FAPESP BIOTA-microorganisms overview

BIOTA annual meeting
São Pedro
2014
João C. Setubal
University of São Paulo
Waste recycling in the Sao Paulo Zoo

- Mangrove
- Amazonian dark earth
- Freshwater reservoir
- Howler monkey feces
- Composting
- Insect gut
- Sugarcane leaves
- Sugarcane waste (filter cake)

Techniques:
- 16S
- Shotgun
- mRNA
Non-metagenomics surveys

Protozoofauna in surface water and sediment samples from the Atibaia River

Ciliated Protozoa  Giardia  Cryptosporidium

Toxigenic species of *Aspergillus* in several foods

Viruses and viroids in plants

Secretome of strains of filamentous fungi
Motivations

• Biotechnology
  – Biomass degradation
  – Herbicide degradation
  – Insecticide degradation
  – Antibiotics (bactericides and fungicides)
  – Tannins and phytates degradation

• Water quality assessment
• Phytopathology
• Human health
• Animal health
Results sampler
Thermophillic bacteria are the primary biomass degraders in the Zoo composting; several probable new strains or species
Termites: **Firmicutes** dominates in *A. euamignathus* and **Proteobacteria** dominates in *C. gestroi*

Citrinin against *Staphylococcus aureus* N315
The Atibaia river has 54 different genera of Ciliated protozoa.
368 plant species found to be infected with more than 370 different viruses
Sugar cane endophytes capable of degrading atrazine

<table>
<thead>
<tr>
<th>Endophytes</th>
<th>Reduction of atrazine</th>
<th>Metabolites ((m/z))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces</em> sp.</td>
<td>85 %</td>
<td>295; 311</td>
</tr>
<tr>
<td><em>Pantoea stewartii</em></td>
<td>70%</td>
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</tr>
<tr>
<td><em>Paracoccus</em> sp.</td>
<td>70%</td>
<td>295; 311</td>
</tr>
<tr>
<td><em>Burkholderia</em> sp.</td>
<td>70%</td>
<td>295; 311</td>
</tr>
<tr>
<td>Control (without bacteria)</td>
<td>0</td>
<td>295</td>
</tr>
</tbody>
</table>
Several new alpha-ARHD gene families found in ADE bacteria

ARHD = aromatic ring-hydroxylating dioxygenase

ADE = Amazonian Dark Earth

Degrader of Polycyclic aromatic hydrocarbons (PAHs)
Toxigenic *Aspergillus* species found in all analyzed foods
Thermo- and solvent-tolerant tannase from *Emericela*

Phytase production using fungal biofilm (*Rhizopus microsporus*)
Should I elaborate?
Microbial diversity in the S. Paulo Zoo

• PIs
  – João C. Setubal
  – Aline M. da Silva
• FAPESP 2011/50870-6
• USP + UNIFESP + Sao Paulo Zoo
• Metagenomics of SP Zoo environments
• 2012-2017 (ongoing!)
Sao Paulo Zoo Environments

- Composting
- A lake (reservoir)
- Howler monkey feces
### Compost sequencing data (# of reads)

<table>
<thead>
<tr>
<th>Day</th>
<th>WGS</th>
<th>16S</th>
<th>Total RNA</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZC3 A (454)</td>
<td>ZC3 B</td>
<td>ZC4</td>
<td>ZC4</td>
</tr>
<tr>
<td>00</td>
<td>-</td>
<td>-</td>
<td>1.151.088</td>
<td>-</td>
</tr>
<tr>
<td>01</td>
<td>520.074</td>
<td>2.270.265</td>
<td>4.106.932</td>
<td>1.014.318</td>
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<tr>
<td>03</td>
<td>-</td>
<td>-</td>
<td>2.481.164</td>
<td>2.623.129</td>
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<tr>
<td>07</td>
<td>-</td>
<td>-</td>
<td>4.556.562</td>
<td>1.495.291</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>3.574.653</td>
<td>2.082.961</td>
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<tr>
<td>64</td>
<td>771.427</td>
<td>1.265.241</td>
<td>5.055.450</td>
<td>2.376.946</td>
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<tr>
<td>67</td>
<td>-</td>
<td>-</td>
<td>2.825.621</td>
<td>1.364.521</td>
</tr>
<tr>
<td>78</td>
<td>1.063.197</td>
<td>2.169.689</td>
<td>11.245.102</td>
<td>1.862.329</td>
</tr>
</tbody>
</table>
### Most abundant species using all samples [Kraken]

