Denmark
(Tom Nielsen)

Objectives

- Improve handling of large catches of small fish at sea.
- Improve the curing and other food processing of small fish.
- Improve and diversify the processing of surplus fish and offal for salmon feeding.

Activities

- Develop methods for improving the quality of small fish chilled by using ice and sea water in insulated containers.
- Study the effect of using carbon dioxide in the designed containers on the quality, yield and storage life of the fish.
- Develop methods for using brine salting of the small fish in the designed containers.
- Compare the traditional anchovy salting with the container salting.
- Use the new container salting to develop marinated products from herring and sardines and an intermediate moisture product of scad.
- Study the use of food grade acid and alkali in addition to salt in order to accelerate the fish sauce preparation.
- Use the new container system and CO_2 treatment on fish for fish meal production.
- Develop fish silage from surplus fish and fish offal for salmon feeding.
- Arrange workshops / seminars in Chile and Peru for dissemination of the results.
- During the first year the catch handling by using insulated containers and carbon dioxide will be improved. The second year the final container will be designed and used for pre-salting and marinating the fish. The third year the final development of marinated products and fish sauce will be carried out, and test productions of fish meal and fish silage for feed will take place.

Expected outcome

The work detailed in the proposal is expected to lead to improved handling and processing of small pelagic fish for human consumption and a better use of the waste for a good quality of fish meal or fish silage for fish feeding.

Partners

DANISH INSTITUTE FOR FISHERIES TECHNOLOGY AND AQUACULTURE - DIFTA
The North Sea Centre
DK-9850 Hirtshals
Denmark

Tom Nielsen
Tel.: +45-98.94.43.00
Fax: +45-98.94.22.26

INSTITUTO DE INVEST. Y TECNOL. PARA LA OCEANOGR., PESCA, Y ALIMENT.
Isla de Txatxarramendi
E-48395 Pedernales
Spain

Rogelio Pozo Carro
Tel.: +34-46-87.07.00
Fax: +34-46-87.00.06

FUNDACION CHILE
Casilla 773
Santiago
Chile

Pablo Herrera
Tel.: +56-2-218.52.11
Fax: +56-2-242.69.00

Contract number: TS3*CT920140

Period: November 1992 to October 1995

DEVELOPMENT OF NOVEL SYSTEMS FOR PLANT PROTECTION AGAINST FUNGAL INFECTION THROUGH GENETIC ENGINEERING OF PLANTS AND MYCOPARASITIC FUNGI

Co-ordinator: Rijksuniversiteit Gent, Ghent, Belgium (Marc van Montagu)

Objectives

- Analysis of the potential inhibitory effect of fungal cell wall degrading enzymes from plants (Nicotiana plumbaginifolia) and fungi (Trichoderma harzianum).
- Transformation of both plants and Trichoderma with the engineered genes encoding the studied enzymes.
- Evaluation of transgenic plants for resistance to fungal pathogens and of transgenic Trichoderma for mycoparasitic activity.
- Improvement of biocontrol activity of Trichoderma by mutagenesis and changed growth conditions.

Activities

* Characterization of hydrolytic enzymes in Nicotiana plumbaginifolia (ß-1,3-glucanases, chitinases,...) and Trichoderma harzianum (ß-1,3-glucanases, ß-1,6-glucanases, chitinases, proteases,...).
* Isolation of Trichoderma strains genetically improved for biocontrol (more adaptable to the environment).
* Construction of chimeric genes, encoding the studied hydrolytic enzymes, and transformation of tomato plants and Trichoderma harzianum.
* Evaluation of transgenic organisms on improved characteristics.
* Optimization of the production and application conditions of the Trichoderma spores.

Expected outcome

The project considers the development of novel strategies to control plant diseases caused by fungi, both at the level of the plant (introducing improved antifungal genes) as at the level of a mycoparasitic fungus Trichoderma, which is already being used as a natural biocontroler. This will lead to increased crop yields, which is important from commercial, nutritional and environmental (reduced need of pesticides) points of view.

Partners

RIJKSUNIVERSITEIT GENT
Laboratorium voor Genetica
K.L. Ledeganckstraat 35
B-9000 Gent
Belgium

Marc van Montagu
Tel.: +32-9-264.51.70/71
Fax: +32-9-264.53.49

CENTRO DE INVESTIGACION Y ESTUDIOS AVANZADOS
Apartado Postal 629
Irapuato, Guanajuato
Mexico

Alfredo Herrera-Estrella
Tel.: +52-46-25.15.41
Fax: +52-46-25.12.82

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Centro de Investigaciones Biológicas
Velázquez 144
E-28006 Madrid
Spain

Carmen Castresana
Tel.: +34-1-561.18.00
Fax: +34-1-562.75.18

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Instituto de Recursos Naturales y Agrobiología
Avenida Reina Mercedes
E-41018 Sevilla
Spain

José Antonio Pintor-Toro
Tel.: +34-54-62.47.11
Fax: +34-54-62.40.02

Contract number: TS3*CT920149

Period: January 1993 to December 1996

THE SUSTAINED AGRICULTURAL DEVELOPMENT OF TROPICAL WETLANDS IN SOUTH AMERICA AND AFRICA

Co-ordinator: Dublin University, Dublin, Ireland (Michael Jones)

Objectives

- Develop a programme for the sustained exploitation of two major freshwater wetland systems in the tropics. These are the floating grass swamps dominated by *Echinochloa polystachya* in Amazonia and the *Cyperus papyrus* swamps of east and central Africa.
- Quantify the role of the wetland systems as carbon sinks by determining the amounts of carbon in the different components of the systems and the cycling of carbon between the atmosphere, the water and these components.
- Produce a mechanistic simulation model of the carbon balance of the wetland systems, and to use this to predict how management practice will alter the carbon balance of the community in the future.

Activities

* Organise a workshop in Essex to establish the methodology for carbon balance measurements;
* Determine the carbon balance of the *E. polystachya* ecosystem in the Amazon basin and investigate how its removal alters the carbon balance of the region;
* Determine the carbon balance of papyrus-dominated swamps in Africa and the effect of harvesting the aerial vegetation on the carbon balance;
* Organise a workshop in Dublin to review with The Biocomposite Centre, Bangor the utilisation of emergent vegetation;
* Determine the extent to which *E. polystachya* may be utilised as an animal feed and provide a sustainable source of fodder;
* Determine how the papyrus vegetation can be utilised when harvested regularly.

Expected outcome

The work outlined in this proposal is expected to show how tropical wetlands can be exploited in a sustainable way. It will also help to quantify the contribution that these wetlands make to global carbon cycling and whether they are a significant carbon sink.

Partners

DUBLIN UNIVERSITY
School of Botany
Trinity College
Dublin 2
Ireland

Michael Jones
Tel.: +353-1-702.17.69
Fax: +353-1-772.694

UNIVERSITY OF ESSEX
Department of Biology
Wivenhoe Park
UK-CO4 3SQ Colchester
United Kingdom

Stephen Long
Tel.: +44-1206-87.33.12
Fax: +44-1206-87.34.16

MAX PLANCK INSTITUT FUER LIMNOLOGIE
Working Group Tropical Ecology
August-Thienemann-Strasse 2
D-2320 Ploen
Germany

Wolfgang Junk
Tel.: +49-45-22.80.21
Fax: +49-45-22.80.22.81

INSTITUTO NACIONAL DE PESQUISAS DA AMAZONIA
Laboratorio Projecto INPA/MAX-PLANCK
Alameda Cosme Ferreira 1756
69011 Manaus
Brazil

Maria Teresa Fernández Piedade
Tel.: +55-92-642.34.40
Fax: +55-92-642.34.40

KENYATTA UNIVERSITY
Botany Department
P.O. Box 43844
Nairobi
Kenya

Francis Muthuri

Contract number: TS3*CT930200

Period: January 1994 to June 1997

CARBON ISOTOPE DISCRIMINATION OF LEAF AND STEM CARBOHYDRATES AS INDICATORS OF DROUGHT TOLERANCE

Co-ordinator: Consiglio Nazionale delle Ricerche, Porano, Italy (Enrico Brugnoli)

Objectives

- Determine the effects of drought, imposed at different stages of growth, on the carbon isotope composition of different plant parts and of carbohydrate pools of rice plants;
- Assess intrinsic differences between rice cultivars in carbon isotope discrimination (Δ) in the presence and absence of drought, and relationships between Δ, water-use efficiency (WUE) and yield;
- Devise a screening test for early selection of genotypes with improved drought tolerance; breeding material, including parents, advanced lines and candidate cultivars.

Activities

- Several field trials and controlled environment experiments were carried out. Field trials were conducted in Brazil over several years starting from 1992 to 1997, though the project was officially started in 1994. Controlled environment experiments were performed to investigate the effects of drought imposed at different developmental stages on productivity and Δ.
- Other experiments were designed to study the effects of drought on panicle water loss, panicle and leaf water potential, abscisic acid (ABA) content and associated changes in spikelet fertility and in grain yield. Experiments were performed to test possible genetic variations in Δ, stomatal conductance, photosynthetic capacity and ABA content in upland rice. Carbon isotope composition of plant samples was determined by isotope ratio mass spectrometers.

Results

Our results demonstrate that Δ in stem carbohydrates is a useful indicator of drought tolerance in upland rice. The most relevant outcomes of the project can be summarised as follows.

⇒ Drought induced at flowering markedly reduced Δ of peduncle sugars extracted at the end of the drought period. Among treatments, peduncle sugar Δwas *positively* correlated spikelet fertility and grain yield. Among genotypes, the correlations between yield and spikelet fertility and □ of peduncle carbohydrates under drought were *negative.*

⇒ Peduncle sugar Δ was correlated with relative growth rate and with WUE measured during flowering and early grain filling.

⇒ It has been demonstrated that Δ in leaf sugars allows to estimate mesophyll conductance (g_m). In rice g_m showed a marked decrease between vegetative and grain filling stages. The decrease in g_m explains, at least partially, the ontogenetic decrease in Δ of the bulk biomass.

⇒ Measurements on intact or excised panicles showed that most (80%) of the panicle water loss is represented by cuticular transpiration. Evidence of genetic variation in panicle water loss was found, but such variation was not clearly associated with the ranking for drought susceptibility.

⇒ Application of ABA to rice plants can induce spikelet sterility, independent of variation in plant water status. Drought caused a marked increase in the endogenous ABA content associated to a decrease in spikelet fertility. Among genotypes, there was a *negative* correlation between leaf ABA concentration and spikelet fertility in fully irrigated controls, while this correlation was *positive* under drought. The drought-induced changes in ABA contents were correlated with changes in peduncle sugar Δ.

Publications

Brugnoli E., Scartazza A., Lauteri M., Monteverdi M.C., Máguas C. 1998. Carbon isotope discrimination in structural and non-structural carbohydrates in relation to productivity and adaptation to unfavourable conditions. In: H. Griffiths (Ed.) 'Stable isotopes: integration of biological, ecological and geochemical processes'. pp. 133-146, BIOS Scientific Publishers, Oxford.

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Partners

CONSIGLIO NAZIONALE DELLE RICERCHE
Istitu per l'Agroselvicoltura
Via Marconi 2
I-05010 Porano
Italy

Enrico Brugnoli
Tel: +39-763-374 689
Fax: +39-763-374 330
E-mail: brugnoli@ias.tr.cnr.it

EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA
Centro Nacional de Pesquisa de Arroz e Feijao
Lab. of Plant Physiology
P. O. Box 179
BR-74001-970 Goiania
Brazil

Beatriz Da Silveira Pinheiro
Tel: +55-62-833 22 06
Fax: +55-62-833 21 00
E-mail: beatriz@cnpaf.embrapa.br

ROGER AUSTIN
Wingate Way 15
Trumpington
GB-CB2 2HD Cambridge
United Kingdom

Roger Austin
Tel.: +44-1223-84 09 30
Fax: +44- 1223-84 09 30
E-mail: r.b.austin@dial.pipex.com

UNIVERSITY OF CAMBRIDGE
Subdept. of Quaternary Research
The Godwin Laboratory
Free School Lane
GB-CB2 3RS Cambridge
United Kingdom

Michael Hall
Tel: +44-1223-33 48 72
Fax: +44-1223-33 47 38

Contract number: TS3*CT930203

Period: January 1994 to December 1997

SUSTAINABLE DEVELOPMENT OF INTENSIVE AQUACULTURE IN THE ANDEAN-PATAGONIA REGION: ENVIRONMENTAL IMPACT AND AGRICULTURAL RE-UTILIZATION OF FISH-FARMING WASTES

Co-ordinator: Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain (Ingrid Walter Ayneto)

Objectives

- Evaluate the environmental impact of intensive salmonid breeding and develop technologies to help sustain the productivity of the Andean-Patagonian ecosystems.
- Evaluate the agronomic value of fish farming wastes for crop production and reduce the use of artificial fertilizers.
- Evaluate cost-benefit of waste removal and reutilization as fertilizer.

Activities

* Lake water (chemical and phytoplankton composition) and sediment (nutrient composition) monitoring in sites affected and unaffected by fish farming. Evaluation of the situation before (1992-1993) and after (1994-1997) the start of fish farming.
* Measurement of phosphorus fluxes from contaminated and uncontaminated lake sediments under laboratory and field conditions (temperature- and light-controlled).
* Reduction of the pollution load through waste extraction: a waste collector for fish farming in floating cages was designed and installed. Wastes were extracted through pumping systems.
* Chemical and physico-chemical characterization of residues (fish farming wastes and biosolids) and soils (Andisols and xeric Mollisols).
* Laboratory incubation to determine rates of N mineralization and P release.
* Greenhouse trials with ryegrass, ornamentals, and seedlings of two native woody perennials.
* Field trial with ryegrass in Andisols amended with collector fish-wastes and biosolids in order to compare different types of wastes available in the region.
* Composting trials of biosolids and fish offal (viscera, skin, heads etc, of salmonids) with sawdust, woodshavings and yard trimmings as bulking agents.

Results

⇒ ***Water quality***

A gradual increase in phytoplankton density and biomass ($p<0.01$) was observed in the area affected by the fish farm. The dominant species during the growing season were diatoms *Aulacoseira granulata* and *Rhizosolenia eriensis*. The global trophic level of the reservoir had not changed after fish farming establishment. Apparently, the main factor that favours such stability is the high rate of water renewal. The eutrophication effect is shown by the higher development of periphyton in the affected areas. The extraction of wastes removed 22-30% of the total P of the wastes. This efficiency could be further improved by adjusting the feeding system and the frequency of waste

pumping from the collector. This would significantly reduce the negative effects of intensive fish farming, and consequently, to increase the fish production volume.

⇒ ***Agricultural reutilization of fish farming wastes.***
Organic residues show a high N and P amendatory value, allowing the transformation of a potential pollution problem into an agronomic resource. Fish-derived amendments, collector fish wastes and fish offal composts, behave as slow-release N fertilizers, reducing N losses by leaching. They also show higher and better quality P contents than biosolids, but rates should be tested carefully in low P-fixing soils to avoid excessive levels of extractable P. Due to their high amendatory value and the costs of waste collection and/or processing, their use should be encouraged in intensive crops, such as ornamentals and seedlings of woody perennials where they show promise.

Publications

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Mazzarino M.J.; Walter I.; Costa G., Laos F., Roselli L. and P. Satti. 1997. Plant response to fish farming wastes in volcanic soils. Journal of Environmental Quality **26**: 522-528.

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Partners

INSTITUTO NACIONAL DE INVESTICAGION Y TECNOLOGIA AGRARIA Y ALIMENTARIA
Dept. Producción y Tecnología de Alimentos
Apartado de Correos 8111
E-28080 Madrid Espaga
Spain

Ingrid Walter Ayneto
Tel.: +34-913 47 67 38
Fax: +34-913 57 22 93
E-mail: walter@inia.es

UNIVERSIDAD NACIONAL DEL COMAHUE
Centro Universitario de Bariloche
Depto Agricultura
Apartado de Correos 1336
8400 San Carlos de Bariloche
Argentina

Fernando Pedrozo
Tel.: +54-944-233 74
Fax: +54-944-221 11

UNIVERSITY OF STERLING
Institute of Aquaculture
UK-FK9 4LA Stirling
United Kingdom

Liam Kelly
Tel.: +44-1786-47 31 71
Fax: +44-1786 47 21 33

Contract number: TS3*CT930205

Period: December 1993 to November 1995

EVALUATION AND MOLECULAR BASES OF LOW COST POSTHARVEST TECHNOLOGIES

Co-ordinator: Instituto del Frío, Madrid , Spain (Carmen Merodio)

Objectives

Analysis of the effect of low-cost postharvest technologies (short-term high CO_2 levels and ethylene removal) on maintaining post-harvest quality of several tropical and subtropical fruits during storage at low temperatures. These technologies offer the advantage of: low investments and reduced exploitation costs, making their use very suitable for developing countries.

Activities and results

Cherimoya (*Annona cherimola* Mill.) cv. "Fino de Jete": Pretreatment with 20% CO_2 and 20% O_2 for 3 days had a long residual effect at low temperature and improved storage life by

- reducing the senescence-like responses,
- slowing fruit softening,
- inhibiting ethylene production and promoting free spermidine and spermine accumulation and a direct effect on the activation of defence responses to low temperature stress.

Cherimoya (Annona cherimola Mill.) cv. "Concha Lisa". This variety can be stored for up to 37 days at 8° C if receiving a pretreatment with 20% CO_2 levels combined with the use of ethylene absorbent ($KMnO_4$-impregnated carrier) at a dose of 3.5g/Kg fruit. CO_2 diffusion in cherimoya fruit under these conditions has been characterized. The applied Fick's diffusion model allows very good predictions for CO_2 penetration.

Lulo (*Solanum quitoenses* L.). We have found out that the most evident damage caused by storage of lulo fruit at chilling temperature was significantly reduced if receiving a treatment of 15% CO_2-2% O_2 for 32 hours. Electrophoretic analysis revealed the accumulation of new polypeptides of high molecular weight in treated fruits. Treatment with CO_2 lowered ethylene production and modified the pattern of sugars and malic acid accumulation in both peel and pulp tissues. Ethylene removal technology improved storage life period (14 days), by preventing fruit softening and weight loss, and maintaining its outstanding eating quality.

Pitahaya (*Acanthocereus pitajaya*). Pitahaya fruit stored at 2°C showed chilling injury symptoms whilce at 5°C no evident damage was observed. No ethylene production was detected during the pitahaya ripening period. In order to overcome damage caused by storage at 2°C, a thermal pretreatment was applied. Regarding witnesses, pretreatment at 25°C previous to storage at 2°C was a useful technology for yellow pitahaya fruit.

Avocado (*Persea americana* Mill.) cv. "Fuerte". Results of hybridization of selected cDNA probes to mRNA from avocado indicated that high CO2 treatment (20%) enhanced changes in gene expression. A clear relationship between softening and increase in the levels of mRNAs encoding for PG and cellulase (Cx) was found. PG and Cx mRNA expression was induced by relative very low increases in ethylene production. The decline in flesh firmness in concordance with the increases in PG and Cx was faster in CO2-treated avocado fruit. Our results confirmed that the action of the ACC oxidase (ACO) was post-transcriptionally regulated. Four novel cDNAs initially screened for differential expression during low temperature or CO_2 treatment were isolated from the avocado cDNA library and sequenced.

Selected publications

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Partners

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
U.E.I. Refrig. de Prod. Vegetales
Instituto del Frío
Ciudad Universitaria
E-28040 Madrid
Spain

Carmen Merodio
Tel.: +34-1-585 69 16
Fax: +34-1-549 36 27

UNIVERSITY OF NOTTINGHAM
Dept. of Physiology & Environmental Sciences
Faculty of Agricultural and Food Sciences
Suttone Bonington Campus
GB-LE12 5RD Loughborough
United Kingdom

Donald Griesson
Tel.: +44-115-951 63 33
Fax: +44-115-951 63 34

UNIVERSIDAD NACIONAL DE COLOMBIA
Proyecto Frutas Tropicals
Departamento de Química
AA 14490 Santa Fé de Bogotá
Colombia

Crisólogo Camargo Hernández (retired)
replaced by:
Luz Patricia Restrepo
Tel.: +57-1-269 91 83
Fax: +57-1-269 35 49

UNIVERSIDAD AUSTRAL DE CHILE
Centro de Estudios en Ciencia y Tecnología de los Alimentos
Avda Bernardo de Chile 3363
Santiago de Chile
Chile

Claudio Romo
Tel.: +56-26-81 13 81
Fax: +56-26-81 21 08

Contract number: TS3*CT930214

Period: January 1994 to June 1997

IMPROVED CONTROL OF BEAN ANTHRACNOSE DISEASE IN LATIN AMERICA AND AFRICA THROUGH INCREASED UNDERSTANDING OF PATHOGEN DIVERSITY

Co-ordinator: Université de Paris-Sud, Orsay, France (Michel Dron)

Objectifs

Ce projet STD3 a pour but une gestion raisonnée de la résistance du haricot commun à l'anthracnose, maladie causée par le champignon *Collectotrichum lindemuthianum*. L'objectif est d'adapter les variétés résistantes de l'hôte à la variabilité du pathogène présent dans les zones de culture intensive du haricot. L'axe principal est à l'étude de la variabilité de *C. lindemuthianum* au point de vue pathologique et moléculaire.

Activités

1. Distribution, caractérisation et identification des isolats pathogènes

   Les collectes seront effectuées par les partenaires du Costa Rica et de Tanzanie (zones où l'anthracnose sévit) dans des régions bien définies (position géographique, altitude, climat, plantes associées). Les isolats seront purifiés (cultures monospores), puis caractérisés selon des critères morphologiques, cytologiques et cytochimiques).

2. Analyse du pouvoir pathogène

   La spécificité pathogène des isolats sera déterminée après infection d'une gamme différentielle de cultivars. La capacité des isolats à attaquer d'autres espèces de plantes sera également testée. Les différents types de processus infectieux seront étudiés. Une fois caractérisés, les isolats retenus seront conservés dans deux collections situées à LARS (UK) et UPS (France).

3. Analyse de la variabilité génétique du parasite

   L'utilisation des méthodes moléculaires ci-dessous permettra de préciser l'importance de la variabilité entre les isolats de *C. lindemuthianum* et leurs relations avec les *Collectotrichum* d'autres espèces parasitant d'autres légumineuses :

   a) RAPD (Random Amplified Polymorphic DNA) permettant d'évaluer la diversité à l'échelle du génome);
   b) RFLP (Restriction Fragment Length Polymorphism) pour étudier la variabilité an niveau d'une région du génome qui n'est pas impliquée directement dans le pouvoir pathogène : le DNA ribosomique;
   c) séquençage de fragments particuliers des gènes ribosomiques (espaceurs internes transcrits, espaceur intergénique non transcrit) après amplification par PCR;
   d) établissement des caryotypes d'isolats sélectionnés en fonction de critères pathologiques et génétiques, après séparation des chromosomes par électrophorèse en champ pulsé.

Résultats attendus

Ces travaux devraient montrer si la variabilité génétique de *C. lindemuthianum* est similaire en Amérique Latine et en Afrique. Cette étude comparative apportera des informations sur la stabilité des gènes impliqués dans le pouvoir pathogène du champignon. Cette analyse permettra de formuler des hypothèses sur l'aptitude de C. *lindemuthianum* à surmonter les gènes de résistance du haricot, de mettre au point un système expérimental pour tester ces hypothèses et de proposer des stratégies pour une meilleure gestion des résistances du haricot à l'anthracnose.

Partners

UNIVERSITE DE PARIS-SUD
Labo. de Phytopathologie Moléculaire
Bâtiment 630
15, Avenue G. Clémenceau
F-91405 Orsay cedex
France

Michel Dron
Tel.: +33-1-6933.6382
Fax: +33-1-6933.6424

UNIVERSIDAD NACIONAL HEREDIA
Escuela de Ciencias Agrarias
P.O. Box 86-3000
Heredia
Costa Rica

German Rivera Coto
Tel.: +506-376.363
Fax: +506-381.585

UNIVERSITY OF BRISTOL
Dept. of Agricultural Sciences
Long Ashton Research Station
Weston Road
Long Ashton
UK-BS18 9AF Bristol
United Kingrom

John Bailey
Tel.: +44-1275-39.21.81
Fax: +44-1275-39.40.07

UYOLE AGRICULTURAL RESEARCH CENTRE
Plant Protection
P.O. Box 400
Mbeya
Tanzania

Frederika Mwalygo
Tel.: +255-65-3081
Fax: +255-65-3087

UNIVERSITY COLLEGE DUBLIN
Dept. of Environmental Resource
General Agriculture
Agriculture Building
Belfield
4 Dublin
Ireland

Bryan Michael Cooke
Tel.: +353-1-706.7194
Fax: +353-1-283.7328

Contract number: TS3*CT930216

Period: May 1994 to April 1998

AMÉLIORATION GÉNÉTIQUE DE L'ADAPTATION À LA SÉCHERESSE DE L'ARACHIDE

Co-ordinator: Institut Sénégalais De Recherches Agricoles, Bambey, Sénégal (Amadou Ba)

Objectifs

L'objectif général est de créer et sélectionner de nouvelles variétés d'arachide adaptées aux conditions de sécheresse du Sahel, du Botswana et de l'Etat du Céara au Brésil.
Les étapes de ce travail sont les suivantes :

- Améliorer la compréhension des mécanismes physiologiques de la résistance à la sécheresse et notamment du maintien de la photosynthèse en condition de stress hydrique et augmenter la capacité de sélection par l'automatisation des tests physiologiques de sélection.
- Achever les sélections en cours de variétés à cycles très courts et de variétés physiologiquement adaptées à la sécheresse.
- Contribuer à mettre au point les itinéraires techniques adaptés aux différents systèmes de production pour les variétés créées.
- Mettre en relation l'effet de la sécheresse et le niveau de résistance à la contamination par *Aspergillus flavus* (champignon responsable de l'aflatoxine).

Activités

- *Création de variétés à cycle très court par back-crosses et sélection généalogique à partir de ces back-crosses.* Le but est de transférer les allèles de précocité d'un géniteur de précocité de 75 jours, Chico, sur les deux variétés de 90 jours, 73-30 et 55-437, actuellement vulgarisées.
- *Obtention de variétés physiologiquement adaptées à la sécheresse par sélection récurrente.* Une population initiale a été créée par croisements en pyramide entre huit génotypes choisis pour leur bon comportement lors des tests agronomiques et physiologiques. La sélection récurrente permet d'améliorer cette population à la fois sur les critères agronomiques et sur des critères physiologiques sans en diminuer la variabilité génétique. Cette population subira son troisième cycle sur le même principe et des sélections généalogiques seront réalisées à partir du deuxième cycle de sélection.
- *Recherche sur la physiologie de l'adaptation à la sécheresse.* Actuellement trois types de tests physiologiques sont réalisés sur la population ci-dessus. Ces tests concernent le développement racinaire, la résistance des membranes protoplasmiques à la dessiccation et la transpiration. On cherchera à préciser le "coût physiologique" d'une fermeture précoce des stomates sur les échanges gazeux de la photosynthèse et à mettre au point le test de sélection correspondant au meilleur équilibre. Par ailleurs, on cherchera à améliorer l'efficience et la rapidité des tests existants par l'automatisation de certaines opérations.
- *Contribution à la mise au point des itinéraires techniques pour les nouvelles variétés par la réalisation d'essais en milieu paysan.*
- *Etude de l'effet de la sécheresse sur la contamination par A. flavus et sur la composition en acides gras des graines.* Les relations sont établies entre cette contamination et le stress hydrique de l'arachide en cours de culture d'une part, et entre la composition en acides gras et la sensibilité à l'aflatoxine d'autre part. On déterminera les interactions entre la résistance à la sécheresse et la résistance au champignon dans une gamme de variétés connues.

Résultats attendus

⇒ Créer et vulgariser des variétés dont le cycle sera plus court que les variétés les plus hâtives actuellement vulgarisées et produisant au moins autant pour les régions dont le cycle de culture a été réduit au cours des vingt dernières années.

⇒ Créer et vulgariser des variétés capables de "supporter" un stress hydrique en cours de culture pour l'ensemble des régions à pluviométrie irrégulière.

⇒ Mettre à profit la compréhension des mécanismes physiologiques de la résistance à la sécheresse pour tenter d'éclairer ceux de la résistance à *A. flavus* en pré-récolte.

Partners

INSTITUT SENEGALAIS DE RECHERCHES AGRICOLES
Centre National de Recherches Agronomiques
Sélection & Adaptation à la Sécheresse
B.P. 53
Bambey
Sénégal

Amadou Ba
Tel.: +221-736050/6051
Fax: +221-736052/6197

DEPARTMENT OF AGRICULTURAL RESEARCH
Division of Crops Research
Private Bag 0033
Gaborone
Botswana

G.S. Maphanyane
Tel.: +267-359.780
Fax: +267-375.204

UNIVERSIDADE FEDERAL DO CEARA
Centro de Ciencias Agrarias
Avenida Mister Hull 2977
60355-000 Fortaleza - Ceara
Brazil

Francisco José Alves Fernandes Tavora
Tel.: +55-85-243.97.30
Fax: +55-85-243.84.42

INSTITUT D'ETUDES & RECHERCHES AGRICOLES
Programme Protéagineux
01 BP 476 Ouagadougou 01
Oouagadougou
Burkina Faso

Didier Balma
Tel.: +226-319.202
Fax: +226-319.206

ESTACAO AGRONOMICA NACIONAL
Quinta do Marques
P-2780 Oeiras
Portugal

Maria do Ceu Matos
Tel.: +351-1-443.15.05
Fax: +351-1-442.08.67

CIRAD - CA
Programme Cultures Vivrières Paysannes
BP 5035
F-34032 Montpellier 1
France

Robert Schilling
Tel.: +33-67.61.56.44
Fax: +33-67.61.56.32

Contract number: TS3*CT930221

Period: January 1994 to December 1996

DEVELOPMENT OF SELECTION AND CLONAL PROPAGATION TECHNIQUES FOR MULTIPLICATION OF ELITE YIELD AND ANTHRACNOSE TOLERANT CASHEW (*ANACARDIUM OCCIDENTALE* L.)

Co-ordinator: Centro de Investigação das Ferrugens do Cafeeiro – CIFC, Oeiras, Portugal
(Carlos José Rodrígues Júnior)

Objectives

The increasing demand by international markets for cashew nut and cashew derivatives has brought together the expertise from Portugal, U.K., Brazil, Guinea-Bissau and Morocco with the following purposes:

- To expand cashew germplasm collection and develop propagation techniques required for the safe introduction of appropriate elite cashew germplasm (yield potential and if possible anthracnose tolerance) into Guinea-Bissau and Morocco.
- To increase general knowledge about clonal propagation techniques.
- To extend and strengthen research and training links between West African countries and Brazilian centres where traditional approaches to cashew breeding and selection are under way.

Conclusions

⇒ In its centre of origin (Brazil) and in producing countries (Guinea-Bissau, Tanzania,India) cashew shows a big biodiversity in potential yield, behaviour towards the main diseases (anthracnose and oidium), nut technological characteristics, etc.;

⇒ Cashew germplasm collections at UFAL (Brazil) and CIFC (Portugal) were increased with new genotypes (wild, semi-spontaneous and improved clones) from different geographic areas;

⇒ Greenhouse and field studies towards anthracnose resistance or tolerance showed different levels of attacks and yield potential, envisaging a future selection of some tolerant material;

⇒ In Guinea-Bissau 4 progeny fields were established with locally selected trees and some introductions that should be followed in the next years for subsequent selection;

⇒ Cashew was introduced in to Morocco and observations show good adaptation to the coastal strips located on the Atlantic Ocean (West) and Mediterranean Sea (North);

⇒ Conventional propagation techniques used in Brazil (side-grafting and budding) were introduced into Morocco and Guinea-Bissau but more systematic studies are necessary to optimise them in these countries;

⇒ The improvement of "in vitro" techniques and the study of several factors affecting its efficiency were carried out following different approaches: the micropropagation of juvenile and adult trees by apical and axillary node culture, micrografting, somatic embryogenesis and thin cell layer. Cashew propagation is possible in well defined "in vitro" conditions (namely node culture and micrografting) and the results obtained represented a big improvement in relation to the past. Neverthless, further experiments seem to be necessary to increase this knowledge as well as to optimise a protocol for a large scale production of this so recalcitrant crop;

⇒ Genetic fingerprinting studies by RAPD experiments showed that striking polymorphisms and reproducible and distinct differences were readily visible within accessions from Brazil, Mozambique and Guinea-Bissau and within a collection of 20 elite Tanzania clones. Despite the promising RAPD polymorphisms observed it is necessary to confirm the "transferability" of the techniques. Strategies for developing robust, reliable and non-random PCR-based markers for detecting and characterising DNA-level polymorphisms were presented;

⇒ A collection of *Colletotrichum gloeosporioides* was established with isolates from different cashew organs of the plant, from different geographic origins and from other tropical fruit species. Some morphocultural diversity regarding fungus growth rates in different media and temperatures was found. Pathogenicity tests on detached cashew leaves of one clone showed also different behaviour of the isolates in terms of lesion area produced. These results showed that cashew isolates of *Colletotrichum gloeosporioides* presented a certain diversity in terms of morphocultural characteristics and virulence. Further studies are needed to characterise better the fungus and the cashew/*Colletotrichum gloeosporioides* relationship.

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Partners

INSTITUTO DE INVESTIGAÇÃO CIENTIFICA TROPICAL - IICT
Centro de Investigação das Ferrugens do Cafeeiro – CIFC
Quinta do Marques
P-2780 Oeiras
Portugal

Carlos José Rodrígues Júnior
Tel.: +351-1-442 33 23/441 35 91
Fax: +351-1-442 30 23

UNIVERSIDADE FEDERAL DE ALAGOAS (UFAL)
Dept. de Química
Laboratório de Pesquisas em Recursos Naturais (LPqRN)
Cidade Universitaria, Tabuleiro
57000 Maceio - AL
Brazil

Enrico Eduardo Pinto de Lemos
Tel.: +55-82-324 12 38
Fax: +55-82-324 13 45

MINISTERIO DO DESENVOLVIMENTO RURAL E AGRICULTURA
Direcção Geral das Florestas e Caça (DGFC)
Caixa Postal 71
Guinea-Bissau

Cipriano Cassama
Tel.: +245-221 780
Fax: +245-221 071

UNIVERSITÉ HASSAN II (IAV)
Département d'Horticulture,
Institut Agronomique et Vétérinaire
Laboratoire de Biotechnologie Végétale
B.P. 6202
Rabat
Morocco

Abdelhadi Abousalim
Tel.: +212-7-77 17 45
Fax: +212-7-77 58 38

UNIVERSITY OF LONDON
Wye College
Unit for Advanced Propagation Systems (UAPS)
Wye
TN25 5AH Ashford - Kent
United Kingdom

Sinclair Mantell
Tel.: +44-1223-81 24 01
Fax: +44-1223-81 33 20

Contract number: TS3*CT930239

Period: March 1994 to February 1998

CLIMATOLOGICAL AND HYDROLOGICAL DETERMINANTS OF AGRICULTURAL PRODUCTION IN SOUTH-AMERICA REMOTE-SENSING AND NUMERICAL SIMULATION

Co-ordinator: Instituto Nacional Tecnología Agropecuaria, Castelar, Argentina
(Cesar Manuel Rebella)

Objectives

The project started in March 1994 and its general objective was to establish a cooperative network in South America for monitoring the impact of climatic and hydrological conditions on agricultural production and increase the local capabilities and inter-relationships among institutions by means of exchanges of methodologies, results and training of their human resources in specific topics.

Particular objectives included :

- Guidelines adaptation of farming systems for varying climatic, hydrological and land use conditions.
- Evaluation of large areas of agricultural production.
- Development of methods to derive hydrological indicators with remote sensing measurements.
- Definition and development of pertinent data bases.
- Assessment of perceptions and preferences about remote sensing based techniques of officers involved in management of agricultural production.

Activities

* Development of a regional cooperative network;
* Generation of satellite data products;
* Land use mapping;
* Evaluation of agricultural and hydrological indicators by means of time series analysis of meteorological satellite data sets;
* Land surface energy balance and evaporation mapping;
* Evaluation of agrometeorology products;
* Collection of agrometeorological, hydrological and statistical data and set up of a database.

Expected outcome

⇒ Periodic workshop meetings;
⇒ Variety of satellite products;
⇒ Land use maps of different areas and guidelines for cost effective use of satellite images;
⇒ Maps of climatic, hydrological and agricultural indicators based on time series analysis of satellite information;

⇒ ET algorithms applicable to different physiographic regions, maps of actual evapotranspiration;
⇒ Detailed design of agrometeorological information system, maps of potential agricultural production;
⇒ Guidelines for product improvement and targeted marketing;
⇒ Digital information system for agricultural planning.

Partners

INSTITUTO NACIONAL TECNOLOGIA AGROPEC.
Centro de Investigación en Recursos Naturales
Instituto de Clima y Agua
Los Reseros y las Cabanas S/N
CC. N0 25
1712 Castelar
Argentina

Cesar Manuel Rebella
Tel.: +54-1-621.0125
Fax: +54-1-481.3032/2360

INST. NAC. DE CIENCIA Y TECNICA HIDRICAS
Centro Regional Andino
Bekgrano Oeste 210
Casilla de Correos 6
5500 Mendoza
Argentina

José Zuluaga
Tel.: +54-61-3825
Fax: +54-61-38251

ORSTOM
Servisio Nacional de Meteorología e Hidrología
Casilla de Correos 20996
9214 La az
Bolivia

R. Bosseno
Tel.: +591-2-355824
Fax: +591-2-391854

UNIVERSIDADE ESTADUAL DE CAMPINAS
Centro de Ensino e Pesquisa em Agricultura
Cidade Universitaria Zeferino Vaz
P.O.Box 1170
13081 Campinas
Brazil

Hilton Silveira Pinto
Tel.: +55-192-393669
Fax: +55-192-394717

UNIVERSIDAD DE SANTIAGO DE CHILE
Facultad de Ciencias Agrarias y Forestales
Laboratorio de Agroclimatología
Santa Rosa 11315
Casilla de Correo 1004
Santiago
Chile

Fernando Santibanez
Tel.: +56-254-12703
Fax: +56-254-17055

UNIVERSIDAD DE VALENCIA
Departamento de Termodinámica y Física
Unidad de Teledetección
Doctor Moliner 50
E-46100 Burjasot
Spain

Vicente Caselles Miralles
Tel.: +34-638-64300
Fax: +34-636-43345

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
Station de Bioclimatologie
BP 91
Domaine Saint-Paul
F-84143 Montfavet
France

Bernard Seguin
Tel.: +33-90.31.61.03
Fax: +33-90.89.98.10

WINAND STARING CENTRE OF INTEGRATED LAND, SOIL & WATER RESEARCH
Dept. of Water Management in Arid Zones
Marijkeweg 11/22
P.O. Box 125
NL-6700 AC Wageningen
Netherlands

Massimo Menenti
Tel.: +31-317-47.42.00
Fax: +31-317-42.48.12

DIRECCION NACIONAL DE AERONAUTICA CIVIL
Dirección de Meteorología e Hidrología
Avenida Mariscal López 1146
Asunción
Paraguay

Miguel Angel Vazquez
Tel.: +595-21-22139
Fax: +595-21-22139

DIRECCION GENERAL DE RECURSOS NATURALES RENOVABLES
Dirección Suelos y Agua
Avenida Eugenio Garzón 456
12900 Montevideo
Uruguay

Alvaro Califra Sanguinetti
Tel.: +598-2-352778

Contract number: TS3*CT930242

Period: January 1994 to September 1997

MANIPULATION OF APOMIXIS FOR THE IMPROVEMENT OF TROPICAL FORAGES

Co-ordinator: Agricultural and Food Research Council, Aberystwyth Dyfed, United Kingdom
(Michael Hayward)

Objectives

- Gain an understanding of the mechanism controlling apomixis in the tropical forage grasses *Brachiaria* and *Paspalum sp.* and to utilize the knowledge and information gained to manipulate the reproductive biology of the species concerned.
- Exploit this information in the development of novel germplasm combining desirable agronomic traits otherwise unobtainable by orthodox breeding procedures leading eventually to the production of improved cultivars.

Activities

- The production of sexual genotypes at differing ploidy levels by the raising of sexual tetraploids from the diploids by the use cell culture systems and the use of androgenetic procedures to develop sexual diploids from the apomictic tetraploids (ORSTOM - France, EMBRAPA - Brazil, IBONE - Argentina and IGER - UK).
- The development of screening tools for the determination of the mode of reproduction by assessment of the distribution of callose around the embryo sac (CPRO - Netherlands and ORSTOM) and the identification of molecular markers associated with the apomictic trait (IGER - UK)
- The manipulation of apomixis by gene isolation and the development of a transformation system for *Brachiaria* and *Paspalum* together with the production of novel variation by this means and by the use of cell culture and protoplast fusion systems (IGER and ORSTOM).
- The training at IGER of young research workers from EMBRAPA and IBONE in the molecular screening and cell culture technologies.

Expected outcome

The programme should provide enhanced technologies for the recognition of sexual versus apomictic plants of the two grass genera involved, together with the generation of novel variation which will be tested for its practical utility in concurrent breeding programmes. By so doing it should ameliorate problems associated with the extensive cultivation of a restricted range of genetic diversity.

Partners

AGRICULTURAL AND FOOD RESEARCH COUNCIL
Plant Genetics & Breeding Department
Plas Gogerddan
UK-SY23 3EB Aberystwyth Dyfed
United Kingdom

Michael Hayward
Tel.: +44-1970-82.82.55
Fax: +44-1970-82.83.57

EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA
National Centre for Research in Beef Cattle
Plant Cytogenetics Laboratory
C.P. 154
79002970 Campo Grande
Brazil

Cacilda Borges Do Valle
Tel.: +55-67-763.10.30
Fax: +55-67-763.22.45

INSTITUTO DE BOTANICA DEL NORDESTE
Grass Genetics Laboratory
Sargento Cabral 2131
3400 Corrientes
Argentina

Camilo Qarin
Tel.: +54-78.32.73.09
Fax: +54-78.32.50.64

CENTRUM VOOR PLANTENVEREDELINGS- & REPRODUCTIEONDERZOEK (DLO)
Arable and Forage Crops Department
P.O. Box 16
NL-6700 AA Wageningen
Netherlands

Marinus Wagenvoort
Tel.: +31-317-47.70.12
Fax: +31-317-41.80.94

ORSTOM
Département Milieux & Activités Agricoles
Rue de La Fayette 213
F-75480 Cedex 10 Paris
France

Yves Savidan
Tel.: +33-1-4803.7645
Fax: +33-1-4035.1713

Contract number: TS3*CT930252

Period: January 1994 to July 1997

REGENERATION AND CONSERVATION OF HARDENED AND BARREN VOLCANIC SOILS IN LATIN AMERICA (CHILE, ECUADOR, MEXICO).

Co-ordinator: ORSTOM, Bondy, France (Paul Quantin)

Objectives

Determine conditions for sustainable regeneration of hardened and eroded volcanic soils:

1. Characterization and mapping of hardened barren ground; dynamics of their extension.
2. Study and improvement of physical, chemical, organic and microbiological fertility factors.
3. Agronomic studies of cultivation systems and assessment of their yield capacity.
4. Economic analysis and evaluation of profitability of sustainable agricultural rehabilitation.
5. Measurement of erosibility, comparing natural soils and rehabilitated soils in adapted and sustainable conditions.
6. Water relations were also studied.

Results

⇒ The strong reversible coherence of "fragipan" (brittle and plastic when humid; hard when dry) tepetates is due to the parallel orientation of clays.

⇒ If the causes of soil infertility (hardness, compactness, impermeability, lack of organic C and assimilable N and P), are remedied, cangahua and tepetate soils are as productive as a natural soil. However, the productivity of small-holder plots does not increase in time, because of a deficiency in mineral and organic fertilizer application.

