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gestió integral
de residus orgànics
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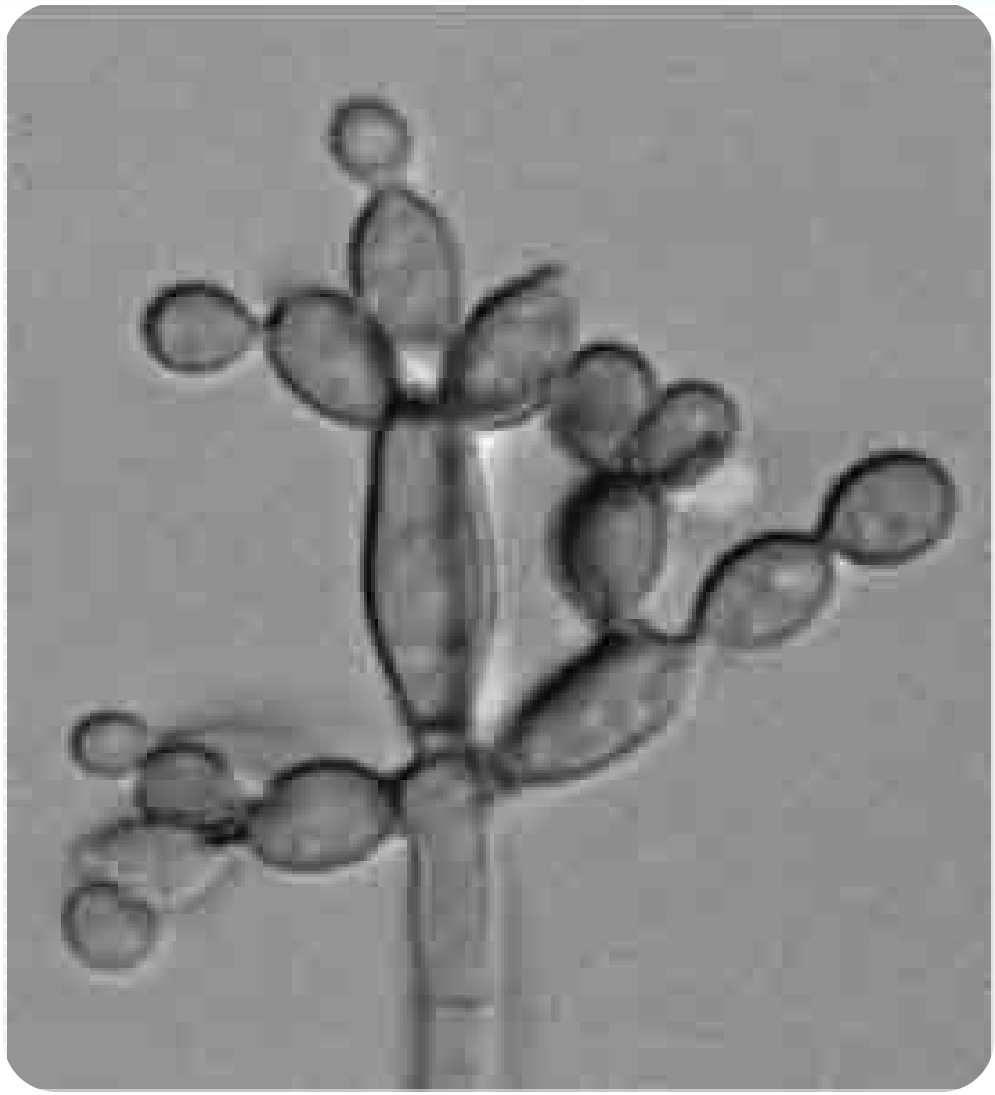
Centre adscrit a:

Enrichment of black yeasts under atmospheres of aromatic hydrocarbons:

Impact on biotechnological applications

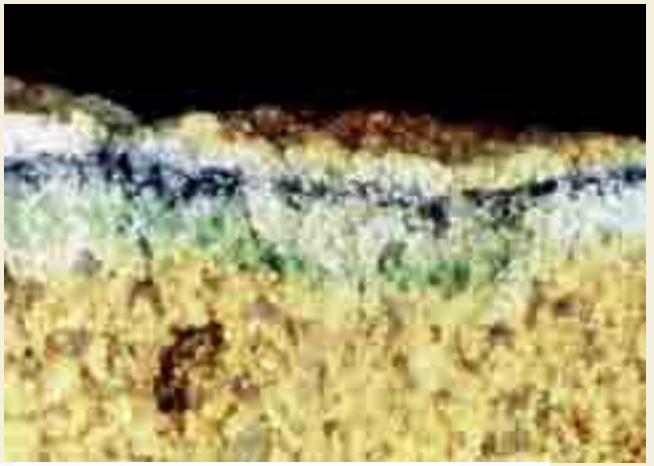
Francesc Prenafeta Boldú



...how extreme is extreme for black yeasts?