<table>
<thead>
<tr>
<th>Species</th>
<th>order</th>
<th>Genome size (bp)</th>
<th># reads (X cov)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermobispora bispora</td>
<td><em>actinomycetales</em></td>
<td>4,189,976</td>
<td>950,821 (45)</td>
</tr>
<tr>
<td>Rhodothermus marinus</td>
<td><em>Rhodothermaceae</em> <em>(bacteroidetes)</em></td>
<td>3,386,737</td>
<td>786,019 (46)</td>
</tr>
<tr>
<td>Thermobifida fusca</td>
<td><em>actinomycetales</em></td>
<td>3,642,249</td>
<td>715,321 (39)</td>
</tr>
<tr>
<td>Sphaerobacter thermophilus</td>
<td><em>Sphaerobacterales</em> <em>(chloroflexi)</em></td>
<td>3,993,764</td>
<td>346,997 (17)</td>
</tr>
<tr>
<td>Pseudoxanthomonas suwonensis</td>
<td><em>xanthomonadales</em></td>
<td>3,419,049</td>
<td>286,846 (17)</td>
</tr>
<tr>
<td>Symbiobacterium thermophilum</td>
<td><em>clostridiales</em></td>
<td>3,566,135</td>
<td>274,424 (15)</td>
</tr>
<tr>
<td>Thermomonospora curvata</td>
<td><em>actinomycetales</em></td>
<td>5,639,016</td>
<td>239,080 (8)</td>
</tr>
<tr>
<td>Thermaerobacter marianensis</td>
<td><em>clostridiales</em></td>
<td>2,844,696</td>
<td>179,158 (13)</td>
</tr>
<tr>
<td>Thermobacillus composti</td>
<td><em>Bacillales</em></td>
<td>4,355,525</td>
<td>128,142 (6)</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td><em>xanthomonadales</em></td>
<td>4,649,035</td>
<td>114,562 (5)</td>
</tr>
</tbody>
</table>
Summary of results so far

• Biomass degradation in composting carried out primarily by thermophilic bacteria (as opposed to fungi)
• Different composting cells seem to have different microbial diversity (richness & relative abundance)
• Rich source of thermophilic enzymes
• There is evidence for seasonality in lake data
Dynamic growth and succession of bacterial and fungal communities during composting of filter cake

2011/50817-8

April, 2012 – March, 2014

Coordinator
Dr. Gustavo H. Goldman
USP – Ribeirão Preto
Joint appointment – CTBE

Brazilian Principal Investigators/PIs
Dra. Juliana Velasco de Castro Oliveira
Research Scientist
Functional Biology, Biotechnology and Biophysics Laboratories
Brazilian Bioethanol Science and Technology Laboratory
1) To know the dynamic growth and succession of this microbial community;

2) To assess the metabolic potential of the microbial community;

3) Identification of lignocellulolytic bacteria and fungi species/new hydrolytic enzymes to improve enzyme cocktails;

4) Identification of yeasts able to metabolize pentoses/new genes/proteins involved in the pentose metabolism.
Filter cake

Time: 6 months
13 time points

Comp 1

Filter cake

Comp 2

Poultry litter

Ash (from boilers)

Time: 45 days
5 time points
Compost samples

Isolation of microorganisms (Avicel, CMC, Xylose, Xylan, LB e YPD)

Total DNA extraction

ITS/16S libraries

Amplicon sequencing

DNA fragmentation

Shotgun sequencing

Identification of isolates

- Lignocellulolytic bacteria and fungi species
- Yeasts able to growth on pentoses

Temperature, humidity, [] of several minerals (P, K, Mg, Ca), pH, C:N rate

Knowledge of dynamic growth and succession of the community/metabolic potential
SUMMARY OF RESULTS

1) First work describing the microbial (bacteria and fungi) communities in two different sugarcane filter cake compost piles;

2) Identification of the metabolic potential of those communities;

3) Isolation and identification of many microorganism and “new” enzymes that would further help to reduce the cost of enzymatic cocktails (breakdown of biomass into monosaccharides);

4) Isolation and identification of many microorganism and “new” genes - genetic manipulation of *S. cerevisiae* (to able the fermentation of pentose).
Diversity, Ecology and Biotechnological Potential of the Symbiotic Bacteriofauna Associated with Insects

Coordinator - Fernando Luis Consoli (ESALQ-USP)

FAPESP: Process 2011-508770

Objectives – To determine the microbial diversity associated with insects and to investigate their ecological role in this association, and to exploit insect microbial symbionts as sources of bioactive molecules for biotechnological application
SUBPROJECT 1 – Insecticide resistant insects as source of bacteria with insecticide degradable potential

5 resistant lines of *Spodoptera frugiperda* to different pesticides were investigated.

