THE VIRTUAL BIODIVERSITY INSTITUTE

OBJECTIVES

The common objective of the BIOTA/FAPESP research projects is to study the biodiversity of the state of São Paulo seeking: a) to understand the processes that generate and maintain biodiversity, as well as those that can result in its harmful reduction; b) to standardize sampling, gathering and making information relevant to biodiversity conservation and sustainable use, available to decision makers; c) to ensure fast and free public access to this information; d) to improve teaching standards on subjects related to conservation and sustainable use of biodiversity (www.biota.org.br). These are not modest goals. The State of São Paulo has an area of approximately 250,000 km^2 covering a large variety of ecosystems, each with very rich biodiversity. This variety is due to the fact that the State is located in a transiction area between a tropical and sub-tropical region.

THE VIRTUAL BIODIVERSITY INSTITUTE

After some six years, there are now eighty five major research projects being developed within the framework of the BIOTA/FAPESP Program. All major Universities (USP, Unesp, Unicamp, UFSCar), Research Institutes (such as the Botanic Institute, the Forestry Institute, the Geological Institute, Inpe), Embrapa Centers and NGOs (Social Environment Institute, SOS Atlantic Tainforest Foundation, Conservation International and CRIA) are taking part in the program. Considering only the researchers linked to these institutions within the State os São Paulo, there are approximately five hundred with a PhD or higher, and six hundred university graduates. There are a further ninety participants from other Brazilian States and roughly fifty participants from abroad.

The research projects linked to the Program are being developed to increase the academic knowledge on the State of São Paulo's biodiversity and to simultaneously produce results that can be use to improve State policies on biodiversity conservation and sustainable use. For this purpose, it was necessary to associate projects aimed at improving biological knowledge with projects seeking to make economic use of certain species.

The program uses the biodiversity definition established by the Convention on Biological Diversity (www.biodiv.org), i.e., biodiversity is the variety of living organisms – fauna, flora and microrganisms – from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity of both genes and populations of either one or several species, as well as the interactions between species and the ecosystems.

All projects are linked through the Environmental Information System of the BIOTA/FAPESP (http://sinbiota.cria.org.br). Developed by the Reference Centre on Environmental Information (CRIA) in collaboration with the State University of Campinas (Unicamp). This system is integrated with an eletronic map base (scale 1:50,000) of the State (http://sinbiota.cria.org.br/atlas), which was digitized by the Forestry Institute of São Paulo using ArcInfo/ArcView. All information is freely available on the Internet and is starting to be used by the State branches in charge of planning its economic development. SinBiota was developed in PostgreSQL using XML and XSLT protocols and na Intel/Linux server.

Last but not least the Program has also launched an eletronic peer reviewed journal, BIOTA NEOTROPICA (www.biotaneotropica.org.br), to publish research results relevant to biodiversity classification, conservation and sustainable use in the Neotropical region, obtained within the framework of the BIOTA/FAPESP Program or not.

As to future developments, the next step is to integrate data produced through the Program with existing information in biological collections (museums, herbaria and microbial culture collections). A distributed information system is being developed to ensure that data remains with each custodian. Inter-operable protocols and standards are being studied and tested. With historical data and standardized sampling associated to a map base, there are a number of tolls that may be developed. At the moment there is work taking place in collaboration with the University of Kansas on geographic distribution models using GARP (Genetic Algorithm for Rule-set Production).

An enormous task to be addressed, concerns authoritative taxonomic information. This is fundamental for data input and for searching purposes. There are a number of international iniciatives that are aiming at implementing a complete catalogue of valid names (including synonyms and common names) and the BIOTA/FAPESP information system intends to collaborate with these initiatives. Examples are GBIF, and the Catalogue of Names (www.gbif.org), Species 2000 (www.sp2000.org) and ITIS, the Integrated Taxonomic Information System (www.itis.usda.gov).

The publications in high level scientific journals are the outstanding point of the BIOTA Program. Up to September 2008, about 800 papers have been published in indexed high impact journals, all available at the Program´s website, highlighting the publications involving researchers from different institutions. In 2006 and 2007, two papers were published at Science and two at Nature (Attachment 1). In 2007, the BIOTA Program has made a great effort to encourage the publication of scientific papers from its several projects. As a result, we published 12 papers, besides scientific notes, reviews and point of views at Scientia Agricola, indexed by INIS and other databases, in volume 64, n 4, from July and August 2007 (www.scielo.br/scielo.php?script=sci_serial&pid=0103-9016&lng=en&nrm=iso).

In 2006 and 2007 many projects were concluded and now their results are to be published at high impact journals.

These concluding projects are also resulting into new projects requests, supporting the knowledge gaps identified during initially developed projects. An important fact during this period was the consolidation of the BIOprospectTA program, with great development within the last years, through the approval of many projects and research support funds.

Regarding the management of BIOTA program, during 2006 and 2007 three main aspects have been focused:

1. Availability of the program's database aiming to support the elaboration of environmental state public policies. For this matter the BIOTA program and other institutions have promoted the "Workshop of priority areas for conservation and restoration in Sao Paulo State", which has resulted on a book entitled: "Baselines for Sao Paulo State's biodiversity conservation and restoration", in press (Attachment 2). This book counts with the participation of over 150 researchers from Sao Paulo State, involved with several subjects.

2. Institutionalization of the BIOTA program. FAPESP's scientific board along with the Program Coordination has defined mechanisms that promote the program's institutionalization for benefited institutions during these nine years. This was thought in order to perpetuate the program and provide a minimum basic infra-structure to support journal management (BiotaNeotropica), database's update (SinBIOTA), help the projects coordinators etc. On November 2007, a partnership was established among three Sao Paulo Universities (USP, Unicamp and Unesp) and FAPESP in order to institutionalize the BIOTA Program during 2008, as shown in the attached documents (Attachment 3).

3. Internationalization of the BIOTA Program through researcher's participation in international events and through knowledge exchange with other countries programs sharing the same purposes. With this objective, the BIOTA Program organized the "Brazilian Nature: mystery and destiny" expo at the Berlin Botanical Museum, on May 2008 (www.fapesp.br/publicacoes/braziliannature).

The BIOTA Program ongoing project abstracts are presented below. They show the variety of research themes developed and the strengthening of the current bioprospection subject. This has always been one of the BIOTA Program objectives.

STUDY OF METAL DISTRIBUTION IN THREE BIOME COMPONENTS OF ATLÂNTICA FOREST: ANT-PLANT-SOIL

André Fernando de Oliveira

OBJECTIVES

Determination of richness and abundance of ants along a transect surrounding of Serra do Itapeti.

Determination of distribution profiles of ants in Atlântic forest with different antropization level and metal contamination: (i) *C. rufipes* (Formicidae: Formicinae), a polymorphic specie; (ii) *S. saevissima* (Formicidae: Myrmicinae). These distribution profiles will be correlated for metal level in plants, soils, nests, and season (raining and dry).

METHODOLOGY

The samples were collected in surroundings of Serra do Itapeti- Mogi das Cruzes/SP, an important area of Atlantic forest between Serra do Mar and Serra da Mantiqueira. A transect through the sampling points represents an antropic activity gradient (Table 1). The last sample site (point 7 – Serra do Itapeti) has the lowest antropic impact while the Point 4 shown a highest one. The later is situated in an area near to Tiete river, where a steel industry worked by 40 years.

Table 1 – Sample site of ants, nests, plants and soil, in Mogi das Cruzes city

Point	Local	Geographic coordinates	Surrounding
1	Parque Leon Feffer	S 23º 32.049'W 46º 14.225'	without vegetation
2	Centro Esportivo Colégio Joana D'Arc	S 23º 31.049'W 46º 13.225'	degraded forest
3	Kosmos Clube	S 23º 30.846'W 46º 12.731'	degraded forest
4	Parque Nagib Najar (COSIM)	S 23º 31.109'W 46º 12.836'	underbrush
5	Estrada Perimetral	S 23º 30.514'W 46º 12.036'	underbrush and degraded forest
6	Área Urbana na Serra do Itapeti	S 23º 30.261'W 46º 11.900'	without vegetation
7	Parque Municipal Francisco Affonso de Mello	S 23º 29.225'W 46º 11.555'	forest with different levels of regeneration

For study of richness and abundance of ants, the samples were taken using pitfall traps due scarceness leaf litter in some places. Each trap had a opening of 9 cm in diameter and 11 cm in height of 11 cm and was buried at ground level and 20 m far one of other. The traps are being kept in ground for 7 days and the specimens were preserved in 70% (v/v).

For obtain a distribution of head capsules sizes of *C. rufipes* and its metal profile, the entire area of *C. rufipes* nests above the surface of the soil, composed of small sticks and leaves of *Poacae*, was collected in the afternoon during the dry season at selected sites (*Table 1*) and then frozen. Afterwards, all of the ants were separated from the interior of the nest.

Ants from each nest were separated in several groups, with 0.2 mm interval head capsule sizes and the frequency distribution was obtained.

Each group was submitted to chemical analysis for metal after cleaning: in ultrasound bath with 5% Extran detergent (Merck). Ants were dried at 105° C for 24 h and ground before 0.4-0.5 g of dried sample was digested with concentrated HNO_3 (and H_2O_2) in a PTFE-closed vessel in a Milestone Ethos Plus microwave workstation (Silva, 2006). were analyzed using acetylene-air flame, and Ca, Mg, and Al were

determined using a nitrous oxide-acetylene flame (Welz, 1985).

The soil fertility analysis was done with procedures described by Raij et al. (2001).

The elements Cu, Cd, Mn, Fe, Ca, Mg, Al and Zn in soil, nest and plants also were analysed using a flame atomic absorption spectrophotometry (3110 Perkin Elmer) (Silva, et al. 2006; Welz, 1985; Kingston & Haswell, 1998).

The results of metal profiles will be submitted to Principal Component Analysis (PCA) or Factor Analysis (Massart et al., 1988; Alfenas, 1998; Brereton, 2002) using the program StatSoft Statistic (1993) while the richness and abundancy of species will be giben by species accumulation curves and the Chao 2 estimator curves, with EstimateS software.

RESULTS AND DISCUSSION

This project was approved in august of 2007. Until now, the samples of *C. rufipes* in raining season from sites in transect were collected. The dry season samples will be collected in july-august/2008.

The specimes in nests from Serra do Itapeti and Tiete Ecological Park were selected by head capsule sizes. Besides those nests, another from Serra do Itapeti are going selected.

Furthermore, it is important to strengthen that the head capsules measures expend a great time and this step must be previous to metal chemical analysis.

The frequency histogram of each nest was obtained. There are similar distributions between dry and raining seasons in same site (Serra do Itapeti and Tiete Ecological Park). Therefore, it is observed a little difference in major groups. A statistical multiple non-linear regression are being used to obtain different groups.

The metal concentration is being determinated in each class of head capsule sizes in all nests collected. By this way, the ants will be separated in groups using factor analysis.

A new procedure will be used with other nests in other sampling points: after to collect the nest, individuals from only four classes will be separated and counted initially classes in range of 1.4 to 1.5 mm; 1.7 to 1.8; 2.1 to 2.3 mm e 3.3 a 3.4 and metal concentration in each class will be determinated. The other twenty classes will be separated subsequently for frequency histogram. We believe these former classes would describe each group in nest.

The soil fertility parameters in each sample point were analyzed. The determination of total metal concentration in soil will be finished before july/08. Additionally, the analytical chemistry lab (LaPeQ) is participating of a IAC Soil Analyses Proficiency Program with intention of guarantee the quality of analytical results.

This work will be able to quantify metal levels on Formicidae while evaluate the correlation between these metal level and those in soil and plant associated for an specific taxon. Besides, we will search a correlation between the richness and soil physicochemical parameters

Once those areas are in Atlantic forest biome but surrounding by a urban net that are growing up very fast, the results of this work will help in the biodiversity monitoring in other similar places.

Prof. Dr. André Fernando de Oliveira
Profa. Dra. Astréa F. Souza Silva
Profa. Dra. Maria Santina de Castro Morini

Pró-Reitoria de Pesquisa, Pós-Graduação,
Extensão e Assuntos Comunitários
Universidade de Mogi das Cruzes (UMC)
ferqa@umc.br

FAPESP 2006/52409-6

EDUCATION, SCIENCES AND CULTURES: TERRITORIES IN BORDERS OF PROGRAM BIOTA-FAPESP

Antonio Carlos Rodrigues de Amorim

In this project considering curriculum as cultural text, we discuss relationships between science education and the pedagogical potential of photographic images and television as well as popular scientific treatment of biodiversity that have been produced by the Biodiversity Program of the State of São Paulo, supported by a grant from FAPESP. On the movements of production of new knowledge, methodologies, practices of investigations and intervention in the reality, the projects of the BIOTA program want to carry through changes, interferences in the scope of the scientific community and in its relations with other cultural fields (for example, the technology, the education, the communication and science popularization, amongst others). It assumes explicit positions to differ from more traditional practice of inquiry, and presents sciences of "biodiversity" integrated in nets of knowledge, spaces (virtual, also), times (interrogating classical methodologies of living beings classification, for example) and experiences.

Discussions regarding biodiversity, in various programs that it aims at, for example, education-related activities, is given a special highlight when measured in its ethical and constituent aspects of nature representations, of human beings and of "sustainable relations". More radically, one could imagine that the discourses on environmental education, backed on sustainability, are all about the relations between human beings and nature, in a constant initiative to overcome/or to evaluate the impact, in general destructive, of these relations. Scientific knowledge, in its educational purposes, is associated with values that seeks, in the elaboration of education curricula, to modify and produce a different citizen/subject. It is exactly in this territory of the production of the *identities for the human*, when it is being discoursed on the environment, that the third set of investigating possibilities of the program BIOTA-FAPESP comes into play. It consists of the perspectives of environmental conservation and preservation related to an extensive characterization of the biodiversity in the State of Sao Paulo and the evaluation, with scientific parameters, of the environmental impacts which have occurred in the State of São Paulo.

