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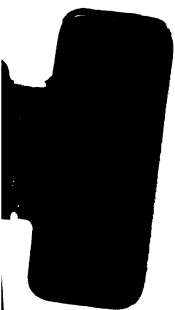


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OF Acetobacter diazotrophicus

Consultant Final Report
IICA/EMBRAPA-PROCENSUL II

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Josef Vanderleyden

Brasília, janeiro de 1989

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA
EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA

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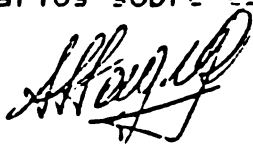
APRESENTAÇÃO

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 Agrícola 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 M. Stagno
Coordenador Contratos IICA/EMBRAPA



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

CONSULTANT FINAL REPORT

1. Consultant's full name: *Josef Vanderleyden*
2. Specialist in: *2.SB.3.*
3. Title of IICA Project:
4. EMBRAPA Program for which consultancy is provided:

PROGRAMA : *PROCENSUL II*
SUBPROGRAMA : *PESQUISA DE SOLOS-06*

IICA Project Activity Code: <i>2.SB.03.06</i>	Administrative Code: <i>R 4845 FIE 0310F</i>
Title of Activity of IICA Project corresponding to this consultancy	<i>Cooperation with EMBRAPA on research on soil management and soil analysis</i>
CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
<i>November 21st. to December 17th.</i>	<i>UAPNFBPS/EMBRAPA</i>
CONTRACT EXTENSION 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

A. Development of genetic tools for the analysis of Acetobacter diazotrophicus.

A new diazotrophic bacterium, identified as A. diazotrophicus, was recently discovered in sugarcane at UAPNPBS/EMBRAPA. Experiments were designed to develop tools for the genetic analysis of this new species, mainly a conjugation system with E. coli and a transposon (Tn5) mutagenesis system. Matings were done on solid medium, applying donor cells (E. coli) and acceptor cells (A. diazotrophicus) at different time intervals. Best results (highest frequency of transfer) were obtained when acceptor cells were allowed to grow for 4hrs on the plate before applying the E. coli cells, to make the conjugation patch. Cells were mated overnight, and subsequently put on selection medium (to counterselect donor cells and acceptor cells). Besides the successful transfer to A. diazotrophicus of a broad host range plasmid (RP-4), surprisingly we found that E. coli plasmids, which do normally not replicate outside members of the Enterobacteriaceae, stably replicate in A. diazotrophicus. At one hand, this can be exploited for future conjugation experiments, in which recombinant plasmids, based on Col E₁ replicons and carrying heterologous genes, can be transferred easily to A. diazotrophicus. On the other hand, it means that a specific suicide plasmid for delivering transposons to A. diazotrophicus has to be developed. A straight forward system to test would be based on the MU - derived suicide plasmids.

B. Analysis of Rhizobium isolates of heterologous origin that give a Fix⁺ phenotype on beans, grown at high temperature.

In February 1989, a collaborative project between UAPNPBS/EMBRAPA - the consultant's laboratory (Belgium, University of Leuven - and two other partners, funded by the EEC/STD program, will start. The project is based on the observation, made at the EMBRAPA station, that some Rhizobium strains, isolated from local legume trees (Leucaena, Lonchocarpus) are able to nodulate Phaseolus vulgaris, resulting in nitrogen fixation even at high temperature (38°C for 8 hrs a day). From a representative collection of strains, including R. phaseoli strains, experiments were started to determine the plasmid profiles through gel electrophoresis, and to isolate the extracellular polysaccharides (EPS). All strains tested produce copious amounts of EPS (precipitation from the culture supernatant with 2 volumes of ethanol) that show fluorescence with the Calcofluor dye, indicating that these exopolysaccharides have α , 1-3 or β , 1-4 linkages. Experiments are in progress to determine the resemblance (or differences) with EPS isolated and characterized from R. leguminosarum var. phaseoli strains. This might give a first indication why these heterologous strains are able to infect P. vulgaris (difference between type I and type II strains). The correlation between heat tolerance and stability /or expression of genetic information of these strains is the subject of an EEC project for the next four years.

6.2 SUPPORT TO RESEARCH UNDERTAKEN BY OTHER EMBRAPA RESEARCHERS

Research activities developed	Results achieved
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Quantification of colonization of wheat roots by Azospirillum spp.