- **Insecticide resistant lines are a source of bacteria capable to degrade pesticides**
- **Some of the selected gut bacteria are commonly associated with natural populations**
- **Larval gut bacteria can be transferred to the progeny either by egg smear or by vertical transmission**
- **Selected gut microbes contribute with the degradation of pesticides by the host insect**
- **Gut microbiota can participate in the process of insecticide resistance**
SUBPROJECT 2 – Microbial diversity associated with fruit flies and their role in host food utilization

Investigated the larval and adult gut microbiota of a lab colony of *Anastrepha fraterculus* and the effects of the host food on the microbial community.

- Larval gut community differs from adult gut community
- Adult bacterial gut community differs from that of the crop
- Larval and adult gut microbiota share few phylotypes
- Microbial community is determined by the host fruit
- Larval community is much more diverse than adult gut community
SUBPROJECT 3 – Microbial diversity associated lower and higher termites

Investigated gut microbial diversity in different castes of lower and higher termites

Gut microbiota of *A. euamignathus* is more diverse than that of *C. gestroi*

Gut microbiota of workers is more diverse than of soldiers

Worker microbiota of *A. euamignathus* is more diverse than of *C. gestroi*, while the opposite is true for the soldier microbiota

Firmicutes dominates in *A. euamignathus*, while Proteobacteria in *C. gestroi*

Microbiota diversities using culturable and non-culturable approaches are quite different
SUBPROJECT 4 – Isolation, characterization and identification of bioactive molecules with biotechnological potential

**Antimicrobials – bactericide and fungicide**

Citrinin against *Staphylococcus aureus* N315

**Enzymes**

Chitinases

**Bioinsecticides**

Several bacterial extracts tested

3 = 100% of insecticidal activity

2 > 80% of insecticidal activity

4 > 50% of insecticidal activity
Biodiversity and Taxonomy of Protozoofauna in Surface Water and Sediment Samples from Atibaia River, Campinas, São Paulo, Brasil.

FAPESP process: 11/50244-8

Coordinator: Regina Maura Bueno Franco

Duration 12/01/11 to 30/11/2014

Other Brazilian Principal Investigators (newly formed group - 2011):
Dra. Isabel Cristina Vidal Siqueira Castro
Maurício Durigan
Msc. Nilson Branco
Msc. Taís Rondello Bonatti
Tenille Filócomo – TT3
Aims: Focus on Ciliated and Pathogenic Protozoa for Assessing Water Quality

Surface water

Biodiversity of ciliated protozoa

Survey Giardia cysts and Cryptosporidium oocysts

Sediment
Methods: Ciliated and Pathogenic Protozoa

Ciliated Protozoa

- Sedgewick Rafter counting chamber (1 mL aliquot): total abundance per species.
- Observations (in vivo): DIC at 400x or 1000x (three hours after sampling).
- Cultures: aliquots of 20 mL: encysted ciliate diversity
- QPS: Quantitative Protargol Staining (12 samples for each site)

Pathogenic Protozoa

- **Surface water:**
  - Membrane filtration
  - Immunomagnetic separation (IMS)
- **Sediment samples**
  - enhanced physical and chemical dispersions
  - IMS
- For both samples: IFA + DAPI + DIC
Main Results

1-Biodiversity of the Atibaia River:
• Ciliated protozoa: 74 taxa belonging to 54 different genera.

2-Pathogenic protozoa:
• *Giardia duodenalis*: *Assemblage A*II evidences contamination by domestic sewage (=human origin).

3-Removal Potential by Predation:
• Cysts and oocysts were predated by some ciliated protozoa species, in experimental conditions.

4-Human resource training:
• Undergraduate students with and without scholarship, Doctoral and Post – doctoral (PNPD process no. 23038.008057/2010-13)

5-Scientific production:
• Paper: “Checklist of ciliated protozoa from surface water and sediment samples of Atibaia River, Campinas, São Paulo (Southeast Brazil).” – submitted
• International congress: 17th International Symposium on Health-Related Water Microbiology (September, 2013) and International Congress of Parasitology (August, 2014).
Project Title: EVALUATION OF THE DIVERSITY OF MOLECULAR PATHOGENS (VIRUSES AND VIROIDS) OF PLANTS IN BRAZIL

FAPESP Process: 2011/50895-9

Duration: 02/2012-07/2014

Principal Investigator: ELLIOT W. KITAJIMA, LFN/ESALQ/USP
ewkitaji@usp.br
As a first step to understand the diversity of plant viruses in Brazil, a detailed listing of these viruses found naturally infecting either cultivated or wild plants were made.