RESULTS AND DISCUSSION

The publication *Olhar amplo sobre a biodiversidade* (in English, "Biodiversity at a glance") was released in 2006 and has as its main objective to publicise, through texts and images, some of the results of projects on biodiversity funded by FAPESP, and highlights the Biota-FAPESP Program. The *looks* in this publication as well as in other images published in the Magazine *Pesquisa FAPESP* (FAPESP Research) in articles on Biota-FAPESP projects are similar to the looks of the taxidermised animals in the film La ville le Louvre, which is about the renovation of the Louvre museum in Paris. In several takes, a filming camera finds the "false" eyes of the taxidermised animals, which watch the renovation of the galleries and the museum. Attentive, anxious, frightened eyes, with expectation, looking at the demolition of the walls, windows, ceilings. Such looks are enunciated, since they represent the emission of singularities, of singular points in a corresponding space. The formations and transformations of the same spaces are important, except for their character of novelty, originality or reproduction, repetition. "What counts is the *regularity* of the enunciated: not an average, but rather a curve. The enunciated, in effect, is not mistaken for the emission of singularities that it supposes, but with the behavior of the curve that passes on its neighbor, and more usually with the rules of the field in which they are distributed and reproduced. The *enunciative* regularity *looks* of the museum, in a special FAPESP publication and in articles of science popularization, places animals and plants as subjects in the third person or as a derived function. Thus their derivative action; it is not of a first person who initiates the discourse. Rather, the discourse initiated in the invisible that the eyes see, but is not shown in the image. The enunciative looks "are not words, phrases or propositions; they are

formations that make the nature beings move a discursive structure, only when they change natures, taking the place of the 'saying', distributing themselves, dispersing in thickness in the language" (Gilles Deleuze, 2005, p. 29). This occurs, as opposed to what was expected, by the multiplicities, only when they reach the human. The enunciative look is the sensory-motor for the entrance of man in the discourse of biodiversity through images.

This insistence on the look could be a way of finding the human in a discursive invisibility. Why continue having this style of thoughts of representation?

Research Projects associated

Images in natural history and biology: senses, durations, aesthetics
Erica Speglich - doctoral student in Education

In this research project, Erica studies images that have been produced by traveler naturalists, present images that are being done by Biota researchers and images used and/or produced for artists in contact with natural sciences. We've organized a collection of images and, for so many times, these images offer us the possibility to think about relationships between paintings, registers and methodologies from natural history studies and the recent images of biology in Biota Program. The attribute of sense, significance for these images is an event because they are not more registers of what happened; they have displacements, cuts, repetitions. These images are historical (and they go to continue being) but these also can be dislocated.

The Biology and its significations of natural
Eugênia Barioni – Undergraduate Student of Biology/Unicamp

Established in 1888, the Bosque dos Jequitibás park is one of the oldest areas of leisure of the city of Campinas and its contribution to knowledge and culture of the city is evident. However, the plurality of discourses that were articulated there during almost all century XX does not exist today. In this assay, we look for to discourse regarding the changes that had occurred in the Bosque dos Jequitibás park, which, currently, receives about a million people for year, approaching an only speech: biology. In the attempt to understand how this new approach has been delineated, some questions had appeared, inside important contexts. The spaces had also changed, or only the discourse? How the visitors can perceive the changes? Is Bosque dos Jequitibás a natural environment? Being a naturalized environment, do contrasts to this biological speech exist or it is totally linear? The search of these answers understood interviews, field registers and images analysis.

Images and School Curriculum of Biology
Marcelo Ananias and Mario Ferreira – Biology Teacher and Undergraduate Student of Biology

Some projects of the Biota/FAPESP Program, which compose the set of discoursive productions of the research to which this research is associated, as it produces senses to nature, men, culture and science, are a field of great interest to think education in Biology, also because they produce thoughts which very quickly suit the logics of stability, the selection of contents as well the relations of power which are historical in the curriculum of Biology in Brazil. In this study, aiming at breaking up with this stability, relationships are sought between curriculum and images of nature produced by researchers of different projects from the Biota/Fapesp Program and/or published in printed material, audiovisual and on the Internet. In them, relationships between biodiversity and public policies, sustainability and conservation, bioprospection and goods are addressed. As an initial step of this research, the Photographic Exhibition – Biota – Biodiversity of the State of São Paulo – Colors and Shadows was performed with high school students of the state public network for the purpose of analyzing the cultural representations which take part in the games of significances of nature in class situations.

Relationships between nature-culture present in the "Herbarium of Artificial Plants" by the Colombian plastic artist Alberto Baraya

Valdemir da Silva - Undergraduate Student of Biology

Since the second half of the 20th century, culture has broadened its spectrum of action within the social practices and traditional disciplines, and this "cultural turnaround" has been shaking the central paradigm of modern science, which is the classical nature/culture separation. As a criticism to this view, in the plastic arts, the "collectionist impulse" has lead artists like the Colombian Alberto Baraya to deepen into the possibilities of production/manufacturing of nature with his work "Herbarium of Artificial Plants". In this context of great discussion about the thin boundary that separates nature (science) and culture, a collection of quotations by this artist has been made digitally, published by several magazines between December 2005 and August 2007, as an attempt to investigate the possible relationships between nature/culture present in Baraya's work. By reading the textual fragments of the interviews with the artist, it is possible to detect different groups of what can be "impulses which move the artist", such as the deconstruction of the naturalist's image, and consequently of the discourse of science. With this, Baraya makes clear the reflective character of his work: his collections are not only aimed at being seen, but also at being thought, analyzed and investigated.

Exhibition 'Dispersed Fragments: Biodiversity images of the Biota-Fapesp Program'

Undergraduate Students of Biology: Aline Rosa Maia, Danilo Fogaca de Macedo, Karla de Abreu Barbosa, Leticia Paiva Silveira, Samantha Maia Meireles, Priscila de Arruda, Vinicius Umaki Morita, Carolina Tognetta Minozzi , Valdemir da Silva, James Alexandre Martins

Secundary Teachers: Terezinha Chagas Carneiro Pessoa and Valquiria Regina de Paula

Visual Plastic Artist: Gustavo Torrezan

Ph.D. Students: Erica Speglich and Alda Romaguera

Go through the curtain and find the biodiversity images of the State of São Paulo. Inside laboratory bottles, cupboard shelves, windows and in the projections of the eyes on the ceiling, the diversity of live beings can only be contemplated through light dispersion. Use a torch to guide your way. The images of nature in this room-laboratory-installation are arranged as a collection of fragments which the visitor can meet and explore. Measuring and quantifying nature are other senses of biodiversity potentized by the utilization of bottles and other material of a scientific laboratory. Exhibition set up and presented to the public in a laboratory of the State Center of Adults Education "Paulo Decourt".

Prof. Dr. Antonio Carlos Rodrigues de Amorim

Faculdade de Educação
Universidade Estadual de Campinas (Unicamp)
acamorim@unicamp.br

FAPESP 2006/00752-9

Grants

MORPHOLOGY AND ANATOMY OF VEGETATIVE ORGANS AND CHEMICAL PROFILE OF *Smilax* L. SPECIES (SMILACACEAE)

Beatriz Appezzato-da-Glória

DESCRIPTION

The aim of this study is to describe the morphology and anatomy of aerial and underground organs of eight species of *Smilax* L., to carry out seeds germination studies to analyze the viability of producing, to analyze the ontogenesis of the underground system of *Smilax polyantha* to explain establishment of secondary structure, to carry out cytogenetics studies and to analyze the chemical profile of *Smilax* species. The genus is known in alternative medicine because of its anti-rheumatic proprieties. The characterization and definition of chemical and anatomical peculiarities of these species could be helpful in identifying the species commonly known as salsaparrilha and greenbrier and avoid the taxonomic confusion that occurs among the species of this genus. For structural analyzes, histological blades are being mounted with sections of vegetative organs obtained through rotary microtome after fixing, dehydrating and infiltrating the vegetal material into synthetic resin. The results are registered through photos and botanic illustrations. To determine the chemical profile, extraction with organic solvents, chromatographic separation and purification and structural identification using spectrometric techniques were performed. Seed germination studies and analysis of rooting and sprouting of buds on stem cuttings are carried out aiming to study the vegetative propagation potential of these species and, consequently, to provide information on its sustainable use.

OBJECTIVES

a) To describe the morphology and the anatomy of aerial and underground organs of eight species of *Smilax* L. The species preliminarily chosen are: *Smilax brasiliensis* Sprengel, *Smilax campestris* Grisebach, *Smilax cissoides* Martius ex Grisebach, *Smilax fluminensis* Steud, *Smilax rufescens* Grisebach, *Smilax oblongifolia* Pohl ex Grisebach, *Smilax goyazana* A. De Candolle and *Smilax syphilitica* Humboldt & Bompland ex Willdenow; b) To carry out seed germination studies to analyze the viability of production; c) To analyze the ontogenesis of the underground system of *Smilax polyantha* Grisebach to explain the secondary structure establishment; d) To carry out comparative karyotype analysis of *Smilax* species; e) To analyze the chemical profile of the *Smilax* species and f) To supply subsidies to reformulate the chapter of salsaparrilha in the Brazilian Pharmacopeia.

METHODOLOGY

The studied species (*Table 1*) were identified by the specialist Prof. Dr. Regina Helena Potsch Andreata and exsiccates were registered to the "Luiz de Queiroz" College of Agriculture Herbarium (ESA) of the University of São Paulo (USP).

Table 1. Studied species, locality and date of collection

Species	Locality of collection	Date of collection
Smilax brasiliensis Sprengel	Itapagipe - MG	Dec 2005, April and Dec 2007
Smilax campestris Grisebach	Porto Alegre - RS	Jan and Dec 2007
Smilax cissoides Martius ex Grisebach	Feira de Santana - BA	July 2006 and Jan 2008
Smilax polyantha Grisebach	Pratânia - SP	Dec 2005 and April 2006
Smilax goyazana A. De Candolle	Rubiataba - GO; Chapada dos Veadeiros - GO	Jan 2007 and Dec 2007
Smilax oblongifolia Pohl ex Grisebach	Ouro Preto - MG	May 2007 and Jan 2008
Smilax rufescens Grisebach	Ilha do Cardoso - SP	March 2006, May 2007, Jan 2008
Smilax fluminensis Steud	Itirapina - SP	Jan and May 2008

For the anatomical study, samples were fixed in FAA 50 (formalin-acetic acid-alcohol) or in Karnovsky solution, dehydrated in a graded ethylic series and then infiltrated in glycol methacrylate resin. Serial sections (5-7 µm thick) were cut on a rotary microtome and stained with toluidine blue O (Sakai 1973). Freehand cross-sections were also cut and stained with astra blue and basic fuchsin and then dehydrated in a graded ethylic series, and 50 and 100% butyl acetate, respectively. Permanent slides were mounted in synthetic resin. Images were captured digitally through a Leica DMLB microscope with a video camera attached to a PC, using IM50 image analysis software. The morphology of the vegetative organs was also registered through photographs and botanical illustrations.

The seed germination experiments were divided into two stages. In the first stage a completely randomized experiment involving six treatments (15-35°C, 20-30°C ,20-35°C, 20°C, 25°C and 30°C) with four replications were used for *Smilax polyantha*. For each replication a germination box (11 cm wide by 3 cm high) with cover was used, where 292g of sand was added, containing 45mL of water (60% of the restrained value) and 20 seeds. Based on the results of the first stage, a completely randomized experiment involving two treatments (20-30°C and 30°C) with four replications were used for *Smilax brasiliensis*, *S. campestris*, *S. cissoides*, *S. polyantha* and *S. rufescens*. All treatments were exposed to daily photoperiod of eight hours of light or in the dark. For each replication a germination box (11 cm wide by 3 cm high) with cover was used, where 292g of sand was added, containing 45mL of water (60% of the restrained value) and 20 seeds. Cytological preparations of root tips with high frequency of metaphase cells showing clear features of chromosome morphology were carried out. For the accumulation of metaphases, as well as prometaphases with well condensed chromosomes, pretreatments of root tips with different combinations of 8-hydroxiquinoline, a mitotic fuse inhibitor and with cycloheximide, a protein synthesis inhibitor, were evaluated in Feulgen stained preparations.

Chemical profile. The methanolic extracts of leaves, rhizophore and roots (30 mg) of *S. polyantha* were submitted to a clean up procedure (RP18 cartridge, eluted with MeOH 80% v/v), filtered through a 0.22 µm membrane filter and analyzed by HPLC-UV-DAD. The chromatographic profile was established using a Jasco HPLC system equipped with a Phenomenex RP18 (250 ? 4.6 mm i.d.; 4 Ìm) column and as mobile phase water (eluent A) and acetonitrile (eluent B), both containing 0.05% of TFA. The gradient program was: 5-15 % B (15 min), 15–20% B (40 min), 20–40% B (48 min), 48–100% B (55 min), at flow rate of 1.0 mL/min. Identification of compounds was performed by retention time and by co-injection with standard compounds under the same conditions.

PRELIMINARY RESULTS

The anatomical and morphological studies have pointed out differences among the studied *Smilax* that could be useful for their diagnosis. Some characters like the frontal view of the epidermis cell wall, the stomata occurrence, the epicuticular waxy deposition and the mesophyll vary among the species. The root analysis had also shown that the parameters described in the Brazilian Pharmacopoeia are insufficient to differentiate the *Smilax* species.

The seeds germinated after 20 to 40 days. In *S. campestris*, *S. cissoides* and *S. rufescens* the germination percentage was more than 80%. However, in *S. brasiliensis* and *S. polyantha* the germination percentage varied from 10% to 20%. The analysis of the development of seedlings and the underground system has being carried out since December, 2006, when the beginning of germination was observed in both experiments. Ten seedlings of each species are being analyzed, photographed and fixed in the following stages: seedlings newly germinated seedlings with expanded eophyll, young plants with four protophyll and plants under one year of age. The development of *Smilax polyantha* Grisebach, through seed germination, starts with the emergence of the primary root and with the development of the cauline axis originated from the plumule. In plants under two months of age, the eophyll, the first metaphyll and the thickening of the cauline branches base are observed. In field gathering, it was observed that the underground organs of *Smilax* species have brown coloring and rigid texture, about ten centimeters of length, horizontal orientation. Erect shoots and adventitious roots arise from knotty and tuberous rhizophores approximately 3-7 cm thick.