⇒ Minimum ploughing is less productive for maize culture without intercrops, than traditional ridging, because of the slopes. The minimum ploughable depth is 40 cm, but for maize and broad-bean cultivation, the optimal depth is 50-60 cm.

⇒ In the cangahua or tepetate regions of Ecuador and Mexico, close to 80% of households own respectively less than 3 or 4 acres/household. These families can survive only if hardened soils are rehabilitated, if they can be salaried by employers, or even if they migrate.

⇒ Cultivated hardened soils are subject to significant erosion (50 to 100 t/ha/year) when they are not managed adequately. However, small plot or terrace trials show that appropriate techniques, such as ridging or terracing reduce erosion to acceptable limits (1 to 6 t/ha/year).

Selected publications

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Partners

ORSTOM
Centre de Bondy
32, avenue H. Varagnat
F-93143 Bondy cedex
France

Quantin Paul
Tel.:+33-1-48 03 77 77
Fax: +33-1-48 03 08 29
E-mail: prat@mpl.orstom.fr

JUSTUS-LIPSIUS-UNIVERSITAET GIESSEN
Wiss. Zentrum Tropeninstitut
Bodenkunde und Bodenerhaltung
Schottstrasse 2
D-35390 Giessen
Germany

Gerd Werner
Tel.:+49-641-702 26 85
Fax: +49-641-702 26 84

COLEGIO DE POSTGRADUADOS
Centro de Edafología
Carretera México-Texoco Km 35,5
56230 Montecillo D.F.
Mexico

José Luís Oropeza Mota
Tel.:+52-595-457 01
Fax: +52-595-457 23

UNIVERSIDAD AUTONOMA DE TLAXCALA
Secretaría de la Investigación Científica
Apartado postal 19
90070 Tlaxcala
Mexico

Antonio Flórez Díaz
Tel.:+53-246-223 13
Fax: +53-246-211

UNIVERSIDAD CENTRAL DE ECUADOR
Facultad de Ciencias Agrícolas
Escuela de Ingeniería Agronómica
Ciudadela Universitaria (U. Central)
A-4607 Quito
Ecuador

Jorge Flor Irigoyén
Tel.:+593-2-543 322 ext. 20
Fax: +593-2-528 704

UNIVERSIDAD DE SANTIAGO DE CHILE
Facultad de Ciencias Forestales
Instituto de Silvicultura
Campus Universitario Isla Techa
Casilla 853
Valdivia
Chile

Ruben Penaloza
Tel.:+56-63-221229
Fax: +56-63-221 227

Contract number: TS3*CT930257

Period: January 1994 to December 1996

BEST MANAGEMENT PRACTICES FOR THE PRODUCTIVE/PROTECTIVE REHABILITATION OF DEFORESTED SLOPING LANDS

Co-ordinator: Conphoebus S.C.R.L., Piano d'Arci-Catania, Italy (Beniamino Morgana)

Objectives

- Work out and validate a methodology to identify, analyze and select suitable management practices for reversing the widespread deterioration of sloping lands, by achieving a balance between poverty alleviation, household food security, and environmental protection.
- Identify suitable management practices for two representative, partially deforested, mountainous or hilly areas (in the order of 10,000 to 100,000 hectares). In Guatemala and Costa Rica, where there are different physical, social, economic, and energetic conditions, the risk of erosion is very high, the existing essential data are nearly sufficient for the development of the project, and pilot interventions can be carried out.

Results

⇒ Integration of the local teams in the international environment. During the development of the project, two Central American researchers were added to the European working groups. Also, a European junior researcher collaborated with the Central American working groups.

⇒ A methodology to identify, analyze and select suitable management practices in deforested sloping areas.

⇒ A model able to describe crop production, including economic outcomes, within a regional context (in respect of real constraints), based partly on the utilization of existing, widely tested models (EPIC, ALES) and in other parts on a further elaboration of their combination.

⇒ A decision-support instrument, helping in the selection of the best management practices, based on multi-criteria analysis, and taking into account the indications given by the partners over the sustainable-management practices for the tropical areas of Central America.

⇒ Selection of the suitable management practices, particularly with regard to the social and economic peculiar conditions in the two sample areas in Costa Rica and in Guatemala. Coffee and sugar cane were selected in Costa Rica, considering different protective practices, while in Guatemala, maize, bean and potato were selected with different protective practices. In the 'risky areas' with steep slopes (i.e. >50% slopes), various forestal species with the related management practices were selected for the dominant and subordinate plane. In those zones, particularly degraded and steep (with >75% slopes), particular indications were given

⇒ Thematic maps concerning the optimization process results.

⇒ Video and photographic documentation showing the stepwise development of field research and the practical application of the suggested management practices both in Costa Rica and in Guatemala.

Selected publications

Stamos C.V., Koullas D.P., Daouti-Koukios E.G. 1996. Development of a decision-support tool for the evaluation of management practices in deforested sloping lands. Proceedings of 5th National Congress on Soft Energies. **Vol. 2**, pp BIO 323-329. Democritos Research Centre, Athens (Greece). November (in Greek).

Stamos C.V., Koukios E.G. 1996. Land management for sustainable development. Presentation. Seminar on the optimal use of biological resources. NTUA. Athens (Greece). November.

Stamos C.V., Diamantidis N.D., Magri S., Sardo V., Mora Camacho J.R., Collado Martínez C.A., Koukios E.G. 1997. Best management practices for the productive/protective rehabilitation of deforested sloping areas. Proceedins of the International Conference on Sustainable Agriculture for Food, Energy and Industry. Federal Agriculture Research Centre, Braunschweig (Germany). June.

Partners

CONPHOEBUS S.C.R.L.
Ist. di Ricerche per le Energie Rinnovabili
Zona Industriale - Passo Martino
Casella Postale
I-95030 Piano d'Arci - Catania
Italy

Beniamino Morgana
Tel.:+39-95-748 91 11
Fax: +39-95-748 92 07
E-mail: Conphoeb@Tin.it

UNIVERSIDAD NACIONAL
Sede Región Brunca
Apartado Postal 34-8000
San Isidro del General
Costa Rica

Juán Rafael Mora Camacho
Tel.:+506-771-68 84
Fax: +506-771-51 41
E-mail: Drueda@irazu.una.ac.cr

NATIONAL TECHNICAL UNIVERSITY OF ATHENS
Dept. of Chemical Engineering – Division IV
Bioresource Technology Unit
Zografou Campus
GR-15700 Athens
Greece

Emmanuel G. Koukios
Tel.:+30-1-772 31 91
Fax: +30-1-772-31 92
E-mail: koukios@orfeas.chemeng.ntua.gr

INSTITUTO DE CIENCIA Y TECNOLOGIA AGRICOLAS
Disciplina de Manejo de Suelos y Agua
km 21,5 Carretera hacia Amatitlán
Barcena, Villa Nueva
01013 Guatemala City
Guatemala

Carlos Adolfo Collado Martínez
Tel.:+502-4-631 20 08
Fax: +502-4-631 20 02
E-mail: ICTA@micro.com.gt

Contract number: TS3*CT940264

Period: June 1994 to May 1998

ETUDE DE LA DIVERSITE BIOLOGIQUE ET DE L'*ATRIPLEX HALIMUS* POUR LE REPERAGE *IN VITRO* ET *IN VIVO* D'INDIVIDUS RESISTANT A DES CONDITIONS EXTREMES DU MILIEU, ET CONSTITUTION DE CLONES

Co-ordinator: Université de Paris-Sud, Chatenay-Malabry, France (Pierre Dutuit)

Objectifs

- Contribuer aux efforts d'amélioration de la production des steppes fourragères à base d'*Atriplex halimus*, afin de permettre une augmentation de la production animale des régions arides méditerranéennes.
- Protéger ces écosystèmes particulièrement fragiles.
- Pays participants à ce projet : Belgique, France, Algérie, Chili, Tunisie.

Activités

* Repeuplement de zones dégradées à l'aide de populations homogènes résistantes obtenues par le clonage *in vitro* d'individus choisis au sein de la diversité biologique de populations naturelles ou créées.
* Le développement des techniques *in vitro* nécessitant l'équipement d'unités de culture autonomes et capables de cloner par microbouturage ou par embryogénèse somatique des quantités importantes d'individus aux qualités de tolérance à la sécheresse, à la salinité et possédant de bonnes performances fourragères.
* Ces unités recevront *in situ* une assistance scientifique de la part des laboratoires européens.

Le projet comporte plusieurs étapes :

Sélection *in situ*

Trois campagnes de repérage par an (soit 12 au total) seront faites dans des régions bien déterminées du point de vue édaphoclimatique pour chacun des trois pays concernés du Sud.

Les populations naturelles d'*Atriplex halimus* sont hautement hétérogènes. De cette diversité biologique sera extraite une cinquantaine d'individus répondant à des critères de résistance à la sécheresse (dix), à la salinité (dix), mais aussi à des critères de productivité fourragère (dix) et de bonne adaptation générale (vingt).

Les prises d'échantillons destinés à la culture *in vitro* se feront sur ces individus mis en défens.

Clonage *in vitro*

* Etude de la micropropagation * Etude des contraintes * Sélection in vitro * Mise au point de tests * Embryogénèse somatique * Semences artificielles

Etude du comportement des clones en écosystèmes naturels

Cette étape consiste au retour aux conditions naturelles des clones produits *in vitro*.

Résultats attendus

Repérage *in situ* des individus performants pour le clonage :
⇒ Résistance à la salinité
⇒ Production de biomasse
⇒ Palatabilité

⇒ Mise au point de tests *in vitro* pour la sélection des individus à cloner pour les critères :
⇒ De résistance à la salinité
⇒ D'activité photosynthétique
⇒ D'absorption de l'azote
⇒ De teneur en oxalate et tannin
⇒ Mise au point de la micropropagation par microbouturage et embryogenèse somatique.
⇒ Réintroduction dans l'aire géographique de l'*Atriplex halimus* de clones sélectionnés.

Partners

UNIVERSITE DE PARIS-SUD
Equipe d'Ecotechnologie
Laboratoire de Botanique
Tour E 1 - Etage 2
F-92296 Chatenay-Malabry
France

Pierre Dutuit
Tel.: +33-1-4683.5419
Fax: +33-1-4683.1303

UNIVERSITE CATHOLIQUE DE LOUVAIN
Laboratoire de Cytogénétique
Place Croix du Sud 4-5
B-1348 Louvain-La-Neuve
Belgium

J.M. Kinet
Tel.: +32-10-47.20.50
Fax: +32-10-47.34.35

UNIVERSITE DES SCIENCES & TECHNIQUES DE BLIDA
Institut d'Agronomie
Département de Phytotechnie
B.P. 270
9000 Blida
Algérie

Fatima Ben Ribiha
Tel.: +213-3-41.58.50
Fax: +213-3-41.78.13

FACULTE DES SCIENCES DE TUNIS
Laboratoire de Biologie Végétale
Campus Universitaire
1060 Tunis
Tunisie

Sadok Bouzid
Tel.: +216-1-51.26.00
Fax: +216-1-88.54.80

UNIVERSIDAD DE SANTIAGO DE CHILE
Departamento de Producción Animal
Fac. des Sciences Agraires & Forestières
Santa Rosa 11 315, La Pintana
Casilla 1004
Santiago
Chili

Sergio Lailhacar
Tel.: +56-2-678.57.32
Fax: +56-2-678.57.00

Contract number: TS3*CT940265

Period: July 1994 to June 1998

IMPROVEMENT OF SYMBIOSIS BETWEEN *RHIZOBIUM MELILOTI* AND ALFALFA IN ACID SOILS FROM ARGENTINA AND URUGUAY

Co-ordinator: Universität Bielefeld, Bielefeld, Germany (Alfred Pühler)

Objectives

- Development of an alfalfa nodulating *Rhizobium* (ANR) collection of native rhizobia isolated from Argentina and Uruguay.
- Identification of strains with increased acid tolerance and/or increased symbiotic properties.
- Identification of genes involved in competition and acid tolerance.
- Development of genetic tools to analyse the plant-microbe-interactions.
- Technology transfer between the participating groups.

Activities

- Isolation of ANR from acid soils in Argentina and Uruguay.
- Screening for acid tolerant ANR using liquid cultures.
- Microbiological characterisation of the ANR (melanin production, antibiotic resistance, growth characteristics, FAME, LPS-profile).
- Genetic characterisation of the biodiversity among the ANR, by using different PCR techniques, IS-fingerprinting, plasmid analysis and 16S rDNA sequencing.
- Development of GFP-labelled ANR for ecological model experiments.
- Plant inoculation experiments with identified strains, by using hydroponic media of different pH and soil samples from Argentina and Uruguay.
- Construction of cosmid gene libraries from acid tolerant strains in order to isolate genes involved in acid tolerance and competition.

Results

- More than 500 ANR were isolated.
- An acid tolerant ANR-subpopulation build by two medium acid tolerant *Sinorhizobium meliloti* strains and 15 acid tolerant *Rhizobium* spp. strains highly similar to the type strain Or191 could be identified.
- The medium acid tolerant *S. meliloti* strains exhibited good symbiotic properties.
- The acid tolerant *Rhizobium* spp. strains showed a poor nitrogen fixation capacity but high competitiveness when coinoculated with *S. meliloti* strains.
- The *S. meliloti* strains were characterised by a high biodiversity.
- The acid tolerant alfalfa nodulating *Rhizobium* spp. population is formed by only one strain type.

Follow up

- Identification of genes conferring acid tolerance and enhanced competition.
- Pilot experiments using soil samples to emulate field experiments.

Selected publications

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Segundo E., Martínez-Abarca F., van Dillewijn P., Fernández-López M., Lagares A., Martinez-Drets G., Niehaus K., Pühler A., and Toro N. Diversity, growth and symbiotic behaviour of Sinorhizobium meliloti strains isolated from acid soils of Argentina and Uruguay. Comparison with other alfalfa nodulating rhizobia. To be submitted.

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Partners

UNIVERSITAET BIELEFELD
Fakultät für Biologie
Lehrstuhl für Genetik
Postfach 100131:
D-33501 Bielefeld
Germany

Alfred Pühler
Tel.: +49-521-106 56 07
Fax: +49-521-106 56 26
E-mail: puehler@genetik.uni-bielefeld.de

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Departamento de Microbiología
Estación Experimental del Zaidín
Profesor Albareda 1
E-18008 Granada
Spain

Nicolas Toro García
Tel.: +34-58-121011
Fax: +34-58-129600
E-mail: ntoro@eez.csic.es

UNIVERSIDAD NACIONAL DE LA PLATA
Instituto de Bioquímica y Biología Molecular
Facultad de Ciencias Exactas
Calles 47 y 115
1900 La Plata
Argentina

Antonio Lagares
Tel.: +54-21-250497
Fax: +54-21-259223
E-mail: lagares@biol.unlp.edu.ar

INSTITUTO DE INVESTIGACIONES BIOLOGICAS
Clemente Estable
Division of Biochemistry
Avenida Italia 3318
11600 Montevideo
Uruguay

Gloria Martínez-Drets
Tel.: +598-2-471616
Fax: +598-2-475548
E-mail: gloria@iibce.edu.uy

Contract number: TS3*CT940269

Period: June 1994 to May 1997

DIAGNOSIS AND CONTROL OF BACTERIAL DISEASES IN PENAEID SHRIMP HATCHERIES – RELATIONSHIP BETWEEN MICROBIAL FLORA, NUTRITION, PRODUCTION TECHNIQUES, AND HEALTH STATUS OF PENAEID SHRIMP

Co-ordinator: Universiteit Gent, Ghent, Belgium (J. Mergaert)

Objectives

Investigate the relations between the microbial flora occurring in hatchery environments, nutrition, and water quality, and the survival and health of shrimp larvae during different developmental stages.

Results

⇒ In the larval stage, an overwhelming predominance of *Vibrio alginilyticus* and *Vibrio harveyi*, both in *P. vannamei* and *P. sinensis*, was observed. The bacterial flora associated with the larvae is unstable and is influenced by the bacterial flora in the food and the environment.

⇒ *V. harveyi* was found to be the major pathogen during larval stages. Through genomic fingerprinting of *V. harveyi* strains, two patterns were found to be related with health problems in shrimp culture. Specific *Vibrio* pathogens are associated with specific shrimp developmental stages.

⇒ ELISA rapid characterization systems were successfully developed for the detection of *V. harveyi* and *Vibrio parahaemolyticus*.

⇒ The probiotic properties of *V. alginloyticus* strain Ili was demonstrated, and this strain has been successfully applied as a probiont on a large scale during larval development.

⇒ The introduction of vitamin-C-enriched rotifer improved the feeding regime and resulted in a faster larval development.

⇒ Results show that a dietary provision of 1% n-3 HUFA is the minimum requirement for post-larval penaeids, and 4% for late post-larvae.

⇒ The supplementation of soybean phosphatidylcholine significantly improved growth, increased the total lipid content of the tissue in *P. japonicus*, and reduced the sensitivity to osmotic stress in *P. vannamei*. The addition of various types of phosphatidylcholine to the diet resulted in a significant increase in the whole body lipid content in both shrimp species.

⇒ The overall biochemical composition of post-larvae revealed few differences in vitamin and astaxantine levels between hatchery and wild post-larvae.

⇒ Fatty acid analysis provided the best tool to differentiate hatchery post-larvae and wild post-larvae, as 16 out of 34 fatty acids were present in clearly distinct concentrations.

⇒ During the project, a total of 2,000 bacterial shrimp isolates were collected and stored at the Laboratory for Microbiology. Databases containing Biolog, FAME and AFLP characterization data are available for comparison and further study at the Laboratory for

Microbiology. ELISA diagnostic systems for the detection of *V. harveyi* and *V. parahaemolyticus* are available at the Heriot-Watt University and at the University of Qingdao.

Selected publications

Kontara E.K., Merchie G., Lavens P., Robles R., Nelis H., De Leeneer A. and Sorgeloos P. 1996. Improved larviculture outputs of postlarval shrimp *Penaeus vannamei* through supplementation of L-ascorbyl_2-polyphosphate in the diet. Aquaculture International. **5**:127-136.

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Vandenberghe J., Li Y., Verdonck L., Li J., Xu H.S., and Swings J. 1998. Vibrios associated with *Penaeus sinensis* (crustacea; decapoda) larvae in Chinese shrimp hatcheries. Aquaculture (in press).

Partners

RIJKSUNIVERSITEIT GENT
Laboratorium voor Microbiologie
K.L. Ledeganckstraat 35
B-9000 Gent
Belgium

Jean Swings
Tel.: +32-9-264 51 16
Fax: +32-9-264 53 46

RIJKSUNIVERSITEIT GENT
Lab. of Aquaculture and Artemisa
Reference Centre
Rozier 44
B-9000 Gent
Belgium

Patrick Sorgeloos
Tel.: +32-9-264 37 54
Fax: +32-9-264 41 93

HERIOT-WATT UNIVERSITY
Dept. of Biological Sciences
RICCARTON
GB-EH14 4AS Edinburgh
United Kingdom

Brian Austin
Tel.: +44-131-449 51 11
Fax: +44-131-451 30 09

OCEAN UNIVERSITY OF QINGDAO
Dept. of Marine Biology
Marine Biotechnology Division
5 Yushan Road
RC-266003 Qingdao, Shandong
P.R. China

Huai Shu Xu
Tel.: +86-532-286 43 61
Fax: +86-532-287 90 91 / 82 49

ESCUELA SUPERIOR POLITECNICA DEL LITORAL
Centro Nacional de Agricultura e Investigaciones Marinas
Microbiology Dept.
P.O. Box 09014519
Guyaquil
Ecuador

Jorge Calderón
Tel.: +593-4-29 64 56
Fax: +593-4-35 46 29

Contract number: TS3*CT940274

Period: October 1994 to December 1998

A NOVEL BASIS FOR PEST MANAGEMENT OF *GLOBODERA* SPP. ON POTATO IN THE CENTRAL ANDES

Co-ordinator: University of Leeds, Leeds, United Kingdom (Howard J. Atkinson)

Objectives

- Identify the extent of decline of potato cyst nematodes (PCN) caused by traditional non-host crops of South America such as quinoa and lupin.
- Screen lines of quinoa and lupin that enhance PCN decline and select them for breeding programmes.
- Develop a reliable basis for determining readiness of PCN to hatch under different cropping regimes.
- Characterize the potential nematicidal activity of lupin and quinoa at the molecular level.
- Investigate the influence of potato cultivars on the survival, infectivity and multiplication of Bolivian PCN populations.

Activities

- Techniques are being developed for monitoring the level of dormancy of field populations of PCN and their readiness to hatch in Bolivian fields under different cropping regimes. Novel techniques will be developed to assess PCN populations; these will include:
  - Measurement of adenylate energy charge using HPLC
  - Changes in the size of the nucleolus in the nucleus of the dorsal pharyngeal gland as an indicator of readiness to hatch (image analysis)
  - Viable egg loss from measurement of eggs number and cyst size (image analysis)
  - Neutral lipid content and fatty acid composition of PCN populations from different field sites (image analysis, gas chromatography).
- Various analytical chemistry techniques, including high-pressure liquid chromatography, gas chromatography, and mass spectrometry, are being used to analyse and provide footprints of host and non-host crop root exudates. This work will provide a reliable basis for screening plant lines for maximum antagonistic activity against PCN.
- Field trials will be conducted in Bolivia to determine the response of PCN populations to different management strategies that currently prevail in local agriculture. Additionally, non-host crops (lupin, quinoa) are being tested to identify lines that contribute to the management of PCN when used in rotation with potatoes. Such lines will form a valuable resource for future plant breeding programmes.

Expected outcome

The work carried out in this project will develop a novel model for improved management of PCN in a sustainable agriculture that is based on traditional agricultural practices. Host and non-host cultivars will be selected that favour enhanced frequency of potato cropping without PCN-related crop loss. This programme should increase potato yield per hectare and free land

for crops such as cereals and legumes that are also used as staple foods by subsistence farmers.

Selected publications

Holz R.A., Troth K. and Atkinson H.J. 1998. The influence of potato cultivars on the neutral lipid content and fecundity of Bolivian and UK potato cyst nematodes, *Globodera* spp. Parasitology (submitted).

Holz R.A. and Atkinson H.E. 1998. Fatty acid composition of lipids from cysts of Bolivian PCN, *Globodera* spp, grown on potato *Solanum tuberosum andigena.* Nematologica (submitted).

Holz R.A., Riga E. and Atkinson H.J. 1998. Seasonal changes in the dorsal pharyngeal gland nucleolus of unhatched second-stage juveniles of *Globodera* spp. In Bolivia. Journal of Nematology (submitted).

Partners

UNIVERSITY OF LEEDS
Centre for Plant Biochemistry & Biotechnology
Woodhouse Lane
Leeds
UK-LS2 9JT West Yorkshire
United Kingdom

Howard Atkinson
Tel.: +44-113-233 29 00
Fax: +44-113-233 31 44
E-mail: h.j.atkinson@leeds.ac.uk

PROINPA
Calle Man Cesped 293
Casilla 4285
Cochabamba
Bolivia

Javier Franco
Tel.: +591-42-495 06
Fax: +591-42-457 08
E-mail: jfranco@papa.bo

CENTRO DE INVESTIGACIONES CIENTIFICAS ISLA DE LA CARTUJA
Instituto de Investigaciones Químicas
Americo Vespucio s/n
Isla de la Cartuja
E-41092 Sevilla
Spain

Manuel Martín-Lomas
Tel.: +34-95-448 95 63
Fax: +34-95-446 05 65

Contract number: TS3*CT940278

Period: October 1994 to March 1998

IMPROVING THE GROWTH OF TROPICAL NITROGEN-FIXING FOREST TREES IN THE GENERA *ACACIA* AND *CASUARINA* THROUGH TISSUE CULTURE AND GENETIC TRANSFORMATION

Co-ordinator: ORSTOM, Montpellier, France (Emile Duhoux)

Objectives

The goal of the project is to develop genetic transformation strategies for nitrogen-fixing species of tropical trees in the genera *Acacia* (*Acacia mangium*, *Acacia mearnsii* and *Acacia crassicarpa*) and *Casuarina* (*Casuarina glauca* and *Allocasuarina verticillata*). Gene transfer technologies were used to introduce agronomically important trraits and to obtain new tree-micro-organism symbioses more adapted to the ecological features of the planting sites:

- Development of techniques for the micropropagation of superior clones of *Acacia sp.* and *Casuarina*.
- Efficient regeneration of whole plants, starting from somatic embryogenesis of *Acacia sp.* and *Casuarina.*
- Using the β-glucuronidase gene and a selection marker, transformation of *Acacia* and *Casuarina* was achieved using either the natural vector *Agrobacterium*, or direct gene transfer techniques (high-velocity microprojectiles); transgenic plants were regenerated.
- Using the β-glucuronidase gene under the control of different known regulatory sequences, constitutive or tissue-specific expression vectors were identified for *Acacia* and *Casuarina*: stability of the transgenes was studied.
- Using the most appropriate vector, a metallothionein gene was introduced into *Casuarina*, and transgenic plants were regenerated.

Activities

- Micropropagation
- Regeneration procedure
- Induction of shoot primordia by *Rhodococcus fascians*
- Identification of a selection marker for the genetic transformation of *Acacia* and *Casuarina*
- Transformation vectors to follow shoot meristem formation
- Agrobacterium-mediated DNA transfer
- Direct DNA transfer by high-velocity microprojectiles
- Analysis of transformed tissues
- Expression vectors for Acacia and *Casuarina*
- Introduction of a metallothionein gene into *Casuarinaceae* trees.

Results

⇒ Identification of superior clones of *A. mangium*, with good organogenic potential.
⇒ Micropropagation of *A. mangium* and *A. mearnsii.*
⇒ Micropropagation of *C. glauca*, using shoots from mature trees

⇒ Using Thidiazuron, differentiation of buds on *A. mangium calli* derived from nypcotyls.
⇒ Regeneration of rooted plants from cotyledons of A. crassicarpa
⇒ Identification of selection markers for *Acacia* and *Casuarina*
⇒ Induction of tumours after inoculation of *A. mangium*, *A. mearnsii* and *A. crassicarpa* by wild-type *A. tumefaciens* strains
⇒ Transfer of the β-glucuronidase gene into A. mangium, using a wild-type A. tumefaciens strain.
⇒ Transient expression of the uidA gene in *Acacia mangium calli* following particle bombardment
⇒ Regeneration of transgenic *C. glauca* trees after transformation of epicotyls with the disarmed *A. tumefaciens* strain C58C1(GV2260; BIN19GUSint).
⇒ Regeneration of transgenic *A. verticillata* plants expressing the β-glucuronidase gene under the control of constitutive or tissue-specific promoters.
⇒ Nodulation by Frankia of transgenic *A. verticillata* and *C. glauca*.
⇒ Isolation and characterization of a metallothionein gene from *C. glauca*.

Outcome

- Gene transfer into *Acacia*, using either disarmed strains of *Agrobacterium tumefaciens* or particle bombardment; growth of transformed tissues
- Stability of expression of the 35S promoter in the tropical nitrogen-fixing *trees A. verticillata* and *C. glauca*.
- Basic knowledge of the expression of several constitutive and tissue-specific promoters in nodulated *Casuarinaceae* trees
- Transformation of A. *verticillata* and *C. glauca* with a constitutively expressed metallothioneione gene.

Selected publications

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Partners

ORSTOM
Laboratoire de Physiologie Cellulaire et Moléculaire des Arbres
911 avenue Agropolis
BP 5045
F-34032 Montpellier cedex 5
France

Emile Duhoux
Tel.: +33-4-67 61 10 12
Fax: +33-4-67 63 82 65

UNIVERSITE MOHAMMED V
Faculty of Sciences
Lab. of Microbiology
Ibn Battouta Street
B.P. 1014
Rabat
Morocco

Abdelkrim Maltouf Filali
Tel.: +212-7-77 54 61
Fax: +212-7-77 54 61

INNOPRISE CORPORATION SENDIRIAN BERHAD
Plant Biotech. Lab.
P.O. Box 60793
90017 Tawau
Malaysia

O. Monteuuis
Tel.: +60-89-77 53 28
Fax: +60-89-76 31 92

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
Instituto de Biología
Departamento de Genética
CCS Bloco A 20 Andar – Sala 098-UFRJ-RJ
Avenida 24 s/n
BR-21941-590 Rio de Janeiro
Brazil

Dulce Eleonora De Oliveira
Tel.: +55-21-590 01 11
Fax: +55-21-590 01 11

TEAGASC
Agriculture and Food Development Authority
Malahide Road
17 Dublin
Ireland

Gerard Douglas
Tel.: +353-1-846 06 44
Fax: +353-1-846 05 24

RIJKSUNIVERSITEIT GENT
Laboratorium voor Genetica
Faculty of Science
K.L. Ledeganckstraat 35
B-9000 Gent
Belgium

Marc Van Montagu
Tel.: +32-9-264 52 05
Fax: +32-9-264 53 49

Contract number: TS3*CT940279

UTILIZATION OF HEMICELLULOSE WASTE FROM AGRICULTURAL AND FOREST INDUSTRIES USING XYLAN-DEGRADING AND XYLOSE-FERMENTING YEASTS

Period: October 1994 to September 1998

Co-ordinator: Rheinische Friedrich Wilhelms Universität, Bonn, Germany
(Milan Hofer)

Objectives

- Isolate new polysaccharide-degrading fungi and xylose-fermenting yeast species from nature.
- Modify genetically available industrial yeast strains by introducing desirable genes from other fungi and/or yeast (protoplasts fusion, chromosome-protoplast fusion).
- Investigate respiratory metabolism of xylose-fermenting strains as affected by mutations in the mitochondrial DNA.
- Investigate transport phenomena in yeasts, both in wild strains and in genetically modified strains, with a view to circumventing glucose repression in the mixture of sugars obtained by degradation of waste materials, and increasing osmotolerance and ethanol tolerance in the production strain.
- Solve the problem of glucose repression of xylose uptake and/or fermentation in chosen yeast and hybrid strains by developing derepressed mutants.
- Construct hybrids between xylose-fermenting and osmotolerant yeasts with higher resistance to elevated osmotic pressures.
- Assess stability of the constructed strains and their industrial potential using laboratory and pilot scale fermenters.

Activities

* Strains with rapid growth rate and high osmotolerance will be isolated from decaying cacti and citrus wastes.
* The genetic modification of yeast strains will be performed by protoplast fusion between living strains and/or between killed cells (by chromosome breaking agents) and suitable recipient strains to obtain hybrids and/or transformed strains able to grow on xylans and to ferment xylose and which, in addition, are tolerant to osmotic pressure and ethanol.
* A range of mitochondrial mutants will be isolated (spontaneously arising or by chemical mutagenesis) and characterized for ethanol/xylitol production.
* Transport studies will involve measurements of H^+/xylose-cotransport as well as assessment of the driving force, of the membrane potential and of _pH.
* Glucose transport-deficient mutants of selected xylan/xylose fermenting yeast strains will be obtained by integrative or substitutive transformation using glucose transporter gene(s) isolated from *Schizosaccharomyces pombe*; the role of hexokinase in glucose repression will be assessed.
* Pilot scale fermenters will be used to evaluate the performance of promising strains from laboratory scale experiments.

Expected outcome

The joint research capacity will be aimed at developing fermentation technology leading to products of high economic value (ethanol, xylitol) from agricultural and forest wastes which otherwise constitute a pollution problem.

Partners

RHEINISCHE FRIEDRICH WILHELMS UNIVERSITAET
Botanisches Institut
Kirschallee 1
D-53115 Bonn
Germany

Milan Hofer
Tel.: +49-228-735 504
Fax: +49-228-735 513

INSTITUTE OF MICROBIOLOGY AND BIOTECHNOLOGY
Meckenheimer Allee 168
D-5300 Bonn
Germany

Herwig Kaspari

PLANTA PILOTO DE PROCESOS INDUSTRIALES
Avenida Belgrano y Pasaje Caseros
RA-4000 San Miguel de Tucumán
Argentina

Danley Callieri
Tel.: +54-81-330 744/330 057
Fax: +54-81-330 087

ST. PATRICK'S COLLEGE
Dept. of Biology
Maynooth
Co. Kildare
Ireland (Rep. of)

Peter Whittaker
Tel.: +353-1-708 38 42
Fax: +353-1-708 38 45

JAWAHARLAL NEHRU UNIVERSITY
School of Live Sciences
New Mehrauli Road
IND-110067 New Delhi
India

Rajendra Prasad
Tel.: +91-11-650 016
Fax: +91-11-686 58 86

Contract number: TS3*CT940298

Period: January 1995 to June 1998

DEVELOPMENT OF METHODS FOR THE CLONAL PROPAGATION OF ELITE, DISEASE-RESISTANT COCONUT PALMS BY SOMATIC EMBRYOGENESIS

Co-ordinator: IRD (ex-ORSTOM), Montpellier, France (S. Hamon)

Objectives

The coconut palm (*Cocos nucifera* L.) is a major agricultural crop in tropical areas. Its importance is due to its role in oil production. It also provides cash and subsistence to small holders. However, the coconut sector has several problems that affect its productivity, particularly the use of unimproved planting material, the old age of existing plantations and various pests and diseases such as Lethal Yellowing (a phytoplasma disease which has devastated coconut crops in the Caribbean Region and continental America). Since coconut palm is generally cross-pollinated and heterozygous, propagation by seeds gives rise to a great variability in hybrid progenies. *In vitro* vegetative multiplication of high-performance individuals thus remains the only short-and medium-term hope for the production of homogenous high-yielding planting material. Cloning would also allow rapid multiplication of selected individuals that exhibit resistance or tolerance to Lethal yellowing. Since coconut is a highly recalcitrant species in *in vitro* culture, the main objective of this project is to overcome present problems in the culture of coconut tissues in order to improve somatic embryogenesis and ensure mass production of plantlets (ramets).

Results

⇒ The collaboration has successfully allowed the regeneration of vitroplants (from plumules, immature inflorescences and leaf fragments) in most laboratories involved in the project. Reliable protocols for plantlet regeneration have been developed from plumules and immature inflorescences.

⇒ This is the first time that prototype regeneration for coconut has become available. This important breakthrough, which is crucial to the future of coconut *in vitro* culture, reflects the quality of the exchange of information, technical know-how and protocols which has taken place between the various partners.

⇒ An International Symposium on Coconut Biotechnology was organised (December 1-5 1997) in CICY, Mérida (Yucatan). 19 countries were represented (6 from Latin America). The symposium included papers in the following areas: biotechnology and coconut industry, genetic improvement, coconut diseases, *in vitro* propagation. This symposium gave to the consortium, an opportunity to widely broadcast the knowledge and some of the results acquired in the framework of this STD3 project.

Significant publications

Chan JL, Saénz L, Talavera C, Hornung R, Robert M & Oropeza C. 1998. Regeneration of coconut (*cocos nucifera* L.) from plumule explants through somatic embryogenesis. Plant Cell Reports, **17** 6-7 (515-521).

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Partners

ORSTOM
Laboratoire de Ressources Génétiques et
d'Amélioration des Plantes Tropicales
Av. Agropolis 911
BP 5045
F-34032 Montpellier
France

S. Hamon and J.L. Verdeil
Tel.: +33-4-67-61-74-96
Fax: +33-4-67-54-78-00
E-mail: hamon @ orstom.fr

PCA ALBAY RESEARCH CENTER
Albay Research Center
Tissue Culture Dept.
Banao
4503 Guinobatan Albay
The Philippines

Erlinda Rillo
Tel.: +639-974-114/9228130
Fax: +639-9216173/9229180

CENTRO DE INVESTIGACION CIENTIFICA DE YUCATAN
Unidad de Propagación Clonal
7 Antigua Carretera a Progreso
Ex-Hacienda Xcumpich
Mex-97310 CODEMEX, Mérida
Yucatán
Mexico

Carlos Oropeza
Tel.: +52-99-44-02-91/71
Fax: +52-99-44-09-07
E-mail: cos@cicy.cicy.mx

UNIVERSITAET HANNOVER
Lehrgebiet Molekulargenetik für Biologie
Herrenhaeuserstrasse 2
D-30419 Hannover
Germany

Hans-Joerg Jacobsen
Tel.: +49-511-769-40-82
Fax: +49-511-762-40-88
E-mail: nhefmek@mbox.lgm.uni-hannover.de

UNIVERSITY OF LONDON
Wye College
Dept. of Agriculture, Horticulture and the
Environment
TN25 5 AH Wye
Ashford
United Kingdom

Jeffrey Moorby
Tel.: +44-1233-81-24-01
Fax: +44-1233-81-33-20
E-mail: shs-rh@wye.ac.uk

CIRAD
Dépt. des Cultures Pérennes
911 avenue Agropolis
BP 5035
F-34032 Montpellier
France

Jean-Luc Verdeil
Tel.: +33-4-67-61-58-00
Fax: +33-4-67-78-51-00
E-mail: Verdeil @ .melusine.mpl.orstom.fr

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Period: September 1994 to June 1998

MULTIDISCIPLINARY STUDY OF THE TRANSFORMATION OF AMAZONIAN FRUITS FOR THEIR COMMERCIALIZATION BY EXISTING ORGANIZATIONS OF SMALL FARMERS

Co-ordinator: Université Catholique de Louvain, Louvain-la-Neuve, Belgium
(Yvan Larondelle)

Objectives

- Generation of the nutritional, technological and socio-economic data needed to promote the production and marketing of food products derived from Amazonian fruits in the Amazon region of Brazil, specifically in the State of Pará. This objective is to be reached by developing and applying technological to be implemented in micro or small firms related to the small farmers' communities of the region.
- The valorization of regional fruits resulting from extraction or fruticulture may then become a factor in sustainable ecodevelopment in the Amazon region.

Activities

The activities have been divided into three phases: a short pluridisciplinary inventory of the existing resources with special focus on fruits; a 30- month phase of biotechnological and socio-economic thorough analysis of four selected fruits with at least one process per fruit, and finally a short phase aiming at the transposition of one selected process at the practical level.

Results

Phase 1 led to the selection of four fruits and of at least one process per fruit.

Brazil nuts (*Bertholettia excelsa*) present an excellent profile in sulphur-containing aminoacids and in unsaturated fatty acids, with high concentration of vitamin E and selenium. Protocols have been set up and optimized for the production of partially skimmed milk (to use as beverage) and fatty milk (to use for cooking). The partially skimmed milk was further studied in terms of emulsion stability and susceptibility to heat treatments. Partially skimmed milk adjusted to pH 8,0 and homogenized at high pressure in the presence of lecithin was selected for indirect UHT treatments.

The flour left after milk production still has good nutritional properties. The humidity content optimal for the storage of that flour was determined, as well as the kinetics of drying. Mixed flours adapted to the nutritional needs of infants and children were formulated on the basis of flours produced in the Pará State (Brazil nut residue, rice, bean, soya, corn and pejibaye - *Bactris gasipaes*).

Cupuassu (*Theobroma grandiflorum*) pulp has an excellent aromatic profile coupled with a high acidity. Its pasteurization was optimized by using *Alicyclobacillus acidoterrestris* as an indicator of quality process. Furthermore, the kinetics of modification of colour, flavour and aroma were modelled. The most appropriate thermal treatments may then be determined for any type of packing and pulp, and for any pH and Brix. Storage studies showed good quality-keeping during at least six months with a major effect of storage temperature but not of pasteurization temperature.

A cupuassu nectar was defined for northern and southern European consumers. Its pasteurization was modelled on vitamin C thermal degradation kinetics. The nectar behaved like a pseudo-plastic fluid.

Economic studies show that mechanical pulp extraction coupled with pasteurization is the most appropriate process for small firms. National market is growing.

The pulp of **assai fruit** (*Euterpe oleracea*) is traditionally extracted with water. This results in a very popular juice, rich in lipids, manganese, and antocyans, but highly perishable because of high microbial contamination and peroxidasic activity. Studies have been conducted on the process of juice making (identification of significant production parameters; conception of a continuous extracting machine), the significance of fruit varieties on production and nutritional quality, and the calorific power of stones. Measures designed to reduce the fruit's microbial charge (washing, sulphating and blanching) and the stabilization of juice by pasteurization (impact on micro-organisms and enzymes) have been thoroughly studied as well.

The flavour (aromatic compounds) and aromatic potential (analysis of heteroside structure and aromatic compounds after acid or enzymatic hydrolysis) of four Amazonian fruits (cupuassu, passion fruit, bacuri - *Platonia insignis* and Barbados cherry - *Malpighia punicifolia*) were determined to foresee the aromatic modifications that may result from technological treatment. Passion fruit showed an important aromatic potential including four glycoside classes. A weak potential with two glycoside classes characterised the three other fruits.

Formulation of blended juices made of **passion fruit**, mixed either with Barbados cherry, papaya and orange, was optimized. Sedimentation problems were solved by centrifugation in the two first cases, while an addition of papaya pulp was necessary in the third one. After pasteurization at 90°C for 10 min, the storage of the mixtures could be extended to 6 months at 30°C.

Follow up

Since 1997, this project is giving a scientific support to a Research and Development field project ("Valorization of fruits by the peasant organizations of the Pará State") based on collaboration between two NGOs (FASE in Brazil and ADRAI in Belgium). Its objective is to validate recommendations made by the researchers in demonstrative experiments in the field.

Partners

UNIVERSITE CATHOLIQUE DE LOUVAIN (UCL)
Faculté des Sciences Agronomiques
Unité de Biochimie de la Nutrition
Place Croix-du-Sud 2/8
B-1348 Louvain-la-Neuve
Belgium

Yvan Larondelle
Tel.: +32-10-47 37 85/35
Fax: +32-10-47 37 28
E-mail: larondelle@bnut.ucl.ac.be

UNIVERSIDADE FEDERAL DO PARA
Centro Tecnológico
Dept. Engenharía Química
Campus Universitario do Guama
Avda. Perimetral
66075-100 Belem PA
Brazil

Maria de Lourdes Soares Oliveira
Tel.: +55-91-249 20 88 ext. 295
Fax: +55-91-229 14 59

EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA
Centro de Pesquisa Agroforestal
Laboratorio de Agroindustria
Bairro do Marco 48
BR-66095-100 Belem-PA
Brazil

Raimunda Fatima Ribeiro de Nazaré
Tel.: +55-91-226 62 29
Fax: +55-91-226 96 80

UNIVERSIDADE CATOLICA PORTUGUESA
Escola Superior de Biotecnología
Rua Dr. Antonio Bernardino de Almeida
P-4200 Porto
Portugal

Cristina Luisa Miranda Silva
Tel.: +351-2-558 00 01
Fax: +351-2-590 351

INSTITUTO DO DESENVOLVIMIENTO ECONOMICO
Coordenadoria Socio-Económica
Avda Nazare 871
66035-170 Belem-PA
Brazil

Teresinha Monteiro Santos
Tel.: +55-91-224 54 14
Fax: +55-91-225 34 14

UNIVERSITE CATHOLIQUE DE LOUVAIN
Faculté des Sciences Agronomiques
Unité d'Economie Rurale
Place Croix-du-Sud 2/15
B-1348 Louvain-la-Neuve
Belgium

Bruno Henry de Frahan
Tel.: +32-10-47 36 74
Fax: +32-10-47 87 06

UNIVERSITE DE MONTPELLIER II
Institut des Sciences de l'Ingénieur
Laboratoire de Génie Biologique et Science des Aliments
Place E. Bataillon
F-34095 Montpellier
France

Jean Crouzet
Tel.: +33-4-67 14 33 12
Fax: +33-4-67 14 42 92

Contract number: TS3*CT940306

Period: January 1995 to June 1998

OPTIMISATION DES TECHNIQUES DE SELECTION DU PALMIER A HUILE A L'EGARD DE LA FUSARIOSE ET PRISE EN COMPTE DE L'INTERFACE RACINE/SOL DANS L'EVALUATION DE LA RESISTANCE

Co-ordinator: CIRAD - Côte d'Ivoire, Abidjan, Côte d'Ivoire, (Hubert de Franqueville)

Objectifs

- Validation de techniques mises au point au laboratoire pour évaluer la résistance à la fusariose du matériel végétal, à différents stades végétatifs.
- Evaluation de la pression parasitaire et des facteurs qui interviennent au niveau de la rhizosphère dans le développement de la fusariose.
- Confronter les résultats obtenus par de nouvelles techniques avec le comportement réel du matériel végétal en zone fusariée et aux résultats des inoculations en prépépinière qui constituent actuellement la base de la sélection précoce du matériel tolérant à la fusariose. Sélectionner des individus (tête de clone, géniteurs) et non plus seulement des populations (croisements).

