AMAZONIA NETWORK

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# Administrative and Scientific Report

CONTRACT INCO-CT-2004-505250

Specific Support Action

(15.02. – 15.08. 2004)

## *Bioprospecting of Amazonian Microorganisms and Plant Secondary Metabolites*

MICRODIV 2

Workshop and Practical Course
at the
Universidad Autonoma Gabriel Rene Moreno
Santa Cruz, Bolivia
15th March – 2nd April 2004

Co-ordination
Guenther Ellersdorfer
Director, Amazonia Network

Regarding to the contract between the Commission of the European communities and the principal contractor of the project, Amazonia Network (ANW), the co-ordinator reports hereby:

**(1) The administrative and organisational activities for preparation and realisation of the project. Inauguration**

**(2) The educational and scientific activity: A three weeks practical course**

**(3) Two one day workshops: International high level scientists and representatives of national governmental organisations**

**(4) Report on the results of the practical course activities (see also CD-Rom)**

**(5) Capacity building impact of the project**

**(6) Sponsoring activities, additional funding**

## (1) Administrative and organizational activities

Guenther Ellersdorfer (ANW), Birgit Zehetmayer (ANW) arrived on march the 6th in Santa Cruz. General co-ordination and last preparations for the event were carried out until the 14th march together with the responsible CIMAR administrative officers, Antonio Gonzales and Eduardo Hinojosa.

On March the 13th arrived the three course instructors: Mauricio Marin Montoya, coming from Medellin, Colombia; Elisabeth Kaltenegger, coming from Vienna, Austria and Alicia Caceres, coming from Caracas, Venezuela.

Because of the excellent work of the persons responsible for the local preparation and organisation of the Santa Cruz Workshop and course, (principal contractor Nr.2 (Centro de Investigacion y Manejo de Recursos Naturales Renovables, CIMAR) it was possible to perform all the following listed activities.

General plan of the whole Project activities in Bolivia

| | Monday 15th March | Tuesday 16th March | Wednesday 17th March | Thursday 18th March | Friday 19th March |
|---|---|---|---|---|---|
| **08:30am to 10:30am** | Inauguration Act of the Course | Practical Course lectures | Field Trip: Excursion for microbiological samples and plant collection from different environmental sites | Lecture: Convention of Biological Diversity (CBD) and the 7th Conference of the Parties in Kuala Lumpur, Malaysia | Practical Course lectures |
| **11:00am to 12:00am** | Official representatives of the Bolivian Academic Institutions | Practical Course lectures | Field Trip | Preparation of field samples, dilution series, incubation of masterplates | Practicial Course lectures |
| **14:00am to 15:30am** | Talks on the theoretical background of the practical course | preparation of solutions, buffer and media necessary for experiments | Field Trip | Preparation of field samples, dilution series, incubation of masterplates | Laboratory Course Session |
| **16.00am to 18.30am** | Discussion | See above | Field Trip | Discussion | Laboratory Course Session |

| | Monday 22nd March | Tuesday 23rd March | Wednesday 24th March | Thursday 25th March | Friday 26th March |
|---|---|---|---|---|---|
| **08:30am to 10:30am** | Plenary lectures of international / national experts | Practical Course lectures | Practical Course lectures | Lecture on the Cartagena Protocol on Biosafety | Practical Course lectures |
| **11:00am to 12:00am** | Plenary lectures of international / national experts | Practical Course lectures | Practical Course lectures | Preparation of axenic cultures and plant extracts | Practical Course lectures |
| **14:00am to 15:30am** | Plenary lectures of international / national experts | Preparation of axenic cultures and plant extracts | Preparation of axenic cultures and plant extracts | Cryoconservation of isolates DNA-isolation | Cryoconservation of isolates DNA-isolation |
| **16.00am to 17.00am** | Plenary lectures of international / national experts | Preparation of axenic cultures and plant extracts | Preparation of axenic cultures and plant extracts | Cryoconservation of isolates DNA-isolation | Cryoconservation of isolates DNA-isolation |
| | **Monday 29th March** | **Tuesday 30th March** | **Wednesday 31st March** | **Thursday 1st April** | **Friday 2nd April** |
| **08:30am to 10:30am** | Plenary lectures of international / national experts | Practical Course lectures | Practical Course lectures | Lecture on the Guadalajara Declaration of the presidential conference ALCUE (bi-regional S&T Cooperation with Latin America and Caribbean), | Examination |
| **11:00am to 12:00am** | Plenary lectures of international / national experts | Molecular biological characterization of isolates | Chemical characterization of plant extracts | Bio assays on fungicide and insecticide activities | Roundtable discussion of proceedings |
| **14:00am to 15:30am** | Plenary lectures of international / national experts | Molecular biological characterization of isolates | Chemical characterization of plant extracts | Phylogenetic analysis of microbial isolates | Emission of course certificates for the students from UAGRM |
| **16.00am to 17.00am** | Plenary lectures of international / national experts | Molecular biological characterization of isolates | Chemical characterization of plant extracts | Phylogenetic analysis of microbial isolates | Closing Act |

Inauguration, Monday the 15th march 2004, at the convention centre of the hotel Cortez in Santa Cruz.

| Time | Speaker |
|---|---|
| 08.00-09.00 | Registration |
| 09.00-09.30 | Inauguration of the event by Dr. Julio Salek Mery, Rector of the Universidad Autonoma Gabriel Rene Moreno |
| 09.30-10.00 | Refreshment |
| 10.00-10.30 | Lic. Maria Rita de Marcos, Direccion General de Biodiversidad, Ministerio de Desarrollo Sostenible y Planificacion, La Paz |
| 10.30-11.00 | Mag. Guenther Ellersdorfer, General Co-ordinator, Amazonia Network |
| 11.00-11.30 | Ing. MSc. Antonio Gonzales, Local Co-ordinator, Centro de Investigacion y Manejo de Recursos Naturales Renovables<br>Universidad Autonoma Gabriel Rene Moreno |
| 11.30-12.00 | Mag. Birgit Zehetmayer, Administrative Co-ordinator, Amazonia Network |
| 12.00-14.30 | Dinner |
| 14.30-18.30 | Presentation of the theoretical lectures on the course contents<br>Mag. Guenther Ellersdorfer, Dr. Mauricio Marin Montoya, Dr. Alicia Caceres, Mag. Elisabeth Kaltenegger, Mag. Birgit Zehetmayer |

## (2) The educational and scientific activity: A three weeks practical course

The Practical Course "Bioprospecting of Amazonian Microorganisms and Plant Secondary Metabolites" took place at the teaching facilities of "El Vallecito", located about 15 km south of Santa Cruz, belonging to the Facultad Ciencias Agricolas, of the Universidad Autónoma Gabriel René Moreno. The practical course was held from 15th of March – 2nd of April 2004 and focused on the following general objectives:

- transfer skilled and practical know-how in basic biotechnological research (microbiological, chemical and molecular biological methods), and bioprospecting strategies in the search for microorganisms and plant secondary metabolites.
- initiate an awareness rising process of the sensitive ethical questions according to the transformation of Amazonian biodiversity into economic value. Discuss the national Bolivian policy and the international mechanisms that regulate the access to genetic resources and the sharing of the benefits arising from their use (ABS), as postulated by the Convention on Biological Diversity (CBD).
- bring together high level experts from Europe and Pan-Amazonia with the aim to form new local capacities in bioprospecting in Bolivia and to establish a sustainable network of scientific cooperation **between Latin America and the European Community** on an equal and, through the exchange of ideas and information, benefit-sharing basis.

The three weeks practical course was structured as follows

**Opening Session**
Theoretical lectures covering the background and methodological tools to exploit microbial and plant biodiversity.

**Practical Course**
Training of fundamental methods for screening, isolation, identification, characterization and conservation of bacterial and fungal species, obtained from different environmental sampling sites.
Training how to design and conduct screening programs, for a variety of biotechnologically important microorganisms.
Training to isolate, fractionize and characterize secondary metabolites from different plants.

The practical course was devided into the following five units:

| UNIT 1 | |
|---|---|
| **ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF BACTERIA FROM ENVIRONMENTAL SAMPLING SITES & SCREENING FOR PLANT GROWTH PROMOTING ACTIVITIES** | |
| Guenther Ellersdorfer<br>*Amazonia Network,*<br>*Vienna Austria* |  |

| UNIT 2 | |
|---|---|
| **MOLECULAR APPROACHES FOR THE CHARACTERIZATION OF PHYTOPATHOGENIC FUNGI** | |
| Mauricio Marin Montoya<br>*Universidad Nacional de Colombia, Sede Medellin, Medellin, Colombia* |  |

| UNIT 3 | |
|---|---|
| **CHARACTERIZATION OF MYCORRHIZA** | |
| Alicia Caceres<br>*Instituto Biologia Experimental (IBE), Universidad Central de Venezuela, Caracas, Venezuela* |  |

| UNIT 4 | |
|---|---|
| **COMPARATIVE AND ECOLOGICAL PHYTOCHEMISTRY & INSECTICIDAL AND ANTIFUNGAL BIOASSAYS** | |
| Elisabeth Kaltenegger<br>*Department of Comparative and Ecological Phytochemistry (DCEP), University of Vienna, Vienna, Austria* |  |

| UNIT 5 | |
|---|---|
| **LEGAL AND SOCIO-ECONOMIC ASPECTS OF BIOTECHNOLOGY IN LATIN AMERICA** | |
| Birgit Zehetmayer<br>*Ludwig Boltzmann Institute for Contemporary Research on Latin America (LBICRLA), Vienna, Austria* |  |

**Closing Session**
Discussion of course results, examinations and emission of certificates.

**(3) Two one day workshops:**

International high level scientists, and representatives from national governmental organisations were invited on the costs of the project to give talks on thematic fields related to the topics of biodiversity and bioprospecting.

1. **Workshop in microbiology on Monday the 22nd march 2004, at the convention centre of the hotel Cortez in Santa Cruz.**

| Time | Speaker, talk title | Country |
|---|---|---|
| Morning | Chairman: Ing. MSc. Antonio Gonzales | |
| 08.30-09.10 | Prof. Dr. Mike Wingfield, Food and Agriculture Biotechnology Institute, University of Pretoria<br>Thema: Fungal tree pathogens increasingly threatening forests and plantations:lessons from Cryphonectria | Pretoria<br>South Africa |
| 09.10-09.50 | Dr. Godofredo Vitti, Luis de Quiroz de Pirasicaba<br>Thema: Microbial diversity of the soil | Sao Paulo<br>Brasilia |
| 09.50-10.30 | Mag. Guenther Ellersdorfer, Amazonia Network<br>Bioprospection strategies and the need for identification | Vienna<br>Austria |
| 10.50-11.30 | Dr. James Robeson, Pontifica Universidad Catolica de Valparaiso<br>Thema: Invertebrate – microorganism interaction in the soil | Valparaiso<br>Chile |
| 11.30-12.10 | Dr. Volga Iniguez, Institute of Molecular Biology, Universidade Major de San Andres | La Paz<br>Bolivia |

| | | |
|---|---|---|
| | Thema: Microbiology in Bolivia | |
| Afternoon | Chairman:Mag. Guenther Ellersdorfer | |
| 14.30-15.10 | Dr. Mauricio Marin Montoja, Institute for plant pathology, Universidad Nacional de Colombia, Sede Medellin<br>Thema: Melecular methods for the identification of plant pathogens | Medellin Colombia |
| 15.10-15.50 | Dr. Gabriel Badillia, Institue of Genetics and Biotechnology, University of Sao Paulo<br>Thema: Secondary metabolites of Streptomycetes | Sao Paulo Brasil |
| 15.50-16.30 | Ing. Carlos Rivadenera, Facultad de Ciencias Agricolas, Universidad Autonoma Gabriel Rene Moreno<br>Thema: Phytopathology in Bolivia | Santa Cruz Bolivia |
| 16.50-17.30 | Dr. Alicia Caceres, Instituto de Biologia Molecular Universidad Central de Venezuela<br>Thema: Applications of Mycorrhiza Technology | Caracas Venezuela |
| 17.30-18.10 | Lic. Miguel Angel Crespo, PROBIOMA<br>Thema: Uso de Microorganismos en el control de plagas | Santa Cruz Bolivia |