The chromosome number 2*n*=32 was observed in *S. polyantha*, *S. rufescens*, *S. fluminensis* and *S. cissoides*. It was not yet possible to obtain a good preparation for *S. brasiliensis*. Variations among the species were observed in their karyotype. In *S. polyantha*, *S. rufescens*, and *S. cissoides* the karyotype was asymmetric with chromosome differences in size. The larger chromosomes have subterminal centromeres, while most of the smaller ones are metacentric or submetacentric. *S. fluminensis* has also assymetric karyotype, but different from the other species especially due to the presence of a large metacentric chromosome. Apparently there are a higher number of chromosomes with median centromere position in this species.

Identification of compounds was performed by retention time and by co-injection with standard compounds under the same conditions. Data were monitored at 254 nm for general aromatics and 360 nm for flavonoid derivatives. This approach led to identification, mainly, of phenolic acids and flavonoids in the extracts. These results could explain the biological properties described to some *Smilax* species.

The anatomical and chemical characterization of medicinal plants is very important for the quality control of the raw material used in the making of phytotherapics and in the elaboration of new Pharmacopeia monographs. This project is integrated with another project coordinated by Prof. Dr. Wagner Vilegas whose process number is 2002/05503-6.

Profa. Dra. Beatriz Appezzato-da-Glória

Escola Superior de Agricultura "Luiz de Queiroz"
Universidade de São Paulo (Esalq/USP)
Home Page: http://www.lcb.esalq.usp.br/lav/smilax/indexenglish.php
bagloria@esalq.usp.br

FAPESP 2005/58964-9

BIODIVERSITY OF ATLANTIC RAIN FOREST CONTAMINATED WITH HEAVY METAL: AN APPROACH FOR BIOREMEDIATION

Elisa Espósito

OBJECTIVES

Evaluate the biodiversity of plant, animals (insects and amphibs) and microrganism from an area of Atlantic Rain Forest contaminated with heavy metals. Also, these results should drive the establishing of a procedure for recovering of this polluted area.

RESULTS AND DISCUSSION

The Atlantic rain forest, in the past covered 1.2 million of km^2, but actually this forest was reduced to 8% of original area and, due because of this is considered the one of the most endangered ecosystems worldwide. The environmental degradation is associated to waste releasing by domestic and industrial sources, and the accumulation of these pollutants is associated with global demographic growth. The increasing in mining and industrial activities has changed the biogeochemical cycle and flux of heavy metal, resulting in more releasing of this elements in the environment (Soares et al. 2002. Diagnóstico e reabilitação de área degradada pela contaminação por metais pesados. In: Simpósio Brasileiro de Recuperação de Áreas Degradadas, Lavras, p. 1-7).

In Brazil there are few studies that evaluate the impact of these metals in environment, as well as studies about recovering of polluted area (Soares et al. 2001. Acúmulo e distribuição de metais pesados nas raízes, caule e folhas de mudas de arvores em solo contaminado por rejeitos de indústria de zinco. *R. Bras. Fisiol. Veg.* **13 (3)**: 302-315), mainly in area such as Atlantic rain forest. For this the determination of the metal concentration is necessary for interpretation of biologic effects, such as toxicity at different trophic level in the ecosystem.

Therefore, the main objectives of the present project is evaluate the interaction between biotic and abiotic components of the environment, and develop a strategy to recovering the impacted area, as well as the recovering of the biodiversity. In this report, the partial results describing the biologic and physical and chemical characterization of the studied area are shown. Also, the microbial, fauna and flora species observed in the polluted and unpolluted points in such area were identified and correlated with the contamination level. However, using these preliminary results, correlation between species and the environment was not observed indicating that more studies should be done to allow positive interference for recovering this area.

The studied area is localized in Mogi das Cruzes, SP (Parque Municipal "Nagib Najar") and present typical Ombrophyle dense forest. In this place, the level of metals was evaluated from 8 points, which showed differences among points, allowing the clustering of the sampling points. Aiming to evaluate the impact of this pollutants, the contamination level was considered according to pattern defined by CETESB (Casarini et al., 2001, Relatório de Estabelecimento de Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo. São Paulo. Série Relatórios Ambientais, CETESB). The total level of Ca, Mg, Fe and Al was lower than that established by CETESB, However, Cu, Zn and Cd were found at higher concentration than that the pattern in some points, such as point "8" (Cd 0,1; Cu 0,5; Cr 4,0; Zn 6,5; Ni 0,5 mg.g-1), and point "2", which are higher than security level. The bioavailability of the metals fluctuated randomly in such points and was different, as expected, from the total level.

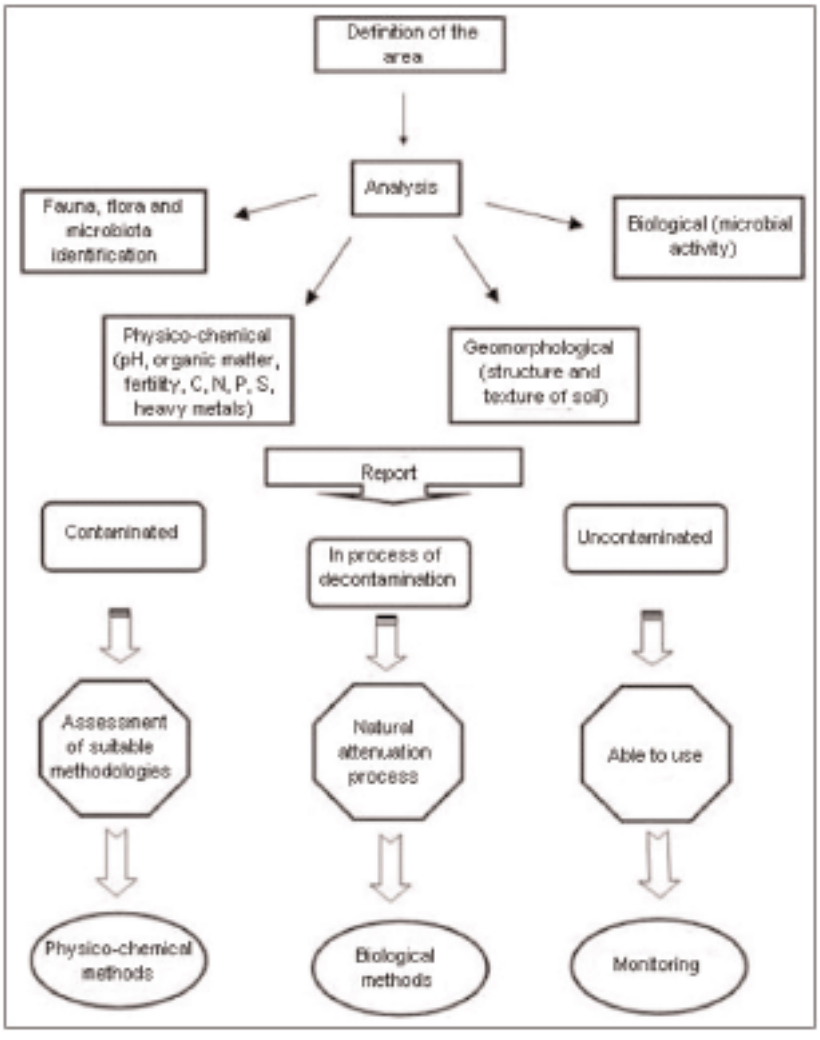

Figure 1. Proposed methodology for determination of the level of environmental impact

The analysis of vegetation showed that in some points the plant species suggest that different succession stages is present, showing in this polluted area species of Poaceae and Cyperaceae family, also in some points an arboreal composition is observed. In perturbed fields are frequently observed species belongs to pioneers families, such as Asteraceae, Cyperaceae, Poaceae, Tiliaceae and Verbenaceae. The partial results showed that 36 famillies with 184 species is present in the studied area, and that the most richness families are Asteraceae (34 spp.), Euphorbiaceae, Fabaceae and Verbenaceae (7 spp.). This result suggests the dominance of the arbustive species, which is associated to initial succession in the most of sampled area.

Eight points, which cover almost all polluted area, were evaluated according to animal presence. For Formicidae all samples were done, including rain and dry season. However, for Amphibia sampling in rain season were not done, since the presence of such group in some area was not detected. The results is still preliminary for both taxa, but for Formicidae, during dry season, 18,743 specimens, belonging to 7 families, 20 genera and 43 species was observed, being the estimated richness (Chao 2) of 45 species. *Camponotus rufipes* (11.93%) was the most frequent group, followed by *Gnamptogenys striatula* (7.82%) and *Camponotus* sp.5 (6.38%). Among these sampled taxa, the presence of poneromorph species (15.84%) was detected. Taking in mind that this group has specialized habits, and due this needs structured environment for nesting and nutrition, we could suggest that this area has been natural recovering. The Shannon-Wiener (H') index and equitability were 3.24 and 0.86, respectivelly, showing na homogenous distribution of the species. For Amphibia, 8 species belonging to 4 families Bufonidae (1 sp.); Hylidae (4 spp); Leiuperidae

(1 sp.) and Leptodactylidae (2 spp), which is associated to perturbed environment, were observed.

Microorganisms from rhizosphere and plant interior (endophytic) has been isolated and further identified bay partial sequencing of 16S rDNA. These communities has frequently been studied for plant growth promotion and some studied has shown that genotypes associated with degradation of xenobiotic compounds may be selected inside the plant, when these plants are cultured in polluted area (Siciliano et al. 2001. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination applied and environmental microbiolog. **Vol. 67, nº. 6**:2469–2475). The results, although partials, showed that the diversity is high, including at least 17 genera in 42 isolates identified. The multivariate analysis will allow to identified the correlation between metal level and microbial diversity. Also, we have suggested that these microorganisms, i.g. bacteria, could be associated with specific plant species and used for recovering of polluted area.

The arboreal species native from Atlantic Forest and evaluated in the present study includ Cedrela fissilis (cedro-da-várzea), *Cecropia pachystachya** (*embaúba*), *Shinus terebintifolius* raddi (aroeira-mansa) and *Eritrina speciosa* *Andrews (mulungu). Also, 3 shrub species *Arachis pintoi*, *Hydrocotyle bonariensis* Lam*. and *Phyllanthus* sp *(quebra-pedra) has been evaluated for bioremediation.

C. fissilis, *C. pachystachya** and *E. speciosa* showed potential for bioremediation, and due this result, fungi and bacteria from rizosphere of *C. fissilis*, *C. pachystachya* were sampled. *C. fissilis* was already essayed for phytoremediation and the microbial community from rhizosphere identified. *C. pachystachya* was also essayed for phytoremediation but the identification of the microbial community from rhizosphere is still going on.

The shrub species, except *A. pintoi*, were sampled from the most polluted area, which presented the higher heavy metal level. The bioaccumulation analysis is still going on, and will provide information about which bacteria-plant species association could be used for recovering of this contaminated area

The metal level in other plant species has been done fr evaluation of the role of environmental variation on metals bioavailability. This project aim to establish a procedure for recovering the biodiversity of this area, including the vegetation close to Tietê River, but for this the biotic and abiotic characterization should be done for driving the biotechnological aspects of these approach.

Profa. Dra. Elisa Espósito

Pró-Reitoria de Pesquisa, Pós-Graduação,
Extensão e Assuntos Comunitários
Universidade de Mogi das Cruzes (UMC)
elisa@umc.br

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CHEMICAL, BIOLOGICAL AND BIOSYNTHETIC EVALUATION OF RED SEAWEEDS SPECIES FROM *Bostrychia* GENUS (RHODOPHYTA, RHODOMELACEAE)

Hosana Maria Debonsi

The project is based on studies of *Bostrychia radicans* Montagne, *B. tenella* and *B. calyptera* species. Also, as we could found *Centroceras clavulatum* (Ceramiaceae) species growing in the same region of the Bostrychia, we decided to perform a parallel study with this species (another financial support). This work has been developed at Faculdade de Ciências Farmacêuticas de Ribeirão Preto (USP), in the Laboratório de Química Orgânica do Ambiente Marinho, under the responsibility of Profa. Dra. Hosana M. Debonsi.

OBJECTIVES

In this project we intend to establish a bioguided chemical study from algae and unialgal culture *in vitro* from *Bostrychia genus*, by means of antifungal, moluscicide, antitumoral and cytotoxic activity evaluation. In this way, the next step is to analyze the secondary metabolites production aiming to establish a biosynthetic correlation in the future. The general objectives are:

1. Bioguided isolation and structural determination of secondary metabolites from red seaweeds of *Bostrychia genus*, by means of antifungal and moluscicide activity evaluation;

2. Citotoxic evaluation from extracts and isolated metabolites using Brine Shrimp lethality (*Artemia salina*);

3. Biosynthetic hypothesis establishment according to the interesting metabolite accumulated.

* The antitumoral, tripanocidal and antileishmanial activities have been evaluated as well, once we get collaborations after the Project FAPESP approval.

METHODOLOGY

Algae material collection: Samples of red algae from *Bostrychia genus* were collected in different locations as in the shore (Ilha das Couves, Praia Brava, Praia Dura, Praia de Cibatel and Praia da Fortaleza, littoral of Sao Paulo) and mangroves (Rio Escuro, Ubatuba), São Paulo state (Brazil). Part of the algal material collected was washed in marine water to remove contaminants and, after that, they were put into plastic recipients and transported (on ice batch) at the Laboratório de Cultura de Algas da Seção de Ficologia do Instituto de Botânica de São Paulo – São Paulo state. In the next step, the algae were cleaned and submitted to unialgal culture *in vitro* production. Besides, the most of material collected (stored in methanol) was transported to Laboratório de Química Orgânica do Ambiente Marinho-Faculdade de Ciências Farmacêuticas de Ribeirão Preto–USP, São Paulo state, where the material was submitted to extractions with organic solvents. The taxonomic characteristics were identified by Dr. Nair Sumiê Yokoya, a researcher connected to the Phycology section of the Institute de Botânica de São Paulo, São Paulo state, Brazil.

Extract fractionation: The extracts prepared using red algae collected from different places were submitted to chromatographic separations. The thin layer chromatography was performed using silica PF_{254} while the separations by means of chromatographic column were performed using Sephadex LH-20 (Merck) or silica. Also, the HPLC (Shimadzu, pumps system SCL-10A VP, detector UV RID-6A and injector SCL-10A VP) was used in separation metabolites and chromatographic profile establishment.