The study of the potential of non-legume nitrogen fixing bacteria, is hampered by the fact that interaction (infection) of these bacteria with their host plants (grasses) does not result in differentiation of the plant root or root hairs. Dr. E. Boddey at EMBRAPA attempts to quantify colonization of cereal roots by a number of selected Azospirillum strains, using a method, recently developed by Anolles and Favelukes (1986). In our laboratory, we constructed mutants of Azospirillum, that are affected in exopolysaccharide synthesis. Some of these mutants were included in the experiment mentioned above. It appears that EPS - mutants of A. brasilense Sp7 attach much less to the root system of wheat, compared to the wild-type strain. This would indicate a role of Azospirillum exopolysaccharides in the interaction process, and justifies further characterization of the exopolysaccharides produced by various Azospirillum isolates. Moreover, previous studies at our laboratory indicated that genes coding for exopolysaccharides are interchangeable between Rhizobium meliloti and A. brasilense. In Rhizobium, the role of EPS for the infection process of legumes is clearly demonstrated. The biochemical analysis of EPS produced by Azospirillum was the subject of another project started in collaboration with Dr. R. Ugalde (Buenos Aires, Argentina) visiting the EMBRAPA station at the same time as the consultant's visit (see Section 6.6).

6.3 TRAINING ACTIVITIES DEVELOPED BY THE CONSULTANT

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

Date: Throughout the consultant's visiting period

Type of event: Short course

Training subject matter: - Extraction of DNA from Rhizobium strains
 - Agarose gel electrophoresis for DNA separation
 - Bacterial matings

Number of beneficiaries from EMBRAPA: 3 (directly involved)

* Short courses, seminars, conferences, etc.

6.4 IN-SERVICE TRAINING PROVIDED BY THE CONSULTANT

In-service training subject matter	Names of counterparts
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None

6.5 ACTIVITIES IN SUPPORT OF RESEARCH STRATEGY AND PLANNING

Research subject matter	Research program to which subject matter is concerned
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Research subject matter: Heat tolerance of Rhizobium inoculants for beans. Development of a strategy to determine the genetic basis of heat tolerance.

Research program: EEC-STD funded project on heat-tolerance, of Rhizobium isolates (see also 6.1.B).

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 recommended for contact
------------------------------------------------	-----------------------------------------------------------

With Dr. R. Ugalde (University of Buenos Aires), it is agreed to join efforts on the analysis on polysaccharides produced by Azospirillum. A collection of A. brasilense mutants (University of Leuven, Belgium) will be sends to Dr. R. Ugalde for biochemical analysis, with emphasis on cyclic glucans. These mutants will also made available to the EMBRAPA station for tests on performance in pot inoculation experimtns. In this way, the genetical, biochemical and microbiological aspects of Azospirillum could be joined in a three-partner project.

6.7 PUBLICATIONS AND REPORTS UNDERTAKEN WITH THE CONSULTANT'S PARTICIPATION

Author(s)*	Title of publication or Report and other bibliographic identification
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None

* Personal, institutional, etc.

6.8 SUPPORT PROVIDED TO EMBRAPA RESEARCHERS IN THESIS AND DISSERTATION WORK

Name of the student	Thesis subject matter and synthesis of advice
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Claudio de Oliveira Cunha See 6.3

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

With the high ranked expertise in soil microbiology, and given the massive collection of diazotrophic bacteria obtained throughout the years at the UAPNPBS/EMBRAPA station, it could be of great benefit to provide funding for the development of basic genetics and molecular biology at the station. This would certainly increase the impact of the highly valuable descriptive work already carried out at the station (microbiology, biochemistry, physiology). Moreover, since a great deal of the activities carried out at the station is focused on the isolation, inoculation and reisolation, and evaluation of diazotrophic bacteria, basic techniques in molecular genetics could be helpful in many other ways. For example, it has been regularly and reproducibly observed at the Station that microbial inoculants undergo changes throughout repeated rounds of inoculation and reisolation. It can be argued that microbial inoculants are subject to different stress conditions depending on soil type, microbial activities present, environmental conditions. It has been clearly demonstrated that the genome of cells exhibits plasticity, that the chromosome and (incoming) plasmids within one cell affect each other in a mutualistic way. Since at the UAPNPBS/EMBRAPA station, throughout all inoculation studies, a careful survey on soil conditions, environmental conditions, history of the planting area, is carried out, I assume that this station would be a unique resource to start analysis of genetic changes within inoculant strains, resulting from DNA transfer from homologous and heterologous host cells.

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

In accordance with suggestions made under item 8, the following is agreed:

- a. a Senior Ph.D. student of the consultant's laboratory will stay at the EMBRAPA station for a prolonged period (starting December 22nd, 1988).
- b. for the future, an arrangement will be sought between EMBRAPA and the University of Leuven (Belgium), for students to have their Ph.D. training at the University of Leuven.

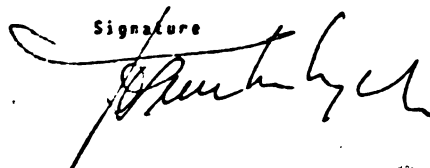
10. CONSULTANT'S COMMENTS ON CIRCUMSTANCES WHICH AFFECTED THE CONSULTANCY WORK

Circumstances for consultancy work were very favorable, despite some minor problems with equipment.

Date:

December 16, 1988

Signature

A handwritten signature in dark ink, appearing to read "G. R. ...", written over a horizontal line.

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 agrônômica 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.