**2. Workshop in phytochemistry on Monday the 29th march 2004, at the convention centre of the hotel Cortez in Santa Cruz.**

| Time | Speaker, talk title | Country |
|---|---|---|
| Morning | Chairman: Ing. MSc. Felix Torrico | |
| 08.30-09.10 | Lic. Rosa Aminta Menendez C. Ministerio de Salud Publica<br>Thema: Investigacion de Plantas de Uso Medicinal | Havanna Cuba |
| 09.10-09.50 | Lic. Veronica Helguero, Ministerio de Desarrollo Sostenible<br>Thema: Sistema de informacion CHM | La Paz Bolivia |
| 09.50-10.30 | Mag. Elisabeth Kaltenegger, Institute for Comparative and ecological Phytochemistry. University of Vienna<br><br>Thema: Insectizide activity of Stemona sp. Secondary metabolites | Vienna Austria |
| 10.50-11.30 | Dr. Stephan Beck, Herbario Nacional de Bolivia, Universidade Major de San Andres<br>Thema: Aspectos Fitoquimicos y de Taxonimia Vegetal | La Paz Bolivia |
| 11.30-12.10 | Ing. MSc. Mario Salidas, Museo Noel Kempff Mercado, Universidad Autonoma Gabriel Rene Moreno<br>Thema: Especies vegetales utiles en Santa Cruz | Santa Cruz Bolivia |
| Afternoon | Chairman: Ing. MSc. Eduardo Hinojosa | |
| 14.30-15.10 | Prof. Dr. Harald Greger, Institute for Comparative and ecological Phytochemistry. University of Vienna<br>Thema: The Diversity of Secondary Plant Metabolites as a Source for the Development of new Ecologically harmless Pesticides | Vienna Austria |
| 15.10-15.50 | Dr. Alberto Gimenez, Instituto de Investigaciones Farmaco Bioquimico, Universidade Major de San Andres<br>Thema: Experiencias locales en Fitoquimica | La Paz Bolivia |
| 15.50-16.30 | Ing. MSc. Felix Torrico, Instituto de Fitoquimica, Universidad Autonoma Gabriel Rene Moreno<br>Thema: Evaluacion de la actividad citotoxica/citostatica, antibacteriana e identificacion cualitativa de metabolitos secundarios en plantas de San Ignacio de Velasco | Santa Cruz Bolivia |
| 16.50-17.30 | Sr. Francisco Mamata, SOBOMETRA<br>Thema: Conocimiento tradicional de medicina natural de la Chiquitania | San Ignacio Bolivia |
| 17.30-18.10 | Dr. Joshua Tewksbury, University of Washington<br>Thema: Why is chilly hot? | Washington USA |

## (4) Report on the results of the practical course activities of each unit

UNIT 1

**ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF BACTERIA FROM ENVIRONMENTAL SAMPLING SITES & SCREENING FOR PLANT GROWTH PROMOTING ACTIVITIES**

Guenther Ellersdorfer
*Amazonia Network, Vienna Austria*

Samples were taken at the Jardin Botanico, Santa Cruz on 17th March 2004, during the MICRODIV2 practical course.
The samples were brought immediately to the laboratory and dilution series of ~1g sample in 0.9% saline with 0.1% tween were made. 100 µl of each dilution step were plated with a Drigalski spatula on the following media: TSA (general purose rich medium), R2A (low nutrient oligotrophic medium) Kings Agar B (enrichment of fluorescent Pseudomonads) and Czapek-Dox Agar (general purpose fungi medium)

Table 1

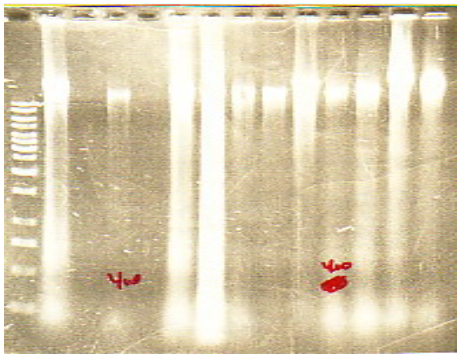
| Sample N° | Sample description | Isolates obtained |
|---|---|---|
| 01 | Soil, 0-10 cm profile under the tree Hura crepitans L. (Euphorbiaceae) - Ochoó, 8m away from the tree trunk (border of the canopy), investigation together with the mycorrhiza sample | 1 |
| 02 | Soil, 10-20 cm profile under the tree Hura crepitans L. (Euphorbiaceae) - Ochoó, 8m away from the tree trunk (border of the canopy), investigation together with the mycrrhiza sample | 6 |
| 03 | Soil, 0-10 cm profile under the tree *Anadenanthera macrocarpa* Benth. (Mimosaceae) - Curupaú (juvenile) | 4 |
| 04 | Soil, 0-10 cm profile under the tree *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | 16 |
| 05 | Soil surface layer with degraded leafs under the tree *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | 2 |
| 06 | Cortex scrapped from the tree *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | 6 |
| 07 | Leafs of *Nicotiana glutinosa* L. (Solanaceae) – Tabacachí. | - |
| 08 | Cortex scrapped from the tree *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | 1 |
| 09 | Cortex scrapped from the tree *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | 8 |
| 10 | Cortex scrapped from the tree *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | 14 |
| 11 | Water and degraded plant material from a small cave in the tree filled with water from the tree *Anadenanthera macrocarpa* Benth. (Mimosaceae) - Curupaú | 3 |
| 12 | Root of an epiphytic plant (Bromeliaceae) | 3 |
| 13 | Soil, 0-10 cm profile under the tree *Mormordica charantia* L. (Cucurbitaceae) - Balsamina. | 4 |
| 14 | Soil, 0-10 cm profile under the tree Papaya | 5 |
| 15 | Soil near the tree Sésaruo (location El Vallecito) | 5 |
| 16 | Root of an epiphytic plant (Araceae) | - |
| 17 | Soil 0-10 cm profile close to the trunk under the tree Caupí (location El Vallecito) Phytopathological and mycorrhiza investigations | 1 |
| 18 | - | 3 |
| 19 | Root and soil of the plant *Nicotiana glutinosa* L. (Solanaceae) – Tabacachí. | 11 |

| | | |
|---|---|---|
| 20 | Root and soil of Pica-pica | - |
| 21 | Soil near the tree Caupí (location El Vallecito) | - |
| 22 | | - |
| 23 | - | - |
| 24 | Soil near the tree Rhedia sp. (location El Vallecito) | - |
| 25 | - | - |
| 26 | - | - |
| 27 | Leaf sample of *Parthenium hysterophorus* L. (Asteraceae) – Chupuruchume, medical plant (secondary metabolites of plant as C-source for bacteria [red] …) | 4 |
| 28 | Soil 10-20 cm profile under a Lemon tree (location El Vallecito) | 4 |
| 29 | Root and soil of a not identified tree | 1 |
| 30 | Root and soil of Acautacea sp. | - |
| 31 | Root and soil of a not identified tree | 5 |
| 32 | Root of Leche-leche | - |
| 33 | Root and soil of the tre Toco –toco (Bignoniaceae) | 2 |
| 34 | Root of the water plant Tarope | 2 |
| 35 | Cortex of Espino blanco (Araceae) | - |
| 36 | Cortex of Conio-conio | - |
| 37 | Root and soil of a Cucurbitaceae sp. | 5 |
| 38 | Root of and Epiphytic plant on a tree of the family Lauraceae | 2 |
| Total Number of pure isolates | | 118 |

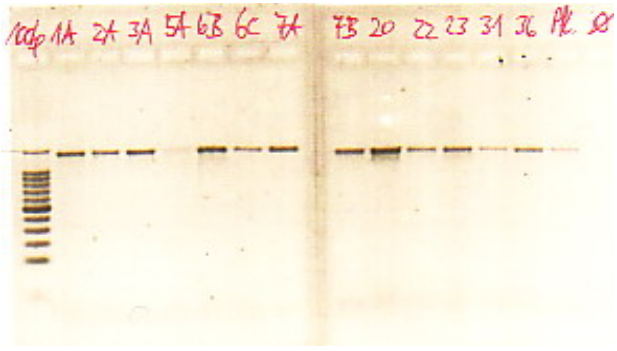
The pure isolates were obtained by sequential streaking of well isolated colonies from the masterplates onto new plates. Some isolates were selected to demonstrate microscopy to describe the cell morphology, others were selected to perform API CH50 and API 20NE tests to obtain a physiological profile of the strains.
From other strains a total DNA extraction was performed by using conventional methods and kit based methods (Qiagen and Promega). The isolated DNA was used to perform a PCR reaction by using the general eubacterial primer 27f and 1369rev to amplify a 1400bp long stretch of the 16S rDNA. A gel electrophoresis was performed to verify the PCR reaction.

Figure 1

a) Isolation of total genomic DNA of selected bacterial strains



b.) PCR products of 16S rDNA, obtained from selected bacterial strains




The 118 newly isolated bacterial strain were cryoconserved in 20% Glycerol and each one copy was sent to the culture collection of the Instituto de Investigaciones Farmaco-Bioquimicas (IIFB), located at the Universidad Mayor de San Andrés in La Paz, under the responsibility of Dr. Alberto Giménez, Director of the IIFB. The complete list of conserved bacterial strains is shown in figure 4.