Activités

* ***Etude des composantes internes de la résistance et outils d'investigation :***
- Perfusion de spores de *Fusarium oxysporum* f.sp. *elaeidis (Foe)* dans les pétioles de têtes de clones déjà caractérisées, puis dans des têtes de clones potentielles. Analyse des extraits de l'endocarpe du fruit. Etude de leurs propriétés fongistatique par chromatographie, densitométrie et autobiographie.
- Etude de la réponse métabolique du palmier à huile et à infection par le *Foe* et nature des substances impliquées; caractérisation des extraits phénoliques.

* ***Etude de l'interface racine/sol dans l'expression de la résistance :***
- Analyse des exsudats racinaires, constitutifs ou synthétisés après inoculation du *Foe*, en fonction de la résistance ou la sensibilité du matériel végétal.
- Etude de la pression de sélection exercée sur l'évolution quantitative et qualitative des populations de *Fusarium* soit par l'outil moléculaire, soit par compatibilité végétative. Identification des sites de pénétration du *Foe* : étude histologique et cytologique.
- Marquage des souches par le gène GUS.
- Etude ultrastructurale de la pénétration et de la progression du pathogène dans la racine.

Résultats attendus

Définir une stratégie globale de lutte contre la fusariose qui tienne compte des facteurs de résistance du palmier à huile et des facteurs intervenant dans l'infection racinaire par *Fusarium oxysporum* f.sp. *elaeidis*. Assurer la durabilité de la culture de plus en plus exposée au parasite et maintenir le potentiel de production d'huile de palme, en Afrique notamment, pour répondre au développement de la région et aux besoins en corps gras des populations concernées.

Partners

CIRAD - COTE D'IVOIRE
Département des Cultures Pérennes
Unité de Recherche Défense des Cultures
01 B.P. 6483
Abidjan 01
Côte d'Ivoire

Hubert de Franqueville:
Tel.: +225-221.869
Fax: +225-214.368

UNIVERSITY OF BATH
School of Biology and Biochemistry
Claverton Down
UK-BA2 7AY Bath Avon
United Kingdom

Richard Cooper
Tel.: +44-1225-826.826
Fax: +44-1225-826.779

UNIVERSITE LIBRE DE BRUXELLES
Faculté de Médecine & de Pharmacie
Laboratoire de Parasitologie Mycologique
Route de Lennik 808
B-1070 Bruxelles
Belgium

Jacqueline Pelseneer- Cooremans
Tel.: +32-2-5556251/ 6505279
Fax: +32-2-5556128/ 6505282

UNIVERSITE DE LYON I
Unité de Biologie Micromoléculaire & Phytochimie
Boulevard du 11 Novembre 1918, 43
Bâtiment 741
F-69622 Villeurbanne
France

Maurice Jay
Tel.: +33-72.44.82.05
Fax: +33-72.43.14.26

INSTITUT DES FORETS
Département des Plantes Oléagineuses
Plantation Expérimentale Robert Michaux
BP 8
Dabou
Côte d'Ivoire

Sekou Diabate
Tel.: +225-302.734
Fax: +225-226.985

EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA
Oil Palm Project
Rodovia AM 010
KM 25 Caixa Postal 319
69.011-970 Manaus - AM
Brazil

Edson Barcelos
Tel.: +55-92-622.20.12
Fax: +55-92-622.11.00

Contract number: TS3*CT940308

Period: October 1994 to May 1998

SUSTAINABLE AGRICULTURE : THE ROLE OF INTEGRATED MANAGEMENT OF ROOT ROT (*PHYTOPHTHORA CINNAMOMI* RANDS) IN AVOCADO (*PERSEA AMERICANA* MILL)

Co-ordinator: Centro de Investigación y Tecnología Agrarias ; La Laguna-Tenerife, Spain
(Luisa Gallo Llobet)

Objectives

The objective of the proposed research was to develop effective, environmentally responsible methods for management of avocado root rot disease caused by *Phytophthora cinnamomi* Rands which is the main limiting factor of this fruit crop. The main objectives were:

- Search for sources of resistance. To evaluate, select, and develop avocado rootstocks resistant/tolerant to *P. cinnamomi*, and to work with fast assay methods for selection.
- Evaluate the agronomic and commercial characteristics of the selected rootstocks with resistance to *P. cinnamomi* in infected soils with different climatic conditions.
- Gain in-depth knowledge of the avocado production system used in Michoacan (Mexico) with a view towards extrapolating data to other avocado-producing regions affected by *P. cinnamomi.*
- Evaluate the effects of host nutrition on root growth and disease resistance.
- Genetic identification of selected resistant-tolerant avocados and *P.cinnamomi* strains using isoenzyme analysis and molecular markers.

Activities

- Targeting of West Indian trees for seed collection and searching for resistance to *Phytophthora* root rot using inoculated nutrient solutions, natural infected soil in pots, field trials and fast assay methods. Resistant rootstocks will be tested and compared with available commercial resistant ones.
- Study of the effect of organic soil amendments and solarization for biocontrol of *Phytophthora* root rot of avocado.
- Classification of suppressive and non-suppressive soils, and isolation and identification of bacteria possibly involved in suppression.
- Determination of the effects of calcium, boron and zinc nutrition on root growth and disease and to evaluate the effect of humic acid on nutrient absorption and disease resistance.
- Genetic identification of selected tolerant-resistant avocado material and of *P. cinnamomi* strains using isoenzyme analysis and molecular markers will be performed. Isoenzymatic characterization for both plant and pathogen material will be done.

Expected outcome

The proposed research developed approaches to plant disease control that will form the core of an integrated management system and will permit successful long-term production of avocado. The work carried out will provide a West Indian avocado resistant germplasm bank. The use of avocado rootstocks with genetic resistance to the disease in combination with other control methods will permit replanting of areas where the disease has occurred. This process will allow

us to transfer the technology obtained to growers to promote understanding and adaption / adoption of integrated avocado crop management.

Partners

CENTRO DE INVESTIGACION Y TECNOLOGIA AGRARIAS
Departamento de Protección Vegetal
Apartado de Correos 60
E-38200 La Laguna - Tenerife
Spain

Luisa Gallo Llobet
Tel.: +34-22-54.01.50
Fax: +34-22-54.29.12

CENTRO DE FITOPATOLOGIA
Colegio de Postgraduados
56230 Montecillo
Texcoco Méjico
Mexico

Daniel Teliz
Tel.: +52-595-45.211
Fax: +52-595-45.211/45.723

UNIVERSIDAD DE COSTA RICA CIPROC
Facultad de Agronomía
2060 San Pedro de Montes de Oca
San José
Costa Rica

Luis Felipe Aarauz Gavallini
Tel.: +506-253.53.23 - Ext. 4214
Fax: +506-234.61.64

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Algarrobo Costa
E-29750 Málaga
Spain

Carlos José López Herrera
Tel.: +34-52-511.000/1
Fax: +34-52-511.186

UNIVERSITY OF BIRMINGHAM
School of Biological Sciences
Edgbaston
UK-B15 2TT Birmingham
United Kingdom

Brian Ford-Lloyed
Tel.: +44-121-414.55.65
Fax: +44-121-414.59.25

Contract number: TS3*CT940314

Period: November 1994 to October 1997

AN INTEGRATED STUDY OF LAND PROPERTIES, THEIR FLORISTIC INDICATIONS AND APPROPRIATE FARMING SYSTEMS IN AN ACKNOWLEDGED BIODIVERSITY CENTRE IN AMAZONIAN PERU

Co-ordinator: University of Turku, Turku, Finland, (Risto Kalliola)

Objectives

Many agricultural efforts in Amazonia have suffered from inadequate knowledge of site properties and appropriate land management systems. The major objective of this work is to gain new scientific understanding of the ecological constrains of agricultural production in this region by:

- determining the geological background for the existence of sites with different production potentials;
- developing a model for the identification of edaphic differences utilizing ecological knowledge of indicator plant species along with remote sensing data;
- promoting the development of appropriate farming systems for each site type to allow continuous production of food plants and/or silvicultural products.

Activities:

The study is a combination of fundamental research and applied research. The former studies have attempted to gain understanding of the variation in site edaphic conditions using aspects of geography, geology and biology. The applied studies try to develop appropriate farming systems for all the different site types.

Results

The results of this study have confirmed the hypothesis that the Amazonian soils can be much more varied than has been previously regarded, and that these variations are relevant in relation to both the ecology of the natural forests and land use planning.

Selected publications

Kalliola R. & Flores Paitán S. 1997. Ecological site conditions and landuse options in Amazonian Peru. In: Usó, J.L., C.A. Brebbia & H. Power (eds.) Advances in Ecological Sciences 1. Ecosystems and sustainable development. Computational Mechanics Publications, Southampton, pp. 254-263.

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Tuomisto H., Ruokolainen K., Kalliola R., Linna A., Danjoy W. & Rodriguez Z. 1995. Dissecting Amazonian biodiversity. — Science **269**: 63–66.

Partners

UNIVERSITY OF TURKU
Dept; of Geography
SF-20500 Turku
Finland

Risto Kallioloa
Tel.: +358-21-633 55 77
Fax: +358-21-633 58 96
E-mail: riskall@utu.fi

INTERNATIONAL SOIL REFERENCE AND INFORMATION CENTRE
P.O. Box 353
NL-6700 AJ Wageningen
The Netherlands

Sjef Kauffman
Tel.: +31-8370-71 718
Fax: +31-8370-244 60

FORSCHUNGSINSTITUT SENCKENBERG
Abteilung für Meeresforschung
Schleusenstrasse 39A
D-26382 Willemshaven
Germany

Georg Irion
Tel.: +49-4421-9475
Fax: +49-4421-9475/50

INSTITUTO NACIONAL DE RECURSOS NATURALES
Remote-Sensing and SIS Directory
Apartado 4992
Calle 17, no. 355
Urb. El Palomar, San Isidro
Lima
Peru

Walter Danjoy Arias
Tel.: +51-14-41 04 25
Fax: +51-14-41 46 06

UNIVERSIDAD NACIONAL DE LA AMAZONIA PERUANA
Departamento de Agronomía
Samanez Ocampo 185
Apartado 496
Iquitos
Peru

Gobert Paredes Arce
Tel.: +51-94-23 41 53
Fax: +51-94-23 89 51

Contract number: TS3*CT940316

Period: November 1994 to October 1997

ASSESSMENT OF GENETIC DIVERSITY OF ECONOMICALLY AND ECOLOGICALLY IMPORTANT TROPICAL TREE SPECIES OF CENTRAL AMERICA AND THE CARIBBEAN: IMPLICATIONS FOR CONSERVATION, SUSTAINABLE UTILIZATION AND MANAGEMENT

Co-ordinator: Institute of Terrestrial Ecology, Midlothian, United Kingdom (Julia Wilson & Amanda Gillies)

Objectives

- Develop tools to assess genetic diversity in tropical tree species using molecular techniques;
- Assess levels and organisation of genetic diversity in populations of economically and ecologically important species, and identify populations with particular value for conservation and breeding;
- Identify biological or anthropic factors contributing to decreases in genetic diversity;
- Promote training in techniques for studying genetic variation in tropical woody plants.

Activities

* Collect *Tabebuia heterophylla* in the Antilles, establish gene banks, assess genetic diversity;
* Map, inventory and collect material from *Swietenia macrophylla* populations in Central America;
* Evaluate relationships between molecular and morphological variation in *Vochysia guatemalensis* and *Cedrela odorata*
* Assess genetic diversity in *Swietenia* and *Cedrela* populations;
* Assess genetic erosion in *Swietenia*, develop conservation and utilization strategies, establish gene banks and provenance tests;
* Collect progenies of *Dicorynia guianensis, Eperua grandiflora, Chrysophyllum sanguinolentum, Virola michelii, Ocotea rubra* and *Vouacapoua americana*, estimate mating system and gene flow parameters and evaluate relationships between life history traits and levels of genetic diversity;
* Develop appropriate molecular techniques, train in molecular methods, sample collection and herbarium techniques.

Results

⇒ *T. heterophylla* is predominantly outcrossing. Trees varied in auto-incompatibility. Results indicate the capacity of the tree to reproduce in both high and low density situations. Populations from northern islands were more diverse than those from southern islands; but northern islands showed less differentiation between them. Results suggest that gene flow between islands is extremely low.

⇒ Populations of *C. odorata* from the Atlantic/Southern Pacific and Northern Pacific regions of Costa Rica were strongly genetically differentiated.

⇒ Heavily logged populations of *S. macrophylla* show less diversity than undisturbed populations.

⇒ Diversity of populations varies according to their proximity to sites of putative Pleistocene Refugia.

⇒ *V. michelii, C. sanguinolentum* and *E. grandiflora* appear to be strictly outcrossing; *D. guianensis* and *V. americana* demonstrated mixed mating systems and were tolerant of selfing.

⇒ Chloroplast DNA studies of *D. guianensis* show clustering (<50 m) of haplotypes at the same scale as the patches of this species in the forest. However, no spatial differentiation was found for nuclear DNA polymorphism, suggesting that there is asymmetry of geneflow by seed and pollen.

⇒ Pollen clouds of *V. michelii* showed genetic differentiation at a scale between 2 and 3 ha, which is close to the neighbourhood size of male trees. For *V. americana,* differences in allelic frequencies of pollen clouds parallel those of adult trees in the same location. Differentiation of allele frequencies in adult trees occurs at a large spatial scale (up to 50 ha).

⇒ *V. michelii* and *C. sanguinolentum* were more phenotypically diverse than *E. grandiflora* and *D. guianensis* (determined by RAPDs). *V. americana* was substantially less polymorphic. Comparing species, outcrossing rate and phenotypic diversity were inversely correlated. Population size of a species was not correlated with diversity.

⇒ *Vochysia guatemalensis* is predominantly outcrossing.

Follow up

Follow up studies will be partly conducted under ERBIC18*CT97-0149. They will include observations on genetic diversity of regenerating seedlings of *S. macrophylla* (to examine the effects of logging on the next generation) and observations of a wider spectrum of Guyanan rainforest species, to confirm the importance of outcrossing in maintenance of genetic diversity. If confirmed, the latter may lead to modifications in sylvicultural and logging operations. Results obtained under this contract have contributed to the formulation of government policy in Costa Rica and Nicaragua regarding *S. macrophylla* and have contributed to revision of the collection strategy in the Antilles for *T. heterophylla.*

Selected publications

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Partners

INSTITUTE OF TERRESTRIAL ECOLOGY
Bush Estate
Penicuik
EH26 0QB Midlothian
United Kingdom

Julia Wilson & Amanda Gillies
Tel: (+44) (0) 131 445 4343
Fax: (+44) (0) 131 445 3943
E-mail: julia.wilson@ite.ac.uk

CENTRO AGRONOMICO TROPICAL DE INVESTIGACIÓN Y ENSEÑANZA
Cartago
Turrialba 7170
Costa Rica

Carlos Navarro
(00) (506) 556 6431, 556 0169
(00) (506) 556 1533
email: cnavarro@computo.catie.ac.cr

INSTITUT DE LA RECHERCHE AGRONOMIQUE
INRA Bordeaux
Station de Recherches Forestières de Bordeaux-Cestas
Laboratoire de Génétique et Amélioration
BP 45
F-33611 Gazinet
France

Antoine Kremer
Tel.: +33-5-57 97 90 00
Fax: +33-5-57 97 90 88
E-mail: antoine.kremer@pierroton.inra.fr

INRA Guadeloupe
B.P. 515
97185 Pointe-à-Pitre cedex
Guadeloupe FW1
Antilles

Patrick Labbé
Tel.: +590-25 59 16
Fax: +590-94 16 63
E-mail: labbe@antilles.inra.fr

INRA Guyane
Station de Recherches Forestières
B.P. 709
97387 Kourou
Guyane Française

Henri Caron
Tel.: +594-92 93 00
Fax: +594-32 69 14
E-mail: caron@antilles.inra.fr

Contract number: TS3*CT940324

Period: January 1995 to December 1998

FOG AS A NEW WATER RESOURCE FOR THE SUSTAINABLE DEVELOPMENT OF THE ECOSYSTEMS OF THE PERUVIAN AND CHILEAN COASTAL DESERT

Co-ordinator: Università degli Studi di Firenze, Firenze, Italy (Mario Falciai)

Objectives

- Experimental verification of the following assumptions:
  - effectiveness of artificially collected fog water in the restoration of vegetation;
  - ability of the plants to sustain themselves by means of water supply derived from the fog they collect and with no more need of man-made collectors;
  - possibility that the surplus of water obtained by means of artificial collectors can be used for subsistence agriculture and for pasture growth.
- Characterization of the ecosystems of the coastal desert;
- Devising possible scenarios of social and economic impact in the field of development projects based on rational utilization of the resources of the coastal desert and on actions to be undertaken to enforce existing laws for environmental protection.

Activities and results so far

- Pilot experiment carried out in a selected area of the coast near the town of Mejía, to verify the assumptions outlined above. In the experimental area, 20 large fog collectors, made up of a polypropylene mesh have been mounted for a total capturing area of 960 m^2. The water obtained from fog collection is being used throughout the year to experiment with the species of plants selected and cultivated in plots in the experimental station area with a drip irrigation system.
- Verification of the possibility of using fog water to restore vegetation in order to allow the development of subsistence agriculture and controlled cattle grazing.
- Verification that some plant species survival can depend on the sole contribution of water coming from fog.
- Collection of amounts of water larger than required by vegetation. This resource can be stored and used in other periods.
- Contribution to the development of a work methodology suitable for applications in other regions of the world, where the advection of marine clouds produces high elevation fogs.

Selected publications

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Partners

UNIVERSITA DEGLI STUDI DI FIRENZE
Dept. Ingegnieria Agraria e Forestale
Via San Bonaventura 13
I-50145 Firenze
Italy

Mario Falciai
Tel.: +39-55-30 23 12 41
Fax: +39-55-31 02 24
E-mail: idrag@cesit1.unifi.it

UNIVERSITA DEGLI STUDI DI PADOVA
Dept. Territorio e Sistemi Agroforestali
Via Gradenigo 6
I-35131 Padova
Italy

Roberto Semenzato
Tel.: +39-49-8277.157
Fax: +39-49-8277.102
E-mail: semenzato@padova.infn.it

UNIVERSITA DEGLI STUDI DI FIRENZE
Istituto di Selvicoltura
Via San Bonaventura 13
I-50145 Firenze
Italy

Gianfranco Calamini
Tel.: +39-55-30231.246
Fax: +39-55-307263
E-mail: calamini@cesit1.unifi.it

UNIVERSITE PAUL SABATIER
Inst. de la Cart. Internat. de la Végétation
Avenue du Colonel Roche 13
F-31062 Toulouse
France

François Blasco
Tel.: +33-61-55.85.43
Fax: +33-61-55.85.44

UNIVERSIDAD NACIONAL DE SAN AUGUSTIN
Inst. Regional de Ciencias Ambientales
Casilla 985
Arequipa
Peru

Percy Carlos Jiménez
Tel.: +51-54-288971
Fax: +-5154-288971
E-mail: ireca@unsa.edu.pe

UNIVERSIDAD CATOLICA DE CHILE
Instituto de Geografía
Avenida Vicuna MacKenna 4860
Correo 22
Casilla 306
Santiago
Chile

Pilar Cereceda Troncoso
Tel.: +56-2-6864721
Fax: +56-2-5526028
E-mail: dcereced@puc.cl

UNIVERSITA DEGLI STUDI DI PADOVA
Dept. Territorio e Sistemi Agroforestali
Agripolis
I-35020 Legnaro (PD)
Italy

Giorgio Franceschetti
Tel.: +39-49-8272.705
Fax:: +39-49-8272.703

Contract number: TS3*CT940333

Period: January 1995 to December 1998

DEVELOPMENT OF MINIMALLY PROCESSED PRODUCTS FROM TROPICAL FRUITS USING VACUUM IMPREGNATION TECHNIQUES

Co-ordinator: Universitad Politécnica de Valencia, Valencia, Spain (Petro Fito Maupoey)

Objectives

- Develop minimally processed products from tropical fruits that have physical, chemical and sensory characteristics as similar as possible to the raw material and are stable at room temperature or on refrigeration, according to local commercial practices.
- Develop products from banana, mango, papaya and pineapple, among other fruits, with two types of properties:
  - high-moisture-content foods (HMF) similar to fresh foods
  - Intermediate moisture-content foods (IMF) such as purees and jams, prepared with mild thermal treatments and showing optimum retention of colour and flavour.
- Model, design and optimize processes to obtain these products, using osmotic dehydration (OD), vacuum impregnation operations (VI), and pulsed vacuum osmotic dehydration (PVOD). These processes will be combined with other treatments, such as pH reduction, use of preservatives, mild heat treatment, high-pressure or refrigeration.

Activities

- Physico-chemical and structural characterization of raw and processed material, and correlation between these parameters.
- Analysis of the sorption isotherms of each fruit in order to find the relationship between water activity and product composition.
- Modelling of mass transfer kinetics and fruit response (impregnation/deformation levels) in vacuum impregnation process.
- Modelling of the effects of temperature, pressure and type of osmotic solution on the kinetics of VOD and PVOD through calculation of kinetic parameters for the transfer of water and solutes between food and osmotic solution.
- Evaluation of combined preservation methods on growth, thermal resistance and inactivation of micro-organisms, considering the effects of water-activity reduction, pH lowering, mild thermal treatment, refrigeration, and oxygen-availability lowering on moulds, yeasts, and bacteria, mesophile anaerobes and aerobes, and psicrophile aerobes. Assessment of changes in texture, colour and nutritional value.
- Design of HMF and IMF products, using impregnation and osmotic-dehydration treatments on the basis of the detailed results of the modelling.
- Study of the native flora (moulds, yeast, and bacteria) development during processing and storage of the products, as well as the quality factors: water activity, pH, texture, colour, vitamin C, degradation of added preservatives and sugar hydrolysis, as a function of the storage temperatures.
- Sensory evaluation of the developed products by a consumer panel, and establishment of correlation between subjective and instrumental evaluation of colour and texture.

Selected publications

Fito P., Chiralt A. 1997. An approach to the modelling of solid-food/liquid operations: application to osmotic dehydration. In: Food Engineering 2000. Ed. P. Fito, E. Ortega, G. Barvosa. Chapman & Hall. 231-252.

Martínez-Monzó J., Martínez-Navarrete N., Chiralt A., Fito P. 1998 Mechanical and structural changes in apple (var. Granny Smith) due to vacuum impregnation with cryoprotectant agents. Journal of Food Science. **63(3)**, 1-5.

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López-Malo A., Alzamora S.M. and Argaiz A. 1997. Vanillin and pH synergetic effects on mould growth. Journal of Food Science. **62**:1-4.

Tapia M.S., Consuegra R., López-Malo A., Corte P. and Welti J. 1997. Minimally processed papaya by vacuum osmotic dehydration techniques. Food Science and Technology International (in press).

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Partners

UNIVERISTAT POLITECNICA DE VALENCIA
Dept. de Tecnología de Alimentos
Camino de Vera s/n
E-46020 Valencia
Spain

Pedro Fito Maupoey
Tel.: +34-96-387 73 64
Fax: +34-96-387 79 56
E-mail: pfito@tal.upv.es

UNIVERSIDAD DE BUENOS AIRES
Facultad de Ciencias Exactas y Naturales
Ciudad Universitaria
1428 Buenos Aires
Argentina

Stella Maris Alzamora
Tel.: +54-1-784-0208
Fax: +54-1-784 02 08/

UNIVERSIDAD CENTRAL DE VENEZUELA
Facultad de Ciencias y Tecnología de Alimentos
Calle Suapure Lomas de Bello Monte
P.O. Box 47097
YV-1041 A Caracas
Venezuela

Maria Soledad Tapia de Daza
Tel.: +58-2-752 44 03
Fax: +58-2-752 38 71

FUNDACION UNIVERSIDAD DE AMERICAS PUEBLA
Dept. de Ingeniería Química y Alimentos
Apartado postal 100
Sta. Catarina Martir
72820 Cholula
Mexico

Jorge Welti Chanes
Tel.: +52-22-29 20 05
Fax: +52-22-29 20 09

UNIVERSIDADE CATOLICA PORTUGUESA
Escola Superior de Biotecnología
Rua Dr. Antonio Bernardino de Almeida
P-4200 Porto
Portugal

Fernanda Oliveira
Tel.: +351-2-558 00 01
Fax: +351-2-59 03 51

Contract number: TS3*CT940335

Period: January 1995 to January 1998

ECOSYSTEMS OF THE IX REGION OF CHILE: INFLUENCE OF LAND USE ON SUSTAINABILITY

Co-ordinator: Universität Bayreuth, Bayreuth, Germany (Klaus-Müller-Hohenstein)

Objectives

- Characterise the main land-use systems (ecosystems) of the Central Valley of the IX region of Chile.
- Examine the sustainability of these ecosystems in the context of biodiversity, nutrient stocks and acidification.
- Establish a Geographic Information System (GIS)-based environmental monitoring and recommendation system, including social and economic aspects.
- Make recommendations for sustainable agriculture and land use in the IX region in strict co-operation with the local population, esp. farmers.

Activities

The project deals with two types of data:
- Descriptors, which characterise the different properties of the respective systems;
- Indicators, which shall describe the degree to which the respective factors or factor-combinations contribute or impede a sustainable use.

Detailed investigation will comprise the following:
- Soil chemistry: detailed description using standard methodologies of total and plant-available elements. As P availability and soil acidification are major problems in this region, adsorption isotherms of P, P fractions, and Al species shall be determined.
- Soil physics: standardized description of soil physical properties as well as erodability (plots and modelling).
- Soil biology: characterisation of soil microbial biomass and soil organic matter by the fumigation/extraction method, classical fractionation, and NMR.
- Phytopathology: evaluation of frequency and intensity of attack of most important diseases and pests by standard methods.
- Hydrology/Hydrochemistry and Pesticide residues: dynamics of small watersheds will be followed and standard water characterisation techniques will be used to evaluate the effect of the different systems on the water regime.
- Biogeography: inventories of existing plant associations, characterisation of the biodiversity and selection of indicator species.
- Agronomy: balances for nutrients, organic matter, energy, and economics will be determined to characterise productivity and its changes over time.
- Agro-sociology/Anthropology: the decision backgrounds of the different groups will be evaluated and recommendations will be elaborated to motivate them to make a better use of natural resources.

Expected outcome

⇒ It is expected that the project will provide a model that could be used to elaborate plans for a more sustainable use of natural resources by means of a GIS. Some factors that prevent the land use from being sustainable have already been identified and discussed with representatives of the local population, of the forest companies and with colleagues from different Chilean universities during a workshop in March 1998, in Temuco.

⇒ Recommendations for an improved use of agricultural and forest lands will be elaborated, as well as publications about the main scientific results of the different working groups.

Partners

UNIVERSITAET BAYREUTH
Institut für Geowissenschaften
LS. Biogeographie- Abt. Agrarökologie
D-95440 Bayreuth
Germany

Klaus Müller Hohenstein
Tel.: +49-921-55 22 70
Fax: +49-921-55 23 15
E-mail: klaus.mueller-hohenstein@uni-bayreuth.de

INSTITUTE FOR ARABLE CROPS RESEARCH
Rothamsted Experimental Station
Soil Science Department
GB-AL5 2JQ Harpenden
United Kingdom

Keith Goulding
Tel.: +44-1582-76 31 33

UNIVERSIDAD DE LA FRONTERA
Departamento Ciencias Químicas
Laboratorio de Química Ambiental
Avenida Francesco Salazar 1145
Casilla 54 D
Temuco
Chile

Itilier Salazar
Tel.: +56-45-252547
Fax: +56-45-252547

UNIVERSIDAD AUSTRAL DE CHILE
Instituto Producción y Sanidad Vegetal
Casilla 567
P.O. Box 567
Valdivia
Chile

Roberto Carrillo
Tel.: +56-63-22 12 32
Fax: +56-63-21 29 53

Contract number: TS3*CT940341

Period: January 1995 to December 1997

NEW FOOD PRODUCTS FROM *PROSOPIS* FRUITS IN LATIN AMERICA: EXTENDING USE AND PREVENTING DESERTIFICATION IN ARID ZONES.

Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
(Fulgencio Saura Calixto)

Objectives

- Obtain gums, syrups and dietary fibre materials form mesquite pods
- Carry out physiological studies on the specific properties of dietary fibres rich in polyphenols
- Check the possibility of cultivating *Prosopis* trees in the dry lands of the regions with the lowest annual rainfall of Spain (south-east)

Activities and Results

⇒ ***Gum from Prosopis seeds.***
Thermal and chemical treatments were applied to seeds in order to remove the tegument, followed by splitting and sorting by colours to separate the endosperm or gum from the seed coat and cotyledon. After milling, a powdered galactomannan was obtained. Molecular weight, galactose:mannose ratio, and rheological properties were tested, as well as other physiological properties (water-holding capacity, glucose retention index, fermentability, etc.)

⇒ ***Syrups and dietary fibres from Prosopis pulp***
Syrups to be used as commerical sweeteners were obtained after water extraction of the sugar-rich mesquite pulp in successive steps using a multistage counter-current system, and centrifugation and vacuum concentration of juices. Clarification of these dark-brown products rendered clear solutions. Physico-chemical and nutritional evaluation of syrups was performed.

⇒ After water extraction, the pulp was pressed, dried (until moisture less than 5%) and milled. A dietary material very rich in insoluble dietary fibre was obtained. *In vitro* and *in vivo* studies, both with animals and healthy human volunteers, were carried out. This fibre material showed a strong bulking capacity, with no adverse nutritional effects. No influence on lipid metabolism could be observed.

⇒ ***Planting Prosopis trees in south east Spain***
Prosopis seeds were germinated, and plantlets kept in nursery until their plantation in three locations: Murcia, Andalucia and Valencia. Excellent growth and adaptation to adverse climatic conditions were observed in trees planted in Murcia. Plantations in the other two locations showed good adaptation but not exceptional growth.

Expected outcomes and follow-up

- New food products have been developed and presented to consumers and industries in Peru. Their introduction in local and regional markets in Latin America is planned.
- A pilot plant has been designed and set up for the obtention of food products from *Prosopis* fruits. Transfer of the technology to local industries is shortly expected.
- Introduction of Prosopis trees in dry areas of Southern Spain could help to alleviate growing desertification. Plantations will be followed-up to check long-term adaptation and productivity.

Selected publications

Bravo L., Grados N & Saura-Calixto F. 1998. Characterization of syrups and dietary fiber obtained from mesquite pods (*Prosopis pallida* L). J. Agric. Food Chem. **46** (4).

Grados N. & Cruz G. 1996. New approaches to industrialization of algarroba pods in Peru. In: "Prosopis: Semiarid Fuelwood and Forage Tree. Building Consensus for the Disenfranchised". Felker P. & Moss J. Eds.

Partners

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Instituto del Frío
Ciudad Universitaria
E-28040 Madrid
Spain

Fulgencio Saura Calixto
Tel.: +34-91-544 56 07
Fax: +34-91-549 36 27

UNIVERSIDAD DE PIURA
Laboratory of Chemistry
Avenida Ramón Múgica s/n
Urb. San Eduardo
Piura
Peru

Gaston Cruz Alcedo
Tel.: +51-74-32 81 71
Fax: +51-74-32 86 45

GENERAL DEL ALGARROBO DE ESPAÑA S.A.
Vereda Real
Correos 1456
E-46184 Valencia
Spain

Carlos Sanjuan Díaz
Tel.: +34-96-135 05 51
Fax: +34-96-135 02 54

UNIVERSITY OF EDINBURGH
Dept. of Medicine
Western General Hospital
Crewe Road
GB-EH4 2XU Edinburgh
United Kingdom

Martin Eastwood
Tel.: +44-131-537 10 00
Fax: +44-131-537 10 06

INSTITUTO POLITECNICO NACIONAL
Departamento de Biotenología
Avenida del Instituto Politécnico Nacional 2508
Apartado postal 14-740
07300 México DF
Mexico

Emma-Gloria Ramos Ramírez
Tel.: +52-5-754 02 00
Fax: +52-5-752 05 90

STD III

Health

Contract number: TS3*CT910024

Period: April 1992 to March 1995

BITES AND STINGS BY VENOMOUS ANIMALS IN BRAZIL: CLINICAL AND LABORATORY INVESTIGATIONS OF ENVENOMING AND THERAPY

Co-ordinator: Liverpool School of Tropical Medicine, United Kingdom
(R.D.G. Theakston)

Objectives

The aim of the project was to assess the problems caused by venomous bites and stings in Brazil.

Activities

- Complete monitoring of patients with severe envenoming were carried out. The effect of antivenom in reducing both the extent of swelling and local necrosis was investigated, as was the problem of possible pituitary and adrenal insufficiency caused by pituitary infarction.
- The use of tourniquets in systemic envenoming was evaluated in 20 patients and 20 controls.
- The prophylactic use of antimicrobial drugs was investigated in patients with moderate and severe systemic envenoming, to determine whether or not organisms present in the oral cavity of venom or on the gangs of wild snakes result in wound infection in the bitten patient.
- An investigation of whether routine prophylactic antihistamine prevents both early and late reactions to antivenom was carried out in both moderately and severely envenomed patients in a randomized double blind placebo-controlled trial.
- Thirty patients with moderate envenoming received half the lowest dose of antivenom given earlier, and 30 received the same starting dose as given in the previous project. Recovery from clinical signs, venom clearance, circulating antivenom levels, coagulation and fibrinolytic, hematological and biochemical profiles were compared.
- Laboratory studies were carried out to assess the significance of the ELISA method for detection of functional molecules rather than immune complexes.
- Studies on patients envenomed by scorpions and spiders were carried out in Sao Paulo and Belo Horizonte to investigate the kinetics of envenoming and therapy.
- The detection of specific venom in lesions caused by *Loxosceles* spider bites was carried out.
- Investigation of the pathogenesis of envenoming by *Crotalus durissus terrificus* in Sao Paulo and Minas Gerais States was carried out to improve patient treatment.
- Determination of the importance of snake bite, to assess the traditional methods of treatment and to try and improve the management of severe cases in indigenous Indian and other associated populations.
- The latest techniques of "molecular genetics" and multivariate analysis of morphological/anatomical features in comparison to venom composition were applied to provide practical guidelines for antivenom manufacturers to ensure adequate neutralizing efficacy of their products throughout Brazil.
- Clone *Bothrops* myotoxins.

Expected outcome

Knowledge of the pathophysiological effects of venom components and establishment of appropriate treatment schemes for patients with envenoming.

Partners

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Venom Research Unit
Pembroke Place
UK-L3 5QA Liverpool
United Kingdom

R.D.G. Theakston
Tel: +44-151-708.93.93
Fax: +44-151-708.87.33

INSTITUTO BUTANTAN
Hospital Vital Brazil
Av. Vital Brazil 1500
05504 Sao Paulo
Brazil

J.L.C. Cardoso
Tel: +551-1-211.83.61
Fax: +551-1-815.15.05

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Hosp. das Clinicas
Av. Professor Alfredo Bolena 110
CEP 13013 Belo Horizonte
Brazil

C.F.S. Amaral
Tel: +553-1-239.71.00
Fax: +553-1-226.82.77

UNIVERSITY OF OXFORD
J. Radcliffe Hospital
Centre for Tropical Medicine
OX3 9DU Headington
UK-Oxford OXDU
United Kingdom

D.A. Warrell
Tel +44-186-522.13.32
Fax: +44-186-522.09.84

UNIVERSITY OF ABERDEEN
Dept. of Zoology
Tillydrone Avenue
UK-AB9 2TN Aberdeen
United Kingdom

R.S. Thorpe
Tel: +44-122-427.20.00
FAx: +44-122-427.43.96

CENTRE D'ETUDES NUCLÉAIRES DE SACLAY
Service de Biochémie des Protéines
Gif-Sur-Yvette
F-91191Saclay
France

A. Menez
Tel: +33-1-69.08.60.00

Contract number: TS3*CT910029

Period: April 1992 to March 1995

A SURVEY OF CHAGAS CYCLES IN URUGUAY BY USE OF GENETIC MARKERS WITH SPECIAL EMPHASIS ON REINFESTATION HAZARDS OF DOMESTIC STRUCTURES BY SYLVATIC CYCLES

Co-ordinator: IRD (ex-ORSTOM) Montpellier I, Montpellier, France (J.P. Dujardin)

Objectives

- Understand why northern and southern areas of Uruguay present different epidemiologically features: domiciliated, more infected insects (*T. infestans*) in the northern departments versus peri-domiciliated, less infected insects in the southern departments: *Triatoma infestans* (Hemiptera, Reduviidae) is almost exclusively domestic in northern departments of Uruguay, such as Artigas, Cerro Largo, Rivera and Tacuarembo, but mainly occupies peridomestic habitats such as chicken coops in the southern departments of Soriano or Colonia. These areas differ in median temperature and humidity, with the northern regions tending to be warmer and drier, but also differ in socio-economic conditions such that rural dwellings in the northern departments are usually constructed of wood and/or adobe blocks, whereas brick and cement houses are more common in Soriano. This parallels higher infection rates of *T. infestans* with *T. cruzi* (causative agent of Chagas disease) in the northern departments. We were interested to examine the environmental and genetic contributions to the observed epidemiologic differences.
- Understand the reinfestation mechanisms of treated areas by the original vector species (*Triatoma infestans*), or by another one (*T. rubrovaria*). Reinfesting populations may represent hidden survivors recovering from the control treatment or they may be bugs immigrating from untreated foci. Operationally, it is important to distinguish between "survivors" (indicating control failure) and "immigrants" (indicating poor geographic coverage). Moreover, localized domestic invasions occurring in Uruguay were due to *T. rubrovaria*, a little-known species of *Triatominae*, providing a certain imperative to identify bug populations that might present a future risk of domiciliation.

This research programme was thus naturally inserted in the activities of the National Control Programme of Chagas Disease in Uruguay.

Results

1st Objective:

⇒ Though they were not distinguishable by isoenzyme electrophoresis, northern and southern populations of *T. infestans* in Uruguay were found to strongly differ at both cytogenetical and morphometrical characteristics. These results were related to the two-ways entry of *T. infestans* in Uruguay. *T. infestans* is believed to have originated from silvatic populations in central Bolivia and to have been dispersed mainly in association with human migrations, particularly during the last century.

⇒ Historical reconstruction suggests that it entered northern Uruguay from southern Brazil at around the turn of this century, but it appears to have entered southern Uruguay from Argentina some 50 years earlier since "vinchucas" (the local term for domestic Triatominae in Argentina and Uruguay) are mentioned in southern Uruguay in the chronicles of W.H.

⇒ Hudson first published in 1865. Biogeographically, the northern and southern departments of Uruguay are separated by the Rio Negro, which was bridged only a few decades ago, so that the apparent cytogenetical and metrical differences between northern and southern populations of *T. infestans* seems to accord both with their different origins and with an ecological barrier between them. In both cases, we can infer development from original founder populations, and assume that these would have differed slightly due to genetic drift which may or may not have been modified by adaptation to the different environments.

2nd objective

⇒ The various data obtained - cytogenetic, morphometric and isoenzymatic - converged on the idea that reinfestant specimens arose from a residual population. Indeed, in case of exchange of individuals between departments, between sectionals or between segments (administrative units in Uruguay), it was unlikely to find such an amount of cytogenetic and morphometric differences between them. Furthermore, when comparing reinfestant specimens with the insects collected before insecticide treatment, it was not possible to detect significant differences. The use of blood meal identification brought further, more defined, information about the mechanisms of reinfestation by *T. infestans*, indicating that the residual population was most probably of peridomestic, rather than domestic, origin.

⇒ Bloodmeal identification in *T. rubrovaria* revealed that this species had no host preference, indicating that its trend to domesticity was probably not due to an anthropophily, but rather to habitat convenience. Human blood was found in 10% of *T. rubrovaria* specimens, but in peridomiciliary conditions only, indicating that in limited geographic areas this silvatic species could make the link between the domestic and silvatic cycles of the parasite.

Partners

IRD (EX-ORSTOM), MONTPELLIER I
Labo de Génétique Moléculaire des Parasites et des Vecteurs
Avenue Agropolis 911, BP 5045
F-34032 Montpellier 1
France
J.P. Dujardin
Tel: +33-1-48.03.77.77
Fax: +33-1-48.03.08.29

UNIVERSIDAD DE LA REPUBLICA
Instituto de Higiene
Avenida Alfredo Navarro 3051
11600 Montevideo
Uruguay
L. Yarzabal
Tel: +598-247.12.88
Fax: +598-280.15.97

UNIVERSIDAD AUTONOMA DE MADRID
Departamento de Biología
Carretera de Colmenar Viejo
E-28049 Madrid
Spain
J.S. Rufas
Tel: +34-91-397.40.13
Fax: +34-91-397.41.23

Contract number: TS3*CT910038

Period: May 1992 to October 1995

IDENTIFICATION OF CANDIDATE PROTECTIVE MOLECULES OF *E. GRANULOSUS* AND DEVELOPMENT OF COMBINED *SALMONELLA* VACCINES

Co-ordinator: Universidad de la República, Montevideo, Uruguay (A. Nieto Cadenazzi)

Objectives

- Identification of vaccine candidate *E. granulosus* antigens (Ags) through analysis of immune evasion mechanisms as well as isolation of recombinant clones encoding the relevant Ags from cDNA libraries. Ags to be investigated will include *glutathione-S-transferase* (GST) and myosins. Immunogenicity and protection will be tested in animal models.
- Analysis of the structure of *E. granulosus* glycans and their influence, as well as that of the idiotype network, on the balance between susceptibility and protection to infection.
- Expression of recombinant antigens in live *Salmonella* vaccines to obtain maximal expression of the recombinant antigens in an integrated and maximally immunogenic form.

Activities

Uruguayan partner (Montevideo:

* purify the *E. granulosus* metalloproteases;
* purify anti-complement molecules obtained from *E. granulosus*;
* identify putative protective antigens from protoscolex (PSC) surface antigens used to protect mice;
* assay the immunogenicity of those antigens; those exhibiting protective capacity will be cloned in the laboratory of the UK partner (Newcastle);
* identify and characterise immunogenic parasite glycans and analyze their role in immune evasion as well as that of anti-idiotypes;
* analyze the influence of *E. granulosus* on Th1/Th2 balance and susceptibility to infection.

UK partner (Newcastle):

* Optimisation of expression of recombinant antigens in aro and htrA *Salmonella* vaccines. The antigens will be expressed both as the whole protein and as immunogenic peptide fused to LT-B;
* Testing of the construct in the mouse model for developing humoral and cell-mediated immunity.

The French partner (Paris):

* Participation in collaboration with Prof. Ehrlich's laboratory in the search of genes coding for proteins essential for parasite survival.

Expected outcome

E. granulosus protease clones will become available for use as immunogens and for sequence analysis. Complement activating glycans will be functionally characterised and the *in vivo* role of complement in susceptibility to infection will be tested. Candidate protective molecules and specific antibodies (Abs) will be prepared. Putative protective molecules will be cloned in

Salmonella and tested as immunogens. Optimisation of expression of recombinant antigens in *Salmonella* vaccines will lead to the development of a putative *Salmonella*-hydrated vaccine, using the recombinant hydatid antigens which become available.