Unialgae culture *in vitro* from Bostrychia genus (Rhodomelaceae): Preliminary biological essays using *Bostrychia* Montagne indicate this genus as potential bioactive metabolite source. In this way, the complementary studies are necessary, as the unialgal culture in vitro establishment at laboratory. So, the unialgal culture become to be obtained from seaweeds collected in Ubatuba/SP. The first procedure step was to cleaning and cutting apical branches of *Bostrychia* different species. The unialgal culture establishment

was obtained using separate apical branches from each species, maintained into snap cap containing marine water and rich medium. The procedure occurs weekly and the snap caps are maintained under controlled irradiance, photoperiod, temperature and salinity. Therefore, this step will give morphological and structural knowledge about the studied algae, which will be very important to future biological studies. The work, performed in collaboration, has been done for doctorate student Cintia Erbert, sob supervision of Prof. Dr. João Luís Callegari Lopes, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (USP).

Chemical evaluation: *B. tenella* diclormethane:methanol (2:1) extract was submitted to chromatographic fractionation, using Sephadex LH-20. Seaweeds from *B. radicans* and *B. tenella* species were analyzed by HS-SPME-GC/MS. A PDMS-coated SPME fiber was exposed for 50 minutes at 60ºC to the vapor phase above 400 mg of fresh algal material contained in a 4 mL-vial. The absorbed substances were thermally desorbed (250ºC, 5 min) from the SPME fiber into the GC-MS (DB-5 column (30 m x 0.25 mm, coating thickness 0.25 µm), temperature was programmed from 60ºC to 240ºC at 3ºC/min). The compounds were identified by comparison of the retention index (RI) with literature date and library mass spectra.

Biological activity evaluation: For the biological analyses were studied populations of *Bostrychia tenella* (J.V. Lamour.) (BTP–Praia Brava, BTC–Ilha das Couves), *B. radicans* (Mont.) (BRP–Praia Brava, BRM-mangrove) and *Centroceras clavulatum* (C. Agardh in Kunth) (CCC-Praia de Cibatel, CCP–Praia Brava).

Cytotoxic evaluation: The activity was evaluated using shrimps from the *Artemia salina* (Leach) species. We performed both a positive and negative control with and solutions without samples, respectively.

Tripanocidal activity evaluation: In this experiment were used epimastigote forms of *Trypanosoma cruzi* Y-strain (cultivated in LIT medium). Profa. Dra. Regina M. B. Cicarelli, Faculdade de Ciências Farmacêuticas de Araraquara, UNESP, has been responsible to *T. cruzi* (epimastigote form) while the Prof. Dr. Sérgio de Albuquerque, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, has been responsible for tripanocidal experiments carried out using LLCK2 cells infected by trypomastigote form.

Antileishmanial activity: The bioassays were performed *in vitro* against promastigote form of *Leishmania. amazonensis*, at Laboratório de Parasitologia, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, São Paulo–Brazil under Prof. Dr. Sérgio de Albuquerque supervision .

Moluscicide activity evaluation: The bioassays have been performed against *Biomphalaria glabrata* (Mollusca, Planorbidae) under supervision of Dra. Toshie Kawano, Laboratório de Malacologia do Instituto Butantan, São Paulo, São Paulo state.

Antifungal activity: The bioassays have been performed using phytopatogenic fungi *Cladosporium cladosporioides* and *C. sphaerospermum*, under supervision of Dra. Maria Cláudia M. Young, Departamento de Fisiologia Vegetal, Instituto de Botânica, São Paulo state.

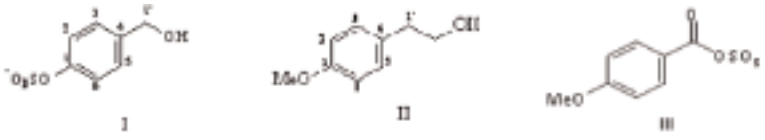
Antitumoral activity: The bioassays have been made using different tumoral cells: HL-60 and CEM (leukemia), HCT-8 (colon), MCF-7 (breast) and B-16 (melanom). The experiments are been performed by Profa. Letícia V. Costa-Lotufo, Prof. Manoel O. de Moraes and Profa. Cláudia do Ó Pessoa, at Laboratório de Oncologia Experimental do Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará.

RESULTS AND DISCUSSION

Chemical evaluation

The fractionation of diclormethane:methanol extract from *B. tenella* yield in the isolation of a new substance potassium 4-(hydroxymethyl)-benzenesulfonate (I), 1-methoxyphenylethyl alcohol (II) and methoxyphenyl sulfate ester (III). In addition, some apolar fractions from B. tenella (Ilha das Couves) were submitted to GC-MS analyses. It was observed 2-Pentanone, n-Hexadecanoic acid (majoritary), 4,6 Cholestadien-3.beta-ol, Colest-5-en-3,-ol, Neoftadiene, 3,5-Colestadien-7-one, 3-Hexene-2,5-diol, 3-methyl-

1,2-Cyclopentadienone, 4-acetylbutiric acid, 3,7,11,15-tetramethyl-2-Hexadecen-1-ol, p-Hydroxybenzaldehyde. The experiments to find peptides lead to BTC-H (hexane sample) and BTC-A (agar sample). The samples will be submitted to techniques affording discovery the peptide sequence. From apolar fraction of methanolic extract from *B. radicans* were isolated hexadecanoic and oleanoic acids, heptadecane, phytol, methyl oleate, squalene, methyl palmitate and cholesterol. Also, the profile chemical of volatile compounds from *B. radicans* and *B. tenella* were obtained by means of solid phase microextraction. The analyses were made by GC-MS. Dihydropseudoionone, (2E)-2-Dodecenal, 1-Pentadecene were observed in B. tenella, and the major volatile constituents of *B. radicans* were ,?-ionone (8.57%), 1-octen-3-ol (8.30%), tridecanal (5.46%), methyl hexadecanoate (4.69%), methyl tetradecanoate (2.76%), (E)-2-octenal (2.32%); (E)-2-nonenal, (Z)-2-octen-1-ol (2.17%) and?-ionone (1.23%). It is known that ,?-ionone has protected action against hepatocarcinogenesis by inhibition HMG-CoA. In addition,?,?,- unsatured aldehydes, for example (E)-2-octenal; (E)-2-nonenal and (E,Z)-2,4-decadienal, were good candidates for employment as antibacterial agents once these compounds caused alterations of membrane permeability properties. For this reason, the results obtained demonstrate that *B. radicans* accumulate compounds with pharmacological potential, besides this work contributed to chemical characterization of this species. From hexanic extract of *C. clavulatum* species, by means of GC-MS analyses, it was possible to identify hexadecanoic acid and 3,5-colestadien-7-one as majority compounds. The isolated compounds from *B. tenella* are showed below:



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Biological evaluation

The results of the experiments for the activity evaluation towards *T. cruzi* show that the BRM, BRP, BTP and CCC extracts presented tripanocidal activity (*Table 1*). We still point out the BTC-H and BTC-D extracts which had 100% of parasite lyse in low concentrations (0.5 µg/ml), indicating that they possess substances with high tripanocidal potential. The activities for *B. radicans* are showed in the *Table 2*.

Table 1. Tripanocidal activity evaluation, epimastigote form (% of lyse) of algae extracts

Conc. µg/ml	BRM	BRP	BTP	BTC-D	BTC-H
0	0	0	0	0	0
0,5	0	0	0	100	100
5,0	0	10.3	28.8	100	100
25,0	20.8	48.2	53.4	100	100
IC_{50}	**58.0**	**25.0**	**21.0**	?	?

Table 2- Trypanocidal (tripomastigote form) and antileishmanial activity of crude extracts from *B. radicans*

	Trypanocidal[a] IC50 (µg/mL)	Antileishmanial[b] IC50 (µg/mL)
Dichloromethanic extract	8,7	0,6
Hexanic extract	60,7	7,9
Methanolic extract	12,0	568,3

[a]Positive control: gentian violet (IC_{50}: 31 µg/mL)
[b]Positive control: Anfotericine B (ED: 304 µg/mL)

It can be concluded that extracts from *B. radicans* can be accumulate substances with high tripanocidal and antileishmanial potential, mainly the dichloromethane one, once it showed to be more potent than the positive controls. Also, the chemical analyses contributed to the chemical characterization of this species. It must be mention that there are few works about chemical composition from genus Bostrychia, justifying

this chemical evaluation. The experiments using *T. cruzi* showed the highest activity for hexane extract (containing phenolic compounds) from *B. tenella*, causing 100% of mortality with 0.5 µg/ml. and, according to *T. cruzi* bioassay, the isolated metabolites will be tested looking for pharmacological properties.

The bioassay of *A. salina* determined the cytotoxity as values of inhibitory concentration (IC_{50}-µg/ml), showing the most cytotoxic potential for the agar from BTP extract with IC_{50} of 52 µg/ml, followed by CCP and BTP extracts (IC_{50} of 278 µg/ml and 404 µg/ml, respectively). The results of *A. salina* bioassay justify future experiments to evaluate antitumoral activity. It is worth noting that the location was an important factor in the pharmacological evaluations verified, since a same species, collected in different locations, presented variations in the IC_{50} values obtained in the two activities tested.

The antitumor assays were performed using the cell lines HCT-8 (colon); HL-60 (leukemia) and SF-295 (CNS). The most active fraction, BRC-H (shore, hexane, 100µg/mL), showed inhibition tumor growing of 104.5 + 0.15 % and 36.1 + 5.0 % in HL-60 and HCT-8, respectively.

Until now, according to the results obtained concerned to unialgal culture *in vitro*, it was possible to infer that the biomass growing do not occur with the speed as hoped. As the growing data is not completely evaluated, it is not possible to conclude that the growing is due to the slow process or due to the small size. In addition, the chemical study was initiated. The material provided by field was submitted to extractions with organic solvent (methanol in proportion of 2:1 (mL methanol:g biomass) and it will be submitted to chromatographic profiles obtain, aiming to compare the results with that obtained from unialgal culture.

Furthermore, the researchers Profa. Dra. Niege Aracari J. C. Furtado, Prof. Dr. Sergio R. Ambrosio and Profa. Dra. Hosana M. Debonsi will be working together aiming to evaluate the antispasmodic potential on cardiovascular smooth muscle of the marine algae and fungal biotransformation extracts. In the future, depending on the obtained results, isolated compounds could be also evaluated. Concerning the natural products isolation, the metabolites could be submitted to biotransformation experiments.

Prize award

Best poster at Natural Products area: Felício R, Yokoya NS, Young MCM, Murakami C, Debonsi HM. Fungicidal activity of extracts from marine red algae *Bostrychia tenella* (Rhodomelaceae). *6th CIFARP – International Congress of Pharmaceutical Sciences*, Ribeirão Preto, São Paulo, Brasil. CD-ROM resumo PN 033, Set/2007.

Publication

4-(Hidroximetil)-Benzenossulfonato de potássio: metabólito inédito isolado da alga marinha *Bostrychia tenella* (Rhodomelaceae, Ceramiales). 2008. *Química Nova*, **Vol. 31, n°. 4**:837-839.

Hosana Maria Debonsi

Faculdade de Ciências Farmacêuticas de Ribeirão Preto
Universidade de São Paulo (USP)
hosana@fcfrp.usp.br

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ANTIMICROBIAL AND ANTI-CANCER ACTIVITY OF CRUDE EXTRACTS AND ACTIVE COMPOUNDS OBTAINED FROM PLANT SPECIES FROM THE STATE OF SÃO PAULO

João Ernesto de Carvalho

The Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA) is located at Betel, 8 Km distant from Unicamp campus, with 8.000m^2 of constructed area and 40 hectares of experimental campus. The work with plant specimens is performed in a unique way, in the search for new drugs presenting anti-cancer, antimicrobial, antiulcerogenic, analgesic and anti-inflammatory activities, integrating the following Divisions: Agrotechnology, Pharmaceutic Chemistry, Phytochemistry, Microbiology and Pharmacology/Toxicology. Due to its multidisciplinary aspect, most of the research projects are developed in an integrated way, involving its various Divisions, making it possible to carry out complete studies from cultivation to isolation, identification, structural modifications, pharmacological mechanisms of action as well as toxicity of the substances. Besides, industrial methods are also developed in order to obtain products from microbial origin through fermentative processes, especially alcoholic fermentation.

Thanks to researches supported by CNPq, FAPESP and funds obtained from rendering services, in collaboration with The National Cancer Institute (USA), CPQBA-Unicamp developes an anti-cancer screening program studying both extracts and active principles obtained from natural resources. Also supported by governmental funds the Microbiology Division studies the antimicrobial activities of both medicinal and aromatic plants cultivated in the region of Campinas.

With the aim of evaluating both antimicrobial and anti-cancer activities of the plant extracts, fractions and active principles present in the State of São Paulo, CPQBA developed a multidisciplinary study in which every Division played an important role.

The Division of Agrotechnology was responsible for the acquisition of the plant species at the following regions: Reserva Biológica do Alto da Serra de Paranapiacaba - SP (Instituto de Botânica / SMA), located in the Serra do Mar with 336 hectares, mountainous country, altitude between 750 and 890 meters; Reserva Ecológica e Experimental de Moji-Guaçu (São Paulo) (Instituto de Botânica/SMA), located in the district of Moji-Guaçú, with 344.42 hectares, showing two different vegetation types: cerrado (woodsy pasture) and forest; Parque Estadual da Ilha do Cardoso - Cananéia, SP (Instituto Florestal /SMA), a 30 Km long and 10km wide area, covering 22,500 hectares. Other plants will be harvested in the experimental campus of the Division of Agro-technology of CPQBA, compiling around 450 species.

The Divisions of Phytochemistry and Organic and Pharmaceutical Chemistry performed the extraction, isolation and identification of the active principles. The grinded plant material was submitted to the process of extraction by maceration with dichloromethane. The filtered material was gathered, mixed and treated with anhydrous Na_2SO_4. After a new filtration, the organic solvent was evaporated under vacuum at 40ºC producing the dichlometanic crude extract (DCE). The plant residue was subsequently submitted to dynamic maceration with ethanol. This procedure was repeated three times with the same powder and after filtration, the plant residue was discarded. The organic solvent was evaporated under vacuum producing the ethanolic crude extract (ECE). The active fraction was partitioned by dry column chromatography on silica gel 60 with chloroform/methanol 5% providing three to five fractions. These fractions were submitted to activity tests and the active fraction went through a new purification process in order to isolate and identify the active principle(s). Structure elucidation of the active principle has been done employing espectroscopical methods (RMN^{1}H, RMN^{1}C^{13}, IR, UV, Mass, etc).