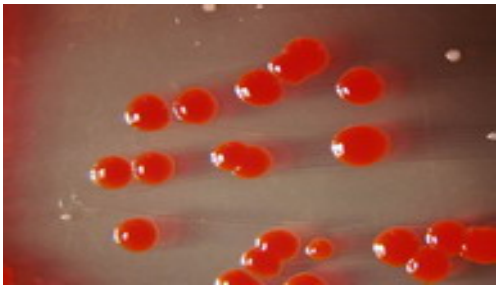
| Figure 2: Unidentified isolate from the rhizosphere of *Mormordica charantia* L. (Cucurbitaceae) – Balsamina, on nitrogen free isolation medium | Figure 3: Unidentified isolate from a rhizosphere sample of *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo |
|---|---|
|  |  |

Figure 4: List of isolates obtained during MICRODIV2

**Lista completada para la verificación de material biológico**

**Microdiv 2**

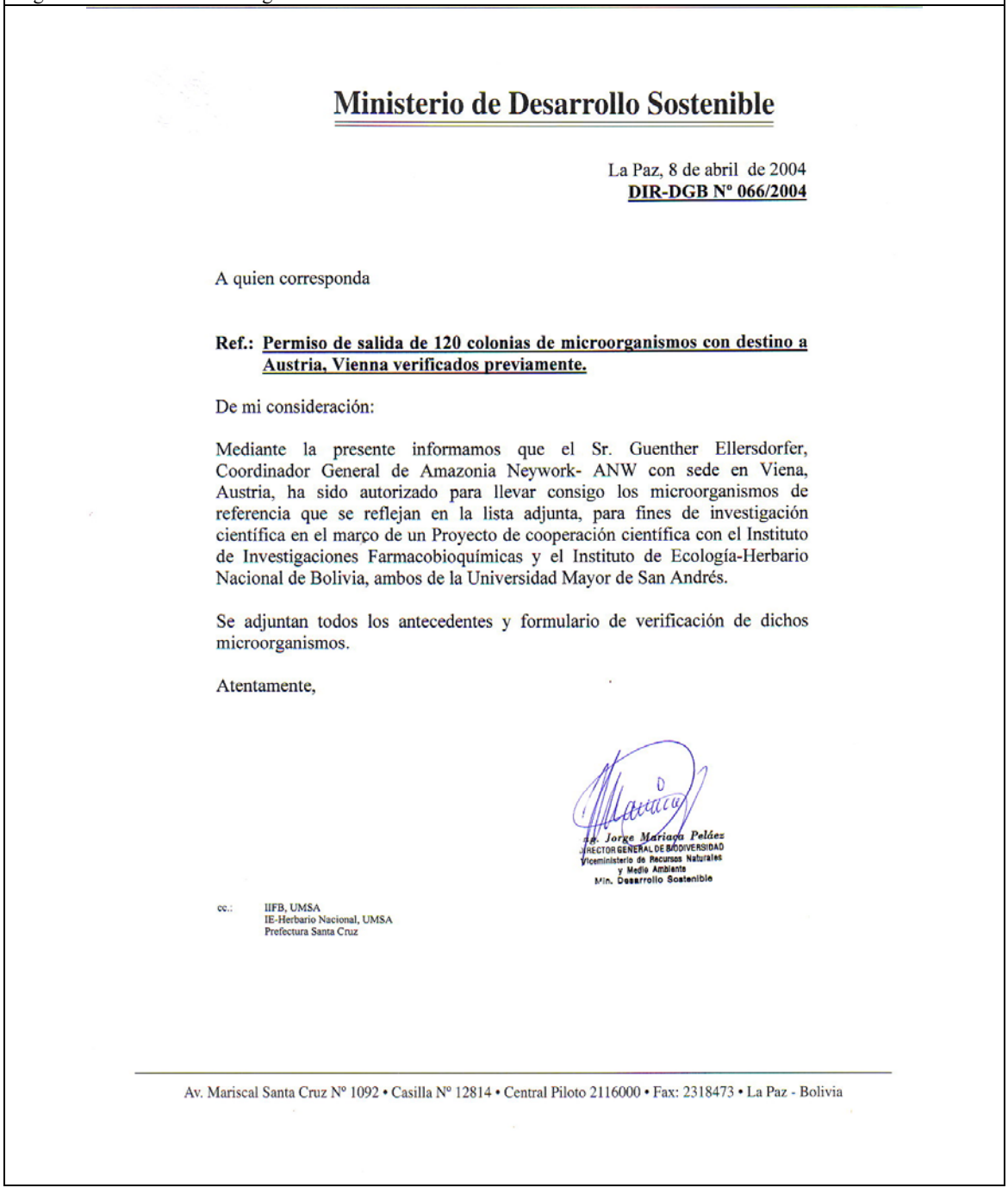
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** | BO1/4 | **31.** | BO6/2 | **61.** | BO11/7 | **91.** | BO19/11 |
| **2.** | BO2/9 | **32.** | BO6/3 | **62.** | BO12/1 | **92.** | BO19/12 |
| **3.** | BO2/11 | **33.** | BO6/5 | **63.** | BO12/4 | **93.** | BO19/14 |
| **4.** | BO2/12 | **34.** | BO6/6 | **64.** | BO12/10 | **94.** | BO27/1a |
| **5.** | BO2/14 | **35.** | BO6/7 | **65.** | BO13/1 | **95.** | BO27/2 |
| **6.** | BO2/15 | **36.** | BO8/5 | **66.** | BO13/4 | **96.** | BO27/4a |
| **7.** | BO2/16 | **37.** | BO9/1 | **67.** | BO13/7 | **97.** | BO27/4b |
| **8.** | BO3/1 | **38.** | BO9/2 | **68.** | BO13/8 | **98.** | BO28/2a |
| **9.** | BO3/2 | **39.** | BO9/3 | **69.** | BO14/1 | **99.** | BO28/4 |
| **10.** | BO3/6 | **40.** | BO9/4 | **70.** | BO14/2 | **100.** | BO28/5 |
| **11.** | BO3/9 | **41.** | BO9/5 | **71.** | BO14/6a | **101.** | BO28/7 |
| **12.** | BO4/2 | **42.** | BO9/6 | **72.** | BO14/6b | **102.** | BO29/1 |
| **13.** | BO4/3 | **43.** | BO9/7 | **73.** | BO14/14 | **103.** | BO31/1 |
| **14.** | BO4/4 | **44.** | BO9/9 | **74.** | BO15/5 | **104.** | BO31/6a |
| **15.** | BO4/7 | **45.** | BO10/2 | **75.** | BO15/6 | **105.** | BO31/7 |
| **16.** | BO4/8 | **46.** | BO10/3 | **76.** | BO15/7 | **106.** | BO31/9 |
| **17.** | BO4/9 | **47.** | BO10/5 | **77.** | BO15/8 | **107.** | BO31/14 |
| **18.** | BO4/11 | **48.** | BO10/7 | **78.** | BO15/9 | **108.** | BO33/1 |
| **19.** | BO4/13 | **49.** | BO10/8 | **79.** | BO17/7 | **109.** | BO33/3 |
| **20.** | BO4/14a | **50.** | BO10/9 | **80.** | BO18/1 | **110.** | BO34/2 |
| **21.** | BO4/14b | **51.** | BO10/10 | **81.** | BO18/2a | **111.** | BO34/3 |
| **22.** | BO4/15a | **52.** | BO10/11 | **82.** | BO18/2b | **112.** | BO37/2 |
| **23.** | BO4/15b | **53.** | BO10/12 | **83.** | BO19/1 | **113.** | BO37/3a |
| **24.** | BO4/16a | **54.** | BO10/14 | **84.** | BO19/2 | **114.** | BO37/3b |
| **25.** | BO4/16b | **55.** | BO10/15 | **85.** | BO19/3 | **115.** | BO37/5 |
| **26.** | BO4/17 | **56.** | BO10/16 | **86.** | BO19/5 | **116.** | BO37/6 |
| **27.** | BO4/18 | **57.** | BO10/19 | **87.** | BO19/6 | **117.** | BO38/1 |
| **28.** | BO5/1 | **58.** | BO10/20 | **88.** | BO19/8 | **118.** | BO38/3 |
| **29.** | BO5/3 | **59.** | BO11/1 | **89.** | BO19/9 | **119.** | BOP13 |
| **30.** | BO6/1 | **60.** | BO11/6 | **90.** | BO19/10 | **120.** | W2667 |

Los abajo firmantes declaran que esta lista corresponde a cabalidad con el material verificado

| | | |
|---|---|---|
| Guenther Ebersdorfer | Antonio Gonzales | Romer Miscerendino S. |
| Presidente | Director | U. Rec.Nat. y Ord.Terr. |
| Amazonia Network | CIMAR – UAGRM | Prefectura Santa Cruz |

An extraordinary success of the MICRODIV 2 project was the achievement of a Material Transfer Agreement (MTA) for Amazonia Network. This MTA, negotiated with and signed by the Director General de Biodiversidad (DGB) and the Viceministerio de Recursos Naturales y Medio Ambiente at the Ministerio de Desarrollo Sostenible, Senior Ing. Jorge Mariaca Peláez, made it possible to bring the 118 newly isolated strains to Vienna, to perform further investigations concerning their identification and characterization as shown in fig.5.

Figure 5: Material Transfer Agreement



**Ministerio de Desarrollo Sostenible**

La Paz, 8 de abril de 2004
**DIR-DGB Nº 066/2004**

A quien corresponda

**Ref.: Permiso de salida de 120 colonias de microorganismos con destino a Austria, Vienna verificados previamente.**

De mi consideración:

Mediante la presente informamos que el Sr. Guenther Ellersdorfer, Coordinador General de Amazonia Neywork- ANW con sede en Viena, Austria, ha sido autorizado para llevar consigo los microorganismos de referencia que se reflejan en la lista adjunta, para fines de investigación científica en el março de un Proyecto de cooperación científica con el Instituto de Investigaciones Farmacobioquímicas y el Instituto de Ecología-Herbario Nacional de Bolivia, ambos de la Universidad Mayor de San Andrés.

Se adjuntan todos los antecedentes y formulario de verificación de dichos microorganismos.

Atentamente,

Ing. Jorge Mariaca Peláez
DIRECTOR GENERAL DE BIODIVERSIDAD
Viceministerio de Recursos Naturales
y Medio Ambiente
Min. Desarrollo Sostenible

cc.: IIFB, UMSA
IE-Herbario Nacional, UMSA
Prefectura Santa Cruz

Av. Mariscal Santa Cruz Nº 1092 • Casilla Nº 12814 • Central Piloto 2116000 • Fax: 2318473 • La Paz - Bolivia

To obtain this MTA was only possible because of the supporting activities of Dr. Stephan Beck, Director of the Herbario Nacional, Dr. Mario Baudoin, Director of the Institute of Ecology and Dr. Alberto Giménez, Director of the Instituto de Investigaciones Farmaco-Bioquimicas, all three located at the Universidad Mayor de San Andrés in La Paz.
For Amazonia Network this MTA means a big chance to work on the microbial biodiversity of Bolivian habitats and will be one of its major areas of activity in the near future.

UNIT 2

# MOLECULAR APPROACHES FOR THE CHARACTERIZATION OF PHYTOPATHOGENIC FUNGI

Mauricio Marin Montoya
*Universidad Nacional de Colombia, Medellin, Colombia*

## Presentation

Plant pathogenic microorganisms cause great losses to the agricultural crops and forestry plantations in the tropics. Due to the lack of adequate technical assistance, poor-resource farmers in the tropics base the management of plant diseases on applications of chemical products, which are frequently badly used. This brings environmental and human healthy problems in addition to the lack of productivity and the increase of pressure over new non-agricultural areas.

Undeveloped countries such as the ones that belong to the Amazonas region require a better academic preparation for their agricultural technicians to accomplish the goal of offering an effective technical advice to the farmers in all the aspects related to plant protection. In this context, unit 2 of MICRODIV2 had as main objective to introduce the assistants to the fundamental aspects regarding the biology of plant pathogenic microorganisms, and specially to present some of the molecular-based techniques used to study this group of organisms.

Specific objectives of this unit were:

- To introduce the main aspects related to the life cycles of plant pathogenic microorganisms
- To present the basic aspects related to the modern theory of molecular biology and its applications.
- To present a current status of molecular techniques used to study plant pathogenic microorganisms.
- To provide students with practical skills to analyse and characterize plant pathogenic microorganisms specially in the following areas:
  - Field symptoms and signs
  - Field sampling of diseased vegetal material
  - Diagnostic analysis in the laboratory – morphological, physiological and molecular tests
  - Preparation of solutions, cultural media and molecular reactives
  - Use of laboratory equipment needed to characterize plant pathogenic microorganisms

## Methodology

An initial diagnosis of the student's basic knowledge regarding the biological aspects of plant pathogenic microorganisms and concepts of molecular biology, indicated a general lack of theoretical basis. Therefore it was necessary to increase the previous planned number of theoretical sessions, which were also supported by a field trip, a two-days workshop and short seminars offered by different instructors of MICRODIV2 on selected topics.