Results

⇒ Uruguayan partner

E. granulosus metalloproteases (EgMP) cleaving human IgG3 and IgG1 were purified. Abs recognizing the 75kDa EgMP band, were found in sera from infected mice and humans but not dogs. Rabbit anti-EgMP was used to screen a cDNA library. A clone was purified, but no sequence homology was found with any known protease. Complement activation at different steps of the cascade by different parasitic preparations was assayed Cyst fluid (CF) produced the maximum TCC levels. CF derived *N*-linked oligosaccharide which produce TCC by fluid phase complement activation were identified. In addition, *in vivo* complement depletion was shown to decrease susceptibility to infection in mice. At least four glucoproteins from the protoscolex (PSC) surface have been identified which are recognised by sera from protected mice and included in ISCOMs which were immunogenic intranassally. Parasite GST and a candidate protective *E. granulosus* clones, one homologous to tropomyosin (EgDf5) and the other to fatty acid binding proteins (EgDf1) were isolated in Prof. Ehrlich's laboratory in collaboration with Prof. Nieto's laboratory. The role of CD4+ and CD8+ T-cells in immunity as well as the IL profiles they produce during *in vitro* proliferation were also analyzed in the mouse in collaboration with Dr. Anders Örn (Karolinska Institute, Stockholm). A MAb (E492) was prepared recognizing GalΒ1-4Gal in PSC and used to isolate a fraction, containing complement-activating, mitogenic, and immunodominant T-independent glycan Ags. Surface PSC glycans obtained by EndoF treatment, were mitogenic *in vitro* and produced hypergammaglobulinemia *in vivo*. They were immunodominant in mouse, eliciting low avidity Abs and an unexpected IgM titer increase after booster. Cyst membranes (CM) glycans were also characterized and found to contain both *N*-linked and *O*-linked oligosaccharides which are immunogenic in infected hosts. A MAb (2B5) recognizing the immunodominant Gal-gicNAc-Man motif in *N*-linked oligosaccharides from CM was prepared. Four major *N*-linked oligosaccharides were found containing GlcNAc, Man, Fuc, Gal and NeuAc. Their structures were further analyzed by FAB-MS in collaboration with

⇒ Prof. Anne Dell (Imperial College, London). The role in immune regulation of anti-idiotypes mimicking CF Ags was analyzed.

⇒ UK partner

A student from Prof. Nieto's laboratory completed his PhD degree in Cambridge performing this research. Methods for the expression of recombinant antigens in *Salmonella* vaccines as fusion proteins to fragment C of tetanus toxin (TetC) were optimised. Preimmunisation with tetanus toxoid did not suppress the response to guest antigens presented as such fusions to TetC. He expressed glycoprotein D of herpes simplex virus in that system and obtained protection of mice from challenge with virus. As fatty acid binding proteins conferred protection from fascioliasis and schistosomiasis, EgDf1 was considered candidate for protection in *E. granulosus*. EgDf1 was cloned and expressed in *Salmonella* and an htrA vaccine expressing it was orally administered to be used for expression of EgDf1 as above and its protective potential in dogs will be tested in Uruguay.

⇒ French partner

Prof. Scazzocchio has participated in collaboration with Prof. Ehrlich's laboratory as described above.

Partners

UNIVERSIDAD DE LA REPUBLICA (URUGUAY)
Fac. Quimica, Cat de Immunologia
Alfredo Navarro 3051
11600 Montevideo
Uruguay

A. Nieto Cadenazzi
Tel: +598-2-47.43.34
Fax: +598-2-47.43.20

UNIVERSITÉ DE PARIS SUD
Centre Universitaire d'Orsay
Inst. Génétique et Microbiologique
Batiment 409
F-91405 Orsay
France

C. Scazzochio
Tel: +33-16-941.63.56
Fax: +33-16-941.78.08
E-mail: scazzocchio@igmors.u-psud.fr

UNIVERSITY OF NEWCASTLE
Dept. of Microbiology
The Medical School
Framlington Place
UK-NE2 4HH Newcastle
United Kingdom

C.E. Hormaeche
Tel: +44-191-222.77.04
Fax: +44-191-222.77.36
E-mail: c.e.hormaeche@ncl.ac.uk

Contract number: TS3*CT910039

Period: July 1992 to September 1995

MOLECULAR APPROACH TO ECHINOCOCCUS DEVELOPMENT

Co-ordinator: Universidad de la República, Montevideo, Uruguay (R. Ehrlich)

Objectives

- Analysis of growth and development processes that take place during the *E. granulosus* life cycle and
- Study of the mechanisms involved in the adaptation to its specific hosts. The leading concept of the proposal was that the knowledge of the regulatory processes controlling *E. granulosus* development, growth and host adaptation could made important contributions to hydatid disease control.
- Furthermore, a complementary objective was proposed: it was focused on the optimisation of the expression of specific parasitic antigens in bacterial vectors, in particular in *Salmonellae*, attempting to contribute to the production of anti-parasitic live vaccines.

Activities

The study of *E. granulosus* growth and development focused on the following aspects:

* Search for transcription factors able to be involved in the regulation of developmental events during the parasite life-cycle;
* Characterization of developmentally regulated genes in order to establish initial molecular markers to decipher regulatory mechanisms;
* Analysis of the parasitic-host adaptive processes through the study of the heat shock response;
* Study of cytoskeletal protein genes;
* Cloning and expression of antigen encoding genes;
* Analysis of the parasite's genome organization;
* As a complementary objective, the improvement of the expression of parasitic genes in attenuated *Salmonella* have been also undertaken.

Expected outcome

Major contributions expected from this project included:

⇒ Contribution to deciphering the basis for the specific host-parasite adaptation and characterising key genetic switches during parasite development;
⇒ The improvement of the expression of Platyhelminth genes in *Salmonella*;
⇒ Training of Latin American scientist in molecular approaches to parasitic diseases.

Results

In a first step we have completed a great deal of descriptive work, mainly involving isolation, cloning and sequencing of several genes. During a second period, the main effort has been centred on expression studies (characterization of promoters, mapping of transcription start sites, improvement of different approaches to study complex formation between promoters and specific transcription factors, spatial and temporal analyses of gene expression). Concerning molecular studies of development, growth and adaptation of *E. granulosus*, the following work has been carried out by our groups:

⇒ Characterization of transcription factors possibly involved in regulation of developmental events during the parasite life cycle: five homcobox- containing genes have been described: Eghbx1-5. The expression of two of these genes has been detected in protoscoteces (Eghbx1-2); in particular Ebhbx1-3 appeared to be expressed specifically in cells associated to calcareous corpuscles;

⇒ Isolation of two protoscolex differentiation markers: EgDf1, a gene coding for a protein related to the fatty acid binding proteins family (FABPs) and EgDf5, coding for a protein related to tropom-yosins. The EgDf1 protein could be involved in the binding and transport of lipids from host origin, a function of crucial importance for an organism like *E. granulosus*, unable to synthesize *de novo* most of its own lipids. Proteins related to the FABI's family were also described in *Schistosoma mansoni* and *Fasciola hepatica*; it was reported that both proteins are able to induce significant protection against experimental infection in animal models. The Egdf5 gene appeared to be expressed specifically in cells of the protoscolex suckers;

⇒ With the aim of studying the mechanisms of parasitic adaptation to the specific hosts, the heat shock response and its time course in *E. granulosus* has been characterized. Several stress proteins were identified by 2D-electrophoresis and a genomic clone containing the complete coding region of a Hsp70 protein, including the 5 regulatory domain was characterized;

⇒ Several genes coding for proteins involved in cytoskeleton organization were characterized; three different actins, the troposyosin-like protein mentioned above, and a gene coding for a putative actin-filament-fragmenting protein.

⇒ Two genes coding for enzymes have been characterized a cytocolic malate dohydrogenase gene and a gene coding for a thioredoxin-like protein.

⇒ A gene coding for calcium-binding protein (EgCaBP) was isolated and its expression has been focalized at the level of calcareous corpuscles.

⇒ Six putative RNA polymerase II transcription regulatory domains have been identified in Porto Alegre and Montevideo labs; they include the proximal promoter domains of EgDf1, Eghbx,1, two actions, malate dehydrogenase and a hsp70 gene. Several targets for general and specific transcription factors were identified and some conserved sequences, that could constitute the binding site for specific protoscolex transcription factors, were detected.

⇒ In relation with *E. granulosus* genome organization, a middle repetitive sequence organized like a mobile element has been reported and the structure of a functional rDNA gene and its regulatory domain have been recently established.

⇒ Finally, with respect to the complementary objective of the proposal, the UK team developed a system which allows expression of recombinant antigens in live *Salmonellae* as either full length proteins or multiple tandem copies of immunogenic epitopes as C terminal fusions to the immunogenic fragment C of totanus toxin, under the control of the anaerobically inducible nirB promoter. Using this system, a trivalent experimental *S. typhimurium* vaccine has been constructed, which protects mise from typhoid, tetanus and schistosomiasis following a single oral dose of the vaccine. The EgDf1 gene from *E. granulosus* has been

expressed using the system described above. Preliminary results indicate that the construct is immunogenic in mice.

⇒ An important number of publications have been produced, from which several correspond to joint articles. The number of young Latin American scientist trainee within the frame of the project was equally important: 5 PhD have been completed either in the L.A. or in the European laboratories and also in "sandwich" programmes including work carried out in both sides; finally 9M. Sc. degrees were completed in the L.A. laboratories. The collaboration between all the partners has proved very fruitful not only in promoting North-South and South-South transfer of expertise, but also in building up research capability in both DC labs. A multidisciplinary collaboration was established through the interaction with another project centred on immunobiological aspects of hydatidosis (TS3*CT910038). The Latin American partners were also involved in the recent launching of a Network for Research and Training in Parasitic Diseases at the Southern Cone of Latin America. Finally, a FAO Collaborating Centre on Epidemiology, Diagnosis and Control of Echinococcosis/Hydatidosis has been created in Montevideo, including the partners of projects TS3*CT910038 and 0039.

Partners

INSTITUTO DE BIOLOGIA,
Facultad de Ciencias
Sección Bioquimica
Tristan Narvaja 1674
11200 Montevideo
Uruguay

R. Ehrlich
Tel: +598-2-48.86.21
Fax: +598-2-40.99.73
E-mail: ehrlich@genetica.edu.uy

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
Centro do Biotecnologia
Av. Bento Gonçalves 9500
91500 Porto Alegre
Brazil

A. Zaha
Tel: +555-1-336.37.77
Fax: +555-1-336.27.79
E-mail: zaha@dna.cbiot.ufrgs.br

UNIVERSITÉ DE PARIS-SUD
Centre universitaire d'Orsay
Institut Génétique et Microbiologique
Bâtiment 409
F-91405 Orsay
France

C. Scazzocchio
Tel: +33-16-941.63.56
Fax: +33-16-941.78.08
E-mail: scazzocchio@igmors.u-psud.fr

UNIVERSITY OF CAMBRIDGE
Dept. of Pathology
The Old Schools
Trinity Lane
UK-CB2 ITN Cambridge
United Kingdom

C.E. Hormaeche
Tel: +44-1223-322.19
Fax: +44-1223-332.51
E-mail: c.e.hormaeche@ncl.ac.uk

Contract number TS3*CT910040

QUANTITATIVE DIAGNOSIS OF SCHISTOSOMA INFECTIONS BY MEASUREMENT OF CIRCULATING ANTIGENS IN SERUM AND URINE

Period: March 1992 to February 1995

Co-ordinator: Rijksuniversiteit Leiden, Leiden, The Netherlands (A.M. Deelder)

Objectives

- Development and optimization of enzyme-linked immunosorbent assays (ELISA) and reagent strips for the quantitative detection of circulating anodic (CAA) and circulating cathodic (CCA) antigens and other adult worm antigens (CA-2), immune complexes, and for the demonstration of several antigens in one assay.
- Evaluation of circulating antigen detection systems for use as sero-epidemiological tools in studies of the chemotherapy, immunology and morbidity of *Schistosoma mansoni*, *S. haematobium*, *S. japonicum* and *S. intercalatum* infection.

Activities

* Monoclonal antibodies directed against schistosome circulating antigens were used in ELISA assays. Once monoclonal antibodies were produced against circulating antigens of *S. japonicum*, an ELISA assay were established and optimized for detection of this species.
* Appropriate, alternative, simple procedures for pre-treating urine specimens were investigated.
* The daily fluctuations of antigen concentration in urine and serum were investigated.
* Reagent strip ("dipstick") assays will be employed in an attempt to develop a simple, field applicable assay.
* Following training and technology transfer, sero-epidemiological surveys and clinical field trials were conducted.

Expected outcome

⇒ Improvement in the sensitivity and specificity of the circulating anodic (CAA) and circulating cathodic (CCA) antigen detection ELISA assays for *Schistosoma mansoni, S. haematobium* and *S. japonicum*.

⇒ Evaluation of antigen detection assays as sero-epidemiological tools.

⇒ The development of a reagent strip assay.

⇒ The successful transfer of ELISA, reagent strip and immunochemical expertise to the DC partners.

Partners

RIJKSUNIVERSITEIT LEIDEN
Dept. of Parasitology
P.O. Box 9605
NL-2300 RC Leiden
The Netherlands

A.M. Deelder
Tel: +31-71-527.68.59
Fax: +31-71-527.68.50

LANDESINSTITUT FÜR TROPENMEDIZIN
Koeningin Elisabeth Strasse 32
D-1000 Berlin
Germany

U. Bienzle
Tel: +49-30-303.27.00
Fax: +49-30-303.27.37

SHANGHAI SECOND MEDICAL UNIVERSITY
Dept. of Parasitology
South Chongging Road 280
200025 Shanghai
China

Z.L. Qian
Tel: +86-21-328.65.90
Fax: +86-21-437.48.61

DR. ALBERT SCHWEITZER HOSPITAL
Lab. de Recherche
B.P. 116
Lambarene
Gabon

J. Prada
Tel: +241-78.11.45

RESEARCH INSTITUTE FOR TROPICAL MEDICINE
Dept. Immunology
Alabang, Muntinlupa
Metro Manila
The Philippines

L.P. Acosta
Tel: +63-2-842.22.45
Fax: +63-2-842.22.45

CENTRO DE PESQUISAS RENE RACHOU
Lab. de Esquistossomose
Av. Augusto de Lima 1715
30190 Belo Horizonte
Brazil

A.L. Teles Rabello
Tel: +55-31-295.35.66
Fax: +55-31-295.31.15

MEDICAL RESEARCH STATION KUMBA
P.O. Box 55
Kumba
Cameroon

P. Enyong

COOPERACION TECNICA ESPANOLA BATA
Bata
Ecuatorial Guinea

P. Simarro

DIAGNOSTISCH CENTRUM SSDZ
P.O. Box 5010
NL-2600 GA Delft
The Netherlands

N. de Jonge
Tel: +31-15-60.30.50
Fax: +31-15-56.81.03

Contract number TS3*CT910042

FACTORS AFFECTING WOMEN'S CHOICES OF HEALTH-CARE PROVIDERS FOR THEIR CHILDREN IN RURAL AND SEMI-URBAN GUATEMALA

Period: May 1992 to April 1995

Co-ordinator: Statens Seruminstitut, Copenhagen, Denmark
(S.C. Sørensen)

Objectives

- Examine:
  - The availability of different categories of health-care providers.
  - Women's choices in providers.
  - The effect of their health-care behaviour on child mortality.
  - Women's expenditure on health-care for children, and
  - the practices and qualifications of lay health-care providers.
- Develop and evaluate a training programme for a group of professionals and lay personnel.

Activities

- Undertaking a survey of availability of health services in each of the selected municipios.
- Conducting a survey of women's choice of health-care providers for their children. The survey will investigate the factors that influence their decisions of choice and how that choice affects the outcome of a disease. It will pay particular attention to women who have not sought professional care for their children who have subsequently died. This survey will be undertaken on a sample of 500 households and information will be gathered on the last illness episode of under-five children.
- In-depth study of health-care behaviour of mothers whose children have died. Mothers with children who have died will be identified from the civil registers and 40 most recent cases of mortality that have occurred among children under five will be entered into the study, and a control group selected.
- A survey on lay practitioners (including "pharmacists") focusing on their educational background, use of modern drugs, preferred treatment for specified diseases and fees charged.
- An important aspect of the project is the development, implementation and evaluation of a training programme of lay and professional health personnel in dealing with women's role in the health care system.
- The data from the different surveys were published in a report.

Expected outcome

The study is expected to elucidate the factors which are related to under-utilisation of health services in cases of severe illness resulting in childhood death. In addition, the project is expected to result in the development and evaluation of a training programme for professional and lay-health care providers, to be used in Guatemala and elsewhere.

Results

The study has, so far, completed four community-based surveys in rural Guatemala with an emphasis on the socio-economic determinants for the utilisation of government health services by women and their children. The results confirm the key role of education of women in improving the health of women and children in rural areas in developing countries.

Partners

STATENS SERUMINSTITUT
Div. of Biotechnology
Lab. of Parasitology
Artillerivej 5
DK-2300 Copenhagen
Denmark

S.C. Sorensen
Tel: +45-3-268.31.88
Fax: +45-3-268.32.28

INSTITUUT VOOR TROPISCHE GENEESKUNDE
Dept. of Epidemiology
Nationalestraat 155
B-2000 Antwerpen
Belgium

P. Van Der Stuyft
Tel: +32-3-247.66.66
Fax: +32-3-216.14.31

CENTRO DE EDUCACION Y INVESTIGACION EN SALUD (CEISAR)
Apartado Postal 476
Antigua
Guatemala

M.E. Valenzuela Delgado
Tel: +502-9-32.33.87
Fax: +502-9-32.33.87

Contract number TS3*CT920044

Period: October 1992 to September 1995

IDENTIFICATION OF A PROMOTER SPECIFICALLY TRANSCRIBED IN THE GUT CELLS OF ANOPHELES MOSQUITOES FOR THE EXPRESSION OF ANTIPARASITIC AGENTS

Co-ordinator: Università di Roma "La Sapienza", Roma, Italy (A. Crisanti)

Objectives

We proposed to perform a series of experiments aimed at the identification of mosquito promoter/enhancer sequences specific for the cells of the intestinal lineage of Anopheles. The candidate genes for conferring a non-vectorial phenotype (anti-gamete/ookinete specific immunoglobulin), against malaria (both human and mouse) was targeted for expression in mosquito intestinal cells by means of a DNA vector containing the specific promoter sequence. The experimental objectives included:

- identification of mosquito genes that are specifically transcribed in the gut cells;
- identification of the upstream regulatory DNA sequences that drive gut specific transcription;
- transfection of mosquito cell lines with DNA constructs containing a reporter gene under the control of the selected promoter sequences;
- development of a suitable *in vitro* and a laboratory scale assay to determine the tissue specificity of the selected promoter;
- cloning of the coding sequences for the binding region of Plasmodium gamete/ookinete antibodies. The gut specific promoter was used to develop transgenic and transplanted mosquitoes secreting in the midgut transmission blocking antibodies. The transmission blocking activity of the antibody produced in the mosquito midgut was assayed in the human *P. falciparum* and in the mouse *P. berghei* models.

Activities

- ***Identification of a promoter sequence specifically transcribed in Anopheles gambiae: gut cells***

  The genes specifically expressed in the gut cell of the *Anopheles* was isolated from a λgt 11 cDNA expression library with the help of an antiserum raised against the secretory protein of the gut. The 5' non transcribed promoter region was isolated from a genomic library using specific cDNA clones. Also the coding sequences of the Aedes trypsin gene was used to screen the Anopheles genomic library to search for the corresponding promoter. The transcription start site was identified by primer extension and S1- mapping and the promoter region was subcloned. The promoter was functionally defined by deletion mapping and *in vivo* assay. In addition the putative regulatory transcription sequences was tested for interaction, in gel shift electrophoresis, with protein from *An. gambiae* nuclear extract.

- ***Cloning of the heavy (H) and light (L) chains of gametocyte monoclonal antibody with blocking activity***

  Mouse hybridomas screening antibodies that both react with gamete/ookinete antigens and have transmission blocking activity were collected from several laboratories. We used antibodies against both *P. falciparum* and *P. berghei* antigens. The variable domains of the heavy and light chains was amplified from cDNA generated from the secreting cells. The

coding sequences of the variable regions H and L were cloned in a eucaryotic expression vector within the invariant regions of the γ and κ chain respectively and expressed in NSO cells, (Orlandi et al 1989). As control the transmission blocking function of the recombination antibody was tested in the supernatant of transformed NSO cells.

* ***In vivo activity of the putative An. gambiae gut specific promoter***
  The upstream sequences of the gut specific transcribed gene were tested on mosquito-derived cell lines for the ability to induce specific transcription and expression. Both a reporter gene (luciferase) and the cloned gamete/ookinete antibody sequence were cloned in a vector, containing long genomic cDNA sequences of *An. gambiae*, downstream of the putative promoter.

Expected outcome

⇒ If the product of the transgene is able to interfere with virus and parasite replication, the resulting mosquitoes should display a non-permissive phenotype for disease transmission.

⇒ The identification of a gut promoter would be particularly relevant for the generation of transgenic mosquitoes to be used in the genetic control of the wild type trains. In fact, to be successful the product of the transgene should not impair the environmental fitness, the fertility and the behavior of the mosquitoes. This would be better achieved if the expression of the transgene is restricted, by a specific promoter, to the organ (gut) were most parasites and viruses undergo replication.

Partners

IMPERIAL COLLEGE
Dept. Biology
Prince Consort Road
UK-London SW7 2BB
United Kingdom

A. Crisanti
Tel: +44-171-594.54.26
Fax: +44-171-594.54.24

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
Dept. of Biology
Prince Consort Road
South Kensington
UK-SW7 2BB London
United Kingdom

R. Sinden
Tel: +44-171-58.95.11
Fax: +44-171-225.82.40
E-mail: r.sinden@bio.ic.ac.uk

FUNDACAO OSWALDO CRUZ
Dept. de Entomologia
Av. Brasil 4365
21040 Rio de Janeiro
Brazil

R. Galler
Tel: +552-1-290.75.49
Fax: +552-1-590.34.95
E-mail: rgaller@gene.dbbm.fiocruz.br

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Molecular Biology Group
Pembroke Place
UK-L3 5QA Liverpool
United Kingdom

J. Crampton
Tel: +44-151-708.93.93
Fax: +44-151-708.87.33
E-mail: p.s.craig@biosci.salford.ac.uk

Contract number: TS3*CT920052

Period: October 1992 to September 1995

SYNTHETIC PEPTIDE ANTIGENS AS A TOOL FOR SPECIES-SPECIFIC SERODIAGNOSIS OF LEISHMANIASIS WITH FIELD APPLICATIONS IN BRAZIL AND COLOMBIA

Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom
(M. Hommel)

Objectives

- The overall objective was to evaluate a series of synthetic peptides, derived from *Leishmania* genes, which can be used, under field conditions, for species-specific serodiagnosis of leishmaniasis. The original rational behind the proposed study was the finding that a synthetic 15 amino-acid peptide, based on the sequence of a *Leishmania donovani* gene, could be used in the laboratory for a specific serodiagnostic test of visceral leishmaniasis.
- The main objectives of the study were to:
  - field-test the existing peptide assay in selected areas of Brazil and Colombia, endemic for visceral leishmaniasis in order to evaluate its predictive value.
  - perform similar studies in areas for (muco-)cutaneous leishmaniasis and Chagas' disease in order to confirm species-specificity.
  - further improve the peptide-carrier construct.
  - use the same rationale and technology to be applied to the *Leishmania braziliensis* complex.
  - include peptide-carrier constructs in assays suitable for use under field conditions.
  - set up a laboratory of molecular biology at the University of Sucre.
  - collect isolates of Leishmania and patient blood and sera from locations in Colombia and Brazil.

Activities

* Improvement of the methodology for the preparation of peptide-carrier constructs by conjugation of peptides to the human serum albumin carrier using thi-oester hetero-bifunctional reagents.
* Design of new mixtures of peptide sequences ("mixotopes") based on consensus and variable motifs of the leishmanial rK39 sequence, an antigen which has proven diagnostic value.
* Testing the potential use of random peptide phage display libraries as a means for the identification of immunodominant leishmanial peptides.
* Investigation of the scientific basis for the direct agglutination test using whole leishmanial promastigotes.
* Collection and PCR characterization of leishmanial isolates from Colombia.
* Development of an antigen-capture assay for the detection of leishmanial antigens in the urine of patients with visceral leishmaniasis.
* Collection of serum from patients with clinical visceral leishmaniasis, cutaneous and mucocutaneous leishmaniasis, Chagas' disease, asymptomatic leishmanial infections and a variety of endemic controls in Brazil.

Results

⇒ Development of a new, ELISA-based, serological assay for the diagnosis of visceral leishmaniasis using a mixture of synthetic peptides linked to a carrier protein (HSA). Publication of the description of the assay and its performance in scientific literature.

⇒ Characterization of new foci of leishmaniasis in Brazil and in colombia (District of Sucre).

⇒ Completion of 3 PhD theses on leishmaniasis (1 from a DC student, 2 from EU students); 2 other PhD theses from DC students are still in the process of completion.

⇒ Setting up of a laboratory for the study of leishmaniasis and the use of molecular biology methods in Sincelejo, Sucre, Colombia. This laboratory has been given the status of Regional Leishmaniasis Reference Laboratory by the Colombian Health Authority.

⇒ Organization in Liverpool of one of the Euroleish Network meetings and editing of the proceedings.

Partners

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Div. of Molecular Biology & Immunology
Pembroke Place
UK-Liverpool L3 5QA
United Kingdom

M. Hommel
Tel: +44-151-708.93.93
Fax: +44-151-708.87.33
E-mail: mhommel@liv.ac.uk

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
Glycobiologie
Centre de Biophysique Moléculaire
Rue Charles-Sadron
F - 45071 Orléans Cédex 2
France

R. Mayer
Tel: +33-238-51.55.62
Fax: +33-238-69.00.94
E-mail: mayer@cnrs-orleans.fr

UNIVERSITY OF KEELE
Dept. of Biological Sciences
UK-Keele ST5 5BG
United Kingdom

R. Maingon
Tel: +44-178-262.11.11
Fax: +44-178-263.00.07
E-mail: bia25@cc.kl.ac.uk

UNIVERSIDADE FEDERAL DA BAHIA
Hospital Universitario Prof. E. Santos
Rua Joao das Botas
Canela
40140 Salvador
Bahia
Brazil

R. Badaro
Tel: +557-1-235.48.66
Fax: +557-1-245.71.10

UNIVERSITY OF SUCRE
Regional Leishmaniasis Reference Laboratory
Sincelejo, AA406
Colombia

P. Blanco-Tuiran
Tel: +575-282.12.40
Fax: +575-282.67.15

Contract number: TS3*CT920053

Period: August 1992 to July 1994

MALARIA PRE-ERYTHROCYTIC STAGES (MPES) EUROPEAN NETWORK ANTIGENS TARGET OF IMMUNE RESPONSES CAPABLE OF INHIBITING *P. FALCIPARUM* PRE-ERYTHROCYTIC DEVELOPMENT

Co-ordinator: Institut Pasteur, Paris, France (P. Druilhe)

Objectives

- Development of the immunology of MPES with the aim of developing an effective MPES vaccine.
- Acquisition of an improved knowledge of the biology of MPES.
- Improved co-ordination and exchanges within and between European and Developing Country teams.

Activities

* Molecular biology studies of pre-erythrocytic antigens from mostly *P. falciparum* and *P. berghei,* and in part, *P. reichenowi* (identification, characterisation, and production of genes and antigens: LSA1 (a major 200 kDa molecule expressed in liver stages), SALSA (a 70 kDa antigen shared between sporozoite and liver stages), LSA3-729 (a pre-erythocytic-stage-specific molecule expressed in sporozoites and liver stages, DG21 (a sporozoite-specific 78 kDa molecule). Antigenic features of these molecules, conservation of epitopes amongst isolates, epitope mapping; immunogenicity in animals, characterization and prevalence of immune responses in humans and in animals, (mice and primates), identification amongst the remaining series of cloned pre-erythrocytic-stage molecules of those that deserve further detailed studies.
* Improvement of the reproducibility of liver infections in Aotus monkeys. Immunization and sporozoite challenges of chimpanzees and Aotus with P. *falciparum* antigens of mice and thamnomys, with *P. berghei*, *P. yoelii* antigens. Analysis of the immune responses developed by immunized animals, and of the type of defence mechanisms operating. Comparison of the type of immunity induced by antigens and by whole parasites (i.e. irradiated sporozoites) in natural versus artificial hosts.
* Analysis of naturally occurring immunity to MPES in field conditions, of the mechanisms regulating parasite loads at MPES level, and of the main antigens inducing such mechanisms. Analysis of the artificial immunity induced by injection of y-irradiated sporozoites, and of the mechanisms and antigens responsible for such immunity.
* Study of the mode of action and the respective importance of antibodies, antibody – cell Cupertino, lymphocyte cytotoxicity, and cytokines using *P. falciparum* and human hepatocytes, under *in vitro* conditions or *in vivo* in SCID mice.

Expected outcome

Improved understanding of the human *P. falciparum* relationship at MPES level, mainly through an analysis of existing regulatory mechanisms developed against those staged by

exposed individuals, and their epidemiological consequences in various areas differing in their vectorial capacity.

Results so far

Molecular biology

Four out of four of the new *P. falciparum* molecules being studied, namely STARP, SALSA, LSA-1 and LSA-3, have been characterised in terms of their full-length DNA sequence and stage-specific expression, and we have initiated the study of three new MPES genes: DG 64, DG 6F and DG 571 (two of them in collaboration with Nijmegen and BPRC). Immunological studies with isolates of *P. falciparum* at the sporozoite stage have shown the consistent expression of those genes, and comparison of sequence data for areas of immunological interest of LSA-1 and LSA-3 have shown a remarkable degree of conservation, in contrast to many other *P. falciparum* antigens. Homologues of these genes have been found in the ape parasite *P. reichnowi* and, for some of them, the sequence (e.g. STARP) determined. Immunological and genetic screening of other plasmodial species led to the identification of an equivalent of LSA-3 in the rodent species *P. yoelii* which, particular, share B- and T-cell epitopes with *the P. falciparum* gene, and open the possibility of performing immunization and challenge with the rodent species in laboratory animals.

For immunization purposes, the genes have been cloned in various types of vectors expressed in prokaryotic and eukaryotic cells, and naked DNA vectors. For instance, a very large range of recombinant-expression systems is now available for LSA-3 as a model system. This, together with the large number of lipopeptides derived from the above molecules, provides a very large range of immunization systems.

Antigenicity studies

Using peptides and recombinants from the four leading molecules, studies in five endemic areas have identified the sequences defining B-cell epitopes, and have showed high to very high sero-prevalences to them (e.g. 97% prevalence to LSA-3 repeats in Senegal, spanning all age groups). Similarly, T-helper-cell epitopes were mapped, and a correspondence between the level of malaria transmission and the proportion of T-cell responders was observed (up to 85% of T-cell responders to most peptides in the Congo region). Many of those epitopes were also found to be able to stimulate the secretion of interferon gamma (the cytokine known to be the most potent in blocking liver-stage development. One LSA-3 peptide induces the secretion of particularly high concentrations of this cytokine. Finally, a very large number of HLA class-1-restricted CTL epitopes were identified in the four genes under study: six in LSA-1, two in SALSA, one in STARP, and eleven in LSA-3.

Immunogenicity and vaccination studies

Chimpanzee and thamnomys were used as model systems to analyze the protection induced per irradiated sporozoites, and the immune response in the 1krad chimpanzee paralleled those recorded in human volunteers immunized in the same manner. However, the CD8 lymphocyte depletion planned in this animal could not be performed. Rodent modelling revealed an inverse relationship between the susceptibility of the host to the parasite and the dose of irradiated sporozoites needed to achieve protection, i.e. protection was very difficult to induce in thamnomys, which is more susceptible than artificial laboratory hosts.

Using the four lead molecules, preliminary immunization attempts were performed in mice of five different H2 haplotypes, with many different synthetic peptides and lipopeptides as well as recombinant proteins, and a large range of adjuvants. One of the most remarkable results was obtained with lipopeptide, which proved able to induce Th and CTL responses without adjuvant, and to increase the immunogenicity to the point of apparently overcoming partially or totally the genetic restriction observed in inbred mice. Therefore, immunizations were performed in chimpanzees, using six lipopeptides injected in PBS, and six non-lipopeptides injected in montanide adjuvant or adsorbed on microspheres. By these means, T-helper-cell responses were induced to all twelve peptides, some of them at very high level (stimulation indices >80). Antibody production was observed towards eleven of the twelve peptides, most of them at very high levels. Moreover, these responses proved to be long lasting, and to be specific to parasite-native proteins. Finally, CTL responses were detected towards six of these peptides. Challenge studies indicated that SALSA and mostly SLA-3 held the best promise in terms of protection.
A large number of immunization schemes were used in mice, with LSA-3 as a model system to assess protection against *P. yoelii* challenge.
Results with QS21, titermax, FCA or FIA were disappointing To date, the best results have been obtained with lipopeptide or with microsphere immunization, which have the advantage of being effective without adjuvant and to induce a full range of immune responses.

***In vitro* study of defence mechanisms**
The P. falciparum human hepatocyte in-vitro system was used to study defence mechanisms. At very low concentration, antibodies to STARP and LSA-3 , and also auto-SALSA antibodies strongly inhibited P. falciparum sporozoite invasion. IgM from irradiated sporozoite volunteers proved more efficient than IgG antibodies. Several attempts to show whether CTL cells could lyse human or chimpanzee infected hepatocytes have been made so far without reaching a conclusive result. However, the conditions necessary to achieve this goal have considerably improved. For instance, a method enabling the raising of CTL-malaria specific CTL lines from healthy volunteers, developed in Oxford, increases greatly the chances of matching the MHC Class-I antigen from the effector cells with that of the target hepatocytes. One drug, already developed and used clinically, proved to be very effective *in vitro upon P. falciparum* liver stages, but not against *P. yoelii* liver stages. G-Oligonucleotide primers derived from LSA-3 were used in a PCR assay, which proved to be the most sensitive diagnostic means available to date to detect very low-grade *P. falciparum* blood infection.

In total, these studies have confirmed the potential of the molecules under study, of which the antigenicity, immunogenicity and conservation among isolates appeared to be remarkable. The vaccination schemes used up to now may not yet be optimal, but they have yielded very encouraging results, and the conditions to assay the efficacy of further immunization schemes have greatly improved.

Partners

INSTITUT PASTEUR
Département Parasitologie Biomédicale
Rue du Dr. Roux 28
F-75724 Paris 15
France

P. Druilhe
Tel.: +33-1-45 68 85 78
Fax: +33-1-45 68 86 40
E-mail: druilhe@pasteur.fr

BIOMEDICAL PRIMATE RESEARCH CENTRE BPRC
Dept. of Parasitology
P.O. Box 5815
NL-2280 HV Rijswijk
The Netherlands

A. Thomas
Tel.: +31-1-584 25 38
Fax: +31-1-584 39 86
E-mail: thomas@itri.avg.mbc.tno.nl

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
Dept. of Biology
Prince Consort Road
South Kensington
UK-SW7 2BB London
United Kingdom

R. Sinden
Tel.: +44-171-594 54 25
Fax: +44-171-594 54 24
E-mail: r.sinden@bio.ic.ac.uk

KATHOLIEKE UNIVERSITEIT NIJMEGEN
Dept. of Medical Parasitology
P.O. Box 0009101
NL-6500 HB Nijmegen
The Netherlands

W. Eling
Tel.: +31-8-061 36 63
Fax: +31-8-054 02 16
E-mail: medpar.jm@aznvx1.nl

UNIVERSIDAD DEL VALLE
Fundación Centro de Primates
AA 25360 Cali
Colombia

S. Herrera
Tel.: +57-2-356 56 21
Fax: +57-2-358 10 61
E-mail: soheva@mafalda.univalle.edu.co

MAHIDOL UNIVERSITY
Fac. of Tropical Medicine – Entomology
Rajvithi Road 420/6
10400 Bangkok
Thailand

A. Asavanich
Tel.: +66-2-246 12 72
Fax: +66-2-246 83 40

INSTITUUT VOOR TROPISCHE GENEESKUNDE
Nationalestraat 155
B-2000 Antwerpen
Belgium

M. Wéry
Tel.: +32-3-247 63 59
Fax: +32-3-216 14 31
E-mail: mwery@itg.be

KATHOLIEKE UNIVERSITEIT NIJMEGEN
Dept. of Molecular Biology
Toernooiveld 1
NL-6525 ED Nijmegen
The Netherlands

R. Konings
Tel.: +31-80-652 25 08
Fax: +31-80-65 21 12

INSTITUTE OF MOLECULAR MEDICINE
Molecular Immunology Group
J. Radcliffe Hospital Headington
UK- OX3 9DU Oxford
United Kingdom

A. Hill
Tel.: +44-186-522 23 01
Fax: +44-186-522 25 02
E-mail: a.hill@well.ox.ac.uk

CENTRE INTERNATIONAL DE RECHERCHE MEDICALE DE FRANCEVILLE
BP 769
Franceville
Gabon

P. Millet
Tel.: +241-67 70 92
Fax: +241-67 72 95
E-mail: Pasmille@club-internet.fr

Contract number: TS3*CT920070

Period: January 1993 to August 1994

COMMUNITY-BASED MALARIA CONTROL UNDER THE GUIDANCE OF HEALTH SERVICES: INTERVENTION STUDY IN ECUADOR AND COLOMBIA

Co-ordinator: Universität Heidelberg, Heidelberg, Germany (A. Kröger)

Objectives

- Describe the epidemiology of malaria in the study areas.
- Measure the impact of several community-based interventions of malaria control on the incidence of malaria attacks and on case management.
- Identify factors favouring or hindering the protective efficacy of specific interventions.
- Measure different cost aspects of such a programme.

Activities

- After the baseline study on malaria incidence and a KAP study in three areas of Ecuador and Colombia regarding self-diagnosis and self-treatment of malaria, the study communities were divided randomly into intervention communities and control communities respectively (randomized community trials).
- The project teams trained staff members of the local health services, who then carried out a series of training workshops in the intervention areas with community representatives and community volunteers.
- After 8 months of intervention, the research team repeated the measurements, both in the intervention and in the control areas, assessing the malaria incidence and people's KAP regarding malaria prevention and treatment.
- Before and after the intervention, health services' staff members (in particular, malaria field workers) were observed when carrying out workshops and when doing community health actions. In particular, DDT residual spraying overall costs of the programme were measured.

Results

⇒ Between the three study areas of the Pacific Coast, there was considerable variation in the socio-economic characteristics of the populations, accessibility of health services, and ethnomedical practices. The demographic structure, housing conditions, and malaria control through vertical programmes, were similar.

⇒ Main malaria vectors were: *A. albimanus* in Ecuador and *A. nuneztovari* in Colombia. The monthly incidence rates of malaria episodes during the wet season were 3.5% in Colombia and 7.0% in Ecuador. The main parasite was *P. falciparum* (92% in Colombia, 86% in North Cost of Ecuador). The remainder was *P. vivax*. Transmission occurred principally inside or around the houses. Users of (unimpregnated) bed nets had the same incidence of malaria episodes as non-users of bed nets. The impregnation of bed nets with lambdacyhalothrin showed a high (71%) protective efficacy against clinical malaria attacks in Colombia. The study in Borbon (North Coast of Ecuador) showed that intensive residual spraying with DDT had the same protective efficacy against malaria episodes as the

impregnation of bed nets with permethrin. However, the costs of the DDT programme were 3.5 times higher than those of the impregnation programme. In Muisne (Ecuador), the protective efficacy of bed-net impregnation was not increased by the additional breeding of larvivorous fish in all large mosquito-breeding places.

⇒ A set of limiting and favourable factors for a community-based programme of bed-net impregnation was identified.

⇒ The educational programme in Ecuador had the following impact (expressed in percentage of increase of correct knowledge and practice): improvement of correct knowledge about malaria transmission (30%), symptoms (25%), and the correct doses of chloroquine for adults (25%). The respective values in Colombia were 28%, 38%, and 48%. Taking correct doses of chloroquine during a clinical malaria attack was improved by 20% in Ecuador, and by 46% in Colombia. The factors related to this were analyzed. The community interventions on mosquito breeding places had no clear effect on vector densities and malaria incidence rates. The cost calculations showed that it was feasible to carry out a large scale impregnation programme.

Selected publications

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Partners

UNIVERSITAET HEIDELBERG
Institut für Tropenhygiene & Oeff. Gesundheitswesen
INF 324
D-69120 Heidelberg
Germany

A. Kröger (now at Liverpool School of Tropical Medicine)
Tel.: +44-151-708 93 93
Fax: +44-151-707 17 02:
Akroeger@liverpool.ac.uk

MUSEO NACIONAL DE MEDICINA DEL ECUADOR
García Moreno 524 y Avda. 24 de Mayo
Quito
Ecuador

M. Mancheno
Tel.: +59-3-257 37 92
Fax: +59-2-227 298

UNIVERSITEIT ANTWERPEN
Epidemiology and Community Medicine
Universiteitsplein 1
B-2610 Antwerpen
Belgium

A. Meehus
Tel.: +32-3-820 25 59
Fax: +32-3-820 26 40

UNIVERSIDAD DEL VALLE
Departamento de Medicina Social
CO-A.A.-034406 Cali
Colombia

Socrates Herrera
Tel.: +57-2-558 19 46

Contract number: TS3*CT920077

Period: October 1992 to September 1995

PHYTOMONAS SPP, TRYPANOSOMES DE PLANTES - RECHERCHES SUR LE METABOLISME, LA VARIABILITE, LA PATHOGENICITE ET L'EPIDEMIOLOGIE, POUR ARRIVER A DES METHODES DE LUTTE NON POLLUANTES

Co-ordinator: CIRAD, Montpellier, France (Michel Dollet)

Objectives

Les trypanosomes de plantes constituent un nouveau domaine de la phytopathologie puisque les recherches ont véritablement débutés dans les années 80. Ce programme consiste à faire progresser les connaissances sur ces microorganismes :

- variabilité,
- pathogénicité,
- épidémiologie,

de manière à identifier des possibilités de lutte non polluantes qui remplaceraient les actuels épandages d'insecticides.

Activities

* Réalisation de primocultures - cultures in vitro - de manière à constituer une large collection d'isolats de trypanosomes de plantes pour étude.
* Etude du métabolisme des *Phytomonas* afin, d'une part, d'améliorer les conditions de culture in vitro - ces organismes se multiplient difficilement dans les conditions actuelles -, et d'autre part, pouvoir intervenir sur ce métabolisme en tant que méthode de lutte possible. (Utilisation des sucres, voies métaboliques, localisation cellulaire des activités enzymatiques) détermination des formes du cycle des *Phytomonas* et rôle de l'AMP cyclique.
* Variabilité des trypanosomes de plantes à l'aide de différentes techniques : immunofluorescence à l'aide d'anticorps monoclonaux, électrophorèse d'isoenzymes, électrophorèse des fragments de restriction de l'ADN kinétoplastique, caractérisation du marqueur génomique de l'ARNr 16 S, séquençage de minicercles de l'ADNk et utilisation en sonde moléculaire.
* Pathogénicité des trypanosomes de plantes : réalisation d'élevage d'insectes vecteurs, acquisition - ou inoculation - des trypanosomes par le vecteur, et transmission à des plantes test. Rôle éventuel de particules virales de trypanosomes dans la pathogénicité.
* Recherche des réservoirs naturels, des *Phytomonas* phytopathogènes, des hôtes naturels de leurs vecteurs.
* Mise au point de méthodes de lutte découlant des résultats obtenus dans les différents domaines évoqués ci-dessus. Recherche de variétés résistantes ou tolérantes aux maladies à trypanosomes.