The Microbiology Division performed tests comprising the following microorganisms: *Bacillus subtilis* CCT 2576, *Escherichia coli* CCT 0547, *Staphylococcus aureus* CCT 2740, *Staphylococcus epidermides* ATCC 12228, *Micrococcus luteus* CCT 2692, *Rhodococcus equi* CCT 0541, *Salmonella choterasuis* CCT 4296,

Pseudomonas aeruginosa ATCC 13388, *Enterococcus faecium* CCT 5079, *Streptococcus faecium* ATCC 10541 and *Candida albicans* ATCC 10231. These antimicrobial tests were carried out as follows: in a sterilized 96-well micro-plate, 100 µL of Mueller-Hinton solution, except in column 12, which used for the controls, was added. In column 1 – line 50µL of the extract to be tested will be added at known concentrations (one different test substance for each column number). Afterwards, 100 ?L of the content in a hole was homogenized with the medium and transferred to the hole of the following line (B). This procedure was repeated until line H, in such a way that a decreasing concentration of the extract was obtained. The last 100 µL was discarded. Then, 100 µL of a recently growing microorganism's suspension (24 hours) was added. The turbidity of this suspension was compared to the McFarland's scale (no 0.5) and diluted to a final concentration of 10^4 cells/mL The plates was sealed with paraffin and incubated at 37°C for 24 hours. After this period, 20 µL of an aqueous solution of 0.5% TTC (tetrazolium triphenil chloride) was added and the plate was incubated once again for 3 hours at the same temperature. The MIC was defined as the minor concentration of the extract or substance capable of blocking the beginning of the reddish coloring (Ellof, Planta Medica 64: 711, 1998.). Only the extracts which presented MIC under 1mg/ml were considered active. As a whole, the following plant species showed the best antimicrobial activities: *Piper aduncun, Piper regnelli, Piper cernuum, Mentha spicata, Vetiveria zizanoides, Mikania glomerata, Mikania laevigata, Spilanthes acmella, Dichorisandra tryrsiflora, Guatteria af. Australis*, and species 07/79 (not identified).

The Division of Pharmacology and Toxicology performed the evaluation of the "in vitro" anti-cancer activities of 198 crude extracts, obtained from 99 plant species according to the methodology developed by the NCI. Human tumor cell lines UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), K-562 (leukaemia), OVCAR03 (ovarian), PC0 3 (prostate), HT-29 (colon), 786-0 (kidney) and NCI-ADR (ovarian expressing multiple-drug resistance phenotype) were kindly provided by the National Cancer Institute (NCI). Stock cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% foetal bovine serum. Gentamicine (50?g/mL) was added to experimental cultures. Cells in 96-well plates (100 µL cells/well) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25 and 250 µg/mL) at 37°C, 5% of CO_2 in air for 48h. Final DMSO concentration did not affect cell viability. Afterwards, cells were fixed with 50% trichloroacetic acid and cell proliferation determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay (http://dtp.nci.nih.gov/branches/btb/ivclsp.html). The results were expressed in graphics presenting the percentages of growth inhibition related to different concentrations of the test substance. Among the tested plant extracts, only 38 species were able to either inhibit or kill the tumor cell lines. The dichlometanic crude extracts presented a far better anti-cancer activity (78%) due to their larger amount of low polarity active principles. Among the most active species, *Bryophyllum calycinum, Calea pinnatifida, Calophyllum brasiliensis, Kielmeyera coriaceae, Lantana canescens, Mikania sp, Nematanthus fritschii* and *Piper arboretum* presented the best anti-cancer *in vitro* profile.

As a sequence, the *in vivo* anti-cancer screening as well as the isolation and identification of active principle were performed with the promising species, taking into consideration the amount of plant material available. One of chosen extracts was the DCE of *Pothomorphe umbellata* (L.) Miq (Piperaceae). Its anti-cancer activity was evaluated on the Ehrlich Ascites Tumor 90-day model. Adult male Swiss mice received 10^5 cells in 0.5 mL, intraperitoneally. On the fourth day after tumor cell inoculation, the first treatment was performed. Additional treatments were carried out on days 14 and 21 post-inoculation. All experimental animals were daily observed and a scored was recorded based on signs of suffering (Wolfensohn & Lloyd, 2003. Laboratory Animal Management and Welfare. 3rd ed.,p.59-73). Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress were humanely killed and that day considered the time of death.

In this study, both the survival number of animals and their life span increased by at least 45% and 50% respectively, demonstrating the *P. umbellata* extract potential pharmacological activity. This crude extract was submitted to dry column chromatography with dichloromethane-methanol (99:1). The column effluent fractions were extracted with methanol, dried under vacuum yielding fractions

FR1 (less polar), FR2 (medium polarity), and FR3 (polar) FR1 demonstrated high potency and cytotoxicity. This result was compatible with the high toxicity of oxalic acid; FR2, containing 4-nerolidylcathecol, presented the lowest cytotoxic activity compared to the other two fractions but showing selectivity for prostate cancer cell line; FR3, containing a mixture of steroids described in the literature as possessing various biological activities, also presented potent anticancer "in vitro" activity. These results suggest that *P. umbellata* DCE *in vivo* antitumoral activity may have been a consequence of the activity of different active principles (Sacoman et al. Braz. J. Medical Biological Res. 41: 411, 2008).

DCE from *Calea pinnatifida* was also evaluated on the Ehrlich Ascites Tumor model increasing the survival time of the experimental animals. This extract was submitted to many cromatographic procedures biomonitored by anti-cancer *in vitro* assay. These chromatographic purifications originated a large number of fractions with antiproliferative activity (Marchetti, 2008-Master Thesis). These results encouraged following up on studies priorizing the identification of active principles, anti-cancer mechanisms of action and studies in other *in vivo* anti-cancer assays.

The purification process of DCE from *Artemisia annua* produced a series of fractions and active principles. Fraction F2S, obtained in larger amounts and showing good selectivity for the ovarian expressing multiple-drug resistance phenotype cell line (NCI-ADR) as well as the leukemia one (K-562) was also evaluated on the Erhlich Ascites Tumor model. It also increased the survival time of the tumor-bearing animals.

Similarly, the purification process of DCE from *Kielmeyera coriacea* L produced various fractions and active principles. The *in vivo* anti-cancer activity of fraction Fr7 was evaluated in the Hollow Fiber experimental model, as follows: fibers filled with human cancer cell lines (MCF-7, NCI-ADR, OVCAR03, $1x10^6$ cells/ml) were implanted in the subcutaneous region of the neck and intraperitoneally of adult male Balbc/c mice. The intraperitoneal treatment was performed after three days. All animals were humane killed on the 14^{th} day and all fibers were excised in order to determine cell viability through MTT assays (Hollingshead et al., Life Sciences. 57: 131, 1995). In this study, the treatment with fraction Fr7 decreased the number of breast and ovarian tumor cells implanted in the abdominal cavity in 61 and 62%, respectively. Doxorubicin, as a positive control, was able to reduce the same cell lines in 40 and 46%, respectively. Neither Fr7 nor doxorubicin were able to affect the ovarian expressing multiple-drug resistance phenotype cell line (NCI-ADR). In the animals submitted to the subcutaneous fiber implants, only the human breast cell line was inhibited by Fr7 (44%) and by doxorubicin (46%), demonstrating the permeability of the fibers used, since both substances reached the animals blood circulation and consequently, the site of the implant (Oliveira, 2008-Doctor Thesis).

This huge research work certainly improved CPQBA's scientists experience in multidisciplinary studies, wrapping up diverse areas as an organized team in the search of Natural Products. We strongly believe that this multidisciplinary study should be extended to other Research Centers involved in the Biota Project in order to increase the number of studied species.

Due to the so many promising results, a new project ought to be submitted to the Biota Program in order to continue the pharmacological screening of the mentioned species. This new project will aim the identification of the selected species' active principles, determine the mechanisms of cell death involved in their activity and develop experimental models where human cancer cell lines will be implanted in immunodeficient mice, as the last step in the Pre-clinical tests.

João Ernesto de Carvalho
Monteiro KM, Ruiz ALTG, Magalhães PM, Rehder VLG, Duarte MCT, Foglio MA.

Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas
Universidade Estadual de Campinas (CPQBA-Unicamp)

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Grants

TAXONOMIC ASPECTS OF *Croton* L. (EUPHORBIACEAE S.S.) OF SOUTHEAST REGION OF BRAZIL

Letícia Ribes de Lima

SUMMARY AND PROPOSAL

The genus *Croton* L. is the second largest and most diverse genus of Euphorbiaceae, with about 1,200 species, grouped in 40 sections. It has a pantropical distribution with the majority of species occurring in the Americas (from Argentina to the United States, as far north as northern Florida and Texas) (Govaerts et al. 2000). Most of the species present in South America occur in Brazil, around 356 (Allem 1979), divided into 26 of the 40 sections acknowledged by Webster (1993). Of them, 172 are found in Southeast region, probably, the center of diversity of the genus. *Croton* is a good example of a "problem genus" – big and taxonomically complex– and that may be the reason it has been neglected in favor of small and clearly delimited genera. Because of its size and diversity, no complete taxonomic treatment in relation to this genus was accomplished since *Prodromus systematis universalis regni vegetabilis* (Mueller, 1866) and, in Brazil, since *Flora brasiliensis* (Mueller, 1873). Therefore, in many Brazilian (and worldwide) herbaria, the existence of great amount of collections of *Croton* non-identified (or wrongly identified) is common regarding level of species, which need an attentive study urgently. Moreover, many species still remain unpublished and others must have their nomenclature reviewed. Besides its taxonomic importance, the genus *Croton* is rich in secondary metabolites, such as alkaloids, terpenoids and cocarcinogens. In a recent study, in which information about medical use was compiled, chemistry and pharmacology of *Croton* species (Salatino et al. 2007) evidenced that the potential of this genus is enormous.

In order to contribute to a better understanding in relation to taxonomic aspects and phylogeny of *Croton*, globally, the proposal of this project is to complete the taxonomic and floristic survey of this genus in Southeast region, seeking a deeper knowledge and delimitation of species, as well as information about morphology, biology, and biogeography. Hence, the object of this study is to obtain the highest number of characters, including molecular, so that they can be used altogether in cladistic analysis of this group. The taxonomic study of the genus *Croton* in Brazil, beginning with Southeast region, also aims at providing reference to studies developed in other Biology areas and that have already been accomplished, such as Professor Dr. Antônio Salatino's research of *Instituto de Biociências of Universidade de São Paulo*, whose objectives are study the secondaries chemical compounds in the genus (process submeted to FAPESP).

Furthermore, this project aims at consolidating a system shared, qualified and updated of data and information about *Croton* species of Brazilian Southeast region, containing approximate geographical coordinates of every place from where specimens were collected, aiming at future studies of ecological niche modeling.

METHODOLOGY

An extensive survey has been accomplished in relation to different species of the genus *Croton* which occur in the states of the Southeast region through analysis of specimens of the main herbaria of such region. In addition, there are attempts to increase collections of herbaria of São Paulo state through specimens picked in the four states of Southeast. They are collected georeferencially in order to contribute to BIOTA-FAPESP (SinBiota) project database; also, their geographical distribution is accurately documented in order to try to understand distribution standards observed, in the future. In such cases, not only branches are collected to produce dry specimens, but also 50g of fresh leaves for phytochemical studies and fragment-samples of leaves in silica gel for later molecular studies – for phylogenetic purposes, as well as for future prospections of sequence(s) that may be used as barcode.

RESULTS – FIRST SIX MONTHS

This project began in January of 2008 and, until now, six expeditions were accomplished in order to obtain more specimens in areas of Grão Mogol (MG), Serra do Cipó (MG), Visconde de Mauá (RJ), Itatiaia (RJ), Campos do Jordão (SP), São Bento do Sapucaí (SP), Moji Guaçu (SP), Itararé (SP), Linhares (ES), and Santa Tereza (ES), comprising all four states of Brazilian Southeast. Moreover, a total of 23 species were collected, representing approximately 14% of diversity of *Croton* in the region. All material obtained was deposited in the herbarium of Instituto de Botânica (in São Paulo). All species have been photographed so that a field guide of the genus will be elaborated.

Material of all taxa was obtained from field for phytochemical experiments, collaborating on Professor Dr. A. Salatino's project. As preliminary results, presence of volatile oils and flavonoids was observed in some taxa analized.

A database with all *Croton* collections was also initiated and has approximately 1000 entries registered proceeding from CVRD, MBML and SP herbaria.

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Profa. Dra. Letícia Ribes de Lima

Instituto de Botânica
Seção de Curadoria do Herbário
+55-11-50736300, extension 201
lerilima@hotmail.com

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SEARCH FOR INHIBITORS OF ANTIOXIDANT PROTEINS FROM *Xylella fastidiosa*

Luis Eduardo Soares Neto

OBJECTIVES

Search for inhibitors of antioxidant enzymes from *Xylella fastidiosa*, using samples obtained from the groups belonging to the BIOprospecTA Network.

RATIONALE

Oxidative stress is an important component of the host response against bacterial infection in both plants and animals. Oxidants can cause damage to macromolecules such as DNA, lipids and proteins. In response to this oxidative stress, several complex mechanisms have evolved in bacteria to detoxify H2O2, superoxide anion radicals and organic hydroperoxides. Therefore, in principle, pathogenic bacteria might be more prone to oxidative stress, if their antioxidant proteins are inhibited.

Our group has characterized in detailed one antioxidant protein (Ohr = "Organic Hydroperoxide Resistance Protein") from *Xylella fastidiosa* that do not share amino acid sequence, nor protein structure with any eukaryotic counterpart (Oliveira e col., 2006). Therefore, Ohr is potentially a promissory target for chemoteraphy. Besides Ohr, two other antioxidant proteins are being characterized, in an attempt to develop assays that can allow us to search for inhibitors.

