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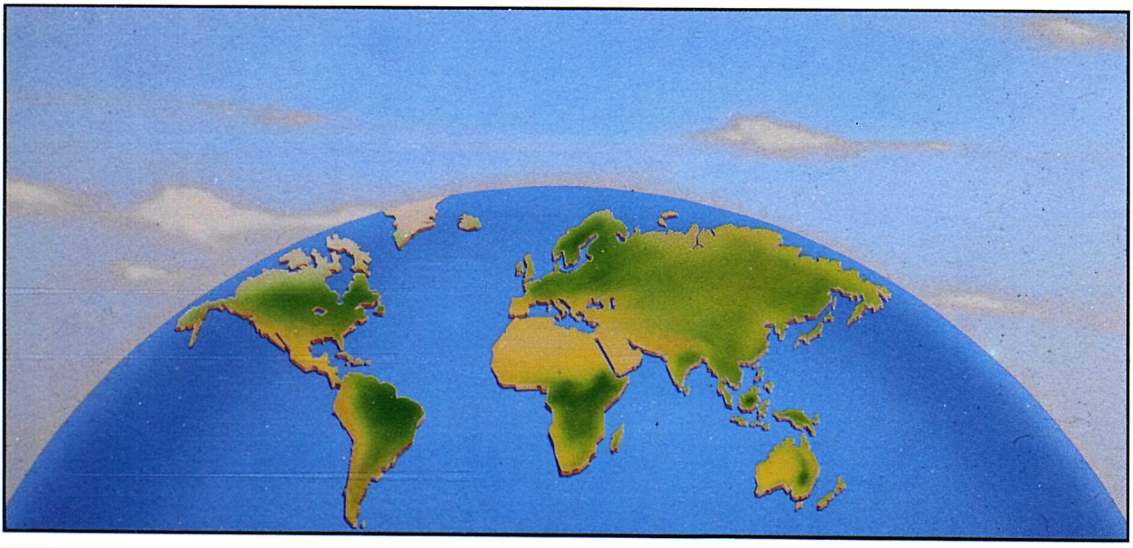
INTERNATIONAL SCIENTIFIC COOPERATION

Consolidated report of activities 1986-90

EC - MEXICO



Report
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International scientific cooperation aims to develop strong and durable links between the scientific communities of certain Asian, Latin American and Mediterranean countries and their counterparts in the European Community. For those countries lacking a substantial body of active scientists, such links allow work to be carried out at an international level but with the advantage of the scientists remaining in their home institutions. For European scientists, such links allow access to a new intellectual environment and the opportunity to apply their skills to a different range of conditions and problems.

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Commission of the European Communities

International scientific cooperation

Consolidated report of activities, 1986-90

EC — Mexico

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Map of Mexico showing principal cities and the location of joint research projects

FOREWORD

International Scientific Cooperation aims to develop strong and durable links between the scientific communities of certain Asian, Latin American and Mediterranean countries and their counterparts in the European Community (EC). For those countries lacking a substantial body of active scientists, such links allow work to be carried out at an international level but with the advantage of the scientists remaining in their home institutions. For European scientists, such links allow access to a new intellectual environment and the opportunity to apply their skills to a different range of conditions and problems.

Joint Research Projects

These are the central International Scientific Cooperation activity and allow research teams to work together on a problem of mutual interest, each team contributing complementary skills, expertise or resources. A minimum of one EC research centre and one Third Country research centre must be included, and participating centres should have an established record in the research field and an adequate level of basic equipment and infrastructure. Thus, permanent staff salaries and infrastructure costs must be supported by the laboratories themselves. The Community normally supports the extra (additional or marginal) costs required to carry out the project on a joint basis; specifically, these may include:

Labour costs - salaries of additional staff such as research assistants or technicians employed to work on the project.

Travel and subsistence - to allow reciprocal working visits between participating centres.

Durable equipment - additional pieces of equipment to complement existing equipment.

Consumables - additional supplies used during the course of the work.

Other expenditure - specific additional costs to be justified and negotiated on a case-by-case basis.

In certain cases, the Community contribution to a joint research project may represent a percentage of the full costs of the research.

Proposals for joint research projects are evaluated on the basis of criteria such as scientific interest, innovative value and the extent to which the joint approach might give added value. For those proposals that are financially supported by the EC, research contracts are established between the Community and the participating centres, one of which is nominated by mutual agreement to manage and coordinate the project and to handle all communications with, and payments by, the Commission. Projects may last from 2 to 4 years. Graduate students may be

incorporated into joint research projects and in this way the projects can have a training function and can enable students to undertake all or part of their work at a second centre.

Postdoctoral Fellowships

These enable qualified Third Country scientists to undertake research in European laboratories and make contact with European scientists; the fellowship may represent a preparatory phase for a joint research project.

Fellowships are normally granted for a period of 6 to 12 months. The Community awards fellows a monthly maintenance grant to cover all costs including food, accommodation and health insurance for the fellow and his/her dependants. Fellows also receive a lump sum to cover travel home at the end of the fellowship period. Cost of travel to Europe is normally borne by the fellow's home institutional or national authority. The European laboratory receiving a fellow is awarded a bench fee to cover research and related costs.

Candidates for fellowships are appraised on the basis of their research and academic records, and on the possibilities for continuing joint scientific research after the fellowship period. For successful candidates, the Community establishes contracts with the fellows for the maintenance grant and with the host laboratory for the bench fee.

Workshops

These deal with topics selected together with the third country national authorities; they bring together up to 10 or 12 European scientists and a similar number of third country scientists to review progress, present results, discuss ideas and develop contacts that might lead to the preparation of joint research proposals. Workshops may also have a regional orientation and include scientists from neighbouring countries participating in International Scientific Cooperation. The Community can cover the travel and subsistence costs of visiting participants and make a contribution towards the publication of the proceedings. The host country covers all local requirements, such as infrastructure, venue, local transport and secretariat.

International Scientific Cooperation activities are always coordinated in conjunction with the third country national science and technology cooperation authorities and in annual meetings, priorities and procedures are established and reviewed as necessary.

International Scientific Cooperation is open to scientists both from the private and the public sectors, from industry, industrial research centres, universities and government research institutes. Research may be carried out on topics from the natural and exact sciences but must be of a precompetitive nature, i.e. further development should be necessary before a product or process is marketed.

INTRODUCTION

The first International Scientific Cooperation activity with Mexico was initiated in 1986, and this volume presents a summary of all activities for which a financial commitment had been made before the end of 1990. It thus covers joint research projects, postdoctoral fellowships and workshops, in various stages of completion: some being totally finished, some in progress and for those projects just starting, a work programme only is presented. These activities conform to the patterns described in the Foreword except for some of the earlier research projects established during the formative stages of International Scientific Cooperation.

The objective of compiling this volume is to show what has been achieved in the framework of International Scientific Cooperation with Mexico. The strength of these achievements lies with their firm scientific foundation and this is reflected in the style of presentation of this volume; however, the contents are orientated towards a wide readership, to allow not only the scientists, both Mexican and European, to place their work in a wider perspective, but also to provide a concise account of the activities to other scientists, government officials, diplomatic representatives and all those interested in science in Mexico and the European Community.

This report covers 35 research projects, 40 postdoctoral fellowships and a workshop, and the summaries included here demonstrate the results of these actions in terms of research findings, productive contacts developed and scientific publications. These results are impressive especially considering that many of the activities are still at an early stage of development and further output will be generated before they are completed.

The subject-matter of the report has been divided by chapters into eight areas, Agricultural Sciences, Biological Sciences, Chemical Sciences, Earth Sciences, Environmental Sciences, Health and Biomedical Sciences, Materials Sciences and Physical, Mathematical and Engineering Sciences, which reflect the subjects covered by International Scientific Cooperation proposal evaluation panels and the need to indicate the spread of activities. Though necessary, this division is somewhat artificial and the main features of each area are described in the introduction to each chapter. Within each chapter, summaries have been arranged in ascending order of project number, an approximately chronological order of starting date.

The successful outcome of the projects is largely the result of the efforts of the scientists involved. In the reports of the joint research projects the names mentioned are those of the principal scientists leading the research groups in the different institutions. It would have been impractical to list all individuals associated with each particular research project; nevertheless, full references have been given to publications resulting from the projects and these indicate some of the other scientists involved. In the reports of postdoctoral fellowships, the fellow's name and institution are given, along with the names of the host scientist and institution.

Another factor in the success of International Scientific Cooperation with Mexico has been the contribution of the Mexican Government's Dirección General de Cooperación Técnica y Científica (Secretaría de Relaciones Exteriores) to promoting the programme and for presenting high-quality proposals to it, and of the Commission staff responsible for the management of the programme.

Giuseppe Valentini,
Director, Science and Technology Cooperation with non-Member Countries, Commission of the European Communities

Further information on International Scientific Cooperation with Mexico is available from:

International Scientific Cooperation,
Directorate General for Science,
Research and Development,
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Dirección General de Cooperación Técnica y Científica,
Secretaria de Relaciones Exteriores,
Homero 213, 2° Piso,
Col. Polanco, México D.F. 11560,
México.

Bibliometric study of Mexican and EC research output

In order to see the work supported by the International Scientific Cooperation (ISC) programme in context, a study was carried out by General Technology Systems Limited in Uxbridge, England, of the extent of co-operation in scientific production between Mexico and scientists of the EC during the years 1986-90.

The source of data was the Science Citation Index (SCI), published by the Institute of Scientific Information in Philadelphia, USA. It covers some 3200 leading scientific journals, the majority of which are international in both authorship and readership and are published in English. (There are two Mexican journals included, *Archivos de Investigación Médica* and *Revista Mexicana de Astronomía y Astrofisíca*). Despite these restrictions it is recognised as providing a good coverage of 'mainstream' science and it has the singular advantage of recording all the corporate addresses for the authors of each paper. This enables papers to be identified when the authors are, say, from Mexico and an EC Member State.

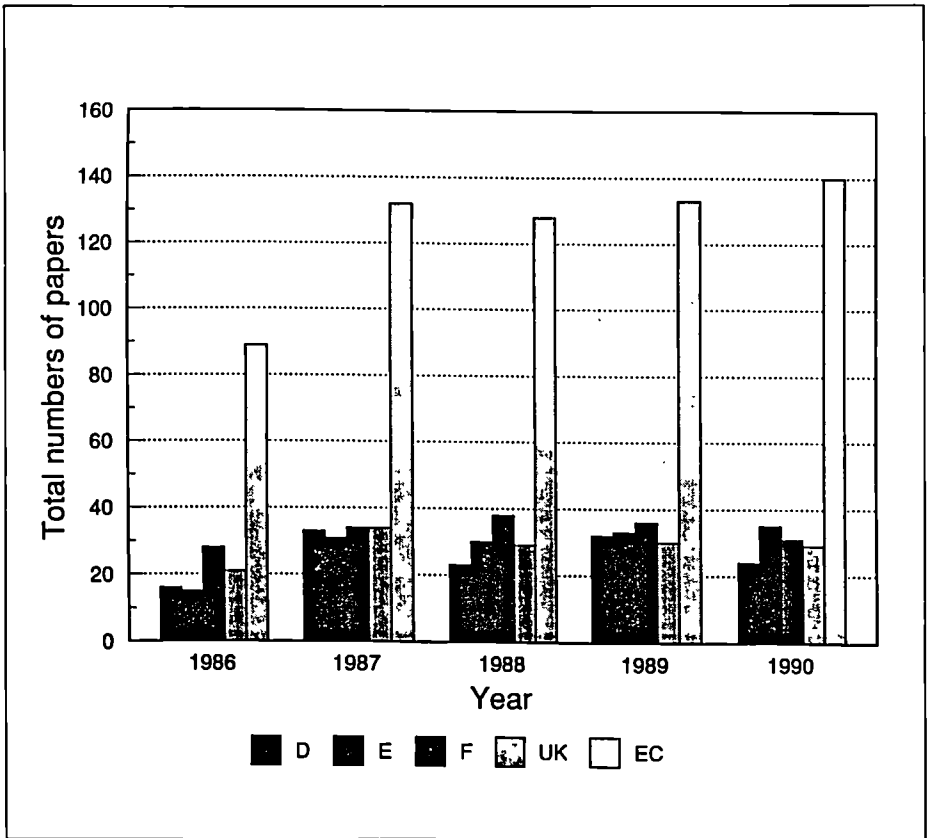

Figure 1: Total numbers of papers in SCI co-authored by Mexican and EC scientists with contributions by four leading EC Member States

During the period under review, Mexican scientists authored or co-authored an average of some 1344 papers per year. The annual total has been rising at about 2% per year, the output from 1986 to 1990 being 1232, 1321, 1477, 1340 and 1353 in successive years. By way of comparison, the total EC annual production averaged 164,000 papers. During the five years, some 624 papers were co-authored by Mexican and EC scientists or 125 per year. The figure rose steadily with time both absolutely and as a percentage of Mexican scientific output, from approximately 8.1% to 10.4% of the total, see Figure 1.

The leading EC Member States in terms of co-operation with Mexico in scientific production were France, Spain, the UK and (the Federal Republic of) Germany. Table 1 shows that, judged in relation to their own total scientific output,. the leading countries were Spain (not suprisingly) with 0.40% and France with 0.11%. Clearly scientists in EC countries are much less likely to collaborate with Mexicans than Mexicans are with EC scientists.

Member State	Papers per year	Percent of output
France	33.4	0.11%
Spain	28.8	0.40%
UK	28.6	0.05%
Germany (FR)	25.8	0.07%
Italy	9.2	0.06%
Belgium	3.8	0.06%
Netherlands	3.6	0.03%
Denmark	2.4	0.05%

Table 1: Mean numbers of papers in SCI co-authored by Mexican and EC scientists, and percent of EC Member State total, 1986-90.

An analysis was made of the eight subject areas in which Mexican-EC scientific co-operation occurred. The classification used was the one adopted for this report but some of the papers were not easy to classify especially within the three life sciences areas (agriculture, biology, medicine). Overall during the five years the main area for co-authorship was physical sciences, with 252 papers (41%). Next came chemical sciences with 99 papers and biological sciences with 97 (both 16%), and health and biomedical sciences with 64 papers (10%). The other four areas had much less co-authorship: earth and materials sciences with 6%, agricultural sciences with 4% and environmental sciences with 1%.

The four leading EC countries all showed rather similar patterns of co-authorship with Mexico. France was strong (relative to the EC as a whole) in earth and chemical sciences (16% and 21% respectively of its output) but weak in physics (29%); Spain had more co-operations in materials(10%) and physical sciences (48%) but fewer in agricultural (nil) and health sciences (7%). Germany was rather strong in physical sciences (52%). The UK was prominent in agricultural (8%) and health sciences (15%) but, surprisingly, weak in biological sciences (10%).

Figure 2 shows the changes in subject areas for co-operation over time. Almost all the overall growth has occurred in the life sciences, whose annual numbers of co-authored papers rose steadily in four years from 18 to 50.

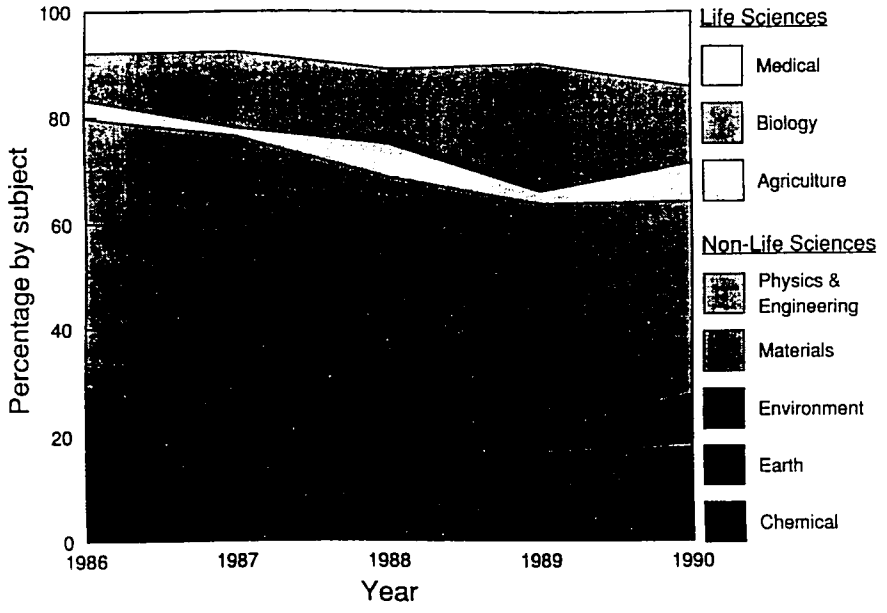

Figure 2: Percentages of papers in SCI in eight scientific areas co-authored by Mexican and EC scientists.

Much of this rise can be attributed to the effects of the ISC programme. Overall, scientists participating in the programme wrote some 92 papers published by, in press in, or submitted to, SCI journals; of these, 68, or almost three quarters, were in the life sciences. Their division by year of publication was as shown in Table 2, overleaf.

Science area	1986	1987	1988	1989	1990	1991	Total
Agriculture		1	3	1	8	17	30
Biology			2	3	3	2	10
Health			2	5	17	4	28
Total Life Sciences		1	7	9	28	23	68
Other				4	7	13	21
Grand Total		1	7	13	35	36	92

Table 2: Distribution by year of Mexican ISC programme publications in SCI by major scientific area listed in this report.

Not all these publications are co-authored by Mexican and EC scientists, although the majority appear to be. The table shows that the scientific results of the programme are now appearing in quite large numbers. It is probable that the total for 1991 will be higher as many joint research projects described in this report are still in progress. The volume of publications in SCI journals represents of the order of 3% of all those with a Mexican author which is a measure of the impact of the ISC programme on Mexican science.

1 AGRICULTURAL SCIENCES

Summary

For the purposes of this report, agricultural sciences are interpreted in the broadest sense, and include not only studies of crop and animal production but also forestry, fisheries and post-harvest processing of agricultural products. Mexico's large land surface and wide diversity of ecological conditions lead in turn to a wide range of agricultural systems and an agriculture industry that plays a very important part in the economy of the country. This is reflected in International Scientific Cooperation with Mexico and this chapter includes about one third of the joint research projects and one quarter of the postdoctoral fellowships covered in this report.

The studies reported in this chapter fall into four main groups: biological nitrogen fixation, pest control, post-harvest processing and fisheries. Despite this wide range of subjects, it is interesting to note that a common feature of many of these studies is that they employ molecular-level investigation of the problem to be tackled.

Biological nitrogen fixation is a very attractive approach to improving crop yield; whilst not new, it is the subject of much research because it is inexpensive and free from unwanted side-effects on the environment, a research centre dedicated to it has been set up by the Mexican National University at Cuernavaca, and it was the subject of an International Scientific Cooperation workshop in Mexico. The biological nitrogen fixation work reported here covers the development of improved bacterial strains, approaches to modifying the symbiosis through genetic engineering and a study of nitrogen metabolism and its genetics.

The major emphasis of the projects relating to pest control summarised here is on the identification and mode of action of naturally-occuring chemicals having pesticidal properties with a view, ultimately, to genetic engineering applications. Such a strategy would allow a specific solution to pest control problems in crops and reduce or eliminate the need for the use of agrochemicals which can have damaging secondary effects on the environment. One project deals with coconut lethal yellowing, a new disease problem for Mexico, and the use of micropropagation to obtain a large number of progeny from selected trees in a short period of time.

The post-harvest processing projects cover fermentation technology to produce new foods and to preserve foods as an approach particularly appropriate for Mexican conditions, the development of new products from sugar, and the food use of safflower and amaranth. The fisheries projects reflect the importance of Mexican marine resources and the possibilities for their more rational exploitation including aquaculture techniques.

Joint research projects

1 Construction of hybrid strains of *Rhizobium phaseoli* with improved symbiotic properties

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Contract number and duration: CI1*/0104, July 1987 to June 1989.

Background

Bacteria belonging to the genus *Rhizobium* form nitrogen fixing nodules in the roots of legumes. Bacterial genes controlling nodulation, host-range specificity and nitrogen fixation have been located on large plasmids (symbiotic plasmids).

In the last few years we have directed our research efforts towards the understanding of the nature of *Rhizobium phaseoli* (the symbiont of the common bean plant *Phaseolus vulgaris*) and its genome. We have found two types of strains able effectively to nodulate *P. vulgaris*. Type I encompasses about 95% of the strains screened, they have a narrow host range of infection and their symbiotic plasmid contains reiterated nitrogenase genes. Type II strains have a broad host range as they are able effectively to nodulate *Phaseolus* as well as other tropical legumes including *Leucaena* and do not present repeated *nif* genes. We have proposed that the two types of strains represent different evolutionary lines. We have preliminary evidence that the symbiotic plasmid of type I and type II strains belong to different compatibility groups. We have recently found that when the symbiotic plasmid of one type II strain is transferred to an *Agrobacterium tumefaciens* devoid of its native plasmids (GMI9023), transconjugants are able to induce nitrogen fixing nodules in both *Phaseolus* and *Leucaena*.

In regard to the nature of the *Rhizobium phaseoli* genome, our studies indicate that it contains a large amount of repeated DNA sequences and that genomic rearrangements are frequent.

Objectives

General Construction of hybrid strains of *R. phaseoli* with improved symbiotic properties by joining plasmids from different strains in the same chromosomal background.

Specific

1 To further characterise *Rhizobium* strains able to nodulate *Phaseolus vulgaris*.

2 To transfer symbiotic plasmids from several type I and type II *R. phaseoli* strains to *Agrobacterium tumefaciens*, and to determine the symbiotic properties of the transconjugant strains.

3 To use *Agrobacterium* transconjugants containing one symbiotic plasmid as recipients of other plasmids and to determine if symbiotic properties can be improved.

4 To construct hybrid *Rhizobium* strains containing plasmids from different native isolates.

5 To use the hybrid strains obtained to define the influence of different replicons in competition between strains.

Figure 1: The laboratory for nitrogen fixation research in UNAM.

Materials and methods

Transfer of symbiotic plasmids of *R. phaseoli* strains to *Agrobacterium tumefaciens*. *Rhizobium* strains were labelled at random with a Tb5-*mob* transposon through a triparental mating using both an *E. coli* strain containing a pBR derivative carrying the transposon and a mobilising helper strain. Labelled strains were selected and conjugated with *Agrobacterium tumefaciens*. *Agrobacterium* transconjugants derived from each strain were inoculated in mass using *P. vulgaris* as host. Strains were purified from nodules and characterised by plasmid profile and by genome *Southern* hydridisation using *nif* genes as probes.

Construction of hybrid *Rhizobium* strains. Different *Rhizobium phaseoli* strains were mated with donor bacteria harbouring Tn5-*mob* on different plasmids. Transconjugants with additional plasmids were verified by the Eckhardt procedure and used as inoculant for nodulation and competition assays at different ratios with parental original strains.

Determination of symbiotic properties. Nodule number, and acetylene reduction were routinely screened in both beans and *Leucaena*. Selected strains were compared by their ability to compete among them.

Results and discussion

Description of two different types of *Rhizobium phaseoli* strains *R. leguminosarum* bv. *phaseoli* strain-collections harbour heterogeneous groups of bacteria where two main types of strains may be distinguished, differing both in the symbiotic plasmid and in the chromosome. Type I strains are characterised by a narrow host range of nodulation and by the presence of *nif*-gene reiterations. Type II strains have a broad host range of nodulation and present single nitrogenase genes. In regard to its symbiotic capability, there is no significant difference in the total number of nodules formed or in nitrogen fixation in both types of strains.

We have recently proposed that type I and type II strains should be considered as two different *Rhizobium* species (Martínez *et al*, submitted).

Competitive abilities of *R. phaseoli* type I and type II strains Seeds of *Phaseolus vulgaris* cv. Negro Jamapa or Negro Angel were inoculated with mixtures of two *R. phaseoli* strains at different proportions. After 16 days nodules were crushed in non-selective media and single colonies were picked and tested for growth in selective media for strain identification. Different combinations of type I and type II strains were analysed. Type I strains showed much higher competitive ability.

At a 1:1 ratio of inoculation, more than 90% of the nodules were formed by type I strains both in the Negro Jamapa and in the Negro Angel bean cultivars. Large inocula of type II strains are needed to increase its percentage of nodule occupation. These differences in competitivity might explain the fact that under natural conditions *Phaseolus vulgaris* is usually nodulated by type I strains.

Construction and symbiotic properties of *Agrobacterium tumefaciens* strains containing symbiotic plasmids from *Rhizobium phaseoli* type I and type II strains. Different type I and type II *Rhizobium phaseoli* strains were used as donors to transfer the symbiotic plasmid to an *Agrobacterium tumefaciens* strain (GMI9023) which had been cured from all its native plasmids. *Agrobacterium tumefaciens* transconjugants containing symbiotic plasmids were obtained from all the *R. phaseoli* strains used. All transconjugants were able to nodulate *P. vulgaris* and conserved the parental nodulation host range: transconjugants containing type II symbiotic plasmids were able to nodulate *Leucaena* in addition to *P. vulgaris*. A fix phenotype was obtained with type I symbiotic plasmids while type II symbiotic plasmids in the *Agrobacterium* chromosomal background were able to induce effective nodules.

An *Agrobacterium* transconjugant containing the symbiotic plasmid from type II *R. phaseoli* CFN299 strain was used to test the effect of other plasmids in regard to symbiotic properties. The original *Rhizobium* strain contains two plasmids in addition to the symbiotic one. *Agrobacterium* transconjugants containing the symbiotic plasmid plus either one of the other or both of them were obtained and symbiotic properties were analysed. The data indicated that one of the plasmids (CFN299 pb) increased the nodule formation and nitrogen fixation in the *Agrobacterium* background.

Increased nodulation-competitiveness of genetically modified R. phaseoli strains Type I *R. phaseoli* strains CFN42, Viking I, TAL182 and CFN279 were genetically modified by the transfer of plasmid CFN299 pb. The transconjugant strains formed on the average 50% more nodules than the original parental strains. Moreover, a significantly larger percentage of nodules were derived from the transconjugants when tested in competition experiments against the non-modified *Rhizobium* strains. These results indicate that, at least under laboratory conditions, symbiotic properties can be improved by genetic manipulation based on plasmid transfer between *Rhizobium* strains.

Conclusions

It is possible to construct hybrid *Rhizobium* strains containing plasmids from different native isolates. Some of these construction show improved symbiotic properties as compared to the parental strains. This approach should be used systematically to search for strains improved in symbiotic properties.

Publications

Brom. S.; Martínez, E., Dávila, G. and Palacios, R. (1988). Narrow and broad host-range symbiotic plasmids of *Rhizobium phaseoli*. *Applied and Environmental Microbiology*, **54**, 1280-83.

Dávila, G.; Brom. S.; Flores, M.; de Lourdes Girard, M.; González, V.; Louzada M.; Martínez, J.; Martínez, E.; Palacios, R.; Piñero, D.; Romero, D. and Valdez, A.M. (1988). The symbiotic genome of Rhizobium phaseoli. *In:* Molecular Genetics of Plant-Microbe Interactions (eds. Palacios, R. and Verma, D.P.S), 187-91.

Martínez, E. and Rosenblueth, M. (1990). Increased bean nodulation competitiveness of genetically modified *Rhizobium* strains. *Applied and Environmental Microbiology*, **56**, 2382-4.

Martínez, E.; Romero, D. and Palacios R. (1990). The *Rhizobium* genome. *Critical Reviews in Plant Science*, **9**, 59-93.

2 Genetics and molecular biology of the *Phaseolus vulgaris* - *Rhizobium leguminosarum* biovar *Phaseoli* symbiotic association

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Contract number and duration: CI1*/0105, August 1987 to July 1989.

Background

The legumes, particularly plants of the genus *Phaseolus*, are a primary source of protein in the diet of the vast majority of the Latin American population. The symbiosis of the legumes with micro-organisms of the genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* is potentially capable of supplying the nitrogen requirements of the plant from atmospheric nitrogen. The research focused on genetical and molecular biology aspects of the *Phaseolus vulgaris* - *Rhizobium leguminosarum* biovar *phaseoli* (to be called *Rhizobium phaseoli* in the rest of the text) - symbiotic association.

Objectives

The general objective of the study was to apply the methodology of genetics and modular biology to define at the molecular level the role of both the plant host and the bacteria during the symbiotic association between *Rhizobium phaseoli* and *Phaseolus vulgaris*.

Specific objectives

1 Construction of two *Rhizobium phaseoli* gene banks in cosmid vectors that are able to replicate in *Rhizobium*. One gene bank was made from a broad-host range strain capable of effectively nodulating *Phaseolus, Leucaena* and siratro, the other will be made from a narrow host-range strain effectively able only to nodulate *Phaseolus*.

2 Identification of symbiotic gene functions by transfer of cloned symbiotic regions from *E. coli* into *pSym* cured *R. phaseoli* strains and position complementation for nodulation and/or nitrogen fixation in *Phaseolus vulgaris* and *Leucaena*.

3 *Rhizobium phaseoli* transposon mutagenesis with generic vectors *(MudlacII)* that generate lac-gene fusions. Those clones turned on by *Phaseolus* root extract will be selected and checked for a symbiotic defective phenotype.

4 Characterisation of *Phaseolus vulgaris* nodule specific proteins and transcripts by SDS-PAGE of nodule extracts and polysomal RNA *in vitro* translation products from nodules induced by wild type and symbiotically altered *R. phaseoli* strains.

5 Isolation and characterisation of nodules specific cDNA clones from *Phaseolus vulgaris* to use some of these cDNA clones as developmental markers to frame the symbiotic interaction (e.g. leghaemoglobin, uricase, glutamine synthetase, xanthyne dihydrogenase, etc).

6 Characterisation of the wild type and altered symbiosis of *Phaseolus vulgaris-Rhizobium phaseoli* by light and electron microscopy.

Materials and methods

The construction of the narrow host range *Rhizobium phaseoli* CE-3 strain genome bank was made in pSup205, a pBR325 derivative with the *cos* site from lambda phage and *mob* site from pRP4 in order to be mobilised from *E. coli* into *Rhizobium* (Cevallos *et al*, 1989).

The broad host range *Rhizobium phaseoli* CIAT 899 library was constructed in pVK102, a low-copy number and broad-host range cosmid vector (Vargas *et al*, 1990). Transfer and identification of symbiotic information from *E. coli* into *Rhizobium* was performed according to the method of Cevallos *et al* (1989). Transposon mutagenesis with genetic vectors MudIIlac was done according to the methods of Cevallos *et al* (1989) and Vázquez *et al* (1991). Nodule extracts and polysomal RNA in vitro translation products from nodules were prepared and separated according to the methods of Campos *et al* (1987) and Sánchez *et al* (1988).

Light and electron microscopy characterisation of nodule tissue were described in Cevallos *et al* (1989) and Soberon *et al* (1990).

Results and discussion

The results from these studies have defined the minimal amount and molecular organisation of the genetic information from *R. phaseoli* that is essential for nodulation. They have also helped us to understand the plant-host response by describing the symbiotic interaction at the ultrastructural and molecular level. The basis to modify by genetic engineering some aspects of the symbiotic interaction between *Rhizobium phaseoli* and *Phaseoli vulgaris* has been created.

The first part described the minimal amount and molecular organisation of the genetic information from *R. phaseoli* that is necessary for nodulation. In this part, specific objectives 1, 3 and 6 were addressed. The construction of the narrow host range *Rhizobium phaseoli* CE-3 strain genome bank was made in pSup205, a pBR325 derivative with the cos site from lambda phage and *mob* site from pRP4 in order to be mobilised from *E. coli* into *Rhizobium*. The construction of this gene bank is described in Cevallos *et al* 1989. Besides partly covering specific objective 1, specific objectives 2 and 3 were addressed since this paper shows that a pSym-cured derivative of the narrow host-range (CFN 2001) strain, incapable of inducing nodulation in *P. vulgaris*, was complemented by CE-3 DNA cloned in pSup205. Recovered transconjugants were able to induce nodulation. Two nodulation regions from the symbiotic plasmid (pSym) of *Rhizobium phaseoli* CE-3 were identified. Two regions were contained in overlapping cosmids pSM927 and pSM991. Analysis of deletion and insertional mutations in the sequences of pSM991 indicated that the genes responsible for the induction and development of nodules in *P. vulgaris* are organised in two regions 20 kb apart. Electron microscopy examinations of nodules induced by the wild type (CE-3) and CFN2001 containing pSM991 or pSM927 or a deletion derivative from pSM991 that still nodulates pSM991-25, were presented.

Recently, by genetic and nucleotide sequence analysis of the two regions involved in nodulation from the CE-3 strain, it was found that the common nodulation genes in *R. phaseoli* have a novel organisation. In spite of forming an operon or mapping physically together, in all specified studied thus far, the functional *nodA* gene is separated from the *nodBC* genes by 20 kb. This novel organisation could be the result of a complex rearrangement, as zones of identity between the two separated *nodA* and *nodBC* regions, were found. Interestingly, despite the separation, the coordination of the expression of these genes seems not to be altered. Two papers, Vázquez *et al*, 1991 and Vázquez *et al*, 1990, describe this work.

The broad host range *Rhizobium phaseoli* CIAT 899 library was constructed in pVK102, a low-copy number and broad-host range cosmid vector. This is described in Vargas *et al*, 1990. This broad host range strain nodulates a wide range of hosts: *Phaseolus vulgaris* (beans), *Leucaena esculenta* and

Macroptilium atropurpureum (siratro). A nodulation region from the symbiotic plasmid was isolated and characterised. This region, which is contained in the overlapping cosmid clones pCV38 and pCV117, is able to induce nodules in beans, *Leucaena esculenta* and siratro roots when introduced in strains cured for the symbiotic plasmid. In addition, this cloned region extends the host-range of *Rhizobium meliloti* (which nodulates alfalfa) and *R. leguminosarum* biovar *trifolii* (which nodulates clover) wild type strains to nodulate beans. A 6.4 kb HindIII fragment contains the essential genes required for nodule induction on all three hosts. A constructed subclone that hydridizes to a NodD probe, when conjugated into a CE-3 narrow hostrange *R. phaseoli* strain, made it able to elicit nodules on *Leucaena esculenta* and siratro roots. These results suggest that this pSym region (1 kb) is involved in the extension of host specificity to promote nodule formation in *P. vulgaris*, *L. esculenta* and *M. atroporpureum*.

The second part involving the description of plant-host response at the molecular level was covered by specific objectives 4 and 5 and partially by 6. We have been working for a longer period of time on this subject and a number of publications have been made. We have constructed two cDNA libraries from beans, one from infected root and other from mature nodules. Several nodulin genes have been isolated from them.

Nodulin genes have been divided in two types. The first of these is early nodulins, when expression is detected in early stages of nodule development and probably related to the nodule formation. We have worked with an early nodulin which was originally isolated from soybean nodules called ENOD2 (Sánchez *et al*, 1988; Lara *et al*, 1988; Padilla *et al*, submitted). Recently, a novel early nodulin with no homology so far discovered to any other gene or protein has been isolated from an infected root bean cDNA library (PvENOD20).

Late nodulins are expressed late in nodule development, at about the onset of nitrogen fixation. Several late nodulin genes have been isolated from bean nodules (Sánchez *et al*, 1988; Lara *et al*, 1988; Padilla *et al*, submitted). Such is the case for leghaemoglobin, uricase II, and a large gene family called nodulin 30.

We have concluded the quantification of the mRNA relative expression levels of early and late nodulins. This was done in nodules induced by wild type and mutant strains of *Rhizobium* as well as an *Agrobacterium* transconjugant strain harbouring the symbiotic plasmid of CE-3 (42d); this bacterium fixes up to 20% of the wild strain. The results of these studies are summarised in Sánchez *et al*, 1988; Padilla *et al*, submitted and Sánchez *et al*, in press.

The nucleotide sequence of the corresponding genomic clone, a member of the nodulin 30 (PvNod30) gene family has been concluded. This nodulin has homology with other genes that contain "Zn finger motives" (Sánchez *et al*, in press).

Publications

Cevallos, M.A.; Vázquez, M.; Davalos, A.; Espin, G.; Sepúlveda, J. and Quinto, C. (1989). Characterisation of *Rhizobium phaseoli* Sym plasmid regions involved in nodule morphogenesis and host-range specificity. *Molecular Microbiology*, **3**, 879-89.

Lara, M.; Ortega, J.L.; Olguin, B.E. and Sánchez F. (1988). Nodulin expression and nitrogen metabolism in *Phaseolus vulgaris* root nodules. *In*: Nitrogen Fixation: Hundred Years After. Eds Bothe, deBruijn and Newton; Gustav Fischer, Stuttgart and New York, 115-17.

Padilla, J.; Miranda, J. and Sánchez, F. Nodulin gene regulation in nodules induced by *Rhizobium* mutants. Submitted to *Molecular and Genetic Plant Microbial Interactions*.

Sánchez, F.; Padilla, J.; Pérez, H. and Lara, M. (1991). Control of nodulin genes during symbiotic nitrogen fixation. *Annual Review of Plant Physiology and Plant Molecular Biology*, **42**, in press.

Sánchez, F.; Quinto, C.; Vázquez, M.; Spaink, H.; Wijffelman, C.A.; Cevallos, M.A.; de las Peñas, A.; Campos, F.; Padilla J. and Lara, M. (1988). The symbiotic association of *Phaseolus vulgaris* and *Rhizobium leguminosarum* bv. *phaseoli*. *In*: Molecular Genetics of the Plant-Microbe Interactions. Eds Palacios, R. and Desh Pal S. Verma; APS Press, St. Paul, Minnesota, 370-5.

Vargas, C.; Martínez, L.J.; Megias, M. and Quinto C. (1990). Identification and cloning of nodulation genes and host specificity determinants of the broad host-range *Rhizobium leguminosarum biovar phaseoli* strain CIAT899. *Molecular Microbiology*, **4**, 1899-1910.

Vázquez, M.; Davalos, A.; de las Peñas, A.; Miranda, J.; Santana, O.; Sánchez, F. and Quinto, C (1990). The nodA gene is physically and transcriptionally separated from the nodBC in *Rhizobium leguminosarum* bv. *Phaseoli* CE-3 strain. In Nitrogen Fixation: Achievements and Objectives. Eds Gresshoff, Roth, Stacey and Newton; Chapman and Hall, New York and London, 597.

Vázquez, M.; Davalos, A.; de las Peñas, A.; Sánchez, F. and Quinto, C. (1991). Novel organisation of the common nodulation genes in *Rhizobium leguminosarum* bv. *Phaseoli* strains. *Journal of Bacteriology*, in press.

Background references

Campos, F.; Padilla, J.; Vázquez, M.; Ortega, J.L.; Enríquez, C. and Sánchez, F. (1987). Expression of nodule-specific genes in *Phaseolus vulgaris L. Plant Molecular Biology*, **9**, 521-32.

Soberon, M.; Membrillo-Hernández, J.; Aguilar, G. and Sánchez, F. (1990). Isolation of *Rhizobium phaseoli* Tb5-induced mutants with altered expression of cytochrome terminal oxidases 1 and aa3. *Journal of Bacteriology*, **172**, 1676-80.

3 Biochemical characterisation of toxic proteins in the defence of plants against insect and microbial pests of importance in Mexico

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Background

Agricultural crops from the tropical and sub-tropical regions of Central and South America are subject to serious pre- and post-harvest damage by a wide variety of insect and microbial pests. Plants contain a range of biochemical compounds which contribute to their overall defence mechanisms against the attacks of these insect and microbial pests. Proteins such as the enzyme (proteinase and α amylase) inhibitors and lectins have been shown to be implicated in the resistance of seeds to predation during post-harvest storage by insects such as *Callosobruchus*, *Tribolium* and *Tenebrio*. Furthermore it has been demonstrated that some of these proteins from seeds can be genetically engineered into other plants where they increase the foliar resistance to feeding by herbivorous insects such as *Heliothis*, *Spodoptera* and *Manduca*. It was our objective to attempt to identify potentially useful proteins of this type which might be employed in plant breeding programmes or in DNA recombinant technology to improve crop resistance.

Materials and methods

We have surveyed a range of existing and potential crop plants (e.g. maize, sorghum, lentil, cow-pea, *Cucurbita fiscifolia*, *Amaranthus cruentas*, Mesquite (*Prosopsis juliflora*) for their content of proteinase (trypsin and chymotrypsin) and α -amylase inhibitors, paying particular attention to those inhibitors most active against the enzymes from the guts of the potential insect pests. We have employed the conventional methods of protein purification (gel-filtration, ion exchange chromatography, etc) in isolating these molecules, but in addition we have also used the modern techniques of affinity chromatography (with Procion red and immobilised enzymes as ligands), FPLC and reverse phase HPLC. The primary structures (amino acid sequences) of such proteins of interest have been determined by automated and manual microsequencing methods. Computer programmes have been utilised in searching for sequence similarities (homologies) as indicators of unsuspected functions.

Results

Maize (*Zea mays*) We have isolated and determined the structure of two potent bifunctional inhibitors of trypsin and insect α -amylase from this import crop plant. One of these, a protein of 22 kD molecular weight was discovered to exhibit a most surprising and unexpected homology with patheogenesis-related (PR) proteins and the intensely sweet protein thaumatin (Richardson *et al*, 1987). The second protein (12 kD) was found to be identical in structure to a trypsin inhibitor previously reported by Prof. Reeck and his co-workers at Kansas State University, who failed to detect its activity against insect α -amylases. We are now collaborating with this group in further studies on the biochemistry and molecular biology of this potential target for protein and genetic engineering (Reeck *et al*, submitted).

Lentil (*Lens culinaris*) We have isolated a number of double headed inhibitors of trypsin and chymotrypsin in from these seeds. Our determinations of the primary structures of these inhibitors have revealed that they belong to the well known family of Bowman-Birk proteinase inhibitors from legumes (Richardson, 1991). One of the isoinhibitors is particularly interesting because it also inhibits proteases from the fungus *Aspergillus niger* and the insect *Sitophilus zeamais*.

Sorghum (*Sorghum bicolor*) In a separate collaboration with workers from the University of Brasilia we have discovered five inhibitors of insect α -amylases in seeds of this important Mexican crop. Two of the proteins belong to the cereal superfamily of enzyme inhibitors, but the other three are small (5 kD) isoinhibitors of locust and cockroach α -amylases, which are currently the smallest known plant inhibitors of this type of enzyme. They have sequence homologies with the purothionins from other cereal seeds and certain other toxic proteins (Bloch and Richardson, 1991).