Ha

Selt



tarctic

06)

Radiotrophic fungi in Chernobil

Dadachova et al. *PLoS One* 2 (2007)

Fungi in creosoted wood

Zhao et al. *Microb Ecol* (2010)

Isolation from the environment

Method	Prevalent genus	Order	Ecology
Animal bait	*Exophiala, Fonsecaea, Cladophialophora*	*Chaetothyriales*	Opportunists
Erythritol	*Exophiala*	*Chaetothyriales*	Opportunists
Mineral oil	*Exophiala, Fonsecaea*	*Chaetothyriales*	Opportunists
Raulin 40°C	*Exophiala*	*Chaetothyriales*	Opportunists
Needle	*Coniosporium*	*Chaetothyriales*	Rock fungi
Alkylbenzene vapors	*Cladophialophora, Exophiala*	*Chaetothyriales*	Xenobiotics
Acidic	*Exophiala*	*Chaetothyriales*	Acidophiles
	Hortaea	*Dothideales*	
Crush	*Coniosporium*	*Chaetothyriales*	Rock fungi
	Hormonema	*Dothideales*	
Low strength	*Exophiala*	*Chaetothyriales*	Oligotrophs
	Sarcinomyces	*Dothideales*	Rock fungi
	Cadophora	*Leotiales*	Oligotrophs
High salt	*Aureobasidium, Hortaea*	*Dothideales*	Halophiles
Suspend	*Cryomyces, Friedmanniomyces*	*Dothideales*	Psychrophiles
Ethanol	*Baudoinia*	*Dothideales*	Ethanophiles

Zhao et al. *Microb Ecol* (2010)

Fungal biofiltration

A lab biofilter treating **toluene** became fully colonized with a dark fungus, then identified as a *Cladosporium sphaerospermum* (the correct identity is *Cladophialophora saturnica*)

This biofilter displayed a very robust and stable performance, despite poor irrigation and relatively high organic loads.

Fungal growth on toluene as sole carbon and energy source was demonstrated

Weber et al. *Appl Environ Microbiol* 61 (1995)

Fungal biofiltration

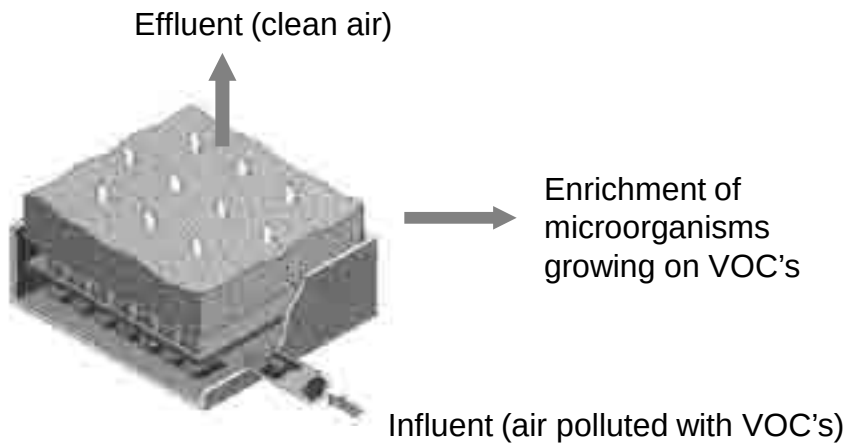
Gas biofiltration techniques are characterized as:

-Relatively inexpensive solution for treating gases polluted with a low VOC’s content

-More sustainable technology than physicochemical alternative processes

-Easy operation and maintenance

-Tendency to clog due to excessive biomass growth or packing deterioration



Active biomass is exposed to:

