



Chaetothyrialean fungi and relatives from oil contaminated areas in Brazil



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Where we are...





Biosciences Institute (IB)

- Graduations → **Biological Sciences, Ecology, Physical Education, Pedagogy.**
- Post-Graduation → **Cell biology, Botany, Zoology, Motricity Sciences, Education, **Applied Microbiology.****



Department of Biochemistry and Microbiology

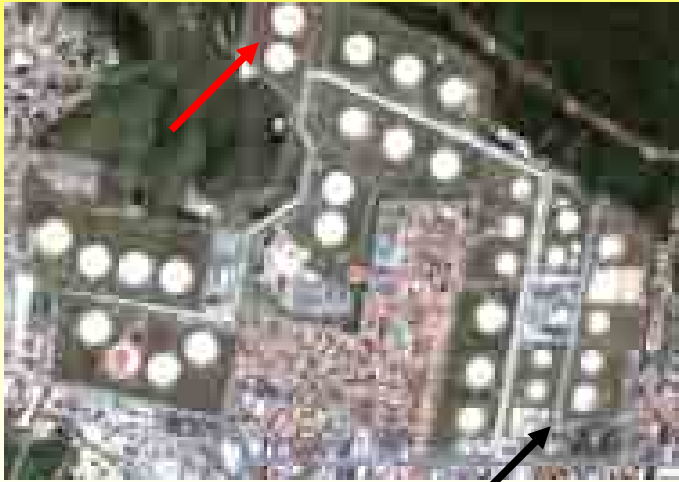


Hydrocarbon-degrading Chaetothyrialean fungi

- **Benzene – priority among oil pollutants.**
- **Benzene-degrading fungi – application in biorremediation and biofiltration.**

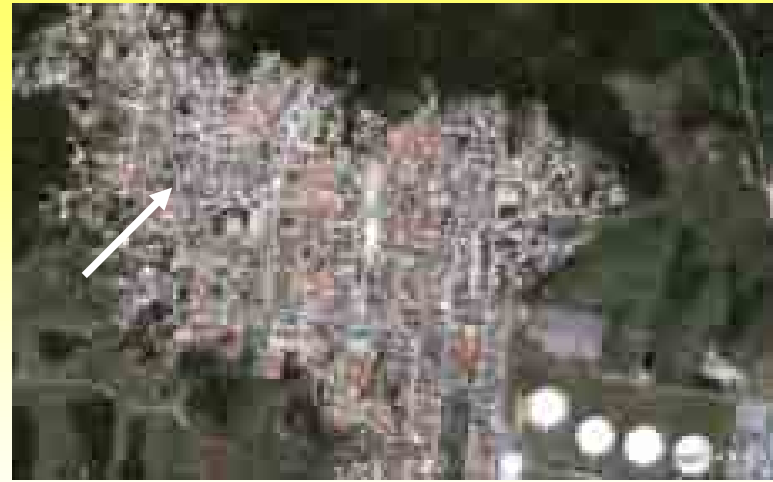


a.



Tebar

b.



Itatinga

c.



Bertioga

d.



Cubatão

Studied areas



Paulínia Refinery – REPLAN – landfarming area





Eucalyptus tereticornis



Garage floor





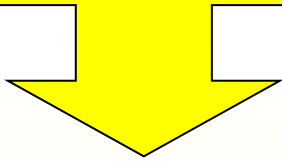
Flotation method for Black Yeast Isolation

SATOW , M.M., ATTILI-ANGELIS, D., HOOG, G. S., ANGELIS, D.F., VICENTE, V. A. Selective factors involved in oil flotation isolation of black yeasts from the environment. *Studies in Mycology*, v. 61, p.157–163, 2008.



Antibiotics addition

**200u/mL Penicillin
200µg/mL Chloramphenicol
200µg/mL Streptomycin
500µg/mL Cycloheximide**



100mL saline



**Samples addition – 10- 20g
Incubation for 30 min – room temperature**



Mineral Oil Addition (20mL)



Vigourous shaking



5 minutes each sample.

Flasks stand for 20 minutes



Look at the concentration of cells on the water-oil interphase.

Plating



- 150 μ L suspension is taken from the water-oil interphase.
- For each Petri dish, the suspension is taken from a different spot of the interphase.
- Mycosel Agar.