Cucurbita fiscifolia We have characterised a small (3.3 kD) but very active trypsin inhibitor from these seeds which clearly belongs to the Squash trypsin/Hageman factor inhibitor family. In addition to being a very potent inhibitor of bovine trypsin, this small protein was also very active against a protease from the insect pest *Prostephanus truncatus*.

Amaranthus Seeds of *Amaranthus hypocondriacus* were shown to contain inhibitory activity against the amylases and proteases in the insect pests of cereals (*Tribolium castaneum, Prostephanus truncatus,* and *Sitophilus zeamais*), the amylases from the legume pests *Callosobruches maculatus, Zabrotes subfasciatus* and *Acanthoscelides obtectus*, and protease activity in the fungus *Aspergillus fumigatus*.

Also assisted by Gerardo Pérez (National Univ. Bogota, Colombia) we have isolated an N-acetyl D-galactosamine specific lectin from the seeds of *Amaranthus cruentus* a Mexican crop plant which is becoming more widely cultivated in Central and South America. So far we have established about 70% of the primary structure of this protein which is completely different from all previously studied lectins. We hope to investigate its potential as a defence agent in insect feeding trials.

Mesquite (Algaroba, *Prosopsis juliflora*) Drought-resistant species of this xerophytic shrub are of widespread occurrence in the dry regions of Central and South America. Some recent studies have suggested that the fruits and seeds of this species might be an acceptable emergency source of foodstuff for humans during famine periods caused by extremes of drought such as those frequently experienced by the north-eastern States of Ceara and Rio Grande do Norte in Brazil. However little is known about the proteinase inhibitors and other anti-nutritional proteins in these seeds. We have therefore collaborated with workers at the Federal Universities in these States in an investigation of these proteins. We have now characterised and sequenced three separate and very different inhibitors of trypsin and chymotrypsin from *Prosopsis* (Negreiros et al, 1991).

Discussion and conclusions

We hope soon to have sufficient information on a variety of toxic proteins of this type to be able to select one or more of the most promising as targets for plant breeding for increased pest resistance. In addition to the financial support, the scientific co-operation has had several benefits for the participants. In particular the European workers have gained an insight into the special problems of tropical and sub-tropical agriculture and ready access to interesting plant materials and the enzymes from important insect pests. Three of the Mexican collaborators have visited Durham where they have gained experience of equipment and techniques not initially available in Mexico. Furthermore the co-operation has attracted the interest and participation of other new collaborators from Brazil, Colombia, the USA and the UK.

Publications

Bloch, C. Jr. and Richardson, M. (1991). A new family of small (5 kD) isoinhibitors of insect α - amylases from seeds of sorghum (*Sorghum bicolor*) have sequence homologies with the wheat γ - purothionins. *FEBS Letters*, in press.

Negreiros, A.N.M.; Carvalho, M.M.; Xavier-Filho, J.; Blanco Labra, A.; Shewry, P.R. and Richardson M. (1991). The complete amino acid sequence of the major Kunitz trypsin inhibitor from the seeds of the Brazilian Mesquite (algaroba) *Prosopsis juliflora*. Submitted to *Phytochemistry*.

Reeck, G.R.; Valdes-Rodriguez, S.; Blanco Labra, A.; Richardson, M. et al. The inhibition of insect gut α -amylases by cereal proteins. Submitted to *Cereal Chemistry*.

Richardson, M.; Valdés-Rodriguez, S. and Blanco Labra, A. (1987). A possible function for thaumatin and a TMV-induced protein suggested by homology to a maize bifunctional enzyme inhibitor. *Nature*, 327, 432-34.

Richardson, M. (1991). Seed storage proteins: the enzyme inhibitors. *In* Methods in Plant Biochemistry, ed. Rogers, L., 5, 261-307. Academic Press, London.

4 Non-dairy lactic fermentations

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Background

Lactic fermentations are very old and efficient ways of preserving foodstuffs. They are also used to increase food varieties and can produce flavours enjoyed by consumers. In Mexico, lactic fermentations have traditionally been used to produce corn-based drinks. More recently, they have yielded fermented dairy products.

This project explored two new ideas for the use of lactic fermentations. The first was to develop a novel food, rather like a yoghurt, from milk whey (often currently thrown away), soya milk and oat flour. The second was to employ lactic acid fermentation in meat as a means of decontamination and to extend its shelf-life. This is a very desirable aim in a hot and humid country like Mexico.

I. Production of novel yoghurt

Introduction

Milk is in short supply in Mexico and is imported in large quantities. There is large-scale cheese production but the unused whey is mostly discarded. It was an objective of this project to utilise this by-product together with inexpensive and widely available plant materials, soya milk and oat flour, to make a food with good nutritive properties and a pleasant flavour.

The nutritive properties of the three components were known from food tables. They could be used to design a starter flora of bacteria that could produce a fermentation and yield a yoghurt-like product. This process should improve the flavour and protein-digestibility of the mixture. It should also eliminate α -galactosidases of soya.

Materials and methods

Two different commercially-used methods for the production of soya milk were used. Both involved cleaning, boiling and peeling the soya beans and then mixing them with water. The first was a method recommended by the American Soy Association and yielded a soya milk with 1:4 soya/water ratio. The second method was that of Wang and Hesseltine (1982) and it gave a 1:10 ratio soya milk. The oat flour was obtained by milling whole oat flakes in an impacting mill and retaining the flour passing through a Tyler 100-mesh sieve. The whey was obtained from Kem Foods of Mexico City in the form of spray-dried cheese whey. The *mixture* comprised 82% soya milk, 11% oat flour and 7% whey solids. As a control, dehydrated cow's skimmed milk of 14.5% solids was employed. Both mixture and control were pasteurised by heating to 80oC for 20 minutes.

Chemical analysis Acid production (reported as lactic acid), pH, total soluble carbohydrates (by phenol sulphuric techniques), sugar consumption (by DNS), proteolysis measured as soluble peptides (by Lowry's technique), apparent viscosity (measured in a Brookfield LVF), water activity (measured in a Decagon CX-10) and colour (measured in a Hunter Lab) were measured. Total solids content of the mixture was 20.6% (soya milk 3.8%, oat flour 9.8%, whey solids 7.0%); lactose was 5.4% and protein 3.9%. In the yoghurt, total solids were 14.3%, with lactose 4.6% and protein 5.0%.

Fermentation A commercial yoghurt starter culture (Rosch) was used. Fermentation took place at 42oC. Initially 12 hours was allowed but it was found that fermentation was complete after 7 hours and this time was therefore used throughout the work. A check was made on the effects of the heat treatment on the microflora. Total available counts, coliforms, yeasts and moulds were all negligible.

Results

The chemical composition of the mixture was obtained from tables, as well as that for the raw components and these compared with the chemical score of an infant food formulation, see table 1.

	Soya	Oats	Whey	Mixt.	Yog'rt	Infant food
Isoleucine	113	95	190	124	155	105
Leucine	111	103	169	121	164	109
Lysine	116	67	205	119	125	102
Methionine+Cystine	83	129	149	115	83	120
Phenylalanine+Tyrasine	137	138	117	133	182	140
Threonine	95	85	210	118	113	88
Tryptophan	130	130	240	155	130	130
Valine	96	102	144	109	138	110

Table 1: Essential aminoacid scores of raw materials, yoghurt and an infant food

Sources: oats, Paul and Southgate, 1978; whey, Hambraens, 1982; infant food, del Valle et al, 1981.

Water availability for the gelatinisation of starch and possible changes in water activity and viscosity during fermentation were monitored with the results shown in table 2. Fermentations were performed with the original mixture and diluted with 50% water and 100% water; milk was used as a control.

	Original mixture	+50% water	+100% water	Milk
Composition:				
Water (%)	79.4	85.3	88.5	85.7
Total solids (%)	20.6	14.7	11.5	14.3
Viscosity (cp):				
80 C, 20 min	1300	40	12	4
80 C, 60 min	1240	105	20	-
Water activity:				
80 C, 20 min	0.986	0.990	0.990	0.980
80 C, 60 min	0.988	0.991	0.992	-

Table 2: Influence of dilution and heat treatment on viscosity and water activity

The buffer capacity of each of the raw materials and that of the mixture were compared to that of milk by the addition of lactic acid to the suspension and measurement of pH. The colour of the raw materials and that of the mixture before and after fermentation was analysed.

Discussion

Yoghurt starters have very complex nutritional requirements: milk supplies them with all of the essential nutrients such as a carbon source (lactose), aminoacids and proteins, vitamins and minerals. These organisms do not grow and produce lactic acid in soya milk as they do in milk, therefore fortification with other sources of carbon and/or nitrogen is needed to enhance fermentation performance. *Streptococcus thermophilus* does not need additional sugars because it can ferment sucrose and glucose, but it is stimulated by nitrogen supplements; on the other hand *Lactobacillus bulgaricus* is unable to utilise sugars present in soya milk, except glucose which reaches low concentrations; it also is stimulated by nitrogen supplementation. In the mixture used in this study, lactose was present at a similar level to that in milk. It was not therefore expected that there would be any limitation due to lack of fermentable sugars. In fact, according to profiles of acid production, pH, carbohydrate consumption and proteolysis, the fermentation mixture is identical to that of milk for yoghurt production.

In the system employed, viscosity changes due to fermentation were negligible. Good viscosity values were obtained because of the gelatinisation of oat's starch during heating. Neither water activity nor milk colour changes during fermentation. Heat treatment has very little influence on the colour. However, the mixture is less white than milk and a little less green than milk. Milk and whey clearly have a green component which could be due to riboflavin as there is a reduction of the vitamin after fermentation by yoghurt cultures. The mixture is in fact a little red (as opposed to green) before fermentation and shifts to green after it. The oat flour suspension alone became darker after heat treatment, but this effect is not noticeable in the mixture.

Further work

A yoghurt-like product with adequate nutritional, physical and chemical characteristics has been obtained from plant foodstuffs and whey. Sensory evaluation of the product is now in progress at UAM-I using three different yoghurt starters. Nutritional studies *in vivo* are also in progress at Oxford Polytechnic.

II. Decontamination of meat

Introduction

Although in healthy animals, muscle tissue is expected to be free from contamination, it frequently becomes infected with microorganisms after the animal has been slaughtered. Poor hygiene and handling practices in the abattoir are responsible. Refrigeration can arrest the process of decay but this is expensive in a country like Mexico. Moreover, many animals are slaughtered in semi-rural municipal abattoirs or rural areas where such facilities are absent. The problem is compounded by the high temperatures encountered in transport, distribution and storage of the carcases: temperatures often reach 35°C and many hours elapse before the meat reaches the consumer.

There is an extensive research literature on the development of the microorganisms in meat. Chemicals are sometimes used to prevent their growth, such as acetic and lactic acid. The latter can be quite effective but it is expensive. An alternative is to use lactic acid bacteria, which some authors have found to be of great value in extending meat shelf-life. Research here has concentrated on the fermentation of added carbohydrates (sucrose) in ground meat in order to develop attractive flavours and extend shelf life, and the changes occurring during meat spoilage.

Objectives

The aims of this project were to study microbial changes and the related chemical composition in meat stored in the semi-tropical conditions found in Mexico, including the effects of extrinsic factors (e.g. air exclusion). A second aim was to study lactic fermentation as a means of increasing meat shelf life. This would involve study of the amount and type of microbial populations in meat cuts so treated.

Materials and methods

The first series of experiments used amines as indicators of spoilage. Beef and pork samples were used, refrigerated and both wrapped in saran film and unwrapped. Total amine nitrogen was determined following exchange chromatography. Because information on slaughter time was not available, some samples of rabbit meat were also used. Counts were made of Pseudomonads, total aerobic viable bacteria and lactic acid bacteria. Results showed that wrapping did not promote the growth of lactic acid bacteria so as to outnumber other types, but the production of diamines was slower than in unwrapped samples. Rabbit meat spoiled faster because of its pre-rigour condition.

In the second series of experiments, inoculation with seven microbial strains of lactic acid bacteria was carried out on the meat samples. The factors studied were oxygen availability (saran-wrapped or unwrapped samples); carbohydrate source (addition of 1% sucrose to half of the samples); storage temperature (15 and 27°C); and strains inoculated. The response variables were: lactic acid concentration; pH; lactic acid bacteria counts and Pseudomonads counts. The results showed lower pH values and Pseudomonads counts for wrapped samples, stored at 27°C and inoculated with *Lactobacillus bulgaricus* and *L. acidophilus*. No significant differences were found between the two species for any of the response variables studied.

Subsequently pork and beef samples on a similar experimental design were treated with: Inoculum of *L. bulgaricus* and *Pediococcus pentosaceus*; a commercial starter inoculum of *M. kristinaevarians* and *L. bulgaricus*; and left uninoculated. The results favoured the samples inoculated with the commercial product in terms of a decrease in Pseudomonads counts and lower pH values, although no significant differences were obtained between species for any response variable. Extrinsic factors such as wrapping, temperature and sucrose addition also affected the quality of the samples regarding low Pseudomonads counts and high lactic acid concentration.

Data were analysed by a SAS package adapted to a PC (HP Vectra 286/12) for analysis of variance and regression analysis.

Discussion

Pseudomonads become the predominant strain in meat stored in air. Dainty (1986) reported that glucose is used exclusively to support the initial phase of growth of Pseudomonads. *Brocothrix thermosphacta* and an *Enterobacter* sp. After glucose depletion, Pseudomonads and the *Enterobacter* sp. metabolise aminoacids (the main source of volatile esters, amines, thiols, sulphide and ammonia) and lactic acid (Edwards *et al*, 1987). However, variation in meat composition, non-enzymatic processes such as lipid oxidation, reactions catalysed by meat and/or bacterial enzymes and analytical methodology may change the pattern of compounds identified in a particular sample. All these compounds produce the fruity, putrid, sulphury and ammoniacal odour characteristic of the type of microbial population present. Liquid oxidation can also be caused by lipases produced by some psychrotrophic bacteria such as *Pseudomonas fragi*.

Brocothrix thermosphacta has been detected also in aerobically spoiled meat, but was thought not to be important except in lamb. This is the only Gram-positive organism found in high numbers on aerobically-stored meat. When glucose is depleted, *B. thermosphacta* metabolises glutamic acid. However, some authors claim that it is strictly saccharolytic. This organism, through an incomplete oxidation pathway of glucose metabolism, produces acetoin (3 hydroxy-2-butanone), diacetyl (2,3-butanone) acetic acid and, if glucose concentration is high enough, 2,3-butanediol.

Non-volatile compounds produced included some biogenic amines such as putrescine and cadaverine, formed from ornithine and lysine respectively. *Pseudomonads* are the major putrescine producers, whereas *Enterobacteriaceae* produce cadaverine. Increasing storage temperature also increases *Enterobacteriaceae* numbers, and hence cadaverine concentration. Other diamines, such as tyramine, spermidine, diaminopropane and agmantine were also detected in meat stored in air. When a semipermeable wrapping film is used, some CO2 is built up in the package, slowing down growth of *Pseudomonads* and encouraging the growth of *B. thermosphacta*, so the latter becomes in some cases the dominant flora. In this situation glucose is converted to acetoin and later aminoacids are converted to short chain fatty acids producing a characteristic sweet, sickly, malty odour.

Further work

Further studies are required in relation to microbial and chemical changes during storage and spoilage stages in the two species under study (beef and pork); the ability of genetically-improved strains to compete successfully with spoilage and pathogenic microorganisms and to produce pleasant flavour and aroma compounds; and the biochemical effect of extrinsic factors.

Other studies will include flavour and aroma production in meat products extended with starch or other amylaceous materials, which decreases the cost of a meat-based product, making it more accessible for low-income populations. This is already done in many countries, sometimes legislated and sometimes not. However, imitating the flavour and aroma of an "original" 100% meat-product is not yet possible.

Publications

Chávez, A. and Guerrero, I. (1990). Detection of biogenic amines as meat spoilage indicators. *Journal of Muscle Foods*, in press.

Chávez, A.M. and Guerrero, I. Efecto de la inoculación de bacterias lácticas en carne en la disminución de la poblacion de *B. thermosphacta*. (Effect of inoculation with lactic bacteria in meat on the reduction of the *B. thermosphacta* population.) *Revista de la Asociación de Tecnólogos en Alimentos de Mexico*, in press.

Galvan Portillo, M.; Wacher-Rodarte, C; Gallardo, F; Farres, A. and Marshall, V (1991). Elaboration of yoghurt with low solid containing milk using polymer producing strains. *Journal of Dairy Research*, in press.

García Garibay, M. and V. Marshall (1991). Studies on polymer production of a strain of *Lactobaciullus delbruckii sp. bulgaricus*. *Journal of Applied Biotechnology*, in press.

Guerrero, I.; Mendiolea, R.; Chávez, A; Ponce, E. and Estrada, P. (1991). Inoculation of lactic acid bacteria on pork and beef surfaces as a means of decontamination. *Meat Science*, in press.

Ponce, E. and Guerrero, I. (1991). Effect of humectants in raw meat. Submitted to *Journal of Muscle Foods*.

Prado, A. and Taylor, A. (1991). Study of volatile compounds produced in meat inoculated with lactic acid bacteria. Submitted to *Meat Science*.

Shirai, K.; Pedraza, G.; Guitierrez, M. and Marshall, V. (1991). Promotion of a yoghurt-like product from soya, oat and whey. I. Fermentation studies. Submitted to *Journal of the Science of Food and Agriculture*.

Shirai, K.; Gutierrez, M. and Marshall, V. (1991). Production of a yoghurt-like product from soya, oat and whey. II. Sensory evaluation studies. Submitted to *Journal of the Science of Food and Agriculture*.

Patents

Mendoilea, R.; Prado, A.; Ponce, E. and Guerrero, I. Procedimiento para descontaminar carne en canal por el uso de fermentación láctica (Means of decontaminating meat by use of lactic fermentations) (Applied for in Mexico, June 1990).

Background references

Dainty, R.H.; Edwards, R.A.; Hibbard, C.M. and Ramantanis, S.V. (1986). Bacterial sources of putrescine and cadaverine in chill stored vacuum-packaged beef. *Journal of Applied Bacteriology*. **61**, 117-23.

Del Valle, F.R.; Villaneuva, H.; Reyes Govea, J.; Escobedo, M.; Bourges, H.; Ponce, J. and Munoz M.J. (1982). Development, evaluation and industrial production of a powdered soy-oats infant formula using a low-cost extruder. *Journal of Food Science*. **46**(1), 192-7.

Edwards, R.A.; Dainty, R.H. and Hibbard, C.M. (1987). Volatile compounds produced by meat pseudomonads and related reference strains during growth on beef stored in air at chill temperatures. *Journal of Applied Bacteriology*, **62**, 403-12.

Hambraeus, L. (1982). Nutritional aspects of milk proteins, *In* Developments in Dairy Chemistry. I. ed. Fox, P.F. Elsevier Applied Science Publishers, London.

Paul, A.A. and Southgate, D.A.T. (1978). *In*: The Composition of Foods. eds. Mac Cance and Widowson. 4th Edition. Elsevier/North Holland Biomedical Press, London.

Wang, H.L. and Hesseltine, C.W. (1982). Coagulation conditions in tofu processing. *Process Biochemistry*, **17(1)**, 7-10.

5 Basic and applied research on glucosyltransferase enzymes for the production of new carbohydrate derivatives from sucrose.

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Background

During the last two decades, the sugar industry has been subject to dramatic changes due to biotechnological developments in this field. The production of high fructose syrups from corn as well as the synthesis of intense sweeteners, such as aspartamine, Alitame^{R}, and Sucralose^{R}, linked with considerable increases in sucrose production and yield from cane and beet roots, are some of the causes of the low international price of sucrose.

In such conditions, there is an urgent need for research in order to find alternative technological applications for sucrose. Moreover, the substitution of sucrose in low calorie foods for synthetic sweeteners requires what has been called "bulking agents", i.e. ingredients with the same texturing properties as sucrose and with low digestibility.

One of the very few examples of enzymatic valorisation of sucrose yielding a high added-value derivative is the synthesis of dextran. Dextran is a D-glucose polymer (glucan) which has found industrial applications in various fields: medical, pharmaceutical and fine chemistry. It is produced by glucosyltransferases of microbial origin (*Leuconostoc* sp.).

Among the enzymes known as glycosyltransferases, dextransucrases are of singular importance. Such microbial enzymes are able to produce glucans, called dextrans, from sucrose. These polymers have wide physical and chemical characteristics, as a function of the dextransucrase source and specificity.

In the fifties, a research group of the US Department of Agriculture did important work on bacterial strains producing various types of dextran (*Leuconostoc sp.* and *Streptococcus* sp.).

After this pioneering work, research concentrated mainly on dextrans produced by one strain of *L. mesenteroides* (strain NRRL B-512f) as well as by some strains of *Streptococcus* sp. The reason is that the former has found an important industrial market (plasma substitutes, chromatography support (Sephadex^{R}), photographic additives, etc), whereas the latter have been found to play an important role in tooth decay.

The structural characteristics of various dextrans were also studied in the sixties by using 13C NMR techniques in particular. This work confirmed that dextrans vary largely in terms of types of glucosidic linkages and degree of branching. The functional properties of dextrans clearly depend on their structure.

It is important to point out that until now no systematic study related to the production and properties of dextrans has been performed in order to develop new applications.

The previous work of dextran classification carried out in the fifties and sixties represents a great potential for further research in order to find new properties and applications of such dextrans.

Objectives

The general objective of this project is to develop, through screening and characterisation of glycosyltransferase activities, a general tool allowing the controlled transfer of carbohydrate moieties onto a variety of acceptors. This will result in the synthesis of oligosaccharides of desired molecular weight, type of linkages, number and position of branching, useful for the food and feed, fine chemistry and pharmaceutical industries; and glycosylated molecules of improved physico-chemical properties or presenting useful characteristics as intermediates in organic synthesis.

This type of product will open new alternatives to sucrose valorisation.

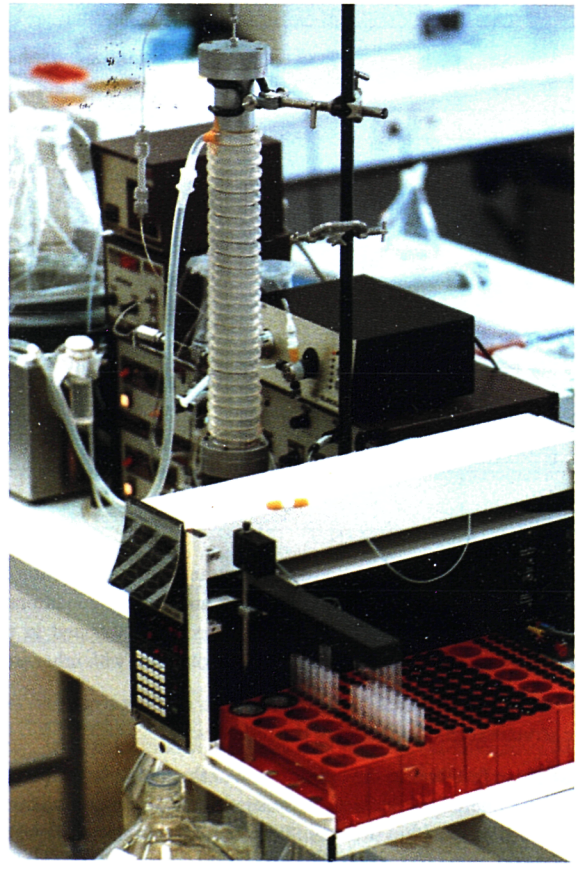
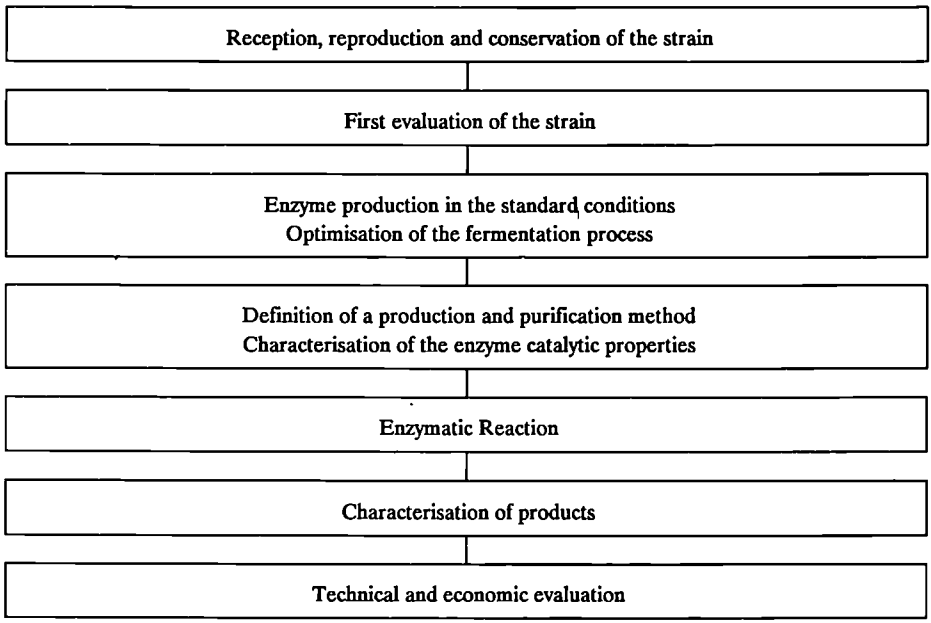


Figure 1: Oligosaccharide purification using semi-preparative HPLC.

Materials and methods

Strains These have been selected from the Northern Regional Research Center Collection in Peoria, Illinois, among the 90 strains, initially described in the fifties, then incorporated in UNAM's and BioEurope's collections, preserved and reproduced using usual methods in microbiology.

Overall strategy The strains have been characterised according to the following strategy:



Results

The classical techniques involved in dextransucrase production have been initially applied for a first evaluation of the strain: culture medium and culture conditions, enzyme activity, acceptor reactions, enzyme purification, etc. The reaction products obtained in the presence of acceptors have been analysed and compared with those already obtained with other strains, as well as the polysaccharides.

This first evaluation has based the selection of a few strains on the glucosyltransferase productivity and the structural characteristics of the products.

With these few strains, the optimisation of the fermentation process has been carried out, based on the usual parameters of the lactic fermentation medium composition and additives, temperatures and pH, aeration, fermentation process. With these strains, a purification procedure of the enzyme has been performed, using phase partition techniques.

Samples of purified glucosyltransferases have thus been produced and then operated under different reaction conditions: the production of polymers; and the production of oligosaccharides in the presence of acceptors such as maltose and oligodextrans.

High molecular weight glucans were characterised using precipitation, chromatographic, and enzymatic techniques. In particular, their resistance to specific hydrolases, such as dextranase and glucoamylase, has been studied. Oligosaccharides were characterised using chromatographic methods (HPLC) such as reverse phase chromatography and ion exchange chromatography.

Up to now, 14 L. *mesenteroides* strains have been studied. Four strains have a potential industrial interest. Strain NRRL B-523 is characterised by a strong production of insoluble glucans, whose properties are under investigation. Strains NRRL B-1299, B-742 and B-1298 exhibit the production of oligosaccharides differing from B-512F oligosaccharides by the nature of glucosidic linkages.

In particular, new oligosaccharides containing α (1-2) glucosidic linkages are developed at pilot scale (1 tonne/month) for food and feed applications. A worldwide patent has been filed by BioEurope, covering these original oligosaccharides.

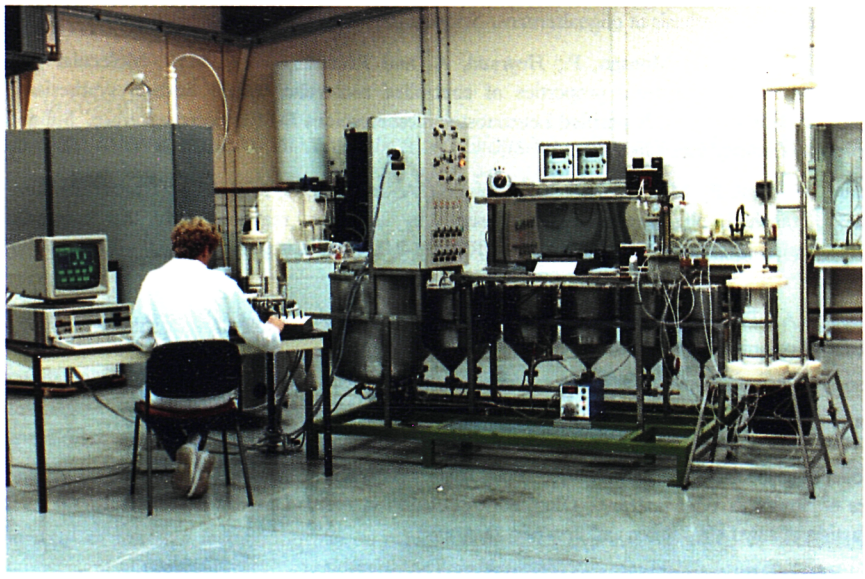


Figure 2: Pilot-scale purification of glucosyltransferase enzyme in BioEurope laboratories

Conclusions

The project has now reached half way. This collaborative work involves the UNAM laboratory doing research in order to find new strains of *Lecuconostoc* sp. capable of producing efficient glucosyltransferases and interesting oligosaccharides. Meanwhile BioEurope is in charge of the evaluation of the potential interest of such enzymes and sugar derivatives in the field of feed additives, dietetic food and pharmaceuticals.

As a first and very promising result of this collaboration between our two laboratories, BioEurope is developing at pilot scale a new oligosaccharide which has recently been patented worldwide as a feed additive having a beneficial effect on intestinal microflora (preprobiotic). Human applications are also under study.

This result encourages us to find other sugar derivatives and applications during the last two years of this project.

Publications

López Munguía, A.; Remaud, M; Pelenc, V; Paul, F.; Monsan, P; Biton, J.; Michel, J.M. and Land, C (1989). Enzymatic synthesis of oligoalternans. *Enzyme Engineering*, **10**, in press.

Monsan, P; Paul F.; Remaud, M. and López, A. (1989). Novel enzymatic modification and production of oligosaccharides. *Food Biotechnology*, **3**(1), 11-29.

Paul, F.; Remaud, M.; Pelenc, V. and Monsan, P. (1989). Enzymatic synthesis of novel oligodextrans. *Enzyme Engineering*, **10**, in press.

Pelenc, V.; López-Munguía, A.; Remaud, M.; Biton, J.; Michel, J.M.; Land, C.; Paul, F. and Monsan, P. (1991). Enzymatic synthesis of oligoalternans. *Sciences des Aliments*, in press.

Remaud, M.; Paul, F.; Monsan, P.; Heyraud, A. and Rinaudo, M. (1991). Molecular weight characterisation and structural properties of controlled molecular weight dextran synthesised by acceptor reaction using highly purified Leuconostoc mesenteroides B-512F dextransucrase. Submitted to *Journal of Carbohydrate Chemistry*.

Remaud, M.; Paul, F.; Monsan, P.; López-Munguía, A. and Vignon, M. (1991). Characterisation of α (1-3) branched oligosaccharides synthesised by acceptor reaction with the extracellular glucosyltransferase from *L. mesenteroides* NRRL B-742. Submitted to *Journal of Carbohydrate Chemistry*.

Valvidia, L.A.; Paul, F.; Monsan, P. and López-Munguía, C.A. (1989). Dextran synthesis in the presence of ozianions, *Enzyme Engineering*, **9**, 390-4.

Patent

Procédé pour préparation enzymatique d'oligodextranes utiles dans la fabrication de substituts de sucres et nouveaux oligodextranes (Enzymatic preparation method for oligodextrans useful for the production of sugar substitutes and new oligodextrans).

- French Patent application no. 8801041, filed 29-01-88.
- European Patent application no. 88403084.2, filed 05-12-88 (Germany, Austria, Belgium, Spain, France, Greece, Italy, Switzerland, Luxembourg, Netherlands, United Kingdom, Sweden).
- US Patent application no. 07/548.938, filed 27-07-90.
- Danish Patent application no. 1794/90, filed 06-12-88.
- Canadian Patent application no. 587/844, filed 10-01-89.

6 The glutamine synthetase isozymes of *Rhizobium leguminosarum* and *R. phaseoli* and their role in nitrogen metabolism.

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Background and objectives

Bacteria belonging to genus *Rhizobium* elicit the formation of symbiotic nodules on the roots of leguminosae. In these nodules a differentiated form of *Rhizobium*, called bacteroids, catalyse the conversion of atmospheric nitrogen into ammonia, which is then used by the plant for the biosynthesis of proteins. The establishment of symbiosis is regulated: in fact when nitrogen fertiliser is present in the soil no nodules are formed.

The study of nitrogen metabolism and its regulation in *Rhizobium* spp. is crucial to understand the establishment of the symbiosis, its regulation and its efficiency. The aim of this project is to study the genes coding for the glutamine synthetase isozymes in *R. leguminosarum* bv. *viciae* and bv. *phaseoli* (hereafter called *R. l. viciae* and *R. l. phaseoli*, respectively). We also study their gene products, the regulation of their expression and the study of some regulatory genes. These studies will contribute to a better understanding of the mechanism of ammonia assimilation in *Rhizobium* spp.

Two forms of glutamine synthetase, GSI and GSII, have been demonstrated in all species tested of the family *Rhizobiaceae*. GSI is similar to the single GS of enteric bacteria and GSII is heat labile and shares extensive homology to a GS of *Phaseolus vulgaris* roots. We have recently described a third gene from *R. leguminosarum* expressing in *Klebsiella pneumoniae* a GS different from GSI and GSII (Espin *et al*, 1990).

We have cloned the gene coding for GSI, glnA, both from *R. l. viciae* and from *R. l. phaseoli*. Upstream of glnA we find the presence of a regulatory gene which we have named glnB. We have also described a regulatory mutant which is completely devoid of GSII activity.

The two group leaders have been collaborating for a long time, see page 65. The Italian group is better organised for studies of molecular biology, while the Mexican group is better organised for studies on plants, Thus, the interaction is proving very fruitful because of the complementary abilities of the two groups. Moreover, exchange of material, bacterial strains and information is very useful. It should be pointed out that *R. l. viciae* interacts with a crop which is important in Europe, *Pisum sativum*, while *R. l. phaseoli* interacts with *Phaseolus vulgaris*, which is important in Mexico. Thus, any application resulting from this basic research project would be beneficial to both regions.

Materials and methods

R. l. viciae and *R. l. phaseoli* wild-type strains, as well as several mutants, are being used or will be isolated. Enteric bacteria like *Escherichia coli* and *K. pneumoniae* are used either as a tool for genetic engineering experiments or as hosts for expression of *R.leguminosarum* genes.

Methods used include: gene isolation, DNA sequencing, construction of mutants, determination of sites for transcription initiation and termination, RNA concentration in different growth conditions, protein purification, amino-terminal sequencing, determination of post-translational modification of the proteins, production of antisera and their use in immunoblots and ELISA assays, and structural analysis of nodules by different microscopic methods.

Results and discussion

We have shown that glnB and glnA genes of *R. l. viciae* are preceded by promoters located upstream of each gene. We find the presence of a glnB-glnA and a glnA mRNA, whose intracellular concentration changes 2- to 3-fold when *R. l. viciae* is grown on different nitrogen sources. Primer extension analysis shows unique transcriptional initiation sites upstream of glnB and glnA. The glnB promoter is rpoN(ntrA)-dependent, while the glnA promoter does not contain a typical consensus sequence for previously-described promoters. In *K. pneumoniae* the glnB promoter requires active ntrC and ntrA genes and a DNA fragment containing 53 nucleotides upstream of the transcription initiation site shows full promoter activity, thus indicating that no NtrC binding sites on the DNA are necessary for activation in the glnB upstream region.

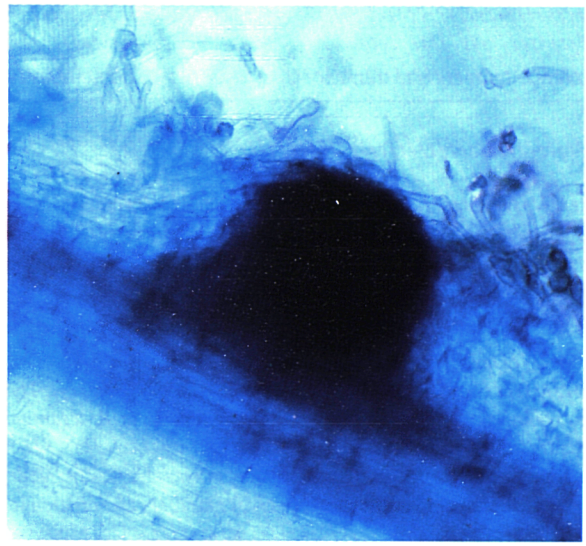

Figure 1: A symbiotic nodule in formation

A root of phaseolus vulgaris was treated with *Rhizobium leguminosarum*. After ten days the root was treated according to a protocol of G. Truchet and stained with methylene blue. This dye reacts with areas of active proliferation.

Using glbT DNA of *Rhizobium meliloti* as a hybridization probe we identified a *R. l. phaseoli* locus (glnT) expressing a glutamine synthetase activity in *K. pneumoniae*. A 2.2 kb DNA fragment of *R. l. phaseoli* was cloned to give plasmid pMW5a, which shows interspecific complementation of a *K. pneumoniae* glnA mutant. The cloned sequence did not show cross-hybridation to glnA or glnII, the genes coding for two glutamine synthetase isozymes of *Rhizobium* spp. While in previous reports on glnT of *R. meliloti* and *Agrobacterium tumefaciens* no glutamine synthetase activity was detected, we do find activity coded by the glnT locus of *R. l. phaseoli*. The glutamine synthetase (GSIII) activity expressed in a *K. pneumoniae* glnA strain from pMW5a shows a ratio of biosynthetic to transferase activity 103-fold higher than that observed for GSI or GSII. GSIII is similar in molecular weight and heat stability to GSI.

A Tn5 insertion mutant, strain CFN2012, of *R. l. phaseoli* devoid of glutamine synthetase II (GSII) activity was analysed. It was shown to contain the Tn5 insertion within a 11 kb BamHI fragment. The corresponding DNA fragment from the wild-type strain was isolated (pSM261) and shown to complement, when introduced into strain CNF2012, the absence of GSII activity. DNA sequencing of the region containing the Tn5 insertion revealed the presence of the ntrB and ntrC regulatory genes with no intercistronic region and that strain CFN2012 carries a ntrC::Tn5 mutation. Further analysis of strain CFN2012 indicated that this mutant has reduced levels of the PII regulatory protein and of GSI adenylylation. In contrast to ntrC mutants of other *Rhizobiaceae*, strain CFN2012 grows on nitrate as the sole nitrogen source.

Work is in progress on: (1) the analysis of the phenotype of a gln A mutant; (2) the characterisation of pure GSII and the changes induced on enzyme activity by ammonia; (3) the characterisation of glnII, the structural gene for GSII; (4) the sequence of the gln I gene and purification and characterisation of GSIII; (5) the analysis of the ntrBC promoter; (6) the preparation and characterisation by electron microscopy of antisera against GSI, GSII, GSIII and against the product of three regulatory genes; (7) the regulation of expression of the PII protein, the product of the glnB gene, and its post-translational modification; (8) the regulation of the glnB promoter in *R. leguminosarum*.

Publications

Chiurazzi, M. and Iaccarino, M. (1990). Transcriptional analysis of the glnB-glnA region of *Rhizobium leguminosarum* biovar *viciae*. *Molecular Microbiology*, **4**, 1727-35.

Defez, R.; Chiurazzi, M.; Manco, G.; Patriarca, E.; Lamberti, A.; Riccio, A.; Lopes, C.; Colona-Romano, S.; Moreno, S.; Meza, R.; Espin, G. and Iaccarino, M. The glutamine synthetase of *Rhizobium leguminosarum* and their regulatory genes. *In*: Nitrogen Fixation: Achievements and Objectives, eds. Gresshoff, Roth, Stacey and Newton. Chapman and Hall, New York and London, in press.

Espin, G.; Moreno, S.; Wild, M.; Meza, R. and Iaccarino, M. (1990). A previously unrecognised glutamine synthetase expressed in *Klebsiella pneumoniae* from the glnT locus of *Rhizobium leguminosarum*. *Molecular and General Genetics*, **223**, 513-16.

Iaccarino, M.; Rossi, M.; Defez, R.; Chiurazzi, M.; Manco, G.; Espin, G.; Lamberti, A. and Riccio, A. (1990). Regulation of nitrogen metabolism in *Rhizobium*. *In*: Inorganic Nitrogen Metabolism. Springer Verlag, 234-40.

7 Development of technological procedures for the production of concentrated and isolated vegetable proteins

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Background and objectives

Safflowers (*Carthamus tinctorius L.*) and amaranth (*Amaranthus hypochondriacus*) are considered to have high agronomic and food potential, especially for arid and semi-arid countries.

In Irapuato, Mexico, Paredes-López and co-workers have studied defatted safflower meal as a means for providing rich protein products (isolates) for human consumption. Little has been done on amaranth. This crop, which was traditionally grown in the Andean highlands, fell into disuse in the American continent after the Spanish conquest. Its resurgence today in Africa, Asia and America, due to its high potential, requires research for development of this ancient but still novel crop.

Some agronomic studies are taking place in Irapuato. However basic information on the protein composition of the seeds is needed both to improve and to increase its use for food. Paredes-López's group had already started some studies in this field. Because of the experience of the INRA laboratory in the field of plant protein biochemistry, it was planned to continue the basic studies on the composition and physicochemical properties of these proteins in Nantes. The work is being carried out by Barba de la Rosa, a student from Irapuato University, in the framework of her thesis.

In Irapuato, most of the studies were focussed on the potential for the incorporation of safflower protein in food products.

I. Safflower protein for food uses

Incorporation of safflower protein isolates into food products. Safflower meal contains high levels of fibre and phenolic glucosides which are associated with a bitter taste and cathartic activity. These factors prevent its use in food formulations. However, safflower has some agronomic advantages over other oilseed crops, such as drought tolerance in arid and semi-arid countries. Isolating safflower protein from the oil-extracted safflower cake is one method of introducing it into the human diet. Moreover, Mexico leads in the production of safflower at a world level. A new procedure (called "micellization", MP) was developed in Irapuato laboratory, and a conventional technique was adapted (called "isoelectric precipitation", IP), to produce safflower protein isolates (SPI). It has been demonstrated that these isolates possess desirable physico-chemical, functional and nutritional attributes. It is pertinent to mention that essentially all protein isolates and concentrates used by the food industry of Latin America are imported.