-Fluctuating concentrations of toxic chemicals

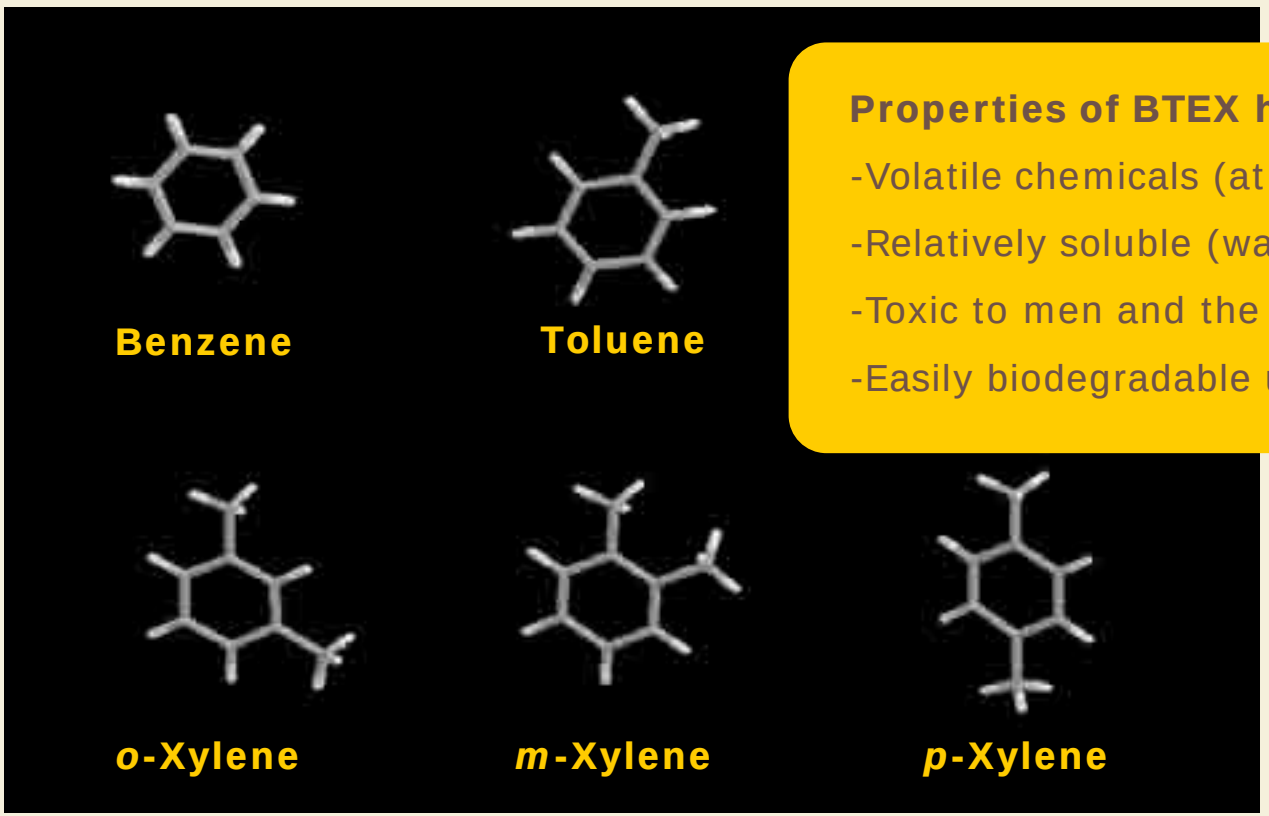
-Dry conditions

-Low nutrient content

-Acidic conditions

BTEX monoaromatic hydrocarbons

Bulk chemicals used in the synthesis of several products (pesticides, explosives, etc.) and materials (plastics), used as solvents (painting and coating), as fuel additives, etc.