METHODOLOGY

Expression and Purification of Antioxidant Proteins from *Xylella fastidiosa*

Initially, genes from *Xylella fastidiosa* were cloned into the vector pET15b for the expression of recombinant proteins in *Escherichia coli*. Expression was performed by standard procedures (Oliveira et al., 2006). Purification of recombinant proteins were performed by metal affinity chromatography as previously described (Oliveira et al., 2006).

Bioassay

All the antioxidant proteins studied here are Cys-based, thiol-dependent peroxidases. Therefore, we search for inhibition by measuring removal of organic peroxides through the "Ferrous Xylenol Orange" assay (see Jiang et al., 1992). Inhibitors were search from several plant and fungi crude extracts that were collect from other groups belonging to the BIOprospecTA network. These extracts were prepared in a 5% (w/v), in DMSO 100%. In the assay, the extracts were at 0,5% final concentration. Appropriate controls (Absence of extracts, Absence of DMSO; absence of extracts and DMSO) were performed. Samples are analyzed in 96-well plates reader (Anthos Zenyth 200st).

RESULTS AND DISCUSSION

Target 1 - Ohr*:* Ohr (Organic Hydroperoxide Resistance Protein) is the protein that has been best characterized by our group so far (Cussiol et al., 2003; Oliveira et al., 2006). As a consequence, until the moment we have only search for inhibitors of this enzyme in crude extracts of both plants and fungi that were collected by groups coordinates by Dr. Mônica Tallarico Pupo e Dr. Jairo Kenupp Bastos (Departamento de Ciências Farmacêuticas da FCFRP-USP) e Dra. Dulce Helena Siqueira Silva e Vanderlan da Silva Bolzani do NuBBE (Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais do Instituto de Química/UNESP/Araraquara). Until this moment, 203 extracts were analyzed, 132 were added into the data bank of the BIOprospecTA network. Not all the samples were added into the system because we could not identify them in the system. So far, 5 crude extracts inhibited Ohr thiol-dependent peroxidase activity. In these

cases, samples were re-analyzed, pre-treating the samples with bovine albumin in order to remove tanines that might be present in the extracts. Furthermore, we also analyzed if the effect could be due to the "prosmicuous effect" (Feng et al., 2007), but his was not the case.

In the case two samples, the crude extracts were fractionated by the group from Dra. Dulce Helena Siqueira Silva and Vanderlan da Silva Bolzani – NuBBE and we could still detect inhibition for some fractions. We are currently trying to isolate the molecule that is probably responsible for this inhibition.

In case we can identify a molecule that inhibits Ohr, we intend to co-crystallize it with Ohr. The elucidation of Ohr-Inhibitor structure might reveal insights into the characteristics required for a molecule to inhibit Ohr.

Target 2 – PrxQ*:* Peroxiredoxins are other group of Cys-based, thiol-dependent peroxidases that contrary to Ohr are ubiquitously distributed. Peroxiredoxin family comprises sub-groups some of them sharing high degree of amino acid sequence similarity. Recently, we have shown that peroxiredoxin proteins from yeast possess high reactivity towards hydrogen peroxide and peroxynitrite (Ogosucu e col., 2007). Among the various subgroups, PrxQ are poorly characterized. We have expressed and purified PrxQ from *Xylella fastidiosa* in *Escherichia coli*. Initial attempts to measure the thiol-dependent peroxidase activity failed. Later, we succeed to measure this enzymatic activity, employing thioredoxin as a reducing substrate. In fact, we were able to reconstitute the thioredoxin system from *Xylella fastidiosa* and it supported the peroxidase activity of prxQ. Preliminary results indicated that PrxQ is equally effective in the removal of hydrogen and organic peroxides. We expect to initiate very soon the search for inhibitors in the plant and fungi extracts.

Target 3 - AhpC: AhpC belong to another sub-group of the peroxiredoxin family of Cys-based peroxidases. We have expressed and purified the recombinant AhpC and measured the DTT-dependent peroxidase activity. In parallel, we are investigating the oligomerization of this enzyme, since it has been reported that homologous proteins in high oligomeric states possess chaperone activity. Soon, we intend to screen AhpC against fungi and plant extracts in an attempt to find an inhibitor.

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Prof. Dr. Luis Eduardo Soares Neto

Instituto de Biociências
Universidade de São Paulo
Departamento de Genética e Biologia Evolutiva
nettoles@ib.usp.br

BIOprospecTA Network
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STUDIES ON BIOTRANSFORMATION OF PENTACYCLIC TRITERPENES BY FILAMENTOUS FUNGI AND EVALUATION OF ANTITUMOR AND TRYPANOCIDAL ACTIVITIES OF THE OBTAINED DERIVATIVES

Niege Aracari Jacometti Cardoso Furtado

OBJECTIVES

The general aim of the present project is to obtain new derivatives of pentacyclic triterpenes by biotransformation processes and to evaluate the antitumor and trypanocidal activities of the obtained products, as well as to furnish extracts and pure compounds to be evaluated by other bioassays of the BIOprospecTA network.

The specific aims are to isolate pentacyclic triterpenes from plants; to determine the best conditions of filamentous fungi to produce spores; to establish culture conditions in the preculture fermentation stage to produce biomass; to evaluate the influence of culture medium and incubation period on biotransformation reactions by chemical profile analysis and biological activities of the obtained extracts; to select two promising fungi capable of biotransforming the pentacyclic triterpenes by chemical profile analysis and biological activities of the obtained extracts; to carry out biotransformations of the pentacyclic triterpenes in preparative scale; to isolate and to characterize pure compounds by spectroscopic techniques (NMR, MS, IR, UV); to evaluate the antitumor and trypanocidal activities of the obtained extracts, as well as to furnish extracts and pure compounds to be evaluated by other bioassays of the BIOprospecTA network; to carry out biotransformations of bioactive natural products furnished by other research groups.

METHODOLOGY

Isolation of the pentacylic triterpenes: the ursolic and oleanolic acids have been isolated from *Miconia rubiginosa*, a species of Melastomataceae, while the pentacyclic triterpene lupeol has been furnished by Prof. Dr. Paulo Cezar Vieira.

Microorganisms: the following microorganisms are being evaluated regarding their ability to biotransform the pentacyclic triterpenes: *Rhizopus stolonifer*, *Mucor rouxi*, *Humicola grisea* variety *thermoidea*, *Chaetomium thermophilum*, *Scytalidium thermophilum*, *Phanerochaete chrysosporium*, *Penicillium roquefortii*, *Aspergillus ochraceus*, *Aspergillus niger* and *Glomerella cingulata*. The selection of the strains was performed considering their potential to make different reactions. Also, thermophylic strains were included because they are an important source of industrial enzymes. All the strains belong to a collection of fungi cultures of the Biology Department of the School of Philosophy and Sciences of Ribeirão Preto of University of São Paulo, except the strain of *Glomerella cingulata* which belongs to the collection of endophyte fungi of the School of Pharmacy of Ribeirão Preto of University of São Paulo.

Conidia production: the microorganisms have been submitted to different media: oat agar (Crotti et al., 1999), malt extract (Tzean et al., 1992) and potato dextrose agar – PDA (Kim et al., 1988). The cultures have been incubated at different temperatures for different periods. The conidia produced in each culture have been harvested using a 2% Tween 80 solution and counted in a Neubauer hemocytometer.

Biomass production: fungi have been grown in 15 mL of seed medium (Jackson et al., 1993) for different periods at 30oC or 40 oC, with shaking at 120 rpm.

Evaluation of the influence of culture medium and incubation period on biotransformation reactions: the mycelia have been transferred to 30 mL of different fermentative media (Czapek and Koch's K1; Atlas, 1995) and the pentacyclic triterpenes have been added to the media at the concentration of 0.1g/L. The cultures have been carried out at different temperatures for 10 days. Samples of each culture have been taken every 24 hours. Control experiments containing either medium with mycelia or medium with substrate dissolved in the organic solvent have been run in parallel. The culture broths have been separated from the mycelia by filtration, followed by partition with ethyl acetate in order to extract lupeol derivatives and with dichlorometane and ethyl acetate to extract ursolic and oleanolic acids derivatives. All the resulting organic solvents have been evaporated under vacuum. The obtained extracts from the cultures developed with lupeol and both ursolic and oleanolic acids have been analyzed by GC/MS and TLC, respectively. Also, the extracts containing biotransformation products are being submitted to evaluation of antitumor and trypanocidal activities.

RESULTS AND DISCUSSION

The cultivation conditions to produce spores and biomass were determined for each fungus, as well as the growth curves in different fermentative media. Therefore, it was possible to determine the best time to add the pentacyclic triterpenes to the cultures considering the strategy of adding substrates only after the growth phase. The choice of this strategy is due to the fact that the enzymes produced during the stationary phase are less specific than those produced during the growth phase. The glycosyltransferases produced in the secondary metabolism, for example, are capable of catalysing reactions with a larger number of substrates when compared with those produced in the primary metabolism (Thiericke & Rohr, 1993).

The abilities of the fungi *A. ochraceus*, *A. niger* and *P. roqueforti* to biotransform the pentacyclic triterpene lupeol were demonstrated only when the fungi cultures were developed in the Koch's K1 medium. On the other hand, the fungi *R. stolonifer* and *M. rouxii* were capable of biotransforming lupeol independently of the culture medium. Also, the biotransformation products were detected only in a specific incubation time displaying the influence of the incubation time on the biotransformation reactions.

The biotransformation of lupeol by *A. ochraceus* and *A. niger* afforded two compounds in each culture which were detected in the cultures developed for more than 7 and 6 days, respectively. The biotransformation of lupeol by *P. roqueforti* afforded only one compound which was also detected only in the culture developed for 7 days. *R. stolonifer* and *M. rouxii* afforded two products of biotransformation of lupeol which were detected in each culture developed in both culture media in different incubation times. The mass spectra of these compounds displayed a series of ions similar to those observed for lupeol that indicated that the main portion of the molecule has not been modified. The GC retention times of the derivatives varied according to the fungus, as well as the relative intensities of some ions, including the molecular ion. The mass spectra were initially obtained in a typical EI source at 70eV. Peaks of the molecular ions of m/z 357, 384 and 424 were observed. However, molecular ion peaks with m/z of 442 and 511 were observed when the mass spectra were obtained at 35eV. Therefore, other experiments of GC/MS using 35eV of electron impact energy are being performed in order to determine the correct molecular formula of the produced derivatives, since the mass spectra obtained at 70eV caused extensive fragmentation.

The biotransformation experiments of the ursolic and oleanolic acids are being undertaken in order to select two promising fungi capable of biotransforming these substrates.

It should be pointed out that the collaboration among research groups can provide better chances of achieving satisfactory results since the extracts of the cultures and pure compounds produced in the biotransformation experiments can be evaluated by other bioassays of the BIOprospecTA network. Also, other bioactive natural products furnished by the other groups can be submitted to biotransformation experiments. Therefore, collaborations with the researchers Sérgio R. Ambrósio and Hosana M. Debonsi are being established aiming to evaluate the antispasmodic activity of the extracts containing biotransformation products on cardiovascular smooth muscle and to carry out biotransformation of the compounds isolated from marine algae. Depending on the obtained results, the derivatives of the pentacyclic triterpenes could be also submitted to the evaluation of antispasmodic activity.

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Prof. Dr. Niege Aracari Jacometti Cardoso Furtado

Faculdade de Ciências Farmacêuticas de Ribeirão Preto
Universidade de São Paulo (USP)
niege@fcfrp.usp.br

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CERRADO BIODIVERSITY: A PROPOSAL OF FIELD WORK FOR TEACHING BOTANY AT THE THREE EDUCATIONAL LEVELS

Osmar Cavassan

GOALS

From Biota/Fapesp: To study and to assess the biodiversity in the state of São Paulo and to spread such knowledge and its relevance.

Of the project: To characterize, under a floristic and phytosociological viewpoint, the native cerrado-associated vegetation from the Legal Reserve in the Bauru campus at Unesp; to describe the florula of Arecacea family present in the Legal Reserve at Unesp and in other native vegetation fragments in Bauru region; to define, within the Bauru campus at Unesp, routes and trails among the native cerrado fragments to be used in field classes of Science, Biology, Botany and Ecology, comprising the three education levels: elementary, secondary and higher education, regarding studies about the regional biodiversity; to propose practical field-oriented activities directed to students from public city and state schools focused on the biodiversity learning related to Sciences, Biology, Botany and Ecology.

METHODOLOGY

1. Knowledge of the biodiversity in the native cerrado vegetation from the Legal Reserve at Bauru campus – Unesp

A floristic and phytosociological characterization was carried out in a cerrado patch and a fragment of swamp forest through quadrat method.

An inventory of the palm species (family Arecaceae) in the Bauru region was organized, including the selected vegetation and comprising morphological, ecological and ethnobotanic aspects.

An inventory of the woody plants reaching more than 1m in height and found throughout the trail was developed, with participation of the undergraduate students involved in the project, in order to be used for field activities. These plants have been described in relation to their morphological, ecological and ethnobotanic aspects, for further utilization in fieldwork offered to students from elementary, secondary and higher education levels which, after scheduling, have the chance to participate in the practical activities.

An inventory of the species of the family Vochysiaceae observed throughout the whole studied area has been performed, where the species are described for their morphology, ecology and ethnobotany.

The plants cited in all fieldwork practices were collected and await identification and/or confirmation by experts to further inclusion in the SinBiota checklist.

For the next stage, we intend to sample epiphytes and climber plant species.

2. Divulgence of the known biodiversity reported for this vegetation

It comprises the development of practical field classes for students attending the three educational levels, using a trail in the cerrado, previously characterized under both botanic and ecological focuses by undergraduate students from Teacher formation in Biological Sciences course, and graduate students in Botany and Science Teaching (either master or doctor levels). Based on the students' participation, such procedure was evaluated prior, during, and after the activity by using questionnaires, interviews with the teachers and spontaneous manifestations from the participants. These data allow analyzing the efficacy of the order of both theoretical and practical activities, the motivation of the students involved, and development of esthetical, cognitive and ethical values.

Furthermore, an additional work has been developed in order to verify eventual differences in the participation between male and female students, based on the formation of classes of spontaneous manifestations.

For the next step, the development of folders and guides about the vegetation surrounding the trail is scheduled, in order to turn it self-explanatory.