Selected publications

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Partners

CIRAD - CP
Laboratoire de Phytovirologie des Régions Chaudes
BP 5035
F-34032 Montpellier
France

Michel Dollet
Tel.: +33-67.61.58.79
Fax: +33-67.63.51.00

CENTRO DE INVESTIGACION EN PALMA DE ACEITE
Apartado Aereo 2548
Villavicencio Meta
Colombia

Fany Albanyl
Tel.: +57-1-255.68.75
Fax: +57-1-217.53.47

FONDO NACIONAL DE INVESTIGACIONES AGROPECUARIAS
Av. Universidad,
Vía El Limón
Maracay
Venezuela

Asdrubal Díaz
Tel.: +58-43-83.12.12
Fax: +58-43-83.14.23

UNIVERSITE DE BORDEAUX II
Labo. Immunologie & Parasitologie Moléculaire
Rue Léon Saignat 146
F-33076 Bordeaux
France

Théo Baltz
Tel.: +33-57.57.16.44
Fax: +33-57.57.10.15

INSTITUT GUSTAVE ROUSSY
Labo. de Pharmacologie Clinique & Moléculaire
Camille Desmoulins 39
F-4800 Villejuif
France

Guy Riou
Tel.: +33-45.59.47.79
Fax: +33-46.78.92.91

INSTITUTO DE INVESTIGACIONES EN INGENIERA GENETICA Y BIOLOGICA
Velto de Oblogado 2490
1428 Buenos Aires
Argentina

Hector N. Torres
Tel.: +54-1-784.55.16
Fax: +54-1-786.85.78

INTERNATIONAL INSTITUTE OF CELLULAR AND MOLECULAR PATHOLOGY
Research Unit for Tropical Diseases
Avenue Hippocrate 74
B-1200 Brussels
Belgium

Frederik R. Opperdoes
Tel.: +32-2-764.74.39
Fax: +32-2-762.68.53

UNIVERSIDAD DE GRANADA
Facultad de Granada
Depto. Bioquímica yBiología Molecular
Campus Fuente Nueva
E-18071 Granada
Spain

Manuel Sánchez Morena
Tel.: +34-58-24.32.63
Fax: +34-58-24.31.74

Contract number: TS3*CT920084

Period: January 1992 to December 1995

ANTIMALARIAL AGENTS WHICH ACT BY AFFECTING THE PHOSPHOLIPID METABOLISM OF THE INTRAERYTHROCYTIC PLASMODIUM. DEVELOPMENT OF A PHARMACOLOGICAL MODEL

Co-ordinator: Centre National de la Recherche Scientifique, Montpellier, France
(Henri Vial)

Objectives

- Study novel antimalarial agents that act upon the phospholipid metabolism in the erythrocytic stages of malarial development,
- Develop appropriate pharmacological models.

Activities and results

* This project aims to find new chemotherapeutic treatments (and, eventually, prophylactic) for malaria. Interruption of phospholipid metabolism of erythrocytic stages of Plasmodium, which is essential for the synthesis of parasite membranes, blocks parasite development. The most promising compound inhibits the choline carrier, a rate limiting in phosphatidylcholine synthesis, a mahor phospholipid in Plasmodium. Compounds such as this step could be effective against parasites resistant to existing antimalarials.
* The programme has provided insight into the nature and site of the choline transporter, and thus for the creation of new molecules that inhibit plasmodial phospholipid metabolism. The programme has also established the therapeutic doses needed to block the multiplication of Plasmodia and to further develop such drugs that have a maximum therapeutic index..

Selected publications

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Partners

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
URA 1856 – Interactions Membranaires
USTL, Case 107
Place Eugène Bataillon
F-34095 Montpellier
France

Henri Vial
Tel.: +33-4-67 14 37 45
Fax: +33-4-67 14 42 86
E-mail: vial@univ-montp2.fr

UNIVERSITÉ DE MONTPELLIER
Centre d'Etude des Matériaux Organiques et Polymères
Case 018
Place Eugène Bataillon
F-34095 Montpellier
France

Louis Giral
Tel.: +33-4-67 14 38 17
Fax: +33-4-67 54 30 79

BIOMEDICAL PRIMATE RESEARCH CENTRE
Medical Biology Laboratoriese
Dept. of Chronis & Infectious Diseases
P.O. Box 5815
NL-2280 HV Rijswijk
The Netherlands

Alan Thomas
Tel.: +31-15-284 25 38
Fax: +31-15-284 39 86
E-mail: Thomas@bprc.nl

INTERNATIONAL INSTITUTE OF PARASITOLOGY
395 Hatfield Road
Winches Farm
St Albans
UK-AL4 OXQ Hertfordshire
United Kingdom

Wallace Peters
Tel.: +44-1727-83 31 51
Fax: +44-1727-86 87 21

INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE
Unité 42
Biologie & Biochimie Parasitaires
Domaine du Certia
B.P. 39
F-59650 Villeneuve d'Asq
France

A. Camus
Tel.: +33-20 91 14 52
Fax: +33-20 05 91 72
E-mail: vial@univ-montp2.fr

UNIVERSIDAD DEL VALLE
Fundación Centro de Primates
A.A. 25360 Cali
Colombia

Socrates Herrera
Tel.: +57-2-356 56 21
Fax: +57-2-358 10 61
E-mail: soheva@mafalda.univalle.edu.co

ORGANISATION DE LUTTE CONTRE LES ENDEMIES EN AFRIQUE CENTRALE
Laboratoire de Biologie
BP 28
Yaoundé
Cameroon

Vincent Mouanda
Tel.: +237-23 22 32
Fax: +237-23 00 61

Contract number: TS3*CT920088

HEALTH AND THE CURRENT ECONOMIC CRISIS IN BRAZIL: THE IMPACT ON THE HEALTH AND CARE OF MOTHERS AND CHILDREN

Period: January 1993 to December 1995

Co-ordinator: Escuela Andaluza de Salud Pública, Granada, Spain (M.García Calvente)

Objectives

- Describe and document the political, economic and health policy changes in Pelotas, Brazil, in the past decade.
- Document levels and trends in maternal and child health status and health care provision and utilisation between 1982 and 1992.
- To make policy recommendations based on the research conclusions.

Activities

Phase 1:

- A study of changes in health policies and health care provision with emphasis on maternal and child care. This study will provide data on recent trends in these areas to document historical changes in the city.
- Anthropological studies based on interviews with members of different groups involved in health care. The aim here will be to investigate the perception of the population and of the health providers regarding changes in health services.
- A study on socio-economic trends that is intended to document the political and economic changes which took place during the decade and how these have affected the quality of life.

These Phase 1 studies will result in a detailed description of changes in the health sector and in the perception of the population and providers relative to these changes.

Phase 2:

- A perinatal study in three maternity hospitals during twelve months.
- A descriptive infant mortality and nested infant mortality case-control study, to identify all deaths among cohort children and to ascertain causes and compare their characteristics with those of control children from the same birth cohort.
- A hospital morbidity study to provide data on the causes of all hospital admissions.
- A follow-up study to trace a 20 per-cent sub-sample of approximately 2000 children at 6-12 months of age and 400 pre-term and/or low birth-weight children.
- A maternal study on health, fertility and family planning utilisation will provide data on past reproductive history.

Comparison of these data to data collected in a similar way in 1982 to assess changes during the decade.

Expected outcome

Increased understanding of changes in health care in the city of Pelotas in Brazil, and the effects of these changes on the health and care of mothers and children.

Results

⇒ Reduction in the number of births: 6.011% in 1982 and 5.04% in 1993, suggesting an increased utilisation of contraceptives or abortions since there was a increase in the number of women of fertile age. A breakdown by socio-economic status shows that the reduction of 707 births in 1993 was not evenly distributed as there were about 1,000 fewer births in the poorest groups and 300 more in the high-income strata.

⇒ Important variations in the nutritional status of the mother: in the decade the mean height increased from 156.4cm in 1982 to 159.9cm in 1993, and weight in the beginning of pregnancy was also substantially higher in 1993, 62.1kg compared to 58kg in 1982. Antenatal care attendances also increased in 1993, with a mean of 7.6 attendances compared to 6.6 in 1982 and medical assistance during delivery increased from 61 per cent in 1982 to 88.3 per cent in 1993. Despite these improvements the proportion of low birthweight (<2,500 g) showed a slight increase in the proportion of pre-term births (5.6 and 7.5 per cent, respectively) and intra-uterine growth retardation (15.0 per cent in 1982 and 17.5 per cent in 1993) The reason for these unexpected findings is still being analysed.

⇒ Important reduction in perinatal mortality: from 32.2/1000 births in 1982 to 22.1/1000 births in 1993, and a reduction of perinatal deaths was equally observed both in the foetal and in the early neonatal periods. Regarding breastfeeding, increase in the proportion of babies being breastfed in the first months of life. At three months of age, for example, the prevalence of full breastfeeding was 53 per cent in 1993 compared with about 33 per cent in the previous decade. As far as nutritional status at 12 months of age is concerned: changes according to the indicator. Thus, there was a slight increase in the proportion of children with low height for age, 6.1 per cent compared to 5.3 per cent in 1982. Reduction in the prevalence of low weight for age, 5.4 per cent in 1982 and 3.8 per cent in 1993, and of weight for height. Important progress in the infant mortality rates, with a drop from 36.4/1,000 live births in 1982 to 21.1/1,000 in 1993.

Partners

ESCUELA ANDALUZA DE SALUD PUBLICA
Apartado de Correos 2070
E-18080 Granada
Spain

M. del Mar Garcia Calvente
Tel.: +34-95-827.50.44
Fax: +34-95-827.05.51

UNIVERSIDADE FEDERAL DO PELOTAS
Caixa Postal 464
96001 Pelotas
Brazil

F. Barros
Tel.: +555-3-271.24.42
Fax: +555-3-271.26.45

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Dept. of Public Health and Policy
Keppel Street
UK-WC1E 7HT London
United Kingdom

P. Vaughan
Tel.: +44-171-927.24.31
Fax: +44-171-637.53.91

Contract number: TS3*CT920092

Period: February 1993 to January 1997

BIOSYSTEMATICS AND ADAPTIVE TRENDS IN THE GENUS *RHODNIUS*

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom
(C.J. Schoffield)

Objectives

The project has addressed a problem of particular relevance to Chagas disease control in Central America, Venezuela, Colombia, Ecuador, Bolivia, Peru, and Brazil, where *Rhodnius* species are important vectors of Chagas disease but, due to morphological similarities between key species, their relative importance and potential for control have been unclear. The project has addressed this problem by:

- ♦ Clarifying the taxonomic status of members of the genus *Rhodnius* with reference to type material
- ♦ Defining the geographic limits and ecological characteristics of the main vector species.
- ♦ Assessing the evolutionary trends within the genus, especially in relation to progressive adaptation to domestic and peri-domestic environments.

Activities

- ✶ Creation of a network of research partners based in eight Latin American countries, with links to research institutes and control organisations in a further five countries.
- ✶ Through the network, field collections of different *Rhodnius* species have been characterised from localities throughout the range of the genus, using morphometric, morphological, and biochemical techniques, including RAPD and mtDNA sequence analysis.

Results

⇒ All species of epidemiological significance have been characterised and established in laboratory colonies, including several related species of potential epidemiological importance. Biosystematic analysis using a large number of morphometric and biochemical characters has provided an outline phylogeny for the group, fully supporting the idea of evolution by radiative adaptation from a discrete source population.

⇒ The distribution of *Rhodnius prolixus* - the species of greatest epidemiological significance - has been clarified. The species now seems to be absent from Mexico, where it had previously been found in abundance in the southern states, and also seems to be of much more restricted distribution in Nicaragua than had previously been thought.

⇒ Historical reconstruction of its dispersal supports the idea that Central American strains of *R. prolixus* may derive from an accidental escape of specimens originally collected from houses in Venezuela some 80 years ago, and this interpretation is fully supported by morphometric and genetic comparisons between Central and South American strains. The analysis indicates that Central American strains of *R. prolixus* now have a very limited genetic repertoire and are now confined to domestic and peri-domestic habitats in parts of Guatemala, El Salvador, Honduras, and northern Nicaragua.

These findings lend strong support to the idea that *R. prolixus* could be completely eliminated from Central America.

Follow-up

- The success of this research network had led to its extension to cover further areas of Latin America where Chagas disease is endemic. The extended network, known as ECLAT, now includes 30 research partners in 19 countries, with numerous associates involved in research and control of Chagas disease vectors. It provides technical support and co-ordination, advanced training, and assistance with field and laboratory work, although most of the research partners also have additional financial support from national sources.
- A key features of the ECLAT network is its ability to address technical problems raised by the vector control services in different areas and to produce detailed operational recommendations in response to control and surveillance requirements (see, for example; contract no. ERBIC18*CT960042).
- The network also provides a forum for improved liaison and discussion between the research community and control service personnel, assisting in the development of new control initiatives such as the recently announced Central American and Andean Pact initiatives for the control of Chagas disease.

Selected publications

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Partners

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Department of Infectious and Tropical Diseases
Keppel Street
UK-London WC1 E7HT
United Kingdom

C.J. Schofield
Tel.: +44-171-927 23 40
Fax: +44-171-636 87 39
E-mail: C.J.Schofield@lshtm.ac.uk

ORSTOM MONTPELLIER
Lab. de Génétique des Parasites et des Vecteurs
Avenue Agropolis 911
F-34032 Montpellier 1
France

J.P. Dujardin
Tel.: +33-1-48 03 77 77
Fax: +33-1-48 03 08 29
E-mail: jpdujard@mail.entelnet.bo

INSTITUTO OSWALDO CRUZ
Depto de Entomología
C.P. 926
21045-900 Rio de Janeiro
Brazil

J. Jurberg
Tel.: +55-21-290 93 39
Fax: +55-21-590 97 41
E-mail: galvao@gene.dbbm.fiocruz.br

Contract number: TS3*CT920113

Period: September 1992 to August 1995

VISCERAL LEISHMANIASIS: EPIDEMIOLOGY AND DISEASE CONTROL

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom
(M.A. Miles)

Objectives

- Establish research facilities at the University of Teresina and a training programme for local staff.
- Perform a thorough comparison of diagnostic serology and diagnostic parasitology for Brazilian and European canine and human visceral leishmaniasis, incorporating innovative diagnostic procedures.
- Assess aminosidine (paromycin) for the treatment of canine visceral leishmaniasis.
- Identify asymptomatic dogs and people and determine whether such carriers can act as a reservoir of infection.
- Recommend and implement improved strategies for disease control based on research findings from the programme.

Activities

* Comprehensive epidemiological data will be assembled from the records held at the Ministry of Health and the University at Teresina. A thorough comparison of the latest appropriate technologies for the diagnosis of canine VL will be undertaken with a minimum sample size of 100 dogs assembled at the Centre for Zoonoses.
* A colony of *Lutzomyia longipalpis* will be established at the University of Teresina; flies will be infected by feeding on dogs with heavy skin infections of *L. chagasi* and the infections will be transmitted to an experimental group of animal imported from a non-endemic area.
* The chemiluminescent probe will be tested for its ability to detect *L. donovani* in experimentally infected and wild-caught *LU. longipalpis*, and compared with detection by microscopy. A prototype diagnostic field kit will be assembled.
* Using dogs with moderately severe VL (without severe wasting) the efficacy of aminosidine treatment, using various regimes, will be investigated. The infection will be monitored using sequential bone marrow and skin biopsy techniques.
* Improved serological techniques will indicate potential asymptomatic human carriers of VL and detailed epidemiological information on suburban visceral leishmaniasis will be available as a result of this study. This epidemiological analysis will be used to recommend improved methods for disease control.

Expected outcome

⇒ A detailed epidemiological description of visceral leishmaniasis will become available as a result of this project.
⇒ The chemiluminescent DNA Probe will be evaluated in the field, and aminosidine trials will have been conducted in dogs.
⇒ Conclusions be drawn on the transmissibility of the disease from asymptomatic carriers.
⇒ Recommendations will be made on the best diagnostic procedures that are currently available and on improved strategies for disease control.

Results

⇒ Data assembled include: incidence of human VL (1981 - 1994); incidence of human VL by age and by sex; suburban distribution of human VL.; suburban distribution of canine seropositivity; records of suburban and periurban sandfly species; suburban distribution of insectivide spraying.
⇒ Clinical, parasitological and serological diagnostic methods have been compared with a cohort of more than 200 naturally infected dogs, and an *L. donovani*-complex specific DNA probe assessed. A DNA-based diagnostic kit was described. An *L. donovani* complex specific colorimetric (visual) PCR assay was developed.
⇒ The *L. donovani*-complex specific probe was shown to be an effective tool for detecting *L. chagasi* infections in wild caught sandflies.
⇒ *L. chagasi* was highly transmissible from dog to dog by *Lutzomyia longipalpis*, infectivity to sandflies was compared with clinical status.
⇒ A cohort of dogs infected experimentally by sandfly bite was established, and parasitological positivity, serological conversion and transmissibility of infection followed.
⇒ A trial of aminosidine for treatment of canine VL was performed: clinical recovery, limited cure, and some adverse effects were obtained.
⇒ A combination of serology and a gamma interferon capture assay detected putative asymptomatic VL among families with index clinical cases. Carrier status is under investigation (in collaboration with Dr. Carlos Henrique Costa). Distribution of selected human genotypic markers within the study cohort have been determined (Oxford, UK).

Results on the diagnosis and transmissibility of canine VL question the efficacy of serological surveys and killing of dogs as efficient means of VL control.

Partners

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Dept. of Medical Parasitology
Keppel street
UK-London WC1E 7HT
United Kingdom

M.A. Miles
Tel.: +44-171-927.23.40
Fax: +44-171-636.87.39
E-mail: m.miles@uk.ac.lshtm

UNIVERSIDADE FEDERAL DO PIAUI
Dept. de Parasitologia e Microbiologia
Campus da Ininga
Teresina, Piaui
Brazil

J.A. Fonseca de Castro
Tel.: +558-6-232.14.17
Fax: +558-6-232.28.12

Contract number: TS3*CT920113

GROUPE HOSPITALIER PITIE-SALPETRIERE
Boulevard de l'Hôpital 47
F-75013 Paris
France

L. Monjour
Tel.: +33-1-45.83.46.84
Fax: +33-1-43.29.70.93

UNIVERSIDADE DO PORTO
Fac. de Farmacia - Microbiologia
Rua Anibal Cunha 164
P-4000 Porto
Portugal

M. Cabral
Tel.: +351-2-200.43.87
Fax: +351-2-200.39.77

FUNDACÍON JIMENEZ DÍAZ
Clínica de la Concepcíon
Div. Enf. Infecciosas
Avenida Reyes Católicos 2
E-28040 Madrid
Spain

M. Gorgolas

Contract number TS3*CT920116

Period: September 1992 to December 1993

REGULATION OF SEXUAL DEVELOPMENT IN MALARIA PARASITES AND THE DESIGN OF LOGICAL INTERVENTION STRATEGIES

Co-ordinator: Imperial College of Science, Technology & Medicine,London, United Kingdom (R.E. Sinden),

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Objectives

Through collaborative studies and associated training programmes, the project aimed to investigate the genetic, molecular, and biological regulations of sexual development of *Plasmodium*. Through the information gained, logical intervention strategies would be investigated.

Activities

Through mutual exchanges of personnel and reagents between the participating laboratories we have integrated the particular expertise of each laboratory in a series of studies, many of which have been published. The diverse methodologies used have been described in these publications and are therefore not repeated here.

Results

⇒ ***Genetic regulation of sexual development***

By comparing the chromosomal location of a large number of genes in different strains of the four rodent species, our data indicated that there is very little gene re-assortment between non-homologous chromosomes. The different chromosomes appear to form stable linkage groups of specific genes in all species. We have initiated a collaboration with the University of Sao Paulo to investigate the genome organization of *P. vivax*. We tested small filters -Plasmodipur; Euro-Diagnostica (The Netherlands) - for removal of white blood cells (wbc) from *P. vivax* infected blood. These filters effectively removed wbc, while the different developmental stages of the parasites were not trapped in the filters. The older blood stages of *P. vivax* (trophozoites, schizonts and gametocytes) could easily be separated using Nycodenz density gradient centrifugation from uninfected erythrocytes. These parasites have been successfully used for separation of the chromosomes in pulsed field gel electrophoresis. To elucidate the mechanism(s) responsible for chromosome-size polymorphism occurring during mitotic multiplication of *Plasmodium* parasites, the possible correspondence between the appearance of karyotype variants, and the loss in the ability to undergo gametocyte differentiation, we characterised a gametocyte-defective clone (HPE) of *P. berghei* that emerged during asexual multiplication of the gametocyte producer clone 8417HP. It exhibited a large subtelomeric deletion of chromosome 5. In *P. falciparum* the effect of a terminal deletion on chromosome 9 (reported to be associated with impaired gametocyte production) on stage specific control of gene expression in sexual differentiation was investigated. Differences in the sexual/asexual pattern of expression of the gametocyte-specific gene Pfg27 were found both at the level of the protein and of mRNA species between the line harbouring the deleted chromosome 9, its parental line 1776, and line 3D7. There is evidence that all genes known so far, which are involved in sexual differentiation and expressed almost exclusively during and after gametocytogenesis,

are clustered on chromosome 5 of rodent parasites. These genes are -tubulin-1, Pbs21, C-type rRNA, and two other characterized genes. To provide evidence of the genetic diversity of the "Pbs21 gene" within other rodent malaria species, attempts were made to clone the equivalent gene from different species. The *P. yoelii* equivalent was cloned and sequenced. Screening of two Ig11 genomic libraries of *P. vivax* with Pbs21 probes resulted in a number of positive clones between 1 and 6 kb in size but none of these appear to be the Pbs21 homologue.

⇒ ***Molecular regulation of sexual development***

To select novel *P. berghei* genes specifically expressed in sexual forms, we devised a subtraction strategy using gametocyte-producer and gametocyte-less clones. A labelled cDNA enriched for sequences selectively expressed in the gametocyte-producer clone 8417HP was obtained after subtraction with a large excess of mRNA prepared from the non-producer clone K173 and used to screen a *P. berghei* genomic library. Among the positive clones, a novel gene that maps to chromosome 5 at a subtelomeric position was selected. Northern analysis using stage-specific RNA preparations from pure cultured sexual forms demonstrated that production of the Pbs21 transcript was initiated in gametocytes; yet translation is evident only after gametogenesis and the transcript is considered to be translationally repressed. Preliminary evidence indicates that the start site of transcription lies - 350nt upstream of the translational start site, and that processing of the transcript occurs at the 3' end of the mRNA molecule. To find regulatory elements for the expression of Pbs21, two larger cDNA clones (1.1 and 1.25 kb) encoding the Pbs21 gene have been sequenced; 639bp of the region upstream the Pbs21 gene have been described. *In situ* detection of mRNA was used to analyze the expression pattern of mRNAs for a number of sexual stage-specific transmission-blocking antigens, including Pbs21, Psf25, Pfs28 and Pfs230, throughout gametocytogenesis of *P. berghei* and *P. falciparum*. The initiation of transcription of these mRNAs occurs in a staged series following commitment to sexual development. Pbs21, Pfs25, and Pfs28 mRNAs accumulate in gametocytes in the absence of detectable translation products (collaboration with NIH, Bethesda) suggesting that post-transcription mechanisms operate to regulate the translation of the protein (see above). We have developed probes which allow the demonstration of transcriptional activity of the two classes of rRNA gene in the highly developed model available in the laboratory. The probes detect the external transcribed spacer (ETS) of the two types of rRNA unit and demonstrate not only transcription but also the degree of conservation between the genes comprising the two types of rDNA unit. To identify cdc2 products in *P. berghei* extracts, immunoblot assays were conducted using a commercial monoclonal antibody directed against the highly conserved domain PSTAIRE. A protein of 30kDa was detected in young trophozoites only. We were able to amplify a 1 kb fragment of a gene, showing a high homology to cdc2, in 5 *Plasmodium*-species: *P. knowlesi, P. berghei, P. vinckei, P. chabaudi and P. yoelii.* These fragments were cloned and sequenced. In cooperation with the University of Sao Paulo, Brazil, the same fragment of the *P. vivax* gene was also cloned and its DNA-sequence determined. In the 1 kb fragment of all species under study, three introns are present at conserved loci. By comparing these introns with respect to a number of characteristics, we were able to draw some conclusions on intron-organisation and evolution within the genus *Plasmodium*.

⇒ ***Biology of sexual regulation***

Mechanisms of transmission blockade in infected hosts. The natural decline of infectivity of gametocytes three days post infection is not antibody mediated since it could be demonstrated that the pattern of infectivity was exactly the same in severe combined immunodeficient mice (scid) as in their intact Balb/c congenic partners. The resulting inhibition of the parasites' sexual cycle within the mosquito occurs within one hour of the

mosquito feed. To date, there is no significant evidence that nitric oxide or its derivatives are involved in the blockade of either *P. berghei* or *P. vinckei* infections. Studies were carried out to test cryopreservation efficacy of sexual stages of *P. vivax* malaria parasite from blood from patients. Zygotes were obtained *in vitro,* and unfrozen and cryopreserved blood infectivity was tested by *An. albimanus* infections. The proportion or recovery was similar to that obtained in cryopreserved asexual stages of the parasite.

⇒ ***Development of logical intervention strategies***

Studies on the expression and immunogenicity of recombinant Pbs21 expressed in a baculovirus system revealed that:

- Expression of the recombinant protein in insect larvae results in higher yields than expression in *in vitro* systems.
- The protein is indistinguishable from native protein by means of conformation-dependent antibodies.
- Deletion of the putative signal sequence prevented protein expression on the cell surface.
- Transmission-blocking activity induced by the full length protein in mice was higher than 90%.

We have evidenced that the protein has either a novel type of GPI anchor, or is an acylated membrane protein. Neither PIPLC treatment nor nitrous acid de-amination had any effect on the molecule, but with hydroxylamine treatment cleavage of the membrane anchor was obtained. Infected blood erythrocytes obtained from *P. vivax*-infected patients, in their original plasma or plasma obtained from uninfected normal donors, were offered to *An. albimanus* and transmission blocking activity was estimated by comparison of the infection rates obtained with the 2 preparations. Protein extracts were prepared from purified *P. vivax* gametocytes and used for immunoblot assays of sera with transmission-blocking activity. Several protein bands were identified. The most frequently observed were protein bands of 113, 103, 94, 85, 68, 47, 41, 37 and 31 KDa.

Partners

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
Dept. of Biology
Prince Consort Road
South Kensington
UK-London SW7 2BB
United Kingdom

R.E. Sinden
Tel.: +44-171-594.54.24
Fax: +44-171-594.54.24
E-mail: r.sinden@bio.ic.ac.uk

RIJKSUNIVERSITEIT LEIDEN
Dept. of Parasitology
Postbus 9605
NL-2300 RC Leiden
The Netherlands

C. Janse
Tel: +31-7-127.68.42
Fax: +31-7-127.68.50

ISTITUTO SUPERIORE DI SANITA
Labo. di Biologia Cellulare
Viale Regina Elena 299
I-00161 Rome
Italy

M. Ponzi
Tel.: +39-06-444.02.70
Fax: +39-06-444.00.18

CENTRO DE INVESTIGACION DEL PALUDISMO
Dept. of Immunoparasitology
Apartado Postal 537
Tapachula 30700, Chiapas
Mexico

M.H. Rodriguez
Tel.: +52-96-26.22.19
Fax: +52-96-26.57.82
E-mail: nrodriguez@hotmail.com

Contract number: TS3*CT920118

Period: December 1992 to November 1993

CELL MEDIATED IMMUNITY TO SCHISTOSOMES. EVALUATION OF MECHANISMS OPERATING AGAINST LUNG STAGE PARASITES, WHICH MIGHT BE EXPLOITED IN A VACCINE

Co-ordinator University of York, York, United Kingdom (R. Wilson)

Objectives

Evaluate cell mediated immune responses to lung stage parasites of *Schistosoma mansoni* in mice and in infected humans.

Activities

* Secreted and soluble antigens derived from schistosomula, cultured *in vitro* for eight days, will be used to expand T-cell clones and lines generated from lymph node populations recovered from mice shortly after intradermal vaccination with day 8 attenuated schistosomula.
* Of the phenotypic and functional assays to be employed, proliferation and interferon gamma production by clones or lines when co-cultured with live lung-stage schistosomula are considered most important. Clones which meet these criteria will be tested *in vivo* for the ability to mediate delayed type hypersensitivity responses and reduce maturation of parasite infections relative to irrelevant T-cell clones.
* Following clinical and epidemiological investigations, including assessment of resistance to reinfection after chemotherapy, lymphocytes will be collected from the peripheral blood of patients from Bela Fama and the profile of their cytokine production (IL2, IL3, IL4, IL5 and Interferon gamma) in response to the same larval antigens will be described.

Expected outcome

⇒ The establishment of T-cell clones which meet the criteria of proliferation and interferon-gamma production, and the measurement of human cytokine profiles in response to lung stage antigens.

⇒ Development of a longer-term project.

Partners

UNIVERSITY OF YORK
Dept. of Biology
Heslington
UK-York Y01 5DD
United Kingdom

R. Wilson
Tel.: +44-190-443.28.30
Fax: +44-190-443.28.60

CENTRO DE PESQUISAS RENE RACHOU
Lab. de Esquistossomose
Av. Augusto de Lima 1715
30100 Belo Horizonte
Brazil

R. Correa-Oliveira
Tel.: +55-3-12.95.35.66
Fax: +55-3-12.95.31.15

INSTITUT PASTEUR LILLE
Centre d'Immunologie & Biologie Parasitaire
Rue du Professeur A. Calmette 1
F-59019 Lille
France

A. Capron
Tel.: +33-3-20.87.79.62
Fax: +33-3-20.87.78.88

Contract number: TS3*CT920123

Period: December 1992 to November 1995

RECOMBINANT ANTIGENS AS SEROLOGICAL TOOLS FOR SPECIFIC AND SENSITIVE TEGUMENTARY AND VISCERAL LEISHMANIASIS DIAGNOSIS

Co-ordinator: Universidad Peruana Cayetano Heredia, Lima, Peru
(Ysabel Montoya)

Objectives

Develop rapid, sensitive and highly specific test based on recombinant peptide antigens for the improved diagnosis of visceral Leishmaniasis (VL) and American tegumentary leishmaniasis (ATL).

Activities

- Sera from patients co-infected with VL and HIV were followed by Western blot using *L.(L) infantum* total proteins by ELISA using rK-39 recombinant protein.
- Construction and screening of *L.(L)infantum* cDNA libraries using sera from patients co-infected with VL/HIV.
- Selection and characterisation of the candidate recombinant proteins for *L.(L.)infantum* and *L.(V) peruviana*.
- Serological assessment of the diagnostic potential of selected *L.(V.)peruviana* recombinant proteins in terms of specificity, sensitivity and predictive value.
- *L(V.) peruviana* synthetic peptides derived after their DNA sequencing from the recombinant protein selected were assessed with ATL and VL sera.
- DNA sequencing of novel genes

Results

⇒ Development of an improved serodiagnostic test in terms of greater specificity, sensitivity and predictive value over conventional tests using recombinant proteins.

⇒ Four novel DNA sequences from *L.(V)peruviana* genes: histone H2B, Hsp70,citochrome oxidase, protein acid ribosomal P2b gene have been sent to the Genebank.

⇒ DNA sequencing of two novel genes from L.(V.)braziliensis ;histone 3 and protein acid ribosomal P2b, gene have been reported to the genebank.

⇒ Three *(L>) infantum* conserved genes have been DNA sequenced, Hsp70 family.

⇒ One PhD, one MSc and seven Licenciate of Biology theses were obtained by Peruvian students. One PhD thesis was obtained in Spain.

Selected publications

Montoya Y., Leon Talledo M., Nolasco O., Padill, Munos-Najar U and Barker, D.C. 1997. Recombinant antigens for specific and sensitive serodiagnosis of Latin American tegumentary leishmaniasis. Trasactions of the Royal Society of Tropical Medicine and Hygiene. **91**:674-676.

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Partners

UNIVERSIDAD PERUANA CAYETANO HEREDIA
Inst. de Medicina Tropical A. von Humboldt
P.O. Box 4314
Lima 100
Peru

Ysabel Montoya
Tel.: +51-1-481 51 77
Fax: +51-1-481 51 77
E-mail: ymontoya@ins.sld.pe

INSTITUTO DE SALUD CARLOS III
Centro Nacional de Microbiología
Servicio Parasitología
Carretera Majadahonda
Pozuelo km 2
E-28220 Majadahonda, Madrid
Spain

J. Alvar
Tel.:+34-916-38 00 11
Fax: +34-916-38 03 41
E-mail: jalvar@iscii.es

UNIVERSITY OF CAMBRIDGE
MRC Outstation of NIMR Molteno
Lab. of Pathology
Tennis Court Road
GB-CH2 1QP Cambridge
United Kingdom

D.C. Barker
Tel.: +44-1223-33 37 37
Fax: +44-1223-33 37 37
E-mail: dcb12@mole.bio.cam.ac.uk

Contract number: TS3*CT920129

Period: October 1992 to September 1996

CLONAL VARIABILITY OF THE PARASITE AS A PREDICTIVE TOOL FOR DIFFERENT CLINICAL MANIFESTATIONS IN TEGUMENTARY LEISHMANIASIS OF PERU AND BOLIVIA

Co-ordinator: Instituut for Tropische Geneeskunde "Prins Leopold", Antwerpen, Belgium
(D. Le Ray)

Objectives

Identification of molecular marker(s) correlating with mucosal compromise of New-World tegumentary leishmaniasis.

Activities

* Selection of patients with active cutaneous or mucocutaneous lesions in two Amazonian regions situated in Bolivia (Ivirgarzama Health District, Cochabamba Department) and in Peru (Pilcopata, Madre de Dios/Cuzco).
* Parasite isolation before chemotherapy.
* Analysis of genetic heterogeneity among sylvatic isolates, using Multi-locus Enzyme Analysis (MLEE). Random Amplification of Polymorphic DNA (RAPD), and PFG karyotyping.
* Comparison with genetic data previously obtained on isolates from the Peruvian *Andes (L. (V.) peruviana*, never associated with mucosal compromise).
* Interpretation of genetic polymorphism in terms of population and evolutionary genetics.

Results

⇒ Implementation of a field laboratory at the Pilcopata Health Post for *in vitro* cultivation, PCR tests, and PC-data entering.

⇒ Isolation of 167 *Leishmania* stocks (Bolivia: 79; Peru: 88), all with well documented clinical records, from cutaneous (n=128) and mucosal cases (n-39).

⇒ *L. (V.) braziliensis* was the most abundant among the sylvatic stocks (90%). *L. (V.) guyanensis* and *L. (V.) lainsoni* were also encountered (respectively 3 and 7%); RAPD was developed and allowed for the first time discrimination of all species in subgenus *Viannia*, including *L. (V.) peruviana*. There was a highly signification correlation between MLEE and RAPD genetic distances.

⇒ Specific chromosomal-size differences (PFG) were correlated with severity of the lesions (size of cutaneous lesions, potentiality of mucosal compromise) and proved to be due to rearrangement of essential genes (gp63, rDNA, and mini-exon).

⇒ The dynamics of these genes led to the development of two PCR-based characterisation methods combining digestion with restriction enzymes (PCR-RFLP) and targeting respectively gp63 and rDNA loci.

⇒ Clonality appears to be the principal reproduction mode in the populations under survey (linkage unbalance of RAPD data), but in rare cases sexual exchange might occur (putative hybrids in the Andean valley of Huanuco). In addition, pseudo-sexual phenomena could be present.

Selected publications

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Partners

INSTITUUT VOOR TROPISCHE GENEESKUNDE „PRINS LEOPOLD“
Lab. of Protozoology
Nationalestraat 155
B-2000 Antwerpen
Belgium

D. Le Ray
Tel.: +32-3-247 63 55
Fax:+32-3-247 63 62
E-mail: dleray@proto.itg.be

UNIVERSIDAD PERUANA CAYETANO HEREDIA
Inst. de Medicina Tropical "Alexander von Humboldt“
Dept. de Bioquímica
Apartado postal 4314
PE-100 Lima
Peru

J. Arevalo
Tel.: +511-4-81 51 77
Fax: +511- 4-81 51 77
E-mail: labc@upch.edu.pe

UNIVERSIDAD MAYOR DE SAN SIMON
Facultad de Medicina
Centro Universitario de Medicina Tropical
Avenida Aniceto Arce
Casilla 3119
Cochabamba
Bolivia

H. Bermúdez
Tel.: +591-42-515 43
Fax: +591-42-515 43
E-mail: cumetrop@pino.cbb.entelnet.bo

IRD (EX-ORSTOM) MONTPELLIER
Laboratoire de Génétique des Parasites
BP 5045
France

M. Tibayrenc
Tel.: +33-6-61 74 97
Fax: +33-6-54 78 00

Contract number TS3*CT920130

Period: September 1993 to August 1995

RISK OF REINFESTATION FROM WILD FOCI OF *TRIATOMA INFESTANS* IN BOLIVIA, A COUNTRY OF THE SOUTHERN CONE PROGRAMME

Co-ordinator: ORSTOM Montpellier, Montpellier, France (J.P. Dujardin)

Objectives

- Evaluate the epidemiological importance of wild populations of *Triatoma infestans* (Hemiptera, Reduviidae). This species is the principal vector of *Trypanosoma cruzi*, causative agent of Chagas disease, throughout the seven southernmost countries of Latin America (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru, Uruguay). In these countries it has become the primary target of Chagas disease vector control programmes.
- Determine the invasive capacity of silvatic *T. infestans*: do they represent a risk for reinfestation ?

Activities

- Using field experiments and laboratory studies, we studied the connections between domestic and wild *T. infestans*.
- Throughout most of its wide distribution, *T. infestans* seems to be exclusively domestic and peridomestic, occupying cracks and crevices in rural dwellings and domestic animal enclosures. True silvatic colonies are known only from the Cochabamba region of southern Bolivia, where the insects can be found amongst rockpiles in association with wild guinea-pigs. The original silvatic focus, some 15 Km south of Cochabamba (Cercado province), was first described in 1946.
- A genetic interpretation of electrophoretic data has so far not revealed differences between the silvatic population and nearby domestic populations so that the degree of isolation between them is unclear. However, in order to apply adequate control and surveillance measures, it is important to understand the relationships between these ecotopes. Control of Chagas disease vectors relies primarily on spraying infested dwellings with pyrethroid insecticides. After the initial intervention however, it is important to continue entomological surveillance so that any new infestations can be selectively retreated.
- Laboratory studies scored different population markers with the idea that, if not panmictic, the ecotopes under study should show some genetic differentiation. Isoenzyme electrophoresis and cytogenetic studies comparing C-banding polymorphism failed to detect significant differences between ecotopes, whereas random amplified polymorphic DNA (RAPD) evidenced different band frequencies. In accordance with this latter genetic marker, morphometric analysis revealed also head and wing differences. The post-ocular region was repeatedly larger in silvatic specimens, either nymphs, males or females, in samples collected at various times (1983, 1992, 1995 and 1996).
- Field experiments explored the dispersive behavior of wild *T. infestans* in Jamach'Uma (Cochabamba, Bolivia). This locality is a small village surrounded by silvatic foci of *Triatoma infestans*. The first experiment used "sentinel henhouses". Artificial, adobe made henhouses were constructed each 100 meters from Jamach'Uma to the wild focus. Though hens are very attractive animals for *T. infestans*, none were found colonizing these

henhouses during a six months survey. Hens were then replaced by rodents, and monthly vigilance was continued for eight months. Again, no *T. infestans* were found, though another species (*T. sordida)* invaded these artificial structures.

- The second field experiment simulated a control programme in Jamach'Uma. In December 1992, infestation by a few nymphs of *T. infestans* was found again which was sprayed in October 1993. They were compared at seven head metrics with 36 fifth instar domestic nymphs coming from Jamach'Uma before treatment, and with two sets of nymphs originating from the surrounding silvatic foci: 8 specimens collected in 1992 and 9 specimens collected in 1995. The results were interpreted in terms of the possible mechanisms of reinfestation whether there was a residual population or reinvasion from surrounding silvatic foci. Metric comparisons strongly supported the hypothesis of infestation resulting from a residual population surviving the insecticide spraying. It is not possible to definitively rule out the idea that some of the infestant nymphs are of silvatic origin, mixed with a residual population. Three arguments were consistent with the hypothesis of no regular migrants, or exceptional migrants, between Jamach'Uma and the wild focus: the delay (ten months) between insecticide spraying and the reinfestation, the stage (fifth nymphs) of the infestant specimens and their metric characteristics.
- On the basis of these laboratory and field data, the silvatic focus of *T. infestans* in Bolivia does not appear to represent a serious obstacle to the application of the Southern Cone Programme in Bolivia.

Partners

ORSTOM, MONTPELLIER 1
Labo de Génétique Moléculaire des Parasites et des Vecteurs
Avenue Agropolis 911, BP 5045
F-34032 Montpellier 01
France
J.P. Dujardin
Tel.: +33-14-803.77.77
Fax: +33-14-803.08.29

BRITISH MUSEUM OF NATURAL HISTORY
Division of Medical & Veterinary Entomology
Cromwell Road
London SW7 5BD
United Kingdom
C. Schofield
Tel.: +44-171-938.89.16
Fax: +44-171-938.93.95

UNIVERSIDAD MAYOR DE SAN SIMON
Centro Univ. de Medicina Tropical
Avenue Aniceto Arce
CAS. 3119
Cochabamba
Bolivia
H. Bermudez
Tel.: +59-1-21.545
Fax: +59-1-042.31.690

Contract number: TS3*CT920155

Period: September 1992 to August 1995

EXPERIMENTAL STUDY OF THE IMPACT OF POPULATION CLONAL STRUCTURE ON RELEVANT MEDICAL AND BIOLOGICAL PROPERTIES OF *TRYPANOSOMA CRUZI*

Co-ordinator: ORSTOM, Montpellier, France (Michel Tibayrenc)

Objectives

- Extensive population genetic analyses have shown that the populations of *T. cruzi*, the agent of Chagas disease, are subdivided into natural clones, stable in space and time. The major clones refer to certain clones that are much more frequently sampled than others; it is suspected that their medical and epidemiological significance is considerable.
- The goal of the present study was to compare some relevant medical properties such as virulence and resistance to drugs of the major *T. cruzi* clones.

Activities

- Studies involved a limited sample of 16 laboratory-cloned stocks representing three major clones selected according to their genetic relationships, ascertained by multilocus isoensyme electrophoresis. Each major clone in the sample was represented by several stocks with extremely variable origins (host, place of isolation). Stocks pertaining to these three clones were studied in order to consider possible interactions between two (or more) different clones. Such mixed infections seem to be common in natural cycles in both triatomine bugs as well as in patients. The impact of clone interactions in a given host could have an important impact on Chagas' pathogenesis. To follow the behaviour of such mixed infections, we used the PCR KDNA probes specific for the major clones. Specific study areas included:
- Differentiation in axenic culture medium, which were estimated from the percentage of trypomastigote forms at given times.
- Differentiation of epimastigote forms to infective metacyclic trypomastigote forms were monitored every day, following morphological changes in a Thomas chamber, in order to obtain at least 20% of metacyclic trypomastigotes.
- Generation of tissue culture trypomastigotes; study of *in vitro* infectivity: experimental mouse infections.
- Histopathological studies of the following organs in mice: heart, brain, liver, spleen, ganglions skeletal muscle, and colon.
- Drug-sensitivity studies were carried out both on *in vitro* and *in vivo* models, using drugs currently applied in the treatment of Chagas' disease: 5-nitrofuran and 2-nitroimidazole.
- Statistical analysis of the results was carried out using commercial software.

Expected outcome

The goal of the project was to define the medical implications of the considerable genetic variability of *T. cruzi*.