Properties of BTEX hydrocarbons:

-Volatile chemicals (atmospheric pollutants)

-Relatively soluble (water pollutants)

-Toxic to men and the environment

-Easily biodegradable under aerobic conditions

Fungal biofiltration

Full-scale biofiltration of volatile aromatic hydrocarbons

Treatment of styrene-containing varnishing emissions

(www.lm-mibi.uni-bonn.de)

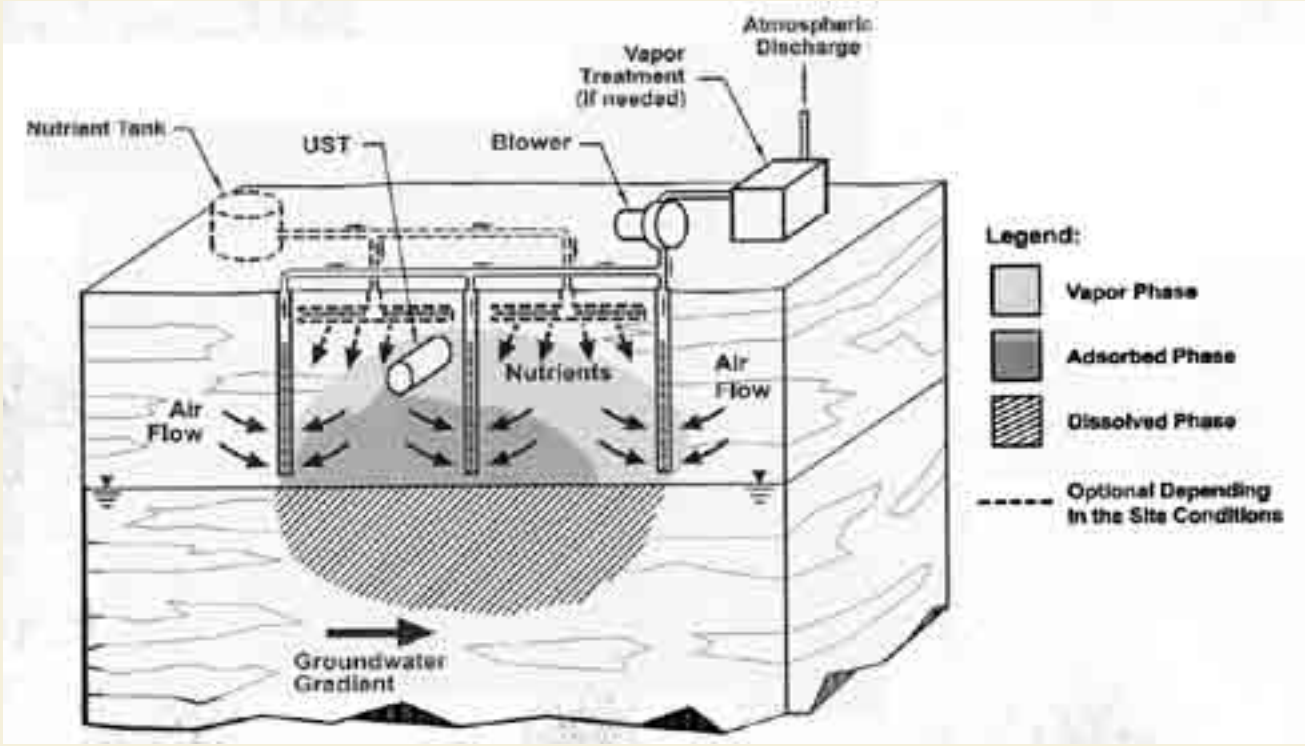
Treatment of solvent vapors of paints in a car manufacturing plant

(Daimler-Benz)