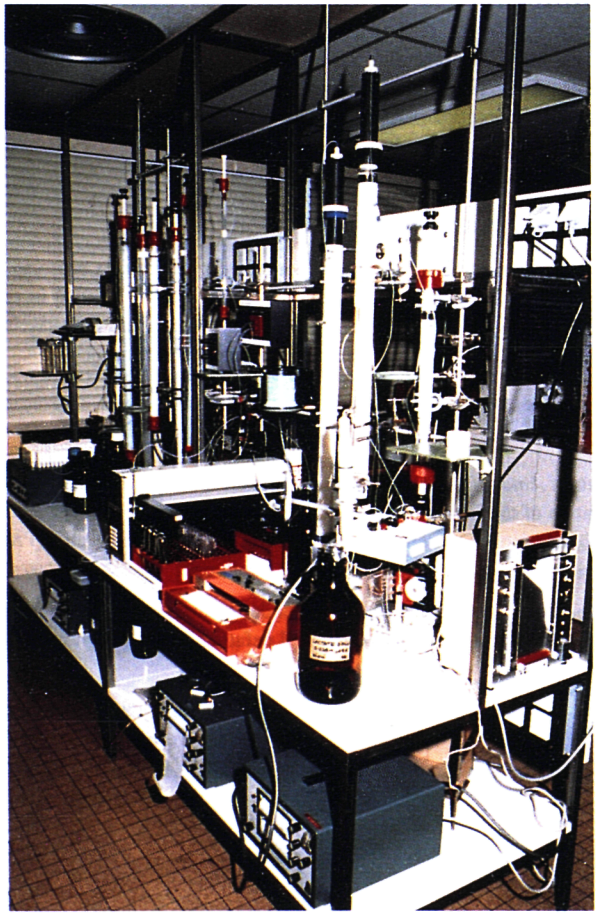

Figure 1: Low pressure protein chromatography

Enrichment of cereal-based food with oilseed protein has received considerable attention. Although snack foods such as cookies and crackers have received less attention than bread, they offer several important advantages including wide consumption, relatively long shelf-life and good eating quality. SPI has not yet been used in this way and the study reports the effects of adding it to wheat-flour based cookies. For this purpose, SPI was obtained by the different procedures mentioned above, using two different safflower meals: one defatted at a pilot plant level and the other one provided by an oil-extracting processing factory.

SPI samples prepared by MP and IP techniques were incorporated into sugar cookies. A commercial soybean isolate was used as a reference. The amount of protein isolate added was that needed to increase the protein content of wheat flour by 20, 40 and 60%. Physical and sensory properties of the products were not significantly ($P > 0.05$) affected by fortification with safflower protein prepared by MP from both sources, whereas some deterioration of these properties was observed when IP and soybean isolates were used.

Safflower proteins for food use. Under this project, scientific and technological information on safflower proteins was collected (Paredes-López, 1990). Safflower ranks at a low level of world production among the major oilseeds, but its availability is now increasing in various countries due to the remarkable performance of new varieties and hybrids. The proteins of the meal have an adequate balance of essential amino acids, lysine being the first limiting amino acid. However, the seed contains phenolic glucosides which are associated with a bitter taste and cathartic activity. The total seed proteins consist mainly of two fractions: 65% with a high molecular weight (260,000 to 290,000) and 26% with a low molecular weight (14,000 to 19,000). Procedures have been developed for the extraction of protein isolates with bland flavours and light colours, which are important sensory characteristics for most food applications. These isolates have high water solubility and acceptable oil binding, whipping and emulsification properties. They may be used as food ingredients in food products.

II. Amaranth proteins: extraction and characterisation

The remarkable nutritional value of amaranth has been well documented. The grain contains 15 to 18% of protein with acceptable lysine and sulphur amino acids, which are usually deficient in cereals and legumes respectively. However there is a lack of information on fractionation and characterisation of amaranth proteins. The studies carried on at INRA Nantes were devoted to these aspects.

Materials and methods

Amaranth samples. Mature seeds of *Amaranthus hypochondriacus* (Mercado cultivar, waxy type) were harvested in the experimental station of the Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP), Chapingo, Mexico. The samples (100g) were milled in a ball mill (Prolabo, percussion mill, Paris). Flour had an average particle size around 100 microns.

The flour was defatted with hexane in a 10% (wt/vol) suspension under continuous stirring during 24h; the flour was then air-dried at room temperature. The whole and defatted flours were stored at 4oC until used.

Protein fractionation procedures. Fractionation of amaranth protein by solubility was carried out on both whole and defatted flours. The efficiency of various protein solvents were compared. For the albumin and globulin fraction the following buffers were assayed:

1 0.1M sodium phosphate buffer pH7;

2 0.1M sodium phosphate buffer pH7 + 5% K2SO4;

3 0.8M NaCl aqueous solution.

4 0.1M sodium phosphate buffer pH7 + 5% 0.8M NaCl.

The prolamins and glutelins were extracted from the freeze-dried residue resulting from the albumin + globulin extraction.

The efficiency of the following solvents was compared:

- Prolamins: 70% aqueous isopropanol;
- Glutelins:
  - 0.1N NaOH aqueous solution
  - 0.1M sodium borate buffer pH 10
  - 0.1M sodium borate buffer pH 10 + 1% SDS
  - 0.1M sodium borate buffer pH 10 + 0.6% ß-mercaptoethanol
  - 0.1M sodium borate buffer pH 10 + 0.6% ß-mercaptoethanol + 1% SDS

Electrophoresis. SDS polyacrylamide gel electrophoresis was carried out according to Laemmli's method with and without reduction of the protein by ß-mercaptoethanol. To characterise high molecular weight subunits of prolamins and glutelins fractions better, a two step one-dimensional electrophoresis was performed. After the first migration in non-reductive conditions, some of the bands, which did not enter the gel, were cut off and transferred to a second gel for a migration in reductive conditions.

Amino acid analysis. This was performed by reversed-phase HPLC chromatography of the phenylthiocarbamyl derivatives (C18 Pico-tag column).

Results

The protein and fat content of the whole flour were 15.8% and 7.2% (on dry basis) and 18% and 1.6% on defatted flour respectively.

Fractionation of the proteins into solubility classes. The highest protein extraction for both whole and defatted flours, 68.5% and 63.8% respectively, was reached with sodium phosphate buffer pH7. The addition of K_2SO_4 did not improve the extraction as was previously noticed by Guegen and Barbot on pea proteins. The yield of protein extraction with these two buffers was in good agreement with the value reported by Konishi *et al* calculated from their data on the basis of 100% recovery: an extraction of 63.6% was found by these authors for the waxy flour sample.

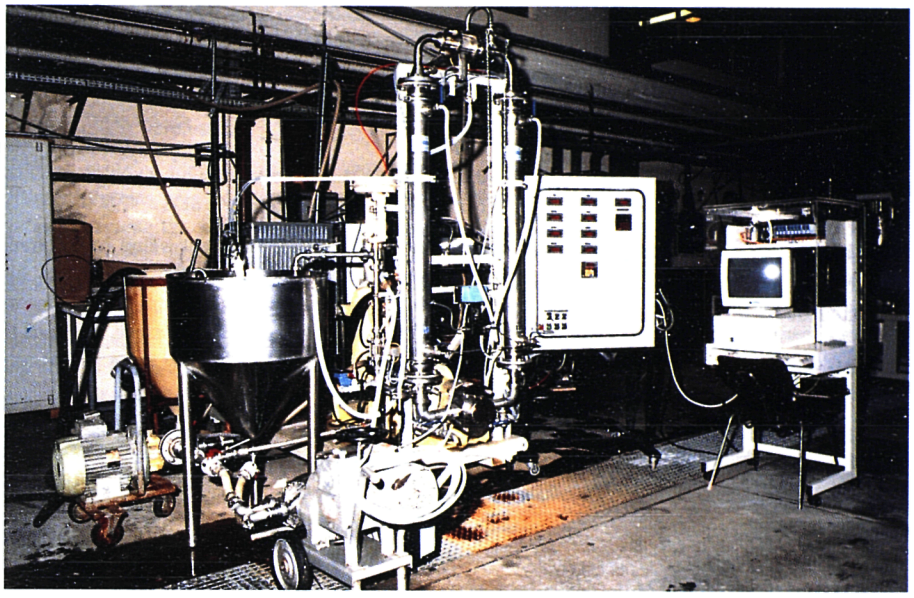

Figure 2: Ultrafiltration pilot plant for the preparation of protein extracts

The most efficient albumin-globulin extraction procedure (0.1M phosphate buffer pH7) was used as the first extraction step for preparing the residual material for prolamin and glutelin extraction. The albumin globulin fraction recovered represented about 66% of the total proteins, the albumin/globulin ratio being around 2. The amount of protein extracted by aqueous isopropanol (70% v/v) was 0.7% and 1.2% for whole and defatted flour respectively.

Similar extraction yields were obtained for glutelins by using either NaOH 0.1N or borate buffer as extracting agents. The addition of SDS slightly increased the extraction yield. On the other hand, the presence of a disulphide cleaning agent did not improve the solubilisation rate of these proteins. It may indicate, as compared to wheat glutelins, that the disulphide bridges are not much involved in the non-soluble characteristic of this amaranth glutelin fraction. The type of material used for the extraction, i.e., defatted or not, has a large influence on the yield of glutelin extraction. About 25% of the total proteins of the flour were found in this fraction when the starting material was the whole flour. On the other hand it gave about 40% when the flour was previously defatted. It is clear that hexane made insoluble some proteins, recovered in the glutelin fraction extracted from the defatted material, that are normally soluble in phosphate buffers.

The influence of defatting on protein extractibility was often noticed by other authors in cereals and shown to affect the proportion of the protein solubility classes according to the Osborne procedure.

An extraction procedure was developed to avoid the denaturation of proteins by hexane. In this procedure, the defatted step was applied to the residue from the albumin + globulin extraction. In these conditions, the presence of globulin-type proteins in the glutelin fraction was eliminated. It led to a proportion of glutelin-type proteins in the range 21 to 24% instead of 40% when defatted flour was

used. However it was observed that the protein content of the final residue tended to increase slightly, indicating that some limited denaturation effects induced by hexane could also occur in the glutelin fraction itself.

Characterisation of the different protein solubility classes. The influence of the extracting buffers on the electrophoretic pattern of the albumins and globulins was studied. It was only slightly modified by the extraction conditions. The main differences were between whole and defatted flours. However they were mainly quantitative and not qualitative. The same protein bands were observed for both, indicating that the denaturation by hexane was not selective.

Because of the poorer quality of the electrophoresis carried out with the whole flour samples and taking into account the qualitative similarity of the patterns obtained with both types of sample, defatted or not, we chose for qualitative studies the albumin and globulin fractions prepared from defatted flours.

In non-reducing conditions the albumin fraction appeared to be composed of some components of relatively high molecular weight (80 kD to 40 kD), of a major polypeptide migrating around 34 kD, of polypeptides in the range 31 to 26 kD and of low molecular weight components corresponding to the doublets 18-16 kD, 13-11 kD. Globulin patterns were composed of high molecular weight components (90-70 kD) bands of polypeptides corresponding the molecular weight around 52 kD, of a doublet at 38-37 kD, bands at 34 kD, 27 kD, 22 kD and 21 kD and of low molecular weight bands in the range from 15 kD to 11 kD. In fact reducing conditions did not alter the globulin PAGE pattern very much indicating that the major proteins in the globulin fractions did not contain an interchain disulphide bridge, Figure 3.

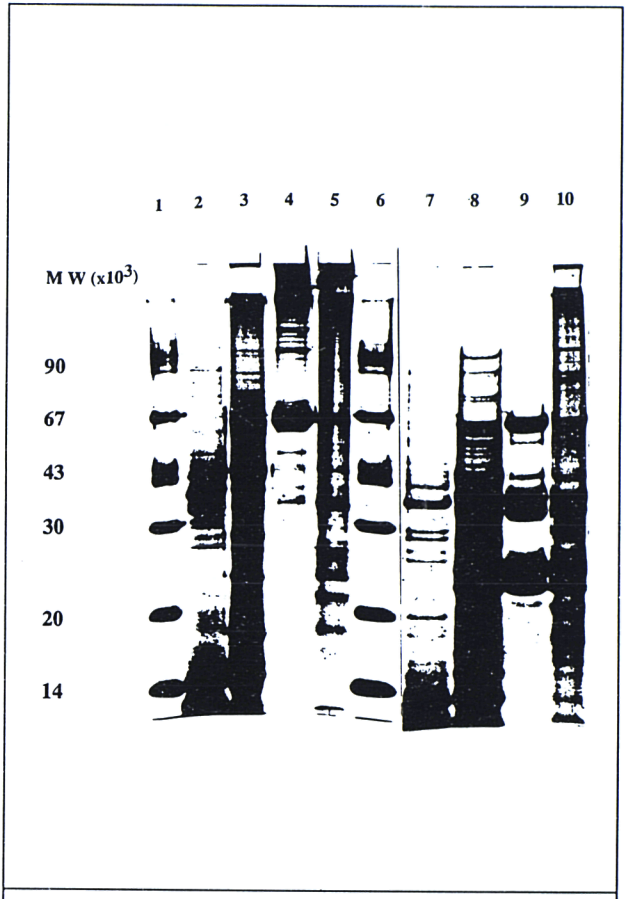

Figure 3: SDS PAGE pattern of the amaranth protein fractions.

In non-reducing conditions (1,2,3,4,5,6) and in reducing conditions (7,8,9,10): Standard (1,6); Albumins (2,7); Globulins (3,8); Prolam ns (4,9); Glutelins (5,10).

SDS-PAGE under non-reducing conditions of amaranth prolamins showed major components of high molecular weight at the top of the gel, many bands of high molecular weight (>90 kD) and a doublet at 67-60 kD. Meanwhile glutelins gave at least seven main bands at 67-60 kD, 47 kD, 34 kD, 25-21 kD, 18 kD and also high molecular weight components which cannot enter the gel. The components that did not enter into the gels were analysed in reducing conditions by SDS-PAGE. They are composed of similar polypeptides in the glutelin and prolamin fractions, which migrated around 35 kD and 22-25 kD.

It was noticeable that the different solubility classes, globulin, prolamin and glutelin shared electrophoretic bands in common, in non-reductive as well as in reducing conditions. In the absence of ß-mercaptoethanol, a polypeptide migrating around 67 kD was always seen. In reducing conditions, bands around 62 kD, 35-33 kD and 25-22 kD were found for the three protein families.

Amino acid composition. Amino acid compositions of the different fractions of amaranth proteins are given in Table 1. All the samples had large amounts of aspartic acid, arginine and glutamine.

Amino Acid	Albumin	Globulin	Prolamin	Glutelin
Aspartic ac.	74.9	88.7	88.4	80.7
Threonine	34.5	41.9	38.0	50.2
Serine	42.1	50.4	59.6	60.0
Glutamic ac.	203.8	176.8	181.0	156.0
Proline	28.6	39.7	64.8	45.8
Glycine	73.2	68.2	65.8	67.6
Alanine	36.2	40.4	41.1	43.6
Cysteine	88.4	39.7	15.4	21.8
Valine	42.9	48.2	51.4	56.7
Methionine	37.0	35.5	25.7	45.8
Isoleucine	39.5	42.6	54.5	49.1
Leucine	45.9	57.5	74.0	77.5
Tyrosine	34.5	39.7	40.1	4.3
Phenylalanine	35.4	51.8	44.2	60.0
Lysine	85.9	68.8	23.6	73.1
Histidine	3.3	10.6	20.5	9.8
Arginine	86.7	98.0	111.0	97.1

Table 1: Amino acid compositions of amaranth protein fractions extracted from defatted amaranth flour

The results are expressed in residue per 1,000 residues.

As in most of the seed, the albumin fraction has a high lysine and sulphur amino-acid content. The very low content in histidine was surprising. The globulin fraction has also a typical globulin composition. However the high content in methionine and cysteine has to be underlined. On the other hand the prolamin fraction and glutelin cannot be compared to the corresponding wheat fractions. These amaranth fractions are comparatively poor in proline and glutamic acid but rich in lysine and sulphur amino acid.

Conclusion

The various extraction procedures developed in the present study show the great influence of the defatting step on the distribution of the proteins in the various solubility classes. The insolubilisation by hexane of some proteins leads to an over-estimated glutelin fraction. To avoid this result, it seems to us that a quantitative evaluation of the solubility protein classes should be carried out on the whole flour, without defatting. On the other hand for qualitative studies we preferred to include a defatting step in the procedure. It significantly improved the technical quality of the electrophoretic pattern and of the chromatography. To avoid a significant modification of the qualitative composition of the fractions. It is important that defatting takes place at the right stage of the extraction procedure. Albumin and globulin fractions have to be prepared starting with defatted flour. For prolamin and glutelin preparation, the defatting step must be done after the extraction of the albumins and globulins. On the other hand, starting with a defatted flour would lead to an incomplete extraction of some denatured globulin and to a great qualitative modification of the glutelin fraction composition. The fractionation of the amaranth protein into solubility classes gave around 66% of the albumin-globulin, 1% of prolamins and 25% of glutelins. The ratio albumin/globulin, which was found to be around 2, was in good agreement with the value previously published by Paredes-López *et al.*

If we compare the present results with those of Konishi *et al* on the qualitative composition of the globulin fraction, we also found electrophoretic bands in the region 32-35 kD and 18-20 kD in reducing conditions. In agreement with this author we can confirm that these patterns as characteristic of subunits from 11S type proteins may indicate the presence of such globulin in amaranth. However, we mentioned that the whole electrophoretic pattern was relatively little affected by the disulphide reduction. We consequently thought that the 11S type protein is not the main globulin fraction. This has to be confirmed by chromatography studies. The poor influence of reduction on the electrophoretic pattern of the amaranth globulin fraction might be explained by the presence of a major 7S type globulin. It is well known that the subunits of the 7S type proteins are not crosslinked by disulphide bridges.

A great similarity between prolamins and glutelins was found in the polypeptides separated in SDS-PAGE with ß-mercaptoethanol. The differences of solubility between these two protein families might be attributed to some variations in the polarity of the similar polypeptides or to the number of disulphide bridges.

Moreover, the prolamins and glutelins proteins, which did not enter the gel in SDS-PAGE in non-reducing conditions, had polypeptides of similar molecular weights around 35 kD and 22-25 kD. It may mean that in amaranth seeds some proteins of high molecular weight are present as soluble and insoluble polymers constituted by similar polypeptides more or less linked by disulphide bridges. Moreover some of these polypeptides around 35 kD and 22-25 kD could be similar in the aqueous soluble and insoluble fractions.

In conclusion, this study confirms the specific composition of the amaranth seed, with some characteristics of both legume and cereal seeds, with important albumin, globulin and glutelin fractions and an interesting amino acid composition.

The composition of these fractions in various oligomeric proteins and in disulphide-linked polypeptides must however be more precisely studied. To complete these studies, we would like to characterise the 11S and 7S proteins of the globulin fraction better, using ultracentrifugation and chromatography techniques.

Publications

Barba de la Rosa, A.P.; Guéguen, J.; Paredes-López, O. and Viroben, G. Extraction and characterisation of proteins from seeds of amaranth. Submitted to *Journal of Agricultural and Food Chemistry*.

Mora Escobedo, R.; Paredes-López, O. and Ordorica-Falomir, C. (1990). Characterisation of albumins and globulins from amaranth. *Lebensmittel-Wissenschaft & Technologie*, 23, 484-7.

Ordorica-Falomir, C., and Paredes-López, O. (1991). Effect of safflower protein isolates on cookie characteristics. *Journal of Food Science and Technology*, in press.

Paredes-López, O. (1990). Safflower proteins in food use. *In* Developments in Food Proteins, ed. B.J.F. Hudson. Elsevier Applied Science Publications, London.

8 The basis for restructuring of the demersal fisheries of the Pacific Mexican coast

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Contract number and duration: CI1*/0431, January 1990 to December 1992.

Background and objectives

The penaeid shrimp fishery in Mexico is the third most important generator of employment and capital of the primary sector. Current management policies have failed to prevent over-exploitation of the resource and to sustain the viability of the fisheries. This fishery activity has a significant impact on the finfish which can be a potential economic resource.

The goal of the project is to improve the management of the Mexican Pacific fisheries by study of the following:

1 To study the penaeid shrimp biology and fishery in coastal lagoons to determine the temporal distribution of recruitment and ecological strategies; and evaluate the impact of culture practices upon the natural populations.

2 To improve the management of the demersal fisheries exploring new areas and evaluating alternative fishing techniques.

3 To determine the reproductive patterns and population structure of the multi-specific penaeid shrimp populations and their relation to the environment conditions at sea.

4 To study the temporal and spatial oceanographic dynamic variations and their relationship with climatic conditions.

5 To evaluate the penaeid shrimp populations and their productivity.

6 To determine the composition and structure of the demersal communities and their relation to environmental conditions.

7 To study in detail the fleet operating in the area and the fishing techniques employed.

Materials and methods

Due to the length of the Mexican Pacific coast, the study is being implemented in two areas, centre (Sinaloa-Nayarit) and south (Oaxaca-Chiapas). the most important shrimp fleet in the Mexican Pacific is at Mazatlan (Sinaloa), while the fleet at Salina Cruz (Oaxaca) is the third largest. The two areas are characterised by differences in their penaeid shrimp fisheries, faunal composition, topography and oceanographic conditions. Operations in Mazatlan started in September 1989 while operations in Salina Cruz started in July 1990.

To satisfy the objectives of the project the following main research subjects are being implemented:

Estuarine studies The following estuarine systems have been identified as study areas: Huizache-Caimanero (23°N, 106°W) in the central area, Laguna Inferior (16°20'N, 94°40'W) and Laguna Mar Muerto (16°20'N, 93°55'W) in the southern area.

The abundance, specific composition, recruitment, biological cycles and organismal influences upon the penaeid shrimp postlarvae are being determined. Data on diurnal variations of the current velocity at the sea surface and bottom, of the tide level, and of the salinity and temperature at the surface and bottom are being collected fortnightly. Monthly salinity and temperature are being registered at five to seven stations in each lagoon, during the same period. Wind strength during three days around full and new moon were collected at one-hour intervals.

Hourly zooplankton samplings are being carried out in the littoral currents of the beaches neighbouring the mouths of the Aguadulce and Botadero estuaries. The composition and specific abundance of penaeid shrimp postlarvae are being determined to establish the difference between the existing organisms in the sea and those that are able to adapt to the estuarine conditions during their migration.

The hydrology and penaeid shrimp postlarval abundance were determined in the southern area on the lagunar systems of Mar Muerto, Laguna Inferior, Laguna Superior and Laguna Oriental, which are interconnected and cover around 140,000 hectares. Fortnightly sampling is being carried out in the mouth of Mar Muerto lagoon, where a good relationship has been developed with the local fishermen in the Paredon and Chiapas cooperatives. In the mouth of Laguna Inferior the sampling is being carried out monthly. The Estuarine Fishing Cooperative of Salina Cruz and the Department of Fishing Promotion of the Oaxaca State Government cooperate in this sampling.

Oceanographic and fishing research cruises Survey cruises are being conducted on research vessels to obtain fishing and biological data about the coastal resources. The species catch composition by weight is determined and the length frequency determined for all the crustaceans and fish captured. The stage of sexual development and other biological parameters are obtained for the most important species. Oceanographic data are determined in each fishing station.

Figure 1: Capture of the first haul with the Mediterranean bottom trawl

This is ten times the yield of the shrimp trawl. Cruise CEEMEX-P4

Alternative fishing methods are used to determine their potential in the area. Long-lines, a Mediterranean demersal trawl, Agassiz net and traps are being tried as well as the shrimp trawler currently employed by the Mexican fleet.

The cruises in the Central area (Sinaloa-Nayarit) have been conducted with rv "El Puma". Starting in 1991, cruises will be carried out with the ship of the Instituto Tecnologico del Mar (ITMAR-Mazatlan) every three months. The Gulf of Tehuantepec will be studied by means of six-monthly cruises with rv "El Puma".

Commercial catch statistics and biological data collection The processing plants are being visited every day during the fishing season to sample the landing catch before it is separated into commercial sizes. Sexual maturity and sizes per species, kind of boat, trip time, gear type, mesh size, net aperture, engine power and fishing zones are all recorded.

During the 1989-90 fishing season, various experiments were carried out to determine the coincidence between the gonadal development state of the penaeid shrimp determined on board commercial shrimp trawlers and at the processing plants. The experiments demonstrate significant differences. Consequently in the current fishing season some fishing masters have been asked to separate the catches by trawling location and only these samples are studied at the processing plants.

The direct observation of the captures, the sale and transfer permits registered by the Fishing Federal Bureau and the relation between the retained and landed catch, will allow us to estimate the real catch of the Mazatlan shrimp fleet. The difference between the retained and landed catch is part of what is called "*rebusca*" (product sold outside the commercial arrangements of the cooperatives).

Sampling on-board shrimp trawlers This is being directed to determine the proportion of penaeid shrimp catch that are sold outside the commercial channels. Measurements are being made of its specific composition, distribution, length frequency and maturity stage and their dependence on the season, geographical area and fishing depth. For each trawl 50 to 200 organisms are studied and their abundance and specific composition are determined as well as the above-mentioned parameters for each species. The sea surface temperature, phase of the moon, sky and sea conditions are also recorded.

Trawling time, engine power and net characteristics are registered for each trawler. During the 1989-90 fishing season an average of 60% of the total trawls per trip were sampled in Mazatlan, representing 352 fishing days and 1420 trawlings.

During the closed season, from March to 15 May 1990, the catches were not landed in the processing plants. However, to obtain data on catches sold outside the market as *"camaron nacional"* or transferred to other states, the Fishing Departments in Sinaloa (Topolambo, La Reforma, El Castillo, Navolato, La Cruz, Mazatlan, El Rosario y Escuinapa) were visited. So far, the data compiled cover the period until October 1990.

In the current fishing season 1990-91, the catch of eight trawlers from Mazatlan fleet has been sampled, covering 461 trawls corresponding to 147 days of fishing operations. In the Tehuantepec area the sampling has covered five trawlers and 223 trawling operations corresponding to 76 days of fishing operations.

Economic and social factors related to the fishery Several research subprojects have been established by the Universidad Autónoma de Sinaloa (UAS) to study the development of the financial situation and organisation of the cooperative societies; the social, economic and labour aspects of the fisherman; and the effect of government through the policies of the public sector. To obtain information about the economic situation and production aspects, two surveys were designed.

The fishing cooperatives were classified in four categories, according to their relative annual yield. To carry out the survey, 34 students of the Escuela de Contabilidad y Administracion, UAS (the School of Accounting and Business Administration) were instructed by means of courses and lectures.

Information on financial sources; trade patterns; generation of indirect employment; and private sector processing plants is being collected. The sources are: the city and state archives, Mazatlan's Chamber of Commerce, Chamber of the trans-formation Industry and Ocean Garden Products, Inc. (the Government shrimp trading office in USA).

Due to the low productivity of the current fishing season and the poor financial status of the cooperative fishing companies, it has been possible to obtain financial information from several companies for some fishing seasons. The economic and physical development of the fleet operating in Mazatlan is the objective of this study.

The total number of cooperative companies for aquaculture and fishing production in the states of Sinaloa and Nayarit; the culture and catching area, economic influences, the number of partners and their seniority, material working conditions, indirect employment and the partners' labour conditions are all being studied.

Collection of data series The collection of historic data series on meteorology, hydrography, hydrometry, catch and effort data, estuarine yield data, etc. is still in progress. Following the start of activities in the southern part (Oaxaca-Chiapas), the collection of the data corresponding to the area has been started. These data will be edited and published by the UAS. The data are being computerised to allow a database needed to implement the objectives of the project to be created.

9 Development of fishing resources in Mexico: species selection and improvement

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Contract number and duration: CI1*/0432, October 1989 to September 1992

Objectives

The aims of this project were established bearing in mind the true socio-economic and geographical position of Mexico. We chose the Yucatan peninsula because it is one of the poorest regions in Mexico, lacking in mineral and oil resources, and also because its 139 800 km2 (over a quarter of the area of France) consist solely of permeable limestone. This signifies that there is no surface fresh water and that intensive agriculture cannot be developed. The only alternative source of development is the sea, especially its coastal shores. This has been clearly perceived by Mexico and, between 1977 and 1987, fishing increased by 64%. But this increased pressure on the salt water environment has given rise to biological imbalances and several species have fallen victim to overfishing.

Our work covers the three states on the peninsula: Campeche, Yucatan and Quintana Roo. The species to be studied are either overfished: the red grouper (or *mero*, the most intensively fished fish in Yucatan), the queen conch (the most extensively collected gastropod in Yucatan and in the Caribbean), or stock management is virtually non-existent as it is with oysters (the most widely eaten mollusc in Mexico). In order to run this programme, specific methods were applied to the three species both with regard to fundamental biology studies and to studies applied to aquaculture.

Materials and methods

Oysters from the Gulf of Mexico are either known as *Crassostrea virginica* or as *C. rhizophorae* according to some authors. We therefore deemed it necessary to carry out a genetic analysis of the different populations distributed throughout the Gulf. We opted for starch gel electrophoresis using enzymatic proteins extracted from the adducent muscle and from the digestive gland. We simultaneously analyzed two control populations, one in Canada (which corresponded to the *Crassostrea virginica*) and the other in Cuba (corresponding to the *Crassostrea rhizophorae*).

With the exception of the Laguna de Terminos in Campeche, no other lagoon in the Yucatan peninsula has oysters. Our aim was, therefore, to transfer seed-oysters (juveniles) taken from Terminos to the Laguna de Rio Lagartos which has no oysters and then experiment with the various primary cultivation techniques used in Europe: suspended and raised frames (on an oyster culture table). We also studied growth, mortality and life cycle.

Figure 1: Biometric data taken from the queen conch

The queen conch (*Strombus gigas*) is so overexploited that its collection has been banned in the State of Yucatan and rigorously restricted in Quintana Roo. It does not exist in Campeche. It was impossible to collect spat and, therefore, the only option open to us was to commence culture from scratch using eggs gathered in the field. For this reason, the procedure used for this species was very different from that used for oysters. We concentrated our studies on larval cultures in order to prepare for the setting up of a hatchery in Mexico. From there, it would be possible to establish localised and controlled populations.

Figure 2: Sampling of stomach contents from juvenile red groupers

The red grouper (*Epinephelus morio*) is a very sought-after fish which represents 88% of fish taken in Yucatan. The size of the catch is dropping which is indicative of overfishing. Many fish taken on the coast are juveniles measuring less than 18 cm and the fishermen do not get much for them. It would be possible to benefit from allowing these juveniles to be cultivated for a while. The aim of this project is therefore the cultivation of this fish either in running water ponds or in floating cages. We also studied the growth, mortality and sexual maturity of the species.

Furthermore, a basic biological study was undertaken since the biology of the species is not well documented.

Results

Oysters. A genetic study provided a complete picture of the populations present in the Mexican lagunas of the Gulf. Through a comparison with *C. virginica* in Canada and with *C. rhizophorae* in Cuba, it can be stated that the Gulf populations belong to the *C. virginica* species with some introgressive characteristics from the *C. rhizophorae* in three populations. This statement is backed by some 2000 electrophoretic analyses performed on samples from the States of Veracruz, Tabasco and Campeche.

Transfers of seed-oysters were implanted into the Laguna de Rio Lagarto. Suspended and raised bed cultures were compared and, generally speaking, were successful with growth to commercial size (80 mm) being achieved within 10 months. *In situ* seed-oysters were taken in Rio Lagartos which demonstrates that transferred oysters will reproduce in a new habitat.

Queen Conches. Histological studies on reproduction enabled us to establish the cycle on the Alacranes Reef (Yucatan) and on the Chinchorro Reef (Quintana Roo). These results were notified to the Ministry of Fisheries in order to enable them to improve their management of these sites. Ecophysiological studies were carried out on larvae. By means of this observation method using epifluorescence, we were able to establish the ingestion and digestion mechanism of larvae with regard to sundry monocellular algae.

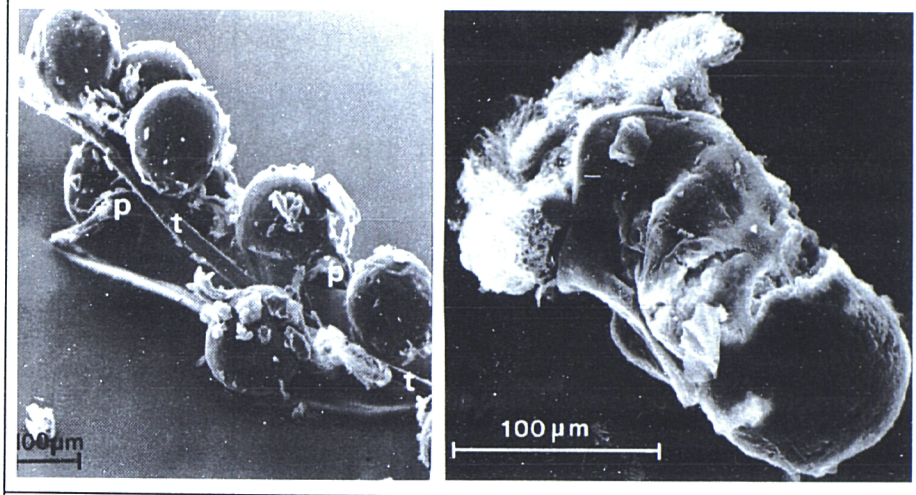

Figure 3: Queen conch eggs (left) and newly hatched spat (right)

The eggs are attached to a central stem (t) by means of a peduncle (p)

Red grouper. Over 1000 adult specimens were examined for the purpose of this study covering growth, diet and reproduction. This is the first really comprehensive study on reproduction which is specific to this hermaphrodite species which is female in its initial phase. Although most individuals become males, it was found that some specimens did not change sex and that others changed sex several times. The diet of the Yucatan population was determined: it included a high percentage of small crustaceans.

Closed-circuit husbandry required detailed adjustments over a period of several months. Within this now operational circuit, initial experiments have enabled us to establish optimal density for the fish and their feeding pattern.

Discussion and conclusions

The first obstacle facing the implementation of this project was the distance separating Merida from the sampling centres. The return trip to the Laguna de Terminos is 1200 km, to Banco Chinchorro (Quintana Roo), 1400 km, to the Tamiahua Laguna (Veracruz), 2500 km. Furthermore, at each sampling centre, we had to make contact with fishermen who had vessels and who knew the habitat concerned. Due to coordination problems between CINVESTAV and local agents, some of the projects failed to take place.

Access to coral reefs to collect queen conches was always difficult. For the Banco Chinchorro, we had to make a 40 km trip to reach the sampling grounds using a flat bottomed boat with an outboard motor. For the Banco Alacranes, the only vessels authorised to visit these were those of the Mexican National Navy and it takes a full day just to get there. Each mission to this site always takes a week because of the rota operated by the Navy's vessels. Clearly, therefore, samples forecast for each month were not always gathered.

Scientific methods for the development of aquaculture have been applied to three species. These species are well known to the consumer and are, therefore, very much in demand. They have widely differing ecologies and life styles. We therefore had to adapt specific methods to each of these species.

These methods were assessed not only locally but also by several trainees in Mexico and Latin America who often stay at the CINVESTAV IPN Unidad Merida.

Publications

Aldana Aranda, D. and Brulé, T. (1990). Maricultura en Yucatan (Aquaculture in Yucatan). *Interface*, 33, 19-22.

Aldana Aranda, D.; Le Pennec, M. and Amador, E. Essais d'acclimatation de l'huitre *Crassostrea virginica* dans les lagunes marines de l'état de Yucatan (Mexique) (Attempts to acclimatise the oyster *Crassostrea virginica* in the sea lagoons of Yucatan State in Mexico). Submitted to *Aquatic Living Resources*.

Aldana Aranda D.; Lucas, A.; Brulé, T.; Andrade, M.; Garcia, E.; Maginot, N. and Le Pennec, M. Observations on ingestion and digestion of monocellular algae by *Strombus gigas* larvae (*Mollusca gastropoda*) using epifluorescence microscopy. *Aquaculture*, in press.

Brulé, T. El pez Mero: estado actual del conocimiento del pez *Epinephelus morio* (mero) en el Banco Campeche (Current knowledge of the red grouper *Epinephelus morio* on the Campeche Bank). *Interface*, 36, 19-22.

Brulé, T. and Deniel, C. Données biologiques sue le mérou *Epinephelus morio* (Valenciennes, 1828) du Golfe du Mexique (Biological data on the red grouper *Epinephelus morio* of the Gulf of Mexico). Submitted to *FAO Synopsis*.

Soegono, D.; Aldana Aranda, D.; Lucas, A.; Moraga, D. and Brulé T. Population genetics of the American oyster *Crassostrea virginica* (Gmelin) along the Mexican coast of the Gulf of Mexico. Submitted to *Biochemical Systematics and Ecology*.

10 Chemical synthesis of indicator compounds for evaluating strategies to engineer virus-resistant plants

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Contract number and duration: CI1*/0438 October 1989 to September 1992

Summary

We want to explore an alternative approach, namely interfering with the translation of some essential viral proteins with the possibility of engineering virus-resistant plants. The biological tests require sufficient amounts of naturally-occurring hypermodified nucleoside queuosine; this compound has shown significant virus inhibition. We therefore have designed a synthetic procedure for the preparation of this compound supported partially by existing procedures.

The synthesis of queuosine consists in the condensation between the functionalised heterocycle preQ and the bulky group diisopropylideneaminocyclopentenediol location in the 5 positions of the heterocycle. We have already prepared the heterocycle preQ characterised by H NMR and C NMR. With respect to the bulky group we have obtained the intermediary I1 characterised by H NMR and the next step (allylbromination of I1) is in progress.

Another aspect of our work involves the preparation of histochemical substrates for the enzyme ß-glucuronidase which is an important gene marker in trangenic plants. In this sense we have prepared the compound sudan-glucuronide which produces orange crystals in the sites of enzyme activity inside the plant tissue. We have also tested its sensitivity by comparing this product with commercially-available analogues. The sudan-glucuronide forms orange crystals whereas X-glucuronide from Sigma Co. forms blue crystals. A disadvantage is that this compound when dissolved in a buffer solution presents a yellow background which makes the detection of low levels of enzyme activity difficult. We are now trying to overcome this problem.

Once we have obtained the antiviral compound queosine and have also purified the product sudan-glucuronide, we shall continue with the biological test and determine its structure through X-ray diffraction techniques. In the framework of this project, M. Brito Arias is preparing a doctoral thesis.

11 Structure, function and regulation of nodulin genes in *P. vulgaris*

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Contract number and duration: CI1*/0628, April 1990 to March 1992.

Background and objectives

Nodulins are a group of proteins that are specifically made in nodules formed on the roots of legumes when a symbiotic relationship is established with bacteria of the genera *Rhizobium*, *Bradyrhizobium* or *Azorhizobium*. The process of nitrogen fixation occurs in these nodules. Nodulins that are involved in the infection process and in nodule morphogenesis are called early nodulins, whereas the nodulins that are first made around the onset of nitrogen fixation are called late nodulins. Several late nodulins are involved in establishing the appropriate environment for nitrogen fixation, ammonium assimilation and transport of fixed nitrogen. In several legumes - alfalfa, pea, sesbania, soybean - the occurrence of nodulins is well established.

The identification and characterisation of *Phaseolus vulgaris* nodulins and nodulin genes has also been initiated. The group in Cuernavaca is especially interested in a family of nodulins of unknown function called nodulin 30 (NOD30).

Characteristics of NOD 30 A cDNA clone encoding NOD30 has been isolated. By hybrid released translation it has been shown that this cDNA clone hybrid selects nodule mRNAs that can be translated in several polypeptides with a molecular weight of 30 kD. The IEPs vary between 4.5 and 7.5. Therefore it is likely that a small NOD 30 multigene family exists. This is confirmed by Southern blot analyses of *Phaseolus* genomic DNA.

When total nodule RNA is translated in the presence of ^{35}S methionine the NOD30 proteins belong to the most abundantly synthesised polypeptides. This suggests that the NOD30 gene is expressed at a high level in the nodule. Nevertheless an in vivo nodulin with a similar molecular weight has not been observed. At the moment information about the *in vivo* NOD30 protein is lacking.

NOD30 mRNA appears just preceding the beginning of nitrogen fixation and is still present on day 28 after infection indicating that it is a late nodulin. In ineffective nodules induced by *Rhizobium* Fix- strains, there is a dramatic decrease in NOD30 mRNA concentration. In contrast, mRNA levels of other nodulins such as leghaemoglobin and uricase are not affected to the same extent. The aim of this project is to characterise in more detail the NOD30 protein and NOD30 genes.

Programme of work

Isolation and characterisation of NOD30 cDNA and genomic clones A set of NOD30 genomic and cDNA clones will be isolated - using the already isolated NOD30 cDNA clones as a probe - from available genomic and nodule cDNA libraries respectively.

The sequences of the cDNA clones will be determined. This will give a first clue about the possible function of the NOD30 protein. In addition we will compare the sequences of the isolated NOD30 cDNA clones to determine whether different NOD30 mRNAs have been cloned. We will especially search for member-specific sequences, since these sequences can be used to pick up the different NOD30 genes from the library. In addition the member-specific sequences will be used to determine the site of expression of the different NOD30 genes (see below). From the isolated genomic clones the sequences of the coding region as well as the promoters will be determined.

In situ hybridization The nodule cell type in which the NOD30 gene is expressed will be determined with the *in situ* hybridisation technique. When member-specific (see above) sequences become available the sites of expression of the different NOD30 mRNAs will be studied, with the *in situ* hybridisation techniques - during *Phaseolus* nodule development. The kinetics of appearance of NOD30 mRNA will be compared with that of other nodulin mRNAs (e.g. leghaemoglobin uricase, glutamine synthetase, ENOD2). The *in situ* hydridisation technique will also be used to study the expression of the ENOD30 genes in ineffective nodules formed by different *Rhizobium phaseoli* mutants.