Theoretical sessions consisted of presentations introducing fundamental aspects related to the main plant pathogenic microorganisms affecting agricultural crops and forestry plantations in the tropics. Furthermore, two sessions were organized to provide the students with skills regarding mathematic operations needed to calculate the amounts of chemicals to prepare chemical solutions, molecular reactives and general buffers.

Practical sessions pretended to introduce the students in the main techniques used to identify and study plant pathogenic microorganisms, including morphological, physiological and molecular aspects. The students had the opportunity to prepare their own solutions and to make their own diagnostic experiments with the material collected in the field trip.

**Activities**

The following subjects were treated in Unit 2 od MICRODIV2, including the theoretical and practical sessions.

## A. Theoretical sessions

| Figure 6: Pathogenic virus on *Nicotiana tabachii* | Figure 7: Microscopic image of fungal hyphae |
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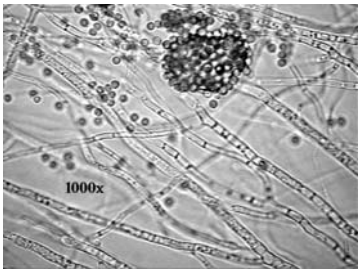
|  |  |
| Figure 8: Lemon fruit covered with mould | Figure 9: Conidiophores of a mould |
|  |  |

1. **Introduction to the study of plant pathogenic microorganisms**
   Within this session the main aspects regarding the study of plant pathogenic microorganisms and the current techniques and biotechnological applications were presented. Special topics included: (a) The basis of modern molecular biology, (b) the main microorganism groups affecting plant production, (c) Thebasis of molecular techniques used to study plant pathogenic microorganisms including PCR, cloning and sequencingm, (d) The current applications used in plant pathology based on molecular techniques (PCR diagnosis, phylogenetic analysis, detection of fungicide-resistant isolates, population genetic analysis, plant selection assisted by molecular markers)

2. **Mycology**
   Due to the fact that nearly 70% of the most important plant pathogens include fungal species, this section consisted of presentations of mycological contents in deep including: (a) General aspects regarding the kingdom Fungi and related kingdoms (Chromistas, Protozoa), (b) The holomorph concept and its importance for fungal taxonomy, (c) Biology and life cycles of each of the taxonomical groups of fungi, (d) Main taxonomic characters used to classify fungi, and (e) Phylogenetic relationships between fungal taxons

3. **Phytobacteriology**
   This section had as main objective to introduce the students in the general aspects of the biology of prokaryotic organisms, specially: (a) The main genera of bacteria causing plant diseases, (b) Aspects related to the morphology of plant pathogenic bacteria, (c) The life cycles of plant pathogenic bacteria, (d) Control measurements against plant bacterial diseases, (e) Concepts of bacterial species, subspecies and pathovars, and (f) Current taxonomy of plant pathogenic bacteria

4. **Virology**
   This section pretended to present aspects related to the biology of plant viruses, making special emphasis in molecular techniques used currently to study and detect viruses. Other aspects treated

comprised: (a) Replication strategies of plant viruses, (b) Taxonomic characters used to classify viruses, (c) Main aspects regarding the characteristics of viral genomes, (d) Symptoms of plant viral diseases, (e) Mechanisms of plant virus transmission, group of vectors and their biological relationship with viruses, and (f) Main taxonomical groups of plant viruses

5. **Chemical calculations**
   Work at the plant pathology laboratory requires a basic knowledge on how to make solutions, buffers, dilutions and preparing molecular reactives. This subject was treated in a four-hour session. The main topics were: (a) Concentration measures, (b) How to prepare buffers and solutions (c) Quantities used in molecular biology and (d) Primer and other reactive dilutions, master mix calculations

6. **Principles of molecular biology**
   Developments of the modern theory of molecular biology have changed the world of biological sciences. Plant pathology has not been an exception; a proof of this is the uncounted number of applications developed to support all the aspects regarding the scope of this discipline. Based on this concept, Unit 2 had special interest on presenting the basis of molecular biology and the tools used to study plant pathogenic microorganisms and interactions with their hosts. The presentations included: (a) The central dogma of molecular biology, (b) Breaking down the dogma: the inverse transcription process, (c) Principles of PCR (d) Regions of the genome used for taxonomic and population studies of plant pathogenic microorganisms( rRNA regions: ITS, small and large subunits,; structural genes; introns based phylogeny), (e) Principles of molecular markers (RFLP, RAPD, SSR,AFLP), and (f) Practical examples used to study plant pathogenic fungi, bacteria and viruses.

## B. Practical sessions

The students of Unit 2 were devided into groups of three members each. During the practical sessions, each group had the responsibility to study and characterize specific plant samples with symptoms or signs of fungal, bacterial and viral origins. The work of each group started from the process of taking the sample in the field trip.

Figure 10: Samples of the field excursion

Figure 11: Microscope image of an unidentified Gram+ bacterial isolate

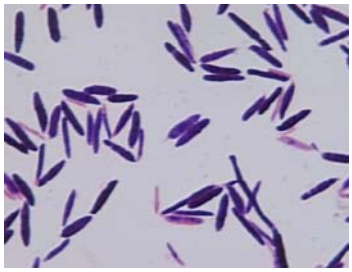
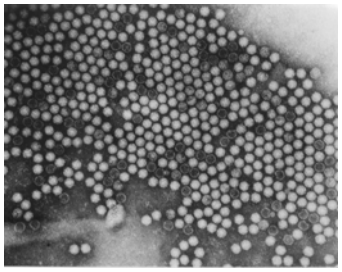


Figure 12: Unidentified plant species. Left virus infection, right non infected specimen

Figure 13: Electron microscopic image of virus capsids



**1.Field excursion**
The field trip related to Unit 2 was made to the Botanical Garden of Santa Cruz, the cultivation area close to "El Vallecito" and a traditional market downtown Santa Cruz. The main purposes of the field trip were: (a) To familiarize the students with the symptoms and signs of plant diseases, (b) To show methods to collect, keep and process diseased plant samples, and (c) To collect plant material to be used in the practical work at the laboratory.
The samples collected included symptomatic tissues of: (a) Maize: dwarf plants with probable symptoms of viral origin, (b) Papaya tree: with symptoms of Papaya ringspot virus, (c) Caupi: plants with symptoms of viral origin; leaves with anthracnose, (d) Several forest trees: showing symptoms of vascular wilt, cankers and signs of Ophistomatoid funi, (e) Tomato: symptomatic plants with Tomato spotted wilt Tospovirus, (f) Bean: plants with leaf spots and bacterial blight, and (g) several fruits, tubers and vegetables with symptoms of bacterial soft-rots, white-rots and several moulds

**2.Preperation of samples and signs observation**
Back to the laboratory, this session included the selectisues for pathogen isolation and signs identification under a stereoscopic microscope. Furthermore the students were trained to prepare: (a) Humid chambers to induce fungal sporulation, (b) Microscopy slides for fungal structure analysis, (c) Slides to observe bacterial movement, and (d) General and selective media to isolate fungi and bacteria (Malt extract agar, Potato dextrose agar, Yeast extract-dextrose-CaCO3, Nutrient agar)

**3.Characterization of plant pathogenic bacteria**
Once pure cultures of bacteria were obtained by sequential streaking of isolates from diseased plants, fruits, tuber and vegetables, each group of students proceeded to make the following analyses: (a) Description of colony characteristics, including morphology, color and period of growth, (b) Gam-stain, (c) KOH test, and (d) Bacterial motility test.
Based on these characteristics each group could affiliate their isolates as possible members of the following taxons: Pseudomonaceae, Rhizobiaceae, Enterobacteriaceae and Clavibacter.

**4.Characterization of fung**
fungi growth in media and humid chambers were studied based on their microscopic characteristics. Students had the opportunity to describe the structiures and to place the observed fungi on each of the following taxons: Oomycetes, Zygomycetes, Ascomycetes, Mitosporic fungi and microscopic Bsidiomycetes.

**5.RNA Virus diagnosis**
Based on the samples collected with symptoms of viral infections, each group made an analysis of the presence of dsRNA elements in the infected material. This analysis is useful to isolate the genome or replicative forms of RNA viruses, including those with single strand and double strand genome or replicative forms of RNA viruses, including those with single strand and double strand RNA. Since this technique requires an important number of reactives and procedures, the practice was very useful to test the incorporation of theoretical knowledge for the development of practical skills for the students. This practice included aspects such as: following complex laboratory procedures, making calculations, preparing buffers and solutions, using special equipment and reactives, including micropipettes, centrifuges, electrophoresis chambers, liquid nitrogen tanks, UV lamps, organic solvents (phenol, chloroform) acids, alcohols, enzymes (DNase, RNase).

**6.DNA extraction**
During this practice assistants applied two kinds of procedures used to extract DNA from microorganisms: one based on a commercial kit and the other on a more elaborated protocol used to extract DNA in high quantities and a low prize.
The "Wizard Genomic DNA purification kit of Promega" was used to extract DNA of selected bacteria proceeding from pure cultures. The DNA extraction protocol was used to extract DNA from fungi growing in liquid media (Malt extract broth). These practices allowed the students to understand the basic procedures used to obtain nucleic acids from microorganisms and included the application of some of the following aspects: (a) Gel electrophoresis (running buffers, loading buffers, DNA ladders), (b) Gel staining with ethidium bromide (c) DNA extraction analysis (integrity and quantity,

characteristics of species of nucleic acids present in gels) (d) Phenol equilibration, (e) Work under sterile semi-conditions.

**7.PCR experiments**

Once DNA was extracted from plant pathogenic bacteria and fungi, students had several sessions regarding the practical use of PCR techniques. Initially the students got familiarized with the reactives necessary to prepare PCR and the equipment used to accomplish if (PCR machine). Special emphasis was made in how to choose primers, how to select PCR conditions and how to interpret PCR results on electrophoresis gels.

Each group prepared a PCR reaction with DNA extracted from each of the studued pathogens. For bacteria, PCR consisted of the amplification of a fragment of 16S rDNA, while for fungal isolates, PCR was developed for two regains: the internal transcribed spacer (ITS) of the rRNA operon and the β-tubulin gene.

Finally, results were analyzed through gel electrophoresis and the results were discussed with the students.

| Figure 14: Fotographic image of a agarose gel showing PCR products derived from different primer sets | Figure 15: Fotographic image of a agarose gel showing RFLP patterns of different 16S rDNA PCR-products |
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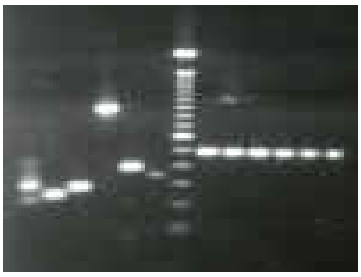
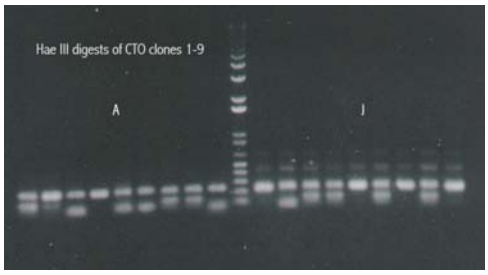
|  |  |

**Conclusions**

MICRODIV 2 was an excellent opportunity for the local assistants to get educated in different aspects of microorganism related investigations. Unit 2 of this course treated aspects regarding plant pathogenic microorganisms and the available tools for their study. Students assisting this unit were very active and recognised the importance to study this group of microorganisms, since they live in the main agricultural region of Bolivia, spread around the city of Santa Cruz..

