IICA

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA — OEA —

Consultant Final Report
IICA/EMBRAPA-PROCENSUL II

DETERMINATION OF THE PRIMARY STRUCTURE (AMINO ACID SEQUENCE) OF THE POLYHEDRYN PROTEIN FROM BACULOVIRUS ANTICARCIA (AgMNPV)

ESCRITÓRIO NO BRASIL

DETERMINATION OF THE PRIMARY STRUCTURE (AMINO ACID SEQUENCE) OF THE POLYHEDRYN PROTEIN FROM BACULOVIRUS ANTICARCIA (AgMNPV)

Richardson, Michael.
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APRESENTAÇÃO

A reprodução e difusão dos Relatórios de Consultores, no âmbito restrito das Diretorias das Unidades do Sistema Nacional de Pesquisa Agropecuária, vinculado à EMBRAPA, tem como objetivo principal o de divulgar as atividades desenvolvidas pelos consultores e as opiniões e recomendações geradas sobre os problemas de interesse para a pesquisa agropecuária.

As atividades de consultoria são realizadas no âmbito do Projeto de Desenvolvimento da Pesquisa Agrtopecuária e Difusão de Tecnologia na Região Centro-Sul do Brasil - PROCENSUL II, financiado parcialmente pelo Banco Interamericano de Desenvolvimento - BID e a EMBRAPA conforme os contratos de Empréstimo 139/IC-BR e 760/SF-BR, assinados em 14 de março de 1985 entre o Governo Brasileiro e o BID.

As opiniões dos consultores são inteiramente pessoais e não refletem, necessariamente, o ponto de vista do IICA ou da EMBRAPA.

A coordenação dos Contratos IICA/EMBRAPA agradeceria receber comentários sobre estes relatórios.

Horacio H. Stagno
Coordenador Contratos IICA/EMBRAPA

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
IICA/EMBRAPA CONTRACT

CONSULTANT FINAL REPORT

1. Consultant's full name: *Michael Richardson*

2. Specialist in: *Purificação de Proteinas e Sequeciamento de Residuos de Amoniácidos*

3. Title of IICA.Project: *2.SB.3.*

4. EMBRAPA Program for which consultancy is provided:

PROGRAMA : PROCENSUL II
SUBPROGARAMA : 05-RECURSOS GENÉTICOS

IICA Project Activity Code: *2.SB.3.05*	Administrative Code: *R 4864 B1B 03105*
Title of Activity of IICA Project corresponding to this consultanc/	*Cooperation with EMBRAPA on reserarch and applications of genetic resources, biotechnology and biologic control of plagues, diseases and weeds.*

CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
12 days january/1989	*CENARGEN - Brasília*
CONTRACT EXTENTION PERIOD (If any)	DUTY LOCATION (Center)

5. Financial support: *PROCENSUL II*

6. ACTIVITIES UNDERTAKEN BY THE CONSULTANT AND RESULTS

6.1 RESEARCH DONE UNDER DIRECT RESPONSIBILITY OF THE CONSULTANT

Research activities developed	Results Achieved

Determination of the primary structure (amino acid sequence) of the polyhedrin protein from Baculovirus anticarcia (AgMNPV)

The protein used was purified by the staff of CENARGEN from the multiple nuclear polyedros virus AGMNPV isolated in 1987 from the caterpillars of Anticarcia gemmatalis in a field of soybeans in Parana (Sothern Brazil) where the virus has been used as an insecticide on more than 10^6 hectares.

i) Preliminary tests (SDS-PAGE) indicated that the protein was of sufficient purity (>95%) for sequence determination

ii) Determination of the N-terminal sequence by the manual DABITC/PITC double coupling micro sequence method also confirmed that the protein was essentially pure as only one sequence was observed. The N-terminal sequence found was PRO-ASP-TYR-SER-TYR-ARG-PRO which is highly homologous with the sequence previously found for the polyhedrin protein isolated from Autographa californica (AcMNPV) and only differed in that the AgMNPV protein has a THR in the fourth position.

iii) The native polyhedrin protein was reduced and S-carboxymethylated to facilitate subsequent digestion with proteolytic enzymes and/or cleavage by chemical means. The carboxymethylated protein was found to be highly insoluble in distilled water, and only solubilized by high pH (addition of >20% pyridine).