Preparation of antibodies against NOD30 NOD30 antibodies will be raised against NOD30 protein made by *E. coli* expression systems. The NOD30 antibodies will be used to determine the *in vivo* NOD30 protein on Western blots. In addition the antibodies will be used to determine the intracellular location of the NOD30 protein with the immunogold technique. We hope that the combination of sequence data, cell type(s) in which the different NOD30 genes are expressed and the intracellular location will give insight on the function of the NOD30 nodulins.

Promoter analyses of a NOD30 gene With the help of the genomic sequences and S1 mapping data, the promoter region of NOD30 gene(s) will be studied. The promoter region (1.5-2kb) will be fused to a reporter gene such as the luciferase bacterial gene and the beta glucuronidase (GUS) gene. *Lotus corniculatus* will be transformed as previously described using vectors derived from the Ri plasmid of *Agrobacterium rhizogenes*. The expression of the NOD30 promoter (luciferase and GUS activities) will be studied in nodules formed on the roots of regenerated lotus plants. The person appointed in Wageningen will go to Cuernavaca to support the work on cytological localisation of these activities.

With deletion studies the regulatory sequences of a NOD30 promoter essential for nodule specific expression will be determined.

12 Molecular biology of dimorphism in *Candida albicans* and *Yarrowia lipolitica*

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Contract number and duration: CI1*/0631, January 1991 to December 1993.

Background

Candida albicans and Yarrowia lipolytica grow normally yeast-like in the laboratory. When subjected to different manipulations, they may grow mycelial. Their combined study will permit the understanding of the role of dimorphism in the pathogenesis of *C. albicans* and other dimorphic fungi. The morphogenetic processes are also useful models for the comprehension of the mechanisms involved in differentiation in eukaryotic organisms. *Y. lipolytica* is amenable to formal genetic analysis and to genetic transformation. Thus, it may help to understand the dimorphic transition of the pathogenic species, *C. albicans*, where these analyses are still not possible to perform, but from which there is a better understanding of the composition and structure of the cell wall and its relation to cell shape. In addition *Y. lipolytica* has been used on a large scale industrially and has industrial potential as host for secretion of foreign proteins.

Programme of work

Three different but overlapping approaches are proposed to tackle the project. A stock of monomorphic mutants from *Y. lipolytica* will be prepared using a screening technique which has already produced two yeast mutants. These mutants will be characterised by formal genetic analyses into complementation groups. Techniques of genetic transformation using different vehicles will allow the cloning of the genes involved in the morphogenetic process.

The second approach is to prepare monoclonal antibodies specific towards antigenic determinants from the yeast or mycelial forms from both fungi. These antibodies will permit the detection of early events in the dimporphic transition; the screening and characterisation of morphological mutants; and the study of the organisation of the cell wall.

The last approach involves the study of the role of polyamine metabolism and DNA methylation in the dimorphic transition of these fungi. Based on previous data, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC) will be analysed and the effect on dimorphism of specific inhibitors of these two enzymes will be tested. Levels of DNA methylation in both morphologies will be measured. If positive results follow, it will be possible to analyse the transcription of specific morphogenetic genes modulated through a methylation-hypomethylation process.

13 Studies on the *in vitro* propagation and cloning of elite and disease-resistant coconut palms

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J. Blake

Department of Agriculture, Horticulture and Environment, Wye College, University of London, Wye, near Ashford, TN25 5AH, United Kingdom.

Contract number and duration: CI1*/0764, February 1991 to January 1994.

Objectives

The link between CICY and Wye will ensure more rapid progress in the development of a successful technique for coconut micropropagation. Fundamental work will be continued at Wye and further development expanded at CICY. CICY will work directly with material such as immature embryos and pollen which cannot be transported to Wye.

The aim will be to obtain greater understanding of the present method of micropropagation and thus improve all stages to maximise yield whilst not increasing the overall time that tissue is in culture. The present 9-12 months from explant to plantlet will not be extended and we hope it will be reduced in order to avoid genetic or epigenetic variation occurring (as has happened with some oil palm cultures). A range of additional techniques will be evaluated in attempts to eliminate a calloid phase, to obtain greater multiplication in a short time and to improve the overall success rate.

The advantages of the combined CICY and Wye effort to establish a successful micropropagation protocol at the two sites, taken in conjunction with Wye's established link in the Philippines, increases greatly the prospect of benefitting coconut production worldwide.

Background

Present techniques of coconut micropropagation. The coconut micropropagation system being developed at Wye College uses immature coconut inflorescence explants which are cultured on sterile medium containing activated charcoal, cytokinin and 2,4-D as essential constituents. A type of organised callus, designated as "calloid", is produced which develops embryoidal structures. Plantlets are then formed when these are subcultured on media with reduced levels of 2,4-D, but the procedure has not been completely successful and only small numbers of clonal plants have been produced. However recent research has given rise to improvements in two of the stages and attention will now be concentrated on particular aspects which remain inefficient. These difficult parts of the protocol will continue to be investigated and alternative techniques, which could provide an improved system, examined.

Development of the present procedure

Embryonic calloid stage. The efficiency of callus and calloid production from floral explants has been much improved following changes in the media preparation procedures but the subsequent stage of embryonic calloid development must be further researched. Wye and CICY will both give priority to investigating methods of obtaining and multiplying embryonic callus in order to increase the yield of plantlets. As the controlling factors for this stage are not known, the effects of cytokinins, gibberellin and changed levels of sugars and nitrogen sources in the media will be followed. The concentration and ratios of ammonium and nitrate ions are of particular importance as ammonium ions are reported to be essential for induction and normal development of embryoids. Further it is necessary to determine whether the cultures lack nitrate reductase and therefore accumulate inhibitory concentrates of nitrate.

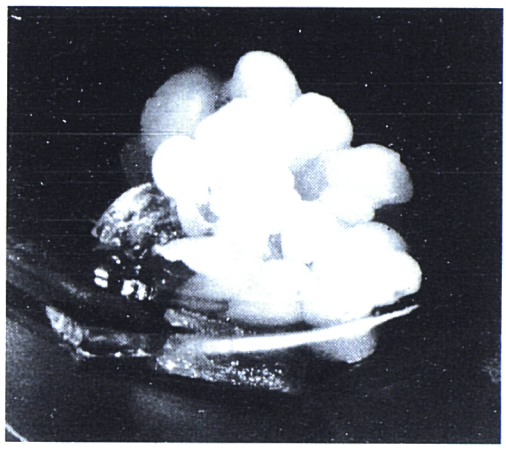

Figure 1: Calloid nodules (after 5 months in culture) from flower meristem cultured with 10^{-4}M 2,4-D

Development of good plantlets. At present embryonic calloid often fails to develop well-balanced shoot and root systems but produces a tissue which resembles the haustorium (or cotyledonary equivalent) of the zygotic embryo. It has now been shown in these laboratories that this unwanted development may be reversed by changing growth regulator levels and Wye will investigate culture systems with varying levels of charcoal in order to achieve the required control of auxin and cytokinin levels. The aim will be so to refine the system that these growth regulators are used at minimal concentrations, and omitted where possible.

Weaning procedures. Early research has been hampered by a shortage of material which has made replicated experiments impossible but now the anticipated increase in efficiency will result in the availability of more clonal plantlets. Weaning procedures using the Nutrient Film Technique (NFT) will be pursued in order to establish the nutritional requirements of plants at this stage.

Alternative techniques to be investigated

The evaluation of methods to obtain direct embryogenesis from other explant tissues. Pollen culture will be attempted at CICY to produce homozygotic (haploid) embryoids and plants. If callus/calloid is produced by pollen or another culture, this would be developed as an alternative source for the development of goof plantlets. Young leaf tissue from seedlings grown *in vivo* will be used at CICY and from *in vitro* seedlings at Wye to investigate methods of achieving direct embryogenesis. Immature zygotic embryos of Tall types will be cultured at CICY to attempt the production of secondary embryos

without a callus phase. The technique, if successful, will be used subsequently to multiply hybrid zygotic embryos obtained by crossing elite Tall and Dwarf parents in ongoing breeding programmes in Mexico which aim to obtain palm with increased resistance to Lethal Yellowing.

Mature, zygotic embryos will be cultured at CICY to give a good supply of *in vitro* plants for experiments to improve the present weaning protocol. This will precede the production of clonal plantlets and minimise losses of the more valuable material.

A procedure to produce shoots directly from crown tissue under high cytokinin conditions will be explored with mature crowns at Wye and with younger crowns at CICY. This follows the highly successful technique established for *Agave* at CICY.

Reversion of flower primordia to give vegetative shoots, thus maintaining organisation without any callus phase, will be investigated at Wye if staff time is available.

Figure 2: Shoot developed from embryoid (after 9 months)

Workshop

Biological nitrogen fixation

European coordinator: J.E. Beringer
School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, UK.

Summary

This workshop was held at Guanajuato from 23 to 26 January 1989. Its objective was to provide a forum in which European, Mexican and other Latin American scientists active in this research field could discuss their results in a constructive atmosphere and could prepare proposals for joint research. Such a gathering enabled proposals to be generated that were original and did not duplicate existing work, that were relevant to the needs of the countries in the region and that were appropriate for the research facilities available.

The main themes developed were nitrogen-fixing trees and shrubs, microbial inoculants, long term nutritional experiments and the identification of nutritional factors limiting biological nitrogen fixation. Although a wide range of biological nitrogen fixation systems are now known, the programme focussed on those systems capable of yielding at least 50 kg of nitrogen per hectare per year on the grounds that other sources, though interesting scientifically, were agronomically unimportant.

There were 28 participants in the workshop, including scientists from 10 European Community countries; the Mexican participants were based at a number of centres, of which the Centro de Investigaciones y de Estudios Avanzados (CINVESTAV) Unidad Irapuato and the Centro de Investigación sobre Fijación de Nitrogeno (Universidad Nacional Autónoma de Mexico, Cuernavaca) were particularly well represented. Other participants came from research centres in Costa Rica, Colombia, Brazil, Uruguay and Argentina.

Apart from the general contacts established and scientific exchanges that took place, the International Scientific Cooperation programme is now supporting three joint research projects that are a direct result of this workshop, one of which is covered in this volume (report 11, pages 55-6).

Postdoctoral fellowships

A. Bravo

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J. Leemans

Plant Genetic Systems, Jozef Platteaustraat 22, 9000 Gent, Belgium.

Receptors for insecticidal crystal proteins (ICP) from *Bacillus thuringiensis* in the midgut of coleopteran insects.

Fellowship period: March 1990 - February 1991.

Summary

We report for the first time the cell damage in the midgut ephithelium of coleopteran insects after *Bacillus thuringiensis* ICP ingestion. As shown in other lepidopteran insects, the toxins penetrate the peritrophic membrane (PM) and affect the epithelial cells. Histopathological changes include hypertrophy of the midgut cells, vacuolation of the cytoplasm, disruption of the microvilli present in the brush border and disintegration of the cells.

The different ICPs used were localised only in the PM and the brush border microvilli (BBM) of the midgut cells from the sensitive insects. We could not detect internalisation of ICP, even at long times after toxin ingestion. The binding of ICP to the BBM was related to toxicity, while the binding to the PM was not specific and not related to the toxic effect.

The only difference in ICP localisations in fed lepidoteran and coleopteran larvae is that in coleopteran insects the CryIIIA toxin is preferentially attached to the BBM of the cells from the posterior part of the midgut. These data suggest that slight differences in the mechanism of action may be involved in the colepteran toxins.

The results presented have strengthened the correlation previously proposed between binding of ICP to the BBM and toxicity in lepidopteran insects as well as in coleopteran larvae. It is also suggested that the cell damage observed in the larvae epithelium depends exclusively on the toxic action of the ICPs at the BBM membrance level.

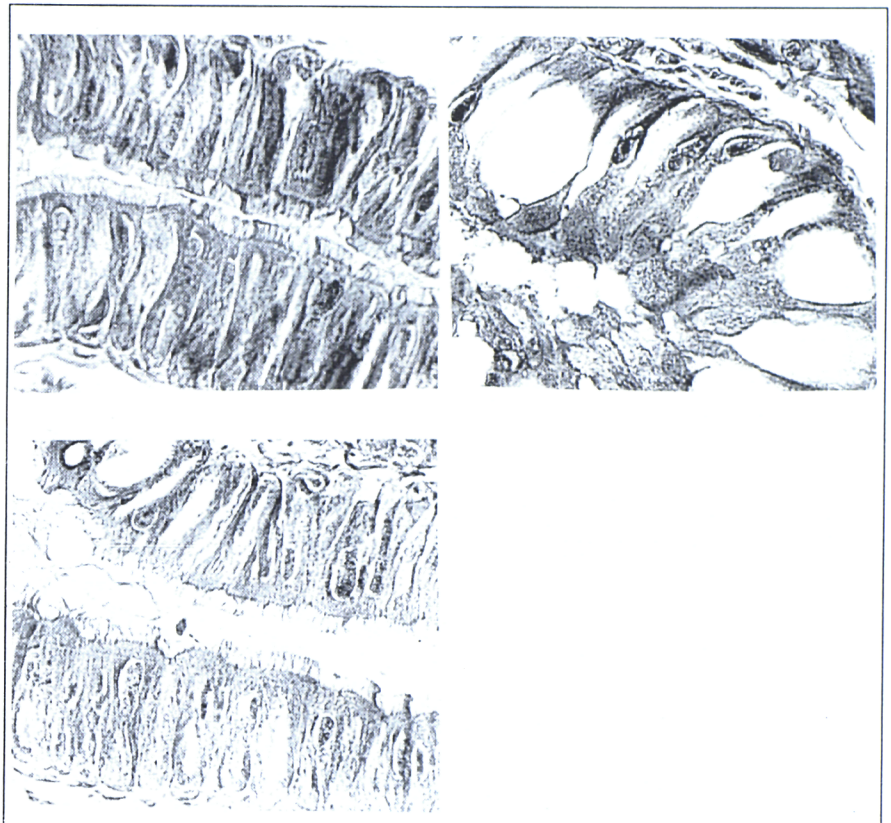

Figure 1: Histopathological effects induced by CryIA(b) and CryIB in the midgut from *Manduca sexta* larvae x300

Midgut from *M. sexta* larvae were isolated after 4h of toxin ingestion. Tissue sections were stained with Heidenhain's Azan staining. Top left, midgut cell from control larvae fed with albumin solution. Top right, midgut cell from CryIA(b) fed larvae. Lower left, midgut cell from CryIB fed larvae. When the larvae were fed with CryIIIA toxin the midgut cells are as at lower left.

An immunocytochemical analysis of *in vitro* binding of ICPs to midgut tissue sections was developed. Staining was observed along all exposed PM and BBM of sensitive insects. No staining was observed in the microvilli present in Goblet cell cavity nor in the Malpighian tubules or in any other membrane in the larvae gut.

The binding of the ICPs to the BBM appear to be highly specific, since the lepidopteran-specific ICPs only bound to the BBM from lepidopteran larvae, while the coleopteran-specific toxin only bound to the coleopteran BBM. The binding to the PM was not related to toxicity.

G. Espin

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M. Iaccarino

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Nitrogen metabolism in *Rhizobium leguminosarum* bv. *phaseoli*.

Fellowship period: June 1988 - May 1989

Summary

The specific objectives of the project were the identification and characterisation of the genes involved in glutamine synthesis. We had already identified a sequence involved in GSII synthesis or activity through the isolation of a *R.l. phaseoli* Tn5 insertion mutant that lacks GSII activity. This mutant was further characterised in Naples.

Another *R.l. phaseoli* sequence already identified was the cloned plasmid pMW5 that codes for a previously unrecognised glutamine synthetase activity (GSIII). This sequence was also further analysed in Naples. The GSIII coding sequence was delimited to a 2.2kb BamH1-HindIII fragment. This sequence did not show cross-hydridisation to the genes coding for the two known rhizobial glutamine synthetase isozymes. The GSIII activity expressed in *Klebsiella pneumoniae* from pMW5 shows a ratio of biosynthetic to transferase activity 10^3-fold higher than observed for GSI or GSII.

The work has been continued in an ISC joint research project (see report 6, page 33).

Publications

Espin, G.; Moreno, S.; Wild, M.; Meza, R. and Iaccarino, M. (1990). A previously unrecognised glutamine synthetase expressed in *Klebsiella pneumonia* from the glnT locus of *Rhizobium leguminosarum*. *Molecular and General Genetics* **223**, 513-16.

Iaccarino, M.; Rossi, M.; Defex, R.; Chiurazzi, M.; Manco, G.; Espin, G.; Lamberti, A. and Riccio, A. (1990). Regulation of nitrogen metabolism in *Rhizobium*. *In*: Inorganic Nitrogen Metabolism, Springer Verlag, 234-40.

Defez, R.; Chiurazzi, M.; Manco, G.; Petriarca, E.; Lamberti, A.; Riccio, A.; Lopes, C.; Collona-Romano, S.; Moreno, S.; Meza, R.; Moreno, S.; Espin, G. and Iaccarino, M. The glutamine synthetases of *Rhizobium leguminosarum* and their regulatory genes. *In*: Nitrogen Fixation: Achievements and Objectives, eds: Gresshoff, Roth, Stacey and Newton. Chapman and Hall, New York-London, in press.

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Formulation of a methodology for the regionalisation of Mexican forests.

Fellowship period: August 1990 - December 1990.

Summary

The study was based on the hypothesis that forestry should be considered not only in terms of traditional regional policy but in its ecological and economic context. Administrative boundaries do not always correspond to the natural boundaries of the resources needed by the local population.

In the first stage, a description of forestry activity in Mexico was prepared. This led to a model whereby forestry activity could be seen as a system. Each of the main elements was treated quantitatively. Three options were developed for solving the problem of dividing up regional units so that an integrated approach to regional management could be made. We then worked out how to ensure that statistics from different regional base units should be prepared on a comparable basis; this should help to provide appropriate training in the regions. Our suggestions for the statistical methodology were prepared to be used in stages.

We hope that this methodology will give regional planners a new tool to establish the main policy lines for the future. In Mexico the forests are often seriously and even irreversibly degraded, or the process of reversal will be long and costly, both socially and economically.

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P. Ballario

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Genetic and biochemical characterisation of glutamate synthase activity (GOGAT) in the yeast *Saccharomyces cervisiae*

Fellowship period: January 1989 - December 1989

Summary

The work has enabled us to clone one of the putative structural genes coding for GOGAT (this is the first eukaryotic GOGAT gene that has been cloned). We were able to determine the presence of two GOGAT activities in this yeast. One of these activities had been previously reported, the other one is a novel activity which has not been described. This finding is interesting since all microorganisms studied so far show only one GOGAT activity.

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M. Van Montagu

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Studies in plant molecular biology.

Fellowship period: January 1988 - December 1989

Publications

Geremia, R.; Jacobs, D.; Goldman, G.H.; Van Montagu, M. and Herrera-Estrella, A. (1990) Induction and secretion of hydrolytic enzymes by the biocontrol agent *Trichoderma harzianum*. *In* Biotic Interactions and Soil-borne Diseases, ed. A.B.R. Beemster. Wageningen, Netherlands Society of Plant Pathology, in press.

Goldman, G.H.; Geremia, R.; Van Montagu, M. and Herrera-Estrella, A. (1990) Molecular genetics of the biocontrol agents *Trichoderma spp. In*: Biotic interactions and soil-borne diseases, ed. A.B.R. Beemster. Wageningen, Netherlands Society of Plant Pathology, in press.

Goldman, G.H.; Van Montagu, M. and Herrera-Estrella, A. (1990) Transformation of *Trichoderma harzianum* by high-voltage electric pulse. *Current genetics* **17** (2), 169-74.

Goldman, G.H.; Villaroel, R. and Van Montagu, M. and Herrera-Estrella, A. (1990) Sequence of the *Trichoderma virida* phosphoglycerate kinase gene. *Nucleic Acids Research*, **18(22)**, 6717.

Herrera Estrella, A; Chen, Z.; Van Montagu, M. and Wang K. (1988). VirD proteins of *Agrobacterium tumefaciens* are required for the formation of a covalent DNA-protein complex at the 5'terminus of T-strand molecules. *EMBO Journal* **7**, 4055-62.

Herrera Estrella, A.; Goldman, G.H. and Van Montagu, M. (1990). High efficiency transformation of the biocontrol agent *Trichoderma spp. Molecular Microbiology* **4**, (5), 839-43.

Herrera Estrella, A.; Van Montagu, M. and Wang, K. (1990). A bacterial peptide acting as a nuclear targeting signal: the *Agrobacterium* VirD2 directs ß-galactosidase into tobacco nuclei. *Proceedings of the National Academy of Sciences of the USA*, **87** (24), 9534-7.

Vernade, D.; Herrera Estrella, A.; Wang, K. and Van Montagu, M. (1988). Glycine betaine allows enhanced induction of the *Agrobacterium tumefaciens* vir genes by acetosyringeone at low pH. *Journal of Bacteriology* **170**, 5822-9.

Wang, K.; Herrera Estrella, A. and Van Montagu, M. (1990). Overexpression of virD1/D2 Genes in *Agrobacterium tumefaciens* enhances T-complex formation and plant transformation. *Journal of Bacteriology* **172** (8), 4432-40.

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Quality of aquacultured shrimp.

Fellowship period: December 1989 - November 1990.

Summary

Research was undertaken on the binding of antibiotics to functional polymers and assessment of leaching from pelleted shrimp feeds; and the accumulation of oxytetracycline in haemolymph and muscle of juvenile *Penaeus monodon* during therapeutic administration of antibiotic-coated pellets.

A comparative study was made of bioassay methods and the Charm 7000 system for determining antibiotic residues in shrimp muscle, and the effect of antibiotics on the survival and metamorphosis of *P. monodon* larvae.

Publications

Brown, J.H. and Higuera-Ciapara, I. (1991). Antibiotics in shrimp culture. Submitted to *Journal of Aquaculture and Fisheries Science.*

Inglis, V.; Solimand, M.K.; Higuera-Ciapara, I. and Richards, R.H. 1991. Amoxycillin in the control of furunculosis in Atlantic salmon *Salmon sala* L. Parr. Submitted to *Veterinary Journal.*

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F. Salamini

Abteillung Pflanzenzächtung und Ertragsphysiologie, Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, Germany.

Expression of drought-induced genes in transgenic plants.

Fellowship period: October 1989 - September 1990

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Studies on physiology, biochemistry and immunobiochemistry for aquaculture development.

Fellowship period: June 1989 - May 1990

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F. Taylor

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An investigation of the movement of radio-labelled plant growth regulators and their metabolites between semisolid media and *Cocos nucifera* tissue explants in sterile culture.

Fellowship period: June 1991 - May 1992

Summary

Many procedures have been developed in tissue culture and plant micropropagation through a largely empirical approach. These have been especially successful when growth and development are achieved comparatively quickly, with few changes in growth regulating levels, and when the minimum of transfers to fresh media are required. However, the method is less suitable when finely controlled changes in auxin and cytokinin concentrations are required over a period of many months. In such cases the number of alternative treatments which are possible becomes too large for direct comparison. An example of a very complex culture procedure is found with the clonal propagation of coconut, and this tissue will be used for the proposed investigation.

The procedures required for the project have already been used in the Wye College laboratories to determine changes in regulator levels in media and in tissue in sterile culture. It is proposed that these proven methods will be used with the regulators 2, 4-dichlorophenoxy ($2^{-14}C$) acetic acid and benzyl ($8^{-14}C$) adenine. Radioactivity will be assessed by liquid scintillation counting (LKB 1211 Rackbeta, Pharmacia Diagnostics) and on TLC plates using a thin layer scanner (Berthold LB 2722-2). TLC and HPLC will be employed for the separation of polar metabolites in extracts of media and plant tissues.

These investigations will give information about the accumulation of unchanged plant growth regulators in the tissue and their conversions (conjugation and hydroxylation) within the tissue will be obtained together with data for the release from the tissue of radio-labelled chemicals into the medium. Preliminary experiments with coconut callus tissue have already been carried out at Wye. Attempts will be made to isolate sufficient quantities of metabolites using HPLC to carry out an assessment of their biological activity. For these experiments tissue would be treated with cold regulators and the metabolites located by "spiking" with ^{14}C. The advantages of the synthesis of conjugates for biological activity studies are recognised and such research in collaboration with the staff of the Department of Biochemistry at Wye will be considered.

It is expected that, by correlating the analytical data concerning levels of regulators and their metabolites with the growth and development of coconut explants, these experiments will establish the conditions required for the successful culture of these tissues. Information of this type has already given practical guidance on how frequently tissue must be transferred to fresh media to keep regulator/metabolite levels within the required limits and so to prevent undesirable changes in tissue types and reductions in growth rates. It is intended to follow up these early successes.

2 BIOLOGICAL SCIENCES

Summary

The two joint projects included in this chapter are still at too early a stage of development for results to be reported; they, and some of the postdoctoral fellowships deal with basic cell and molecular biology studies such as gene expression, the biogenesis of membrane proteins and transport mechanisms of cells and membranes. Other postdoctoral studies include an ecological study of natural selection in plants, the biological significance of nitric oxide and a study of engineering aspects of xanthan fermentation, this latter demonstrating the multidisciplinary nature of biotechnology as well as the difficulty of assigning certain studies to particular chapters of this book.

Joint research projects

14 mRNA processing and gene expression

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Contract number and duration: CI1*/0790, May 1991 to October 1993

Objectives

The goal of the research is to understand the different aspects governing the stability and the processing of bacterial and phagic mRNAs. Decay of transcripts is often controlled by a rate limiting first endonucleolytic cleavage by RNase III followed by rapid exonucleolytic degradation. Two exonucleases have been identified: polynucleotide phosphorylase and RNase II.

The project will be focused on the role of the elements (secondary structure and sequence) responsible for the specificity of RNase III and stability of the mRNA. This will be examined in three different systems (integrase λmRNA where RNase III site is located 3' to the structural gene of the integrase, polynucleotide phosphorylase (PNPase) mRNA where the RNase III cleavage site is located 5' to the gene for PNPase, the messenger of the operon coding for initiator tRNA Met f2, a termination factor NusA and the initiator IF2, where the RNase III site is also located 5' to the gene coding for NusA. This will be investigated *in vivo* by selecting different mutants as well as analysing *in vitro* the degradation of mutant and wild type transcripts using purified RNase III.

Primary transcripts from the three systems discussed above are much more stable (half life in the range of 5 to 15 minutes) than the average mRNA and than the corresponding RNA processed in the 5' leader by RNase III (half life 30-45 seconds). We will investigate whether a mRNA RNase III site has any effect in RNase III ($^+$ and $^-$) wild type and mutant strains when fused to the 5' end of a stable or unstable mRNA.

Finally, the role and specificity towards secondary structure of two exonucleases, polynucleotide phosphorylase and RNase II, will be investigated both *in vivo* and *in vitro*. The role of these enzymes will also be investigated on the expression of their own mRNAs, as well as on others' mRNAs.

We hope that the results obtained will allow us to understand how a messenger is inactivated and degraded.

Work programme of G. Guarneros in Mexico

Isolation of deletion mutants at the 5' end of the *sib* site. All the experiments necessary to identify the 5' extent of the RNase III site of *int* mRNA will be done by Dr Guarneros' team. This includes *in vitro* deletion just upstream the t1 terminator, sequence analysis of the corresponding mutants and biological assays after transfer to the plasmid assay system constructed by G. Guarneros.

Characterization of this *sib* site. The isolation of numerous point mutants in the sib region will be continued by selecting λ phages able to promote integrative recombination in the absence of cII function. The effects of these mutations on the mRNA cleavage and degradation rate will be realized by using the plasmid pUS6.

Physiological role of PNPase and RNase II. The stability of the *int* mRNA mutated in the *sib* region, towards PNPase and RNase II, will be examined by Northern blot analysis and enzymatic determinations of galactokinase and β-galactosidase activities in strains harbouring pnp- and mb- mutations.

Work programme of M. Grunberg-Manago in France

In vitro analysis of the mutant *int* mRNA. The *int* messengers from the different mutants isolated by Dr Guarneros' team will be analyzed *in vitro* for their sensitivity to the PNPase, RNase II and RNase III. Radioactive messengers will be synthesized and, after action of these nucleases, analysed on polyacrylamide gels to determine their sizes.

Characterization of the RNase III sensitivity site. Translational mutants of the *pnp* and *mc* messengers uncleaved by RNase III will be selected as growing colonies on lactose media by using a fusion between the 5' part of the studied messengers to the 3' end of the lacZ gene. These mutants will be sequenced and the half lives of the corresponding mRNA measured by hybridization with a specific probe on filters or by Northern analysis. Comparisons between the 3' degradation mode and the 5' processus will be realised by transporting the RNase III site into the 5' leader of other messengers and by determining any variation in their half lives.

The PNPase site specificity. The presence of an autocontrol would indicate that the PNPase can identify a specific site on its mRNA. Therefore, the different mutants isolated will be also analysed for their PNPase autocontrol capacity by using *pnp-lac*Z fusions integrated into the chromosome via a λ vector. The operator will be localised and characterized. Comparisons of its structural motif with another target, the *int* messenger, will be made and affinities between these different targets analysed.

15 Trafficking of lysosomal integral membrane proteins: molecular mechanisms involved in the processes of sorting and transport to lysosomes

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Contract number and duration: CI1*/0860, May 1991 to April 1995

Objectives

The study of the molecular mechanisms involved in the targeting of integral membrane proteins (LIMPs) to lysosomes will consist of two parts.

Part 1: Study of the signal(s) involved in the targeting of LIMPs to lysosomes. The search for the signal(s) involved in the transport of LIMPs from the Golgi system to lysosomes will focus on the structure of the proteins, since recent studies have shown that the only post-translational modification of the proteins is the acquisition of N-linked carbohydrates, and the transport of LIMP to lysosomes is independent of these carbohydrates. The study of the signal(s) will begin with the cloning of the cDNAs coding for LIMPS I, II and III. The cloned cDNAs will be sequenced, and the sequences analyzed to determine the translated and nontranslated segments; the stretches coding for the luminal, transmembrane and cytoplasmic domains of the proteins; the N-glycosylation sites; and the target sequences for restriction enzymes. Subsequently, the sequences of the three LIMPs will be compared to identify any shared sequence(s). The role of the sequence(s) in the targeting of LIMPs to lysosomes will then be tested by deleting the sequence(s) from the cDNAs, trans-fecting mouse cells with the truncated cDNA and studying the localization of the expressed LIMPs by immunofluorescence microscopy. The sequence(s) whose deletion results in altered localization of the LIMPs will be used to construct cDNAs with the cDNAs of the rat asialoglycoprotein receptor and transferrin receptor, plasma membrane proteins which like the LIMPs have a leader sequence, but lack any other transport signal. Finally, the cDNA constructs will be expressed in mouse cells and the ability for the LIMP sequence(s) to target the plasma membrane proteins to lysosomes studied by immunofluorescence microscopy.

Part 2: Study of the LIMP targeting device(s). The targeting device(s) is (are) expected to consist of a protein or group of proteins binding specifically to the targeting signal(s) and producing the sorting and subsequent transport of LIMPs for Golgi to lysosomes. The characterization of the device will begin by testing the binding of radiolabelled proteins obtained from cytosolic, Golgi and lysosomal fractions to LIMPs immobilised on Sepharose. The protein(s) binding to more than one LIMP (LIMP-BP) will be characterized and the requirements (pH, ionic strength, ions) and properties (Km) of the binding studied. If antibodies against LIMP targeting sequences are available (part 1) the effect on the binding

of LIMP-BP to LIMPs will be tested. Alternatively, if the identification of LIMP-BP precedes the characterization of the LIMP targeting signal(s), the LIMP domains (i.e. chemically or proteolitically derived fragments) recognizing LIMP-BP will be identified and N-sequenced and the site in the sequence of the protein localized to identify the target signal(s). Finally, the pathway of transport of LIMPs to lysosomes will be morphologically characterized by immuno-electron microscopy using mouse and rabbit antibodies raised against LIMP and LIMP-BP respectively.

Work programme of I.V. Sandoval in Spain

Study of the expression of complete and truncated LIMP cDNAs, as well as of cDNAs of the analoglycoprotein and transfer in receptors containing lysosome targeting signals: L-M (TK^-) mouse cells will be cotransfected with the corresponding cDNA and purified TK^+ gene. The TK^+ cells will be selected and the expression and localization of the LIMP and quimeric proteins studied by immunofluorescence and immunoelectromicroscopy using specific antibodies.

Characterization of the LIMP targeting devices: A first selection of LIMP binding proteins (LIMP-BP) will be performed by examining the binding of radiolabelled proteins obtained from soluble and membrane fractions from cytosol Golgi and lysosomes to LIMPs linked to Sepharose. The selected proteins(s) will be physically and chemically characterized and the conditions for optimal binding to LIMPs determined. Special attention will be paid to the binding requirement for ions and pH conditions, which could play an important role in the physiology of the LIMP/LIMP-BP by determining their recycling and dissociation. If the LIMP targeting sequence(s) is (are) already identified, antibodies against the sequences will be developed and the effect on the binding of LIMPs to LIMP-BP(s) studied. On the contrary, if any LIMP-BP is identified before that, they will be used to characterize the targeting sequence(s). For this purpose LIMPs will be enzymatically or chemically digested and the fragments binding LIMP-BP(s) identified by gel overlaying or immunocoprecipitation. Then, the reactive fragments will be N-sequenced and the sequences used to analyze the target sequences or domains within the LIMPs.

Study of the cellular localization of LIMP-BP(s) and morphological characterization of the pathway of LIMP transport to lysosomes: Polyclonal anti-LIMP-BP(s) antibodies will be raised in rabbits and used to study the cellular localization of the protein(s) in NRK cells at the electron microscopy level. The study will be performed with pre- and post-embedding techniques, using peroxidase (qualitative studies) and gold (qualitative studies) labelled anti-rabbit antibodies. Double labelling using mouse anti UMP and rabbit anti UMP-BP antibodies will be performed at the optical and electron microscopy levels. The pathway of transport of the LIMP/LIMP-BP complexes to lysosomes will be studied by double immunofluorescence and immunoelectron microscopy, using mouse and rabbit antibodies labelled with fluorescine/rhodamine and 3/5 nm gold, respectively.

Work programme of E. Lamoyi Velazquez in Mexico

Cloning of LIMP-cDNAs. For this purpose we have already developed monospecific antibodies against LIMPs II, III and IV purified by affinity chromatography, as well as cDNA complementary oligonucleotides. The polyclonal antibodies will be used for screening a rat kidney library enriched for cDNAs coding membrane proteins, made in the expression vector λ gtll. The oligonucleotides should help to identify any cDNA clones isolated from the λ gtll library coding for the N-terminus of the proteins, and if needed to screen a rat kidney cDNA library made in the vector λ gt10.

Sequencing of the LIMP cDNAs and characterization of the sequences. The LIMP cDNAs clones will be sequenced and the complete sequences studied by computer analysis to determine the translated and nontranslated segments, which stretches code for the terminal, transmembrane and cytoplasmic domains of the proteins, the sites for post-translational modification (i.e. glycoylation) and the target

sequences for restriction enzymes. Subsequently, individual LIMPs will be compared to determine the existence of identical and homologous sequences.

Manipulation of the LIMP cDNAs. The manipulation will be conditioned by the data generated from the analysis of the sequences (i.e. restriction maps; existence and localization of shared sequences and N-glycoxylation sizes). The sequence(s) shared by different LIMPs will be deleted and the effect of the deletion upon the transport of LIMP to lysosomes studied. If no such sequence(s) is identified the stretches of cDNA coding for the luminal and cytoplasmic domains will be subjected to short random deletions. The effect of the deletions on the transport of LIMPs to lysosomes will be studied by immunofluorescence in cDNA transferred L-M(TK^-) cells. Sequences whose deletion do not alter the transport of the LIMP through the RER and Golgi, but cause the accumulation in a prelysosomal compartment or expression on the cell surface will be selected. Such sequences will be used to construct quimeric cDNAs with the rat plasma membrane proteins asialoglycoprotein and transferrin receptors. The constructs will be expressed in L-M(TK^-) cells and the ability of the LIMP sequences to target the plasma membrane proteins to lysosomes examined. Sequences with this property will be subjected to nucleotide substitutions and the minimum effective length determined. Finally, the effect of such manipulations on the localization of both LIMPs and quimeric proteins will be studied.

Postdoctoral fellowships

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The biological significance of nitric oxide.

Fellowship period: November 1987 - December 1988

Publications

Amezcua, J.L; Dusting, G.J.; Palmer, R.M.J. and Moncada, S. (1988). Acetylcholine induces vasodilatation in the rabbit isolated heart through the release of nitric oxide, the endogeneous nitrovascodilator. *British Journal of Pharmacology* **95**, 830-4.

Amezcua, J.L.; De Souza, B.M.; Palmer, R.M.J. and Moncada, S. Inhibition of nitric oxide synthesis inhibits endothelium-dependent vasodilation in the rabbit-isolated heart. Submitted to *British Journal of Pharmacology.*

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Molecular mechanisms of the phosphate transport in yeast cells. Cloning of the yeast mitochondrial transporter.

Fellowship period: May 1990 - April 1991

Summary

The mitochondrial phosphate transport protein (PTP) was purified in a reconstitutively active form from *Saccharomyces cerevisiase* and *Candida parapsilosis* by Guérin. The rate of unidirectional phosphate uptake into reconstituted proteoliposomes was stimulated about 2.5 times by Δ pH (i.e: pH external 6.8 versus internal pH 8.0). The Km and Vmax values were 2.2 mM and 170 nmol.min^{-1}. μg^{-1} protein respectively. These values are similar to the low affinity phosphate transport system of the yeast plasma membrane Km + 1.53 mM and Vmax + 16.99 nmol. min^{-1}.mg^{-1} dry weight of cells.

The cloning and characterisation of the mitochondrial PTP gene that was proposed as part of the project to be performed was already being performed by Wohlrab in Boston. Therefore it was decided to initiate studies for the cloning and characterisation of the high affinity transport system of phosphate but in the yeast plasma membrane. In January 1991, the characterisation of the mitochondrial PTP from *Saccharomyces cerevisiae* was published by Wohlrab's group.

Guy Lauquin made contact with Oshima from Japan to obtain the pho T mutants (high-affinity phosphate transport) of *Saccharomyces;* it took around 3½ months. Two strains bearing the pho 84 mutation arrived from Japan. Since the mutants segregated secondary mutants, most of them were unable to produce suppressible acid phosphatase which allowed us to select the pho T mutation by a straining method on plates. The strains were purified several times and the acid phosphatases characterised by their sensitivity to heat inactivation.

Once purified, the KCY-64 strain (mat α , pho 3-1, pho 84-1, Trp-1) was utilised for transformation with a cDNA bank prepared in *E. coli.* Two techniques were used: transformation by LiCl and spheroplasting-PEG. Although the number of transformants was better by spheroplasting-PEG, the number of colonies with possible PH084 was reduced.

It has been suggested that the high affinity phosphate transport system is repressed by phosphate and since the yeast was grown in a rich medium, the amount of specific mRNA could be relatively scarce. Therefore, the correspondent cDNA prepared in *E. coli* was highly limited. Transformation will then be performed with a yeast DNA bank. The yeast DNA bank was prepared and from the original KCY-164 strain a new ura-minus strain was constructed by

crossing the KCY-164 strain with the DBY-747 strain (mata, leu2, his3, Trp1, ura3). Sporulation and tetrad analysis was performed, the ura-minus cells selected and the phenotype analysed. One of the strains named KCY-164 (2) (mat α , pho3, pho84, leu2, his3, Trp1, ura3) has been selected for transformation.

Publications

Cerbón, J. and Calderon V. (1990). Proton-linked transport systems as sensors of changes in the membrane-surface potential. *Biochimica Biophysica Acta*, **1028** (3), 261-7.

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Natural phenotypic selection of floral characters: a study of ecological and phylogenetic components.

Fellowship period: January 1991 - December 1992

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Mixing and oxygen transfer in xanthan fermentation.

Fellowship period: September 1990 - August 1991

Summary

Nowadays, xanthan gum is the most commercially important microbial polysaccharide. Additionally, because of the interesting rheological changes that occur during its culture, xanthan gum production is a good model for studying viscous fermentations.

Although various impeller geometries have been characterised in order to improve the mixing of model fluids (simulated broths), practically no experience has been reported from actual cultures. This is also true for power consumption and oxygen transfer.

The present project aims to study aspects of the rheology, power consumption, mixing and mass transfer, during xanthan fermentation. The main task is to propose suitable agitation systems to maximise the objective function of the fermentation (i.e: the final gum concentration). Accordingly, we shall carry out experiments both in a fermenter (equipped with accurate power input measurement devices as well as with oxygen transfer measuring instruments) and in open mixing tanks (where accurate mixing studies can be performed).

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I - The role of polynucleotide phosphorylase in the regulation of *int* gene expression.

II - Sequencing of the *E. coli* PTH-RAP DNA region

Fellowship period: December 1988 - November 1989

Summary: I

My main objective was to investigate the role of the 3'-exonuclease polynucleotide phosphorylase (PNPase) in the regulation of the int gene expression. I had previously observed that the expression of a gene int fusion increased in a background mutant for PNPase when the sub site was at the 3'-end of the fused gene. Mutations in the gene for RNaseII, another 3'-exonuclease, did not affect the int-fusion expression. Our working model to explain these results assumed that the exonucleolytic activity of PNPase degraded specifically and processively the "free" 3'-end of the int mRNA. Eventually the degradation inactivated the coding region in the messenger reducing int gene expression.

We measured *in vivo* int RNA. We found that the levels of mRNA correlated inversely with the PNPase activity thus suggesting a direct participation of the enzyme in RNA degradation. The transcription terminator tI at the 3'-end of int mRNA contains a stem-and-loop structure. Exonucleolytic degradation *in vitro* by PNPase, and RNaseII, is inhibited by secondary structure in the RNA. I had previously proposed that the terminated RNA has a positive effect on int gene expression by protecting the mRNA against 3'-degradation.

Our *in vitro* results confirmed this proposal; int transcripts ended at mutant terminators that reduce the stability of the stem structure and are degraded faster than the wild type terminated transcripts. This lability depended largely on an active PNPase. Consistent with these data, in vitro digestion of RNA with purified PNPase resulted in faster degradation of mutuant than wild type-terminated RNA. RNaseII degradation appears equally inhibited by both wild type and mutant-terminated structures. It is possible that the terminated int mRNA stability depends largely on the protection of 3'-ends by the stem-and-loop terminator structure.