Besides the usual logistic problems related to the lack of infrastructure, and the students preparation about the subject, time was efficiently managed and the main planned aspects to be addressed in this unit were fully covered.

Protocols, procedures, bibliography and supporting material used in Unit 2 were given as hand-outs to the students and are compiled in the CD-ROM prepared for MICRODIV2

UNIT 3

# CHARACTERIZATION OF MYCORRHIZA

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The term "mycorrhiza" is referred to the symbiotic mutualistic association between some soil fungi and the roots of a great majority of the vascular plants. These associations are presented in nature under a variety of forms, depending upon the type of organisms involved: Arbuscular, Ectomycorrhiza, Ectendomycorrhiza, Arbutoid, Monotropoid, Ericaceae and Orquideaceae. The arbuscular mycorrhiza (AM) represents a universal symbiosis present in more than 80 % of the plant species, with more of 150 fungi described species. In this symbiotic association normally a nutrient and metabolite exchange between the fungi and the host plant occurs, with profits for both symbiotic organisms. The host plant increases its ion uptake rate (mainly P and other elements like Zn and Cu), and the fungi obtains carbon derived compounds produced by the plant. Additional benefits in improvement of mineral nutrition, pathogen resistance, increase in photosynthetic rate and tolerance to water stress also have been reported.

In a plant community, the propagation structures of AM fungi (AMF) present in the soil are considered as a native inoculum. These propagules could be spores, mycellium, roots fragments or any other fungi-colonized organic material. Temperature, humidity, presence of host species, sand proportion and pH of soil, changes in P and organic matter concentration in soil, are environmental factors that can affect the distribution of the native inoculum of AM fungi and the number of AM associations formed, altering the soil infection.

Commonly, the mycorrhiza propagules are concentrated in the first layer of the soil, being susceptible to perturbations depending upon its intensity and the propagule kind. In many habitats, the extra-root hyphae network and the infected roots are probably the most important source of inoculum, but also the most susceptible ones in special to physical damage or mechanical rupture. The spores are resistant structures which can remain in the soil for long time reflecting the soil infectivity after intensive perturbations.

The AMF root colonization process is developed trough infective hyphae growing from any of the above described propagules. During this process the plasmatic membrane of the host cell is surrounding the penetrating hyphae without any perforation, forming a close contact surface. At the same time, the penetrating hyphae repeatedly branch in dicotomic way until the formation of an arbuscule. The life-cycle of an arbuscule is of 2-4 days depending upon the species. In culture species with high-growth rate, this pattern of life-cycle is very common, whereas in low-growth rate tree species the arbuscules seems to have longer life-cycle). After the arbuscule formation, globular structures with lipids called vesicles are formed, which constitutes an energetic reserve for the fungi. These structures can be found in intra- and extra-cellular spaces.

From the study of the plant-fungi symbiotic relation the concept of "mycotrophy" was derived, defining the plant ability to nutrient uptake through the association with the fungi. This fact also defined three different grades of MA plant-dependence: non-mycothropic, facultative and obliged. Facultative species can reach maturity without AM association, showing low colonization rate in fertile soils. Obliged species does not reach reproductive stages without AM association. Commonly, plants with low P-uptake ability and other nutrients are more dependent for association. Non-mycothropic species are HMA-independent in all its growth stages.

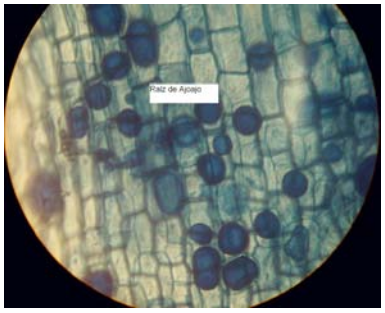
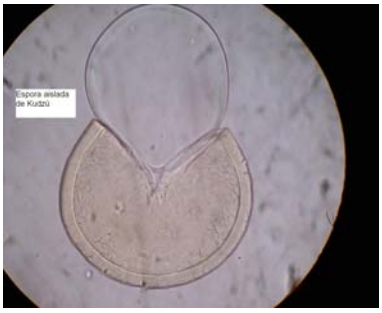
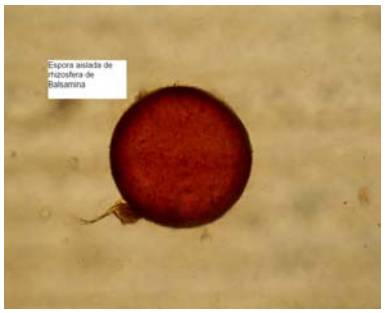
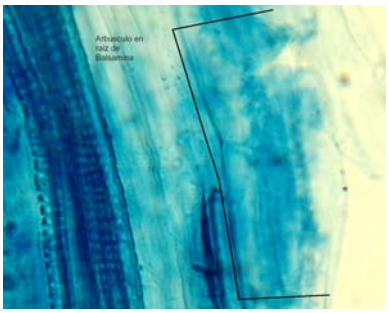
Environmental factors acting on dependence are the same that influences the P nutrition. Dependence is a function of P deficit within the plant, which in turn will depend upon the plant demand of P and its supply. The P supply is related to the different P forms present in the soil and plant physiological and morphological mechanisms, like root growth rate and morphology. The demand of P is related with the minimal P concentration in tissues, required to obtain the maximal growth rate. Commonly, species associated to low-nutrient environments show several common attributes like low nutrient uptake rate, low biomass turnover and photosynthetic rate.

Plants with fine and branched roots (i.e. herbaceous plants), depending upon the P concentration in soil, are facultative in regard to AM association. Studies on tropical grasses (*Brachiaria sp* and

*Paspalum sp),* with a well branched root system, showed high growth response to AM association, probably related to high P demands in early stages. The low proportion of AM found in plants associated with fertile soils, suggested low-cost adaptations for nutrient uptake, i.e. abundance of radical hairs, branched and fine root system, organic acid secretion, tolerance to low P concentration, low growth rate.

AMF must be considered as physiological obliged symbiotic organisms, since its life-cycle is completed only when are associated to the host plant. No specificity exists between the fungi or the host. A radical system can be simultaneously colonized by several fungi species and, conversely, one fungi species can simultaneously colonize roots of several proximal hosts. However, different degrees of susceptibility have been found in host species or cultures. The host genotype is critical to define the maximum level of AM colonization, but several fungi species can exhibit the same potential on one host species, which is interpreted as different degrees of compatibility between hosts and colonizers.

The net profit of symbiosis for the host is depending upon the nutrient uptake increase and the maintenance cost of the fungi and its carbohydrate consumption. Between 4-20% of photosynthetic fixed C can be exported to infected roots, and this additional drainage influences on transitory reductions in plant growth. Different varieties of cultured Sorghum from several soil fertility conditions inoculated with the same species of fungi, show different cost/profit balance.

| | | |
|---|---|---|
| Fig.16: Mycorrhization of *Gallesia integrifolia* Spreng. (Phytolaccaceae) – Ajo-ajo. | Fig.17: Isolated spore from *Pueraria phaseoloides* (Roxb.) Benth. (Fabaceae) - Kudzú | Fig.18: Isolated spore from *Pueraria phaseoloides* (Roxb.) Benth. (Fabaceae) - Kudzú |
|   |   |   |
| Fig.19: Isolated spore of *Mormordica charantia* L. (Cucurbitaceae) - Balsamina. | Fig.20: Isolated arbusculus of *Mormordica charantia* L. (Cucurbitaceae) - Balsamina. | Fig.21: Arbusculus in the root of *Mormordica charantia* L. (Cucurbitaceae) - Balsamina. |
|   |   |   |

UNIT 4

# COMPARATIVE AND ECOLOGICAL PHYTOCHEMISTRY & INSECTICIDAL AND ANTIFUNGAL BIOASSAYS

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Summary
In the scope of the practical course, crude extracts of seven different plant samples, collected in Santa Cruz, were prepared and subsequently tested in several insect and antifungal bioassays. Additionally, a root extract of *Stemona curtisii* Hook.f. with well known chemical composition and pronounced insecticidal as well as antifungal activities (KALTENEGGER et al., HARTL, 2003; 2003; PACHER et al., 2002) was included in all bioassays as positive control. Moreover, this extract was further fractionated by column chromatography and the fractions again tested in selected bioassays to demonstrate the principle of bioassay guided fractionation.
To characterize the extracts and to monitor the separation by column chromatography analytical TLC was performed. Insecticidal activity was evaluated using larvae of the pest insects *Spodoptera eridania* Cramer as test organism while antifungal properties were assayed against the filamentous microfungi *Cladosporium herbarum*.

| ***Program of the practical course*** | |
|---|---|
| *16th March* | Preparation of laboratory equipment |
| *17th March* | Field Trip and collection of plant material |
| *18th March* | Extraction of plant material |
| *19th March* | Liquid-liquid extraction |
| *23rd March* | Column chromatography, Thin-layer chromatograpy |
| *24th March* | Preparation non-choice artificial diet feeding assay |
| *25th March* | Finalisation non-choice artificial diet feeding assay |
| *26th March* | Agar diffusion assay |
| *30th March* | Thin-layer bioautography |
| *31st March* | Contact Toxicity |
| *1st April* | Leaf disc assay; Artificial diet feeding assay - evaluation |
| *2nd April* | Agar diffusion assay and bioautography – evaluation |

## Plant Material

The Plant material was collected in the Botanical Garden, Santa Cruz (Bolivia) and identified by a local botanist. Species are listed in table 1 and photos of selected species are given in Fig.1 and Fig.2.

**Table 1**. Plant species collected in the Botanical Garden

| species | common name | plant part | family |
|---|---|---|---|
| *Anadenanthera macrocarpa* Benth. | Curupaú | cortex | Mimosaceae |
| *Hura crepitans* L. | Ochoó | cortex (stem) | Euphorbiaceae |
| *Gallesia integrifolia* Spreng. | Ajo – ajo | cortex | Phytolaccaceae |
| *Erythroxylum ulei* O.E.Schulz | Coca camba | leaves | Erythroxylaceae |
| *Nicotiona glutinosa* L: | Tabachachi | leaves | Solanaceae |
| *Mormordica charantia* L. | Balsamina | upper parts | Cucurbitaceae |
| *Parthenium hysterophorus* L. | Chupurujume | upper parts | Asteraceae |


**Fig. 1** *Hura crepitans* (Mimosaceae). **A**. Pistillate flower. **B**. Pistillate flower, premature state. **C**. Pistillate and staminate flowers. **D**. Immature fruit. (photos A, B, D by Kaltenegger E., botanical garden, Santa Cruz, Bolivia. C: internet, http://ctfs.si.edu/webatlas/english/huracr.html)

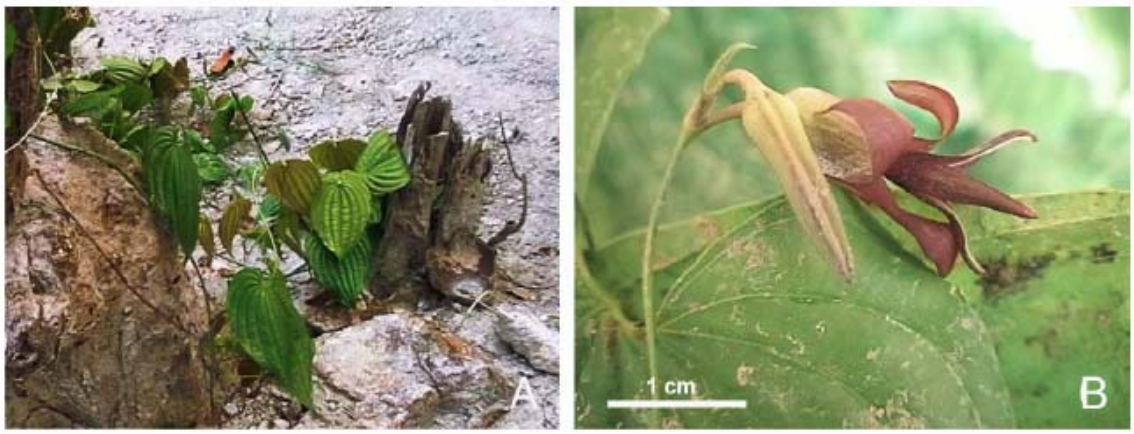


**Fig. 3** *Stemona curtisii* (Stemonaceae). **A**. Habitus. **B**. Flower. (photos by Greger H., natural location, Ko Lipe, South Thailand)

Additionally, a lipophilic crude extract of roots from *Stemona curtisii* (Stemonaceae), Fig. 3, was made available from the Department of Comparative and Ecological Phytochemistry (Institute of Botany, University Vienna, Austria). This extract served as positive control as well as demonstration sample.