Incubation at 35°C



Sample: contaminated soil with hydrocarbons.
a) 5-day-incubation plate (Mycosel).
b) up to 25-day-incubation plate (Mycosel + cycloheximide).



Results

348 black fungal strains were isolated in total

- 285 from contaminated soil samples
- 63 from the plant material

Name	Number of isolates	Source	Region
<i>E. xenobiotica</i>	06	Contaminated soil	Paulínia – SP
<i>E. angulospora</i>	01	Contaminated soil	Paulínia – SP
<i>C. immunda</i>	13	Contaminated soil	Paulínia – SP
<i>C. minourae</i>	02	Contaminated soil	Paulínia – SP
<i>A. pullulans</i>	01	Contaminated soil	Paulínia – SP
<i>R. apiculatum</i>	01	Contaminated soil	Paulínia – SP
<i>E. dermatitidis</i>	13	Garage floor	Rio Claro - SP
<i>E. dermatitidis</i>	10	<i>Eucalyptus tereticornis</i>	Rio Claro – SP
<i>E. bergeri</i>	02	<i>Eucalyptus tereticornis</i>	Rio Claro – SP
<i>C. devriesii</i>	03	<i>Eucalyptus tereticornis</i>	Rio Claro – SP
<i>P. americana</i>	01	<i>Eucalyptus tereticornis</i>	Rio Claro – SP

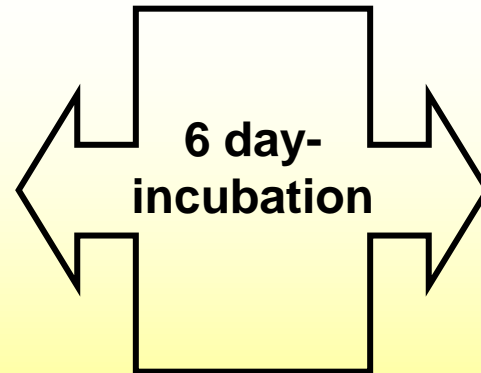
16% Identified taxa based on ITS sequencing: 54 strains in 348 isolates (16 out of 63 plants; 37 out of 285 soil samples).