This listing was based on published records (regular articles from Brazilian or international journals, abstracts from meetings, dissertations and thesis), and for that purpose, these publications were catalogued from 1911 to 2013.

An annotated list of these viruses was produced, according to the host plant species in which they were found. A parallel list based on the species (genus, family, order) of the virus was prepared, as well as a list of the distribution of these viruses in the 27 Brazilian states.
RESULTS:

1. A TOTAL OF 368 PLANT SPECIES, BELONGING TO 69 FAMILIES HAVE BEEN REPORTED AS BEING INFECTED BY 169 RECOGNIZED SPECIES AND 71 UNASIGNED PLANT VIRUS SPECIES, AND 6 VIROIDS SPECIES. ADDITIONALLY THERE WERE 133 CASES OF PLANTS INFECTED BY UNIDENTIFIED VIRUSES.

2. THERE HAVE BEEN REPORTS OF PLANT VIRUSES IN ALL 27 BRAZILIAN STATES, TOTALLING ABOUT 1400 CASES.

3. STATE OF S.PAULO HAD MORE REPORTS: 430, INVOLVING 192 PLANT SPECIES INFECTED BY 199 DIFFERENT PLANT VIRUSES AND 3 VIROIDS SPECIES.

NEXT STEPS

1. UPDATE YEARLY THE EXISTING LISTS

2. ANALYSE GENOMIC VARIABILITY OF SELECTED GROUP OF VIRUSES (BEGOMO-, POTY-, TOSPOVIRUSES, P.EX.) TO UNDERSTAND THEIR EVOLUTION.
Prospection of endophytic microorganisms for the degradation of toxic compounds used in the sugar cane cultivation

Coordinator: Natalia Reiko Sato Miyasaka
Universidade São Francisco
Bragança Paulista – SP
FAPESP 11/50917-2
Objectives

• Isolate endophytic microorganisms from leaves of sugar cane (mineral medium with atrazine as the sole carbon source)
• Identification of endophytes by sequencing of the gene16S rRNA (Dr. VM de Oliveira, CPQBA, UNICAMP)
• Quantify the biodegradation of atrazine (in vitro) (HPLC)
• Evaluate the biodegradation products of atrazine by UHPLC-MS/MS (Dr. ACF Sawaya, IB, UNICAMP)
• Characterization of metabolites by High Resolution Spectrometry (Dr. M. Eberlin, IQ, UNICAMP).
• Acute ecotoxicity test of metabolites with Daphnia similis (Dr. D. D'Angelis, UNESP, Rio Claro)
Table 1. Reduction of atrazine by endophytes and detection of metabolites

<table>
<thead>
<tr>
<th>Endophytes</th>
<th>Reduction of atrazine</th>
<th>Metabolites (m/z)</th>
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<td><em>Streptomyces sp.</em></td>
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<td><em>Paracoccus sp.</em></td>
<td>70%</td>
<td>295; 311</td>
</tr>
<tr>
<td><em>Burkholderia sp.</em></td>
<td>70%</td>
<td>295; 311</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>295</td>
</tr>
</tbody>
</table>

* Nutriente broth with atrazine (30 mg/l), aerobic conditions, after 12 days.
Conclusions

• the endophytic bacteria isolated from sugar cane leaves reduced the concentration of atrazine in vitro.
• the herbicide detoxification process occurred through the biotransformation of atrazine ($m/z$ 216) into a more polar compound with $m/z$ 311 and a proposed molecular formula of $C_{14}H_{27}O_2N_6$.
• acute ecotoxicity test with *Daphnia similis* indicated that the metabolite $m/z$ 311 was non toxic.
Conclusion

• The mechanism of detoxification of atrazine by endophytes was different from other free-living microorganisms that inhabit the soil or rhizosphere. However, it resembles the mechanism of detoxification described for plants. The results showed new aspects of atrazine detoxification in sugar cane.
THE MICROBIOME OF AMAZONIAN DARK EARTH: STRUCTURE AND FUNCTION OF THE MICROBIAL COMMUNITIES FROM RHIZOSPHERE AND BIOCHAR ASSOCIATED TO THE BIOGEOCHEMICAL CYCLES