Summary: II

E. coli rap mutants are known to grow λ wild type phage and *E. coli* pth mutants are defective in protein synthesis. Both pth and rap sites map very close in the bacterial chromosome. In fact I found that they are tightly linked in a 1.6 kb segment of bacterial DNA. Both pth and rap mutants share phenotypes. The pth mutant shows the rap phenotype under certain conditions and according to Menninger the rap mutant is partially defective in the hydrolase activity. The rap mutant can shown protein synthesis inhibition under conditions where no effect was found in wild type bacteria. Taken together these results indicate that rap and pth affect one and the same gene.

To help the understanding of the rap-pth relationship we analysed the nucleotide sequence of the bacterial DNA segment that encodes pth and rap complementing functions. We determined a 1620 bp sequence which contains an open reading frame of 194 aminoacids preceded by putative transcription-translation signals. A series of deletions were made at both ends, and mutations by insertion of small oligonucleotide sequences, all generated *in vitro*. Complementation results of plasmid constructs harbouring these mutants in the rap or pth mutants confirmed the assignment of the gene to the above open reading frame.

The calculated molecular weight of the pth-rap protein, about 21 kD, is in agreement with the experimental value of 22 kD found in the maxicell system for a polypeptide which correlates with the pth-rap complementing activities. However the definitive assignment of the 21 kD protein to the proposed open reading frame and the identity of rap and pth mutations need more experimental evidence.

The work described here led to a joint research project (see report 14, page 74).

Publications

Guarneros, G.; Kameyana, L.; Orozco, L. and Velazquez, F. (1988). Retroregulation of an int-lacZ gene fusion in a plasmid system. *Gene* **72**, 129-30.

Guarneros, G. and Portier, C. (1990). Different specificities of ribonuclease II and polynucleotide phosphorylase in 3' mRNA decay. *Biochimie*, in press.

Guzmán, P. and Guarneros, G. (1989). Phage genetic sites involved in λ growth inhibition by the *Escherischia coli* rap mutant. *Genetics* **121**, 401-10.

Guzmán, P.; Rivera Chavira, B.; Court, D.L.; Gottesman, M.E. and Guarneros, G. (1990). Transcription of a λ DNA site blocks *E. coli* growth. *Journal of Bacteriology* **172**; 1030-4.

Perez Morga, D. and Guarneros G. (1989). A short DNA sequence from λ phage inhibits protein synthesis in *E. coli* rap. *Journal of Molecular Biology* **216**, 243-50.

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Microscopic theory of biological ion channels.

Fellowship period: January 1990 - December 1990

Summary

The electrostatic interactions between membrane-embedded ion-pumps and their consequences for the kinetics of pump-mediated transport processes have been examined. We showed that the time course of an intrinsically monomolecular transport reaction can become distinctly nonexponential, if the reaction is associated with charge translocation and takes place in an aggregate of pump molecules. First we considered the electrostatic coupling of a single dimer of ion-pumps embedded in the membrane. Then we applied the treatment to the kinetic analysis of light-driven proton transport by bacteriorhodopsin which forms two-dimensional hexagonal lattices. Finally, for non-ordered molecules, we also considered a model in which the pumps are randomly distributed over the nodes of a lattice. Here the average distance is equal to that deduced experimentally and the elemental size of the lattice is the effective diameter of one single pump.

This latter model was then applied to an aggregate of membrane-embedded Na, K- and Ca-pumps. The electrostatic potential considered was the exact solution calculated from the method of electrical images for a plane membrance of finite thickness immersed in a infinite aqueous-solution environment. The distributions of charges (ions or charged binding sites) were considered homogeneous or discrete in the membrane and/or in the external solution. For discrete distributions we compared the results from a mean field approximation and a stochastic simulation.

Publication

Nieto-Frausto, J.; Läuger, P. and Apell, H-J (1991). Electrostatic coupling of ion pumps. Submitted to *Biophysical Journal.*

3 CHEMICAL SCIENCES

Summary

Mining is a major industry in Mexico and chemical extraction of metals from ore is included amongst the studies reported in this chapter. One project deals with new metal extraction techniques for sulphide ores containing metals in low concentrations which are highly-priced or have strategic or technological value. In addition these ores may contain toxic heavy metals which would be environmentally hazardous if dumped as residues. Another study concerns the extraction of uranium from natural phosphates. Other subjects included in the chapter are the synthesis of amino acids, metallic carbenes and sulphide catalysts for hydrotreatment in fine chemical applications.

Joint research projects

16 Leaching, separation and recovery of some metal values present in the sulphide minerals: sphalerite, galena and chalcopyrite

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Contract numbers and duration: CI1*/0516/0552/0553, February 1990 to May 1993.

Background

Sulphide ores have long been exploited as leading sources of metals of commercial value such as copper, zinc, silver and some noble metals. The two main problems associated with these ores are low metal concentrations and the presence of poison metals which affect the separation processes and cause impurities in the metals recovered. On the other hand, sulphide ores may also contain metals of increasing technological interest such as germanium, gallium and indium.

Objectives

The project is intended to study the three main steps of sulphide ore treatment, namely flotation, leaching and metal separation and concentration. J. Bessière in France is determining appropriate conditions for the flotation agents used for the selective separation of galena, chalcopyrite and blenda when mixed together and is examining how high frequency dielectric techniques can explain the behaviour at solid/liquid interfaces. J. de Gyves in Mexico is establishing appropriate physicochemical conditions for the selective leaching of spharelite, chalcopyrite and galena and characterising the electrochemical reactions responsible. She and M. Valiente in Spain are studying and optimising solvent extraction processes for metals present in these ores. The latter is developing liquid membranes and new water-insoluble polymers to selectively absorb particular metals.

Methods

A range of techniques is available in the three participating laboratories. They include:

electrochemistry: d.c. polarography, differential pulse polarography, voltamperometry, steady state potentio-static polarisation, h.f. dielectric measurement;

spectroscopy; atomic absorption (flame and graphite furnace), X-ray fluorescence, inductive-coupled plasma; on-line spectrophotometry analysis (FIA) for continuous process monitoring;

membrane separation (with flat membrane support);

infra-red spectra for polymer analysis are needed because of their insolubility.

Results

Effect of nickel ions on galena. The conditions for hydroxide depression have been determined from the thermodynamic data. The effects of pH and the physico-chemical characterisation of the depressing agent have been measured both with and without the presence of xanthate.

Dielectric characterisation of ore mixtures. A new dielectric cell for flow measurements has been designed. The effects of frequency, ionic concentration and temperature on their dielectric properties have been measured before and after absorption by the collector in the presence of nickel ions.

Leaching. The mechanism and kinetics of the leaching of pyrite and galena are being studied at different concentrations of HCl and H_2SO_4, to determine solution rate as a function of acid concentration by means of voltamperograms. Pyrite and galena electrodes were constructed; chalcopyrite will be used shortly.

Electrodissolution kinetics and mechanism of the leaching process for galena in the presence of a xanthate. The anodic oxidation of potassium ethyl xanthate, X^-, has been studied with a galena substrate. The Tafel slope was 62.8 mV and the order of reaction, 0.96, in agreement with the reaction:

$$PbS + 2X^- = PbX_2 + S + 2e^-.$$

The work will be continued to study the mineral-solution interface in flotation conditions by Electrochemical Impedance Spectroscopy.

Ionic flotation. These studies involve aqueous solutions, either acid (HCl and HNO_3) or alkaline (NaOH). Precipitation tests have been carried out with cations Ag^+, Zn^{++}, Ge (IV) and Ga (III) using several common reagents such as oxine, cupferron, chloranilic acid, aluminium ammonium pyrolidinethiocarbamate, sodium lauryl sulphate and sodium octylbensylsulphonate. The ionic flotation of cations will be studied in the future.

Solvent extraction. High molecular weight tertiary amines dissolved in kerosene have been used to extract iridium and gallium from HCl solution. The variation of extraction rate with Cl^- and amine concentration has been determined. The electrochemical behaviour of iridium in HCl media gave information about the stochiometry of the InCl species so formed. Other studies concerned the use of tributyl phosphate, TBP, dissolved in cumene to extract Ti(IV) and Fe(III) from chloride media. For Cl^- concentrations up to 5M, Fe(III) is extracted while Ti (IV) remains in solution.

Other extracting agents tried were mixtures of quaternary ammonium bases, trioctylphosphine oxide, KELEX 100 or Cyanex 471x (tri-isobutylphosphine sulphide).

Liquid membranes. The solvent extraction work led to the design of a liquid membrane system in which the co-transport of Ti (IV) in the presence of Fe (III) could characterise difficult metal separations. The liquid membranes consisted of organic solutions of tributylphosphate, TBP, in cumene supported on a microporous flat teflon membrane. Cl^- concentration was high and this

provided the driving force for the mass transfer of Ti (IV). Separation of Ti (IV) and Fe (III) was accomplished by proper regulation of Cl^- content in the feed solution.

Synthesis and characterisation of new co-ordinating polymers bearing phosphine sulphide groups. An important project objective is to develop metal co-ordinating polymers that can selectively absorb metal ions, especially noble metals and those of the platinum group. The standard of comparison is the performance obtained by the use of novel metal extracting reagents in conventional solvent extraction such as tri-isobutylphosphine sulphide (Cyanex 471x) which has shown excellent results in selectively extracting Ag(I), Au (III) and Pd (II).

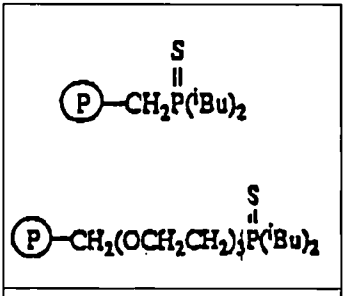

Figure 1

A modified polystyrene was made with 2% of divinylbenzene cross linking by the insertion of di-isobutylphosphine sulphide. Two different polymers of general formula shown in Figure 1 were obtained. The difference between the first and second is the inclusion of the alkyl group $(OCH_2\ CH_2)_3$ as a spacer between the functional group and the polymer. The capacity and rate of metal absorption for both polymers were studied using Pd(II) and Au(III) in chloride solutions. The polymers with the spacer (lower diagram) have between 60 and 100% higher absorption capacity than those without, but the rates are not significantly different.

Publications

Aguilar, J.L; Rodríguez de San Miguel, E. and de Gyves, J. Estudio del comportamiento electroquímico del indio en medios concentrados en ácido (Study of the electrochemical behaviour of indium in highly concentrated acid solutions). Submitted to *Revista de la Societa Química Mexicana.*

Huerta, A. and Genescá, J. (1991). Mecanismo y cinítica de la reducción del oxígeno sobro galena (Mechanism and kinetics of the reduction of oxygen from galena). *Afinidad*, in press.

Huerta, A. and Genescá, J. Mechanism and kinetics of the xanthate oxidation over a PbS electrode. Submitted to *Electrochimica Acta.*

Muñoz, M. and Valiente, M. (1991). Separation of titanium and iron by a solid supported liquid membrane using tributylphosphate, TBP, as carrier. *Analytical letters*, **24** (10).

17 Use of chiral derivatives of ß-aminopropionic acid in the asymmetric synthesis of optically pure ß-amino acid

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Background and objectives

In addition to their fundamental biochemical and physiological significance, amino acids are important in human and animal nutrition and as flavourings, taste enhancers and sweeteners. Both natural and artificial amino acids are also components of many therapeutic agents, agrochemicals and cosmetics; and in basic research some of them are valuable tools to elucidate the mechanism of enzyme reactions. As a result of the wide spectrum of the applications of amino acids, their economic impact is quite significant and has accordingly led to the development of a variety of procedures for their extraction from natural sources and for their chemical synthesis.

The synthetic organic chemist must face the fact that most amino acids are biologically active only in one enantiomeric form. Desired amino acids must, therefore, be synthesized as enantiomerically pure compounds. Indeed, several methods are now available for the preparation of amino acids of high enantiomeric purity.

ß-amino acids, although much less abundant than their α analogues, are also present in peptides, and in free form show interesting pharmacological effects. Furthermore, ß-amino acids can be cyclized to ß-lactams which are potentially biologically active and of current interest. In this respect, a fair number of methods for the synthesis of racemic ß-amino acids have been developed, but very few for the preparation of enantiomerically pure compounds.

Encouraged by the enormous potential of non-racemic derivatives of glycine as precursors of optically active α-amino acids, we decided to explore the usefulness of chiral ß-alanine enolates as starting materials for the preparation of (R)- or (S)-ß-amino acids. In particular, in view of the successful development of the imidazo-lidinone 1 for the preparation of (R)- or (S)-α-amino acids, it was considered that tetrahydro-pyrimidinone 2 might serve as an effective reagent for the synthesis of the analogous ß-amino acids.

Methods

The heterocycle rac-2 was prepared from ß-alanine by initial conversion of its methyl ester to the corresponding N-methylamide (3), which formed a Schiff base with pivalaldehyde (azeotropic removal of H_2O). Cyclization of imine 4 was possible under severe conditions: treatment with benzoic anhydride and heating to 180°C for 8 hours afforded the desired heterocycle, with an overall yield of 44%.

The alkylation products 5-10 are formed by treatment of enolate 2-Li, generated with lithium diisopropilamide (LDA) in THF, with halides RX at -75°C.

Results

X-ray diffraction study of 1-benzoyl-2-*tert*-butyl-3-methylpyrimidin-4-one (2) Because of the present interest in the understanding of the precise structure of oxygen- and nitrogen-containing heterocycles and because such information can be important in order to ascertain the factors responsible for the stereoselectivities observed, we carried out an X-ray analysis with a suitable crystal of perhydro-pyrimidinone 2.

A view of the solid-state structure of 2 is provided in Figure 1. The pyrimidinone ring is rather flat and has a sofa conformation with five of the six atoms approximately in a plane, and the "acetal" carbon C(2) out of plane.

The most interesting feature of the crystal structure is, however, that the six-membered ring adopts a conformation with axial tert-butyl group! The axial orientation of the bulky substituent could be necessary to maintain conjugation of both ring nitrogens with the carbonyl groups, which would cause stringent steric repulsion with an equatorial *tert*-butyl group at C(2). Six-membered rings bearing axial *tert*-butyl groups are rare, so this finding is of general interest; nevertheless, the practical consequences are also significant; if the enolate would still have a conformation with an axial *t*-butyl group, one of its faces would be predicted to be sterically hindered for attack by electrophiles. This was indeed the case, as described in the following section.

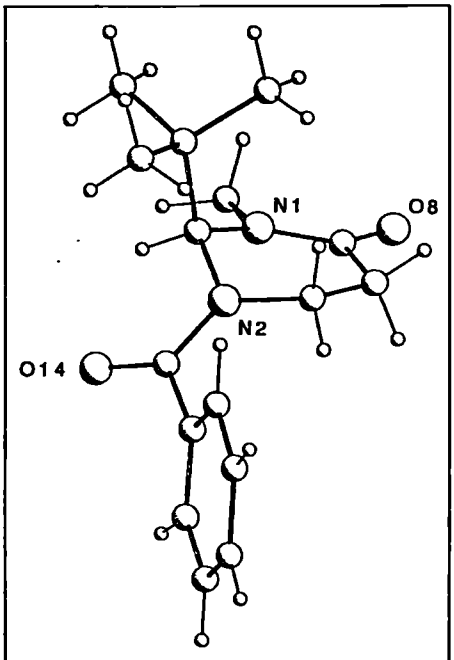

Figure 1. Crystal structure of 2.

Diastereoselectivity of alkylation of enolate 2-Li. High diastereoselectivity (ds = 86-97%) was found as indicated by integration of the C-13 NMR spectra of the crude products. That addition took place preferentially from the side opposite to the *tert*-butyl group, to afford the *trans* products, was determined by NMR spectroscopy. Finally, an X-ray diffraction study of the benzylated derivative confirmed its relative configuration as *trans*. (Figure 2).

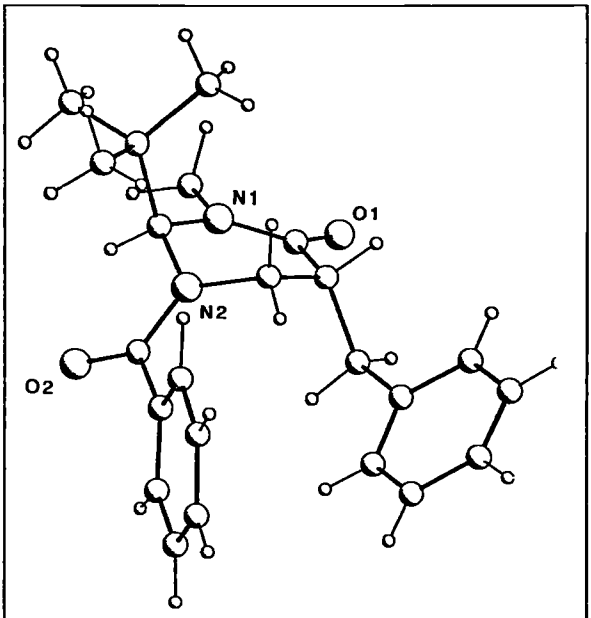

Figure 2. Crystal structure of the benzylated derivative.

Hydrolysis of the pyrimidinone adducts to give the α-substituted ß-amino acids. The final step of the overall conversions involves the hydrolysis of the heterocyclic products with cleavage of the ring and regeneration of a carboxylic acid. Hydrolysis is best achieved under acidic conditions, which are less likely to cause epimerization at the stereogenic centre C(2) of the desired amino acid (important for non-racemic analogues). Nevertheless, drastic conditions were required for the hydrolysis of adducts (6N HCl, 160-180°C, sealed tube) which proceeded in excellent yields to furnish a mixture of salts of the α-substituted ß-amino acids and methyl ammonium hydro-chloride.

Conclusions

ß-alanine, an inexpensive and achiral amino acid was converted efficiently into the racemic N,N-acetal 2. Unexpectedly, an X-ray crystallographic structure of perhydro-pyrimidinone 2 revealed the axial orientation of the *tert*-butyl group, which is probably responsible for the high *trans* diastereoselectivity found in the addition of enolate 2-Li to electrophiles. In this way, the chirality centre at C(2) induces the stereoselective formation of the new stereogenic centre at C(5) of the heterocycle.

The hydrolysis of the resulting adducts proceeds with 6N hydro-chloric acid to afford the desired *α*-substituted ß-amino acids in good yields.

Discussion

This work provides the basis for the preparation of optically-pure α-substituted ß-amino acids via enantiomerically pure derivatives of type 2. The preparation of such starting materials is being actively pursued.

The implementation of these studies in Mexico is necessary because there are no other research groups working in this field at the present time. The required amino acids are currently isolated from natural sources, or imported at great cost.

Particular benefits of the collaborative approach are the following:

1. Our colleagues in Barcelona (C. Miravitlles, E. Molins) are expert crystallographers who will determine the 3-dimensional structure of key compounds obtained in this work.

2. Interaction between the research groups in Mexico and Spain enriches the project since more ideas can be put into practice and complementary approaches lead to a more comprehensive study.

3. Exchange visits between the researchers in both countries lead to additional collaborations and further interactions with other chemists.

Publications

Juaristi, E; Quintana, D; Lamatsch, B. and Seebach, D. (1991) Asymmetric synthesis of ß-amino acids. 1. Highly diastereoselective addition of a racemic ß-alanine enolate derivative to electrophiles. *Journal of Organic Chemistry*, 56, 2553.

Juaristi, E.; Escalante, J; Lamatsch, B. and Seebach, D. Asymmetric synthesis of ß-amino acids. 2. Preparation of enantiomerically pure *a*, ß-disubstituted ß-amino acids. Submitted to *Journal of Organic Chemistry*.

Juaristi, E.; Rizo, B.; Natal, V.; Escalante, J. and Regla, I. (1991) Alternative method for the resolution of 1-benzoyl-2-tert-butyl-3-methyl-1,3-imidazolidin-4-one. *Tetrahedron-Asymmetry*, in press.

Postdoctoral fellowships

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Synthesis of heterocycles of biological interest by means of carbene complexes of transition metals.

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Synthesis and characterisation of new non-conventional sulphide catalysts based on coordination compounds of heavy metals.

Fellowship period: December 1990 - November 1991

Summary

During the last few years, a major effort has been made at IRC in Villeurbanne to design new sulphide catalysts for hydrotreatment in fine chemicals applications. In particular it has been shown that sulphides with a pyrite-type structure like RUS_2 possess remarkable properties for hydrogenation and hydrolysis. Even with unsupported catalysts, these results are obtained with members of the systems $Ni_x Ru_{1-x} S_2$ and $Co_x Ru_{1-x} S_2$. The project seeks to prepare supported catalysts using bimetallic clusters.

The methods will involve preparation of precursors for CoRu and NiRu sulphides based on the recent discovery of the Co-Ru heterodimetallic sulphur cluster. These new compounds will be characterised by spectroscopy, NMR and XPS. They will be used to prepare both supported and unsupported catalysts. Particular attention will be paid to the impregnation step and the activation procedure. The catalysts will be characterised by XRD, HREM and TPR. Study of the interaction of organic reagents with the precursors or with unsupported samples will help us to understand the $Ni_x Ru_{1-x} S_2$ system. Finally the catalytic activity of these new materials will be compared with that of conventional sulphides in different reactions carried out in flow microreactors, e.g. hydrogenation, hydrodesulphurisation.

4 EARTH SCIENCES

Summary

The reports in this chapter cover two broad areas, the resources of the arid lands of northern Mexico and seismology and earthquake engineering.

The first project has used satellite remote sensing, a tool of great utility for such a vast and sparsely populated area. It has been used for vegetation survey which can help livestock management and the utilisation of pasture; it has also been used to detect sodium carbonate efflorescences which might have economic value and to provide insights into geological processes. Another study has examined in more detail the soil-plant-erosion relations of the natural vegetation used for extensive cattle ranching and has provided some ecologically-based guidelines for the management of this fragile resource.

The importance of seismology and earthquake engineering for Mexico, and particularly for Mexico City, does not need emphasising. The two subjects are considered together here because of the continuum that exists between seismic source and the shocks received by a building. Every effort needs to be made to increase the safety of the public threatened by natural disasters and this subject is a priority for International Scientific Cooperation with Mexico. The planned seismological study involves tracing the pattern of seismic shocks between artificial explosions made at sea off the Pacific Coasts and Mexico City, while the earthquake engineering study will consider the effects of local ground conditions and the response of a selected, instrumented building in Mexico City during earthquakes.

18 Inventory of the Sonora Desert resources by remote sensing

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Contract number and duration: CI1*/0065, March 1986 to August 1989.

Summary

This project consisted essentially of the setting up of a low-cost image processing system appropriate for resource inventory in Mexico. During the course of the project the system was used to study vegetation composition and the extent of sodium carbonate efflorescences.

Vegetation The general objective of this study was to undertake an inventory of the vegetation resources of the extensive arid lands of northern Mexico. During the period of the project, processing and interpretation of LANDSAT-4 Thematic Mapper data relating to the southwestern edge of the Gran Desierto de Altar was undertaken.

An initial comparison between summer and winter data indicated the superior potential of winter data owing to reduced cloud cover at that time. Using image processing, enhancement and classification techniques, 10 false colour classes representing paloverde (*Cercicium microphyllum*), creosote bush (*Larrea tridentata*), burr-sage (*Franseria dumosa*), halophyte and dune vegetation types were established.

Detection of sodium carbonate efflorescences Using LANDSAT data of the Bahía de Adair region of the Mexican Sonora Desert and processing to distinguish between sea water and sodium carbonate deposits, previously unmapped sodium carbonate efflorescences were located.

In addition to possible economic importance of these deposits to the chemical industry, the pattern of deposits identified by this study suggests that the salts have originated at the Pinacate volcano and have been swept down to emerge in the sands of Bahía Adair. Further study is required to confirm this and, possibly, to suggest the existence of an aquifer under the desert.

19 Contribution to the study of soil water-plant production-erosion relations in the northern Mexico arid zone.

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Contract number and duration: CI1*/0087, September 1986 to August 1989

Summary

This study concerns water - soil - vegetation relations in a natural environment exploited for extensive cattle production but having a fragile ecological balance. The objective of the work was to propose rational management scenarios with resource conservation as a primary aim, taking special account of the soil and climatic constraints imposed by the environment. The results obtained have allowed a better understanding of the basic science of the environment, knowledge of which was lacking but which is, however, essential. Nevertheless, it must be pointed out that two of the years of the study, 1988 and 1989, were years of exceptionally low rainfall and so, for the sake of completeness and representativity, further study is required.

Background

In Mexico, zones with a mean annual rainfall of less than 500 mm cover 55% of the land surface and two thirds of the surface suitable for agriculture. These low-rainfall zones are mainly located in the north of the country.

The principal constraint to agriculture in these zones is climatic and is characterized by the low level, and variability in time and space, of precipitation. In addition, most rain falls in summer, when the high levels of solar radiation cause an important fraction of the water to be lost directly through evaporation. Soil factors represent another important constraint: the clay texture of the soil reducing infiltration and the storage of an available reserve of water; the presence of salts and low nutrient levels further reduce agricultural potential.

As a result of these constraints, agricultural cropping in these environments is limited and the traditional economic activity of the region is extensive cattle production. Such activity, based on natural rainfall, occupies 90% of the area of the north of Mexico, the rest being dedicated to irrigated agriculture. The utilization of the natural grazing resources of these arid and semi-arid zones needs to be managed on a rational basis since their ecological equilibrium is fragile and they are being subjected to an uncontrolled intensification of their exploitation which could lead to irreversible degradation of their productive potential. This problem has been recognised by the scientific community, which, amongst other initiatives, has undertaken research at a test site in the arid zone of the north of Mexico at the Mapimi Biosphere Reserve, located on the borders of the states of Chihuahua, Coahuila and Durango.

Objectives

With the objective of proposing utilization scenarios for the arid north Mexico environments, it was necessary to make up for the deficient scientific knowledge of the environment and in particular to characterise the function and dynamics of the basic ecological units identified in the Mapimi reserve. In the arid ecosystem studied, water is the principal factor conditioning the existence of plant life, agricultural activities and human occupation. In such an environment, as exact an understanding as possible of the quantity of water available as well as its distribution and dynamics in the soil and in the landscape appeared indispensable.

With this perspective, we were particularly interested, on the one hand, in the study of the storage of rainwater in the soil and its availability for plant species of forage value in the arid zone of the north of Mexico and, on the other hand, in the redistribution of water in the landscape. It is essentially the links between the hydrodynamics of the principal soils, the discontinuous spatial distribution of the plant cover, the water movement in space in the form of surface runoff, along with the attendant risk of erosion, that we have tried to establish.

In addition, we have tried to determine the ecophysiological conditions for the production of the principal plant species of forage value as a function of the climatic conditions and in relation to the characteristics of the soil cover and its spatial variability.

Methods

Rainfall was measured using standard rain gauges to record amount, frequency, intensity and spatial distribution. Rainfall simulation by sprinkler was used to study the roles of vegetation cover, slope and soil type. The roles of rainfall intensity and frequency and coefficients of run-off and infiltration were determined. Run-off micropits, natural rain run-off and erosion plots (500 m^2 and 1,000 m^2) were created. Microcatchments (6.6 ha) and catchments (15.6 km^2 and 11.5 km^2) were selected to study the dynamics and redistribution of water and other elements in the landscape.

Soil humidity was measured using gravimetric, tensiometric and neutron scattering techniques. Vegetation cover was estimated using point quadrats, photography from a mast and helium balloons, aerial photography and satellite imagery. Soil characterization and mapping was carried out. Pressure bomb measurements of ecophysiological behaviour of the principal plant species of value as forages were made.

Conclusions and recommendations

The number of grazing animals should be maintained in equilibrium with forage resources to avoid overgrazing and soil compaction. Water harvesting techniques using small dams and landscape features which would reduce evaporation to a minimum, could be practised. The two principal factors affecting surface runoff appear to be the presence or otherwise of vegetation and a surface crust and these two factors have opposing effects.

The two grasses studied in detail, *Hilaria mutica* and *Sporobolus airoides*, both remain dormant during prolonged drought. However, after rain *S. airoides* grows and exhausts soil water rapidly, whilst in *H. mutica* growth is less rapid, but water potential remains high for longer, with water being conserved in its cells owing to their rigidity. The different strategies are consistent with the observation that *S. airoides* is found in low-lying areas where water accumulates while *H. mutica* is found in dryer places. Because of these ecological differences and the fact that *H. mutica* is not consumed by cattle once dry, a grazing strategy involving the use of *H. mutica* when green and reserving the pastures as a forage reserve for the dry season might be proposed.

20 Study of seismic risk in Mexico City associated with the crustal structure in seismically active zones by means of deep seismic sounding

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Contract number and duration: CI1*/0635, December 1990 to November 1992

Preparation of the experiment

Two months before the experiment takes place the compilation of topographic maps and aerial photographs necessary for the field work will begin. An experienced scientist will be contracted by the Institute of Geophysics (UNAM) for a period of two years to help the experiment preparation and to collaborate in the following stages of the work. The ship carrying out the seashots has to be prepared for the transportation of explosives. In addition, containers for the explosives and signposting buoys will be constructed.

The Institute of Geophysics will deal with the logistics of the experiment. This includes keeping in touch with the local authorities and with the radio stations which will transmit time signals and messages to the field operators during the experiment. At present, Spanish digital seismic stations can only receive time signals at a frequency of 77.5 kHz. In order to use these stations in Mexico, it is necessary to modify them to receive time signals at other frequencies available in the area under study.

Preliminary field work

This task will be performed for a period of one month before the experiment. Each recording point will be inspected and located on topographic maps. This is a very important matter, because many recording points have difficult access due to the rough topography of this region. This task will be carried out by experienced scientists with a wide knowledge of the geological and geographical characteristics of the region under study. The transport of equipment and operators to the field will be effected by means of cars to be rented in Mexico City.

Field work

The experiment will be carried out in a period of 21 days. A total of 31 shots at sea and 2 shots on land, in a quarry located in Mexico City, will be recorded by 21 digital and 10 analog portable seismic stations. Three profiles are planned, one following the coastline from Acapulco (Guerrero State) to Lazaro Cardenas (Michoacan State) with a length of 380 km. Two other profiles will be 300 km long and will go from the coast to Mexico City. Seismic stations will be operated by specialists from each of the participating institutions. The distribution of profiles and shots can be seen on the maps of southern Mexico below, Figures 1 and 2.

Figure 1. Location map

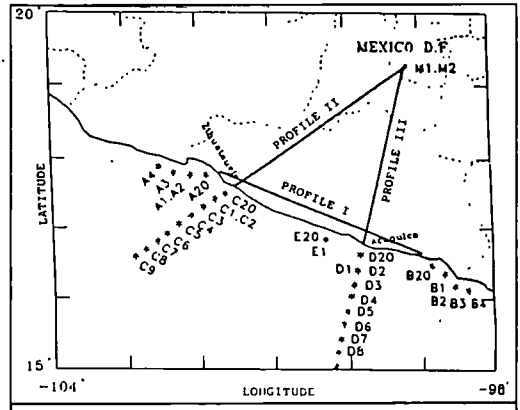

Figure 2. Distribution of profiles and shots in the area

During the experiment a demodulation centre will be established in the area under study. This centre will be connected by telephone to the shotpoint and to the operators and it will inform all participants about possible changes in the time schedule during the experiment. In the demodulation centre, stations will be checked daily in order to ensure that the fieldwork is properly carried out. This centre will be occupied by two experienced scientists.

Data processing and interpretation

Data processing will be carried out for a period of two months after the experiment. Data corresponding to the analog stations will be digitized electronically using an A/D converter. The result of the digitization of each seismic signal and the data registered by digital stations will be recorded together on a magnetic tape in a compatible format so that they can be analyzed by all the participant

institutions. This task will be undertaken separately in Mexico and Spain, because data recorded by Mexican stations cannot be processed in Spain and data recorded by the Spanish stations cannot be processed in Mexico. Due to the large quantity of data to be recorded in this experiment it will be necessary to acquire a workstation (HP9000-series 300, or similar) to process and analyze the data stemming from this project.

In a first stage, data interpretation will be carried out separately in Mexico and Spain by each one of the working groups of the participant institutions. In a second stage, results will be compared to obtain a common global model for the region under study. This stage requires frequent contact between the different working groups in order to facilitate the interchange and discussion of results. For that reason, several trips for the scientists of each working group have been planned. These trips will take place throughout the period of data interpretation.

21 Study of site effects and building response in Mexico City during earthquakes

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Contract numbers and duration: CI1*/0673/0674, January 1991 to December 1994

Objectives

The project has the following four main objectives:

1. Analyze "free-field" data of strong-motion earthquakes recorded in Mexico City, to study seismic wave amplification in the lake-bed zone.
2. Extend the capabilities of existing theoretical models for ground response analyses; after model calibration, the predictions will be used for the microzonation of specific areas.
3. Instrument with accelerometers one carefully-selected building in the lake-bed zone of Mexico City to study soil-structure interaction and structural response to earthquake ground shaking. The data will be collected over 4 years.
4. Analysis and interpretation of the structural response data; comparison of observations with predictions yielded by standard engineering models.

Objective 1 can be pursued at small extra expense since many strong motion instruments are already installed, and software for seismic signal processing and analysis is available.

Objective 2 to be carried out mainly in Milan, covers a major part of the theoretical work of the project. The existing software for 2D seismic analyses developed at the Politecnico will be greatly improved in efficiency and flexibility, for application to specific configurations in Mexico City and elsewhere, and a 3D extension will also be considered.

Objective 3 requires purchase, installation and maintenance of instruments over a period of 4 years, and will be the main responsibility of the Mexican team. Finally, objective 4 will provide the main practical output of the project from the viewpoint of earthquake engineering applications, with a significant potential for improvement of building codes in Mexico and elsewhere.

Work programme

Initially, work will begin in Milan on an existing computational model for 2D seismic analysis with the aim to improve substantially the treatment of the source (incident wave) and of the absorbing boundaries. The model uses the pseudo-spectral method limited to SH waves and the previous improvement is expected to result in greatly reduced computer memory occupation, as well as in a

significant scientific contribution. This will require most of the full-time work of a junior researcher, plus the contribution of a senior scientist for a limited period of time. Work on fully nonlinear, 1D seismic ground response analyses on Mexico City data will also be initiated, to interpret the observed response in particular areas of the lake-bed zone.

At the same time, a number of candidate buildings for instrumentation will be identified in Mexico City. When the Italian team leader visits the city, the final choice will be made based on appropriate optimality criteria. Once the building has been selected, the actual structural and geotechnical properties needed for modelling its seismic response will be obtained by: (a) gathering the original drawings and documentation concerning its structural design, the soil mechanics study, and construction; (b) in-situ verification of its structural properties by non-destructive tests; (c) ambient vibration testing of the building and (d) soil borings in the vicinity of the structure.

The final design of the instrumentation will also be performed including the selection of instruments and the definition of their exact location in the structure and in the ground (free-field), as well as the design of the interconnections between them and the choice of the data acquisition system.

This work is expected to take about one year.

Phase 2. During the second phase, work will take place in Milan aimed at constructing 2D dynamic subsoil models. There will be soil boring at the building site and adjacent areas calibrated with project observations as soon as they become available (the instrumentation will include free-field accelerometers). Ground motions of previous earthquakes (especially 1988 and 1989 events) recorded by the existing network will be used in the meantime. This will require extending the computational model to handle also P- and SV-waves, as well as exploring the feasibility of 3D analyses.

Secondly, the calculation of dynamic soil-structure interaction parameters will be performed, based on the soil data available and the detailed plans of the existing foundations. Validation of current methods for interaction analysis is going to be one of the salient contributions of the project.

A 3D model of the structure will be prepared for computing its seismic response, including the soil structure interaction. This model will be calibrated with the results of the ambient vibration tests. At the same time, a methodology for seismic identification will be designed for the determination of the properties of a simplified model of the structure and of the soil-structure interaction from the measured response of the building to the actual earthquakes.

The instrumentation in the building and in the ground will be installed, calibrated and tested. The programme for the operation and maintenance of the network will begin.

The duration of the second phase could be between one and two years, assuming that the instrument network of the building will start operating 8-12 months after initiation of the project.

Phase 3. The third phase will begin with the interpretation of the measurements as soon as the data are available (events with $5.5 < M_S < 7.0$ are recorded in the Mexico City area roughly once a year), while its end will coincide with that of the project. Most of the work in this final phase will be performed together by the two teams, since it will involve the overall calibration of the models (free-field ground response, plus soil-structure interaction, plus structural response) with the recorded data. The initial models of structural response will be modified and improved. Of special significance also appear to be the aspects related to the predictability of the elastic response spectrum, the significant duration, the amount of nonlinear soil response, the fundamental site response frequency and the amount of interaction damping. Important for microzoning applications will also be the comparison of predictions yielded by 1D vs. 2D ground response analyses.

Conclusions on the differences between the response predicted by present design practice and what is actually measured will be obtained. Modifications to common practice will be proposed.

5 ENVIRONMENTAL SCIENCES

Summary

The studies reported in this chapter involve wastewater treatment and the fate of agrochemicals washed from agricultural land into coastal lagoons. With a large proportion of the country having an arid climate, water resources are critical and water research is a major priority for International Scientific Cooperation with Mexico.

A number of novel techniques for wastewater treatment with particular advantages for Mexican conditions have been studied or are to be studied in the projects reported here. These techniques involve anaerobic digestion; the use of water hyacinth to remove nutrients and heavy metals from effluent, the excess water hyacinth itself being disposed of in an anaerobic digestion process; the root zone method in which wastewater flows through reed-beds *(Phragmites)* where bacteria oxidise impurities and protozoa can remove bacteria, including pathogens; and the use of biofilms for phosphate removal.

The project on the fate of agrochemicals is particularly interesting since it deals with a highly productive tropical coastal environment where agricultural and horticultural production demand a high level of agrochemical use; however, many of these agrochemicals are washed by rain from the soil and into coastal lagoon ecosystems where they pose a threat to important fisheries and the rearing grounds for offshore fishery stocks.

Joint research projects

22 Anaerobic digestion and water hyacinth in wastewater treatment

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Contract number and duration: CI1*/0198, May 1988 to April 1990

Background and objectives

This project was originated to answer the need in Mexico to develop low cost and efficient wastewater treatment systems. It was proposed to study at laboratory and pilot scale a system integrating advanced anaerobic digestion processes as a secondary treatment and a water hyacinth pond as a tertiary treatment. Later the use of algae ponds was introduced.

A second objective was the formation of a group of professionals in anaerobic wastewater treatment capable of undertaking both fundamental as well as applied research. Equally important was the transfer of this technology to wastewater consultancy firms.

Specific objectives were the installation and operation of pilot plants; the study of factors that influence the granulation process of the anaerobic sludges and their adaption to specific wastes; and the study of the post-treatment of the anaerobic effluents in algal and water hyacinth ponds.

Materials and methods

Laboratory studies were performed under controlled conditions in 4 litre reactors treating different kinds of wastes (both synthetic and real) to understand the ecophysiology of anaerobic sludges as well as the treatability of the wastewaters. From these reactors sludges were sampled and cultured to perform microbial population, methanogenic activity and granulation studies (Guyot *et al*, 1990). Water hyacinth and algae were cultured in a greenhouse to study the kinetics of the removal of heavy metals and nutrients.

Semipilot scale studies were carried out in 120 litre upflow anaerobic sludge blanket (UASB) reactors to study *in situ* the treatability of different wastes and obtain mass and energy balances.

Pilot scale studies in 40 and 50 m^3 UASB reactors were performed treating wastewaters from a yeast-producing industry and from the university (domestic with chemicals). Residence time distribution, scaling up, construction and operational data were obtained from them. The wastewaters were characterized according to the standard methods (APHA 1985) to determine process parameters.

Results and discussion

Development of design and consultancy services. The most important result is the formation of a group of specialists which regularly advises small enterprises on wastewater treatment processes. Projects between UAM, industries and other research institutions are in progress and together with our partner group at the Engineering Institute at UNAM (II-UNAM), we have become an important reference for anaerobic digestion in wastewater treatment in Mexico.

A patent, developed and owned by UAM-UNAM, for the design of UASB reactors has been transferred to two companies (Descontaminación and IMASA) which will build two full-scale UASB reactors, to treat yeast and municipal wastewaters, respectively. A third transfer contract is on its way with a new consultancy firm (GTSA) formed by former students of this group.

Another patent, owned by UAM-UNAM-ORSTOM, providing a procedure for the production and adaption of anaerobic sludges, will be transferred once there are more full-scale UASB reactors.

Pilot scale experiments on the treatment of municipal wastewater A scheme for municipal wastewater treatment has been adopted as a model for study. Screened municipal wastewater is processed in a UASB reactor. This effluent is rich in nutrients and heavy metals which are removed in the water hyacinth pond. Excess water hyacinth is disposed of in a two-stage anaerobic digestion process. Studies on the fate of heavy metals and the role of microalgae are in progress to complete the process. To work out the design basis, several experiments were undertaken to validate at a small pilot scale the feasibility of the anaerobic digestion - water hyacinth system to bring wastewaters to a tertiary quality.

Oscar Monroy, Sofia Sarquis and Catalina Reyna studied the influence of the harvesting rate and organic matter on the removal of nitrogen and heavy metals to find design criteria for the operation of water hyacinth ponds. A dimensionless number, which is a measure of the Nutrient Removal Capacity of the system, was found and this helped them operate the ponds at a constant removal efficiency. They also found that healthy growing water hyacinth will absorb heavy metals, accumulating them mainly in the roots (60-80% of the total metals were absorbed). After five days the metals in the roots will start leaching back to the solution. At ten days, the fraction accumulated there is down to 5%, whereas the total accumulation within the plant at ten days is 42% of that at five days.

Oscar Monroy and Manuel Fuentes made a kinetic characterization of water hyacinth degradation in a two-phase anaerobic reactor. Difficulties in the first acidogenic phase were found. They tried first with a packed bed leaching reactor and found that a continuous operation was not practical. A different process is being tried in which juice and solids are treated separately. The juice is treated in a two-stage anaerobic process involving an upflow acidogenic sludge blanket reactor followed by a UASB reactor. The solids are being treated by dry anaerobic fermentation; a preliminary experiment performed by Beatriz Schettino has shown that this process is a better alternative to solid aerobic fermentation. Inoculating with bovine ruminal juice, a 20% solids water hyacinth (0.98 A.) had a 25% degradation producing 18 g of volatile acids per 100 g of dry water hyacinth.