iv) An attempt to digest the carboxymethylated polyhedrin with trypsin in the presence of 20% pyridine was unsuccessful, but the protein was hydrolyzed by this enzyme in 0.2M Tris/HCl buffer (4hr at 37°C) pH8.1).

v) Many of the tryptic peptides of the polyhedrin protein were purified by reverse phase HPLC on a column of Vydac using a prolonged (120 min) gradient of acetonitrile in 0.1% trifluoroacetic acid, and were produced in sufficient quantity and purity for subsequent sequence analysis by the DABITC method.

vi) The carboxymethylated polyhedrin was also digested with the GLU-specific protease from S. aureus V8 in 0.2M Tris/HCl buffer pH8.5 containing 2M guanidine HCl for 24hr at 37°C. Some of the resulting peptides were purified the reverse phase HPLC method described above.

vii) Analysis of the purified tryptic and V8 peptides yielded the sequence of approximately 120 amino acids (50% of the total sequence of about 247 residues). The sequence found differed in 17 positions from the known sequence of the AcMNPV polyhedrin protein.

viii) Dr. Richardson has taken back to England sufficient samples of the polyhedrin protein to enable him to complete the sequence determination in collaboration with the work which continue at CENARGEN.

6.2 SUPPORT TO RESEARCH UNDERTAKEN BY OTHER EMBRAPA RESEARCHERS

Research activities developed	Results achieved
Attempts to purify Globulin proteins from Inhame (*Colocasia* spp)	
A mixture of partially purified globulin (salt soluble) proteins was examined by the DABITC sequence method.	At least four protein sequences observed. Two major, and two minor which correspond well to the results obtained by two dimensional PAGE methods employed by CENARGEN researchers. One of the sequences appeared similar to sequence observed for albumin protein in Sept. 1987.
Ion-exchange chromatography on DEAE Trisacryl resin in presence of 6M urea	No separation obtained as proteins failed to bind to support. Results suggested that the pI's (isoelectric points) of the proteins were too close to operating pH (8.3) of column.
Suggestion that CM-Sepharose or weak cation ion-exchange HPLC column should be employed at approx pH 5-6 for separation in future (using 6M urea to maintain solubility of proteins).	

6.3 TRAINING ACTIVITIES DEVELOPED BY THE CONSULTANT

Date	Training subject matter	Type of event*	Number of beneficiaries	
			From EMBRAPA	From other institutions

NONE

* Short courses, seminars, conferences, etc.

6.4 IN-SERVICE TRAINING PROVIDED BY THE CONSULTANT

In-service training subject matter	Names of counterparts
Manual DABITC/PITC double coupling microsequencing method for proteins and peptides.	
Methods for reduction/S carboxymethylation of proteins; enzymatic digestion, reverse phase HPLC purification of peptides.	

6.5 ACTIVITIES IN SUPPORT OF RESEARCH STRATEGY AND PLANNING

Research subject matter	Research program to which subject matter is concerned
Discussions with Kjell-Ove Holmstrom and others in Molecular biology group on the possible improvement of 25 storage proteins by amino acid replacements in sequence positions indicated by homology and secondary structure predictions (computer aided). Suggested possible role of these proteins as enzyme inhibitors (or proteinases and/or α-amylases) should be investigated.	

6.6 ACTIVITIES IN SUPPORT OF OTHER CENTERS AND UNIVERSITIES IMPROVING THE RESEARCH CENTERS LINKS WITH ABROAD

Subject matter on which links were recommended	Persons, centers and universities recomended for contact
Development of automatic gas-phase protein sequencing facility at Universidade de Brasilia (Prof. Lauro Morhy, Depto. Biologia Cellular)	Suggestion that Mestrado student from this laboratory (Mr. Carlos Andre Ornellas Ricart) who has some limited experience of protein sequencing by manual methods should do his Ph.D. with Prof. C.J. Bailey, Department of Biochemistry, Trinity College, Dublin Eire, who has good experience of automatic gas-phase sequencing using the same equipment (Applied Biosystems Inc.) as proposed for Universidade Brasilia
Provision of national service for Brazil. Technical training for personnel.	
Plant Molecular Biology group at CENARGEN	Prof. Alejandro Blanco Labra, Dr. Luiz Herera Estrela, CINVESTAV Inst., Irapuato Guanuato, Mexico
Protein Chemistry group at CENARGEN	Dr. Lila Castellanos, Head of Department of Protein and Peptide Chemistry, Centro de Ingeneria Genetica y Biotecnologia, P.O. Box 6162, La Habana, Cuba.