We proposed a general model of parasitic protozoa population structure that strongly suggests that many parasites (e.g. *T. brucei* and various species of Leishmania) have typical clonal population structures like *T. cruzi*. Hence, the approach and experimental design proposed could be the basis for further studies involving other parasites.

Results

⇒ A set of 21 parasites stocks representing four major natural clones (clonal genotypes that are widespread and constitute most part of the stocks isolated from chagasic patiens) have been selected on the basis of genetic charactezrization invloving 15 isozyme loci.

⇒ Since the beginning of the project, the following goals have been reached:

⇒ Improved genetic characterization of the 21 stocks: a more accurate picture of the actual genetic variability of our sample has been provided by:
- increasing the number of isozyme loci from 15 to 23
- using random primer amplification of polymorphic DNA or RAPD.

⇒ During biological characterization of the 21 stocks, the following main parameters were screened:
- virulence on Balb/C mice
- in vitro culture kinetics (pure clones and mixtures of clones), epimastigote/ trypomastigote transformation rate, *in vitro* drug sensitivity, transmissibility by the vector *Triatoma infestans* (pure clones and mixtures of clones).

⇒ All these parameters have been quantified. A highly significant correlation was found beetween biological variability and genetic diversity: the stocks that are genetically closely related have a strong statistical tendency to have similar biological behaviours, while the opposite is true for the distantly related clones. Major clones tend to behave like distinct taxa for these biological parameters. The stocks attributed to the clonal group 19/20 generally grow faster, transform more actively into trypomastigotes, are more virulent to mice and less sensitive to drugs. Moreover, in experiments dealing with mixtures of clonal genotypes (a situation that is quite frequent in chagastic patients), we noted in some cases, indications for interactions between clones. For instance in mice, the mixture of a strongly virulent clone and a poorly virulent one proved to be more virulent than the more virulent clone. We proposed the hypothesis that mixtures of clonal genotypes in the same patient could play a role in Chagas pathogenicity.

⇒ Complementary analyses from anatomopathology and in vivo experiments on three different mouse strains confirmed considerable biological diversity among *T. cruzi* stocks pertaining to the different major clones.

Conclusions

- Most of the work outlined at the start of the project has been completed, together with additional experiments on *T. cruzi* transmissibility by *Triatoma infestans*, and the biological behaviour of mixtures of clones.
- All results fully confirmed the working hypothesis of the project, i.e. that clonal diversity of *T. cruzi* has a major impact on this parasite's biological diversity, including medically relevant parameters such as virulence, resistance to drugs, and transmissibility by triatomine bugs.

Selected publications

De Lana M., Pinto A., Da S., Barnabé C., Quesney V., Noël S. and Tibayrenc M. Trypanosoma cruzi: compared vectorial transmissibility of three major clonal genotypes by Triatoma infestans. Exp. Parasitology. In press.

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Partners

ORSTOM – MONTPELLIER 1
Labo. de Génét. Des Parasites et des Vecteurs
BP 5045
F-34032 Montpellier 01
France

Michel Tibayrenc
Tel.: +33-467-61 74 97
Fax: +33-467-54 78 00
E-mail:
michel.tibayrenc@cepm.mpl.orstom.fr

UNIVERSIDAD AUTONOMA DE MADRID
Unidad de Parasitología
Carretera de Colmenar Viejo
E-28049 Madrid
Spain

Dediego
Tel.: +34-91-397 53 11
Fax: +34-91-315 00 75

UNIVERSIDAD DE CHILE
Depto. de Bioquímica
70086 Santiago 7
Chile

A. Solari
Tel.: +56-2-737 00 81 ext. 5724
Fax: +56-2-735 55 80
E-mail: ngalanti@med.uchile.cl

Contract number: TS3*CT930219

Period: January 1994 to December 1996

FIELD EVALUATION AND FURTHER CHARACTERIZATION OF AN INVASIVE SPECIFIC MONOCLONAL ANTIBOBY AGAINST *ENTAMOEBA HISTOLYTICA*

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom
(D. Warhurst)

Objectives

- Application and development of techniques for the distinction of *Entamoeba histolytica and E. dispar*, with a view to clarifying the epidemiology of amoebiasis in different areas of the world, and focussing the treatment effort.
- Training and transfer of technology to enhance research potential of scientists from countries with endemic diseases.

Activities

- Training visits.
- Collaboration
- Joint publications

Results

⇒ A colorimetric PCR technique (Solution Hybridisation Enzyme-Linked Assay) (SHELA) has been developed for differentiation of faeces containing *E. histolytica* and *E. dispar*. A comparison of the zymodeme of isolate cultures with SHELA results on cultures in Bangladesh was in agreement with the detection of *E. histolytica* in 23/23 cases (13 zymodeme 2 and 10 zymodeme 14). However, three cultures identified as zymodeme 1 were tested, and 2 reacted in the SHELA *as E. histolytica* while the other one reacted *as E. dispar*. It is clear that more testing of non-pathogenic material from Bangladesh is needed to determine whether this degree of non-agreement is significant, since it has not been seen in materials from other areas. It is encouraging to note that zymodeme 14 reacts as well in PCR-SHELA as zymodeme 2.

⇒ The parallel examination of the original faecal specimens using the *Entamoeba* and *E. histolytica*-specific ELISA technique of Petri and colleagues gives some contradiction with zymodeme and with the PCR-SHELA. The common occurrence of apparently mixed infections is notable. However, given that the original faeces are being examined and culture is inevitably selective, this is not so much of a problem as the rather low sensitivity of the immunological technique for the detection of *E. Histolytica* itself. To investigate this, further 40 more faecal specimens from Bangladesh are being tested in the UK laboratory and will be compared with the results of the cultures and of the Petri ELISA.

⇒ Improvements to the protocol and kit for the PCR-SHELA have been developed, using a modified system names Semi-Nested PCR-SHELA. This has been successful for both amoebiasis and malaria contexts. The advantage of the technique is that the labelled internal probe, which normally needs to be added to the PCR product after the reaction, is

incorporated in the sealed tube and acts as a primer of a PCR nest, and as the detector for the 1st and 2nd products of the PCR.

⇒ All that needs to be done at the end of the single PCR run is to dilute the product in buffer and apply it to the micro-titer plates for the avidin capture and digoxigenin enzymatic detection procedure. This substantially reduced the time taken for the technique.

Selected publications

McNerney R., Aguirre A., West B., Stoker N., Miles M., and Wilson S. 1995. Customizing PCR detection. Presented at "Nucleic acid-based technologies: current challenges. Future strategies and end-user perspectives". Cambridge Health Institute, San Francisco. May 1995.

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Partners

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Dept. of Medical Parasitology
Keppel Street
UK-London WC 1E 7HT
United Kingdom

D. Warhurst
Tel.: +44-171-927 23 41
Fax: +44-171-636 87 39
E-mail: d.warhurst@lshtm.ac.uk

HOSPITAL CLINICO Y PROVINCIAL DE BARCELONA
2182 Villaroel 170
E-08036 Barcelona
Spain

M. Corachan
Tel.: +34-93-45 46 000 ext. 2182
Fax: +34-93-45 15 272

UNIVERSIDAD DE LOS ANDES
Depto de Ciencias Biológicas
Carrera 1 Este N. 18A-10
4976 Santa Fé de Bogotá
Colombia

F. Guhl
Tel.: +57-1-286 75 93
Fax: +57-1-284 18 30
E-mail: fguhl@uniandes.edu.co

INTERNATIONAL CENTRE FOR DIARRHOEAL RESEARCH
P.O. Box 128
1000 Dhaka
Bangladesh

R. Haque
Tel.: +880-260 01 71 78
Fax: +880-288 31 16
E-mail: rhaque-cholera@external.ait.ac.th

UNIVERSITY OF LEIDEN
Lab. for Parasitology
Medical faculty
P.O. Boc 9605
NL-2300 RC Leiden
The Netherlands

A.M. Polderman
Tel.: +31-71-527 68 45
Fax: +31-71-527 68 50
E-mail: parasito@rullf2.leid.nl

Contract number: TS3*CT930226

Period: January 1994 to December 1996

ROLE OF INSECT HOST DEFENCES IN TRYPANOSOME DEVELOPMENT IN CHAGAS' DISEASE VECTORS WITH EMPHASIS ON THE ACTIVITY OF IMMUNE DEPRESSION AGENTS

Co-ordinator: University College of Swansea, Swansea, United Kingdom (N. Ratcliffe)

Objectives

- Determine the presence and role of insect vector immune defence factors in insect-parasite interactions.
- Determine the effect of parasites on the vector immune defence reactions.

Activities

Year 1

* Lectin staining.
* Hemolymph and crop lectins purification initiated.
* Infectivity studies on different parasite strains.

Year 2

* Lectin staining.
* Hemolymph and crop lectins purification.
* Effect of immunosuppressive agent.
* Prophenoloxidase.
* Blocking experiments commenced.

Year 3

* Lectin staining.
* Blocking experiments.
* Test pure lectin against parasites.

Exchange of scientists and training.

Expected outcome

⇒ To learn how *T. cruzi* and *T. rangeli* evade the normal insect defence mechanisms and colonize the host.

⇒ To discover vector molecules responsible for transformation of the parasites from one stage to another.

⇒ Eventually to show whether compounds that induce immune-depression in the vectors can facilitate the control of Chagas' disease transmission.

Contract number: TS3*CT930226

Partners

UNIVERSITY COLLEGE OF SWANSEA
School of Biological Sciences
Singleton Park
UK-SA2 8PP Swansea
United Kingdom

N. Ratcliffe
Tel. :+44-179-229.54.54

FUNDACAO OSWALDO CRUZ
Dept. de Entomologia
Av. Brazil 4365
21045-900 Rio de Janeiro
Brazil

E. De Souza Garcia
Tel. :+21-2-90.75.49

RUHR UNIVERSITAET BOCHUM
Dept. Special Zoology & Parasitology
D-44780 Bochum
Germany

G. Schaub
Tel. :+49-23-470.04.563

Contract number: TS3*CT930227

Period: January 1994 to December 1996

IMMUNOLOGICAL CORRELATES OF RESISTANCE AND SUSCEPTIBILITY TO INFECTIONS WITH GASTRO-INTESTINAL NEMATODES IN NORTH EAST BRAZIL

Co-ordinator: University of Nottingham, Nottingham, United Kingdom (D. Wakelin)

Objectives

The overall objective of the proposal was to make a detailed study of the immune responses to infection with gastro-intestinal nematodes and to identify causal correlates of resistance and susceptibility to these important parasites. The specific objectives were to:

- Determine the prevalence and intensity of gastro-intestinal nematode infections across an appropriate age range in populations living in communities where these parasites are endemic.
- Follow the patterns of reacquisition in these populations after effective chemotherapy.
- Identify and characterize individuals showing resistance or susceptibility to infection and re-infection.
- Analyze the antibody, cellular responses to each infection, correlate these with parameters of resistance and susceptibility, and identify target antigens of the parasites concerned.

Activities

Infections with the major gastro-intestinal nematodes of man are endemic in the Recife area of N.E. Brazil, a pilot study showing high prevalence in poor urban and rural communities, and the excellent laboratory facilities at CPq AM, Recife offer a unique opportunity to examine the parasitology and immunology of these infections. Collaboration with the laboratories in Nottingham and Marseille provides an unrivalled combination of expertise in the immunoparasitology of helminth, specifically intestinal nematode, infections. The proposal is to carry out a detailed study of immune (serological, cellular and cytokine) responses in individuals of known infection/reinfection status. The data obtained will make it possible to correlate resistance or susceptibility to infection with the capacity to mount particular responses, and throw light on those mechanisms which regulate the development and expression of host protective immunity. In this context definition of T helper subset responses, and of target parasite antigens are seen as key priorities.

Expected outcome

- The project has provided the first detailed survey of intestinal nematode infections in N.E. Brazil. Extensive clinical data on the surveyed communities have been collected and are being analyzed. The populations have been extensively surveyed, blood and stool samples have been taken on several occasions. It is clear that the parasites *Ascaris*, Hookworm and *Trichuris* have high prevalence, particularly in children.
- Worm burdens: particularly in the case of *Ascaris*, can reach very high levels and are therefore likely to cause a number of clinical problems. The populations have been treated with anthelmintic and the patterns of reinfection followed. Lymphocytes and sera from

individuals shown at each survey to be repeatedly heavily of lightly infected are being analyzed for antibody and cytokine responses in order to look for immunological correlates of resistance and susceptibility. The data collected have already contributed to increased public health awareness of the importance of intestinal nematode infections in the Recife area. The research capabilities of the Brazilian partner have been considerably strengthened. Surveys carried out in poor urban and rural areas in the Recife area showed a high prevalence of gastrointestinal (GI) nematode infections.

- In the urban area *Ascaris lumbricoides* and *Trichuris trichiura* were the commonest species (57 and 62%), whereas in the rural area hookworm (*Necator*) was commonest (79%). Infections were present in all age groups, but were most prevalent and most intense in children below 16 years. Only Ascaris infections occurred at very high intensity, faecal egg counts of more than 20,000 eggs per gram being recorded. The study groups have all received chemotherapy, and this proved largely successful in eliminating infection, but with time since treatment levels of infection are again rising. Sera taken before the first chemotherapy have to date been analysed for total and parasite specific IgE responses, as this isotype is considered to give the greatest degree of parasite specificity. High levels of total and parasite specific IgE have been recorded in individual infected with *Ascaris* and with *Trichuris*. Although the first result was unexpected, high anti-*Trichuris* IgE responses were not. Assays for IgG isotypes are now in progress, and it is hoped soon to have data on cytokine responses from individuals who have consistently shown either high or low worm burdens. All of these data will then be analysed for association with level of, and predisposition to, infection.

Partners

UNIVERSITY OF NOTTINGHAM
Dept. of Life Sciences
University Park
UK-NG7 2RD Nottingham
United Kingdom

D. Wakelin
Tel. :+44/160/251.32.32

CENTRO DE PESQUISAS AGGEU MAGALHAES
Av. Moraes Rago
Caixa Postal n□7472
50670-420 Recife
Brazil

H. Coutinho
Tel. :+551/81/271.40.00

CNRS
Lab. d'Immunogénétique Parasitaire
Parc Scientifique de Luminy
Case 906
F-13288 Marseille 9
France

A. Dessein
Tel. :+33/561/691.269.400
E-mail: alain.dessein@medecine.univ-mrs.fr

Contract number TS3*CT930229

Period: January 1994 to March 1996

REGULATION OF SEXUAL DEVELOPMENT IN MALARIAL PARASITES AND THE DESIGN OF LOGICAL INTERVENTION STRATEGIES

Co-ordinator: Imperial College of Sciences, Technology, and Medicine, London, United Kingdom (R. Sinden)

Objectives

Through collaborative studies and associated training programmes, the project aimed to investigate the genetic, molecular and biological regulation of sexual development of *Plasmodium*. Though the information gained logical intervention strategies would be investigated.

Activities

Through mutual exchanges of personnel and reagents between the participating laboratories we have integrated the particular expertise of each laboratory in a series of studies, many of which have been published. The diverse methodologies used have been described in these publications and are therefore not repeated here.

Results

⇒ ***Expression and immunogenicity of recombinant transmission-blocking antigens***

A preliminary comparative study assessing the immunogenicity of full length Pbs21 and Pbs21 lacking the anchor region has shown that antibodies reactive to native Pbs21 were induced in both groups of immunized mice, however pronounced differences in the titre of antibody responses in Western blot analyses and in the transmission-blocking activity of immune sera were observed. Three recombinant baculovirus containing the full length coding region of Pfs28, the *P. falciparum* homologue of Pbs21, were purified and patterns of protein expression in insect cells was determined by Western blot analysis, and optimal conditions for antigen production were determined. The immunogenicity of the recombinant protein will be tested in immunization experiments and compared to recombinant Pfs28 expressed in yeast (obtained from NIH, Bethesda). A plasmid for DNA vaccination containing the Pfs28 gene was constructed. Plasmid mediated protein expression will be tested using a mammalian cell system. A novel *P. berghei* sexual stage specific protein was identified. Native protein was purified by electroelution. Preliminary data suggest that the protein is expressed in zygotes and ookinetes starting approximately 6 h after activation of gametocytes. Immune sera against the protein and monoclonal antibodies are currently being developed to further characterize the protein and to screen an ookinete specific cDNA library.

⇒ ***rRNA regulation***

In situ hybridization confocal laser scanning microscope studies on the regulation of rRNA during sexual development are described elsewhere in the report. The identification of proteins that are phosphorylated/dephosphorylated. The phosphorylating and H1 kinase activities of extract preparations of *P. chabaudi* and *P. berghei* parasites were analyzed

along the erythrocytic cycle. In experiments using *P. berghei* extracts, histone was intensively labelled in young trophozoite extracts; this activity decreased in mature trophozoites and disappeared in schizonts. Labelling of non-histone proteins was parasite stage specific: a band of >200 kDa was phosphorylated in young trophozoites, a band of 45kDa was labelled in mature trophozoites and a 40 kDa protein was phosphorylated in schizonts. Quantitative analysis of ^{32}P-ATP incorporation was carried out in *P. chabaudi.* Major histone kinase activity occurs in mature trophozoites. In the absence of histone, no incorporation of ^{32}P- was detected in schizonts. When H1 histone was added, stimulation of the phosphorylation occurred in all asexual parasite stage extracts. Experiments to study phosphorylation in *P. berghei* sexual stages have produced contradictory results mainly because of asexual stage contaminations. We have standardized purification techniques for micro- and macro- gametes, zygotes and ookinetes and are currently using these preparations. cdc-2 like kinase activity; and expression of cyclin during the different phases of the parasite life cycle. The peptide sequence reported for a highly conserved region of the cyclin molecule of several species, between positions 201 and 335, named the "cyclin box" and the codon usage of *P. falciparum* were used for PCR-amplification of *P. chabaudi* DNA. The products obtained were cloned in the Small site of pBluescript plasmid for their characterization. The sequencing and analysis of the inserts obtained are in progress. Preliminary comparisons of the 400 bp band indicated sequence homology with human G protein coupled receptor; human gene 1AC; and several anonymous sequences of the *P. falciparum* genome (including genomic clone 0433). The amino acid sequence shared homology with yeast cell division control protein 10 (20% in a 38 aa strand); mouse tyrosine receptor FLT4 (66% in a 10 aa strand), a putative serine/threonine kinase R107.4 (83% in a 6 aa strand) and a similar degree of homology (40% in 18 aa strands) with zinc fingers of rat, mouse and human. We are currently sequencing the other clones.

⇒ ***Molecular karyotype changes related to gametocytogenesis in P. berghei and in P. falciparum***

In *P. berghei* several sexual-specific genes have been characterized, which map to chromosome 5. Moreover, rearrangements which affect this chromosome correlate with defects in the gametocytogenesis. Its structural organization has been studied in detail in collaboration with the University of Leiden. A long-range map of chromosome 5 from the gametocyte-producer clone 8417HP, taken as a reference clone, has been constructed and genes hybridizing to it positioned. Subtelomeric portions of this chromosome have been studied in more detail. They are characterized by the presence of a region, extending 60 kb at most, shared by both the extremities and symmetrically located. This region is involved is most large-scale rearrangements affecting this chromosome. In *P. falciparum* the role of the terminal portion of chromosome 9 in gametocyte differentiation has been investigated. By analyzing a synchronous parasite population of line HB3, heterogeneous for the size of chromosome 9, it was shown that the small fraction of full-length chromosome 9 was preferentially retained in those parasites developing into gametocytes. Studies on isogeneic parasite lines (derived from isolate 1776) differing for the size of chromosome 9 demonstrated that parasites harboring the deleted forms do not enter the earliest detectable step of sexual differentiation. This suggests a role of functions encoded in that region of chromosome 9 in the regulation of early events of sexual differentiation.

⇒ ***Regulation of sexual/asexual gene expression***

A novel gene family, which maps to the terminal portions of *P. berghei* chromosome 5 (one member was selected by subtraction procedures), has been characterized. It contains three partially homologous genes which share the N-terminus of the deduced proteins. The genomic region of one of these three genes is involved in large scale subtelomeric

rearrangements observed in two characterized gametocyte-defective mutants. A *P. berghei* gene (pbB7) conserved within the Plasmodium genus is also being characterized The coding sequence exhibits significant blocks of similarity with a class of nucleosome assembly proteins. The nuclear localization of pbB7 gene product has been demonstrated both in *P. berghei* and *P. falciparum* using specific monoclonal antibodies. A significant difference in the size of the corresponding transcripts has been observed when comparing sexual and asexual parasites. Introns have been detected positively and a possible mechanism of alternative splicing is under investigation. In order to describe the promoter of Pfg27 gene of *P. falciparum* and its regulation, a structural and functional analysis was undertaken on the genomic region upstream the gene which is active in early stages of gametocytogenesis. Northern blot analysis, RNAse protection and nuclear "run-off" experiments on sexual and asexual stages permits us to state that the expression of this gene occurs at the level of transcription initiation. The gene is transcribed in the first 2 to 3 days of sexual differentiation, while it stops afterward.

⇒ ***Genetic transformation***

- The successful development of a drug selectable system for the genetic transformation of the rodent malaria parasite, *Plasmodium berghei*, based upon the donation of drug resistance to the antimalarial drug pyrimethamine. A copy of the homologous dhrf/ts gene containing a Ser/Asn110 mutation has been engineered into *E. coli* plasmids creating transfection vectors. A series of vectors have been constructed that are designed to integrate in a site specific fashion into the parasite genome.
- The use of the system to transform and select transformed parasites that maintained the vectors as episomes. The further characterization of the biological properties of the plasmid DNA maintained in the transformed parasites.
- The successful and site specific introduction of foreign DNA into subtelomeric regions of three *P. berghei* chromosomes demonstrating that subtelomeric structures can support expression of RNA polymerase II transcribed genes.

⇒ ***Gene mapping***

Collaborative studies revealed that the location and linkage of genes on chromosomes of rodent malaria parasites are highly conserved. The link between chromosome 5 and sexual development has been investigated. In different non-gametocyte producing parasite clones a specific rearrangement in the subtelomeric regions of this chromosome has been observed and genes involved in sexual development appear to cluster on this chromosome. In one of our collaborations a long range restriction map of the chromosome has been produced and the size reduction shown to consistently involve rearrangements in a single region of the chromosome. A YAC library of a gametocyte producing parasite clone has been produced and to date YACs covering 60% of chromosome 5 have been isolated.

⇒ ***Gene expression***

The cloning and mapping of the 5' regions of model genes has resulted in identification of the promoter regions of both A-type rRNA genes and the Pbs21 gene. The latter was shown by *in situ* mRNA hybridization on bloodstage parasites to be transcribed only in female gametocytes. Transient transfection technology for the functional analysis of the structure of these promoters is under development. The precise pattern of expression of the stage specific rRNA genes has been determined throughout most of the *P. berghei* life cycle (collaborations with Imperial College). In collaboration with University of Sao Paulo, the cell cycle gene crk2 has been cloned and characterized from a number of different malaria species (*P. berghei, P. knowlesi* and *P. vivax*) as have the genes encoding the ribosome associated EF-1 alpha protein. The genetic and biochemical characterization of crk2 is in hand. This collaboration also initiated studies on the organization of the genome of *P. vivax* involving a 6 week visit of a student from USP.

Partners

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
Dept. of Biology
Prince Consort Road
South Kensington
UK-London SW7 2BB
United Kingdom

R. Sinden
Tel.: +44-171-594.54.25
Fax: +44-171-594.54.24
E-mail: r.sinden@bio.ic.ac.uk

RIJKSUNIVERSITEIT LEIDEN
Dept. of Parasitology
Postbus 9605
NL-2300 RC Leiden
The Netherlands

C. Janse
Tel.: +31-71-27.68.42
Fax: +31-71-27.68.50

ISTITUTO SUPERIORE DI SANITA
Labo. di Biologia Cellulare
Viale Regina Elena 299
I-00161 Roma
Italy

M. Ponzi
Tel.: +39-06-444.02.70
Fax: +39-06-444.00.18

CENTRO DE INVESTIGACION DEL PALUDISMO
Dept. of Immunoparasitology
Apartado Postal 537
Tapachula 30700, Chiapas
Mexico

M. Rodriguez
Tel.: +96-26.22.19
Fax: +96-26.57.82
E-mail: nrodriguez@hotmail.com

Contract number: TS3*CT930234

Period: December 1993 to December 1995

REDUCING MATERNAL MORTALITY AND MORBIDITY IN BOLIVIA: APPROPRIATE BIRTH PRACTICES IN THE FORMAL AND INFORMAL SYSTEMS OF PERINATAL CARE

Co-ordinator: Dublin University, Dublin, Ireland (B. Bradby)

Objectives

- Identify culturally appropriate practices and technologies of birth for women in highly traditional rural (Quechua-speaking), modernising rural (Aymara-speaking) and urban marginal (rural migrants).
- Study the range of factors which prevent childbearing women from participating in formal health care services.
- Produce baseline data on knowledge and practices in relation to pregnancy and birth, which can then be monitored and updated.

Activities

The project carried out data collection on childbirth practices, and on complications of pregnancy and birth, that laid the basis for identifying the factors and circumstances that have such an adverse effect on the reproductive health of women. Existing knowledge was based almost entirely on statistics from the formal health care system. However, in rural Bolivia, 80% of births take place outside this system, and the project has elaborated methodologies that will allow data to be collected from the informal sector of birth care, and collated with similar data from the official care sector. Three case study localities have been identified, with differing socio-economic characteristics:

* Highly traditional rural (Quechua-speaking)
* Modernising rural (Aymara-speaking)
* Urban marginal (rural migrants)

In each area, the project will work through local health personnel and through local research organisations which have themselves built up good networks in urban and rural communities.
The baseline data collected will enable women and health care personnel to initiate concrete actions at the local level to improve service delivery in ways that are appropriate to the social and cultural circumstances of women and their caregivers.
The project involves six partner institutions, three from Bolivia, and three from Europe, in a complex pattern of co-operation, using specific professions and skills developed in the different contexts, which must be used together if such a project is to be successful. These include anthropology, linguistics, medicine, and midwifery, as well as popular-educational and communications skills, necessary for adequate dissemination in non-literate cultures. The project includes a training element, both for local fieldworkers, in methods of qualitative and quantitative data collection and analysis, and for community promoters, who will attend short orientation courses on the aims of the project and in communication skills.

Expected outcome

- It is hoped that in relation to the problem of maternal mortality, the qualitative study will lead to greater understanding of the reasons for under-utilisation of existing maternity services in Bolivia, and to recommendations for ways of decreasing cultural barriers to service delivery. It is also hoped that the findings will help programmes for training traditional birth attendants to develop in ways that are culturally appropriate, and which can ultimately empower, rather than deskill, local people.
- The baseline data collected during the quantitative phase of the fieldwork should enable women and health care personnel to initiate concrete actions at the local level to improve service delivery in ways that are appropriate to the social and cultural circumstances of women and their caregivers. The baseline study will also enable local networks of statistical collection to be put in place so as to enable to monitoring and updating of the practices and problems encountered in the study.

Results obtained

⇒ The project's objectives were to identify appropriate birth practices for rural and migrant women in Bolivia, and to look at the factors leading to under-use of existing health services, in the light of concerns about rates of maternal mortality which are high by international standards.

⇒ The team of three Bolivian non-governmental organisations and two European institutions carried out studies in rural and peri-urban areas, using a combination of qualitative methods and a questionnaire survey.

⇒ Part II of the report documents traditional understandings of birth and of birth care, setting fertility and birth within the complex cosmic vision of the relationships between earth and sky. Principles such as upright positions in birth and the clothing and warmth of the mother relate to these understandings of rain, earth, sun and regeneration. The metaphors are particularly elaborated around the placenta, and birth is seen as a dual process, involving the birth of both baby and the placenta, its "soul". Hospital birth is then approached through the eyes of these traditional understandings. Fear of the Caesarean structures and migrant women's approach to hospital birth, leads to both passive resistance to hospital procedures by giving birth alone, and to active negotiation with hospital staff for other kinds of "help". The traditional prioritising of care in the birth of the placenta carries through into women's expectations of attention in hospital.

⇒ Part III of the report examines quantitative data from the project in relation to international health planners' agendas. It looks at the emergence of maternal mortality as a global problem in the last decade, and presents the current situation in Bolivia. It reviews the biomedical arguments on risk factors, and discusses four principal causes of illness and death in childbirth. The view of traditional birth attendants in international programmes is examined in the light of the Bolivian government's training programmes. The results of a questionnaire administered to 298 women are assessed in terms of the effectiveness of reported practices from the points of view of women themselves and of current international recommendations.

⇒ Finally, there is an assessment of data on obstetric practices collected from institutional medical personnel, which are evaluated for their effectiveness in preventing complications and in addressing women's needs.

⇒ The project's recommendations fall into five areas: furthering dialogue between traditional and biomedical services; arresting the decline of traditional midwives; allaying cultural

fears of hospital birth; systemic response to emergency care; and further research, including a large-scale study of different systems of placental management.

Partners

DUBLIN UNIVERSITY ,TRINITY COLLEGE,
Dept. of Sociology
Dublin 2
Ireland
B. Bradby
Tel.: +351-1-702.12.96

CENTRO DE INVESTIGACION, EDUCACION Y SERVICIOS
Arturo Costa de la Torre 1322
Casilla 9935
La Paz
Bolivia
S. Rance
Tel.: +591-239.00.11

INSTITUTO DE LENGUA Y CULTURA AYMARA
Casilla 2681
La Paz
Bolivia
Y. Yapyta
Tel.: +591-234.31.95

TALLER DE INVESTIGACION Y FORMACION ACADEMICA Y POPULAR
Calle Argentina 37
Sucre
Bolivia
P. Nina
Tel.: +591-642.22.53

UNIVERSITY OF ST. ANDREWS
Dept. of Social Anthropology
St. Salvator's Building
North Street
UK-KT17 9AL St. Andrews
United Kingdom
T. Platt
Tel.: +44-133-46.29.79

Contract number: TS3*CT930243

Period: January 1994 to December 1995

RAPID DETECTION OF MULTIDRUG-RESISTANT MYCOBACTERIA

Co-ordinator: Institut Pasteur, Paris, France (Stewart Cole)

Objectives

- Elucidation of the molecular bases of drug-resistance in *Mycobacterium tuberculosis.*
- Development of rapid methods for detection of drug resistance.

Activities

- Development of a standard protocol for performing polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP) analysis of genes associated with resistance to isoniazid, rifampicin and streptomycin. Prospective study of drug resistance in which the results obtained by PCR-SSCP were compared with those obtained by the proportion method.
- Implementation of molecular detection strategies in clinical and reference laboratories.

Results

⇒ Resistance to isoniazid and rifampicin results from alterations to key chromosomal genes and can be readily detected by means of DNA sequencing or PCR-SSCP analysis of selected mutational hotspots. A standardized PCR-SSCP protocol for the detection of isoniazid and rifampicin resistance was developed and optimized. A blind, prospective, longitudinal study was then conducted in which the results obtained by PCR-SSCP of cultured samples of *M. tuberculosis*, from ± 100 specimens obtained from recent tuberculosis cases, were compared with the drug susceptibility patterns established by the generally accepted reference technique, the proportion method.

⇒ In the case of rifampicin, excellent sensitivity and specificity were observed, and the results were concordant for 99% of the samples. Furthermore, identical results were obtained by a second-generation molecular test for drug susceptibility employing solid-phase reverse hybridization. For 87% of the strains, good agreement was seen between the isoniazid resistance profiles predicted by PCR-SSCP and those determined microbiologically. The difference between the two data-sets can probably be explained by the existence of an additional minor resistance mechanism that has not yet been uncovered.

⇒ In summary, the molecular methods developed and evaluated during this project proved robust, reliable and, above all, considerably quicker as they allow resistance patterns to be determined in 2 days. This compares very favourably with the two-four weeks required for conventional microbiological procedures.

Selected publications

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Partners

INSTITUT PASTEUR
Unité de Génétique Moléculaire Bactérienne
28, rue du Dr. Roux
F-75724 Paris
France

Cole Stewart
Tel.: +33-1-45 68 84 46
Fax: +33-1-45 68 89 53

INSTITUTO DE SALUD CARLOS III
Centro Nacional de Microbiología
Servicio de Parasitología
Sinesio Delgado 10-12
E-28029 Madrid
Spain

Arturo Ortega-Calderón
Tel.: +34-1-314 08 07
Fax: +34-1-733 66 14

INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS
Centro de Microbiología y Biología Celular
Laboratorio de Genética Molecular
Carretera Panamericana - km 11
Apartado 21827
Altos de Pipe, EDO. Miranda
Venezuela

Howard Takiff
Tel.: +58-2-572 74 46
Fax: +58-2-572 74 46

UNIVERSITAET BERN
Institute for Molecular Microbiology
Friedbuehlstrasse 51
CH-3010 Bern
Switzerland

Amalio Telenti
Tel.: +41-31-648 7017
Fax: +41-31-260 063

Contract number TS3*CT930247

Period: January 1994 to December 1996

MOLECULAR TECHNIQUES FOR VECTOR AND PARASITE IDENTIFICATION APPLIED TO A PILOT VECTOR CONTROL STUDY OF LEISHMANIASIS

Co-ordinator: University of Keele, Keele, United Kingdom (Rhaiza D.C. Maingon)

Objectives

The overall goal is to use previously established molecular techniques to investigate the potential preventive value of permethrin-impregnated curtains from reducing man-biting sandfly rates in a pilot control study in a selected highly endemic focus of cutaneous leishmaniasis. The present study is a pre-requisite for further intervention vector control trials aimed at reducing the incidence of the disease in areas of domestic transmission.

Specific objectives:

- Detemine the vectorial capacity of anthropophilic sandfly species in two ecologically different regions (Lara and Miranda states).
- Gather epidemiological information in the Guayamure/Rio Claro of Lara and Miranda state, using molecular techniques combined with classical field methods.
- Evaluate the efficacy of permethrin-impregnated curtains against endophilic phlebotomine sandflies with respect to a reduction of biting rates in a highly endemic pilot area in Lara and Miranda states.

Activities

- Extensive field work indicated a high level of endophilic transmission of cutaneous disease in El Ingenio (Miranda state) and - to a lesser extent- in Guyamure and Rio Claro villages in Lara state (Venezuela). El Ingenio village consists of 54 house with a population of 254 inhabitants of predominant agricultural occupation. This, and other features such as high prevalance of cutaneous cases or long-term surveillance of these villages made them suitable to investigate the efficacy of insecticide-impregnated curtains in reducing intra-domiciliary sandfly number and their biting rates.
- During the first year, the entomological analysis of the densities and sandfly species inside vs. outside house in El Ingenio, provided the base-line data for selecting the study and control houses for a pre-pilot vector control trial. This aimed to gain an insight into the relative value of all variables involved in an effective trial. Deltamethrin at a dose of 15 mg/m^2 was used to soak curtains (6 mm mesh size).
- A number of issues related to the El-Ingenio trial have been examined to identify specifically the cause(s) for the apparent lack of vector control:
  - Sandfly trapping methods used for monitoring the trial
  - Variables affecting sandlfy susceptibility to a number of insecticides such as insecticide source, insecticide concentration, curtain fabrics, and curtain mesh size
  - Changes in the local sandfly population density and/or sandfly behaviour

- Changes in the community behaviour, particularly of those people living in the selected houses.

Results

⇒ The vectorial capacity of anthropophilic sandflies in El Ingenio and Altagracia de Orituco (North-central Venezuela) has been partially elucidated. Since dissection to find out natural infection rates is currently being carrie dout, it will be important to implement the PCR technique qith pooled sandflies (of a given species, i.e. *ovallesi* with *Le. Braziliensis* specific primers MpL 1 and MP 3 H).

⇒ Epidemiological information int eh Rio Claro and Guayamure foci of Lara and Altagracia de Orituco (Guarico state) a,d El Ingenio (Miranda state) has used classical and molecular techniques except in the northern region. Implementatin of human DNA detection by PCR in sandfly bloodmeals would enhance the sensitivity of ELISA detection.

⇒ For evaluating the efficacy of permetrin-impregnated materials against endophilic sandflies, a second three-way trial is required, comparing both vector control methods: insecticide-impregnated curtains with insecticide wall-spraying inside houses in El-Ingenio. Due to weather pattern changes, there has been a very reduced number of sandflies in both places throughout 1995. The three-way trial is scheduled to take palce as soon as the sandfly density increases.

Partners

UNIVERSITY OF KEELE
Dept. of Biological Sciences
Centre for Applied Entomology and Parasitology
Keele
GB- ST5 5BG Staffordshire
United Kingdom

Rhaiza D.C. Maingon, R. Ward, A. Wheele
Tel.: +44-1782-62 11 11
E-mail: bia25@keele.ac.uk (R. Maingon)
bia40@keele.ac.uk (R. Ward)

UNIVERSIDAD DE CARABOBO
Ciencias Salud
La Morita 4944
Apa'rtado 4876
Maracay – Edo Aragua
Venezuela

D. Feliciangeli-Pinero, J. Arrivillaga
Tel.: 58-4-333 36 56

ISTITUTO SUPERIORE DI SANITÀ
Labo. di Biologia Cellulare
Viale Regina Elena 299
I-00161 Roma
Italy

M. Maroli
Tel.: +39-6-499 03 02

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Parasite and Vector Biology Division
Pembroke Place
Liverpool L3 5QA
United Kingdom

H. Townson
Tel.: +44-151-708 93 93

UNIVERSIDAD DE VENEZUELA
BIOMED
San Nicolas a Providencia
Area Hospital Vargas, San José
YV-4043 Caracas
Venezuela

N. Rodríguez
Tel.: +58-2-81 46 30
Fax: +58-2-861 12 58
E-mail: nrodriguez@hotmail.com

UNIVERSIDAD LISANDRO ALVARADO
Facultad de Medicina
Laboratorio de Parasitolog ùia
Barquismeto, Edo Lara
Venezuela

R. Bonafante Garrido

Contract number TS3*CT930255

Period: October 1993 to September 1995

ORAL VACCINE AGAINST CHOLERA WITH "BUILT-IN" ADJUVANTICITY

Co-ordinator: Istituto Ricerche Immunobiologiche Siena, Siena, Italy (S. Rappuoli)

Objectives

- Development of new vaccines against diarrhoeal diseases (such as cholera and enterotoxigenic *E. coli*), based on the immunization with live-attenuated strains of *V. cholera* and *Salmonella* expressing non-toxic derivatives of cholera and heat-labile toxins.
- To further increase the immunogenicity of the antigens expressed, *in vivo,* by the attenuated strains, we have engineered the *Salmonella typhimurium* strain to express a peptide derived from IL-1β peptide that has been proposed to be a good adjuvant.

Activities

✶ ***Design, construction, purification and characterization of LT and CT mutants***

Heat-labile toxin (LT) is a bacterial protein with ADP-ribosylating activity, produced by enterotoxigenic *E. coli* strains, structurally and functionally related to Cholera toxin (CT). These two toxins share 80% sequence homology and the same 3D structure. LT and CT are organized as AB_5 hexamers, where the homopentameric B subunit binds the receptor on the membrane of eukaryotic cells, while the A subunit is responsible for the ADP-ribosylation of the α subunit of G_s a GTP-binding protein. Using the known 3D structure of LT and computer modelling analysis, we have identified residues previously not known to be important for enzymatic activity, and provided the rationale to probe their function by changing them by site-directed mutagenesis. We have generated a number of different mutants of LT and CT, expressed them in *E. coli* and *V. cholera* strains, respectively, purified and characterized. Some of them have been found to be completely devoid of enzymatic activity, both, *in vivo* and *in vitro*. We have analyzed the biochemical and immunological properties of the non toxic mutants to define the influence of the mutations in the A subunit on the assembly, stability and immunogenicity of each of the mutant molecules. We have obtained mutants in which the amino-acid substitution had not affected the toxicity; mutants in which aminoacid substitutions had dramatically reduced the toxicity and mutants in which the substitution had prevented the formation of the A/B holotoxin. By the study of the biochemical and immunological properties of the nontoxic mutants, we have found that, single amino-acid substitions in the A subunit may affect not only the enzymatic activity, but may also have profound effects on the ability to form the AB_5 structure, on the stability during long-term storage, and on the trypsin sensitivity and immunogenicity of the assembled mutant molecules. Among the non toxic mutants tested, the CT-K63 and the analog LT-K63 mutants, proved to be non toxic, well assembled, stable to trypsin treatment, and able to induce neutralizing antibodies against both the A and B subunit. This property suggests that the A subunit plays an important role in protective immunity and raises the possibility of using these molecules to improve vaccines against LT and cholera.

- ***Expression of CT-K63 in a Vibrio cholera attenuated strain: IEM101***

  The *V. cholera* IEM 101 strain is an attenuated EI Tor strain isolated in China. This strain does not contain the genes encoded by the entire virulence cassette. IEM 101 has been used both in rabbits and humans to study its immunogenicity and toxicity. In rabbits, IEM 101 was able to induce protection against the challenge with a virulent strain after immunization with a single dose. In humans it has been shown to be safe, able to colonize the gut and to induce a strong immune response. We have studied the ability of IEM 101 to produce, correctly assemble and secrete into the supernatant the wild-type CT as well as the CT-K63 mutant protein, and we have tested the toxicity, *in vivo*, of the recombinant strains. The results showed that IEM 101 was able to produce and secrete into the supernatant, the wild-type toxin, as well as the mutant toxin. The results of the toxicity *in vivo*, in a Rabbit ILeal Loop assay showed that IEM 101 expressing wild-type CT was able to induce fluid accumulation, while IEM 101 expressing CT-K63, did not. The insertion of the mutated gene into the chromosome of IEM 101 is underway.

- ***The interleukin-1β peptide***

  The nonapeptide sequence VQGEESNDK, corresponding to the aminoacids 163-171 of IL-1β, and the pentapeptide GEESN, has been reported to retain the immunoenhancing properties and to be devoid of proinflammatory activity of the entire IL-1β molecule. To increase the ability of *Salmonella* strains to induce immunity against recombinant antigens, we have engineered the strain to express this peptide, derived from IL-1□, using as carrier proteins the flagellin of *Salmonella*, LamB and MalE of *E. coli*. The level of expression of the recombinant proteins and the immunogenicity induced by the recombinant strains, or by the purified protein, has been evaluated. The results showed that all the recombinant proteins maintained their functionality and that the amount of each of the native proteins produced were comparable to that of the corresponding chimera proteins. The recombinant strains were used to immunize (i.p.) BALB/c mice. The results showed that the presence of the nonapeptide in the flagellen expressed by *Salmonella* led to an increase in immunogenicity of about 2.5-fold, confirming previous data obtained with the purified protein. In the case of LamB, the immune response induced in mice immunized with *Salmonella* strains carrying the recombinant LamB gene was similar to that induced in mice immunized with the strain carrying the native LamB gene. In the case of malE, the presence of the nonapeptide had a weak immunadjuvant effect, which could only be detected using low doses of antigen, and a low-responder strain of mice. With higher doses or after a booster, and with a high-responder mouse strain, no difference could be seen.

- ***Expression of LT-K63 in attenuated Salmonella typhimurium straius***

  Four attenuated *S. typhimurium* strains of different serotypes, three of them carrying the virulence plasmid and one which does not contain the virulence plasmid, have been used for the expression of the LT-K63 mutant protein. The gene coding for LT-K63 has been cloned in different plasmids with low, moderate, medium and high copy numbers. The correlation between the copy number of the different plasmids combined with the presence of the virulence plasmid, and the *in vitro* expression level of LT-K63 has been evaluated. The recombinant strains have been used for oral immunization in mice and the immunoresponse induced has been assessed. The results showed that the different *Salmonella* strains were able to produce, assemble and secrete the mutant protein into the periplasm. The *in vitro* expression level of LT-K63 showed a good correlation with the copy number of the different plasmids used, so that the amount of LT-K63 produced was higher when the high copy number plasmid was used. The results of the immunogenicity experiments showed that after a single oral immunization, the mice immunized with the strain carrying the virulence plasmid and expressing the highest level of LT-K63 mount a high anti-LT IgG

response in the sera, as well as an IgA response in the mucosa, starting from the second week after immunization.