Amparo Ramos, Margarita Salazar and K. Ilangovan worked on a new idea in the UASB-water hyacinth system by introducing algae to aerate the effluent, further reduce the organic matter content and remove the inorganic nutrients and heavy metals from the anaerobic secondary effluent. The effects of transition heavy metals and their interactions was studied in the algal, water hyacinth and anaerobic sludge. In the three systems, the antagonistic effect of Zn against Cd and Cu was established. If the former was absent, the other two would inhibit growth and removal of substances from the water, while its presence would significantly reduce the toxic effects of Cd and Cu. The metals accumulate mainly in the vacuoles and chloroplasts which appear to be disorganized.

Behaviour of activated sludge

Adalberto Noyola and Jean P. Guyot studied the factors that influence the granulation and the ecophysiology of the anaerobic sludges in the UASB reactors. They found that glucose and egg albumin favoured granule formation, if compared with VFA. In this respect, granule formation was faster with egg albumin. In the end (7 months), the largest mean granule diameter was 1.7 mm. It was also found that $FeSO_4$ reduced start-up of reactors, so a faster and more stable operation was achieved with this salt using VFA as substrate.

Guyot, Noyola and Monroy found that uncoupling between growth and energy production of acetoclastic bacteria is a new explanation for the low sludge generation in anaerobic reactors. They suggest that in order better to compare results among researchers, the bacterial concentrations in sludges should be presented per gram of volatile suspended solids. Furthermore, since there is no relationship between substrate disappearance and methane production in granular sludge, it is not worthwhile to make an analysis of substrate consumption on the basis of methane production curves.

J.P. Guyot and F. Ramirez found that formate, a major product of the anaerobic degradation of organic matter, decreases reactor performance by inhibiting the acetoclastic reaction of the *Methanosarcina* sp. bacteria. They conclude that a ratio *Methanosarcina/Methanothrix* might help predict the ability of a culture to be inhibited by accumulations of formate or hydrogen.

Fajardo and Guyot worked on the anoxic adaptation of activated sludge in a batch to provide anaerobic inocula for digesters. They observed the evolution of the microbial activity and composition and the physical characteristics of the sludge. During the adaptation process the sludge was fed with acetate. When compared with unfed sludge, a higher volatile suspended solids (VSS) and a lower sludge volume index (SVI) was obtained. All different groups of strict anaerobes involved in anaerobic digestion (methanogens and OHPA) were present in the fresh activated sludge, indicating a great potential to develop anaerobically. During incubation of the sludges, microbial counts increased in both (acetate-fed and unfed), with a significantly higher final count in sludge fed with acetate. In both cases, methanogens represent the dominant group. In the acetate-fed sludge, counts of hydrogenotrophic methanogens are 100 times higher than those of acetoclastic methanogens.

Construction of pilot plant Since the start-up of the project, pilot-scale facilities in a sugar factory and at IMEXA have been designed. A 50 m^3 UASB reactor has been constructed to implement the treatment scheme described above at a pilot scale on the university campus. A team formed by Octavio Gonzalez, Adalberto Noyola, Oscar Monroy and students undertook the basic design and supervised the final engineering work of the consultants (Descontaminacción and Constructors Anahuacalli). This design was also used to form the basis of a 40 m^3 UASB pilot reactor treating a yeast effluent at IMEXA.

Treatment of industrial wastewater

Wastewater from the terephthalic acid industry. Herve Macarie, a doctoral student from France, Jean Pierre Guyot and Adalberto Noyola worked with the effluent of a factory in Mexico, Tereftalatos Mexicanos. A downflow fixed-film reactor achieved 75% COD removal with a 3.4 days HRT and organic loading of 1.9 $kgCOD\ m^{-3}d^{-1}$. No inhibitory effects were noticed as compared with when this same effluent was treated in a UASB reactor. A treatment process comprising primary settling, anaerobic and aerobic secondary treatment was proposed as an option to their aerobic activated sludge process. Unfortunately, the company declined to continue with the project and no pilot-scale experiments were done.

Wastewater from the yeast production industry. Oscar Monroy and David Rodriguez, a former undergraduate student from UAM, have been working with the effluents of a yeast production factory both at bench and pilot scale. The objective is to design a full-scale treatment plant based on anaerobic digestion of the wastewater, in order to use it for irrigation purposes. Removal efficiencies of 60% were obtained at organic loadings of 12 kg $m^{-3}d^{-1}$. Further work is needed to remove the effect of K^+ which is thought to prevent further biological treatment. A 40 m^3 UASB pilot reactor has been constructed and the basic engineering design of the full-scale plant has been done.

Anaerobic treatment of a synthetic effluent containing *p*-toluic acid. Herve Macarie and J.P. Guyot studied the anaerobic biodegradation of *p*-toluic acid as an example of a difficult and persistent pollutant. With this type of effluent, it was found that the biodegradability of *p*-toluic acid was not sufficient to promote adequate REDOX conditions during the start-up. It was demonstrated that $FeSO_4$, a chemical which, through the sulphate reducing bacteria, is transformed to a reducing substance (FeS), provides a REDOX potential suitable for the bacteria to break down the *p*-toluic acid. It was found essential to add this reducing agent continuously.

Treatment of the university campus wastewater with anaerobic digestion and water hyacinth. With the aim of evaluating the year-long feasibility of tertiary biological treatment by microalgae, a semi-pilot plant consisting of a 1000 litre balance tank, 110 litre and 5000 litre UASB reactors, a 450 litre algal tank and 760 litre water hyacinth pond is working with the university wastewaters. The anaerobic secondary effluent from the UASB reactor is used for the tertiary treatment with algae and water hyacinth. The system can work in series or in parallel to compare the results. Technical difficulties in the operation of the system have caused large differences in nutrient removal efficiencies (between 25-70%) and biomass production as they depend strongly on the physico-chemical and climatic conditions. We are currently working on the optimization of the system's operation. Some results have been obtained, one of the most significant being that the system UASB-water hyacinth can remove up to 81% of the organic load. The algal system can remove up to 85% of the ammonium ion and 70% of phosphates.

Conclusions

The need for anaerobic digestion processes for wastewater treatment is becoming apparent in Mexico. Basic investigations and applied research have shown its technical and economic feasibility both at municipal and industrial levels. Diffusion of the technology will be easier now with the two pilot plants which have been built. The next step now is to introduce process control schemes to improve the reliability of anaerobic digestion and the post-treatments. More fundamental research will be needed to find control variables in the reaction steps of the degradation paths and in the response of the microorganisms and water hyacinth to the presence of persistent and toxic pollutants. Applied research is also needed to develop control systems for the pilot plants and assess their reliability.

Publications

Noyola, A.; Guyot, J.P. and Monroy, O. (1987) El tratamiento anaerobio de aguas residuales (The anaerobic treatment of wastewater). *Interface*, **23**, 15-18.

Guyot, J.P.; Noyola, A. and Monroy, O. (1988) Actividades metanogicas de lodos granulares de un digestor de lecho de lodos (Methagenic activities of granular sludges in an activated-sludge reactor). *Revista AINSA* (Medellin, Columbia) **A98**, 23-32.

Guyot, J.P.; Noyola, A. and Monroy, O. (1988) Comparacion experimental de dos tipos de reactores anaerobicos avanzados (Experimental comparison of two types of advanced anaerobic reactors). *Revista AINSA* (Medellin, Colombia), A98, 81-92.

Guyot, J.P. and Ramirez, F. (1989) Inhibition of the anaerobic acetate degradation by formate. *Biotechnology Letters*, 11, 365-8.

Guyot, J.P.; Macarie, H. and Noyola, A. (1990) Anaerobic digestion of a petrochemical wastewater using the UASB process. *Applied Biochemistry and Biotechnology*, 24/25, 579-89.

Guyot, J.P.; Noyola, A. and Monroy, O. (1990) Evolution of microbial activities and population in granular sludge from an UASB reactor. *Biotechnology Letters*, 12(2), 155-60.

Guyot, J.P.; Fajardo, C.; Noyola, A. and Barrera, C. (1991) Study of the transition of activated sludges to an adapted anaerobic inoculum for anaerobic digestion. *Biotechnology Letters*, in press.

Guyot, J.P.; Fajardo, C. and Noyola, A. Transition of an activated sludge state to an anaerobic adapted inoculum. *In* Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer, ed. J.P. Belaich. Plenum Publishing Corporation, 385-6.

Ilangovan, K.; Fuentes, D.M. and Monroy, H.O. (1990) Transport of heavy metals during anaerobic digestion of water hycinth. *Biotechnology Letters*, 5(12), 387-92.

Ilangovan, K.; Salazar, M.; Sudakar, D. and Ramos, A. (1990) Interaction of transition heavy metals in *Chlorella pyrenoidosa*. Submitted to *Environmental Technology Letters*.

Monroy, H.O. and Sarquis, S. (1990) Design considerations for wastewater treatment with water haycinth *E. crassipes*. *Environmental Technology Letters*, 11, 669-74.

Noyola, A.; Macarie, H. and Guyot, J.P. (1990) Treatment of terephthalic acid plant wastewater with an anaerobic fixed film reactor. *Environmental Technology*, 11, 239-48.

Patents

Noyola Robles, A. (UNAM-UAM). Design of a UASB reactor.

Noyola Robles, A. (UNAM); Monroy Hermosillo, O. (UAM); Guyot, J.P. (ORSTOM). Technology related to the process for the production of anaerobic sludges for UASB reactors.

23 Fate of agrochemicals in tropical coastal lagoon ecosystems

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Background

There is increasing global concern about the fate of persistent agrochemicals, particularly chlorinated hydrocarbon biocides, which are introduced into the coastal marine environment as a result of runoff (land drainage). Intensive studies during the 1970s revealed extensive damage to some terrestrial and marine ecosystems (particularly bird populations) ascribed to DDT pollution. DDT production was banned by most developed countries, but production and use continues in many developing countries.

A wider arsenal of agrochemicals is now employed including other organochlorines, organophosphorus, carbamate and pyrethroid pesticides and also herbicides. In developing countries, economic constraints often force farmers to apply the most cost-effective treatments despite their adverse environmental consequences. Where intensive agriculture (particularly horticulture) is practised in tropical countries, large quantities of agrochemicals are almost universally applied. This often occurs on the coastal plain in which runoff from agricultural land is rapidly discharged into the marine environment. Over one third of the world's tropical coasts are dominated by highly productive and shallow lagoons which support important fisheries (fish, crustaceans and bivalves) and act as nursery grounds for even larger offshore stocks.

Mexico is rather typical of many tropical countries in that both intensive agriculture and aquaculture are developing side by side but not in unison. In the State of Sinaloa for example, over 50 shrimp farms are under construction or have been authorized. Shrimps traditionally represent one of Mexico's principal exports and coastal lagoons are the major nursery grounds for postlarval growth. Shrimp (and oyster) culture are, however, very recent developments. The coastal plains of the same State also produce two thirds of the national horticultural exports (principally tomatoes), representing 10% of the country's total agricultural exports (60% of the USA's total imports of these products). The coastal human population consumes large quantities of fish from the lagoon ecosystem as well as shrimp and bivalves. The lagoons are also important habitats for an enormous diversity of marine animals and a detailed catalogue and reference collection has been made of these over the past decade at UNAM's

Mazatlan field station. This type of information, together with a number of publications on ecology and contamination of the lagoons of Sinaloa, provide valuable background material. The site selected for the present work (the Altata-Ensenada El Pabellon lagoon system with an area of about 360 km^2 - see Figure 1) is particularly well suited for this study, receiving effluents from the River Culiacan and drainage from 600,000 hectares of mechanized, artificially irrigated, agriculture (principally horticulture and sugar cane).

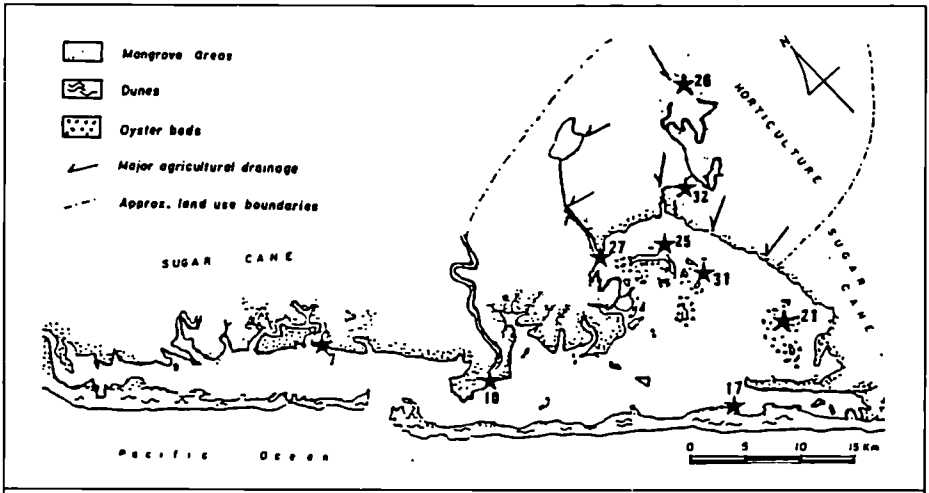

Figure 1. Locations of sampling sites within the Altata-Ensenada el Pabellon lagoon system

Objectives

The aim of the research is to evaluate the fate of agrochemicals in tropical lagoon environments (using the Altata-Ensenada el Pabellon lagoon system in Sinaloa, Mexico, as a model). Pathways, reservoirs and degradation of a variety of agricultural chemicals are being examined in order to quantify fluxes.

Methods

The project was designed to combine the expertise and facilities offered by both partners. UNAM have acquired a substantial data base on the tropical lagoon system selected for study and also offer excellent research facilities at their Mazatlan Field Station. MESL-ILMR have extensive experience in analytical chemistry and in environmental chemistry research. By integrating these attributes, a unique opportunity has been created to study the fate of agrochemicals in tropical lagoon ecosystems.

To identify the agrochemicals of relevance to the area (and hence those for investigation), an in-depth study was conducted to estimate quantities of the compounds used within the basin draining into the Altata-Ensenada el Pabellon lagoon system.

Sediment and selected biota samples have been collected for analytical screening of agrochemicals during the period of the project. Analytical methods have been developed and fine-tuned as necessary. Whilst the majority of analyses have been performed at MESL in Monaco, the relevant equipment and expertise have now been introduced at UNAM in Mazatlan and all future analytical work will be centred on this new laboratory.

A particularly innovative aspect of the project, which is currently being introduced, relates to the use of radiochemically labelled pesticides to track pathways and the degradation of selected compounds more easily and precisely. These will be used under controlled experimental conditions to quantify rates of transfer and breakdown. It is envisaged that these experiments will culminate with "mesocosm" studies to quantify interactions between competitive processes and thus afford the knowledge to model environmental behaviour.

Results and discussion

Estimated quantities of the major agrochemicals used annually in the drainage basin of the Altata-Ensenada el Pabellon lagoon system are listed in Table 1. The data relate to the agricultural year 1987/88, which is the most recent period for which a comprehensive data set could be compiled. The data illustrate the considerable changes in agrochemical usage that have occurred in recent years. Organophosphorus pesticides are the most commonly used compounds followed by the carbamates. Regarding the classical and highly persistent organochlorine pesticides (the DDTs and the "drins"), consensus of opinion was that their usage had ceased within the area and it is unlikely that they have been applied in significant quantities by farmers/horticulturalists during the last five years. Of the organochlorine pesticides, the only compounds currently used are toxaphenes and endosulfan.

Distribution of organochlorine agrochemicals Analyses of sediments and biota for organochlorines revealed comparatively low levels of DDTs and "drins" compared to those reported from elsewhere throughout the world. The distribution of DDTs reveal a high concentration at only one station, with lowest concentrations located furthest from agricultural inputs.

If the ratio of DDT to its metabolites (e.g. DDE and DDD) is examined, metabolites are shown to dominate DDT. This implies that little "fresh" pp'-DDT is entering the system and demonstrates cessation of usage of the compound. This suggestion is further supported from analyses by MESL of sediments from different Central American countries where DDT is dominated by its degradation products. The sedimentary distribution of dieldrin in the area was measured and again low concentrations were found. Although the distribution differs from that identified for DDTs, by far the highest concentration is recorded at station 26 where the maximum total DDTs value was also reported.

As previously stated, toxaphenes and endosulfan are the only organochlorine pesticides currently being used in the area. Toxaphenes are difficult to analyse and our efforts continue to quantify them. Results of endosulfan measurements at three sampling points lead to the conclusion that relatively large sedimentary reservoirs exist for all forms of endosulfan but only endosulfan sulfate is readily bioaccumulated and it can reach high concentrations, particularly in mussels.

Samples taken at different times of the year have all revealed the presence of endosulfan. Work is now being carried out to elucidate further its chemistry in this system.

Agrochemical	Quantity used (1987/88)	Application	Compound class	Approximate environmental half-life (months)
Malathion	541m^3 *	insecticide/ acaricide	organophosphorus	n.d.
Maneb	230 t *	fungicide	dithiocarbamate	n.d.
Chlorothalonil	106 t *	fungicide	phthalimide	2-3
Aldicarb	66 m^3 *	insecticide/ acaricide	carbamate	2
Parathion	61 t	insecticide/ acaricide	organophosphorus	n.d.
Methamidophos	42 t	insecticide	organophosphorus	n.d.
Bensulide	36 t *	herbicide	organophosphorus	4-12
Monocrotophos	32 t *	acaricide	organophosphorus	n.d.
Chlorpyriphos	30 t	insecticide	organophosphorus	3-4
Methonyl	22 t	insecticide/ acaricide	carbamate	rapidly degraded
Paraquat	18m^3 *	herbicide	bipyridylium	n.d.
Dimethoate	6 t	insecticide/ acaricide	organophosphorus	n.d.
Permethrin	5 t	insecticide	pyrethroid	rapidly degraded
Carbaryl	5 t	insecticide/ growth regulator	carbamate	n.d.
Metribuzin	4 t *	herbicide	triazine	1-2
Zineb	2 t *	fungicide	dithiocarbamate	n.d.
Toxaphene	1 t	pesticide	organochlorine	n.d.
Fenvalerate	<1 t *	insecticide	pyrethroid	n.d.
Atrizine	<1 t *	herbicide	triazine	5-7
Simazine	<1 t *	herbicide	triazine	7

Table 1. Estimates of major agrochemicals used (1987/88) within the basin draining into the Altata-Ensenada el Pabellon lagoon system.

Quantities are expressed as tonnes for solids and m^3 for liquids; the value given refers to the active ingredient only except where starred when the weight or volume refers to the concentrated product because no data on the amount of active ingredient are available. Environmental half-lives are taken from the Agrochemicals Handbook, 2nd edition, Royal Society of Chemistry, Cambridge, England.

Distribution of organophosphorus agrochemicals Sediment samples have also been analysed at MESL for organophosphorus pesticides including malathion, parathion, monocrotophos and chlorpyrifos. Of these compounds only chlorpyrifos was shown to have a widespread distribution. Chlorpyrifos was generally shown to be present at higher concentrations than either pp'-DDT or dieldrin reflecting the change away from use of the persistent organochlorine compounds. Highest concentrations of chlorpyrifos are associated with riverine inputs carrying agricultural runoff. Dilution and/or

degradation result in gradients of declining concentrations away from these points of entry. Preliminary mass balance calculations indicate that of the chlorpyrifos used in agriculture within the drainage basin, approximately 0.03% becomes incorporated into the lagoon sediment.

The physical chemistry of chlorpyrifos differs from that of most other organophosphorous pesticides. It is generally less soluble in water (0.4 $mg.dm^{-3}$ compared, for example, to 24 $mg.dm^{-3}$ for parathion). The log K_{ow} of 5.11 contrasts with that for malathion (2.89) and more closely resembles the log K_{ow} of pp'-DDT (6.19). Whilst this would enhance partitioning of chlorpyrifos onto sediments compared to that of most other organophosphorus compounds, it is likely that the identification of only chlorpyrifos in the sediments is a function of rapid degradation, as well as solubilisation, of the others. The high K_{ow} would also enhance biological uptake of chlorpyrifos.

Chlorpyrifos is extremely toxic. The 96 hour LC_{50} for a sensitive species of tropical shrimp has been reported as 10 $ng.dm^{-3}$. Population changes have been demonstrated within plankton communities exposed to 100 $ng.dm^{-3}$. Our results demonstrate that chlorpyrifos is sufficiently stable to contaminate marine systems significantly. Although within the lagoon system discussed the compound appears dispersed and at relatively low concentrations, most of the discharge from land occurs during sporadic rainfall events where concentrations are likely to be substantially elevated. Within the Altata Ensenada el Pabellon lagoon system, this also coincides with the penetration of the post larvae of migrating shrimp to low salinity regions of the lagoons. The association between fish kills and pesticides transported by rainfall events has already been demonstrated in temperate environments. Moreover, little is known about the effects on benthic fauna from prolonged exposure to sediment-bound pesticides. Further research is required into these topics.

Radiochemical experiments The results obtained from the agrochemical surveys directed that the choice of radiolabelled pesticides should include endosulfan, pp'DDT, chlorpyrifos and parathion. These compounds, labelled with ^{14}C, have now been purchased by MESL-ILMR.

Initial experiments within ILMR were performed using the ^{14}C chlorpyrifos. Processes of partition/sorption, volatilisation, photodegradation, and ^{14}C-CO_2 production have been investigated in order to "tune" the experimental design. A chromatographic procedure was also developed to separate polar degradation products of the chlorpyrifos. Experiments continue.

At UNAM's Mazatlan field station a laboratory equipped for ^{14}C experimentation is currently under construction. A mesocosm experiment is also underway and in equilibration prior to the addition of labelled agrochemicals. This will enable the most realistic emulation of environmental conditions.

Conclusions

The partnership forged during this research project has offered both participants unique opportunities for research. Results from the study have yielded and will continue to develop a model for pesticide dispersion and transfer in tropical lagoon ecosystems. This can then be used to assess and predict the impact of pesticide discharges in similar environments and should help coastal planners to design integral strategies for coastal zone management, to protect the population at large from the effects of pollution and contribute to the conservation of well-balanced lagoon ecosystems.

24 Studies on the root zone method of wastewater treatment

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Summary of proposal

The Root Zone Method (RZM) is a new, low cost method of wastewater treatment which depends on the flow of wastewater along the annular spaces between surrounding soil and rhizome of the reeds (normally *Phragmites*) where bacteria oxidise the impurities. Protozoa are important in the removal of bacteria, including pathogens, by their predatory activities.

Pilot-scale RZM performance. Pilot-scale reed beds will be constructed from plastic containers following the design of test tanks introduced by the Water Research Centre, Medmenham Laboratory. The test tanks will be filled to 0.6 m depth with either soil or pea gravel and planted with the reed *Phragmites*. The performance of these will be compared with the performance of full-scale reed beds in England operating at the same organic and hydraulic loadings by monitoring the removal of BOD_5, ammonia, COD and total organic carbon. The biological parameters studied will be mainly bacteria and protozoa. The latter will be used as indicator organisms through the saprobic system to assess the water quality of effluents. Samples of planting medium will be taken using a core-sampler and the protozoan populations will be counted and identified to species level. Results from such experiments will provide the data necessary to determine, firstly the magnitude of any scaling-down effects that are usually experienced when operating small-scale treatment plants and secondly the importance of the planting substrate or medium in the design of reed beds.

RZM performance in Mexico. Pilot-scale test tanks will be constructed and operated in tropical and sub-tropical areas of Mexico using the same design and materials as those used in the UK. Effluent quality will be monitored using the same techniques and similarly the protozoan and bacterial populations will also be determined. Information gathered from pilot-scale and full-scale plant in the UK will be compared with the data gathered from the experimental work carried out in the tropical and sub-tropical regions of Mexico. The comparisons will enable performance of full-scale plant in tropical and sub-tropical climates in Mexico to be predicted.

Removal of pathogens. In other aerobic biological waste-treatment processes it is well established that protozoa play a vital role in the removal of bacteria including pathogens. The ecology of protozoa in the RZM will be investigated using multivariate analysis and the role of protozoa in the elimination of pathogenic bacteria will be determined. In the UK, total viable bacteria and coliforms will be sufficient

for this purpose but in Mexico the presence or absence of *Salmonella* and *Shigella* will be monitored also.

Not all protozoa are beneficial, some are harmful to man since certain species are capable of causing human disease. The amoeba *Entamoeba histolytica* which causes amoebic dysentery and hepatic and cerebral abcesses is a well-known example and this causes a very large medical problem in Mexico. However, there are also some free-living amoebae such as *Naegleria fowleri* and *Acanthamoeba* spp. which cause primary amoebic meningoencephalitis (PAM) and granulomatous encephalitis (GAE) respectively. While *E. histolytica* is not a problem in the UK there have been several reported cases of PAM both in Mexico and the UK. Treatment of these diseases is difficult and prevention is still the best approach. Therefore some research will be directed towards estimating the numbers of potentially pathogenic amoebae which emerge in effluents from reed beds. Amoebae will be isolated from core samples and inoculated onto non-nutritive agar plates seeded with *E. coli*. Isolates will be axenised in modified SCGEYM medium and later identified to species using isoelectric focusing on gels. Pathogenicity tests will be carried out in white mice following the inoculation of axenic amoebae into the nose and into the brain.

RZM cost estimation. Finally, the construction and running costs of reed beds will be compared with those for the treatment of sewage in Mexico using oxidation ponds. The total data will provide excellent scientific and economic baselines for deciding whether or not full-scale reed beds should be introduced into Mexico.

Work programme of C.R. Curds in England

To coordinate the activities of staff at the Natural History Museum and a Ph.D. student who will work towards the objectives of the project by comparing pilot-scale RZM's with full-scale RZM's in order to ascertain whether there are any significant scaling-down effects; ascertaining the magnitude of scaling-down effects; and comparing performance of RZM's grown on different substrates in terms of colonization by protozoa, removal of pathogenic bacteria, removal of pathogenic free-living amoebae and oxidation efficiency.

Work programme of F. Rivera in Mexico

To coordinate activities of staff of UNAM and a Ph.D. student who will work towards the objectives of the project. The latter will determine the efficiency with which pathogens (bacteria, free-living pathogenic amoebae and *Entamoeba histolytica*) are removed from wastewaters by the root zone method; assess the performance efficiency of RZM's using saprobic indicators and physico-chemical parameters; evaluate whether the RZM is more cost-effective than oxidation ponds for treating wastewaters in Mexico; and determine how the root zone method is applicable to the treatment of wastewaters in tropical and sub-tropical climates.

25 Biological phosphate removal in a biofilm reactor operated in the sequencing batch reactor mode

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Contract number and duration: CI1*/0782, November 1991 to April 1994.

Summary of proposal

The Sequencing Batch Reactor (SBR) process has become an interesting technology for developing countries because it allows performance of different tasks in just one reactor basin. Cost effectiveness; ease of operation and maintenance; and flexibility are the major advantages of the SBR technology. So far, only activated sludge SBR systems have been applied. Problems of sludge settleability could be minimized by application of proper process strategies. In several cases, however, growth of settleable sludge flocs could not be controlled properly.

To eliminate sludge settlability problems, development of a biofilm SBR system is proposed. The aim of this research is to study the feasibility of this idea and to demonstrate the advantages and disadvantages - if any - of the novel technology. Special attention is to be directed to the process of biological phosphate removal. The question is by which means microorganisms capable of accumulating excess amounts of phosphate can be enriched in a biofilm reactor operated in a SBR mode. Successful operation of biofilm SBR systems would be of particular interest with respect to eutrofication control in developing countries.

The research will be carried out mainly in Mexico. Pilot-plant studies are planned to be conducted at the treatment plant of the University of Mexico. In parallel, laboratory studies will be executed in Hamburg under controlled conditions. Scientific and technical advice and exchange of experience and ideas will be provided by reciprocal visits of the project leaders and students.

Two of the pilot-plant reactors are planned to be built as fixed-bed reactors. Two other reactors will be equipped with rotating substrata and operated as twin-chamber RBCs. By systematic variation of the process conditions (hydraulic retention time, cycle duration and frequency, fill rate, etc.) proper process strategies will be defined. Advanced removal of organic pollutants and of phosphate are to be achieved.

Included in the programme are an economic and operational investigation including studies on process stability, an investigation of biofilm growth and sloughing of biofilm, an investigation on support material (substrata for biofilm adhesion) and the development of design procedures.

Postdoctoral fellowships

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Phosphate removal in a biofilm reactor

Fellowship period: January 1988 - December 1989

Summary

The main objective of this research project was to demonstrate the ability of biological films to perform phosphate removal. The implies the necessity of a biofilm accumulating phosphate in quantities greater than the stoichiometric ones required for growth and maintenance. Attention had to be paid to those operational parameters that can affect that ability.

A laboratory reactor was built and operated after the sequencing-batch-reactor procedure. The biofilm was grown as submerged biofilm and the anaerobic/anoxic and aerobic conditions were managed through turning on and off an air pump for bubble aeration, see Figure 1 (overleaf). The laboratory reactor consisted of a glass cylinder, open at the top and filled with 14 cylinders of a support material (Bio-Net, 155 m^2/m^3) specially made for biological wastewater treatment by the company Norddeutsche Seekabelwerke AG. The reactor was operated after the fill-and-draw procedure.

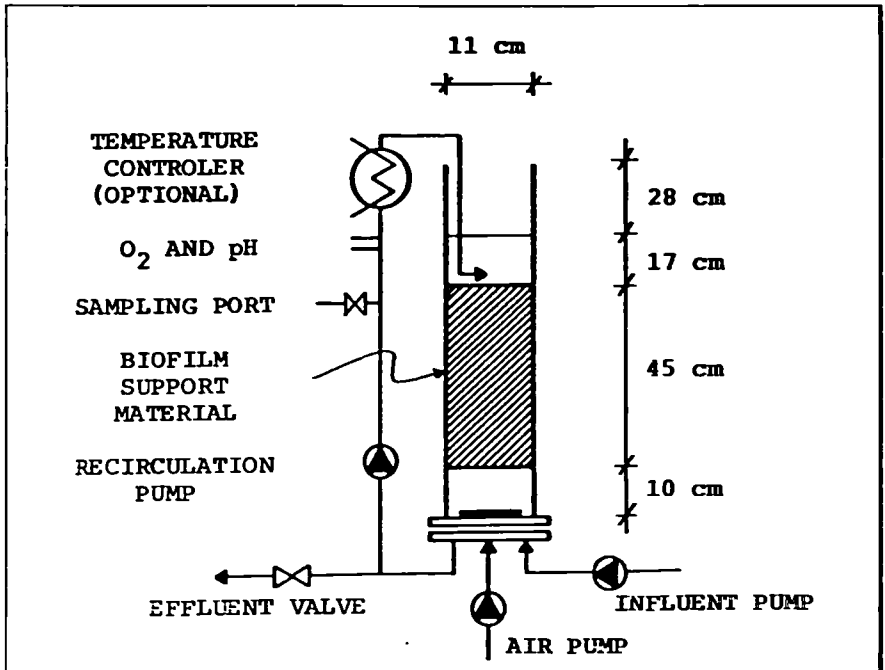

Figure 1 The laboratory reactor

Liquid volume (without biofilm), 6.67 l. Liquid volume (with biofilm, average), 5.5 l. Total surface area, 1.194 m^2. Specific surface area, 175 m^2/m^3. Recirculation (average), 1.0 l/min

Conclusions

The biofilm-SBR manages C, N, and P removal efficiently. Up to 91 percent P removal was achieved with an inflow concentration of 9 mg/litre. During start-up, for P and N removal, the biofilm stabilisation is time-dependent because of ecological adjustments in the microbial populations, independent of quantity or quality of inoculation sludges.

Phosphate P removal increases with the duration of the anaerobic phase. In order to get good P removal, P release for endogenous requirements (after substrate sequestration) during the anaerobic phase has to be permitted. Better P uptake during the subsequent aerobic phase corresponds to longer anaerobic endogenous P release. Longer cycle duration allows a more stable operation of the reactor: with a 12-hour cycle it is possible to predict with confidence the behaviour of the transformation processes involved in C, N, and P removal. Even though the aerobic phase is absolutely necessary, its duration does not significantly affect the P removal process. During the aerobic phase, after a minimum P concentration has been reached, a

prolongation will not cause changes in that concentration. Sludge production increases inversely to the duration of the anaerobic phase.

Substrate inflow concentration does not directly affect the P removal process but it does affect the time required for substrate sequestration and, as a consequence, the time for endogenous P release. Temperature affects P release and P uptake rates but does not significantly affect the overall P removal process or the P effluent concentration. Temperature effects (between 15 and 25^{o}C) on nitrification are also negligible. The anaerobic uptake of sodium acetate and peptone have a linear (stoichometric) relationship with P release: it is an energy-consuming reaction. Glucose sequestration is an energy-producing reaction and the uptake rates are higher than those of acetate and peptone.

This work is now being continued in a joint research project (see report 25, page 120).

Publication

Gonzalez-Martinez, S. and Wilderer, P.A. (1991). Phosphate removal in a biofilm reactor. *Water Science and Technology*, **23**, 1405-15.

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Studies on the treatment of domestic wastewater for human consumption

Fellowship period: February 1989 - July 1989

6 HEALTH AND BIOMEDICAL SCIENCES

Summary

After agricultural sciences, health and biomedical sciences represent the second most important area of this report. However, in common with the agricultural sciences projects described in chapter 1, many of the studies described here involve the molecular approach.

The largest group of studies covered by this chapter concerns diseases of public health importance in Mexico. Studies are included of the rabies virus and of the differences in virulence between virus strains isolated from different hosts; of immunochemical differences between pathogenic and non-pathogenic strains of *Entamoeba histolytica*, a cause of dysentery; and of immunological research for application to diagnosis of or prophylaxis against the *Taenia solium* tapeworm which can cause the life threatening disease, neurocysticercosis. Study of another intestinal parasite, *Giardia lamblia*, focuses on the impact of the parasite on the nutrition of the host, which can be very serious in children.

Other reports include an investigation of infant mortality in Mexico as an indicator of the health and development status of a population, research on stimulation of enzyme production by arterial tissue and some pharmacological studies. Two studies deal with traditional approaches to medicine; they emphasise that traditional medicine is widely practised in Mexico, and that it often coexists with and represents an alternative to "scientific" or "institutional" medicine and conclude that there is much mutual benefit to be derived from improved understanding and cooperation between the two approaches.

Joint research projects

26 Importance of virus-host coadaptation for the rabies virus

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Contract number and duration: CI1*/0089, September 1986 to December 1990

Background

The most recent data from viral pathology have tended to demonstrate and to generalise an extremely important phenomenon within epidemiology which is the adaptation of the pathogenic agent to its host. Up until recently, this specificity did not seem to exist for the rabies virus. However, recent experiment results have suggested, on the contrary, that the rabies virus is indeed an excellent example of virus-host adaptation.

Vulpine rabies which is currently endemic in Western Europe would appear to be a prime example of this coadaptation: in effect, bovines are 10 000 times more resistant to the vulpine rabies than foxes and cats, and at least 100,000 times more resistant than dogs. On the other hand, the fox has proved to be extremely resistant to viruses isolated in the skunk, in the African dog or in the South American vampire. Furthermore, the emergence of the monoclonal antibody has enabled us to demonstrate the antigenic variability of the rabies virus. The old myth of the perfect uniqueness of the rabies virus has now been disproved.

Arrays of monoclonal antibodies directed against nucleocapsid or viral glycoprotein antigenic determinants have already enabled us to define differences between the rabies virus and other extremely similar viruses (Lyssavirus) between rabies viral strains of different geographical sources and has also enabled us accurately to identify some attenuated vaccine strains.

These results incontrovertibly demonstrate the potential for biological and antigenic variation of the rabies virus and this now raises a number of new questions to which the epidemiologist must find answers in order to arrive at prophylactic methods which best suit the true situation and which are, consequently, the most effective.

The following points, *inter alia*, merit in-depth investigation:

1. In parallel to what has been stated with regard to vulpine rabies in Europe, in Latin America and specifically in Mexico, is there an ancestral adaptation of the rabies virus along two different types of vector, the vampire and the dog and, if so, what are the interactions between these types of virus?

2. What is the degree of antigenic variability in Mexican strains whether among themselves or in relation to vaccine strains used? Failures recorded in attempts to vaccinate bovines against vampire-transmitted rabies using traditional attenuated vaccine strains (Flury) tend to favour the existence of a rabies virus strain which is specific to vampires and which is antigenically separate from traditional strains.

Chiroptera and dogs are the major carriers of rabies in Mexico. These species inhabit geographical zones which are distant from each other or which are ecologically different which signifies that contact between them is unlikely.

Objectives

The objective of this research is to study the virulence in the mouse of some pathogenic and vaccinal Mexican strains of rabies virus and to develop some diagnostic and differentiation immunological tests.

Materials and methods

Figure 1: Vampire bats used for isolation of the virus

Virus strains. Virus were isolated from human beings, dogs, pigs, bovines, and vampire bats from different geographical areas of Mexico. Attenuated vaccinal strains (Roxane, ERA, ID, V319) and CVS11 reference strains were also used.

Rabies diagnoses were made by immunofluorescent antibodies techniques and inoculation tests on mice brains.

Mice. CD1, BALB/C and albino Swiss mice of different sex, age and weight were used for virulence studies. Mouse inoculation, virus titration and serum-virus neutralization tests were done by conventional methods.

Immune serum production in Guinea pig and chicken. Adolescent (1 month) male guinea pigs and young Rhode Island (1 month) chickens were immunized with 9 intramuscular injections at intervals of 2 weeks with ERA attenuated vaccine or with "Fuenzalida" type inactivated vaccine. One week after the last injection, blood samples were collected for serum-virus neutralization tests in mice, against CVS11 and vaccinal strains of rabies virus.

Production and characterization of monoclonal antibodies (MCabs). MCabs were produced by the adoptive transference technique (Lara *et al.*, 1989). Briefly, spleen lymphocytes from donor mice immunized against rabies virus were transferred into non-immunized cyclophosphamide-treated syngenic mice, before the subsequent fusion of the splenocytes with mouse mieloma SP2 cells. The immunization of BALB/C mice was carried out with a V319 vampire bat origin, vaccinal strain. The characterization of MCabs was made by the ii direct immunofluorescence, ELISA, immunoblotting and mice neutralization techniques.

Tests with a reduced panel of monoclonal antibodies (MCabs). A reduced panel of 8 MCabs was selected because they fulfilled the required criteria for identifying each lyssavirus serotype and European bat virus (Montano Hirose *et al.*, 1990). They were kindly offered by Lafon from Institut Pasteur, Paris.

The panel was tested by immunofluorescence assay on rabies-infected brain smears, with 15 isolates from different species from different geographical areas of Mexico.

Results

Virulence studies in mice. Any vaccinal attenuated strain could produce deaths or clinical signs to any adult mouse by the intraperitoneal (I.P.) route. Nevertheless, in this research, by the employment of the intracerebral route (I.C.) a significant difference in the time of appearance of rabies signs (paralysis and prostration) was found in CD1 mice (15 g), between the vaccinal-attenuated strains (Roxane, ERA, ID, V319) and two pathogenic and CVS11 reference strains ($P < 0.01$). On the other hand with the I.P. route, a significant difference in the time of appearance of signs could be established in adult mice (CD1 and BALB/C) between the CVS11 reference strain and one pathogenic vampire bat origin strain ($P < 0.01$).

The adult CD1 female was found more resistant than any other mice species to the I.P. inoculation of any strain of rabies virus in this research.

The isolation by the I.C. route of 9 dog-origin strains and 4 vampire bat-origin strains in adult albino Swiss mice show that vampire strains are less virulent than dog strains for this species ($P < 0.025$).

Diagnostic and differentiation immunological tests. Polyclonal antibodies. The development of virus-neutralizing antibodies titres against CVS11 and vaccinal strains in guinea pigs and chickens are given in Table 1. Immune chicken sera show neutralizing activity against the CVS11 strain. Chicken sera also showed less neutralizing antibodies against vaccinal strains than guinea pig sera. Chickens react better to the immunization with inactivated Fuenzalida type vaccine than with ERA-attenuated vaccine.

Strain *	Guinea pig pool 1 **	Guinea pig pool 2	Chicken pool 3	Chicken pool 4
CVS11	1:488	1:625	0	0
CVS11	1:437	>1:625	0	0
ERA	>1:625	>1:625	1:7	1:21
ID	>1:625	>1:625	1:5	1:107
ROXANE	>1:625	>1:625	1:45	1:501
ROXANE	>1:625	>1:625	1:78	1:478

Table 1. Neutralizing antibodies production (Guinea pigs and chickens)

* In all cases 50 DL 50%

** Titres found in each 4 animals pool

Pool 1 and Pool 3, animals immunized with ERA vaccine

Pool 2 and Pool 4, animals immunized with Fuenzalida vaccine

Monoclonal antibodies (MCabs) production. With the adoptive transference technique, more positive splenocytes were obtained (800) than with the traditional immunization method (340) ($P < 0.01$). We selected 6 hybridomas because of their clear response in the immunofluorescence technique (hybridomas 1-6, 1-10, 1-11, 8-2, 8-3 and 8-5). All selected hybridomas were negative *vis-a-vis* neutralisation tests, positive *vis-a-vis* "ELISA" tests and there was no difference between different strains. The immunoblotting test showed that selected hybridomas were directed to nucleocapsid protein of rabies virus.

Reduced panel test of monoclonal antibodies (MCabs) with rabies isolates. The great majority of isolated strains in Mexico can be classified as serotype 1 with two exceptions. A virus isolated in the Mexican state of Oaxaca from a bovine and virus isolated in Mexico City from a pig were clearly recognized by MCabs which react with the serotype 1 lyssavirus as well as the monoclonal antibodies which react only with lyssavirus serotypes 2, 3, 4 and EBL1.