6.7 PUBLICATIONS AND REPORTS UNDERTAKEN WITH THE CONSULTAT'S PARTICIPATION

Author(s)*	Title of publication or Report and other bibliographic identification

Amino acid sequence of Baculovirus anticarcia polyhedrin protein will be published (in FEBS Letters, or Biochim. Biophys. Acta) when it is completed. Expected date of submission April 1989.

* Personal, institutional, etc.

6.8 SUPPORT PROVIDED TO EMBRAPA RESEARCHERS IN THESIS AND DISSERTATION WORK

Name of the student	Thesis subject matter and sinthesis of advice

NONE

6.9 OTHER ACTIVITIES DEVELOPED BY THE CONSULTANT

NONE

7. OTHER NATIONAL SYSTEM CENTERS, APART FROM DUTY STATION CENTER, ASSISTED BY THE CONSULTANT

Research center	Area of assistance provided by the consultant
Fundacao Ezequiel Dias (Belo Horizonte/July 1988)	Protein purification/amino acid sequence determination
Dept. Biochemistry & Molecular Biology Universidade Federal de Ceará (Fortaleza, Jan. 1982, July 84, July 1988)	" " Training of Ph.D. students in England.
Universidade Federal do M.G. do Sul (Corumba, Sept. 1987)	Course on Plant protein inhibitors of enzymes.
Univ. Fed. R.G.N. (Natal, July 1988)	Advice on research programmes in crop improvement/protein chemistry
Universidade Fed. Brasilia (present)	Training of Ph.D. students in England.

8. CONSULTANT'S SUGGESTIONS AND TECHNICAL OR INSTITUTIONAL RECOMMENDATIONS FOR THE IMPROVEMENT OF THE RESEARCH SERVICE

The facilities/equipment available at CENARGEN for the purification and sequencing of proteins are generally satisfactory, but could be improved greatly by the provision of the following additional items.

i) Two small dry ovens (at 52° and 80°C) for the DABITC method. At present the researchers at CENARGEN are using water baths which hinder the subsequent drying in vacuo of the reactants and occasionally lead to the loss of samples inadvertently exposed to water vapour.

ii) A long column (1cm x 200cm) of Biogel P-6 for preliminary fractionation of tryptic/V8 peptides by gel filtration would greatly facilitate their subsequent purification by reverse phase HPLC.

iii) Two or three additional small 'dry-seal' vacuum dessicators would speed up the drying phases of the DABITC method. Also the single sliding top/vacuum greased dessicator used at present could constitute a safety hazard.

iv) A vacuum-line manifold with 3/4 taps/outlets attached to the vacuum pump via the liquid nitrogen trap would also increase the possible rate of sequencing and would help to protect the pump against damage caused by the reactants.

v) Some problems were encountered during the operation of the reverse phase HPLC equipments. These were caused by gas bubbles released from the solvents. This problem can be overcome by keeping all of the solvents flushed with helium during the operation.

9. AGREEMENTS OR COMMITMENTS ESTABLISHED WITH EMBRAPA RESEARCHERS IN-SERVICE OF THE FUTURE DEVELOPMENT OF RESEARCH IN THE CONSULTANT'S FIELD OF SPECIALIZATION

Dr. Richardson has agreed to continue with the sequencing of the polyhedrin protein in his laboratory at the University of Durham in England and will also assist with any other proteins of interest to CENARGEN. Dr. Richardson is also prepared to host any of the staff of CENARGEN who require further training in England, and is himself prepared to visit CENARGEN again in the future, should this be necessary. The most optimal times would be January 1990 or March 1990 when he would be available for at least three or four weeks.