"Hydrocarbonophilic" black yeasts

Fungal biofiltration

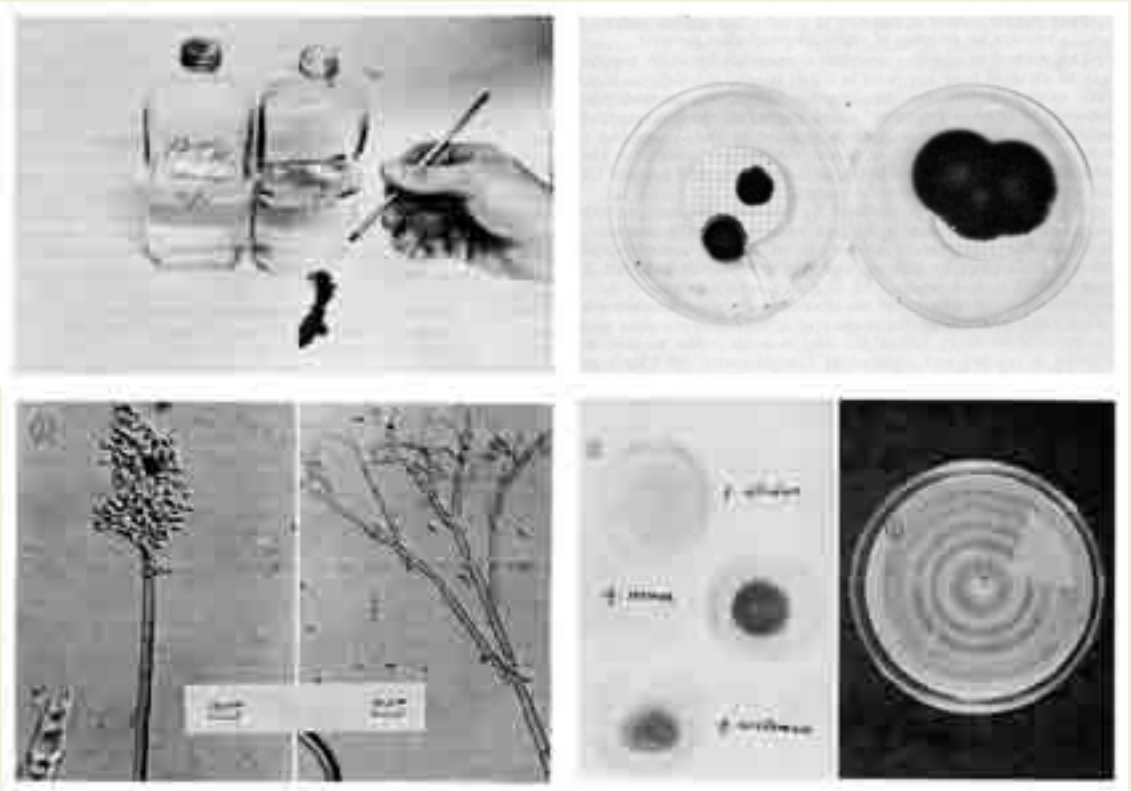
Bioventing of BTEX polluted soil due to leakage of underground storage tanks of fuels and petroleum derivates (i.e. former gasoline stations)



Black yeasts in hydrocarbon-rich environments

Kerosene fungus (*Amorphoteca resinae*)

Interest in this fungus arised by reports of its occurrence in storage and aircraft fuel tanks containing aviation fuel in the early 1960's



Sheridan et al. *TUATARA* 19 (1971)

Fungal metabolism of hydrocarbons

Assimilative metabolism of aliphatics and phenols

Different black-yeasts in the *Dothideales* assimilate aliphatic hydrocarbons and phenolic compounds (i.e. *Amorphoteca resinae*, *Aureobasidium pullulans*, *Cladosporium sphaerospermum*, etc.)

Co-metabolic degradation of aromatic hydrocarbons

Biodegradation of BTEX in a biofilters inoculated with the white-rot fungus *Phanerochaete chrysosporium*.

Substrates were not used as carbon and energy sources, and biofilters were supplemented with straw

Braun-Lüllemann et al. Battelle Press (1995)