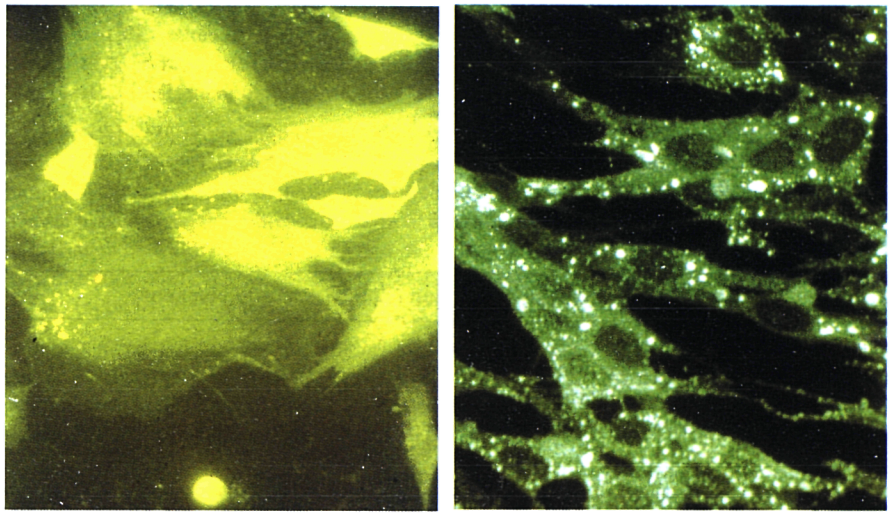

Figure 2: Images of immunofluorescence with a conventional polyclonal antibody (left) and with clone 8-2 (right)

Discussion

The results obtained from mice virulence studies show that the inoculation of this species (15g or adult mice by I.C. or I.P. routes) can be a simple preliminary test of differentiation of strains mainly when suspicion of post-vaccinal rabies exists.

Because it is of no public health or economic significance, there exists limited information about the immune response of non-mammal animals against the rabies virus. The immune sera obtained in traditional species (rabbit, goat, guinea pig, horse, etc.) are practically unable to differentiate strains of rabies virus. Nevertheless, our results suggest that it is possible to differentiate rabies virus strains with immune serum obtained in non-mammal animals like chickens.

With the adoptive transference technique, anti-nucleocapside MCabs were produced for the general diagnosis of rabies virus infections by the immunofluorescence technique. These MCabs were more efficient than traditional polyclonal antibodies used in Mexico for this purpose.

With the selected MCabs offered us by M. Lafon of the Institut Pasteur, two atypical isolated strains were detected. This may explain why some failures occur in rabies vaccination.

Acknowledgements

We are indebted to the "Instituto Nacional de Investigaciones Forestales y Agropecuarias" and "Productora Nacional de Biologicos Veterinarios", who kindly housed us in their laboratories, after our own laboratory had to be demolished following the earthquake in Mexico City of 19 September 1985.

Publications

Aguilar-Setien, A. and Garza Ramos, J. (1989) La rabia: una enfermedad antigua y un nuevo paradigma. (Rabies: an old disease and a new paradigm). *Ciencia y Desarollo*, **15** (88), 33-9.

Lara, S.V.; Ciprian, C.A. and Aguilar-Setien, A., (1989). Increase of rabies virus antibody-producing lymphocytes by means of intrasplenic adoptive transference in mice treated with cyclophosphamide. *Archivos de Investigación Medica*, **20**, 262-3.

Background reference

Montano Hirose, J.A.; Bourhy, H. and Lafon, M., (1990). A reduced panel of anti-nucleocapsid monoclonal antibodies for bat rabies virus identification in Europe. *Research in Virology*, **141**, 571-81.

27 Immunochemical differences between surface antigens of pathogenic and non-pathogenic *Entamoeba histolytica* zymodemes

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Contract number and duration: CI1*/0090, September 1986 to August 1988

Results

An axenic culture of trophozoites of *Entamoeba hystolica*, strain HM-2, pathogenic zymodeme II, after centrifuging, was used to obtain a lipopeptidophosglycane (LPPG) by the phenol-water method. This LPPG was then used to develop an ELISA test for use as an indicator of the differences between pathogenic and non-pathogenic strains. The results of this test have shown a sensitivity of 100% and a specificity of 69%.

The isolation, cloning and culture of a non-pathogenic zymodeme of *Entamoeba hystolica*. The trophozoites were isolated from the faecal matter of persons without a clinical record of amoebiasis and placed in a Robinson culture. After 60 days the trophozoites were cloned by a limiting dilution and transferred to the medium TY1-S-33 in the presence of amikacin and cephotoxim in order to eliminate bacterial growth in the medium.

The zymodeme of one of the clones was type I; this was considered to be non-pathogenic according to the Sargeaunt classification. This non-pathogenic clone, called MAV-1 was compared with a clone, called A, obtained from the pathogenic strain, zymodeme II, HM1:IMSS.

Different tests *in vivo* and *in vitro* showed that the clone MAV-1 was not pathogenic and was zymodeme I. In addition, no adhesion of 112 kDa was detected on its surface. This 112 kDa protein is found on the surface of pathogenic trophozoites of the clone A and had been considered as a protein contributing to the virulence of the amoeba. Earlier results had established that there are at least antigenic differences associated with the surface proteins between pathogenic and non-pathogenic strains of amoeba.

To continue the work on the differences in polyosidic surface antigens an LPPG was obtained from a virulent strain and another from a non-virulent strain. ELISA test results show that the LPPG from the virulent clone A when treated with serum from persons suffering from an amoebic hepatitis abcess (AHA) was the same as that from the original pathogenic strain, HM1. On the other hand, the behaviour of the LPPG of the non-virulent clone L-6 when treated with the AHA serum was the same as when treated with normal sera.

These results show for the first time that a polyosidic surface antigen behaves differently when treated with serum from persons suffering from AHA depending on whether it comes from a virulent or non-virulent clone. This demonstrates that the ELISA technique can discriminate between virulent and non-virulent strains.

To be able to work with a homogeneous polyosidic molecule starting from a glycolipid phosphate derived from trophozoites of *Entamoeba hystolica*, several operations were necessary. A test sample was made from a homogeneous fraction after hydrolysis with dilute acid. An oligosaccharide was found; in biogel P-2 chromatography this eluted between the tetra- and the dodecasaccharide references. In silica thin film chromatography it presents a unique trace whose migration is intermediate between the same references. Antigenicity has been envisaged through the use of this oligosaccharide as an inhibitor during an indirect ELISA test. Gas phase chromatography analysis of the purified oligosaccharide indicates that it contains 1 part mannose, 1 part galactose and the remaining 9 to 10 parts of glucose.

Publications

Vargas, M.A.; Isibasi, A.; Kumate, J.; and Orozco, E. (1990). Non-pathogenic *Entamoeba hystolica*: functional and biochemical characterization of monoxenic strain. *Molecular and Biochemical Parasitology*, **40**, 193-202.

Isibasi, A.; Blanco, F.; Arreguín, C.; Martínez, G.; Pelayo, R.; Orozco, E. and Kumate, J. (1990). Differencias immunoquímicas de polisacáridos de superficie obtenidos de *Entamoeba hystolica* cepa HM1-IMSS y sus clonas C-A virulenta y L-6 no virulenta. (Immunochemical differences between surface polysaccharides obtained from *Entamoeba hystolica* type HM1-IMSS and its clones C-A (virulent) and L-6 (non-virulent). *Archivos de Investigacion Medica* **21** (suppl).

28 The importance of traditional medicine in health care among the rural population

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Contract number and duration: CI1*/0091, March 1987 to February 1988.

Background

Beginning in 1975, when the Work Group on Traditional Medicine was created by the World Health Organization, many countries started developing policies and programmes geared at setting in motion the community's health resources. They gave special importance to promoting and developing traditional medicine. However, by the middle of the 1980s Latin America was still lagging behind considerably on this subject and the policies that the Panamerican Health Office issued insisted on applying institutional preventive and healing programmes rather than traditional medicine. This type of care was focused exclusively on gynaecological and obstetric care in rural areas.

Mexico's example is more complex than other Latin American countries, especially since Mexican and foreign research workers began many investigations in the 1940s about the different ethnic groups that inhabit the Mexican territory. It became clear how important traditional medicine can be in caring for the health of these groups. On the legal side, flexible legislation tolerated midwives, healers, herbalists and other traditional therapists. But perhaps the most important aspect may reside in cultural traditions - in the use of medicinal plants, for example, which are part of the traditions and ideas of many of the country's population.

Therefore, the genéral and well-attested fact that traditional medicine represents one of the basic resources of Mexico's and Latin America's population when they need to care for health problems became a sufficient reason to undertake this project.

The starting point was one of the largest surveys on traditional medicine ever carried out in Latin America, the IMSS-COPLAMAR programme, conducted by the Traditional Medicine Unit of the Mexican Social Security Institute in 1984. The result of the survey was a list in order of importance of the ten main conditions for which traditional medical care is requested.

Objectives

The purpose of this research project was to provide a more extensive and better understanding of the first five of these conditions, namely the evil eye, indigestion, fright, fallen fontanelle and dysentery.

Research objectives were, first to describe the five main conditions where traditional medicine is requested; local names, Spanish synonyms, etiological concepts, symptoms, therapeutic procedures, and prevention. The second objective was to identify the most relevant data in terms of frequency distribution of the five main conditions for traditional medicine among the population under study and

the third was to identify the main vegetable and material resources that are used in preventing and healing the diseases under study; also to collect and analyse botanical specimens as accurately as possible.

Methods

The methodology followed during the investigation was based on three basic standards. The first was how to apply all products under study; in other words, to ensure that information on the subject would be readily available to both non-specialized readers on traditional medicine or on complex research procedures, but at the same time be useful to experts. The second standard was to concentrate on the approach rather than on the format and to use a pragmatic rather than a speculative approach. This involved the widespread application of qualitative analysis techniques to the way institutions and governments carry on their task regarding primary health care. Thirdly, we sought a stronger inter-cultural production link between the two kinds of medicine, traditional and modern. In other words, we wanted to develop better understanding and communication between institutional health service personnel and the main people involved in traditional medicine.

Therefore, the following framework was developed:

Method	Technique	Device
Observation by participants	Record of observations	Guidelines for observations
Selected key data	Organized interview	Guidelines for the interview
Bibliographical summary	Bibliographical system	Record cards
Botanical identification	Botanical collection	Botanical press

The study area was based on the regions covered by nine rural medical units. We took their location into consideration and we also selected two other regions in the same area. The latter are described as "areas of intensive activity". The work was carried out in twenty-seven locations in the states of Puebla, Oaxaca and Veracruz. Sixty-two traditional therapists were interviewed in the same working area. The investigation took place from March 1987 to March 1988.

Results

Regarding the objectives, the research resulted in a detailed description of the five conditions under study, including synonyms, infected population, seasonal variations, causes, evolution, diagnosis, treatment and prevention, and the following plants were gathered and identified:

Disease	Number of plants collected	Number botanically identified
Evil eye (*Mal de ojo*)	35	25
Indigestion (*Empacho*)	46	38
Magical fright (*Susto*)	58	41
Fallen fontanelle (*Caída de mollera*)	11	10
Dysentery (*Disentería*)	41	33
All diseases	191	147

Table 1. Plants used to treat various diseases.

Note: Since some of the plants that were collected are used to treat more than one of the diseases under study, the actual total number of plants botanically identified is 86.

Discussion

In the field of traditional medicine, there still are more questions than answers. In spite of all that has been achieved so far, certain basic questions are still unanswered. Thus positive medical and academic explanations suggest that an appropriate interpretation of the signs and symptoms of most traditional diseases would lead in itself to an unquestionable equivalence between diseases treated by traditional medicine and those accepted by medical science. However, parallel statements that "physicians do not cure" the evil eye or magical fright are still subject to analysis in connection with an ideological and cultural framework, whereby a disease is more than an isolated expression of the body.

In a multi-cultural environment like Mexico's, traditional medicine has been kept for many centuries at the margin of officially-acknowledged resources for health care. On the other hand, and for an equal time, traditional medicine has been the most important resource for the health requirements of millions of Mexicans. This research project has also proved that traditional medicine and medical science have become alternatives that coexist and are in practice combined. Nevertheless, mechanisms capable of inter-relating them in order to enhance the effectiveness of both medical systems have not been developed. We feel that it will not be possible to do this unless certain basic questions that still prevail are answered regarding internal coherence in the traditional medical system. For example, the relationship between certain beliefs and treatments; an epidemiological evaluation of disease; and the therapeutic effectiveness of specific procedures and plants: all need further study.

Conclusions

The final result of the research project represents a considerable achievement in the study and understanding of Mexican traditional medicine, which is used by millions of Mexicans. However, there is still a need for further research aimed at strengthening the knowledge we now have about the subject. The experience obtained during the study, as well as the results achieved, show the need to undertake clinical research on several diseases that have been reported to have significant importance for the population and that are not listed on institutional morbidity and mortality records. It is clear that traditional diseases do not disappear if they are simply ignored, since each day thousands of people in both rural areas and cities state that they suffer from the evil eye, magical fright, indigestion, a fallen fontanelle, or paralysis due to a draught, etc.

In our opinion, medical science can face this health problem as long as it takes into consideration the following three premisses. First, traditional medicine is a field that deserves to be investigated; it is scientifically interesting and demands specific strategies, methods and techniques to be developed. Many of them were inherited from other non-medical fields of science (anthropology, sociology, phytochemistry, history) or they have remained in a complicated relationship with the medical field (psychoanalysis, for example).

The second premise is that there is a cultural and social range within disease that is not just organic. In Mexico it is deeply complicated due to the country's history, because the background of the health-disease-healing process is affected by Pre-hispanic, Colonial, Spanish, African, Positive and Modern influences, etc. Thirdly, this social, economic, cultural and psychological side of the disease is not too far from organized medical health services and has considerably favoured certain sectors while excluding others. Due to the above, it is essential that the desire for change that Mexican health institutions have expressed be led towards a search for alternatives based on the country's characteristics.

Although the project gave positive results, it also uncovered gaps in information about the diseases under study. It would therefore be useful to continue researching the matter, and in particular to study indigestion and fallen fontanelle in their epidemiological, chemical, ethnic and botanical aspects.

Publications

Zolla, C.; Del Bosque, S.; Tascón, A.; Mellado, V. and Maqueo, C. (1988) Medicina Tradicional y Enfermedad (Traditional medicine and health care); CIESS, Mexico.

29 Pregnancy and childbirth care in Mexican rural areas

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Contract number and duration: CI1*/0092, March 1987 to February 1988.

Background

One of the most significant examples of coexistence between traditional and institutional medicine in Mexican rural areas is the way pregnancy, childbirth and postpartum are treated. Health institutions concerned with expanding and improving their coverage of maternal and child care are taking advantage of the community's human resources represented by traditional midwives. The same situation can be seen in other countries where traditional medicine is important. That is the reason why the World Health Organization has pointed out the need to outline the activities that traditional midwives perform in health care and to determine how the health system can help them to carry out their tasks more safely. The result of WHO's interest is that policies to integrate empirical midwives have been abandoned and instead policies to acknowledge, assist and advise traditional midwives have been developed.

In Mexico, traditional midwives form the majority of total traditional therapists and are an important source of health care. Mexican health institutions have stated that approximately 66% of all childbirths in the country are treated by traditional midwives and trained empirical midwives.

An analysis of the organisation of the overall health system, with its institutional and traditional resources and so called "home medicine", needs to adopt research and planning schemes for health activities that are appropriate to different types of services. However, there has been little interdisciplinary research aimed at building a traditional system of concepts, beliefs and practices. What has been done lacks an evaluation of the impact that these practices have had on maternal and child health because there has not been a system to register the problems associated with it nor the therapy that was applied.

Objectives

The main objective of this project was to achieve a thorough knowledge of the traditional system of beliefs, concepts and practices and to find out the kind of care given by empirical therapists during pregnancy, childbirth and postpartum in rural areas in the State of Morelos.

Detailed objectives were to register the therapeutic resources that are used, to gather the species of plants employed, and to record the diseases and complications associated with pregnancy, childbirth and postpartum in the population under study. We also aimed to prepare an exhaustive bibliography on the subject.

Methods

A full-time team of researchers was assigned to carry out the investigation. They were familiar with the area and had previously researched traditional medicine, medical anthropology and ethnic/botanical medicine. The team remained in the chosen areas in the State of Morelos between the months of April and October 1987 and carried out field work until December.

Observation guidelines and ethnic/botanical procedures were designed to obtain information relevant to the practical use of herbs. Also, photographic and recording material was used along with means for ethnic/botanical collection and surveys. The botanical samples that were gathered were pressed and dried; subsequently they were identified by the Herbal Centre at the Mexican Social Security Institute (IMSS).

Organized interviews were used as a means to obtain information about the most relevant subjects on conception, sterility, pregnancy, childbirth, postpartum and abortion; diseases that women suffer during pregnancy and postpartum; diseases that newborns and nursing babies suffer; the social status of traditional, trained and non-trained empirical midwives; and the material resources used for each of the above.

Another technique that was used was active observation in order to obtain information by sharing the activities of the subject under study. This technique was quite relevant, since it increased contact between researchers and midwives.

Finally, complementary information was obtained about geographical, socio-economic and cultural aspects, as well as information on maternal and child care and on gynaecological and obstetric care supplied by the health institutions. Information was also obtained from the available bibliography on the areas under study.

Ethnic/botanical information, photochemistry, and pharmacology in connection with the plants that traditional midwives use became important tools to help us to understand the types of treatment and decide which plants should be analyzed subsequently. The analysis was based on the information from traditional therapists in the State of Morelos (specifically, traditional midwives); persons identified as knowledgeable on the subject; and health institutions in the State of Morelos.

Results

It was confirmed that in the area under study there is a multiple pattern of care for pregnancy, childbirth and post-partum, which is represented by traditional medicine (trained, non-trained and traditional midwives) and by health institutions. Supply and demand differ according to the urban influence, culture and levels of income of the individuals who use these services and the geographical location of institutional health services.

The characteristics of three types of midwives (trained and non-trained empirical, and traditional) were identified, confirmed and explained. A detailed description was provided of the concepts, beliefs and traditional practices in connection with pregnancy, childbirth and postpartum, as well as maternal and child diseases. Ninety five medicinal plants used by different midwives were collected and 88 of these plants were botanically identified.

An extensive bibliography about different, scientific fields on pregnancy, childbirth and postpartum has been provided.

Discussion and conclusions

Empirical midwives are the most representative of all traditional therapists. They have been the only community health resource officially included in some institutional health programmes. However, the knowledge, practices and beliefs that distinguish them are lagging behind and are subordinated and screened by a prevailing interest to obtain their cooperation for solid health policies. This fact has delayed any possibility of building a clear and solid scheme on procedures that midwives use in healing and its cultural essence.

This research project is a significant achievement in providing a detailed description of pregnancy, childbirth and postpartum from the point of view of traditional medicine; that is, based on the system of beliefs, practices and concepts of traditional medicine. It is now possible to undertake further research in which the approach of a certain practice or belief compared to others can be outlined.

The following types of midwives were identified in this research project: trained and untrained empirical midwives and traditional midwives. However, a system of beliefs was registered in this classification that, in general terms, seems to be a common denominator for all three types. The matters left for discussion at the end of this project concern the impact of official training and urban development and the elements of change in the ideological nature of pregnancy, childbirth and postpartum among the Mexican rural population. Further research will enable us to visualize the direct relationship between beliefs, concepts and specific practices.

This research project has confirmed once again the kind of co-existence and interaction that obtains between traditional and institutional medical care during pregnancy, childbirth and postpartum. However, the plans aimed at optimising both systems involve severe judgements regarding the appropriate relationship between the two types of medicine. For a long while, it was considered that midwives could be included in institutional tasks. However, this type of approach induced a strong culture shock mainly because the academic medical system was being overrated and traditional medicine was being rejected. In view of this failure, the policy for integration changes to an attempt to make the two medical fields complementary. But it is still not clear how this is to be done. This research project seems to confirm that only an increase in our knowledge about the actual and symbolic efficiency of traditional procedures will create true complementarity.

Publication

Mellado, V.; Zolla, C.; Castañeda, X. and Tascón, A. (1989). La atención al embarazo y el parto en el medio rural Méxicano. (Pregnancy and childbirth care in the Mexican rural environment). CIESS, Mexico.

30 Research on the neurocrinal and paracrinal bases for control of hypophyseal hormone secretion

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Summary

Regulation of thyrotropin releasing hormone (TRH) metabolism in vivo. Previous studies have shown that TRH is involved in the control of TSH, PRL and GII secretions. The purpose of these experiments was to determine if and how TRH metabolism is regulated under physiological conditions where its secretion rate from median eminence is presumed (or was shown) to be altered. Various experimental paradigms were analysed:

1 Continuous lactating rats: TRH mRNA levels in paraventricular nucleus (PVN) were higher during gestation than during lactation. No change occurred during lactation or at weaning. On the contrary TRH levels in the mediobasal hypothalamus (MBH) dropped between the end of gestation and the beginning of lactation, increased during lactation and dropped again at weaning. These data suggest that a TRH surge occurs between the end of gestation and the beginning of lactation and that lactation affects translational or post-translational processing.

2 Controlled suckling of lactating rats induces a rapid (55 min) and reversible (= 15 min) drop of TRH levels in the MBH. TRH mRNA levels in the PVN are transiently increased (maximal value at 30 min succion).

3 Cold stress: TRH (in MBH) and TRH mRNA levels in the PVN are modified as described for controlled suckling, except that TRH mRNA peak values are observed later (at one hour). Anterior hypothalamic TRH and TRH mRNA levels increase during cold stress: however, the kinetics of TRH mRNA variations is different from that in PVN.

4 Oestrous cycle: TRH mRNA levels in the hypothalamus fluctuate extensively with maximal values during D2. Under these circumstances degradation of TRH by pyroglutamate amino peptidase II (PPII) in the adenohypophysis is regulated (lower levels during D2 and P).

5 Thyroid status: previous studies showed that TRH mRNA levels in the PVN are regulated downward by thyroid hormones, while PPH levels in the adenohypophysis are regulated upward. We have determined the cellular specificity of this control by studying TRH. TRH mRNA, and PPII in various brain regions and peripheral tissues. The data suggest that those parameters are only sensitive to hormone changes in the hypothalamus, circadian fluctuations

of TRH mRNA parallel those of in vitro basal or K^+ stimulated release of TRH. In the olfactory bulb, a similar cycle in the basal release is observed, but is not accompanied by changes in TRH or TRH mRNA. PPII levels in hypothalamus or olfactory bulbs do not change during the cycle.

Regulation of TRH metabolism in vitro. In order to determine the first and second messengers involved in the regulatory events observed *in vivo*, we have studied TRH metabolism in primary cultures of hypothalamic, cortical or adenohypophyseal cells. Cells were incubated in the presence of various neurotransmitters, such as TRH, dopamine (DA), angiotensin II (AII) and somatostatin (SRIF). PPII activity was measured by following the degradation of ^{3}H-TRH after chromatographic separation of its catabolytes. Incubation of pituitary cells for 16 hours with TRH (10^{-6} M) decreased PPII activity by 25%; DA has a similar, but more rapid action. In contrast, manipulation of second messengers (stimulation of cAMP by forskolin, use of CA_2 ionophores, stimulation or protein kinase C by TPA, activation of the arachidonic cascade) stimulated PPII activity. Desensitization of protein kinase C, as well as chronic treatment with estradiol, also modulate the enzyme activity. We observed that stimuli that increase TRH release by hypothalamic cells (increased cAMP levels or membrane depolarization) also increase TRH mRNA levels.

PPII activity in cortical or hypothalamic neurons or brain slices is not modifed by short term (minutes) or long term (hours) stimulations with either TRH, cAMP or TPA, a protein kinase C activator. On the other hand, long term stimulation of adenohypophyseal cells with TRH decreases PPII activity. The effect is mediated by TRH receptor activation and can be mimicked by TPA. It is independent of the sex of the animal or thyroid hormone levels. A short term inhibitory effect of TPA on PPII activity has also been observed.

Role of arachidonic acid in prolactin regulation. Pituitary cells in culture well loaded with 3H arachidonic acid and stimulated were challenged with either DA, TRH or AII. Radioactive material released into the medium was analysed and assessed. It was found that DA induces a parallel inhibition of AA and PRL release, under basal conditions as well as after TRH or AII stimulation. One interesting finding is that, in contrast to prolactin secretion, inhibition of AA is insensitive to pretreatment with pertussis toxin, an uncoupling of *aO* and *ai* GTP binding proteins. This indicates that coupling mechanisms involved in the regulation of AA release are distinct from those responsible for other second messengers connected with prolactin secretion.

The interrelationships of the AA pathway with other second messengers were also investigated. Initial results show that forskolin is unable to affect AA, although it stimulates both cAMP production and prolactin secretion. AA is also insensitive to calcium channel agonists or antagonists. In contrast, TRH and AII, two peptides which affect intracellular calcium mobilization, induce also a parallel increase in AA and PRL release.

Finally, dopamine inhibition on AA production was also found on membrane preparations, an observation which suggests the existence of a direct negative coupling of the D2 receptor with phospholipase A2, the enzyme responsible for AA production. Such negative coupling of a receptor with PLA2 had not been described so far.

Role of target cells on development and differentiation of hypothalamic neurons. Cells of the intermediate lobe (IL) of the pituitary constitute an homogeneous cell population (98% are melanotrophs), which are innervated during development by neurons originating in the basal hypothalamus. Consequently, they provide an adequate model to identify and characterize target cell factors responsible for terminal differentiation. We have established a serum-free culture medium which permits coculture of melanotrophs with foetal hypothalamic cells sampled in day 15 of gestation. We found that coculture specifically increases the speed of differentiation of dopaminergic (DA) neurons and induces the formation of cell clusters connected by thick bundles of neurites. Both processes appear independent; they have been established on biochemical (rate of tritiated dopamine

uptake, expression of differentiation markers) as well as neuroanatomical parameters. The factor(s) responsible for these effects are not expressed by adenohypophyseal cells, in particular corticotrophs, in spite of the fact that neurointermediate cells and corticotrophs share expression of the POMC gene. Specificity of the interaction is further suggested by the fact that a different set of dopaminergic neurons (mesencephalic DA neurons) are insensitive to our coculture condition.

Conclusions

In the different aspects of this research project concerning neuroendocrine and paracrine control of adenohypophyseal hormone secretions significant results have been obtained illustrating the pleotropism of the mechanisms involved at different levels.

TRH biosynthesis is altered during stimulation of TRH release. This correlates with changes of TRH mRNA levels, and possibly also with translational or post-translational processing efficiencies.

Hormones regulate TRH synthesis in a tissue specific manner; however under some circumstances several hypothalamic nuclei are regulated in a coordinated manner.

TRH extracellular degradation in the adenohypophysis is regulated by hormonal status in a coordinated fashion with TRH biosynthesis and release from PVN neurons.

TRH biosynthesis can be regulated by alterations of second messenger levels. The same is true for PPII activity; however this regulation appears restricted to the adenohypophysis. It may modulate the effectiveness of TRH on lactotroph cells.

Beside coupling with other transduction mechanisms, neurohormone receptors (TRH, angiotensin II, dopamine) are also coupled with phospholipase A2. For dopamine, it represents the first demonstration of a direct inhibitory coupling with this enzyme. Such a regulation of the arachidonic acid cascade may be involved in paracrine regulations in the anterior pituitary.

Cells from the pituitary intermediate lobe secrete a diffusible factor which selectively enhances neurite growth and differentiation of fetal hypothalamic neurons in culture, in particular phone which synthetize dopamine.

Publications

Uribe, R.M.; Joseph-Bravo, P.; Pasten, J.; Ponec, G.; Méndez, M.; Covarrubias, L. and Charli, J.L. (1991) Some events of TRH metabolism are regulated in lactating and cycling rats. Submitted to *Endocrinology*.

31 Nutritional impact of *Giardia lamblia* on growth, body composition, energy utilization and energy expenditure in children and adults in northern Mexico

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Introduction

The effects of parasites on the nutritional status of the host can result in a series of disturbances of the nutritive process by affecting digestion and absorption. Often because of the illness, the person is inactive and his physical performance is impaired. When the host is undernourished, the situation can be complicated by the unknown priorities given to these needs in such a way that the host may be capable of accommodating the extra demand for energy caused by a moderate parasite burden by reducing the discretionary energy expenditure. On the other hand, other components of energy expenditure such as the basal metabolic rate could be affected by an alteration of body temperature. Parasitic infections do not generally result in a high fever; however, *Giardia* may cause a slightly elevated temperature.

In Sonora, Mexico, a high incidence of intestinal parasitosis of up to 61% has been detected in the pre-school population. Of the pathogenic parasites, *Giardia lamblia* has been the most prevalent. Recently a study in Sonora showed negative effects on vitamin A status and growth. In this study more undernourished children were found in the infected group (based on vitamin A status and by the relationship of weight for age and height for age).

The purpose of this research is to study the nutritional impact of *Giardia lamblia* on growth, body composition, energy utilisation and energy expenditure in children and adults in a population with a high and sustained prevalence of the parasite. To accomplish this, specific studies will be conducted both in children and adults, testing different hypotheses in each case.

Materials and methods : study I

Subjects. Ten boys infected by *Giardia lamblia* were selected through the health clinics (Junta para el Progreso y Bienestar de Hermosillo) using a specific protocol which included a urinary test for indoxyl phosphate (Indican) to assure the absence of bacterial infection. They were selected from an original sample of 99 boys in the age range 6-10 years by exclusion of 40 non-infected boys and 41 infected by other parasites. The boys were their own controls for all the parameters measured before and after treatment with a specific antiparasitic agent.

Study Protocol. The 10 boys recruited for the study underwent two 7-day periods of measurement separated by a 7-day period for treatment of the *G. lamblia* infestation. Before the first week of the study the mother was instructed how to weigh and record the food intake of the child, and the Basal Metabolic Rate (BMR) of the child was measured using a Deltatrac ventilated hood. Pilot studies of BMR measurements of children of similar age were previously carried out to assess the need for a trial measurement with the Deltatrac to avoid raised energy expenditure due to anxiety raised by the procedure.

On the first day of the 7-day period, each child was given an oral drink of water containing a known amount of stable (non-radioactive) isotopes of hydrogen and oxygen. This 'doubly labelled water (DLW)' method gives information on both the body composition of the child and on the free-living energy expenditure over the following 7 days. During the 7 days a sample of the second urine specimen of the day was collected, and all food eaten was weighed and noted to assess the amount of protein, carbohydrate and fat in the diet (this is used to calculate the food quotient ('FQ') needed for the DLW method). After 7 days each child was treated with the anti-parasitic agent Tinidazole for 2 days. Stools collected several days later were used to confirm whether treatment had been successful or whether a further course of treatment was required. The BMR, DLW and food intake were then repeated to give values after treatment to compare with those obtained before treatment in the same children.

Figure 1: Measurement of Basal Metabolic Rate (BMR) in a young boy

As a complement to these studies, 20 children from the same area were studied through the year to assess water intake and water loss from the body by evaporation. These factors can improve the validity of the DLW technique under different conditions of temperature and humidity.

Preliminary results

The results of RMR and BMR for the pilot test are presented in Table 1. There were no significant differences between the three measurements. However, the difference between the two BMR's was less than BMR1 compared to the RMR, suggesting a slight training effect but more important the effect of the standardisation of a more controlled measurement such as the BMR.

	Age	Energy expenditure, kJ/day			
		RMR	BMR 1	BMR 2	BMR(FAO)
Mean	4.6	4125	4034	3989	3829
SE	0.2	102	90	80	60

Table 1. Resting and basal metabolic rate in pre-school children

The analysis of variance did not detect a difference between BMR's 1 and 2 (P=0.713). Nevertheless the RMR values seem to be consistently higher, which would indicate the need for having the training session as a standardised procedure before the actual BMR measurement, as well as the need for applying the BMR protocol. No significant difference was observed between the measured BMR and the FAO/WHO/UNU predicted values in the range of 3-10 years of age (P=0.09). Gas recoveries in propane gas calibrations within 4.2 and 5.3 kJ/d of energy expenditure were 99.8 (S.E.=1.3) and 99.6 (S.E.=1.4) per cent for O_2 and CO_2 respectively. However, because we think we are at the limit of the capabilities of measurement by the Deltatrac system we decided for the final study to increase the body size of the children..

Materials and methods : study II

The second part of the study, to be carried out in 1991 and 1992, will look mainly at problems of malabsorption by studying metabolisable energy, nitrogen and fat within the context of a normal but controlled diet for the subjects. For this purpose we will use adults who are heavily infected with *Giardia lamblia* who will be resident in the metabolic unit (approximately three weeks), before and after treatment in order to test the effect of the parasite on the parameters.

Based on the regional diet, various menus are being prepared for a fixed composition in terms of the energy contribution by carbohydrates, protein and fat; trying not to exceed 30% from fat, 50-55% from carbohydrate and 15-20% from protein.

Preliminary results

Calorimetry studies. Basal metabolic rate and postprandial thermogenesis will be determined in the subjects before and after treatment by means of the Deltatrac metabolic monitor as for the children. There will be no DLW in this section since the subjects will be confined to the unit.

In preliminary studies, basal metabolic rate was determined by ventilated hood indirect calorimetry in 32 male adults divided into four body mass index groups: <20;20-24.9;25-29.9 and 30-39.9 in the age range of 18-30 years. The weight range was 51.75 to 118 kg and the height 1.61 to 1.89 m. Basal metabolic rate was analysed with body weight by regression and compared to the values predicted using

the Schofield equations (Schofield, 1985). These equations overestimated BMR by 6.71, 8.44, 5.68 and 4.39% in the four BMI groups respectively (Table 2); however, the overall difference of 6.3% in all groups was not statistically significant when analysed by a paired t test (P=0.07).

Subjects		Weight, kg	BMI, kg/m^2	Meas. BMR, MJ/d	Est'd. BMR MJ/d	Difference, %
1-8	Mean	58.49	18.21	6.14	6.74	+6.71
	SD	6.48	1.40	0.39	0.71	2.92
9-16	Mean	67.71	22.99	6.59	7.17	+8.44
	SD	8.01	1.40	0.87	0.51	5.73
17-24	Mean	86.08	27.40	7.75	8.26	+5.68
	SD	6.72	1.25	0.95	0.36	11.18
25-32	Mean	101.45	33.94	8.70	9.13	+4.39
	SD	11.45	2.39	0.83	0.66	9.70
All	Mean	78.43	25.64	7.29	7.82	+6.30
	SD	18.65	6.11	1.26	1.09	7.82

Table 2. BMR and percentages by which Schofield equations overestimate the actual measurements.

The BMR prediction equation based on weight for 28 of the 32 subjects in the 18-30 years of age range is presented in Table 3. If we compare our equation to Schofield's by covariance analysis the slope is the same but the intercepts differ significantly ($p<0.001$).

Men 18-30	N	Equation	r	Mean weight
This study	28	BMR=0.063 (wt) + 2.29	0.93	75.8
Schofield	2879	BMR=0.064(wt) + 2.84	0.65	63.0

Table 3. Regression of BMR (MJ/day) on weight compared with Schofield equations

The results show the same overall tendencies as the analysis of BMR in different population groups reported by Henry and Rees (1988). However, the percentage overestimation of the Schofield equations seems to be higher in the major of those ethnic groups than that found in this study. Our data comply with the basic controls of a BMR determination stipulated in the latter report. Furthermore, as an additional control, the subjects spent the night at the unit and were given an evening meal equivalent to $^1/_3$ of their estimated energy requirement. All subjects went through a training resting metabolic rate measurement before the final BMR determination.

Figure 2: Estimation of lung and body volume during body composition assessment

At this stage our sample size is too small to conclude that our results differ from the estimation obtained using the Schofield equations. However, there appears to be an overall tendency to have lower values. It would be very useful to expand the sample size to improve the overall predictions and decrease the error.

This preliminary part of the study has allowed us to test our basic calorimetry procedures and at the same time has produced useful information regarding BMR under controlled conditions for a group of the population that has not been previously reported. It has also expanded data banks currently available for future estimations of energy requirements in different populations.

Background references

Henry, C.J.K. and Rees, D.G., 1988. A preliminary analysis of basal metabolic rate and race. *In*: Blaxter, K. and MacDonald, J. (eds). Comparative Nutrition. John Libbey. London.

Schofield, W.N., 1985. Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition: Clinical Nutrition.* **39C**(1):5-41.

32 Application of recombinant DNA expression technology for the study and control of *Taenia solium* cysticercosis in Mexico.

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Background and objectives

Cysticercosis is caused by the establishment of *Taenia solium* larvae, mainly in the central nervous system, the eye and the skeletal muscle of humans and pigs after ingestion of eggs shed in human faeces by the adult tapeworm. The life cycle of epidemiological importance includes the human carrier of the adult tapeworm, free living eggs and pigs with cysticercosis. Nevertheless, the importance of this parasitic disease lies in the individual with neurocysticercosis, because, due to the frequency and severity of the disease, it is now recognized as a priority in public health in many developing countries.

Figure 1: Taenia eggs seen under the microscope

This is the only method actually in use to detect the taenia carrier. It is not specific because all taenid. eggs have the same morphology.

Neurocysticercosis, although a life-threatening disease in severe cases, is generally a long-lasting infection affecting the quality of the patient's life and social environment. The disease has socioeconomic importance because 75% of patients with neurocysticercosis are at productive ages and are frequently unable to work soon after the onset of symptoms. Calculations of costs for medical care, such as hospitalization, chemotherapy, neurosurgery and computer tomography, show that US $ 14.5 million were spent in Mexico during 1986 to treat only the 2700 new hospitalized cases of neurocysticercosis.

Recent evidence indicates that the main risk factor for acquiring cysticercosis is the close contact with the tapeworm carrier. An adult worm may remain in its host for up to 20 years releasing proglottids and inducing minimal symptomatology. *Taenia* carriers seldom know, if ever, that the eggs found in the proglottids, when ingested by another person, a pig, or even themselves, may cause cysticercosis, figure 1.

The main objective of this project is to incorporate new advances in immunology and recombinant DNA technology to the study of cysticercosis and the causative organism, *T. solium*, which will result in the production of novel reagents with both immunodiagnostic and immunoprophylactic value. Production of *T. solium* antigens by genetic engineering and the construction of *T. solium* specific DNA probes are the first goals. The application of these molecules in immunodiagnosis, seroepidemiological surveys and as vaccines can instigate a powerful new approach to the prevention, treatment and control of cysticercosis in Mexico. Furthermore, the availability of these reagents will facilitate new approaches for studies of the biology of *T. solium* and the host-parasite interaction in man and pig. The existing infrastructure, the institutional and national commitment to research in Mexico and the awareness of the Mexican authorities of the immense problem presented by

cysticercosis provide a unique opportunity for structured epidemiological studies, collection of parasite materials and of sera, and for field testing of immunological reagents, including genetically engineered antigens.

In recent years there has been a significant proliferation of scientific research in developing countries related to the recognition by scientists and politicians alike of the necessity of applying science to the great health problems that still face the developing world. Since modern biotechnology offers great promise for facilitating this important development, the joint venture nature of the present project will favour the fulfilment of its objectives, because the rapid pace of change and the expense of new equipment limit the possibilities for laboratories in developing countries fully to participate in these advances. One route to solve this problem is through collaboration of scientists from developed and developing countries. The Liverpool School of Tropical Medicine with the collaboration of McManus at the Queensland Institute for Medical Research in Brisbane, Australia, will provide the expertise and appropriate training in molecular and immunological techniques to the Mexican partner; while the Instituto de Investigaciones Biomédicas at the National University of Mexico will provide the expertise in the study, handling and provision of the parasite selected, *Taenia solium*, to the English partner.

Results

Detection of *Taenia* eggs by a DNA hybridization technique. *Taenia* eggs were identified by a DNA hybridization technique using whole *Taenia* DNA as a probe. When *T. saginata* total genomic DNA was used as a probe, the sensitivity with *T. saginata* eggs was very high because even one egg could be detected; furthermore, this assay was very specific using the same probe with *T. pisiformis* eggs or DNA (obtained from a dog) but with *T. solium* there was cross hybridization (Figure 2). Thus we decided to use a specific DNA sequence as a probe in order to improve the specificity of this assay and, preferentially, a repetitive sequence because it has been demonstrated that these sequences are in general highly species-specific and also increase the sensitivity of the assay.

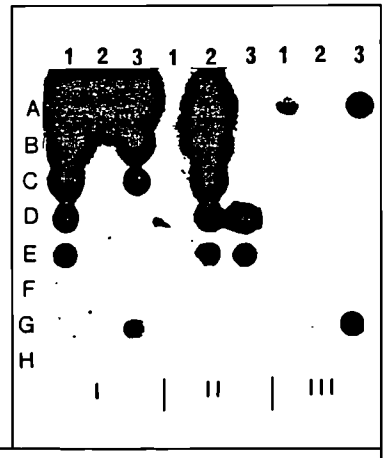
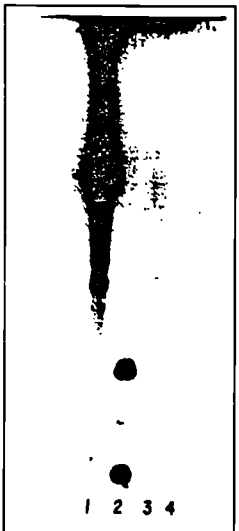


Figure 2: *T. saginata* (1) and *T. pisiformis* (2) eggs made up in 200 µl water containing 5000 (A), 1000 (B), 500 (C), 100 (D), 50 (E), 10 (F), 5 (G), or 1 (H) eggs. Total *Taenia* genomic DNA (3) was also dotted in 100 ng, 10 ng, or 1 ng amounts (*T. saginata* A, B, C; *T. pisiformis* D, E, F) or in 100 ng and 10 ng and 10 ng amounts (*T. solium* G, H). Radioactive probes were prepared by nick translation of 1 µg Taenia DNA (I = *T. saginata*, II = *T. pisiformis*, III = *T. solium*).