BIOTA/FAPESP: MICROORGANISMS (2011/50914-3)

Coordinator: SIU MUI TSAI

Cell and Molecular Biology Laboratory
Center for Nuclear Energy in Agriculture/ USP
Piracicaba – São Paulo
Terra Preta Da Amazonia

Amazonian Dark Earth

- Elevated Organic Matter
- High pH
- Presence of black carbon = charcoal

- Deep Horizon A with dark color
- Litic artifacts and ceramics
- High levels of P, Ca, Zn, Mg
Community Structures
Who are in soil charcoal?
- Phylogenetic analyses
- Sequencing the 16S rRNA gene
- Image analyses of communities

Genetic Potential and Gene Expression
What can the microbes do?
- Metagenome
- Functional genes
- Metaproteomics
- Metatranscriptomics

Functional Characterization
- New methods of cultivation
- Stable isotope probing (SIP)
- The multidimensional approach: Gene/Protein Neural network

ADE: The Black Gold of Amazonia
TPN = Terra Preta Nova (Soil Reconstruction)
Two 16S rRNA gene libraries were constructed from samples taken from a site that was undisturbed for over two decades and sequenced.

Ribosomal Database Project (RDP)
FUNCTIONAL DIVERSITY OF ALPHA-ARHD GENE IN AMAZONIAN DARK EARTH AND BLACK CARBON

ARHD primers for large subunit (catalytic domain) of aromatic dioxygenases.

P. putida F1 (NC_009512.1) (todC1)

Rieske center

Fe$^{2+}$ binding site

α-ARHD 2F

Clone library (~300bp)

α-ARHD 2R

BLACK CARBON

AMAZONIAN DARK EARTH

N° observed OTUs vs N° of valid clones graph.
FUNCTIONAL DIVERSITY OF ALPHA-ARHD GENE IN AMAZONIAN DARK EARTH AND BLACK CARBON

ARHD = aromatic ring-hydroxylating dioxygenase
150 Isolates from ADE

Siderophore production:
- 35 Pseudomonas – 50% +
- 30 Arthrobacter – 20% +
- 14 Bacillus – 65% +

Antibiotics: Q-TOF (inhibition halos)
- Phenazine (Pseudomonas sp.)
- Pseudomononine (P.putida) – 330 Da
SECRETOME FROM FILAMENTOUS FUNGI: SEARCHING ENZYMES (PHYTASES AND TANNASES) AND PROTEINS OF BIOTECHNOLOGICAL INTEREST

FAPESP (MicroBiota): 2011/50880-1
(01/02/2012 a 31/01/2014)

Responsible researcher: Prof. Dr. Luis Henrique Souza Guimarães

Students: 04 DR; 03 MS; 02 SI
Published Articles: 07
Submitted articles: 04

Global Industrial Enzymes Market

2015: US$ 8 billions

- Diagnostic
- Research and Biotechnology
- Animal feed
- Food
- Beverage
There are many strains of filamentous fungi isolated and stored in many culture collections in the São Paulo state, which would be used for secretome analysis, especially for enzyme production, as highlighted in this project.

The aim was to study the secretome of different strains of filamentous fungi under diverse culture conditions (Submerged and Solid-State Fermentation, and Biofilms), especially the production of phytases and tannases, as for as the production of other proteins involved in the tannins and phytates degradation.

It is important to reinforce that this project is in agreement with the MicroBiota aim and it will attend 3 main subjects, Biotechnology, Biochemistry and Bioprospection.

Some examples of results obtained:

*Metarhizium anisopliae* secretome;
Phytase production using *Rhizopus* biofilm;
Thermo and solvent tolerant tannase from *Emericela*.

Many fungal strains were analyzed for production of secreted enzymes
Metarhizium anisopliae Secretome

Culture media with chrysalis as inducer

Secreted proteins

IBCB 167

IBCB 360

IBCB 384

IBCB 425

Example of identification: endo-N-acetyl-β-D-glucosaminidase

Importance: Virulence factor

Biotechnological application – biological control
## Phosphatase and Phytase Production