Assimilation of aromatic hydrocarbons

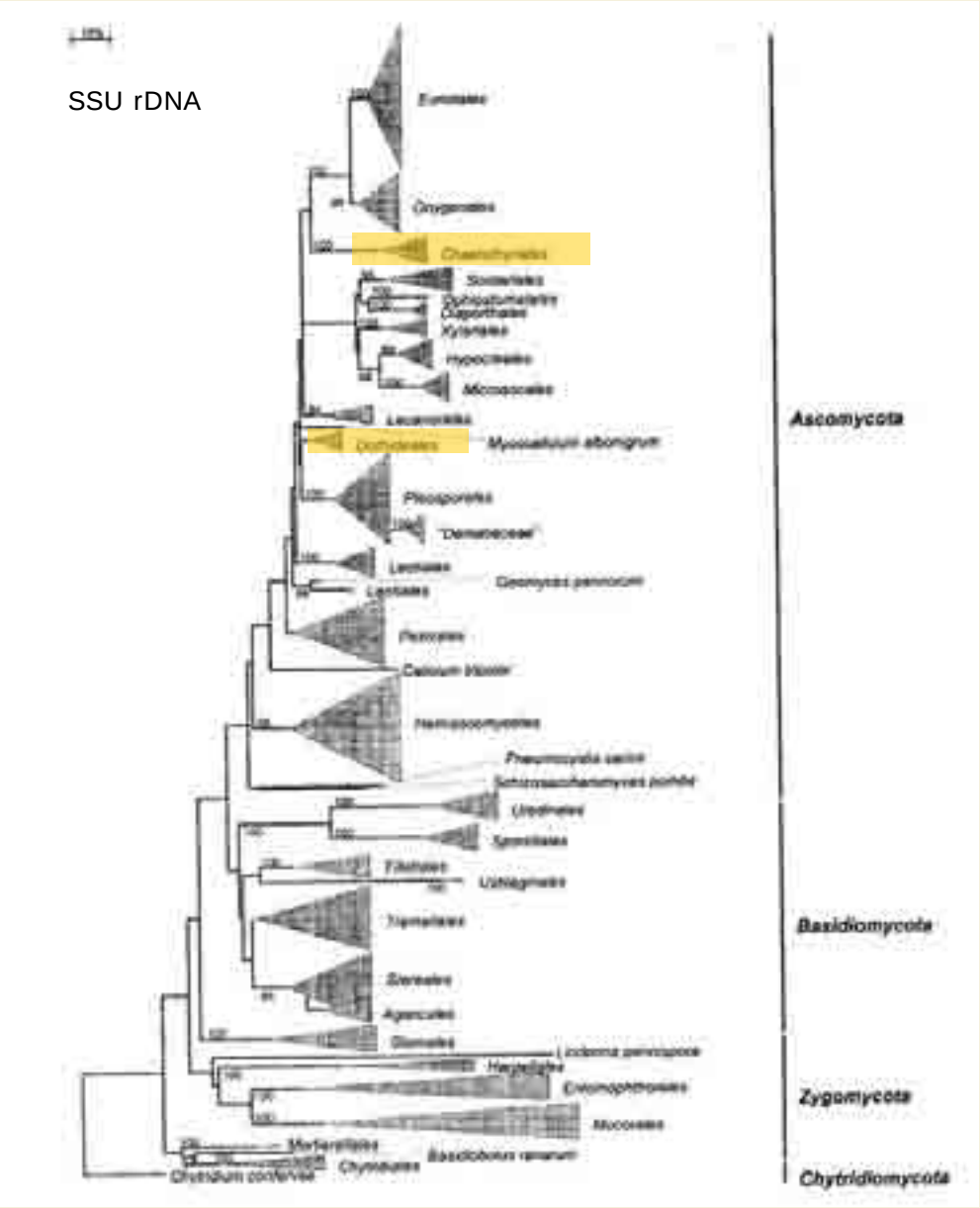
Several species of the *Chaetothyriales* are able to grow on BTEX hydrocarbons as the sole source of carbon and energy

Prenafeta et al. FEMS Microbiol Rev 30 (2006)

"Hydrocarbonophilic" black yeasts

Phylogeny of black yeasts

Eukaryota
Fungi/Metazoa group
Fungi
Dikarya
Ascomycota
Pezizomycotina
Eurotiomycetes
Chaetothyriomycetidae
Chaetothyriales
Herpotrichiellaceae
...
Dothideomycetes
Dothideomycetidae
Dothideales