The publishing of an illustrative book, presenting the results of the project, describing the botanic, ecological and ethnobotanic aspects of the local flora is also planned.

RESULTS AND DISCUSSION

1. Floristic aspects of the Cerrado

The survey about the floristic composition of vascular species in the cerrado fragment at UNESP reported 187 species belonging to 136 genera and 57 families.

181 out of the total number refer to angiosperms, distributed into 132 genera and 55 families. From the total number of angiosperm species, 30 have been successfully identified at genus level, three are under identification and/or confirmation by experts.

2. Floristic aspects of the swamp forest

In the alluvial area studied, 107 species were sampled, from 53 families, amongst woody, bushy, herbaceous, epiphyte and climber species. Two species are under identification and/or confirmation by experts.

3. Inventory of species of the family Arecaceae

Ten palm species have been identified in the Bauru region, belonging to 6 genera, all of the subfamily Arecoideae; the tribe Cocoeae is represented by 8 species distributed into the subtribes Attaleinae with 6 species and Bactridinae with 2 representatives; the tribe Areceae is represented by one species of the subtribe Euterpeinae and the tribe Geonomeae comprises a single species. The genus with the highest number of species was *Syagrus*, comprising four species, followed by the genus *Acrocomia* represented by two species; the genera *Allagoptera*, *Butia*, *Euterpe* and *Geonoma* are represented by one species each.

4. Inventory of species of the family Vochysiaceae

Five species have been reported: *Qualea grandiflora* Mart., *Qualea multiflora* Mart., *Qualea parviflora*, *Vochysia cinnamomea* Pohl. and *Vochysia tucanorum* Mart. Such species have been described in relation to their morphological, ecological and ethnobotanic aspects.

5. Inventory of species found along the trail in the cerrado area

Overall, 390 individuals comprising 33 families, 58 genera and 68 species were reported. The most representative families were: Fabaceae (52 specimens), Annonaceae and Burseraceae (47 specimens), Rubiaceae (30 specimens) and Myrtaceae (28 specimens).

Note: The inventory of epiphytes and climbers has been just initiated.

Prof. Dr. Osmar Cavassan

Faculdade de Ciências de Bauru
Universidade Estadual Paulista (Unesp)
cavassan@fc.unesp.br

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Thematic Project

DISCOVERY AND DEVELOPMENT OF POTENTIAL CHEMOTHERAPEUTIC AGENTS FROM MARINE INVERTEBRATES AND ASSOCIATED MICROORGANISMS

Roberto Gomes de Souza Berlinck

SUMMARY AND PROPOSAL

Marine natural products isolated from both marine invertebrates and microorganisms have received increased attention during the last 50 years due to their structural complexity and biological activities. In particular marine sponges, ascidians, octocorals, bryozoans, as well as marine fungi and bacteria, have shown to be prolific sources of potently active and structurally unique secondary metabolites. Although Brazil has a 8.000 km long coastline, the knowledge of marine natural products from Brazilian marine organisms is still limited.

The proposal of this project is the chemical investigation of biologically active crude extracts obtained from marine invertebrates and marine microorganisms collected in different regions of the Brazilian coastline. Extracts of marine invertebrates active already tested in assays of cytotoxic and antituberculosis activities, as well as of antimicrobial activity against both antibiotic-sensitive and antibiotic-resistant human pathogenic microbes and of inhibition of specific enzymes implied in the life cycle of pathogenic protozoa, will be subjected to dereplication by LC-PDA-MS before a chromatographic fractionation toward the isolation of biologically active compounds. In parallell, marine fungi and bacteria isolated from marine invertebrates (sponge and ascidians) will be grown in optimized artificial media in order to produce secondary metabolites. The marine microbe growth media will be extracted by adsorption on polymeric resin and by liquid-liquid extraction. Extracts from marine microbes will be subjected to the same above mentioned bioassays. Additionally, a new set of bioassays related to the inhibition or stimulation of chemical mediators implied in immunomodulation processes will be applied to the whole crude extract collection (> 500), in order to search for active extracts. Active extracts will be subjected to a dereplication by LC-PDA-MS analysis, in order to get information about crude extracts chemical profiles and prioritize the isolation of novel bioactive compounds. After dereplication, marine microbial extracts will also be subjected to fractionation aiming the isolation of pure active metabolites. Pure chemical entitites obtained from marine invertebrates and microorganisms will be spectroscopically identified and evaluated in the bioassays in which the original crude extract was active, aiming to obtain information on their mechanism of action.

METHODOLOGY

Collections of marine invertebrates by scuba diving are scheduled at least one/year, at different diving locations. Animals are either immediately frozen or preserved in EtOH. Frozen animals are freeze dried at the laboratory before be stored at – 20°C. Animals preserved in EtOH are also stored at – 20°C. Vouchers are deposited at the Museu Nacional (Rio de Janeiro) or at the Departamento de Biologia (Universidade Federal do Paraná). An aliquot (50 g) of freeze dried animals is extracted with MeOH, the extract is filtered, evaporated and stored at -20°C. These extracts constitute the extract library. Aliquots are send to bioassays.

Small pieces of selected marine invertebrates are incubated in Petri dishes with seven different growth media. Microbe strains are purified exhaustively until a single axenic strain is isolated. Pure strains are growth in 250 mL of agar-free media. After growth, the mycelia + growth media are extracted either with EtOAc or using solid phase extraction on C18 (gradient of MeOH in H2O). Aliquots of extracts are send for bioassays.

Bioassays included in the screening are: a) cytotoxicity against cancer cell lines; b) antimicrobial against oral pathogens, including resistant to antibiotics; c) antituberculosis against *Mycobacterium tuberculosis* H37Rv; d) inhibition of enzymes isolated from *Leishmania* sp. and *Trypanosoma* sp.; e) imunomodulatory (including anti-inflammatory).

Active extracts are dereplicated and prioritized using LC-PDA-MS and RMN-^{1}H. Prioritized extracts are fractionated using usual chromatographic techniques and HPLC. Compounds isolated are identified by NMR, MS, UV, IR and circular dichroism spectroscopy techniques.

Pure compounds are re-evaluated in the original bioassay on which the crude extract was active. Promisingly active compounds are further investigated toward the understand of its mechanisms-of-action.

RESULTS – FIRST YEAR

Marine Invertebrates

1. Sponge *Plakortis angulospiculatus* – One new and five known polyketides were isolated. Plakortide P (1) was the most active in antineuroinflammatory and antiparasitic assays.

2. Sponge *Pachychalina alcaloidifera* – The major metabolite isolated from this sponge, ingenamine G (2), was further investigated in order to understand its action as a cytotoxic agent.

3. Nudibranch *Tambja eliora* – Tambjamine D (3) previously isolated from this animal was shown to be cytotoxic, and was further investigated to establish its cytotoxic and genotoxic profile.

4. Sponge *Aplysina fistularis* – an extensive chemical investigation of this animal led to the isolation of 9 bromotyrosine derivatives, of which 11-oxoaerothionine (4) was shown to be anti-inflammatory and 11-ketofisturalin-3 (5) displayed antituberculosis activity (manuscript in preparation).

5. Ascidian *Didemnum sp.* – a series of modified diketopiperazines (6 – 9) were isolated from the anti-*Pseudomonas aeruginosa* active extract of this animal. Currently the absolute configuration of the phenylalanine residue in 6 and 7 is under investigation. Both 6 and 7 displayed potent anti-*Pseudomonas* activity.

Marine Microorganisms

1. Fungus *Penicillium oxalicum* – investigation of the cytotoxic and antituberculosis active crude extract of this fungus led to the isolation to the known alkaloids oxaline (10) and neoxaline (11). Currently the major compound isolated, oxaline (10), is under evaluation as antituberculosis agent, since no such activity has been reported for this compound.

2. Fungus of yet unknown taxonomy (possibly *Penicillium*) – the known 8-methoxy-3,5-dimethylisochroman-6-ol (12) was isolated from the antimicrobial crude extract of this strain. We are currently investigating the antimicrobial activities of this compound, since no such activities have been reported for it.

3. *Penicillium citrinum* – a completely novel metabolite skeleton was found in the antibiotic active crude extract of this strain. The major metabolite (13) was identified. We are currently investigating its absolute stereochemistry and biological activities.

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Prof. Dr. Roberto Gomes de Souza Berlinck

Instituto de Química de São Carlos
Universidade de São Paulo
http://www.iqsc.usp.br/pesquisa/qopn
rgsberlinck@iqsc.usp.br

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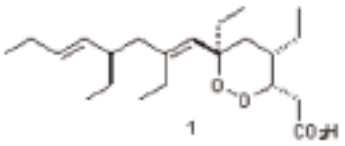

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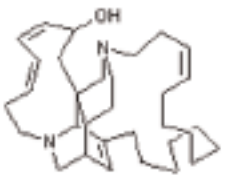

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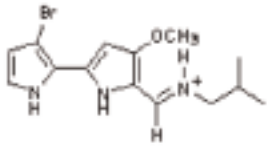

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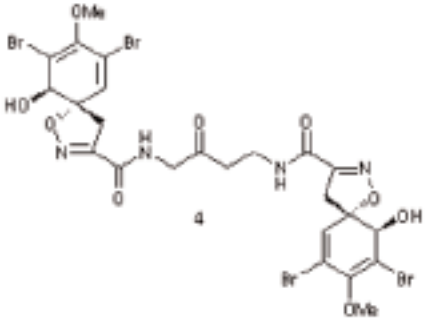

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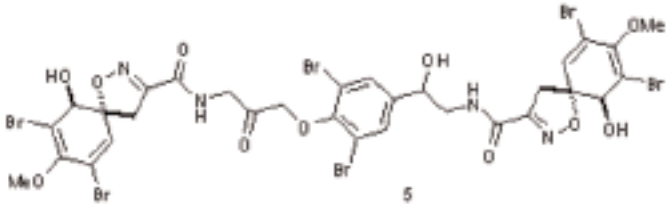

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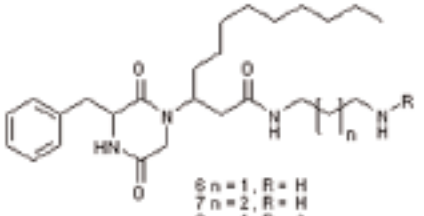

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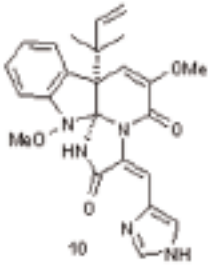

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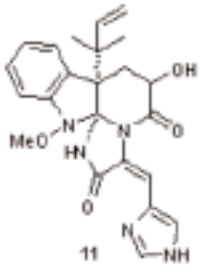

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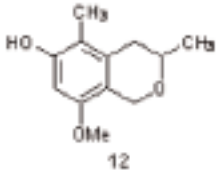

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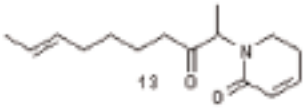

13

EFFECTS OF FOREST FRAGMENTATION ON THE FIG TREE POPULATIONS AND ON THE FIG-FIG WASP MUTUALISM IN SÃO PAULO STATE

Rodrigo Augusto Santinelo Pereira

OBJECTIVES

Understand the effects of forest fragmentation on fig tree populations, investigating the following questions:

1. The diversity, equitability and species richness of fig wasp communities associated with *Ficus* species vary according to landscape fragmentation levels?
2. Intensively fragmented landscapes are pollen constrained?
3. Parasitism by non-pollinating fig wasps is stronger in more fragmented landscapes?

METHODOLOGY

To answer these questions we will estimate fig population parameters and collect samples of wasp bearing figs from several *Ficus* species growing on forest fragments localized in landscapes with different levels of fragmentation. Fig frugivory by birds will be studied in these areas as a related subproject.

Study areas

Table 1. Characteristics of the study areas. Landscape area represents the study region delimited by a radius (buffer) of 10 km around the main fragment

City	Fragmentation level	Main fragment	Area (ha)	Landscape area (ha)	% of forested area
Ribeirão Preto	More	Estação Ecológica de R. Preto and other fragments	200 or less	~24,000	7.5
Gália	Medium	Estação Ecológica de Caetetus	~2,000	~42,000	12
Teodoro Sampaio	Less	Parque Estadual Morro do Diabo	~33,000	~135,000	30

Data sampling

Fig tree populations – all individuals of fig inside rectangular plots (125 x 20 m = 0.25 ha) will be measured. Sampling units will be allocated in the main fragments as well in smaller ones inside each landscape. A total of 19.5 ha will be surveyed on the three areas.

Fig wasp communities and pollen limitation – about 30 figs per fig tree will be collected and individualized in plastic flasks for wasp emergence and further analyses. Seeds, pollinating wasps and non-pollinating wasps will be counted to assess the correlation of these variables with fragmentation level. Pollen limitation will be inferred by the number of foundress pollinating wasps that enter each fig to pollinate/oviposit. About 50 figs per tree will be collected just after pollination. As pollinating fig wasps die inside the fig after pollination, theirs bodies can be counted.

Frugivory by birds – in each area fig trees with ripe fruits will be used as focal tree to observe the visit and consumption of figs by birds. Observations will occur on the early morning and on the end of afternoon for 3-4 days in each tree.

RESULTS AND DISCUSSION

Fig tree populations

The data sampling are in course, therefore we will present preliminary data.

We reported seven *Ficus* species in the study areas (*Table 2*). Less fragmented areas presented higher number species and lower densities of fig trees (*Table 3*). As fig species have pioneer features, more fragmented landscapes might provide more establishment opportunities for them due to higher proportion of forest edges and gaps. However, the loss of habitat might explain the lower diversity in the more fragmented landscapes.

Table 2. Species occurrence on the study areas

Species	Ribeirão Preto	Gália	Teodoro Sampaio
Ficus citrifolia	x	x	x
Ficus crocata		x	x
Ficus eximia	x	x	x
Ficus insipida	x	x	x
Ficus luschnatiana		x	x
Ficus obtusifolia	x	x	x
Ficus trigona	x	x	x

Table 3. Diversity and population parameters of the study areas

Parameters	Ribeirão Preto	Gália	Teodoro Sampaio
Total of registered species	5	7	7
Sampled area (ha)	0.5	1.0	2.0
Density of fig trees in the plots (ind/ha)	16	5,7	2,8
# of species sampled in the plots	2	5	4
% of forested area	7.5	12	30

Fig wasp communities and pollen limitation

We collected 18 fig crops of 6 fig species for wasp sampling (*Table 4*). However these materials are preserved in the deep freezer for future analyses.