**Fig. 2** *Mormordica charantia* (Cucurbitaceae). **A**. Flower. **B**. Flower, lateral view. **C**. Immature fruit. **D** and **E**. Fruit. (photos by Kaltenegger E., botanical garden, Santa Cruz, Bolivia)

**Extraction**

The fresh plant material was hackled separately and extracted with MeOH at room temperature for 24 hours. The methanolic crude extracts were filtered, concentrated and the aqueous residues partitioned between CHCl3/H2O by liquid-liquid extraction. The organic CHCl3 fractions were evaporated to dryness, dissolved in MeOH and subsequently used as lipophilic crude extracts for comparative TLC as well as insect and antifungal bioassays.

**Bioassay guided fractionation**

The lipophilic crude extract of *Stemona curtisii* was roughly separated by open column chromatography (CC) using solvent mixtures of increasing polarity (hexane, EtOAc, MeOH, see table

2). The CC fractions were concentrated and subsequently tested in selected bioassays to evaluate insecticidal and antifungal activities.

**Table 2**. Solvent mixtures of open column chromatography.

| | hexane | EtOAc | MeOH |
|---|---|---|---|
| I | 100 | | |
| II | 70 | 30 | |
| III | 50 | 50 | |
| IV | 25 | 75 | |
| V | | 100 | |
| VI | | 75 | 25 |
| VII | | 50 | 50 |
| VIII | | | 100 |

**Thin Layer Chromatography**
TLC was performed on analytical Silica gel F254 60 plates (20x20 cm, 0.25 mm, Merck); detection under UV (366 nm) and application of reagent solutions: Mobile phase CH2Cl2-EtOAc-MeOH, 70: 30: 5; spraying with Dragendorff´s reagent (Merck), selective reagent for N-containing compounds, e.g. alkaloids. Mobile phase Hexan-Diethylether, 4:6 spraying with Anisaldehyde-sulfuric acid reagent, detection of aliphatic substances.

**Insect bioassays**
Bioassays were carried out with larvae of the polyphagous pest insect *Spodoptera eridania* (Lepidoptera, Noctuidae), which were made availably by Christopher Pruett, Head of Agricultural Entomology and Integrated Pest Management, Instituto de Investigaciones Agrícolas, Universidad Autónoma "Gabriel René Moreno": Adults were captured with light traps and forced to oviposit on paper. The egg masses were stored in the fridge (+4°C) until further use. Hatching of the larvae was induced by incubating the egg masses at ambient temperatures. To obtain third instar larvae, a population of *Spodoptera eridania* was reared on camote leaves (sweet potato, *Ipomoea batatas* L., Convolvulaceae*)* at ambient conditions (26 ± 2°C, 60 – 70 % relative humidity).

**Artificial diet feeding assay**.
Non-choice feeding studies were carried out with neonate larvae of *S. eridania* (n = 20). Crude extracts, dissolved in MeOH, were incorporated into the artificial diet following standard procedures (KUBO, 1991). Test insects were kept on artificial diet portions containing different concentrations of the crude extracts (2500, 1000 and 100 µg/g fresh wt) and after 5 days (moist chamber, 26 °C, darkness) the survival and growth rates of larvae were determined in comparison to a control group, exposed to a diet treated with solvent only. From the dose-response curves LC50 and EC50 values were calculated by probit- log analysis (NORUSIS, 1999).

**Leaf Disc Choice Test.**
The leaf disc assays were carried out with third instar larvae of *S. eridania*. Leaf discs of a standard size (1.33 cm2) were cut from camote leaves (*Ipomoea batatas*) with a cork borer and used as substrates for the presentation of plant extracts to the test insects. The leaf discs were treated on their upper surface with either 20 $\mu$L of methanolic solutions of plant extracts (concentration 0,22 mg/cm2)

or with solvent only. After the solvent was evaporated, two leaf discs impregnated with plant extracts and two solvent control discs were placed on moist filter paper in a Petri dish and presented to two larvae. The behaviour of the larvae was observed and journalised to detect antifeedant or repellent activities.

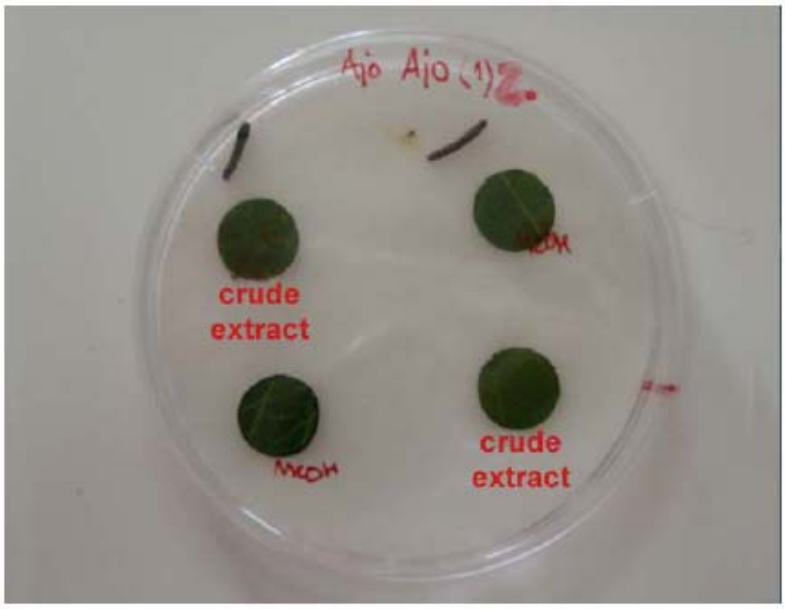


Fig.4. **Leaf Disc Choice Test**. Sample *Gallesia integrifolia*, Ajo Ajo. Two leaf discs were either treated with plant extracts (concentration 0,22 mg/cm2) or solvent only. After solvent evaporation, leaf discs were placed on moist filter paper in a Petri dish and presented to two third instar larvae of *S eridania*.

**Contact Toxicity.**

The inner walls of glass vials (area = 0.75 dm2) were coated with methanolic solutions of plant extracts, using a concentration of 2.7 mg/dm2. Negative controls vials were treated with solvent only. After evaporation of the solvent, 20 neonate larvae of *S. eridania* were transferred into the vial for a 3h period followed by administration of a camote leaf to stop the experiment and to provide food for surviving larvae. After 24 h the survival rate was monitored. The *Stemona curtisii* extract as well as a commercial Pyrethrum extract, latter dissolved in acetone, served as positive control groups (conc. 2.7 mg/dm2).

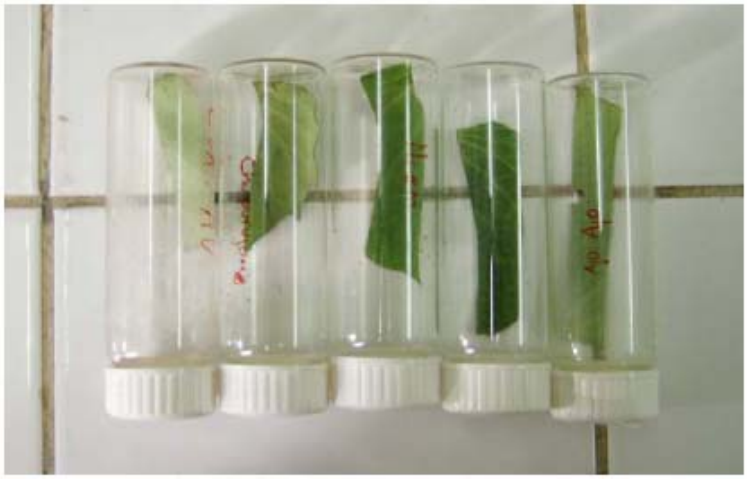

Fig.5. **Contact Toxicity.** 20 neonate larvae of *S. eridania* were exposed to plant extract coated surfaces of glass vials. Following a period of 3 hours, kamote leaves were transferred into the vial to stop the assay.

**Antifungal bioassay**
The plant extracts as well as CC fractions were assayed in selected bioassays against the filamentous microfungi *Cladosporiu herbarum*.

**Fungal inocula**
*Cladosporium herbarum* was cultured on 4% (w/v) malt extract–agar medium (MEA; Merck, Darmstadt, Germany) in 9 cm Petri dishes at room temperature and in darkness. In order to obtain conidia, fungi were cultured for 3–10 days. Harvesting was carried out by suspending conidia in 1% (w/v) sodium chloride solution containing 1% (v/v) Tween 80. A sterilized Drigalski spatula was used to separate spores from hyphae. The spore suspension was transferred into 1.8 mL cryotubes and stored at -20°C until further use.

**Bioautography on thin-layer plates**
Plant extracts as well as CC-fractions were applied as small spots on TLC plates (Silica gel F254 60 plates, 20x20 cm, 0.25 mm, Merck) and the plates developed with a mixture of CHCl3-hexane-MeOH, 8 : 2 : 1; after evaporation of the organic solvent TLC plates were homogeneously sprayed with about 30 mL of 4% (w/v) malt extract broth containing conidia (108 CFU/mL). Plates were incubated for 3 days in a moist chamber at 25°C in the dark and the appearance of blank zones in the mycelium layer indicated antifungal activity.

**Agar diffusion assay**
Plant extracts (40 mg) were dissolved in 1 mL MeOH twofold diluted from 40.0 to 20.0 and 10.0 mg/mL; 25 µL aliquots were applied to 9 mm diameter paper discs (no. 2668/2; Schleicher and Schuell, Dassel, Germany). After evaporating the organic solvent, discs were placed in 9 cm diameter agar plates (MEA) previously inoculated with 0.5 mL spore suspension. After 3 days, growth was of the fungi was monitored.

***Results***

**Artificial diet feeding assay**
The toxicity and growth inhibition of plant extracts to neonate larvae of *Spodoptera eridania* exposed to artificial diet containing different concentrations of lipophilic crude extracts, is recorded in Table 2. None of the in the botanical garden collected plant species showed pronounced toxicity against *Spodoptera eridania* with LC50 values >> 2500 µg/g and almost no mortality of larvae even at highest concentration of extracts incorporated in artificial diet. Furthermore, only *Anadenthera macrocarpa* and *Erythroxylum ulei* inhibited the growth of larvae, but compared to *Stemona curtisii* only at very high concentrations.