Partners

ISTITUTO RICERCHE IMMUNOBIOLOGICHE SIENA
Via Fiorentina 1
I-53100 Siena
Italy
R. Rappuoli
Tel.: +39-57-729.33.73

UNIVERSIDADE DE SAO PAULO
Av. Prof. Lineu Prestes 1374
005508-900 Sao Paulo
Brazil
S. Newton
Tel.: +551-1-818.74.08

INSTITUT PASTEUR
Dept. Parasitologie Biomédicale
Rue du Dr. Roux 25
F-75724 Paris 15
France
M. Hofnung
Tel.: +33-1-45.68.88.30

Contract number: TS3*CT930259

Period: February 1994 to January 1997

EPIDEMIOLOGICAL, CLINICAL AND SERO-VIROLOGICAL STUDIES OF HEPATITIS C IN GABON AND BRAZIL

Co-ordinator: Institut de Médecine et d'Epidémiologie Africaines/INSERM U13, Paris, France (Bernard Larouzé)

Objectives

- Evaluate the importance of hepatitis C virus (HCV) in terms of public health in two countries with different levels of endemicity in order to design preventive strategies.
- Collect information on the variability of HCV strains and serological patterns in order to improve diagnostic procedures and contribute to vaccine design.
- Describe the distribution of HCV infection, identify risk factors, and study transmission modes.
- Investigate relationships with chronic liver diseases and hepatocellular carcinoma.
- Compare the structures of HCV strains circulating in these countries and related serological patterns
- Investigate serological cross-reactions with related viruses.

Activities

In order to evaluate the importance of hepatitis C virus (HCV) in terms of public health in two countries with different levels of endemicity (Gabon: high level; Brazil: low level), we studied, in each country, the distribution of hepatitis C infection in different geographical settings in general and high-risk populations. Additional studies will be dedicated to risk factors in a community-based study in Gabon where the prevalence of antibody to HCV is much higher (7%) than in Brazil (1-2%). As a complement of this last study, a clinical and virological study of anti-HCV positive subjects was performed in order to evaluate the clinical impact of HCV infection. In addition, case-control studies in Gabon and Brazil allowed the determination of risk for cirrhosis and HCC attributable to HCV. From the same material, sero-virological studies of HCV will be designed using PCR techniques to compare the structures of HCV strains circulating in these countries and related serological patterns, and to investigate serological cross-reactions with related viruses. These studies provided information to elaborate prevention strategies (including HCV blood screening in blood banks), to improve serodiagnostic techniques, and to contribute to the development of future HCV vaccines.

Expected outcome

⇒ By knowing the epidemiology and clinical impact of HCV in Gabon and Brazil, and after the interpretation of the sero-virological studies, much will be gained in terms of introduction of mandatory testing in blood banks and immunoprophylaxis when a vaccine becomes available. These studies will provide a basis for decision-making by health authorities from these countries and from countries with similar HCV patterns. Insight into HCV prevention would complement the effort developed in Africa and in Brazil (programme developed by the Federal Government) to control HCV infection.

⇒ The sero-virological studies will allow the design of serological tests adapted to the regional variability of HCV strains and taking eventually into account cross-reactivities with related viral agents? These tests will be used for epidemiological investigations, clinical diagnosis and, if the health authorities decide to screen blood donors, will be used for this purpose. In the long run, results of the sero-virological studies will contribute to the design of anti-HCV vaccines. The implementation of this protocol will be based on a scientific network, which will reinforce links between participants and improve their scientific skills.

Partners

INSTITUT DE MEDECINE ET D'EPIDEMIOLOGIE AFRICAINES /INSERM U 13
Hôpital Bichat
46 rue Henri Huchard
F-75019 Paris
France

Bernard Larouzé
Tel.: +33-1-40 36 37 51
Fax: +33-1-40 36 16 99

UNIVERSITA DEGLI STUDI DI MILANO
Istituto di Medicina Interna
Via Pace 9
I-20122 Milano
Italy

M. Colombo
Tel.: +39-02-55 03 54 31

INSERM U.370
CHU Necker
Rue de Vaugirard 156
F-75730 Paris cedex 15
France

C. Brechot
Tel.: +33-1-40 61 56 41
Fax: +33-1-40 61 55 81

INNOGENETICS N.V.
Viral Diseases Dept.
Industriepark Zwijnaarde 7/4
B-9052 Gent
Belgium

G. Maertens
Tel.: +32-9-241 07 11
Fax: +32-9-241 09 07
E-mail: geertmae@mail.com

ARISTOTELIAN UNIVERSITY OF THESSALONIKI
School of Medicine
Dept. of Microbiology
GR-54006 Thessaloniki
Greece

A. Antoniadis
Tel.: +31-99 13 46
Fax: +31-20 03 92

ESCOLA PAULISTA DE MEDICINA
Disc. Doenças Infecciosas
Rua Napoleão de Barros 715
7 Andar
04023 São Paulo
Brazil

C. Granato
Tel.: +55-11-572 63 48

UNIVERSITE DE LIBREVILLE
Faculté de Médecine
Département de Parasitologie, Mycologie et Médecine Tropicale
B.P. 794
Libreville
Gabon

J. Perret
Tel.: +241-77 24 17

Contract number: TS3*CT940263

Period: January 1994 to August 1998

ANALYSIS AND CHARACTERIZATION OF PHOSPHOFRUCTOKINASE AND PYRUVATE KINASE OF *LEISHMANIA*, POTENTIAL TARGETS FOR NEW DRUGS

Co-ordinator: Christian de Duve Institute of Cellular Pathology (ICP), Brussels, Belgium (P. Michels)

Objectives

- Study the structure and kinetics of phosphofructokinase (PFK) and pyruvate kinase (PYK) of *Leishmania*, key enzymes in the metabolism of the parasite, and determine differences with the corresponding mammalian enzymes.
- Design and synthesize selective inhibitors of the *Leishmania* enzymes, based on their differences with the mammalian enzymes

Activities

- Cloning and sequence determination of the *Leishmania* PFK and PYK genes.
- Overexpression of the *Leishmania* enzymes in bacteria (*Escherichia coli*) or yeast (*Hansenula polymorpha*).
- Purification of the recombinant enzymes.
- Kinetic analysis of the purified enzymes.
- Structure modelling of the *Leishmania* enzymes, using the X-ray coordinates of the crystal structures of homologous enzymes.
- Structure-function analysis of residues potentially important for inhibitor design by site-directed mutagenesis.
- Crystallization trials of recombinant *Leishmania* PFK and PYK.
- Synthesis of potentially selective inhibitors of *Leishmania* PFK and PYK.

Results so far

⇒ *Leishmania* PFK and PYK genes have been cloned and characterized.

⇒ *Leishmania* PFK and PYK have been overexpressed in *Escherichia coli*, purified and kinetically characterized.

⇒ Well-diffracting crystals of *Leishmania* PYK have been obtained and are being used for resolution of the enzyme's three-dimensional structure.

⇒ Fructose analogues have been synthesized that inhibit *Leishmania* PFK.

Follow-up

- Resolution of the three-dimensional structure of *Leishmania* PFK and PYK.
- Design and synthesis of highly selective and potent inhibitors of the *Leishmania* enzymes.

- Use of inhibitors selective for *Leishmania* PFK and PYK for the development of compounds with antiparasitic activity.

Selected publications

Michels et al. 1997. The glycosomal ATP-dependent phosphofructokinase of *Trypanosoma brucei* must have evolved from an ancestral pyrophosphate-dependent enzyme. Eur. J. Biochem., **250**, 698-704.

Ernest et al., 1998, Protein Expression and Purification, in press.

Partners

CHRISTIAN DE DUVE INSTITUTE OF CELLULAR PATHOLOGY
Research Unit for Tropical Diseases
Avenue Hippocrate 74
B-1200 Brussels
Belgium

P. Michels
Tel : +32-2-764.74.63
Fax : +32-2-762.68.53
E-mail : michels@trop.ucl.ac.be

UNIVERSITY OF EDINBURGH
Department of Biochemistry
George Square
GB-Edinburgh EH8 9XD
United Kingdom

L. Gilmore
Tel : +44-131-650.37.28
Fax : +44-131-650.37.11
E-mail : lag@holyrood.ed.ac.uk

UNIVERSIDAD CENTRAL DE VENEZUELA
Instituto de Biología Experimental
Grupo de Genética Molecular
Apartado Postal 47525
YV-1041 A Caracas
Venezuela

J. Ramírez
Tel : +58-2-751.05.44
Fax : +58-2-753.58.97
E-mail : jramirez@neblina.reacciun.ve

UNIVERSITE PAUL SABATIER
Groupe de Chimie Organique et Biologique
Route de Narbonne 118
F-31062 Toulouse Cedex
France

M. Willson
Tel : +33-561-55.68.07
Fax : +33-561-25.17.33
E-mail : willson@iris.ups-tlse.fr

Contract number: TS3*CT940266

Period: June 1993 to May 1996

CHARACTERIZATION OF THE IMMUNE RESPONSE AGAINST *TRYPANOSOMA CRUZI* ANTIGENS (GP 50/55 AND URINARY ANTIGEN) INVOLVED IN IMMUNOPATHOLOGY AND THEIR POTENTIAL USE IN DIAGNOSTICS

Co-ordinator: Universidad Autónoma de Madrid, Madrid, Spain (M. Fresno)

Objectives

- Improve the understanding of the immune responses involved in protection and pathology in Chagas' disease in order to help control it.
- Characterize two antigens that may be involved in pathology and also may be good candidates for diagnostic.
- Study the cellular immune response against both antigens.
- Study the potential use of these two antigens as diagnostic tools.

Activities

* Functional and biochemical characterization of the *T. cruzi* antigen GP 50/55 which shares an epitope with a lymphocyte activation antigen and induces crossreactive antibodies in Chagasic patients which suppress lymphoid activity.
* Functional and biochemical characterization of a *T. cruzi* 80 kDa urinary antigen (UA).
* Cloning of the genes coding for the GP 50/55 and the UA proteins.
* Testing the reactivity of chagasic sera from patients with different clinical status, with purified natural or recombinant GP 50/55 and the UA proteins.
* Testing the reactivity of chagasic sera from patients with different clinical status, with purified natural or recombinant GP 50/55 and its possible relationship to differential diagnostic.
* Studying the cellular immune response to *T. cruzi* and the role *in vivo* and *in vitro* of several cytokines. A special interest will be devoted to the study of the humoral and cellular response against the GP 50/55 protein and its role in pathology.
* Developing simple and highly sensitive methods for detection of parasite circulating antigens in urine to improve Chagas' disease diagnosis and follow-up of treated patients.

Results

⇒ We have investigated the biochemical and functional properties of *T. cruzi* GP50/55, a glycosyl-phosphatidylinositol (GPI)-anchored membrane antigen. Some of the properties (e.g. molecular mass, susceptibility to degradation) were reminiscent of those displayed by the *T. cruzi* lysosomal cysteine proteinase (GP57/51). A 50-52 kDa proteinase activity, specifically inhibited by thiol protease inhibitors, was immunoprecipitated with monoclonal antibodies (mAb) to GP50/55 (mAb C10), migrating slightly faster than the enzyme precipitated by mAbs to GP57/51. Moreover, the GP50/55 antigen detected by mAb C10 is expressed in the parasite membrane whereas the GP57/51 is not. The protein GP50/55 has been purified to homogeneity. We have found that the cystein protease activity copurifies

with the GP50/55 protein (defined by reactivity with our monoclonal antibodies). However, the cysteine protease may be in fact a protein very tightly bound to GP50/55.

⇒ This has led to the identification of a mucin-like protein complex of 30,40 and 50kDa (AgC10) as the one recognized by mAb C10. The aminoacid composition and the structure of sugar chains have been elucidated. The epitope recognized by Mab C10 has been defined as well.

⇒ This purified protein is able to suppress the immune response against *T. cruzi* and selectively alters the production of tumor necrosis factor (TNF) but not interlukin - 1 (IL-1) by macrophages. These strategies may contribute significantly to the survival of the parasite.

⇒ On the other hand, the amino acid sequence of the N-terminal portion of an 80-kDaTrypanosoma cruzi urinary antigen (UAg) affinity - purified from the urine of infected dogs showed high degree of homology with human and dog transferrins. Whereas polycolonal antibodies were unable to discriminate between the parasite antigen and transferrin, some MAbs specifically and selectively recognized an 80kDa UAg but not host transferrin, and also reacted against a *T. cruzi* lysate.

⇒ Immunoprecipitation analysis showed that UAg specific antibodies bind to several trypanosome antigens including an 80 kDa polypeptide co-migrating with UAg. This UAg is a form of the host transferrin taken up and modified by the parasite. The nature of this modification is under investigation.

⇒ In agreement with those results it was not a surprise that we were unable to isolate the cDNA clone for a transferrin related UAg, by immunoscreening with polyclonal antibodies to transferrin and by PCR with degenerate oligonucleotides of conserved regions of transferrins. However, we succeeded to isolate a series of clones expressing the C-terminus portion of the tubulin protein of *T. cruzi*. This finding was also supported by immunoprecipitation experiments showing that the anti-UAg antibodies referred above (used for screening of library) were capable of recognizing tubulin. Simultaneously, we have determined the existence of a 50-55 kDa tubulin as a minor component of the purified UAg preparation, therefore secreted in the urine of infected dogs. This recombinant antigen can therefore be used in the development of urine tests for diagnosis.

⇒ We have found that 100% of all human chronic chagasic sera reacts with this AgC10 complex which underlines the importance of this antigen as a potential candidate for diagnostics. Moreover, we have found that AgC10 induces cross-reactive antibodies that react with a 70kDa protein of lymphocytes. By screening of a human T-cell cDNA library with human chagasic sera, we isolated a couple of cDNA clones. One of those human clones (Cha 9.1.2) have homology with the repetitive region of the *T. cruzi* antigen SAPA, thought to be involved in the evasion of immune response. This clone is recognized by a large percent of chagasic sera having cardiomyopathy. We have mapped to this site the reactivity of all autoantibodies in the chagasic sera.

⇒ Those results further expand our previous work, indicating that sera from chagasic patients recognize antigens present in human T and B lymphocytes. Moreover, the characterization of autoantibodies against lymphocytes may lead to the definition of a prognostic antigen for predicting the outcome of the disease.

Partners

UNIVERSIDAD AUTONOMA DE MADRID
Cons. Sup. de Investigaciones Cientificas
Centro de Biologia Molecular
Canto Blanco
Madrid
Spain

M. Fresno
Tel.: +34-91-397.84.13
Fax: +34-91-397.47.79
E-mail: MFRESNO@mvax.cbm.uam.es

GESELLSCHAFT FÜR BIOTECNOLOGISCHE GENE EXPRESSION
Mascheroeder Weg 1
D-3300Braunschweig
Germany

J. Mc Carthy

HOSPITAL DE NIÑOS
Lab. de Virologia
Gallo 1330
Buenos Aires 1425
Argentina

S. Grisntein

Contract number TS3*CT940272

Period: August 1994 to July 1997

STUDIES ON HUMORAL AND CELLULAR IMMUNE RESPONSES IN HUMANS TO PREVIOUSLY DEFINED MALARIA VACCINE CANDIDATES

Co-ordinator: Institut Pasteur, Paris, France (L. Pereira da Silva)

Objectives

Identification of B and T cell epitopes present in recombinant *P falciparum* antigens recognised by the human immune system in relation to naturally acquired protective immunity; search for human genetic factors (in particular HLA) involved in the development of immunity to malaria infection.

Activities and Results

* ***Field studies on anti-disease and anti-parasite premunition***

  Our analysis of the protective role of parasite antigens is based essentially on the comparative studies of immune responses in protected and susceptible individuals exposed to malaria infection. This is performed by a longitudinal clinical-parasitological survey with a permanent follow-up of human populations from endemic areas of Senegal and Brazil: a) inhabitants of two villages (Dielmo and N'Diop) in the holo- and hyper-endemic area in Senegal and b) the Candeias, Urupa and Porto Chuelo sites in Rondonia, Brazil (hypoendemic malaria with epidemic episodes). Previous studies from various research groups (including our own) have been done using the criteria of age to define the development of premunition. However, the field studies developed by our groups in Senegal and Brazil, in the last year show the limitation of these simplified criteria. The following recent results illustrate these limitations and make a case for a more precise definition of premunition. Our longitudinal survey allows a comparative clinical and parasitological analysis of the populations from N'Diop and Dielmo villages, which are situated only 5 km apart in the Side Saloum area of Senegal. The presence of a permanent stream running through Dielmo provides permanent breeding sites for *Anopheles* mosquitoes and an intense and perennial malaria transmission (around 200 infective bites per person per year). In N'Diop transmission occurs intensively only in the rain season (around 20 infective bites per year concentrated in the four months rainy period). The annual incidence of malaria attacks as well as parasite index differs considerably in both villages: adults from N'Diop present a higher number of malaria attacks; children under 5 years old from Dielmo present twice as many malaria attacks than children from N'Diop. However, in N'Diop children over 5 continue to present a high frequency of malaria attacks until they are 12 - 14 years old. Another interesting observation concerns the evolution of clinical immunity: it is accepted that premunition is characterised by a decrease in the number of malaria attacks. We tried to verify if this age dependent immunity corresponds also to a decrease in intensity of symptoms in immune adults. However, detailed studies of symptoms and quantified signs (temperature, sudoresis, vomiting) indicates that the only clear difference is found in the duration of the symptoms. Other observations concern the non-specificity of clinical immunity conferred by malaria parasites. In Dielmo it is observed that clinical attacks by *P malariae* are quite rare in spite of the high prevalence of

parasites of this species in the blood of children. In Rondonia, Brazil, where clear premunition was not observed in various cross-sectional surveys. Interference between *P falciparum* and *P vivax* infections is also observed. In the light of these and other observations, the immune status (level of clinical and anti-parasite immunity) is now defined individually, in relation to the evolution of the infection in the child or in the adult (asymptomatic or symptomatic; stable or unstable parasitemia) in the periods preceding and succeeding the time when samples of sera and/or cells are taken for analysis.

- ***HLA typing***

  Following previous analysis of HLA, class I antigens typing of Dielmo habitants we have performed analysis of HLA-A, B, C, DR and DQ in 116 habitants of Dielmo from the Serere ethnic groups. No statistically significant differences were observed in the frequency or distribution of the 25 different alleles identified in the Dielmo Serere in relation to the results described by other authors concerning the Mandinka groups from Senegal and Serere and the Mandinka groups of the Gambia.

- ***Immunological studies in the endemic areas***

  In the last year we have concentrated our studies in Dielmo on characterisation of the isotope specific antibody responses against total and specific *P falciparum* antigens. This was justified by previous results showing the protective antibodies are not neutralising antibodies, but are cytophilic antibodies (bind to Fc receptors of macrophages). The anti-parasite activity depending on mechanisms of ADCI and/or opsonization/phagocytosis. Serum samples from three cross-sectional surveys in the Dielmo village (145 habitants of all age groups) were used for analysis of antibody isotypes. In a first approach, total antigen of *P falciparum* was used for measuring total anti-malarial antibodies of the IgM and IgG class and of IgG sub-classes in a ELISA assay. Adults had higher levels of specific antibodies than children. With IgM, IgG2 and IgG3 accounting for the difference. Differences in antibody levels were significant for IgG1, IgG2, IgG3 and IgG4 between the lowest and the highest transmission seasons (while infective bites/person/night increased around 20 fold). No particular isotype distribution pattern could be found to be associated with any given parasitemia level. The relationship between the OD values of each isotype and the risk of clinical malaria attack (in the period following the serum sampling was tested using a Poisson regression model. Only the IgG3 OD increases were found to be associated with a significant reduced risk of malaria attack. These seroepidemiological data suggest that, whereas the total IgG specific activity is not indicative of any given level of protection against malaria, the level of IgG3 was significantly associated with the relative susceptibility to clinical malaria attacks. The analysis of antigen specific IgG3 levels is underway. Preliminary data indicate an increase in anti R45 and anti MSP-3 antibodies of the IgG3 isotope in individuals with reduced risk of malaria attacks. Studies are also in progress on the measurement of antibodies against the different fragments of the C terminal part of the MSP-1 antigens concerning the 42 Kd and 19 Kd processing of products. MSP-1isolated and characterised by the MRC laboratory. The Rondonia samples show an increase in the level of antibodies against EB200 (Pf332) and Pf72 antigens as a function of age and exposure to malaria infection. However, no correlation could be observed with any premunition. Isotype analyses are now in progress.

Partners

INSTITUT PASTEUR
Unité de Parasitologie Expérimentale
28 rue du Dr. Roux
F-75724 Paris Cedex 15
France

L. Pereira da Silva
Tel: +33-1-45.68.86.27
Fax: +33-1-40.61.31.85

INSTITUT PASTEUR DAKAR
Unité d'Immunologie
BP 220 Dakar
Senegal

J.L Sarthou - C. Roussilhon - C. Rogier - A. Dieye
Tel: +221-23.51.81
Fax: +221-23.87.72

ORSTOM DAKAR
Laboratoire de Paludologie
Dakar
Senegal

J.F. Trape
Tel: +221-32.09.62
Fax: +221-32.16.75

NATIONAL INSTITUTE FOR MEDICAL RESEARCH
Mill Hill
London
United Kingdom

A. Holder
Tel: +44-181-959.36.66
Fax: +44-181-913.85.93

UNIVERSIDADE DE SAO PAULO
Departamento de Parasitologia
Cidade Universitaria
Sao Paulo
Brazil

A.M. de Aguirra Massola
Tel: +55-11-815.07.36
Fax: +55-11-815.04.27

Contract number: TS3*CT940277

Period: August 1993 to July 1996

CONTROL OF *TAENIA SOLIUM* CYSTICERCOSIS THROUGH SPECIFIC DIAGNOSIS, SYSTEMATIC EPIDEMIOLOGY AND DEVELOPMENT OF A RECOMBINANT VACCINE

Co-ordinator: University of Edinburgh, Edinburgh, Scotland (L.J.S. Harrison)

Objectives

Taenia solium cysticercosis is responsible for serious public health problems, in addition to creating financial losses to pig producers in countries where the parasite is endemic. While control of the parasite can be achieved to some extent through improvements in public health, sanitation and pig management/husbandry practices, the development of reliable and sensitive diagnostic procedures would greatly assist control through facilitating the execution of reliable epidemiological surveys. Such surveys not only form the basis for pinpointing and evaluating control measures, but are also essential for the design of environmentally appropriate control strategies, including the introduction of a recombinant vaccine. The proposal therefore aims 1) to improve diagnosis of human and porcine cysticercosis 2) to conduct epidemiological surveys as a prelude to selecting appropriate study areas for assessing control via drug treatment and 3) select potentially protective antigens, for use in the design of a recombinant vaccine in a second phase of this project proposal. Of particular importance will be the detection of neurocysticercosis in man. Specific objectives were:

- Transfer established diagnostic procedures from Europe to Mexico, via a training programme.
- Conduct epidemiological surveys for porcine and human cysticercosis: the former in order to identify areas for control (e.g. by drugs such as praziquantel) and the latter as a guide to appropriate medical treatment.
- Clone, sequence and express metacestode excretory/secretory proteins of diagnostic potential.

Activities

* To transfer established procedures from Europe to Mexico via a training programme. A Mexican student will be trained in the use of a monoclonal antibody based antigen detection ELISA assay developed in UK. The assay will then be transported to Mexico where it will be standardised for use in the detection of human and porcine cysticercosis followed by epidemiological studies. A follow up visit by a member of the European component is then to be carried out to Mexico once the student has returned to the laboratory.
* Existing DNA probes for the differentiation of *T. solium* and *T. saginata* will be sequenced and developed into a PCR diagnostic test for use in the field.
* To conduct epidemiological studies for porcine and human *T. solium* infection. The immediate objectives of this study are to carry out a survey in pigs reared under different management systems, comparing the results obtained with the ELISA assay with the presently used meat inspection procedures and detailed tongue examinations in pigs. At the same time studies will be conducted to determine the efficiency of the assay and to determine sero-prevalence in hospital patients.

* To clone, sequence and express potentially protective oncospheral genes. Due to the known extensive cross reactions between *T. solium* and *T. saginata*, and to the hazards of working with *T. solium* oncospheres, the identified potentially protective oncospheral antigens will be cloned from (λ-Zap (Stratagene)) cDNA libraries of *T. saginata* oncospheres.
* To clone sequence and express excretory/secretory proteins of diagnostic potential. The first activity, which was already achieved, was to identified the protein antigens which were to be cloned; the second to prepare or collect suitable serum samples for use in the primary and secondary screening of the cDNA library; the third activity is to prepare a cDNA library from *T. saginata* metacestodes. Finally, any identified clones from this library and the library prepared from *T. saginata* oncospheres will be recloned into a suitable vector for more efficient expression. Clones first identified using the *T. saginata* system will be subject to secondary screening in order to identify that sub-set is also reactive with *T.solium.*

Results

⇒ ***Training***

The newly prepared HP10 monoclonal antibody reagents were titrated and standardise prior to shipment. The Mexican student trained in the conduct of the ELISA assay and lyophilised reagents were transported back to Mexico, lyophilised for use in the screening work. DNA probes were grown up and sent to Spain for sequencing and further analysis.

⇒ ***Epidemiological studies for porcine and human T. solium infection***

A collection of sera from pigs and human either *T. solium* infected or non-infected were collected in order to evaluate the sensitivity and specificity of the diagnostic ELISA assay.

A group of 293 sera from non-infected (49) and experimentally infected pigs (244) were obtained (from pigs experimentally infected and maintained in the Veterinary School in UNAM). Pigs lightly or heavily infected were bled at different times during the infection and, after the number of cysticerci were determined in a representative sample of each pig (to obtain sera from rustically bred pigs), we examined different slaughter houses near Mexico city, and identified one in Zacatepec, Morelos, which introduces a considerable amount of rustically bred pigs from the states of Puebla and Morelos (Mexico). This is of a special interest considering that this is the population exposed to the higher risk to the infection. With the support of the authorities of this abattoir, we collected 200 ml of sera for each pig and also their tongues. Tongues were maintained in formol saline and the parasite number determined by slicing the tongue to count all the cysts present. Cysticerci collected were conserved for confirmation by an immunopathologist. In addition, a panel of 32 sera from a slaughter house in which only pigs from technified farms were included were used to test serological assays, sera from rustically bred pigs from Tianguizolco, Guerrero.

A panel of 43 sera from Tianquizolco were collected. These 43 pigs were randomly selected and completely necropsied to determine the presence of cysticerci or other disease.

A collection of 112 CSF were obtained from the Institution Nacional de Neurologia y Neurocirugia, Mexico, D.F. For each patient the diagnosis was confirmed based on the clinical examination, nuclear magnetic resonance and tomography. The pathology and type of infection was also recorded. Finally we prepared a collection of human sera from a neurological institution (Instituto Nacional de Neurologia y Neurocirugia, D.F.). For this, we collected 392 sera from patients that consulted the institution for the first time. For each the sex, age, clinical diagnosis, AIDS, NMR, TC, and other pathology and infections were recorded. All the results obtained in the evaluation of the assays based on the detection of antigen HP10 and antibodies against vesicular fluid antigens indicate that both assays are appropriate to cysticercosis diagnosis in pigs maintained in technified conditions. However, both assays showed a lower specificity and sensitivity for the detection of cysticercosis in

rustically bred pigs and infected humans. Several clones have been identified and sequenced from the *T. saginata* oncospheral library including the gene encoding the principal 18kDa secreted antigen of activated oncospheres of *T. saginata*. The sequence and immunogenicity of *T. saginata* ferritin has been established. Various expression systems have been examined with a view to selecting the most promising for the larger scale expression of *T. saginata* proteins. The selected systems are now functional in the IAH Pirbright laboratory, where preliminary experiments have been initiated.

⇒ For the cloning, sequencing and expressing of excretory/secretory proteins of diagnostic potential, groups of calves were infected with *T. saginata* metacestodes in order to produce 4 week old metacestodes. These were extracted and used in the preparation of a □ZAP (Strategene) cDNA libraries on three separate occasions. Libraries are constructed according to routine procedures. Metacestodes of this age have been shown to produce diagnostically important excretory proteins. The intention is to use these protein antigens as the trapping layer in ELISA assays designed to detect anti-parasite antibody in the serum of infected cattle.

⇒ The initial extraction and preparation of the RNA was conducted at CTVM while the cDNA preparation and titration of the resultant libraries was conducted at IAH Pribright. Once the libraries were prepared they were screened with sera in Madrid.

Partners

UNIVERSITY OF EDINBURGH
CTVM
Roslin,
UK-EH25 9RG Midlothian, Scotland
United Kingdom

Leslie JS Harrison
Tel: +44-131-650.62.17
Fax: +44-131-445.50.99
E-mail: leslie.harisson@ed.ac.uk

INSTITUTO DE SALUD CARLOS III
Servicio de Parasitologia
Centro Nacional de Microbiologia
Carretera de Majadahonda-Pozuelo
Majadahonda 28220
Madrid
Spain

T. Garate
Tel: +34-1-638.00.11
Fax: +34-1-639.18.59
E-mail: tgarate@isciii.es

INSTITUTE FOR ANIMAL HEALTH
Department of Immunology
Pirbright Laboratory
Ash Road, Pirbright, Woking
UK-Surrey GU24 ONF
United Kingdom

RME Parkhouse
Tel: +44-1483-23.24.41
Fax: +44-1483-23.24.48
E-mail: chris.chrisholm@bbstc.ac.uk

UNIVERSIDAD NACIONAL DE MEXICO
Dept. of Immunology
Instituto de Investigaciones Biomedicas
A.P. 70228
04510 Mexico, D.F.
Mexico

E. Sciutto
Tel: +52-5-550.39.82
Fax: +52-5-550.00.48
E-mail: edda@sedvase1.dgsca.unam.mx

Contract number: TS3*CT940294

Period: October 1994 to September 1997

INTEGRATION MULTIDISCIPLINARY STUDY OF HUMAN FASCIOLIASIS IN THE BOLIVIAN NORTHERN ALTIPLANO

Co-ordinator: Universidad de Valencia, Valencia, Spain (S. Mas-Coma)

Objectives

Characterization of human fascioliasis in the Bolivian Northern Altiplano (in humans endemic with very high prevalence rates, very high altitude) in preparation for control measures.

Results

⇒ The endemic zone appears to be isolated between the Lake Titicaca and La Paz: about 250,000 people live in the zone, more than 2 million people in the neighbourhood, and a large livestock population is at risk;

⇒ The parasite has developed several strategies to adapt to the altitude conditions, which favour transmission.

⇒ Drinking water is an important additional mode of infection.

⇒ The transmitting snails proved to belong to only one species: the European species *Lymnaea truncatula*, imported by European settlers.

⇒ Sheep and cattle are the main reservoirs, because of their high prevalence rates and degree of intensity. Pigs and donkeys, with high prevalence rates and intensity, also represent efficient reservoir. Other potential definitive hosts present (goats, horses, llamas, alpacas, wild lagomorphs, and rodents) are not active transmission agents.

⇒ Man has proved to be a very effective and viable definitive host (human isolates show very high transmission rates at molluscan level) with very high prevalence rates (maximum prevalence rates of 68.2% in schoolchildren and 65.4% in total population according to coprological-method calculations, which can be increased by 20% according to immunological-method calculations). Very high individual infection levels (more than 1000 eggs/g faeces are common in children; sometimes up to 5.064 eggs in extreme cases), with up to 12 different protozoan species and 5 helminth species concomitantly affecting fascioliasis-infected children, including well known pathogens, like *Entamoeba histolytica*, *Cryptosporidium sp.*, *Giardia intestinalis*, *Ascaris lumbricoides*, or *Trichuris trichiura*.

⇒ Andean inhabitants have several customs related to transmission (eating aquatic or semi-aquatic vegetables by adults; swallowing or chewing of aquatic plants stems and roots by children; defecating outdoors; family tradition of breeding own livestock including sheep, cattle, pigs and donkeys; a custom of great social settledness among Aymara Indians, life in dispersal communities).

⇒ Control measures used normally against human fascioliasis are not sufficient in the Bolivian Altiplano and must be enlarged. Human fascioliasis must no longer be

considered merely as a secondary zoonotic disease but be included in the list of important human parasitic diseases.

Selected publications

Estebán J.G., Flores A., Aguirre C., Strauss W., Angles R., and Mas-Voma S. 1997. Presence of very high prevalence and intensity of infection with *fasciola hepatica* among Aymara children from the northern Bolivian Altiplano. Acta Tropica. **66**: 1-14.

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Partners

UNIVERSIDAD DE VALENCIA
Departamento Parasitología
Facultad Farmacía
Avenida Vicente Andrés Estelles s/n
E-46100 Burjassot
Spain

S. Mas-Coma
Tel.: +34-96-386 42 98
Fax: +34-96-386 47 69
E-mail: s.mas.coma@uv.es

DUBLIN CITY UNIVERSITY
Parasitology Unit
School of Biological Sciences
Glasnevin
Dublin 9
Ireland

J.P. Dalton
Tel.: +353-1-704 54 07
Fax: +353-1-704 54 12

UNIVERSITE DE PERPIGNAN
Département de Biologie Animale
Avenue de Villeneuve
F-66860 Perpignan cedex
France

J. Jourdane
Tel.: +33-4-68 66 20 50
Fax: +33-4-68 66 22 81
E-mail: jourdane@univ-perp.fr

INLASA
División de Prasitología y Mycology
Pasaje Rafaél Subicta 1889
Miraflores
La Paz
Bolivia

R. Angles Riveros
Tel.: +59-2-22 66 70
Fax: +59-2-22 82 54
E-mail: reangles@mail.entelnet.bo

Contract number: TS3*CT940296

Period: September 1994 to August 1997

GENETIC AND IMMUNOLOGICAL FACTORS IN HUMAN RESISTANCE TO *SCHISTOSOMA MANSONI*

Co-ordinator: INSERM U399, Marseille, France (A. Dessein)

Objectives

- Determine to which extent genetic factors control susceptibility to infection and to disease in subjects living in an endemic area (Brazil) and in subjects who recently migrated from a non-endemic area of *S. mansoni* (Kenya).
- Identify and characterize the mode of action of susceptibility genes.
- Evaluate two schistosome antigens as vaccine candidates.
- Develop a strong group of immunology at the Faculty of Medicine of Uberaba.
- Strengthen the expertise of the group of Salvador in the field of schistosomiasis.

Activities

* Epidemiological studies to evaluate the weight of environment and behaviours on infection and morbidity.
* Search by segregation analysis of gene(s) with a major effect on infection and morbidity.
* Mapping of these genes by linkage analysis using the microsatellite method.
* Analysis of the anti-parasite immune response of susceptible and resistant subjects to uncover an immunological deficit in genetically susceptible individuals.
* Analysis of the lymphokine production pattern of T-lymphocytes in subjects with various degree of fibrosis.
* Purification, cloning and production of the active antigen in F28 fraction. Mapping of antigenic determinants of Sm37.
* Evaluation of the cellular and antibody response of resistant and susceptible subjects to recombinant protein and to peptides.

Expected outcome

⇒ The demonstration that genetic predisposition to infection and disease accounts, to a large extent, for the heterogeneous distribution of clinical phenotypes in endemic area of *Schistosoma mansoni*.

⇒ The demonstration that such a genetic predisposition results from the action of a few genes (major genes) controlling infection and regulating Symmer's fibrosis.

⇒ The identification of immune mechanisms that play a critical role in human defences against *S. mansoni*.

Progress toward vaccine through the identification of:

⇒ The protective immune mechanisms to be stimulated by the vaccine;

⇒ The immunological deficit that must be "overcome" by the vaccine in genetically susceptible subjects;

⇒ Vaccinating antigens.

⇒ Strengthening of research capabilities of two Brazilian laboratories: This project is based on a long standing collaboration between our groups; it is grounded on observations made by us in Brazil on a major gene in human resistance to *S. mansoni* and on the immunological mechanisms that are critical to human resistance to infection.

⇒ A similar immunological approach has been taken in Kenya, interactions between the partners will allow the determination of whether the observations on the genetic control of resistance can be extended to another endemic area. This project has a major training component for young scientists.

Results

⇒ Evidence has been obtained by segregation analysis for the existence of a major gene or a major locus in the control of infection intensities.

⇒ This major gene (Sm 1) has been located on chromosome 5q31-33.

⇒ Analyses of the immune response of homozygote resistant and susceptible subjects have demonstrated differences in lymphokine production. Resistance is associated with a Th0/2 type lymphokine production and susceptibility is associated with a Th0/1 type of lymphokine production pattern.

⇒ The active molecule in F-28 has been purified and cloned. It is referred to as Sm10. Antigenic determinants have been mapped on Sm37.

⇒ Cellular and antibody response to these antigens are being evaluated in resistant and susceptible subjects.

Partners

INSERM U399
Faculté de Médecine
Dept. Immun. and Gen. of Parasite Diseases
27 bd Jean Moulin
F-13385 Marseille Cedex 5,
France

A. Dessein
Tel: +33-4-91.83.44.52-53
Fax: +33-4-91.79.60.30
E-mail: alain.dessein@medecine.univ-mrs.fr

UNIVERSIDADE DA BAHIA,
Hospital E. Santos
Lab. Immunologia
Rua Joa das Botas s-n Canela
Salvador 40000 Bahia,
Brazil

E.M. Carvalho
Tel: +55-7-12.37.73.53
Fax: +55-7-12.45.71.10

FACULDADE MEDICINA TRIANGULO MINEIRO
Dept. Tropical Medicine
Caixa Postal 118
38001 Uberaba - MG,
Brazil

A. Prata
Tel: +55-3-43.12.10.80
Fax: +55-3-43.12.66.40

UNIVERSITÉ PARIS VI, PITIÉ-SALPÉTRIÈRE
Dept. Biostatistics and Medical Informatics
91 bd de l'Hôpital
F-75013 Paris,
France

L. Abel
Tel: +33-5-45.86.56.84
Fax: +33-5-45.86.56.85
E-mail: abel@biomath.jussieu.fr

INSERM U155
Université de Paris VI
Genetic Epidemiology
Château de Longchamps
Bois de Boulogne
F-75016 Paris,
France

J. Feingold
Tel: +33-5-45.20.77.91
Fax: +33-5-46.47.95.01

KENYA MEDICAL RESEARCH INSTITUTE
Biomedical Sciences Research Centre
Mbagathi Road, P.O. Box 54840
Nairobi,
Kenya

K. Gachuhi
Tel: +254-2-72.25.41 (ext 243)
Fax: +254-2-71.51.05-72.00.30

CAMBRIDGE UNIVERSITY
Microbiology and Parasitology Division
Tennis Court Road
Cambridge CB2 1QL,
United Kingdom

A.E. Butterworth
Tel: +44-1223-33.37.41
Fax: +44-1223-35.34.92

Contract number: TS3*CT940299

IMMUNE RECOGNITION OF A NOVEL 45KDA ANTIGEN, SPECIFIC TO *MYCOBACTERIUM LEPRAE*, AND EVALUATION AS A POTENTIAL VACCINE CANDIDATE

Period: January 1995 to June 1997

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom (H.M.Dockrell)

Objectives

- Compare T cell recognition of the 45kDa antigen in leprosy patients, contacts and controls.
- Identify immunodominant epitopes in the 45kDa antigen recognised by T cells
- Investigate whether immune recognition of the 45kDa antigen identifies leprosy patients at risk of developing reactional complications

Activities

- Preparation of a panel of 45kDa reagents, including recombinant 45kDa antigen and overlapping peptides spanning its sequence.
- Establishment of a functional cellular immunology laboratory at Centro Medico, Mexico City, allowing the assessment of T cell immunity in leprosy, and training of Mexican scientists in appropriate methodology.
- Assessment of lymphocyte transformation and cytokine secretion induced by the 45kDa antigen in T cells from 59 leprosy patients, 20 leprosy contacts and 17 endemic controls in Mexico. Isolation of human T cell lines recognising the *M. leprae* 45kDa antigen from an UK BCG vaccinated donor.
- Mapping of immunodominant epitopes in the 45kDa antigen, recognised by sera from leprosy and tuberculosis patients, using synthetic peptides. Comparison of anti-45kDa antibodies in leprosy patients with or without a history of *erythema nodosum leprosum*.
- Exchange visits between scientists in London and Mexico totalling 38 men-weeks.

Results

⇒ Proliferation responses to the *M. leprae* 45kDa antigen were higher in tuberculoid leprosy patients (92.8% positive) than in lepromatous leprosy patients (60.6%); responses were also much higher in household leprosy contacts (88.2%) than in endemic controls (10%). Mexican tuberculosis patients did not show positive lymphocyte proliferation to the *M. leprae* 45kDa antigen, although positive responses were detected in UK BCG vaccinated controls.

⇒ Interferon-γ production was also induced by the 45kDa antigen (tuberculoid leprosy>lepromatous leprosy>contacts>endemic controls), and by T cell lines recognising the 45kDa antigen, isolated from an UK BCG vaccinated donor.

⇒ Antibodies to the *M. leprae* 45kDa antigen were present in sera from 71% of the leprosy sera; although none of the control sera from the same leprosy endemic area were positive, antibodies were detected in 33% of pulmonary tuberculosis sera. Epitope mapping using synthetic peptides identified both leprosy specific and cross-reactive epitopes in the 45kDa antigen which were recognised by IgG antibodies.

⇒ These results suggest that the 45kDa antigen is an immunodominant leprosy antigen, which contains both leprosy-specific and cross-reactive T and B cell epitopes.

Follow-up

Professor F. Vega-Lopez's laboratory in Mexico is now a partner in a new EU contract (ERBIC18*CT97-0235) investigating mechanisms of protective immune responses to pathogenic mycobacteria, which started in November 1997.

Publications

Dr Gabriela Jimenez Diaz. 1997. Reconocimiento del antigeno de 45kDa de *Mycobacterium leprae* por anticuerpos de pacientes con lepra. Post-graduate thesis in Dermatology. Universidad Nacional Autonoma de Mexico.

Rafael Mondragon-Gonzalez. 1998. Reconocimento immune de un nuevo antigeno de *Mycobacterium leprae* por cellulas de pacientes con lepra y controles endemicos sanos Master in Science thesis submitted to Universidad Nacional Autónoma de Mexico.

Partners

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE
Dept. of Clinical Sciences
Keppel Street
London WC1E 7HT
United Kingdom

H.M. Dockrell
Tel: +44-171-927.24.66
Fax: +44-171-637.43.14
E-mail: h.dockrell@lshtm.ac.uk

RIJKSUNIVERSITEIT LEIDEN
Dept. of Immunohaematology & Blood Bank
University Hospital
Building 1E3-Q
P.O. Box 9600
Leiden
The Netherlands

R.R.P. De Vries
Tel: +31-71-26.38.00
Fax: +31-71-21.67.51
E-mail: pdvries@ab.dlo.nl

INSTITUTO MEXICANO DEL SEGURO SOCIAL
Dept. de Dermatologia & Micologíia Medical
Ave. Cuauhtemoc 330
Colonía Doctores
México D.F.
Mexico

F. Vega-Lopez
Tel: +52-5-761.03.52
Fax: +52-5-761.03.52

Contract number: TS3*CT940303

Period: October 1994 to September 1997

CELL MEDIATED IMMUNITY TO SCHISTOSOMES: EVALUATION OF MECHANISMS OPERATING AGAINST LUNG STAGE PARASITES, WHICH MIGHT BE EXPLOITED IN A VACCINE

Co-ordinator: University of York, York, United Kingdom (R. Wilson)

Objectives

- Identify and clone the antigens mediating lung phase immunity to *Schistosoma mansoni* in mice vaccinated with irradiated cercariae.
- Use the recombinant antigens for evaluation of T cell responses in mice exposed to the irradiated vaccine, and for protection experiments.
- Examine the responses of peripheral blood lymphocytes from human patients with different clinical forms of schistosomiasis, to recombinant lung stage- antigens.
- Analyze selected lung stage antigens at the molecular level by mapping B and T cell epitopes using synthetic peptide constructs.