10. CONSULTANT'S COMMENTS ON CIRCUMSTANCES WHICH AFFECTED THE CONSULTANCY WORK

Dr. Maria Jose Amstalden Sampaio and the other staff at CENARGEN did everything possible to make sure that my regretably short visit to the Institute was a success. The forward planning and prior preparation at CENARGEN was excellent. All of the necessary equipment and chemicals were readily availeble and no significant delays or hindrances were encountered. The hospitality received was, as always in Brazil, first class. I am most grateful to all of those involved, and to IICA I say thank you very much.

Michael Richardson

Date: 16th January 1989.

Signature

Programa II. Geração e Transferência de Tecnologia

O Programa de Geração e Transferência de Tecnologia é a resposta do IICA a dois aspectos fundamentais: (i) o reconhecimento, por parte dos países e da comunidade técnico-financeira internacional, da importância da tecnologia para o desenvolvimento produtivo do setor agropecuário; (ii) a convicção generalizada de que, para aproveitar plenamente o potencial da ciência e da tecnologia, é necessário que existam infra-estruturas institucionais capazes de desenvolver as respostas tecnológicas adequadas às condições específicas de cada país, bem como um lineamento de políticas que promova e possibilite que tais infra-estruturas sejam incorporadas aos processos produtivos.

Nesse contexto, o Programa II visa a promover e apoiar as ações dos Estados membros destinadas a aprimorar a configuração de suas políticas tecnológicas, fortalecer a organização e administração de seus sistemas de geração e transferência de tecnologia e facilitar a transferência tecnológica internacional. Desse modo será possível fazer melhor aproveitamento de todos os recursos disponíveis e uma contribuição mais eficiente e efetiva para a solução dos problemas tecnológicos da produção agropecuária, num âmbito de igualdade na distribuição dos benefícios e de conservação dos recursos naturais.

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA

O Instituto Interamericano de Cooperação para a Agricultura (IICA) é o organismo especializado em agricultura do Sistema Interamericano. Suas origens datam de 7 outubro de 1942, quando o Conselho Diretor da União Pan-Americana aprovou a criação do Instituto Interamericano de Ciências Agrícolas.

Fundado como uma instituição de pesquisa agronômica e de ensino, de pós-graduação para os trópicos, o IICA, respondendo às mudanças e novas necessidades do Hemisfério, converteu-se progressivamente em um organismo de cooperação técnica e fortalecimento institucional no campo da agropecuária. Essas transformações foram reconhecidas oficialmente com a ratificação, em 8 de dezembro de 1980, de uma nova convenção, que estabeleceu como fins do IICA estimular, promover e apoiar os laços de cooperação entre seus 31 Estados membros para a obtenção do desenvolvimento agrícola e do bem-estar rural.

Com um mandato amplo e flexível e com uma estrutura que permite a participação direta dos Estados membros na Junta Interamericana de Agricultura e em seu Comitê Executivo, o IICA conta com ampla presença geográfica em todos os países membros para responder a suas necessidades de cooperação técnica.

As contribuições dos Estados membros e as relações que o IICA mantém com 12 Países Observadores, e com vários organismos internacionais, lhe permitem canalizar importantes recursos humanos e financeiros em prol do desenvolvimento agrícola do Hemisfério.