**Bioassay guided fractionation**
Column chromatography (CC) led to a roughly separation of the crude extract from *Stemona curtisii* in fractions of different polarities. The lipophilc hexane fractions (I1-III1) contained mainly tocopheroles and triterpenoids, whereas stilbenoids where found in fractions of medium polarity (III2-IV2). Polar fractions eluted with mixtures of EtOAc and MeOH included mainly mixtures of alkaloids. In subsequent antifungal as well as insect bioassays activities of CC fractions were compared to those of the crude extract to find active principles. The antifungal activity of the crude extract was then found in fractions of medium polarity in a bioautography assay (see fig. 6) leading to stilbenoids as active principles whereas pronounced insecticidal activities of the crude extract could be attributed to characteristic stemona alkaloids. In an artificial diet feeding assay, alkaloid containing fractions showed strongest activity, leading to a 100% mortality of larvae in fractions with highest amount of alkaloids (VII1-VIII2). Furthermore, pronounced inhibition of growth rates was observed in alkaloid containing fractions (V1-VI2).

**Table 3.** Toxicity and growth inhibition of lipophilic crude extracts to neonate larvae (n=20) of *Spodoptera eridania* in comparison to a lipophilic crude extract of roots from *Stemona curtisii.a)*

| Plants | Conc. [µg/g] | Survival rate [%] | Weight per larvae [mg] | Growth rate [%] | LC50 [µg/g] | EC50 [µg/g] |
|---|---|---|---|---|---|---|
| *Anadenthera macrocarpa* | 2500 | 80 | 0.4 | 8 | >>2500 | 782 (620-929) b) |
| | 100 | 70 | 5.2 | 105 | | |
| *Hura crepitans* | 2500 | 80 | 8.2 | 166 | | |
| | 1000 | 93 | 5.3 | 107 | >>2500 | >>2500 |
| | 100 | 80 | 6.8 | 137 | | |
| *Gallesia integrifolia* | 2500 | 70 | 4.1 | 83 | | |
| | 1000 | 90 | 4.5 | 91 | >>2500 | >>2500 |
| | 100 | 90 | 4.7 | 95 | | |
| *Erythroxylum ulei* | 2500 | 100 | 0.7 | 14 | | |
| | 1000 | 100 | 2.6 | 53 | >>2500 | 1074 (884-1248) b) |
| | 100 | 90 | 9.7 | 196 | | |
| *Nicotiona glutinosa* | 2500 | 85 | 6.6 | 133 | | |
| | 1000 | 90 | 7.3 | 147 | >>2500 | >>2500 |
| | 100 | 70 | 8.4 | 170 | | |
| *Mormordica charantia* | 2500 | 90 | 5.9 | 119 | | |
| | 1000 | 95 | 7.2 | 146 | >>2500 | >>2500 |
| | 100 | | 8.1 | 164 | | |
| *Stemona curtisii* | 2500 | 0 | 0 | 0 | | |
| | 1000 | 0 | 0 | 0 | 9 c) | 4 c) |
| | 100 | 40 | 0.1 | 2 | | |

a) Nonchoice feeding studies were conducted with neonate larvae of *Spodoptera eridania* (n=20) that were reared on artifcial diet spiked with various concentrations of plant extracts. After 5 days of exposure, survival and weight of the surviving larvae were determined and compared to controls that had been exposed to diet treated with solvent (MeOH) only. From the dose–response curves LC50 and EC50 values were calculated by probit-log analysis.

b) Fiducial limits.

c) LC50 and EC50 values according to Kaltenegger et al. (2003).

The survival and growth rates of neonate larvae of *Spodoptera eridania* in this assay are given in table 3.

**Table 4.** Survival and growth rate of neonate larvae (n=20) from *S. eridania*, reared on artificial diet portions spiked with CC fractions of *Stemona curtisii* compared to the lipophilic crude extract. *a)*

| CC fractions *Stemona curtisii* | Conc. µg/g | Survival rate [%] | weight per larvae [mg] | Growth rate [%] |
|---|---|---|---|---|
| $II_1$, $II_2$, $III_1$ | 2500 | 85 | 5.6 | 113 |
| $III_2$, $IV_1$ | 2500 | 100 | 6.4 | 129 |
| $IV_2$ | 2500 | 80 | 1.6 | 32 |
| $V_1$ | 2500 | 100 | 2.5 | 51 |
| $V_2$, $VI_1$, $VI_2$ | 2500 | 55 | 0.5 | 10 |
| $VII_1$, $VII_2$, $VIII_1$, $VIII_2$ | 2500 | 0 | 0 | 0 |
| crude extract | 2500 | 0 | 0 | 0 |

a) Nonchoice feeding studies were conducted with neonate larvae of *Spodoptera eridania* (n=20) that were reared on artifcial diet spiked with CC fractions of *Stemona curtisii*. After 5 days of exposure, survival and weight of the surviving larvae were determined and compared to controls that had been exposed to diet treated with solvent (MeOH) only.


Fig. 6. **Bioautography** *Stemona curtisii* CC fractions

**Leaf Disk assay**

Neither antifeedant nor repellent properties could be observed for *Anadenthera macrocarpa*, *Hura crepitans*, *Erythroxylum ulei*, *Nicotiona glutinosa*, *Mormordica charantia*. Larvae feed on leaf disks treated with extracts of these plants without any effect and without preference of the control disks. Also for *Gallesia integrifolia*, in summary no antifeedant activity was detected and control disks as well as treated disks were eaten after 6 hours. But larvae feeding on treated disks acted after oral uptake with an noticeable and extended period of total inhibition of motility. However, after this period, larvae recovered completely and showed no abnormity or loss of fittness. Finally, besides the positive control *Stemona curtisii,* only *Parthenium hysterophorus* exhibited antifeedant activity on the third instar larvae of *S. eridania*.

**Contact toxicity**

In contact toxicity assays, extracts of *Gallesia integrifolia* and *Parthenium hysterophorus* showed pronounced contact toxicity with survival rates of neonate larvae of only 25 and 10%, respectively. From the remaining plant samples, *Stemona curtisii* included, only the commercial pyrethrum extract proved to be active and achieved 100% mortality of the test insects. Thence, *Stemona curtisii* posseses insecticidal properties when orally consumed whereas *Gallesia integrifolia* and *Parthenium hysterophorus* showed especially after oral uptake no activity. The active principles of the latter plants are possibly detoxified as a result of mixed function oxidases, located in the midgut tissues when ingested. But in this assay, substances are assumed to penetrate through the cuticula and/or via the tracheal system into the larvae, thence avoiding detoxification in the midgut tissue and displaying activity. In table 5 survival rates of neonate larvae of *Spodoptera eridania* after contact with plant extracts are summarized.

**Table 5**. Contact Toxicity of lipophilic crude extracts from different plants against neonate larvae of *Spodoptera eridania* compared to *Stemona curtisii* and a commercial Pyrethrum extract [a)]

| Plants | Survival rate [%] |
|---|---|
| *Anadenthera macrocarpa* | 95 |
| *Hura crepitans* | 90 |
| *Gallesia integrifolia* | 25 |
| *Erythroxylum ulei* | 100 |
| *Nicotiona glutinosa* | 95 |
| *Mormordica charantia* | 100 |
| *Parthenium hysterophorus* | 10 |
| *Stemona curtisii* | 80 |
| Pyrethrum extract | 0 |
| Control | 95 |

[a] Contact toxicity was assessed using glass vials (area = 0.75 $dm_2$) coated with methanolic solutions of plant extracts (2.7 mg/$dm_2$). After evaporation of the solvent, 20 neonate larvae were placed into the vials, and survival rates were assessed after 48 h.

**Bioautography**

Only *Anadenanthera macrocarpa* revealed weak antifungal activity against *Cladosporium herbarum* in thin layer bioautography, leaving a small inhibition zone on the plate as a result of inhibition of spore germination. The other plant extracts showed no inhibition zones in the mycelial layer.

**Agar diffusion**

In agar diffusion assays on malt extract agar, neither repression of germination nor growth inhibition of *Cladosporium herbarium* could be detected, except the case of *Gallesia integrifolia.* Here, application of impregnated paper discs on the agar plate surface previously inoculated with the test organism resulted in dose-dependent growth inhibition of the mycelium leaving inhibition zones around the paper discs.

***Literature***


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UNIT 5

**LEGAL AND SOCIO-ECONOMIC ASPECTS OF BIOTECHNOLOGY IN LATIN AMERICA**

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General Introduction:

The Workshop started with a talk giving a general overview about the political history of The Convention on Biological Diversity (CBD), The "Struggle for Compensation" ("benefit sharing" to Indigenous Peoples in return for access to biodiversity on their lands (e.g.: bioprospecting) as kind of a Stocktaking of a still open-ended discussion. Due to the fact, that the 7th Conference of the Parties to the CBD (COP7), having taken place in Kuala Lumpur, Malaysia, between 9th and 20th of February 2004 – only some two weeks before Microdiv 2 (Workshop and Course) - Unit 5 has put special emphasis on the actual results (so far then published) and the ongoing processes within the CBD-Working Group on Art. 8j.. as well as on the position, the European Union is taking in this forum, having proposed in Kuala Lumpur for example a compromise text for the implementation of *sui generis* systems with regard to Article 8(j) of the CBD as necessity to putting special emphasis on the obvious link between environment and cultural diversity.

| Fig.22: Children of the Chictano people in the east of Bolivia (MICRODIV2 field trip) | Fig.23: Students group of unit 3 at the excursion to the botanical garden Santa Cruz, Bolivia |
|---|---|
|  |  |

At the very beginning of the event, the need soon became obvious to give a general explanation, why it is of high importance to know about sociological (socio-political, legal and socio-economical) aspects of biotechnology when dealing with genetic resources whilst promoting and training biotechnological techniques to students of developing countries.

Workshop:

The introductory talk at the opening session of the Workshop, entitled "Aspectos socio-economicos y socio-politicos de la Biotecnología" was later also published in the University Journal of the University Gabriel René Moreno (a Bolivian sociologist !) of Santa Cruz. It also focussed on the close relationship between economic underdevelopment and its foundations in rough inequality (in income and living standards) and exclusion (from access to public services like health- and education systems, just to give an example), saving prosperity, wealth and welfare traditionally only in the hands of few. It was one aim of this presentation to make clear that the fight against poverty, the current new concepts of sustainable development, the search for alternative economic models, such as "Solid Economy" revitalized in todays Brazil, etc. can not have a mere technical solution (f. ex. biotechnology as tool for development)

Given, that those lectures generally until today never have been included in the education of students in microbiology in Bolivia, the participation in Unit 5 lectures therefore was obligatory to all the

participants (diploma students, graduate students, post graduate students, professionals as well as University teachers) in the practical course. (for more details and general evaluation of the impact of Unit 5 please see chapter "summary").

Course Unit 5 - Lectures:

The lectures of Unit 5 were blocked to 2 hours seminars on three days of the whole practical course.

1.) 18th of March 2004, Thursday:
This session especially focussed on the Convention of Biological Diversity (CBD) and the 7th Conference of the Parties in Kuala Lumpur, Malaysia, between 9th and 20th of February 2004 and reflected especially those topics and discussions, which appear relevant for developing countries such as Bolivia giving also emphasis to the actual situation of indigenous people and their worldwide enforced rights.