Activities and Results

★ ***Molecular Biology of Schistosome Antigens***

Experimental work performed during this project has sought to use several different techniques to pinpoint potential vaccine antigens. This has involved the identification of cDNAs encoding such molecules, their sequence analysis, and the subsequent production and purification of recombinant proteins in *E. coli*. A secondary aim has been to identify and subsequently characterize genes expressed uniquely by lung-stage schistosomula using the technique of RAP-PCR. We have also investigated the possibility of identifying cDNAs encoding putative transmembrane or secretory proteins from expressed sequence tags produced as part of the schistosome genome project. Serum raised in rabbits against proteins released by lung-stage schistosomula during *in vitro* culture has been used to screen both lung-stage and adult worm DNA libraries. A novel clone coding for a protein of Mr 16.4kDa (A26) has been identified, sequenced, expressed in a pET vector, and purified. A monospecific serum has been produced against the recombinant A26 protein and used to identify the "native" parasite protein by probing Western blots of soluble preparations of cercariae (SCAP), lung-stage schistosomula (SLAP) and adult worms (SWAP). Preliminary results indicate that a protein of approximately 31kDa is detected in all three preparations, but appears to be most abundant in cercariae. The same screening procedure also identified three previously described vaccine candidates, paramyosin, myosin and calpain, the last of which we are pursuing further. In addition to the clones identified by screening lung-stage and adult worm DNA libraries with sera raised against lung-stage proteins, secretory proteins from other life-cycle stages have been sought. A schistosome calcium-binding protein and a 21.7kDa antigen have been identified from a cercaria DNA library, plus a putative cytochrome C and four unknown clones. Screening of an adult worm library with antibodies directed against released proteins resulted in the isolation of another four unknown clones, plus previously sequenced *S. mansoni* HSP70, cathepsin and a known, secretory protein, LGG. A number of the unknown clones have been sequenced more extensively and

subsequently expressed in the pQE or pET vectors. As well as being vaccine candidates, such proteins may have potential use as markers of disease progression in human patients, and will enable the immunogenicity of proteins released at different life-cycle stages to be compared in various assays. The contribution of the Lille Pasteur Institute group to the project has involved the subcloning and expression of cDNAs encoding some of the proteins potentially involved in the protective immune response directed against lung schistosomula of *S. mansoni*, namely calpain, the tegumental antigens Sm22.6 and Sm21.7, and a DNA encoding an *S. mansoni* homologue of mammalian epididymal secretory protein I. Attempts to express these four cDNAs are currently underway in Lille but significant difficulties have been experienced with all of the clones. A variety of vectors are therefore being tried to circumvent the problems. In a new departure, the lung stage library constructed in York has been transferred to our partners in Belo Horizonte for screening with sera from patients in the acute phase of the disease and five clones (HL-1 to HL-5) were obtained. All five have been sequenced and expressed in either pET or pQE vectors and are now at the purification stage.

✶ ***Assessment of immunogenicity***

Now that we have developed a panel of recombinant proteins representative of molecules released by parasites at various stages throughout the life-cycle, the immunogenicity of each can be assessed. Since parasite-specific Th1 cells play a pivotal role in the effector response in once-vaccinated mice, most attention will focus on assays of T cell proliferation and cytokine production. The secretion of cytokines by cells recovered from the lungs of mice 17 days post-vaccination will be our main indicator of protein immunogenicity since it is this sub-set which is responsible for challenge parasite elimination. In particular, we shall be seeking proteins which stimulate high levels of IFN□ production. We are also developing an *in vivo* assay of antigen reactivity, by injecting recombinant proteins into the pinnae of mice previously exposed to the irradiated vaccine to measure delayed-type hypersensitivity responses. In collaboration with our Brasilian partners, we are currently testing the ability of peripheral blood mononuclear cells (PBMC) from patients with different clinical forms of schistosomiasis to proliferate *in vitro* in response to each recombinant protein. Four recombinant antigens have been tested so far; all elicited responses above background but considerably lower than those to SWAP and SEA preparations (this is not unusual with recombinants and represents an obstacle to their evaluation as vaccine candidates by direct probing of human responses). In an alternative screen, we are using an ELISA to determine total IgG responses to each recombinant of patients in the various clinical categories of schistosomiasis, as an indicator of preceding T helper cell responses.

✶ ***Human schistosomiasis in Brazil***

Field surveys of schistosomiasis in the vicinity of Belo Horizonte have continued, concentrating on the district of Sabara. Stool sampling of 1413 residents revealed an overall prevalence of 27%, with a mean egg count of 58 epg (range 5 - 633). Of these infected individuals, only 12% presented egg counts above 500 epg which might be considered a heavy infection; few showed evidence of advanced hepato-splenic disease. For this reason, we have continued our studies of human responses to *S. mansoni* in an endemic area of Northern Minas Gerais state, centered on Corrego Bernardo, using a field laboratory in the city of Governador Valadares. Both cellular and humoral responses to SLAP, SWAP, and SEA have been evaluated. *In vitro* stimulation of PBMC with SLAP leads to a significant proliferative response in patients with the different clinical forms of the schistosomiasis, except in hepatosplenic individuals. Neutralisation of cytokines in PBMC cultures reveals that the proliferative response to SLAP is differentially regulated from that to SEA and SWA, with no effect observed following the addition of antibodies to IL-4, IL-5 and IL-10. On the other hand, addition of anti-IFN□ antibodies to the PBMC cultures readily decreased the proliferative response to SLAP as it did for SWAP and SEA. Special emphasis has been given to the development of methods for the intracytoplasmic

staining for cytokines that would allow for their simultaneous identification together with the cell type secreting them, using flow cytometry. Initial intracytoplasmic staining data was obtained for IL-2, IL-4, IL-5 and IFN□ in PBMC. Patients were divided into IgE^{hi} and IgE^{lo} groups on the basis of their serum responses to SWAP. The results demonstrate that the IgE^{hi} group had a significantly higher frequency of IL-4 and IL-5 positive cells than the IgE^{lo} group. The frequency of IFN□ positive cells was the same in both groups but, whilst in the IgE^{hi} group the ratio for IFN□/IL-4 was 13, in the IgE^{lo}group it was 35. Taken together, these results correlate IL-4 directly and IFN□ inversely with serum IgE levels. It is important to note that in this study all patients were age-matched, thus no influence of age can be attributed to the observed results. Furthermore, all individuals were from the same area and to date, the differences in the two groups of patients cannot be attributed to different levels of water contact. We have also demonstrated that exposed, "normal endemic" individuals have a high IgE antibody response which is elevated only to schistosomula tegumental antigens. We have previously demonstrated that PBMC from "normal endemic" individuals secrete significant levels of IFN□ when stimulated *in vitro* with SWAP or SEA. The data obtained with the intracytoplasmic staining for cytokines demonstrated that infected patients with low levels of anti-SWAP IgE have the highest frequency of INF□ positive cells. We have postulated that the effective immune response to a *S. mansoni* infection is multifactorial and that it is site-dependent, i.e. different compartments may develop distinct effector responses to the invading parasite. In this context, it is possible that the early attrition may be mediated by IgE antibodies and the later, as the parasite migrates through the lungs, the immune effector mechanism becomes mainly IFN□-dependent. The correlation between the intracytoplasmic staining of cytokines and development of resistance to infection, or pathology is not yet adequate to determine whether there is a cause/effect relationship and whether one can distinguish between the roles of Th1 and Th2 responses in these situations. One of the major criticisms of work on human immune responses to *S. mansoni* infection has been that the studies are always performed using PBMC, which may not reflect the events in the lymphoid organs. To address this question we have compared the phenotype of cells present in the peripheral blood and in the spleens of hepatosplenic patients. We observed an increase in $CD4^*HLADR^+$, $CD5^+CD19^+$, $CD8^+HLADR^+$ and NK cells in both compartments, relative to that in normal non-infected/non-exposed controls (accident victims). These results demonstrate that analysis of the peripheral blood reflects the findings in a lymphoid organ, such as the spleen.

Partners

UNIVERSITY OF YORK
Dept. of Biology
Heslington
UK-York Y01 5DD
United Kingdom

R. Wilson
Tel: +44-1904-43.28.30
Fax: +44-1904-43.28.60
E-mail: raw3@york.ac.uk

CENTRO DE PESQUISAS RENE RACHOU
Lab. of Cellular & Molecular Immunology
Av. Augusto de Lima 1715
30100 Belo Horizonte
Brazil

R. Correa-Oliveira
Tel: +55-3-12.95.35.66
Fax: +55-3-12.95.31.15
E-mail: correa@dec001.cict.fiocruz.br

INSTITUT PASTEUR LILLE
Centre d'Immunologie & Biologie Parasitaire
Rue du Professeur A. Calmette 1
F-59019 Lille
France

A. Capron
Tel: +33-3-20.87.79.62
Fax: +33-3-20.87.78.88

Contract number: TS3*CT940305

Period: November 1994 to October 1995

HEALTHY PEACE? REHABILITATION & DEVELOPMENT OF THE HEALTH SECTOR IN POST-CONFLICT SITUATIONS

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom, (A. Zwi)

Objectives

Analyse the long-term impact of conflict on health with particular reference to disability and childhood diseases in two post-conflict societies (Ethiopia and El Salvador) and using these conditions as tracers to:

- analyse the development of health-related policies, plans and programmes aimed at rehabilitating the health system in the post-conflict period, and to identify the key factors determining policy choice and influencing implementation.
- analyse the financing of health sector rehabilitation and development in the post-conflict period and assess existing strategies with respect to their sustainability and equity.
- analyse the role of international aid in post-conflict rehabilitation and development of the health sector, and to assess the long-term implications of alternative patterns of international assistance.

Activities

This project represented the first phase of a planned 3-year project, and lasts for a period of 1 year. During this period a situation analysis were prepared in both countries comprising three components:

- Health needs in post-conflict situations:
  This component of the study will assess the impact of conflict on health status with particular reference to disability and immunisable diseases, and will analyse trends in patterns of morbidity. The emphasis will be on collection and comparative analysis of existing secondary data. In addition, rapid and participatory methods of health needs assessment will be used to build health profiles in at least two districts, which have been differently affected by war.
- Health policy analysis:
  This component of the study will provide an historical analysis of the development of the health system in the two countries. Particular attention will be focused on the impact of conflict on the health policy environment during the periods of conflict, and its implications for the functioning of the health system. The focus for this aspect of the study will be on events since peace has been secured, and on the role of international agencies in the design and implementation of rehabilitation programmes.
- Health financing components:
  This component of the study will provide a comprehensive review of changes in the health financing system during and immediately after the wars. It will provide the basis for an assessment of the key financing issues facing the health sector in both countries, with particular respect to the future role of international aid.

Expected outcomes

⇒ Reports and publications documenting the research findings were drafted and circulated widely as a contribution to current international debates concerning post-conflict recovery.

⇒ It is anticipated that more detailed research proposals will be prepared to follow up on specific issues in the future.

Partners

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Dept. of Public Health and Policy
Keppel Street
London
United Kingdom
A. Zwi
Tel: +44-171-927.24.04

ESCUELA ANDALUZA DE SALUD PUBLICA
Apartado de Correos 2070
Granada
Spain
A. Ugalde Blasco
Tel: +34-5-827.50.44

UNIVERSIDAD CENTROAMERICANA JOSE SIMEON CANAS
Dept. de Salud Pública
Autopista sur 01 168, Jardines de Guad
Apartado (01) 168
San Salvador
El Salvador
E.A. Selva-Sutter
Tel: +50-5-73.44.00 ext 119

UNIVERSITY OF ADDIS ABEBA
P.O. Box 1176
Sidist Kilo Campus
Addis Ababa
Ethiopia
A.B. Kello
Tel: +25-1-112.32.30

Contract number: TS3*CT940319

Period: January 1995 to December 1997

DISSECTION OF THE MECHANISMS LEADING TO THE SELECTIVE TRIGGERING OF PROTECTIVE AND NON PROTECTIVE MURINE T-CELL RESPONSES FOLLOWING INFECTION WITH *LEISHMANIA*: RELEVANCE FOR THE INDUCTION AND DETECTION OF HUMAN PROTECTIVE IMMUNITY

Co-ordinator: Institut Pasteur, Paris, France (G. Milon)

Objectives

- Define the early priming conditions that may commit naive/virgin T-cell populations within lymphoid organs draining *Leishmania*-loaded sites to produce a given set of cytokines upon subsequent reactivation in the non lymphoid parasite-loaded tissues such as the dermis. The terms of priming conditions refer to different cellular components: mast cells, mononuclear phagocytes, dendritic leucocytes (mast cells, NK leucocytes, neutrophils, eosinophils, either infected or loaded with parasite extracts as sources of/or exposed to priming cytokines such as IL4, IL12, IFNγ, INFα later named "polarizing cytokines".
- Specify the relative contribution of the different cells (a) able to process and present parasite-derived peptides to naive T-cells, i.e. dissect the roles of infected mononuclear phagocytes versus dendritic leucocytes, and (b) able to release a given set of polarizing cytokines. If a peculiar combination of cytokines and antigen-presenting cells is recognized as critical, a logical consequence is to ask whether such priming conditions will be rapidly set in motion *in vivo*, in both the skin site of infection and draining lymph nodes depending upon the genetically dependent ability of mice to control (resistance) or not (susceptibility) the parasitic/pathogenic processes initiated by *Leishmania spp. (L. major, L. brazillensis, L. panamensis)*.
- Define more relevant correlates of a protective versus non protective immune response within a very well studied polymorphic human population exposed to *Leishmania braziliensis, L. panamensis* while extending the training of scientists in the domain of endemic human leishmaniasis/asymptomatic parasitic processes.

Activities

- Pursue *in vitro* studies using naive/virgin and experienced mouse or human T-cells to specify the priming/reactivation conditions that determine whether T cells will produce IL4, IL10, IL13 or interferon γ (IFNγ) upon subsequent rechallenge, namely cytokines which deactivate mononuclear phagocytes rendering them permissive to *Leishmania spp.* growth (IL4, IL10, IL13) or which activate mononuclear phagocytes rendering them no more permissive to *Leishmania spp.* growth (IFNγ).
- Pursue *in vivo* studies in conditions allowing access to both the *Leishmania*-loaded dermis and draining lymph nodes: (ears of mice are optimal sites to inject *Leishmania spp.* and from which to recover all the cells presently thought to be directly or indirectly critical for T cell priming, polarization and for T cell reactivation namely in addition to dendritic

leucocytes, mononuclear phagocytic leucocytes and Natural Killer cells, mast cells, neutrophils, eosinophils, keratinocytes).

Expected outcome

A better understanding of the T-cell priming and reactivation conditions set in motion within clinically/epidemiologically defined susceptible and resistant human populations exposed to *Leishmania spp.* The Brazilian and Colombian teams have studied for several years areas where humans are continuously exposed to *Leishmania.* These areas are unique places to study the influence of T-cell responses on outcome of the parasitic process toward either and asymptomatic process or a disease of variable severity.

Partners

INSTITUT PASTEUR, PARIS
Unité d'immunophysiologie cellulaire
Rue du Docteur Roux, 25
Paris Cedex 15
France

G. Milon
Tel: +33-14-568.86.69
Fax: +33-14-061.31.69
E-mail: gmilon@pasteur.fr

FUNDACAO OSWALDO CRUZ
Lab. of Cellular & humoral immunity in protozooses
Avenida Brazil 4365
Manguinhos
Rio de Janeiro-RJ
Brazil

S.G. Coutinho
Tel: +55-21-280.15.89
Fax: +55-21-590.35.45

CIDEIM
Fundacion Centro Internacional de Entrenemiento E Investigaciones Medicas
Avenida 1 Norte 3-03
5390 Cali
Colombia

N. Saravia
Tel: +57-2-361.49.31
Fax: +57-2-667.29.89
E-mail: cideim@cali.cetcol.net.co

UNIVERSITE DE LAUSANNE
Institute of Biochemistry
Chemin des Boveresses, 155
Lausanne
Switzerland

J. Louis
Tel: +41-21-692.57.03
Fax: +41-21-692.57.05

Contract number: TS3*CT940321

Period: November 1994 to October 1997

DEVELOPMENT OF SILOS MANAGERIAL SKILLS IN BRAZIL: RESEARCH AND IMPLEMENTATION OF SUITABLE TOOLS FOR INTER-SECTORAL AND PARTICIPATIVE ACTIONS IN DEALING WITH MAIN LOCAL HEALTH PROBLEMS

Co-ordinator: Università Commerciale L. Bocconi, Milano, Italy (M. Meneguzzo)

Objectives

- Develop an innovative model for managing local health units to support strategies directed at decentralising health services in developing countries.
- Specific attention will be given to:
  - developing the institutional framework for local health units;
  - planning and programming processes at local level;
  - resource allocation;
  - relationship between decentralisation strategies and utilisation of health care.
- Evaluate the role of decentralisation and institutional strengthening as a tool for solving the general crisis in public welfare in Brazil and in the two European countries involved in the project, Italy and Spain.
- Set out training programs for public managers.

Activities

- Analysis, comparison and systematisation of SILOS (Local Health Systems) managerial skills development, methodologies and tools in each specific national context; identification of a methodological approach for the three countries.
- Analysis and comparison between the different decentralisation experiences in the National Health Systems of Brazil, Italy and Spain, in particularly selection of planning, management and controls tools on which experimentation must be concentrated; choice of SILOS for research activity; introduction of research in local process, and definition of project task forces and working laboratories in SILOS selected by the participating centres. Three different interventions will be implemented:
  - Training and education of human resources directly charged with management responsibility on a SILOS level.
  - Consulting, aimed at accompanying, analysing and systematising the decision-making responsibility on a SILOS level.
  - Evaluation of the research projects in their specific context.
- Evaluation of the research project in the context of the National Public Health Care Systems in transition decentralisation processes, and settlement of a proposal regarding the reproduction of the experience on a large scale, in different contexts, particularly in Latin American Countries.

Expected outcome

⇒ Improvement of managerial effectiveness for the SILOS which are involved in the research in terms of capacity of intersectoral response to community health problems and quality of health and social well-being. Strengthening of managerial methodologies and tools and requirements to their effective implementation at a local level.

⇒ Preparation of a guideline regarding the evaluation of managerial skills on a SILOS level in order to allow for comparison between the different national contexts.

⇒ Definition of methodological options which allow for transformation of local experiences in health policy for National or Mixed Health Systems.

UNIVERSITA COMMERCIALE L. BOCCONI
Centro. di Ricerche Gestione Ass. Sanitaria
Via Bocconi 8
Milano
Italy

M. Meneguzzo
Tel: +25-8-36.62.03

UNIVERSIDADE DE SAO PAULO
Av. Dr. Arnaldo 715
Sao Paulo
Brazil

C. Vieira de Sousa Unglert
Tel: +551-1-881.24.51

UNIVERSIDADE DA BAHIA
Faculty of Medicine
Rue Padre Feijo 29
4 Andar C.H.R.
Salvador - Bahia
Brazil

C. Fontes Teixeira
Tel: +557-1-245.01.51

UNIVERSIDAD DE BARCELONA
Dept. CC Polit. Idret Public
Edifici B
Bellaterra
Spain

J. Subirats Humet
Tel: +34-3-581.60.54

Contract number: TS3*CT940343

Period: January 1995 to December 1997

BIOTECHNOLOGICAL APPROACHES TO THE TOTAL UTILISATION OF CRUSTACEAN SHELLFISH AND SHELLFISH WASTE

Co-ordinator: University of Nottingham, Nottingham, United Kingdom (John Peberdy)

Objectives

- Enhance the processing of crustacean shellfish, especially krill and shrimps, to improve the recovery of tail meat.
- Produce added value products from krill tail meat through the development of novel processing methods.
- Recover added value products from peeling and wash water waste and from "head" meat waste.
- Exploit shell waste as a source of chitin and chitosan, and a substrate for solid state microbial fermentation.

Activities

- Development of technologies for handling and processing shellfish.
- Changes to freezing procedures monitored in the context of modifications to muscle protein.
- Protocols established for the recovery of fatty acids, pigment and proteins from waste water; constituents purified/tested for use as emulsifying and foaming agents and food colorants; enzymes from head tissue investigated for biomedical uses.
- Micro-organisms with the capability of using the chitinous shell waste were isolated/identified for their chitinase and chitin deacetylase activities and the most active screened to facilitate utilisation of chitinous shell waste in solid state fermentation.
- Procedures for recovering chitin from shell waste were developed to exploit enzymes derived from existing and novel microbial strains.

Outcomes

- ⇒ A fast pre-chilling procedure has been developed for shrimp, krill and langostinos to increase the storage period to 8 days.
- ⇒ Improved procedures for shrimp peeling have increased yield.
- ⇒ Offal and shell waste, which comprised 70-80% of the catch, is now being utilized for fish/animal feed and for inclusion in snacks.
- ⇒ A canning procedure has been developed to use Antarctic krill as human food.
- ⇒ A range of processing methods, based on minced tail meat, have been cultivated to present opportunities for the development of novel food products.
- ⇒ Due to the low functionality of krill muscle the addition of binders and co-enhancers was deemed to be of major significance.
- ⇒ Development of alternative processing procedures has facilitated the recovery of commercially valuable products from wash/defrosting waters.
- ⇒ A variety of proteins, amino acids and lipids have been identified for use in processing.

⇒ An ultrafiltration system for the recovery of proteins from wash waters has been developed.
⇒ A range of chitinolytic micoorganisms have been isolated and characterised to facilite production of chitinase enzymes.
⇒ Preliminary work suggests the components of these systems can be purified and the enzymes have potential for the modification of waste.
⇒ A range of bacteria has been isolated which produce the deacetylase enzyme.
⇒ The shell waste can be exploited for use as a substrate for microbial fermentation leading to its use in developing countries to produce value added products.
⇒ Microbial cultures have been used for demineralisation and hydrolysis of chitin-protein-complex of prawn shell waste.
⇒ A biotechnological approach to waste shell utilisation as been demonstrated and waste minimisation has been achieved.
⇒ In Chile the chemical procedure for chitin extraction from krill shell has been optimised and the technology transferred to a company in Ecuador. In the UK joint industrial and government funding and been secured for a pilot operation for a biotechnological approach to waste processing.

Partners

UNIVERSITY OF NOTTINGHAM
Dept. of Life Science
University Park
GB-NG7 2RD Nottingham
United Kingdom

John Peberdy
Tel.: +44-115-951 32 31
Fax: +44-115-951 32 31

UNIVERSITY OF THE PHILIPPINES AT LOS BANOS
National Institute of Biotechnology & Applied Microbiology
4031 Los Banos
The Philippines

Ernesto J. del Rosario
Tel.: +63-94-27 21 / 2
Fax: +63-94-27 21

PESQUERA FRANCIS DRAKE S.A.
Casilla 56 V
Ruta 68 Km 104
Valparaiso
Chile

Poul Hansen
Tel.: +56-32-29 11 69
Fax: +56-32-29 11 79

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Instituto del Frío
Ciudad Universitaria s/n
E-28040 Madrid
Spain

Pilar Montero
Tel.: +34-91-549 23 00
Fax: +34-1-549 36 27

QUEEN'S UNIVERSITY OF BELFAST
Dept. of Chemical Engineering
David Keir Building
Strandmillis Road
GB-BT9 5AG Belfast
United Kingdom

Michael Healy
Tel.: +44-1232-24 51 33
Fax: +44-1232-38 17 53

UNIVERSIDAD DE SANTIAGO DE CHILE
Centro de Estudios en Ciencia y Tecnología de Alimentos
Avda Libertador O'Higgins 3363
Santiago
Chile

Claudio Romo
Tel.: +56-2-681 63 64
Fax: +56-2-681 63 60

Contract number: TS3*CT940345

Period: January 1995 to December 1997

ASSESSMENT OF IMMUNE RESPONSES INDUCED IN PRIMATES IMMUNIZED WITH LIPOPEPTIDES DERIVED FROM *P.FALCIPARUM* MPES ANTIGENS

Co-ordinator: Institut Pasteur, Paris, France (P. Druilhe)

Objectives

Construct single and multiple epitope peptides containing a lipid moiety using the sequences already identified in LSA1, LSA3, SALSA and STARP molecules, and analyze the immune response induced in primates by such peptides.

Activities

Syntheses of novel peptides and lipopeptides were performed, particularly with the antigens SALSA, STARP, and mostly LSA3. Immunization studies were carried on in mice of various haplotypes, *Aotus trivirgatus* and chimpanzees in BPRC (The Netherlands) and in CIRMF (Gabon). These formulations were used either alone, injected in PBS, or with various adjuvants, or in schemes of administration including either a recombinant first followed by a lipopeptide, or a lipopeptide first followed by recombinant or naked-DNA immunization. The study of immune responses induced in this manner was extremely detailed, including classical antibody, Th, CTL assays, and novel assays such as a Class-I restricted IFN-g Elispot responses, and homing of lymphocytes in the liver and in-situ analysis of parasitological and cellular events following challenge. A lipo-Mixo-lipopeptide was synthesized so as to represent all existing and possible degeneracies within the Starp repeat sequence.

Results

The main result is that full, sterile protection could be achieved by immunization with simple, non-toxic, very well tolerated lipopeptide formulations injected in saline in chimpanzees, i.e. without any adjuvant. The protection achieved in this manner proved to be reproducible on successive challenges, including massive ones of up to 10 million *P. falciparum* sporozoites. Results confirm the remarkable potential of this means of immunization and suggest that clinical experiments can be performed with them, particularly in view of parallel experiments performed with similar lipopeptide formulation made with antigens derived from Hepatitis B and HIV viruses in humans. Indeed, addition of a palmitoyl chain can dramatically increase T-helper cell responses in a wide range of MHC-Class II haplotypes, and induces B, Th and CTL responses. Large lipopeptides can be endogeneously processed and associated with Class-I. Lipopeptides are safe, perfectly tolerated, and highly immunogenic in chimpanzees, whose immune system is the closest to human one. Moreover, they induce responses that are protective. Improved antigenicity and immunogenicity were obtained through theStarp Mixotope-peptide that consists of a convergent combinatorial library of peptides, or mixotope, obtained in a single synthesis by introducing sequence degeneration, as compared to the consensus sequence peptide inducing sequence degeneration. In view of the proper internalization of the lipid-tailed peptide by cell membranes, we attempted mucosal immunization with the same formulations. These proved also highly effective and sometimes

induced even greater responses than when the same peptide was injected by subcutaneous route. This mode of systemic immunization is of interest for vaccine delivery in tropical countries as it requires neither any syringe nor trained personnel.

Selected publications

Aidoo M., Lalvani A., Allsopp C.E.M., Plebanski M., Meisner S., Krausa P., Browning M., Morris-Jones S., Gotch F., Fidock D.A. Takiguchi M., Robson J.H., Greenwood B.M., Druilhe P., Whittle H.C., and Hill A.V.S. 1995. Identification of conserved antigenic components for a cytotoxic T-lymphocyte-inducing vaccine against malaria. Lancet., , 345:1003-07

BenMohamed, L., Gras-Masse, H., Tartar A., Daubersies P., Brahimi K., Thomas A., and Druilhe P. 1997. A lipopeptide-based, adjuvant-free, malaria vaccine induces potent and long-lasting B, Th and CTL responses in mice and chimpanzee. Eur. J. Immunol., **27**, 1242-1253.

Pasquetto V., Fidock D.A., Gras H., Badell E., Ballou W.R., Eling W.,Tartar A., and Druilhe P. 1997. *Plasmodium falciparum* sporozoite invasion is inhibited by naturally-acquired or experimentally-induced polyclonal antibodies to the STARP antigen. Eur. J. Immunol. **27**, 2502-2513

Perlaza B.L., Herrera S., Tartar A., Druilhe. P. B- and T-cell responses produced in Aotus by lipopeptides and peptides from the malaria antigens LSA1, SALSA, STARP and LSA3. Infec. Immunity, (in press).

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Aidoo M., Lalvani A., Gilbert S.C., Whittle H.C., Daubersies P., Druilhe P., Hill A.V.S. CTL epitopes for HLA-B53 and other HLA types in the malaria vaccine candidate liver-stage antigen 3. Eur. J. Immunol.(submitted).

Partners

INSTITUT PASTEUR
Biomedical parasitology
28 rue du Docteur Roux
F-75015 Paris
France

Pierre Druilhe
Tel.: +33-1-45 68 85 78
Fax: +33-1-45 68 86 40
E-mail: druilhe@pasteur.fr

UNIVERSIDAD DEL VALLE
Fundación Centro de Primates
AA 25360
Cali
Colombia

Socrates Herrera
Tel.: +57-23-56 56 21
Fax: +57-23-58 10 61
E-mail: soheva@mafalda.univalle.edu.co

KATHOLIEKE UNIVERSITEIT NIJMEGEN
Subfaculty of Biology
Dept. of Molecular Biology
Toernooiveld 1
NL-6525 ED Nijmegen
The Netherlands

G.J. Wullems
Tel.: +31-80-61 61 61
Fax: +31-80-55 34 50

INTERNATIONAL CENTRE FOR MEDICAL RESEARCH
Dept. of Immuno-Parasitology
Franceville
Gabon

P. Millet

UNIVERSITY OF OXFORD
Institute of Molecular Medicine
Dept. of Molecular Immunology
John Radcliffe Hospital
Oxford
United Kingdom

Adrian Hill
E-mail: a.hill@well.ox.ac.uk

Contract number: TS3*CT940346

Period: August 1992 to July 1994

MALARIA PRE-ERYTHROCYTIC STAGES (MPES) EUROPEAN NETWORK ANTIGENS TARGET OF IMMUNE RESPONSES CAPABLE OF *INHIBITING P. FALCIPARUM* PRE-ERYTHROCYTIC DEVELOPMENT

Co-ordinator: Institut Pasteur, Paris, France (P. Druilhe)

Objectives

- Development of the immunology of MPES with the aim of developing an effective MPES vaccine.
- Acquisition of an improved knowledge of the biology of MPES.
- Improved co-ordination and exchanges within and between European and Developing Country teams.

Activities

- Molecular biology studies of pre-erythrocytic antigens from mostly *P. falciparum* and *P. berghei,* and in part, *P. reichenowi* (identification, characterisation, and production of genes and antigens: LSA1 (a major 200 kDa molecule expressed in liver stages), SALSA (a 70 kDa antigen shared between sporozoite and liver stages), LSA3-729 (a pre-erythocytic-stage-specific molecule expressed in sporozoites and liver stages, DG21 (a sporozoite-specific 78 kDa molecule). Antigenic features of these molecules, conservation of epitopes amongst isolates, epitope mapping; immunogenicity in animals, characterization and prevalence of immune responses in humans and in animals, (mice and primates), identification amongst the remaining series of cloned pre-erythrocytic-stage molecules of those that deserve further detailed studies.
- Improvement of the reproducibility of liver infections in Aotus monkeys. Immunization and sporozoite challenges of chimpanzees and Aotus with P. *falciparum* antigens of mice and thamnomys, with *P. berghei*, *P. yoelii* antigens. Analysis of the immune responses developed by immunized animals, and of the type of defence mechanisms operating. Comparison of the type of immunity induced by antigens and by whole parasites (i.e. irradiated sporozoites) in natural versus artificial hosts.
- Analysis of naturally occurring immunity to MPES in field conditions, of the mechanisms regulating parasite loads at MPES level, and of the main antigens inducing such mechanisms. Analysis of the artificial immunity induced by injection of y-irradiated sporozoites, and of the mechanisms and antigens responsible for such immunity.
- Study of the mode of action and the respective importance of antibodies, antibody – cell Cupertino, lymphocyte cytotoxicity, and cytokines using *P. falciparum* and human hepatocytes, under *in vitro* conditions or *in vivo* in SCID mice.

Expected outcome

Improved understanding of the human *P. falciparum* relationship at MPES level, mainly through an analysis of existing regulatory mechanisms developed against those staged by exposed individuals, and their epidemiological consequences in various areas differing in their vectorial capacity.

Partners

INSTITUT PASTEUR
Département Parasitologie Biomédicale
Rue du Dr. Roux 28
F-75724 Paris 15
France

P. Druilhe
Tel.: +33-1-45 68 85 78
Fax: +33-1-45 68 86 40
E-mail: druilhe@pasteur.fr

BIOMEDICAL PRIMATE RES. CENTRE BPRC
Dept. of Parasitology
P.O. Box 5815
NL-2280 HV Rijswijk
The Netherlands

A. Thomas
Tel.: +31-1-584 25 38
Fax: +31-1-584 39 86
E-mail: thomas@itri.avg.mbc.tno.nl

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
Dept. of Biology
Prince Consort Road
South Kensington
GB-SW7 2BB London
United Kingdom

R. Sinden
Tel.: +44-171-594 54 25
Fax: +44-171-594 54 24
E-mail: r.sinden@bio.ic.ac.uk

KATHOLIEKE UNIVERSITEIT NIJMEGEN
Dept. of Medical Parasitology
P.O. Box 0009101
NL-6500 HB Nijmegen
The Netherlands

W. Eling
Tel.: +31-8-061 36 63
Fax: +31-8-054 02 16
E-mail: medpar.jm@aznvx1.nl

UNIVERSIDAD DEL VALLE
Fundación Centro de Primates
CO-AA 25360 Cali
Colombia

S. Herrera
Tel.: +57-2-356 56 21
Fax: +57-2-358 10 61
E-mail:
soheva@mafalda.univalle.edu.co

MAHIDOL UNIVERSITY
Fac. of Tropical Medicine – Entomology
Rajvithi Road 420/6
T-10400 Bangkok
Thailand

A. Asavanich
Tel.: +66-2-246 12 72
Fax: +66-2-246 83 40

INSTITUUT VOOR TROPISCHE GENEESKUNDE
Nationalestraat 155
B-2000 Antwerpen
Belgium

M. Wéry
Tel.: +32-3-247 63 59
Fax: +32-3-216 14 31
E-mail: mwery@itg.be

KATHOLIEKE UNIVERSITEIT NIJMEGEN
Dept. of Molecular Biology
Toernooiveld 1
NL-6525 ED Nijmegen
The Netherlands

R. Konings
Tel.: +31-80-652 25 08
Fax: +31-80-65 21 12

INSTITUTE OF MOLECULAR MEDICINE
Molecular Immunology Group
J. Radcliffe Hospital Headington
GB- OX3 9DU Oxford
United Kingdom

A. Hill
Tel.: +44-186-522 23 01
Fax: +44-186-522 25 02
E-mail: a.hill@well.ox.ac.uk

CENTRE INT'L DE RECH. MEDIC. DE FRANCEVILLE
BP 769
Franceville
Gabon

P. Millet
Tel.: +241-67 70 92
Fax: +241-67 72 95
E-mail: Pasmille@club-internet.fr

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Life Sciences and Technologies for Developing Countries (STD III)
1991–1994
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(in numerical order of contracts)

Agriculture

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TS3*CT910003	Influence of cultivation on organic nitrogen status in tropical soils. Adjustment of a mathematical model to nitrogen fertility
TS3*CT910004	Extractivism in Central Amazonia: Viability and optimization
TS3*CT910010	Coat protein mediated resistance of *solanum tuberosum* and *nicotiana tabacum* towards Andean potato mottle virus
TS3*CT910014	Genetic improvement of banana for local consumption and for export, with reference to cercosporiosis resistance
TS3*CT910021	Nutrient cycling and sustainability in alley-cropping systems in the humid tropics. II: phosphorus, labile soil, organic phosphorus and base cations
TS3*CT920017	Farmer strategies and production systems in fragile environments in mountainous areas of Latin America
TS3*CT920061	Irrigation-water management and salinization: intercomparison of simulation models in Argentina and Egypt
TS3*CT920069	Genetic improvement of phaseolus food legumes for the lowland and highland tropics of Colombia and Peru
TS3*CT920071	Adaptation of maize to acid soils of the tropics
TS3*CT920091	Evaluation of local poultry resources for creating genetic stock with improved adaptability, productivity and disease resistance for tropical environments
TS3*CT920093	Thermochemical upgrading of biomasses to gaseous and liquid fuels and feedstocks
TS3*CT920094	Development of an integrated system to control bean diseases in tropical and subtropical regions
TS3*CT920098	Biocontrol of damaging root-knot nematode (Meloidogyne Spp.) pests of staple food and cash crops by inducing suppressive soils with the bacterial parasite *Pasteuria penetrans*
TS3*CT920106	Definition and conditions of use of field immunodiagnostics for parasitic diseases prevailing in extensively bred cattle
TS3*CT920109	Estudios bioquímicos e histológicos de los cefalópodos relacionados con la aplicación de tecnologias convencionales y nuevas y con el control de calidad
TS3*CT920110	Adding value to products, by-products and waste from small- and medium-sized cassava-processing industries in Latin America
TS3*CT920111	Intégration de stratégies d'amélioration de la résistance du riz à la pyriculariose (*Magnaporthe grisea*) dans les nouveaux programmes de création variétale

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TS3*CT920125	Biological management of irrigation channel weed problems in irrigated semi-arid agriculture
TS3*CT920128	Conservation and regeneration of soil fertility in tropical agricultural systems by the manipulation of earthworm communities (macrofauna project - second phase)
TS3*CT920131	Organisation of information systems on production inputs, catches and characteristics of small-scale fishery in Ecuador
TS3*CT920134	A project to significantly improve the handling and processing of small pelagic fish for aquaculture and food use
TS3*CT920140	Development of novel systems for plant protection against fungal infection through genetic engineering of plants and myco parasitic fungi
TS3*CT920149	The sustained agricultural development of tropical westlands in South America and Africa
TS3*CT930200	Carbon isotope discrimination of leaf and stem carbohydrates as indicators of drought tolerance
TS3*CT930203	Sustainable development of intensive aquaculture in the Andean-Patagonia region: environmental impact and agricultural re-utilization of fish-farming waste
TS3*CT930205	Evaluation and molecular bases of low-cost post-harvest technologies
TS3*CT930214	Improved control of bean anthracnose disease in Latin America and Africa through increased understanding of pathogen diversity
TS3*CT930216	Amélioration génétique de l'adaptation à la sécheresse de l'arachide
TS3*CT930221	Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose-tolerant cashew (*Anacardium occidentale* L.)
TS3*CT930239	Climatological and hydrological determinants of agricultural production in South-America remote-sensing and numerical simulation
TS3*CT930242	Manipulation of apomixis for the improvement of tropical forages
TS3*CT930252	Regeneration and conservation of hardened and barren volcanic soils in Latin America (Chile, Ecuador, Mexico)
TS3*CT930257	Best management practices for the productive/protective rehabilitation of deforested sloping lands
TS3*CT940264	Etude de la diversité biologique et de *l'Atriplex halimus* pour le repérage *in vitro* et *in vivo* d'individus résistant à des conditions extrêmes du milieu, et constitution de clones
TS3*CT940265	Improvement of symbiosis between *Rhizobium meliloti* and alfalfa in acid soils from Argentina and Uruguay
TS3*CT940269	Diagnosis and control of bacterial diseases in penaeid shrimp

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TS3*CT940278	Improving the growth of tropical nitrogen-fixing forest trees in the genera *Acacia* and *Casuarina* through tissue culture and genetic transformation
TS3*CT940279	Utilization of hemicellulose waste from agricultural and forest industries using xylangdegrading and xylose-fermenting yeasts
TS3*CT940298	Development of methods for the clonal propagation of elite, disease-resistant coconut palms by somatic embryogenesis
TS3*CT940300	Multidisciplinary study of the transformation of Amazonian fruits for their commercialization by existing organizations of small farmers
TS3*CT940306	Optimisation des techniques de sélection du palmier à huile à l'égard de la fusariose, et prise en compte de l'interface racine/sol dans l'évaluation de la résistance
TS3*CT940308	Sustainable agriculture: the role of integrated management of root rot (*Phytophthora cinnamomi* rands) in avocado (persea americana mill)
TS3*CT940314	An integrated study of land properties, their floristic indications, and appropriate farming systems in an acknowledged biodiversity centre in Amazonian Peru
TS3*CT940316	Assessment of genetic diversity of economically and ecologically important tropical tree species of Central America and the Caribbean: implications for conservation, sustainable utilization, and management
TS3*CT940324	Fog as a new water resource for the sustainable development of the ecosystems of the Peruvian and Chilean coastal desert
TS3*CT940333	Development of minimally processed products from tropical fruits using vacuum impregnation techniques
TS3*CT940335	Ecosystems of the Ix region of Chile: influence of land use on sustainability
TS3*CT940341	New food products from prosopis fruits in Latin America: extending use and preventing desertification in arid zones

Health

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TS3*CT910024	Bites and stings by venomous animals in Brazil: clinical and laboratory investigations of envenoming, and therapy
TS3*CT910029	A survey of Chagas cycles in Uruguay by use of genetic markers with special emphasis on reinfestation hazards of domestic structures by sylvatic cycles
TS3*CT910038	Identification of candidate protective molecules of *E. granulosus*, and development of combined Salmonella vaccines

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TS3*CT920053	Malaria pre-erythrocytic stages (MPES) European network antigens target of immune responses capable of *inhibiting P. Falciparum* pre-erythrocytic development
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TS3*CT930229	Regulations of sexual development in malarial parasites and the design of logical intervention strategies
TS3*CT930234	Reducing material mortality and morbidity in Bolivia: appropriate birth practices in the formal and informal systems of perinatal care
TS3*CT930243	Rapid detection of multi-drug-resistant mycobacteria
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TS3*CT930255	Oral vaccine against cholera, with "built-in" adjuvanticity
TS3*CT930259	Epidemiological, clinical, and sero-virological studies of hepatitis in Gabon and Brazil
TS3*CT940263	Analysis and characterization of phosphofructokinase and pyruvate kinase of Leishmania, potential targets for new drugs
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TS3*CT940272	Studies on humoral and cellular immune responses in humans to previously defined malaria vaccine candidates
TS3*CT940277	Control of *Taenia solum* cysticercosis through specific diagnosis, systematic epidemiology, and development of a recombinant vaccine
TS3*CT940294	Integration multidisciplinary study of human fasciolasis in the Bolivian northern altiplano
TS3*CT940296	Genetic and immunological factors in human resistance to *Schistosoma mansoni*
TS3*CT940299	Immune recognition of a novel 45 KDA antigen, specific to *Mycobacterium leprae*, and evaluation as a potential vaccine candidate
TS3*CT940303	Cell-mediated immunity to schistosomes: evaluation of mechanisms operating against lung stage parasites, which might be exploited in a vaccine
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TS3*CT940321	Development of silos managerial skills in Brazil: research and implementation of suitable tools for inter-sectoral and participative actions in dealing with main local health problems
TS3*CT940343	Biotechnological approaches to the total utilisation of crustacea shellfish and shellfish waste
TS3*CT940345	Assessment of immune responses induced in primates immunized with lipopeptides derived from *P. falciparum* MPES antigens

Contract number	Title
TS3*CT940346	Malaria pre-erythrocytic stages (MPES): European network antigens target of immune responses capable of inhibiting *P. falciparum* pre-erythrocytic development

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Avenue du Roi 202/Koningslaan 202
B-1190 Bruxelles/Brussel
Tél. (32-2) 538 43 08
Fax (32-2) 538 08 41
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J. H. Schultz Information A/S
Herstedvang 10-12
DK-2620 Albertslund
Tlf. (45) 43 63 23 00
Fax (45) 43 63 19 69
E-mail: schultz@schultz.dk
URL: http://www.schultz.dk

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