DCPIP technique

redox indicator
2,6-dichlorophenol
indophenol



Pre-inoculum





*Cladophialophora
minourae*

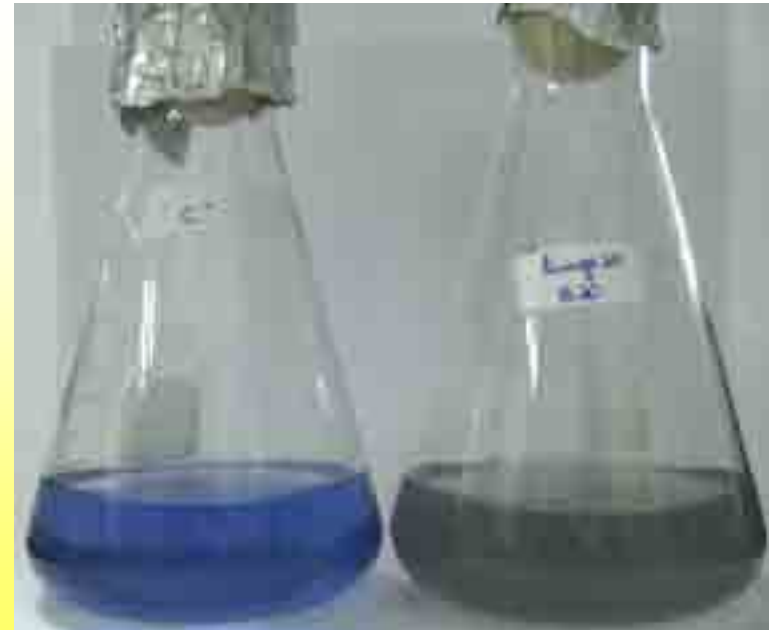


- 20mL pre-inoculum
- 80mL BH
- 0,5% benzene

Exophiala xenobiotica



C. immunda



BIOSCREEN C MBR

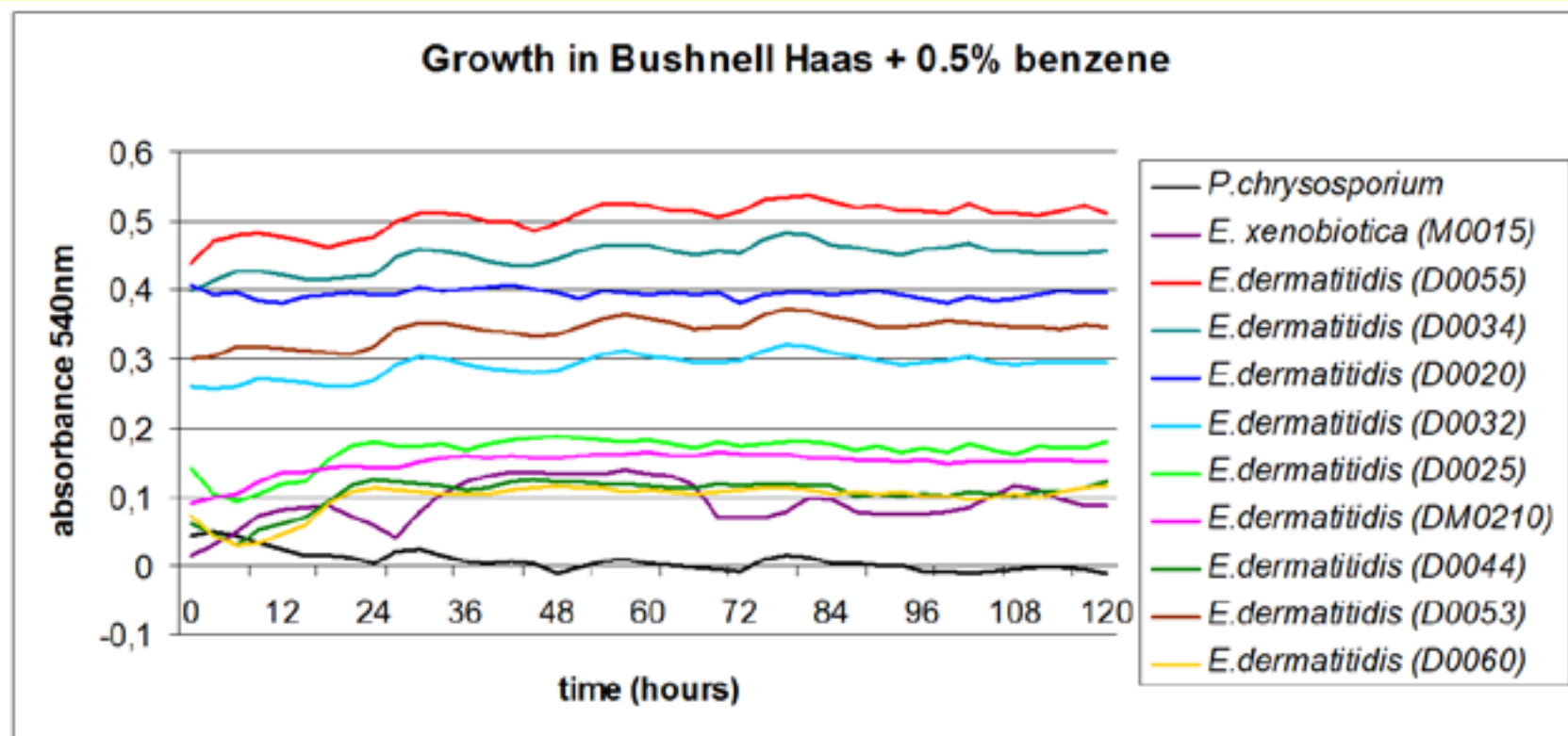
Biosceen C is a system designed for automating routine microbiology work. It uses a unique micro-plate format (10x10 wells), so called "Honeycomb" format, which is especially well suited for highly accurate temperature control. It features two covered honeycomb plates making it possible to run 200 samples simultaneously.

Biosceen C MBR is a computer-controlled incubator/reader/ shaker equipped with 8 filters from 405nm to 600nm including a wide band filter. It has a sensitive reader that measures turbidity, a technique more sensitive than a spectrophotometer. It also includes a linear shaker to assure good mixing in each well. The test length can be set from one single measurement to up to a year.

Its unique patented incubator system features temperature control maintaining the set temperature with a 0,1 degree centigrade accuracy, while avoiding condensation of liquid on the inside of the micro plate lid. It can cool down the samples 6 degrees centigrade below the ambient temperature and its working range is from 1 to 60 degrees in steps of 0,1 degree centigrade.