**Importance:** Microbial nutrition; biotechnological application (molecular biology and feed)

<table>
<thead>
<tr>
<th>Filamentous Fungi</th>
<th>Phosphatase activity (Total U)</th>
<th>Phytase activity (Total U)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaline</td>
<td>Acid</td>
</tr>
<tr>
<td></td>
<td>Intra</td>
<td>Extra</td>
</tr>
<tr>
<td>Cinza Novo</td>
<td>61,4&lt;sup&gt;F&lt;/sup&gt; ± 0,3</td>
<td>1,3&lt;sup&gt;G&lt;/sup&gt; ± 0,9</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>53,2&lt;sup&gt;G&lt;/sup&gt; ± 0,9</td>
<td>0&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>162,0&lt;sup&gt;A&lt;/sup&gt; ± 0,7</td>
<td>38,5&lt;sup&gt;B&lt;/sup&gt; ± 0,7</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>113,7&lt;sup&gt;C&lt;/sup&gt; ±0,3</td>
<td>31,0&lt;sup&gt;C&lt;/sup&gt; ± 0,7</td>
</tr>
<tr>
<td><em>A. niveus</em></td>
<td>97,8&lt;sup&gt;D&lt;/sup&gt; ± 0,3</td>
<td>0&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. phoenicis</em></td>
<td>150,3&lt;sup&gt;B&lt;/sup&gt; ± 0,3</td>
<td>60,6&lt;sup&gt;A&lt;/sup&gt; ± 0,9</td>
</tr>
<tr>
<td><em>P. variotii</em></td>
<td>22,2&lt;sup&gt;H&lt;/sup&gt; ± 0,3</td>
<td>0&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>R. microsporus</em></td>
<td>64,3&lt;sup&gt;F&lt;/sup&gt; ± 0,3</td>
<td>8,3&lt;sup&gt;F&lt;/sup&gt; ± 0,7</td>
</tr>
<tr>
<td>var. <em>microsporus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma sp.</em></td>
<td>22,4&lt;sup&gt;H&lt;/sup&gt; ± 0,9</td>
<td>19,7&lt;sup&gt;D&lt;/sup&gt; ± 0,6</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>22,6&lt;sup&gt;H&lt;/sup&gt; ± 0,6</td>
<td>10,8&lt;sup&gt;E&lt;/sup&gt; ± 0,7</td>
</tr>
</tbody>
</table>
Phytase production using fungal biofilm (*Rhizopus microsporus*)

- **Maltodextrin**
- **Soy Flour**
- **Corn Bran**
- **Corn Flour**

**Spray Drying**

- **Enzyme Purification**
- **Enzyme characterization**

**Characterization**

- **Added to feed composition for chickens**
Tannase Production: Example *Emericela nivea*

Enzymes with different properties as high stability, Solvent tolerance, temperature and pH of activity, etc.

Importance: bioremediation; food and beverage; pharmaceutical

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Acetone</td>
<td>130.37 ± 2.47</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>149.85 ± 2.04</td>
</tr>
<tr>
<td>Ethanol</td>
<td>148.47 ± 2.59</td>
</tr>
<tr>
<td>Glycerol</td>
<td>138.50 ± 2.94</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>150.00 ± 6.87</td>
</tr>
<tr>
<td>Methanol</td>
<td>139.57 ± 1.13</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>140.95 ± 1.96</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative activity (%)</th>
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<tr>
<td></td>
<td>0.01%</td>
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<tr>
<td>SDS</td>
<td>138.96 ± 2.55</td>
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<td>Triton X-100</td>
<td>121.63 ± 2.59</td>
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<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative activity (%)</th>
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<tr>
<td></td>
<td>1 mM</td>
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<tr>
<td>β-Mercaptoethanol</td>
<td>110.12 ± 0.56</td>
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<tr>
<td>EDTA</td>
<td>109.51 ± 0.98</td>
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<tr>
<td>Control</td>
<td>100</td>
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</table>
1. Microbial diversity in the São Paulo Zoo
2. Dynamic growth and succession of bacterial and fungal communities during composting of filter cake
3. Diversity, Ecology and Biotechnological Potential of the Symbiotic Bacteriofauna Associated with Insects
4. Isolation, characterization and identification of bioactive molecules with biotechnological potential
5. EVALUATION OF THE DIVERSITY OF MOLECULAR PATHOGENS (VIRUSES AND VIROIDS) OF PLANTS IN BRAZIL
6. Prospection of endophytic microorganisms for the degradation of toxic compounds used in the sugar cane cultivation
7. Amazonian Dark Earth Microbiome
8. Biodiversity of Toxigenic *Aspergillus* Species in Brazil: Occurrence, Polyphasic Taxonomy and Distribution
9. SECRETOME FROM FILAMENTOUS FUNGI: SEARCHING ENZYMES (PHYTASES AND TANNASES) AND PROTEINS OF BIOTECHNOLOGICAL INTEREST