Starting out with the Rio Declaration 1992 of the United Nations and its 23 main principles, those declared "intention" was combined with an actual study of the World Bank focussing on the living standards of indigenous peoples in Latin America, stating that 50 % of the whole Bolivian national population and 64 % of its indigenous population earn less than 2 US$ per day (definition for absolute poverty). Absolute poverty in this context means that indigenous peoples have bad conditions of health, illnesses, hunger, bad alimentation standards, illiteracy, degraded natural resources, bad housing conditions, minimal employment rates, no access to public services like fresh water, electricity, canalization, schools, health system, medicine, etc.. This is the definition of poverty – and its contrary the definition of welfare – designed by "Western" development policies. But how is the specific definition of the afflicted indigenous peoples, what are their major concerns, when reflecting about poverty, welfare, benefit, share, etc. ?

However, this "crude" comparison between international declarations based on western knowledge and the World Bank Study had the didactic aim to initiate a discussion between the participants of the course and to initiate a reflection and awareness-rising process with regard to the interrelation and the strong links between politics, economy and sociology and then to enter the next complex scene - prepared essentially by the CBD - of an intercultural dialogue on "Benefit-Sharing".

The group discussion was followed by the presentation of the main objectives and articles of the CBD, giving special emphasis to the suggestions of the design of Access and Benefit sharing Models with indigenous peoples as well as focussing especially on the needs and legal frameworks of conservation of biodiversity *in situ* and *ex situ* (referring explicitly to one of the main goals of MICRODIV2) in developing countries and the propagation of educational programs, scientific research and technical capacity building activities.

Due to the fact that a tendency of Latin American representatives in most of the (even academic) discussions can be clearly observed, to look at biodiversity as kind of naturally growing source of richness ("green oil"). They often tend to draw a line of tradition between the former exploitation of the silver mines by foreign forces (Conquista) and the evolving interest especially on the Amazon biodiversity. However, in order to concentrate those prejudices the coordination of Unit 5 decided to focus especially on article 15 of the CBD (Access to Genetic Resources), stating that the sovereign rights of the states on their natural resources is recognized and will be regulated by national legislation, BUT has to create conditions in order to facilitate to other contracting parties of the CBD the access to genetic resources for its environmentally adequate utilization and MAY NOT not put contrary restrictions.

Although there evolved a rather long-lasting discussion about the meaning and the actual potential of this article, even giving examples of practical implementations in national legislations of contracting parties already having ratified the CBD, those regulations were interpreted as "unfair". The question "if a state, having ratified the CBD, may restrict access to its genetic resources to

other contracting parties of the CBD" therefore was included in the final exam: Only five of nearly fifty students and course participants understood, that the state has to enter into negotiations with the other contracting party but may not put *ex ante* restrictions to those willing to have access to national genetic resources. Although three permanent professors and university teachers of the academic staff of the University of Santa Cruz also passed the exam, their prejudices prevailed in answering "Yes, the state, although having ratified the CBD, may put restrictions to other contracting parties willing to access its genetic resources".

2.) 25th of March 2004, Thursday:

This session focussed on general outlooks on Biosafety and the Cartagena Protocol on Biosafety giving special emphasis to the legal regulation procedure in Bolivia.

This session started with the "classical" article of Garret Hardin (Science, vol. 162, 13th of Dec. 1968) "The Tragedy of the Commons" and tried to sharpen awareness in applying its principles to the current discussions of development aspects, the relation between environment and biosafety as well as of "(traditional) knowledge". "How is it possible to manage problems, which do not have technical solutions?" If it is continued to search technical solutions to the big concerns and challenges of the developing world only in the area of science and technology, the result will be to worsen the situation. It was shown, that in this very context the European DG Research therefore is focussing in its actual research framework program on inter-, multi- and trans-disciplinary research approaches in order to find answers to important questions always considering at least three foci of investigation (a.) political analysis in order to determine the effective conditions for sustainable development, integrating the perspectives of the state, the market and the society; b.) system analysis, including the components of interaction (legal frameworks and effective rights), focussing on conservation and management of biological resources, as well as strategies of socio-economic development; c.) solution of specific technological problems in generating useful instruments for sustainable development.

Discussion of the lections of the "Green Revolution" (more food production as well as more poverty and underdevelopment in the same time frame of observation), public research versus private research (the problem of the production of knowledge: public or private? Definition of public science as creativity and dynamic support of a given society to find solution to concrete necessities at the national, regional and local level)); The international instrument of Intellectual Property Rights mainly as economic tool leading to the conclusion that there is no scientific method available which serves as solution to all the problems. There has to be at least the consideration of alimentation traditions and its changes (urbanization processes), the need to understand the cultural value of food, to understand the impacts of inequality (in income and living standards) and exclusion on development, to mobilize existent socio-political networks (also of civil society), to open up canals of communications and interaction, to establish new forms of organization (cooperatives), etc.

Presentation of the Cartagena Protocol on Biosafety of the CBD, its main goals and principles as well as an overview of the most important contracting parties and those states which refused signature or/and ratification. Given, that the Spanish Version of the Cartagena Protocol is still in some of its articles in urgent need of improvement (some articles are simply missing in its Spanish translation) and most of the course participants, although holding or attending academic degrees in life sciences, are not capable to read or understand English, there was the need to discuss those significant differences between the original English and the translated Spanish version.

Also in this context a significant lack of information had to be observed. Neither students nor university teaching staff had any pre-knowledge about the main principles of biosafety (based on gene manipulation or not), risk assessment, risk regulation and management.

3.) 1st of April 2004, Thursday:

In this session the focus was put on the actual goals (Guadalajara Declaration) of the EU's bi-regional S&T Cooperation with Latin America and Caribbean (ALCUE), with special emphasis on the issues of *Competitive Growth in the Global Environment* : agro-industry and integrated sectorial development in areas of special socio-economic importance, including clean technologies in agriculture and industry; on *Cultural Heritage* : S&T research for the knowledge, conservation and valorisation of cultural heritage (traditional knowledge) and its contribution to human sustainable development, connecting it to education but also to economic activities such as sustainable tourism. In addition, those certain cross-cutting areas were discussed as a 6th priority theme, with emphasis on the establishment and the strengthening of *innovation* capacities in those above mentioned specific domains and finally education & training of *human resources* (academic or not) including trans-national and inter-sectorial mobility.

Preparatory discussions with regard to the final examination.

Outlook, Summary and Policy Recommendations:

Most universities in developing countries show an obvious lack of modern laboratory equipment. However, it is our considered professional observation, that this lack of equipment is accompanied by a significant low level of academic education in life sciences. The general students "scientific" knowledge does not exceed the knowledge of European laboratory technicians. Prejudices with regard to biotechnological applications prevail, matters of environmental biosafety, different security steps of setting free (genetically modified or manipulated) microorganisms are simply not known (laboratory experiments, controlled longer term green-house experiments, controlled long-term free-land experiments, etc.). Scientists being also in the position of University teachers, having recently applied large-scale field experiments using newly composed bioregulators against varmints, are completely astonished by the simple question if a proof against human pathogenicity of its constituents was realized before or if its potential impact could also harm symbiotic or other insects. Until today in most latinamerican countries are no respective instructions or even general guidelines regulating those issues of biosafety available. We realized the fact, that academic University Personal, having been trained also in Europe or the United States by means of academic exchange programs show a clearly higher level of expertise. Unfortunately those University teachers often have the itch to leave their country of origin given the limited opportunity to really apply their know-how and the exhausting costs and personal efforts to make things change. Those who decided to come back and stay and took over departments or even chairs show a definite long-term impact on their students and show a high potential as solid partners in the future.

In order to change the situation for the better, there is the need of adaptation of the respective national university education programs (specific policy recommendations to national ministries of education) which may lead to the elaboration of a specific teaching plan which covers not only the needs of fundamental academic education (e.g. courses in English should be obligatory to all the students of life sciences, where all important scientific publications are only performed in English) but also covers the tasks of national concern. It is suggested to strengthen international, national, regional university initiatives (such as the realization of specific international scientific conferences in developing countries, international workshops and courses at universities of developing countries, the exchange of university teaching staff, etc.), to take measures of strengthening existent or initiating new interdisciplinary relationships with other (European) universities, etc.

Having focused on the declarations of intent published in the documents of the several presidential conferences between Latin America, the Caribbean and the European Union (ALCUE), the idea of a stronger collaboration in academic and private research projects emerged early and was intensified throughout the last years. This interest does not only focus on the field of biodiversity conservation but primarily in investigating its economic potential.

Therefore we give the policy recommendation to focus on the support of academic education (performing workshops and courses in the relevant fields) in latinamerican partner institutions which are interested in becoming participants in FP7 as a accompanying measure to such research projects

searching for applications of life sciences. Given that the European Union generally holds a very high level of academic expertise in the relevant fields and already has announced its interest to become a stronger partner of Latin America and the Carribean, the latter shows a highly visible biodiversity but the urgent need of the theoretical and practical know-how. Acknowledging the results of the present Specific Support Action of the MICRODIV 2 workshop and course it has to be outlined, that it only was a first step in the correct direction. However, due to its defined short time-frame the impact of the SSA is limited. On the other hand ALPHA Programs tend to focus only on theoretical University seminars and expert exchange, which may appear far too narrow, given that safe laboratory work has to be trained practically.

On the other hand there is the utmost need, to apply internationally agreed guidelines of biosafety, risk assessment, risk regulation and -management by experts and to promote the practical legal implementation of the Cartagena Protocol on Biosafety on the different levels in those latinamerican countries, which already ratified the protocol.

## (5) Capacity building impact of the project

Throughout the 3 weeks of the practical course the Team of instructors educated 32 students from the Universidad Autónoma Gabriel Rene Moreno in the contents as reported under point 4.

| Fig.24: Programming the PCR machine | Fig.25: Inoculation of bacterial isolates |
|---|---|
|  |  |
| Fig. 26: Prof. Wingfield gives a lecture on fungal diversity | Fig. 27: Evaporation of plant extracts for non-choice artificial diet feeding assay |
|  |  |
| Fig. 28: Students writing the final exam | Fig.29: Happy with the diploma |
|  |  |

During the two workshop sessions 9 national Bolivian and 11 international scientists gave presentations of their scientific work to an audience of ~150 local scientists and students. Also the participation of governmental representatives, academic representatives and private enterprises gave the MICRODIV 2 event a representative frame.

The intense participation of institutions from, the UAGRM and UNAMAZ during the activities performed during MICRODIV 2 enabled the dissemination of the research and education activities throughout the Latin American UNAMAZ network.

The performance of the PRACTICAL COURSE and WORKSHOP in the facilities of the Universidad Autónoma Gabriel René Moreno (UAGRM) in Santa Cruz de la Sierra was also recognised by the public because of the participation of Antonio Gonzales, Alicia Caceres, Birgit Zehetmayer and Guenther Ellersdorfer in a life Television interview in a local Santa Cruz University television broadcasting enterprise.

## (6) Sponsoring activities

During the preparation period for the MICRODIV2 event, Amazonia Network was approaching several enterprises in Austria and Germany. The following list shows the sponsors which were donating consumables, chemicals, media, DNA extraction kits, PCR purification kits, API tests, enzymes, dNTPS and buffers for PCR, Micropipettes and plastikware. All this donations were used to support the Santa Cruz laboratories in "El Vallecito"at the UAGRM, which are "traditionally" poorly equipped with consumables due to very high prices at the local suppliers, or its is even impossible to purchase e.g.:specigic enzymes or fine chemicals in Bolivia.

### List of Project Sponsors

| | |
|---|---|
| bioMérieux Austria Industry<br>Eduard Kittenberger Gasse 97,<br>1230 Wien, Austria<br>www.biomerieuxx.com | |
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Responsible for the elaboration of the report

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