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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

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Collaborative Research Programme

TERMINAL EVALUATION REPORT

UNIDO contract # 92/057

ICGEB ref. #: CRP/BRA 91-02

Project initiation: June 1992

Project termination: June 1994

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TERMINAL EVALUATION REPORT

Part 1

Title of Project	
RFLP Analysis of Entomopathogenic Fungi	
Keywords: Paecilomyces sp; Metarhizium sp; entomopathogenic fungi; molecular markers	
UNIDO contract # 92/057	ICGEB ref. #: CRP/ BRA 91-02
Project initiation: June 1992	Project termination: June 1994
Principal Investigator's name: Myrian S. Tigano Milani	
Affiliate Centre mail address : CENARGEN/EMBRAPA	
Telephone no. (061) 274.2717	Telex no.
Fax no. (061) 274.3212	Email address myrian@cenargen.embrapa.br

Abstract:

The entomopathogenic fungi from the genera Metarhizium and Paecilomyces have great potential as biological control agents of crop pests. However, little is known about the extension of genetic variation among strains within the morphologically defined species. Arbitrarily primed PCR and PCR with tRNA consensus primers were used to analyze variability among 27 P. fumosoroseus isolates, 15 of which came from the same host, Bemisia tabaci, 9 previously unidentified Paecilomyces isolates and 29 P. lilacinus isolates, 21 of which originated from soil of differents regions of Brasil. Phenetic and cladistic analysis were used to analyse respectively arbitrarily primed PCR and PCR with consensus tRNA gen primers. Both techniques were powerfuls tool for molecular characterization of Paecilomyces isolates at different taxonomic levels. Regarding fungi from the genera Metarhizium, arbitrarily primed PCR was applied to analyze the genetic relationships among 33 isolates of M. anisopliae, 2 isolates of M. flavoviride and 1 unidentified Metarhizium.

OBJECTIVES/METHODOLOGY
(proposed at the time of the submission of the research proposal)

The main goal of this project was to obtain molecular markers for fingerprinting Metarhizium and Paecilomyces strains used in field tests and evaluate the populational genetic variability of these fungi using the following models:

- P. fumosoroseus strains isolated from whitefly geographical populations;
- P. lilacinus strains isolated from nematodes and soil in different Brasilian regions;
- M. anisopliae strains isolated from homopteran in Brazil;
- M. anisopliae strains isolated from scarabaeidae in different countries.

To attain these objectives P. fumosoroseus strains, P. lilacinus strains, and M. anisopliae strains were selected from CENARGEN and USDA (USA) collections.

The initial proposal of this project was to use RFLP markers, but we proposed laler (in the first report) the use of another technique to analyze the strains, arbitrarily primed PCR, also known as random amplified polymorphism DNA (RAPD).

The use of RFLPs for studies of germoplasm organization has been limited by the expense and difficult of data collection. Recently, scientists have begun to used RAPD markers as a tool for characterizing germoplasm. Because the data can be generated efficiently and inexpensively, this technique can be applied routinely to specific germoplasm questions. We proposed for this project the use of arbitrarity primed PCR characters markers to estimate the genetic relationship among individuals in the mentioned populations of entomopathogenic fungi.

Another additional technique, PCR using consensus tRNA gene primers (tRNA-PCR) was used in order to investigate the phytogenetic validity of the phenetic groups revealed by analysis of arbitrarity primed PCR characters.

RESULTS
(compare against the set objectives)

1. Studies with Paecilomyces fumosoroseus

- 322 arbitrarity primed PCR characters and 107(53 informative) tRNA-PCR characters revealed polymorphism withim isolates morphologi cally classified as P. fumosorosues;
- Three distinct groups were observed from phenetic and multivariate analysis of arbitrarity primed PCR characters: Two of these groups formed a monophyletic groups in a cladistic anaylsis using products amplified by tRNA consensus primers. Isolates from B. tabaci were represented in 2 of the 3 groups, and different genotypes were identified even when they were isolated from an epizootic population.
- The classification of some morphologically unidentiified isolates was clasified.

2. Studies with P. lilacinus

- 293 arbitrarity primed PCR characters and 112 tRNA-PCR characters revealed polymorphism within P. lilacinus isolates analyzed;
- No clear phenetic groups were observed in cluster or multivariate analysis of the arbitrarily primer PCR data;
- Phylogenetic hypothesis based on tRNA characters failed to find monophyletic groupings for a P. lilacinus isolates;

3. Studies with Metarhizium anisopliae

- 236 scorable binary characters were obtained by fingeprinting with 12 oligonucleotide primers. Amplified fragments ranged from 21 kb to 170 bp;
- One closely related group was observed, including all the strains isolated from spittle bugs in Brazil,
- Arbitrarily primed PCR moved to be a powerful technique to clarify the identification of the species, and to analyze the variability of endemic populations of Metarhizium.

For move details the manuscripts of the three papers originated from this project (see publications) will be sent to ICGEB, as soon as they are approved by the journals.

Work plan and time schedule (originally envisaged)

1) Definition of strains to be analysed based ont host range, place of origin and pathogenicity;

2) Evaluate and optimize methods of DNA extraction specific for each species and/or strain;

3) Construction of a genomic and/or cDNA libraries to be used as sources of DNA probes for the RFLPanalysis;

4) Use the probes described above, as well as others (isolated genes, ribosomal DNA probes, heterologous probes) provided by other institucions. For the detection of polymorphisms between the species adn strains;

5) Select the probes which best characterize the different species and strains. Try to corretale RFLP patterns with characteristics of interest to biological control.

Time schedule

The work elements numbered below refer to those in the work plan

work elements	months 0	3	6	9	12	15	18	21	24
1)	———								
2)	———								
3)		———							
4)		———							
5)		———							

Work plan and time schedule (actual)

Project landmarks, duration of individual tasks (use bar charts); evaluation criteria (publications, patents, services, training)

The work plan and the time scredule were adapted to the new techniques used in the project.

Work plan

1) Definition of strains to be analysed, and growing cultures;
2) DNA extraction;
3) Arbitrarily primed PCR;
4) PCR using consensus tRNA genes primers (tRNA-PCR)
5) Preparation of publications

Time schedule

Work elements

Months

0 3 6 9 12 15 18 21 24

1) ———————
2) ———————————
3) ———————————
4) ———————————
5) ———————

NETWORKING

Scientific collaboration in this project was perfomed with:

Dr. Bruno W.S. Sobral - CIBR, La Jolla, USA

Dr. Rhonda J. Honeycutt - CIBR, La Jolla, USA

Dr. Corby Kistler - University of Florida, Gainesville, USA

Dr. James Maruniak - University of Florida, Gainesville, USA

Dr. Donald Roberts - Boyce Thompson Institute, Ithaca, USA

Dr. Lawrence A. Lacey - USDA, Montpellier, France

PUBLICATIONS

Tigano-Milani, M.S., Honeycutt, R.J., Lacey, L.A., Assis, R., McClelland, M., and Sobral B.W.S. Genetic variability of Paecilomyces fumosoroseus isolates revealed by molecular markers. Submitted to Journal of Invertebrate Pathology.

Tigano-Milani, M.S., Martins, I., and Sobral, B.W.S. DNA markers for differentiating isolates of Paecilomyces lilacinus. Submitted to Journal of General Microbiology.

Tigano-Milani, M.S., Gomes, A.C.M.M. and Sobral, B.W.S. Genetic variability of Brazilian isolates of the entomopathogenic fungus Metarhizium anisopliae. Submitted to Journal of Invertebrate Pathology.