Development of probes made of repeated sequences of *T. solium* and *T. saginata* DNA. We developed genomic libraries from *Taenia solium* in pBluescript KS(+/-). Recombinant clones containing repeated sequences from both species were analyzed in Southern blots. Several *T. solium* clones were found to be related because they hybridized among themselves; clone pTs9 was specific for *T. solium* since it did not hybridize with *T. saginata*. This sequence was termed the HaeIII repeat and is around 200 bp (Figure 3). We also detected at least two different and not related *T. saginata* specific clones. The *T. solium* and *T. saginata* repetitive DNA probes were highly specific since they did not hybridize with each other or with genomic DNA from other taeniid species including *T. hydatigena*, *T. pisiformis*, *T. taeniaeformis* and *Echinococcus granulosus* or genomic DNA from other eukaryotes including *Saccharomyces cereviciae*, *Candida albicans*, *Entamoeba histolytica* and *Giardia lamblia* in a dot-blot assay. The usefulness of these species-specific probes will now be evaluated in dot blot assays using purified eggs from several *Taenia* species and human faecal samples.

Figure 3: Southern blot of *T. solium* (1,2) and *T. saginata* (3,4) DNA digested with HaeIII (2,4) and Hind III (1,3) restriction enzymes. pTs 9 DNA was labelled with ^{32}P and used as a probe.

Standardization of *T. solium* antigen detection in faecal samples. An immunodiagnostic test for *Taenia* specific faecal antigen based on polyclonal rabbit antisera against *Taenia saginata* or *Taenia solium* proglottid extracts in capture ELISA was developed. Taenia specific antigen was detected in detergent-solubilized faecal extracts from *T. solium* and *T. saginata* infected hosts. Coproantigen from *T. solium* infected hamsters did not cross-react with faeces from rodents infected with *Hymenolepis diminuta*, *H. citelli*, *H. microstoma*, *Necator americanus*, *Strongyloides ratti* or *Nematospiroides dubius* and faeces from uninfected animals. Application of the test to a series of more than 20 human taeniasis (*T. solium* or *T. saginata*) cases and over 200 stools from people with other intestinal helminth infections indicated excellent sensitivity, with apparently no false negatives, and specificity to the genus level, since no discrimination between *T. solium* and *T. saginata* cases could be achieved.

The *Taenia* coproantigen test became completely negative by 6 days following successful treatment of patients for intestinal taeniasis (Figure 4). Anti *T. solium* adult monoclonal antibodies are currently being produced against somatic and excretory-secretory preparations in order to try to obtain species specificity of the coproantigen test.

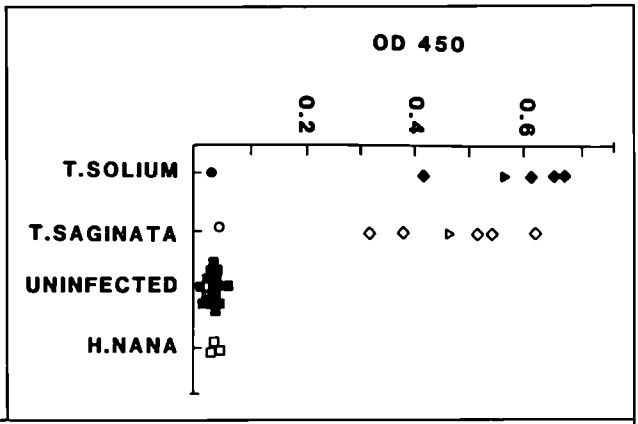


Figure 4: Specificity of *Taenia solium* ELISA with human faecal supernatants. *T. solium* group containing positive infected samples (◆), pre- (▸) and 6 days post- (●) drug treatment samples, as does the *Taenia saginata* group (infected ◇; pre-treatment ▷, and post-treatment, ○). Control uninfected individuals (■) and *Hymenolepis nana* infected samples (□) were also tested.

Construction and evaluation of cDNA libraries from *T. solium*. The production of up to 2 μg of mRNA from 0.5 x 10^6 *T. hydatigena* oncospheres was achieved which represents a major improvement on other methods which required 30 x 10^6 oncospheres. *In vitro* translation of *T. solium* adult RNA was good and protein products were immunoprecipitated by anti-adult sera including a 14 kD antigen which was dominantly expressed. cDNA fragments were produced from adult and cysticercal mRNA and expressed in lambda gt11 and lambda zap systems. These ranged from 0.5 to 3.5 kb and after cloning produced 10^5 to 10^6 recombinants. Libraries were screened with hyperimmune experimental sera and with pools of sera from patients with neurocysticercosis; specificity was monitored by eliminating the clones positive to sera from patients with other parasitic diseases such as hydatid disease. Actually, attention is directed towards the optimal production of the selected fusion proteins for application to immunodiagnosis and seroepidemiology and to the production of a cDNA library from *T. solium* oncospheres.

Immunocharacterization of native antigens. Native *T. solium* antigens were identified by the use of clinically defined human sera from neurocysticercosis and disseminated cysticercosis patients (Mexico and China), from human taeniasis tapeworm carriers and from patients with other parasitic helminth infections, and by the use of pig sera from experimental cysticercotic infections (Mexico) at various times post-infection (1-5 weeks) and rabbit hyperimmune sera raised against oncosphere, metacestode and adult *T. solium* extracts in the immunoelectrotransfer blot technique. Sera from humans and pigs infected with *T. solium* cysticerci recognised the same major antigens in both oncosphere (egg derived) and cysticerci (metacestode) extracts. However, more antigens were detected by post-infection pig sera especially by 3 weeks post infection. As well as these common antigens, both oncosphere and metacestode exhibited putative stage-specific antigens, including an immunogenic "doublet" of 14 kD in metacestodes. Oncosphere specific antigens appeared also as a low molecular weight component (under reducing conditions) at approximately 8-10 kD. In general, stage specificity was more associated with the metacestode stage and there was an apparent time-related antibody response in pigs during metacestode development associated with sequential increase in antigen recognition.

Publications

Allan, J.C. & Craig, P.S. (1989). Coproantigens in gut tapeworm infections: *Hymenolepis diminuta* in rats. *Parasitology Research* 76, 68-73.

Allan, J.C.; Avila, G.; García-Noval, J.; Flisser, A. and Craig, P.S. (1990). Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* 101, 473-7.

Flisser, A.; Reid, A.; García-Zepeda, E. and McManus, D.P. (1988). Specific detection of *Taenia saginata* eggs by DNA hybridization. Letter to *Lancet*, 17 December II:1429-30.

Flisser, A.; Plancarte, A.; Correa, D.; Rodríguez-del-Rosal, E.; Feldman, M.; Sandoval, M.; Torres, A.; Meza, A.; Parkhouse, R.M.E.; Harrison, L.J.S.; Wilson, M.; Avila, G.; Allan, J.; Craig, P.S.; Vallejo, V.; Ortiz, D.; García, E. and McManus, D.P. (1990). New approaches in the diagnosis of *Taenia solium* cysticercosis and taeniasis. *Annales de Parasitologie Humaine et Comparée* 65, suppl. 1:95-8; and also published in the Proceedings of the International Workshop of Helminth Basic Research and Second Regional Workshop on Basic Research and Hydatid Disease, Solis 1989, Ediciones Logos, Montevideo Uruguay.

McManus, D.P.; García-Zepeda, E.; Reid, A.; Rishi, A.K. and Flisser, A. (1989). Human cysticercosis and taeniasis: molecular approaches for specific diagnosis and parasite identification. *Acta Leidensia* 57, 81-90.

33 Health, infant mortality and differential fertility in Mexico.

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Contract number and duration: CI1*/0458, November 1989 to October 1992

Background

Infant mortality is often regarded as a sensitive indicator of health in a population. It is related to fertility but the relationship is complex and involves both socio-economic factors and biological ones. Family formation patterns also play a role, involving the age, gender and birth intervals of the children and have different effects on perinatal, infant and child mortality.

However, indicators based solely on mortality statistics are not satisfactory. A new conceptual framework for the description of population health is needed that takes account of family circumstances and the use made of the existing health-care system.

Methods

The main work so far has been an extensive bibliographic study by a doctoral student, C.J. Echarri Canovas. He began with the 1987 Mexican National Fertility and Health Survey, and then extended his search to cover household patterns (rather than families) and health-care use. The focus is national, using large-scale data bases, rather than based on field work with small populations and groups. Attention is paid to the constitution of nuclear or non-nuclear households, the gender of the head of household, the life-cycle stage and the economic activity of women in the household.

The bibliographic search has extended to Belgium, France and Spain. It has used the preliminary results of the Mexican 1990 Census and the data base of the National Directorate of Family Planning. A presentation has been given in Guadalajara and a further one will be given in August 1991 in Washington DC.

34 Characterisation and regulation of protease synthesized by arterial endothelial cells.

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C.G. Knight

Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 4RN, United Kingdom.

Contract number and duration: CI1*/0434, July 1989 to June 1992

Background

The maintenance of endothelial cell monolayer integrity in blood vessels is a crucial factor for blood cell circulation and the prevention of platelet adhesion to the subendothelium matrix. The loss of this monolayer integrity is considered as one of the principal causes in the development of cardiovascular diseases like atherosclerosis. In physiological circumstances platelets do not adhere to an intact endothelial cell monolayer; however, they could play an important role in keeping endothelium by secreting endothelial cell growth factors and inhibitors of replication.

Endothelial cells are known to secrete a number of proteases acting on several macromolecular constituents of subendothelium. Among these enzymes are the metalloproteases (collagenases and gelatinases), and stromelysin. These cells also produce plasminogen activators important for fibrin clot lysis, tissue remodelling, anglogenesis and tumour growth dissemination. The loss of integrity of endothelium could be related to an abnormal regulation of proteinase and fibrinolytic activities expressed by endothelial cells. In spite of the important participation of different components from endothelial cells and platelets (e.g.urokinase-type plaminogen activators, plasminogen and their receptors) in fibrinolysis and proteolytic cascade activation, no studies focused on the cooperation between endothelial cells and platelets in this event have been undertaken.

Results

We have recently shown that the morphology of endothelial cells in culture is modified when they are in presence of platelets, Figure 1. This observation also suggested that platelets could induce important modifications in the protein expression of endothelial cells.

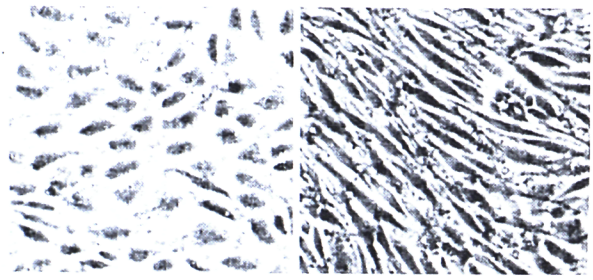

Figure 1 Morphology of endothelial cells: left- untreated; right-incubated with blood platelets (1 x 10^8/ml) for 24 hours at 37°C.

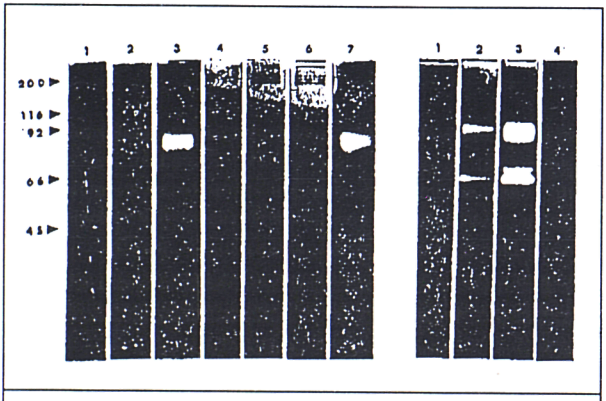

Figure 2 Identification of casein and gelatin degrading activity by $NaDodSO_4$- substrate gel analysis.

We have also shown that interaction between platelets and endothelial cells in culture produce the activation of a new proteinase observed at 85 kDa level in a polyacrylamide gel impregnated with casein, Figure 2. Modulation of endothelial cell gelatinase activity by platelets has also been demonstrated.

In gel (left) casein degrading activity of: 1, platelets extract; 2, conditioned medium of endothelial cell (EC); 3, conditioned medium of EC incubated with platelets (10^8/ml). After separation by electrophoresis of conditioned medium of EC incubated with platelets, the gel was incubated in presence of serine proteases inhibitors; 4, di-isopropylfluorophosphate 1mM; and 4-(2-aminoethyl)-benzenesulfonyl fluoride 1mM; 5, aprotinine 0.1 mM; and metalloproteases inhibitor; lane 7; 1, 10 phenantroline 2 mM.

In gel (right) gelatin degrading activity of: 1, platelets extract; 2, conditioned medium of EC incubated with platelets (10^8/ml). After separation by electrophoresis of conditioned medium of EC incubated with platelets, the gel was incubated in presence of 1,10 phenantroline. Molecular weights of protein standards are indicated in kilodaltons on the left.

Discussion

These observations have shown the importance of ceullular serine and metalloproteases in platelets - endothelial cells interaction. Our understanding of this interaction will be greatly aided by the development of synthetic substrates and inhibitors. This will be the subject of our future studies.

In the framework of this project, G. Flores Delgado is preparing a doctoral thesis.

Figure 3: G. Flores Delgado at Créteil

Publications

Menashi, S.; Flores-Delgado, G.; Hornebeck, W. and Legrand, Y. (1990). Protéases des cellules endothéliales et leur modulation par les plaquettes. (Endothelial cell proteases and their modification by platelets). *Pathologie Biologie*, **38**, 1015-1019.

Menashi, S.; Flores-Delgado, G. and Legrand, Y. (1990) Endothelial proteases stimulated by blood platelets. *Nouvelle Revue Française d'Hematologie*, **32**, 453-4.

Postdoctoral fellowships

G. Castañeda-Hernandez

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T. Godfraind

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Pharmacology of arterial hypertension and cardiac insufficiency

Fellowship period: July 1988 - September 1988

Publications

Castañeda-Hernández, G. (1989). Evidence for the existence of the same endogenous digitalis-like factor in several mammalian species. *Comparative Biochemistry and Physiology C: Comparative Pharmacology and Toxicology*, **94**, 49-53.

Castañeda-Hernández, G. (1990). Interaction of guinea pig heart extracts with antidigoxin antibodies, ouabain binding site and Na, K-ATPase. *Proceedings of the Western Pharmacological Society*, **33**, 9-13.

Castañeda-Hernández, G.; Bravo, G. and Godfraind, T. (1991). Chloropromazine treatment increases circulating digoxin-like immunoreactivity in the rat. *Proceedings of the Western Pharmacological Society*, **34**, in press.

A. Flisser

Instituto de Investigaciones Biomédicas,
Universidad Nacional Autónoma de México,
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Research on human and swine cysticercosis

Fellowship period: October 1987 - November 1988

Summary

This work led to a joint research project (See report 32, page 149) but with a different UK partner.

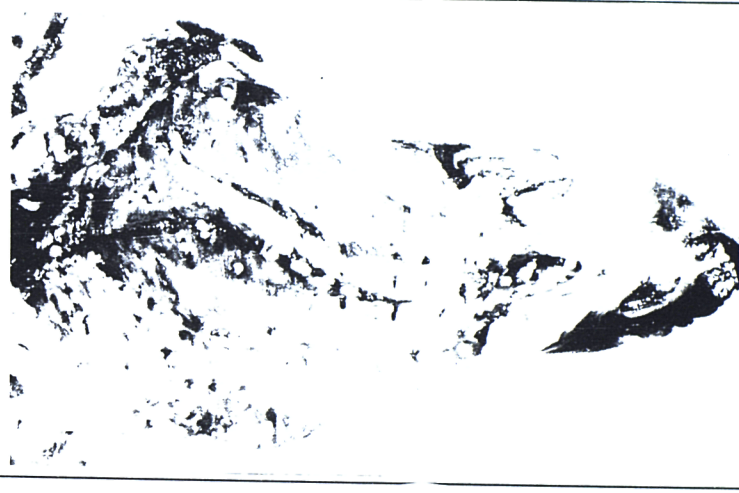

Figure 1: Tongue of a pig full of cysticerci

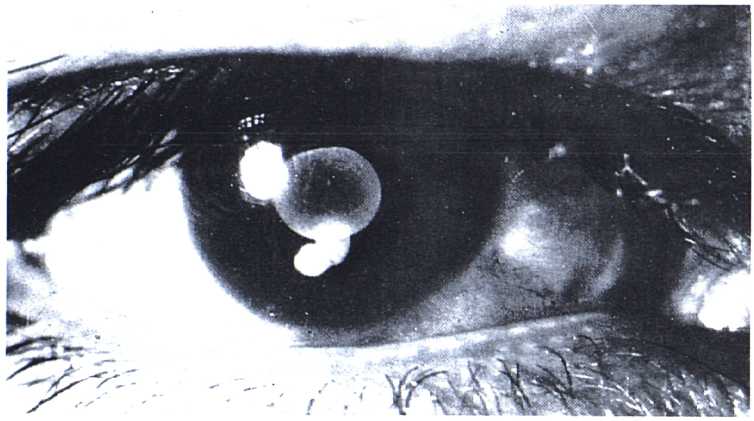

Figure 2: An evaginated cysticercus in the anterior chamber of the eye

Publications

Allan, J.C.; Avila, G.; García-Noval, J.; Flisser, A. and Craig, P.S. (1990). Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **101**, 473-7.

Flisser, A.; Reid, A.; García-Zepeda, E. and McManus, D.P. (1988). Specific detection of *Taenia saginata* eggs by DNA hybridization. Letter to *Lancet*, 17 December II:1429-30.

Flisser, A. (1988). Neurocysticercosis in Mexico. *Parasitology Today*, **4**, 131-7.

Flisser, A.; Plancarte, A.; Correa, D.; Rodríguez-del-Rosal, E.; Feldman, M.; Sandoval, M.; Torres, A.; Meza, A.; Parkhouse, R.M.E.; Harrison, L.J.S.; Wilson, M.; Avila, G.; Allan, J.; Craig, P.S.; Vallejo, V.; Ortiz, D.; García, E. and McManus, D.P. (1990). New approaches in the diagnosis of *Taenia solium* cysticercosis and taeniasis. *Annales de Parasitologie Humaine et Comparée* **65**, suppl. 1:95-8; and also published in the Proceedings of the International Workshop of Helminth Basic Research and Second Regional Workshop on Basic Research and Hydatid Disease, Solis 1989, Ediciones Logos, Montevideo Uruguay.

McManus, D.P.; García-Zepeda, E.; Reid, A.; Rishi, A.K. and Flisser, A. (1989). Human cysticercosis and taeniasis: molecular approaches for specific diagnosis and parasite identification. *Acta Leidensia* **57**, 81-90.

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Cell injury and intercellular communication

Fellowship period: January 1989 - December 1989

Publications

Mourelle, M.; Beales, D. and McLean, A.E.M. (1990). Electron transport and protection of liver slices in the late stages of paracetamol injury. *Biochemical Pharmacology*, 40 (9) 2023-8.

Mourelle, M.; Beales, D. and McLean, A.E.M. (1990). Prevention of paracetamol induced liver injury by fructose. *Biochemical Pharmacology*, in press.

R. Pérez Montfort

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V. Brade

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Studies on the activation of complement and resistance to complement-induced lysis in *Entamoeba histolytica*

Fellowship period: September 1989 - August 1990

Summary

Studies were focused on the culture and maintenance of the axenic culture of *E. histolytica*; and the production of axenic cultures resistant to lysis by 10% human serum. Initial studies were made on the binding and processing of serum C9 by amoebas susceptible or resistent to lysis by serum. Studies were then made of functional inactivation of C3 and C9 by subcellular fractions of *E. histolytica* in a) normal human serum b) EGTA-Mg serum, c) serum deficient in C2 and C4, d) purified human C3 and C9, and e) in the presence of cysteine proteinaise inhibitors (p-hydroxymercuribenzoate, E-64). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was made of the action of subcellular fractions of *E. histolytica* on human C3 and C9 both in purified form and in normal human serum.

The main conclusions of this work are that *E. histolytica* can consume complement by two mechanisns: with purified components direct proteolysis of C3 seems to play the most important role, while in serum the activation of the whole cascade by membraneous structures appears to be the most relevant.

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Host 1, P.J. Aggett
Host 2, G. McNeill

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Human Nutrition Unit, The Rowett Research Institute, Greenburn Road, Bicksburn, Aberdeen AB2 9SB, United Kingdom.

Energy expenditure studies in human subjects

Fellowship period: December 1987 - November 1988

Summary

This work led to a joint research project (see report 31, page 144) with the Rowett Research Institute.

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Circulatory effects on serotonin and of antiseratogenic agents

Fellowship period: August 1989 - July 1990

Publications

Bom, A.; Villalón, C.; Verdouw, P. and Saxena, P.R. (1989). The 5-HT_1-like receptor mediating reduction of porcine carotid arteriovenous shunting by RU 24969 is unrelated to either 5-HT_{1A} or 5-HT_{1B} subtype. *European Journal of Pharmacology*, **171**, 87-96.

Den Boer, M.O.; Villalón, C.; Heiligers, J.; Saxena, P.R. and Humphrey, P.P.A. (1990). The craniovascular effects of sumatriptan in the pig., *British Journal of Pharmacology*, **99**, 37.

Den Boer, M.O.; Villalón, C.; Heiligers, C.M.; Saxena, J.H.P. and Humphrey, P.P.A. (1990). The effects of sumatriptan on the carotid circulation of the pig. *European Journal of Pharmacology*, **183**, 1275-6.

Den Boer, M.O.; Villalón, C.M.; Heiligers, J.P.C.; Humphrey, P.P.A. and Saxena, P.R. (1991). Reduction of cranial arteriovenous anastomotic shunting by sumatriptan, a new antimigraine drug, is mediated by a 5-HT_1-like receptor. *British Journal of Pharmacology*, in press.

Heligers, J.H.P.; Villalón, C.M.; Bom, A.H. and Saxena, P.R. (1990). Effects of S9977, a potential antimigraine drug, on arteriovenous anastomoses in the carotid vascular bed of the anaesthetised pig. *European Journal of Pharmacology*, **183**, 1279-80.

Saxena, P.R. and Villalón C. (1990). Cardiovascular effects of serotonin agonists and antagonists, *Journal of Cardiovascular Pharmacology*, **15** (Suppl. 7), S17-S34.

Saxena, P.R. and Villalón, C. (1990). Brain 5-HT_{1A} receptor agonism: a novel mechanism for antihypertensive action. *Trends in Pharmacological Sciences*, **11**, 95-6.

Villalón, C.; Bom, A.; Heilgers, J.; Saxena, P.R. and Humphrey, P.P.A. (1990). Reduction of porcine carotid arteriovenous shunting by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtype. *British Journal of Pharmacology*, **99**, 36.

Villalón, C.M.; Den Boer, M.O.; Heilgers, J.P.C. and Saxena, P.R. (1990). Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. Special report. *British Journal of Pharmacology*, **100**, 665-7.

Villalón, C.M.; Heiligers, J.; Den Boer, M.O. and Saxena, P.R. (1990). Putative 5-HT_4 receptor mediates 5-HT-induced tachycardia in the anaesthetised pig. *European Journal of Pharmacology*, **183**, 1160.

Villalón, C.M.; Bom, A.H.; Heiligers, J.; Den Boer, M.O. and Saxena, P.R. (1990). The 5-HT_1-like receptor involved in the indorenate-induced reduction of porcine cephalic arteriovenous shunting seems unrelated to either 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtype. *European Journal of Pharmacology*, **190**, 167-76.

Villalón, C.M.; Den Boer, M.O.; Heiligers, J.P.C.; and Saxena, P.R. (1991). Further characterisation, using tryptamine and benzamide derivatives of the putative 5-HT_4 receptor mediating tachycardia in the pig. *British Journal of Pharmacology*, **102**, 107-12.

7 MATERIALS SCIENCES

Summary

Materials sciences is a fast-moving area in which a substantial amount of research worldwide is leading to technological applications of new materials. The subject has recently been given priority status in International Scientific Cooperation with Mexico and this chapter covers two postdoctoral fellowships, being the only actions to have been implemented so far.

Postdoctoral fellowships

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Defects in materials by conventional and high resolution electron microscopy

Fellowship period: October 1988 - September 1989

Publications

Krekels, T.; Shi, T.S.; Reyes-Gasga, J.; Van Landuyt, G. and Amelinckx, S. (1990). On the structure responsible for the 2 2AoX2 2Ao diffraction pattern in $YBa_2Ci_3O_x$. *Physica C* **167**, 677.

Reyes-Gasga, J.; Krekels, T.; Shi, T.S.; Van Tenderloo, G.; Van Landuyt, G.; Amelinckx, S.; Bruggink, W.H.M. and Verwey, H. (1989). 3D vacancy ordered superstructures in homogenous $YBa_2Cu_3O_x$. *Physica C*, **159**, 831.

Reyes-Gasga, J.; Krekels, T.; Van Tenderloo, G.; Van Landuyt, G.; Bruggink, W.H.M.; Verwey, H. and Amelinckx, S. (1989). 3D oxygen vacancy ordered superstructures in $YBa_2Cu_3O_x$ prepared by the constant stochiometry cooling method. *Solid State Communications*, **70**, 269.

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Characterisation of magnetic impedances

Fellowship period: May 1991 - April 1992

8 PHYSICAL, MATHEMATICAL AND ENGINEERING SCIENCES

Summary

Physical, mathematical and engineering sciences cover a wide range of subjects, basic and applied, and in this chapter twelve postdoctoral fellowships and one joint research project are reported. Subjects studied include computer simulations of the dynamical properties of protein and drug molecules, of physical properties of minerals, and of large industrial plant accidents; analysis of the transport in pipes of gas-liquid mixtures for geothermal applications; astronomy and space studies; lasers; and basic mathematics and physics.

35 Dynamical properties of biomolecules: proteins and drugs. Monte Carlo and molecular dynamics computer simulations.

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W.F. Van Gunsteren
Laboratory of Physical Chemistry, University of Gröningen, Nijenborgh 16, 9747AG Gröningen, The Netherlands.

Contract number and duration: CI1*/0347, January 1989 to December 1991

Background and objectives

Protein, polypeptides and nucleic acids are particularly important among the molecules essential to life. Their importance stems from the impressive diversity of their functional roles. Because of their fundamental role in living organisms, peptides and proteins as a class of molecules have been intensively studied. Their function is intimately related to their structural and dynamic properties and a variety of experimental methods have been used to study these properties. Many important questions are, however, inaccessible to these experimental methods. For example, what forces drive a peptide to adopt its particular conformation? Why does a peptide pack in the observed packing mode in a crystal? How much strain is imposed on the molecule by the crystal lattice? Are the solid state crystal conformations the most probable ones that the molecule can show in solution? Can we predict the equilibrium conformations in solution that play a predominant role in the activity of molecules? In an attempt to answer such questions, some theoretical methods have been used, including energy minimisation and molecular dynamics, to study the structural energetic, entropic and dynamic properties of peptides and proteins.

Since the internal dynamics of proteins and polypeptides, as well as other important molecules in biology, play an essential role in their function, it is fundamental to understand the origin and detailed nature of the motions that occur at the atomic level. Although the functional properties of proteins and peptides are now fairly well understood from the structural viewpoint, the molecular specificities responsible for their remarkable dynamic behaviour are still largely a matter of discussion and research. Since the actual functional proteins have evolved through gradual selection, possible malfunctional polypeptides should warn us that some specific structural or dynamic features might be of decisive importance in the control of biochemical processes. These considerations explain why the theoretical approach of protein dynamics, resting upon a description as accurate as possible of the macromolecule at the atomic level, is now accepted by most researchers in the field. Whenever comparisons have been possible between experiment and such theoretical treatments, fair agreement was achieved and the theoretical work has brought more insight than an experiment alone. The major problem in such a theory is the enormous gap between the timescale of atomic motions in the femtosecond range and the characteristic times of biochemical events, which extend from picoseconds to minutes.

In this project we emphasize the use of molecular dynamics simulations with special application to the study of the conformations and dynamical properties of polypeptides and proteins. Naturally-occurring biologically active peptides have become one of the fastest growth areas of research for future pharmacotherapeutic drugs. Among these, the neuropeptides are of special interest owing to their implications for animal and human behaviour. We initiated our studies taking a polypeptide, the Leucine-Enkephaline, which is one of the brain pentapeptides with significant morphine-like apioid activity. This system has been investigated by several research groups both experimentally and theoretically, in particular its conformational characteristics, with the aim of arriving at a structural framework that could provide the basis of its recognition by the same apioid receptor as morphine.

Due to the molecular flexibility of most biologically active peptides, the study of their structure-dynamics-function relationships is somewhat difficult. A polypeptide chain can assume an extremely large number of conformations because of the possibility of rotations about the single bonds of the backbone and side chains. We can presume that the high flexibility of the peptide has something to do with the variety of functions attributed to those enkephalins. In a theoretical analysis of the preferred conformation of a polypeptide, cognizance must be taken of the fact that the macromolecule is a dynamic object in a constant state of fluctuation because of thermal excitation, i.e. the polypeptide exists in an ensemble of conformations. To determine the thermodynamically preferred conformation, the structure with the lowest free energy must be identified, in other words the global minimum energy has to be found.

On the other hand the problem of predicting the tertiary structure of a globular protein, given the primary sequence of amino acid residues that constitute it, is one of the most important and most exciting problems in biochemistry and many attempts recently have been made to solve this problem. While a wealth of information on the three-dimensional structure of globular proteins exists, remarkably little is known about the essential factors governing the folding of proteins to their native conformation.

Optimization procedures are required for an ultimate understanding as to how interatomic interactions lead to the folded, most-stable conformation of a protein from a linear polypeptide chain. A major problem in locating the global minimum of the empirical potential function that describes the conformations of a protein arises from the existence of many local minima in the multi-dimensional energy surface: the multiple minima problem. This problem exists even for a system as small as a terminally blocked amino acid and becomes worse as the size of the system increases.

Whereas algorithms are available for minimizing a function of many variables, none exist for passing from one local minimum, over an intervening barrier, to the next local minimum - and ultimately to the global minimum - in a many-dimensional space. Several procedures have been developed to overcome this problem. The multiple minima problem is not unique to protein folding but arises in many other fields of biology, chemistry and physics whenever complexity appears.

Since it is generally accepted that enkephalin molecules can present different equilibrium conformations in solution, we decided to study those systems in a systematic way, using the Molecular Dynamics Techniques to explore the multiple wells that stabilize. For detecting the multiple minima we have used different techniques that are mentioned in the next section. The other problem we would like to explore is the effect of the solvent on the multiple minima stabilization.

One of the benefits of the collaborative research initiated with the Dutch group of H.J.C. Berendsen (and, formerly, W.F. van Gunsteren), for this project, are the facilities we had to use the latest version of GROMOS for doing multiple minima searches. In this way we can complete the minima search we initiated using CHARMM with the minima obtained with GROMOS. The other two problems in which we will collaborate with the European group are the introduction of the solvent to the study of multiple minima and the determination of the thermodynamic (free energy) change between the most important conformations of the system.

Materials and methods

Since it is possible to employ small peptides as good models for exploring general domains of local ordered structures and since there is evidence that large proteins consist of folding domains that can behave as independent structural entities we have been addressing the multiple minima problem in peptides looking for any stable conformation arising from a multidimensional energy surface with many local minima.

We have used three different techniques to overcome the multiple minima problem inherent in finding the global minimum of the pentapeptide Leu-enkephalin: Minimisation Techniques, Simulated Annealing and Molecular Dynamics Methods. We have combined the two last techniques to find multiple minima, since the simulated annealing process consists of heating the system at a very high temperature, then lowering the temperature by slow stages until the system freezes and no further changes occur. At each temperature, the simulation must proceed long enough for the system to reach a steady state.

We used two different algorithms to do the dynamics simulation, CHARMM and GROMOS in a recent version, for the minima search. The last algorithm was developed by the Dutch group, and the programme was provided to us in the framework of the collaborative research initiated last year.

Results and discussion

The pentapeptide Leu^5-Enkephalin, *Tyr*^{1} - *Gly*^{2} - *Gly*^{3} - *Phe*^{4} - *Leu*^{5} (Figure 1) has opiate properties similar to morphine and appears to bind two different δ - and μ -opiate receptors. From the study of many δ - and μ - selective analogues it has been suggested that the δ - active form should be an extended form of the peptide chain whereas a folded form should be required for μ - activity.

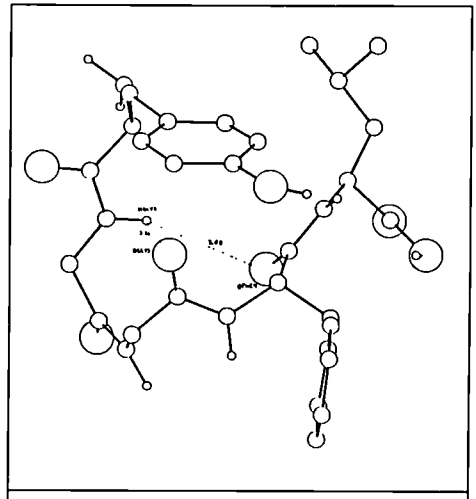

Figure 1

For exploring the different conformations of the pentapeptide we did an annealing simulation, first heating up to 1000°K and then freezing down to 300°K. At room temperature we did the molecular dynamics simulations, having as a statistical mechanical reference system a microcanonical one, with fixed total energy, volume and number of particles. We have done this for both the neutral and the zwitterion forms. The results for the zwitterion showed a folded conformation, since the net charges on the terminal residues favoured this conformation, whereas the neutral form shows different minima which we are trying to characterize according to their relative stability.

Postdoctoral fellowships

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Multiwavelength laser action in metal vapour lasers

Fellowship period: March 1991 - February 1992.

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Computer simulation studies of physical properties of minerals

Fellowship period: September 1988 - August 1989

Summary

The aim of the work was to study ionic diffusion in glassy systems. The applications are in the glass industry and to aid understanding of the transport mechanisms of electric charge in minerals and igneous rocks. Molecular dynamics was used as the principal method since it permitted us to impose extreme thermodynamic conditions on the system being studied and allowed detailed observation on a microscopic scale of phenomena that would otherwise have been impossible to observe.

The following activities were undertaken. First, implementation of a molecular dynamics simulation program. This code is totally vectorisable and exploits to a great extent the supercomputing capabilities of the Cray XMP-48, from the Atlas Supercomputer Centre in Oxfordshire, UK. The main characteristics of the program include: the calculation of two-body forces including a coulombian term, plus a repulsive exponential term. The coulombic forces are calculared using Ewald's sums; three-body interactions; temperature; pressure; order parameter; radial distribution functions; autocorrelation functions and diffusion coefficients, among other quantities.

Second, a few samples of pure SiO_2 and SiO_2 with various concentrations of Na were prepared. They are currently being used to study the structure of glassy systems and diffusion processes.

Publication

Alvarez, L.J.; Alavi, A. and Siepmann, J.I. (1989). A vectorisable algorithm for calculating three-body interactions. Submitted to *Computer Physics Communications* for the thematic issue on Molecular and Brownian Dynamics.

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Homomorphisms of vector bundles over a Riemann surface

Fellowship period: October 1989 - October 1990

Publications

Brambila Paz, L. (1991). An example of an indecomposable semistable vector bundle with non-commutative algebra of endomorphisms. *Aportaciones Matematicas, Sociedad Matematica Mexicana.*

Brambila Paz, L. Vector bundles of Type T_3 over a curve. Submitted to *Journal of Algebra.*

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Algorithms for recovery of functions of more than one variable

Fellowship period: November 1989 - October 1990

Publication

González Casanova, P. (1991). Quasi-interpolant elastic manifolds. Submitted to *Journal of Approximation Theory.*

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Study of CP violating phenomena in the context of B, K and D physics, in presence of low energy supersymmetry (SUSY)

Fellowship period: February 1991 - January 1992

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Space stations: construction, operation and potential applications

Fellowship period: April 1989 - March 1990

Summary

Neri-Vela is a Mexican astronaut and flew in the US space shuttle in 1985. During his study at ESTEC he worked on the Columbus programme which is preparing for the placing in orbit in the late 1990s of the Columbus Attached Laboratory, Columbus Free Flying Laboratory and Coloumbus Polar Platform. The Columbus programme is the European contribution to the international programme agreed between the US, Canada, Japan and Europe, known as "Freedom".

Publications

Neri-Vela, R. (1990). La Agencia Espacial Europea (The European Space Agency) *Ciencia y Desarrollo*, **XVI (91)**, 23-40.

Neri-Vela, R. (1990). La estación espacial *Freedom* y el nuevo concepto de teleciencia. (The space station *Freedom* and the new concept of remote science). *Ciencia y Desarrollo*, **XVI (92)**, 17-28.

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Simulation of large industrial plant accidents. Application to nuclear-reactor systems

Fellowship period: June 1988 - May 1989

Summary

The main objective of the work was to develop, implement and test a new numerical procedure with the following characteristics a) be more efficient than an existing, extensively tested numerical procedure, and b) be applicable to the simulation of accidents involving large industrial plant. As a result of the investigation, a numerical procedure based on a cyclic reduction method was developed and implemented in a general purpose computer code. The algorithm, which is referred to as a "chain solver", proved to be more accurate and economical than the original one, was applied successfully to the simulation of the hydrodynamics occurring in a nuclear-reactor system and fulfilled the above conditions.

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Administrative information systems in state-public enterprise relations

Fellowship period: February 1988 - January 1989

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Mathematical aspects of the quantum field theory related to holonomic models of matter interactions

Fellowship period: February 1990 - January 1991

Publications

Ebner, D.W. and Rodríguez-Romo, S. A bosonic model for relativistic spinors. Submitted to *Physical Review D.*

Ebner, D.W. and Rodríguez-Romo, S. A unified treatment of fermions and bosons. Submitted to *Physical Review Letters.*

Keller, J. and Rodríguez-Romo, S. (1990). A multivectoral Dirac equation. *Journal of Mathematical Physics*, **31(10)**, 2501.

Rodríguez-Romo, S. and Ebner, D.W. A Clifford approach to Yang-Baxter algebras. Submitted to *Physical Review D.*

Rodríguez-Romo, S. and Ebner, D.W. Quantum group symmetry of the Pauli principle in quantum superspace. Submitted to *Physical Review Letters.*

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Study of bubbles and supernova remants in Magellanic clouds

Fellowship period: November 1990 - October 1991

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Martingales and differential stochastic calculus

Fellowship period: March 1989 - February 1990

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Transport of gas-liquid mixtures in geothermal applications. The bubble flow regime in horizontal and near horizontal pipes.

Fellowship period: September 1988 - August 1989

Bubble flow in horizontal and near horizontal pipes

An experimental investigation of concurrent bubble flow in horizontal pipes was performed. Liquid and air mass velocities ranged from 2800 to 4850 and 0.08 to 2.1kg/m^2-s respectively and liquid holdups from 0.65 to 1.0 were studied. Two-ring conductance probes were used for the liquid hold-up measurement and a quick closing valve for their calibration. A conductance needle probe was employed for the bubble size and the local void measurements. The results compare well with those obtained with an optical probe. A two-needle conductance probe was used to measure the single bubble velocity. Pressure drop was also registered in order to compare the different theories in the technical literature.

Bubble growth was the criterion for the transition to slug flow. The results were compared with Barnea's and Taitel-Dukler's model and an important discrepancy was found. No substantial pipe diameter influence on bubble size was observed. A new correlation which fits the experimental data well was proposed. On the other hand the homogeneous model was found to underpredict the pressure drop but it was possible to correct it by a correction factor determined by the non-slip hold-up. This deviation was thought to be provoked by the slip between bubbles and the continuous phase which was proved to exist by the hold-up measurements.

Two-phase air-water flow in a vertical venturi meter

The objective of this work was to study the throat length influence on the pressure drop and the hold-up distribution. The experiments were carried out in a 50 mm vertical perspex pipe with air-water mixtures at low qualities (0 to 4) %, in a low pressure rig (2 bar). Liquid and gas mass velocities ranging from 400 to 1800 and 1.1618 to 14.667 kg/m^2-s respectively were studied. The results show that there is a good correlation between the quality and the hold-up in the inlet and the throat of the venturi meter. There is also a good correlation between the quality and the pressure drop multiplier. A simple modified venturi model fits the mass flow rate for a standard throat device rather well.

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F. Lehner

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The first international scientific cooperation activity with Mexico was initiated in 1986, and this volume presents a summary of all activities for which a financial commitment had been made before the end of 1990. It thus covers joint research projects, post-doctoral fellowships and workshops, in various stages of completion: some being totally finished, some in progress; and for those projects just starting, a work programme only is presented.

The objective of compiling this volume is to show what has been achieved in the framework of international scientific cooperation with Mexico. The strength of these achievements lies with their firm scientific foundation and this is reflected in the style of presentation of this volume; however, the contents are oriented towards a wide readership, to allow not only the scientists, both Mexican and European, to place their work in a wider perspective, but also to provide a concise account of the activities to other scientists, government officials, diplomatic representatives and all those interested in science in Mexico and the European Community.

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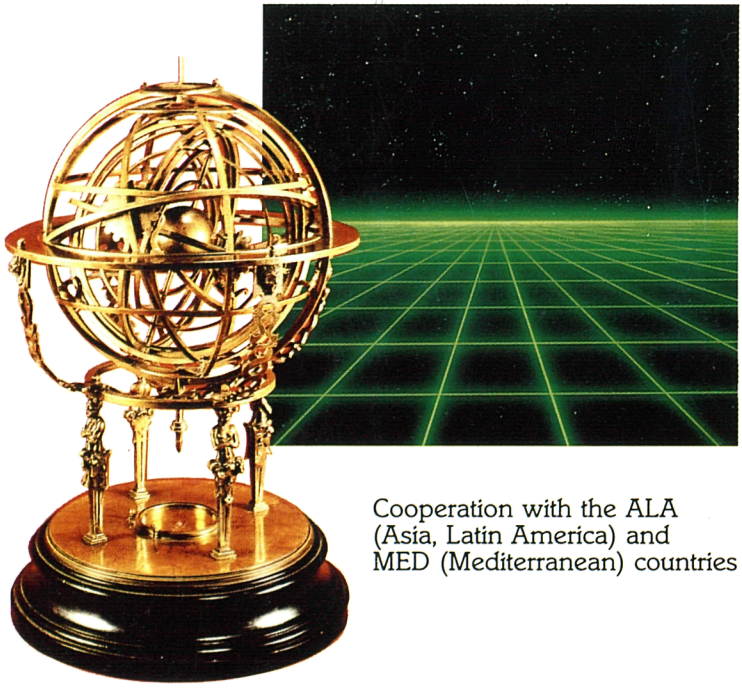

Cooperation with the ALA
(Asia, Latin America) and
MED (Mediterranean) countries

COMMISSION OF THE EUROPEAN COMMUNITIES
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