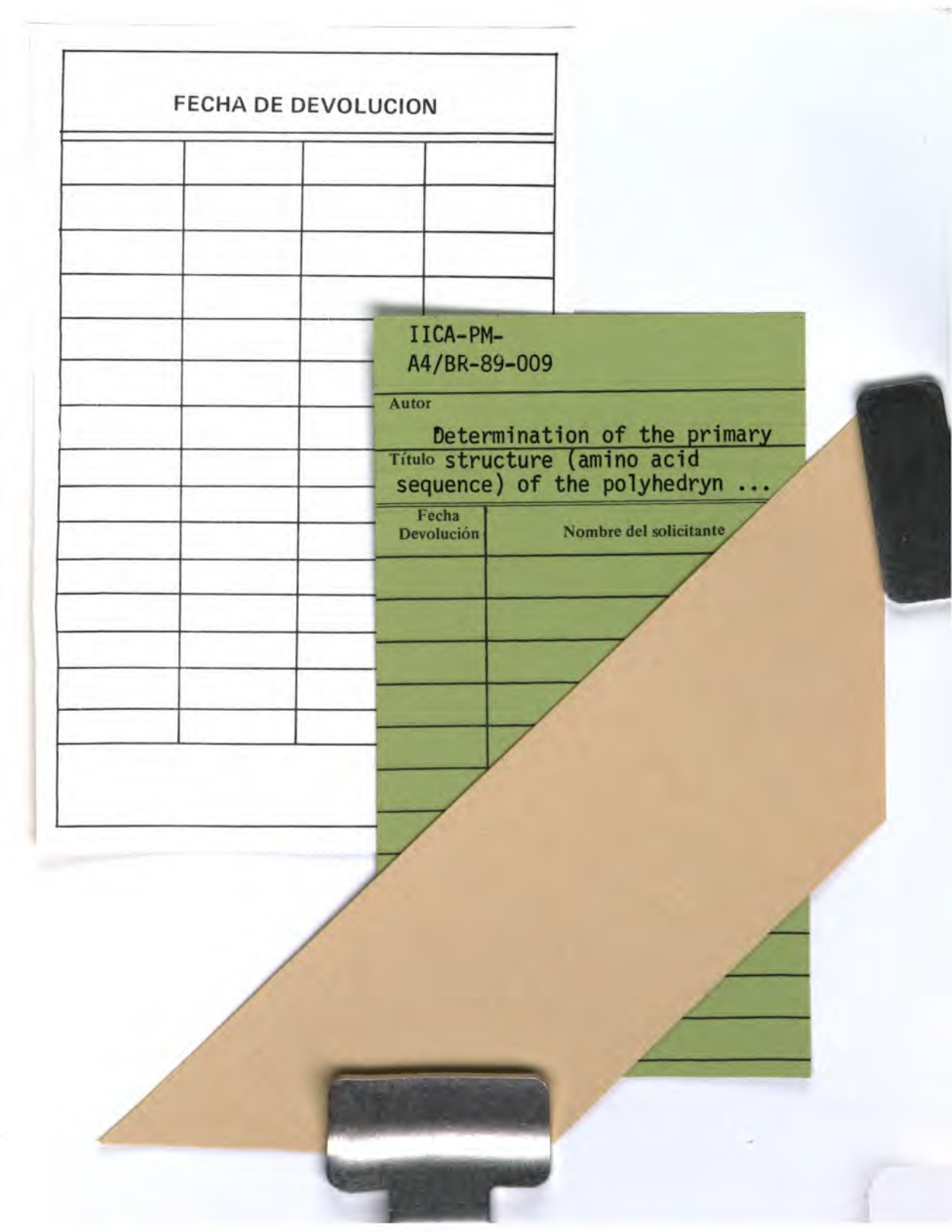
O Plano de Médio Prazo 1987-1991, documento normativo que assinala as prioridades do Instituto, enfatiza ações voltadas para a reativação do setor agropecuário como elemento central do crescimento econômico. Em vista disso, o Instituto atribui especial importância ao apoio e promoção de ações tendentes à modernização tecnológica do campo e ao fortalecimento dos processos de integração regional e sub-regional.

Para alcançar tais objetivos o IICA concentra suas atividades em cinco áreas fundamentais, a saber: Análise e Planejamento da Política Agrária; Geração e Transferência de Tecnologia; Organização e Administração para o Desenvolvimento Rural; Comercialização e Agroindústria, e Saúde Animal e Sanidade Vegetal.

Essas áreas de ação expressam, simultaneamente, as necessidades e prioridades determinadas pelos próprios Estados membros e o âmbito de trabalho em que o IICA concentra seus esforços e sua capacidade técnica, tanto sob o ponto de vista de seus recursos humanos e financeiros, como de sua relação com outros organismos internacionais.

Esta publicação foi reproduzida na Gráfica do Escritório do IICA no Brasil, em Brasília, em janeiro de 1989, numa tiragem de 100 exemplares.

Responsáveis pela reprodução: Jadir José dos Santos e Murillo Sodré da Silva.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Nombre del solicitante

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