Across areas, the lesser fragmented landscapes presented higher number of foundress wasps per fig, suggesting lower pollen limitation (X ± SE - Ribeirão Preto: 1.4 ± 0.1, n=4 crops; Gália: 2.8 ± 0.1, n=13; Teodoro Sampaio: 3.6 ± 0.2, n=8). Across species within areas our data are not complete to perform comparisons. Only *F. citrifolia* was collected on the three landscapes (*Table 5*).

Table 4. Fig species and number of crops sampled for wasp community analyses

Area	Species	# of crops
Ribeirão Preto	*F. citrifolia*	1
	F. trigona	2
Gália	*F. citrifolia*	3
	F. eximia	1
	F. insipida	1
	F. obtusifolia	4
Teodoro Sampaio	*F. citrifolia*	3
	F. crocata	3
Total		18

Table 5. Mean ± SD of foundress number per fig species. N = 50 figs per crop

Species	Area	# of crops	# of foundresses
F. citrifolia	Ribeirão Preto	3	1.5 ± 1.1
	Gália	3	5.4 ± 3.3
	Teodoro Sampaio	2	2.9 ± 2.9
F. crocata	Teodoro Sampaio	2	5.2 ± 4.8
F. eximia	Gália	2	2.7 ± 1.7
F. luschnatiana	Gália	1	0.9 ± 0.3
	Teodoro	2	1.2 ± 0.6
F. obtusifolia	Gália	6	2.2 ± 2.1
	Teodoro Sampaio	1	4.4 ± 2.6
F. trigona	Teodoro Sampaio	1	5.6 ± 5.7
F. insipida	Gália	1	0.2 ± 0.5
	Ribeirão Preto	1	1.0 ± 1.4

Frugivory by birds

Our data suggest that fig frugivory by birds is very limited in the most fragmented landscape (*Table 6*). Indeed, in the Estação Ecológica de Ribeirão Preto medium and large frugivorous birds are not observed anymore (M. E. Lapate, unpublished data). In Teodoro Sampaio we observed only one seed predator species. However, sample size is small in this area and more samples are required for a more confident interpretation.

Table 6. Frugivorous birds observed in the study areas

Area	Fig species	N (h)	#of bird species	Main bird species	Visit rate (#.10h^{-1})	Consumption (Figs. min^{-1})
Ribeirão Preto	*F. citrifolia*	3 (65)	1	*Thraupis sayaca*	0.8	0.5
Gália	*F. citrifolia*	1 (29)		*Penelope superciliaris*	2.5	0.4
	F. eximia	1 (17)	7	*Thraupis sayaca*	8.5	0.6
	F. insipida	1 (28)		*Pteroglossus aracari*	1.5	0.4
Teodoro Sampaio	*F. crocata*	1 (23)	2	*Pyrrhura frontalis*	6.1	0.7

Preliminary data suggest that forest fragmentation negatively interfere on fig tree communities, decreasing species diversity, pollinator availability and dispersion by birds.

Rodrigo Augusto Santinelo Pereira

Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto
Universidade de São Paulo (USP)
Home page: sites.ffclrp.usp.br/ficus
raspereira@ffclrp.usp.br

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Young researcher

BIOTRANSFORMATION OF PIMARADIENOIC ACID BY FILAMENTOUS FUNGI AND EVALUATION OF THE INHIBITORY EFFECT ON RAT AORTA CONTRACTION AND ON THE *Trypanossoma cruzi* DIHYDROOROTATE DEHYDROGENASE ACTIVITY OF THE DERIVATIVES OBTAINED

Sergio Ricardo Ambrósio

OBJECTIVES

The general aim of the present project is to obtain derivatives of *ent*-pimara-8(14),15-dien-19-oic acid (pimaradienoic acid, PA) by fungal biotransformation and to evaluate their inhibitory potential on the phenylephrine or KCl induced-rat aorta contraction and on the *Trypanossoma cruzi* dihydroorotate dehydrogenase activity. For these purposes, the specific aims are:

- Isolation of the PA from the dichloromethane extract of the roots of *Viguiera arenaria* Baker;
- Determination of the best conditions (culture medium and incubation period) for the filamentous fungi to produce spores;
- Determination of the incubation period in preculture fermentation stage to produce biomass;
- Evaluation of the incubation period on biotransformation reactions by HPLC (high performance liquid chromatography) analysis;
- Biotransformation of PA in preparative scale;
- Isolation and structural determination of the derivatives;
- Evaluation of the derivatives inhibitory potential on the phenylephrine or KCl induced-rat aorta contraction and on the Trypanossoma cruzi dihydroorotate dehydrogenase activity.

METHODOLOGY

Isolation of the PA: the PA was isolated from the dichloromethane extract of the roots of Viguiera arenaria according to Ambrosio et al. (2004).

Microorganisms: the biotransformation processes have been carried out using the following microorganisms: *Aspergillus ochraceus*, *Glomerella cingulata*, *Mucor rouxii* and *Rhizopus stolonifer*. All the strains belong to the Collection of fungi of the Laboratory of Enzymology of the Faculty of Pharmaceutical Sciences, USP.

Production of the spores: the microorganisms have been inoculated in different media (oat agar, malt extract and potato dextrose agar) and incubated at 30°C during different periods of time (7 and 15 days). The production of spores has been analyzed using a Neubauer hemocytometer. For the production of the fungi biomass it has been used approximately 1.107 spores/mL.

Production of the biomass: the production of the biomass for the fungi has been evaluated during 3 days using 100 mL of the seed medium (Jackson et al., 1993) at 30°C and 120 rpm.

Biotransformation processes: the mycelia have been transferred to a flask containing 100 mL of fermentative media (Czapek, Atlas, 1995) and PA (0.1 g/mL, solubilized in dimethyl sulfoxide) has been added. The concentration of dimethyl sulfoxide has been not exceeded 2.5% and samples (1 mL) have been taken every 24 hours, during 10 days. The culture broths have been partitioned with ethyl acetate and the obtained extracts have been analyzed by HPLC to evaluate the incubation period on biotransformation reactions. After this stage, the biotransformation of PA has been done in preparative scale aiming the isolation of the PA derivatives.

Evaluation of the derivatives inhibitory potential on the phenylephrine or KCl induced-rat aorta contraction: The thoracic aorta (male Wistar rats weighing between 200 and 250 g) has been quickly removed, cleaned of adherent connective tissues and cut into rings (5–6 mm in length). Two stainless-steel stirrups have been passed through the lumen of each ring. One stirrup has been connected to an isometric force

transducer to measure tension in the vessels. The rings have been placed in a 5 mL organ chamber containing Krebs solution gassed with 95% O2/5% CO2, and maintained at 37 ºC. The rings have been stretched until an optimal basal tension of 1.0 g and then equilibrate for 60 min. Endothelial integrity has been assessed qualitatively by the degree of relaxation caused by acetylcholine (Ach, 10-6 mol/L) in the presence of contractile tone induced by Phenylephrine (10-8 mol/L). After the ACh test, the rings have been washed out for 30 min in Krebs solution gassed with 95%O2/5%CO2. After returning to the resting tension, the rings have been incubated with the PA derivatives at concentration of 20 ìg/mL for 30 min. In a second set of experiments, the same experimental protocol has been used to investigate the effects of pre-incubation with PA derivatives on the contractile response induced by KCl (45 mmol/L). The inhibitory potential of these metabolites will be analyzed comparing our results with the data previously described for PA.

Evaluation of the derivatives inhibitory potential on the *Trypanossoma cruzi* dihydroorotate dehydrogenase activity: the evaluation of the derivatives inhibitory potential on the *Trypanossoma cruzi* dihydroorotate dehydrogenase activity will be done in collaboration with the Profa. Dra. Maria Cristina Nonato at the Laboratory of Crystallography of Proteins, USP.

RESULTS AND DISCUSSION

PA (2.8 g) was isolated from the dichloromethane extract of the roots of *Viguiera arenaria* according to Ambrosio et al. (2004). The cultivation conditions to produce spores and biomass have been determined for each fungus. However, results about the biotransformation of PA and the biological bioassays have not yet been obtained. In addition, the researchers Niege Araçari Jacometti Cardoso Furtado, Sergio Ricardo Ambrosio and Hosana Maria Delbonsi will be working together aiming to evaluate the antispasmodic potential on cardiovascular smooth muscle of the marine algae and fungal biotransformation extracts. In the future, depending on the obtained results, the isolated compounds could be also evaluated. Concerning the natural products isolation, some metabolites could be submitted to biotransformation experiments.

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Prof. Dr. Sérgio Ricardo Ambrósio

Pró-Reitoria Adjunta de Pesquisa e Pós-Graduação
Universidade São Francisco (Unifran)
http://www.unifran.br
sergioambrosio@unifran.br

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BIOLUMINESCENT BEETLES (COLEOPTERA: ELATEROIDEA) FROM THE ATLANTIC RAIN FOREST: BIODIVERSITY AND USE AS BIOINDICATORS FOR ENVIRONMENTAL IMPACT

Vadim Viviani

INTRODUCTION

Among terrestrial organisms, bioluminescence is predominantly found among insects. In beetles, the richest group, bioluminescent species occur mainly in the Elateroidea superfamily, which includes three main bioluminescent families: Lampyridae (fireflies), Phengodidae (railroad worms) and Elateridae (click beetles). About 2000 species of luminescent beetles are described around the world (Lloyd, 1978). Brazil has the richest biodiversity of luminescent insects, with about 500 species which correspond to roughly 23% of the described species in the world, but many more remain to be described (Viviani and Bechara, 1998; Viviani, 2001). The three main families of luminescent beetles as well as luminescent species of Staphylinidae (Costa et al., 1986) are found in Brazil. They are distributed among three main ecosystems: the Amazon rain forest, the Atlantic rain forest and Cerrado (savannas). During the last two decades we investigated the fauna of luminescent beetles occurring mainly in the Central and Northern region of São Paulo State Atlantic rain forest, one of the richest and most threatened tropical forests in the world. More recently we expanded our studies to the Southern region of São Paulo State Atlantic Rain forest and in areas of intersection with Cerrado (savannas) of Rio Claro and São Carlos. Here we compare the diversity of luminescent beetles in three main regions of São Paulo state Atlantic rain forest: (1) EBB: Estação Biológica de Boracéia at Salesopolis, which is a large contiguous reservation; (2) Faz. Santana (Campinas), a small remnant of semi-decidual forest; (3) Rio Claro, an area of intersection between Cerrado and Atlantic Rain forest, and (4) Sorocaba city which is located in an intersection between Atlantic Rain forest and Cerrado.

OBJECTIVES

1. Catalog bioluminescent species occurring in the Atlantic Rain Forest
2. Investigate the effect of artificial night lighting on the occurrence of bioluminescent species
3. Investigate the occurrence of bioluminescent species in environments under anthropogenic effect
4. Investigate the evolution of bioluminescent species of Neotropical region
5. Contribute to the studies of structure and function of beetle luciferases and their biotechnological applications ongoing in our laboratories

METHODOLOGY

Collecting sites

I. Campinas Metropolitan area (Campinas, SP; Latitude: 22º 53' 20".5 S; Longitude: 47º 04' 40".4 O; 1.500.000 people)

Faz. Santana, Sousas. This is a remnant of semi-decidual Atlantic rain forest (Fig.3A) surrounded by Atibaia's river. Surrounding the forest, there are open fields, horse pastures (Fig.3B) and marshies.

II. Rio Claro (22° 05' e 22° 40' S; 47° 30' e 47° 55' O; 200.000 people)

UNESP Campus. It is constituted mostly by open fields that periodically are burned.

Washington Luis Highway Marsh (Fig. 3C). It is a marsh surrounded by sugar cane fields.

III. Sorocaba (Latitude: 23º 29' 56".3 S; Longitude: 47º 27' 30".1 O; 600.000 people)

UFSCAR Campus (Fig.3D). It is constituted mostly by cattle pastures, surrounded by secondary growths and marshies.

Votorantim Marsh. A marshy field surrounded by cattle fields in an increasingly urbanizing site.
Votuparanga shore. Several remnants of Atlantic rain forest and marshy areas at the basis.

IV. Biological Station of Boraceia (Salesopolis, SP; 23º33' lat. S 45º50' long. W)

It is the largest and most contiguous area of preserved Atlantic rain forest located on the sea shore.

Collecting techniques

Adult fireflies and click beetles were collected at dusk and night over the grass and flying. Adult phengodid males were collected using ultraviolet light trap, and also on the grass. Larval fireflies were collected on the grass and on the ground. Larval phengodids we collected mainly on the ground at night. Larval click beetles were collected on decaying logs and on the soil.

RESULTS AND DISCUSSION

The Atlantic rain forest has one of the richest fauna of bioluminescent beetles in the world. This study shows a prospection of bioluminescent species performed during the past 20 years in Eastern São Paulo state. Some of the studied areas shown here were recently included in our investigation. According to this investigation, the eastern region of São Paulo state, which is the most populated and industrialized, accounts with at least 50 different species. A total of 37 species in 14 genera of Lampyridae, 7 species in 4 genera of Elateridae and 6 species in 4 genera of Phengodidae were found (Table 1). An underestimation showed that Biological Station of Boracéia accounted with the highest diversity (31 species) followed by Faz. Santana in Campinas municipality (24 species), Sorocaba municipality (20 species) and Rio Claro municipality (18 species) (Table 2). The highest diversity is found in forest environments, whereas the largest population densities of fireflies are found in open marshy environments (Table 3). As expected, the more preserved sites accounted with the highest number of species. Small sites close to the cities under anthropogenic influence have a few persistent species such as the lampyrids *Aspisoma lineatum* and *Bicellonychia lividipennis*.

Prof. Dr. Vadim Viviani
Cleide Costa
Priscila Tanioka
Oskar Hagen
Rogilene Prado

Universidade Federal de São Carlos (UFSCar) – Campus Sorocaba
viviani@ufscar.br

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