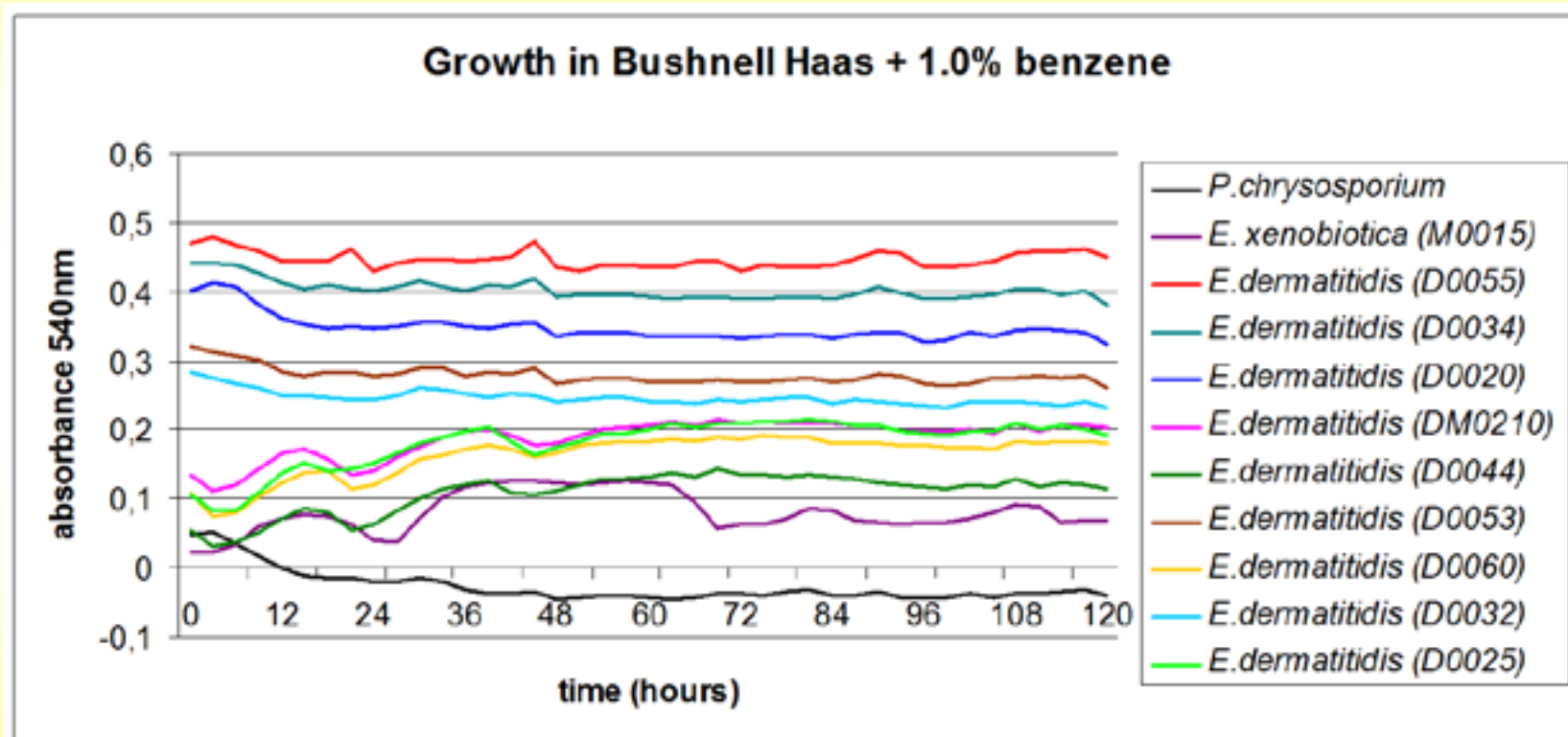


Results



Growth curves of 10 selected strains in Bushnell Haas + 0.5% benzene, 5-day incubation at 25°C, continuous shaking.

Results



Growth curves of 10 selected strains in Bushnell Haas + 1.0% benzene, 5-day incubation at 25°C, continuous shaking.

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Future plans

- *Exophiala dermatitidis*
- *Cladophialophora* sp.
- *Cladophialophora devriesii*
- Confirm biodegradation ability with other tests.

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CENTRO DE ESTUDOS DE INSETOS SOCIAIS

**Muito
obrigada!**



Laboratório de
Microbiologia