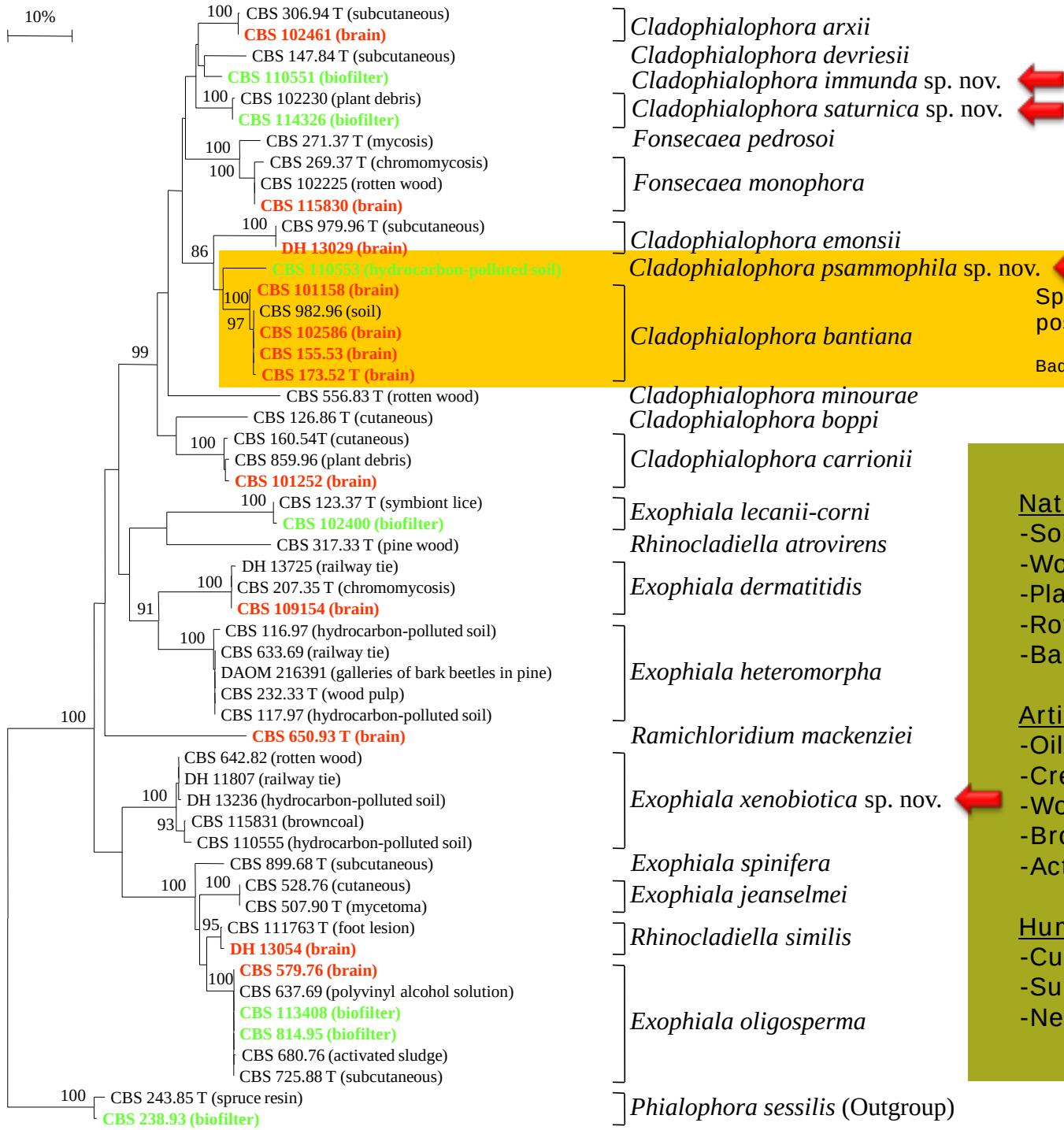
Potential biohazard of fungal biofilters

Are air biofilters treating aromatic hydrocarbons actually breeding for pathogenic fungi?

BSL-2 Toluene-degrading bacteria:

Pseudomonas putida
Pseudomonas aeruginosa
Mycobacterium vacae
Mycovacterium spp.
Nocardia spp.

Neighbor Joining
ITS1-5.8S-ITS2



Specific intron (558 bp) at position 1768 in the SSU rDNA

Badali *et al*. (submitted)

Natural environments
-Soil
-Wood/resin
-Plant debris
-Rotten wood
-Bark insect galeries

Artificial environments
-Oil contaminated soil
-Creosoted wood
-Wood pulp
-Browncoal
-Activated sludge

Human pathogens
-Cutaneous
-Subcutaneous
-Neurotropic

Prenafeta *et al*. *FEMS Microbiol Rev* 30 (2006)

"Hydrocarbonophilic" black yeasts

Natural sources of monoaromatic hydrocarbons

Myroxylon balsamum (*Fabales*)
tolu tree

Styrax benzoin
(*Ericales*)
styrax

J. Buckingham, *et al*. (1995) *Dict natur Prod*

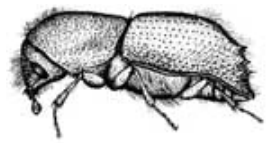
Helianthus annuus
(*Asterales*)
sunflower

Pinus sylvestris
(*Coniferales*)
scots pine

A.C. Heiden *et al.* (1999) *Geophys Res Let*

Quercus ilex
(*Fagales*)
evergreen oak

Quercus robur
(*Fagales*)
oak



Ips pini
(*Coleoptera*)
pine engraver beetle

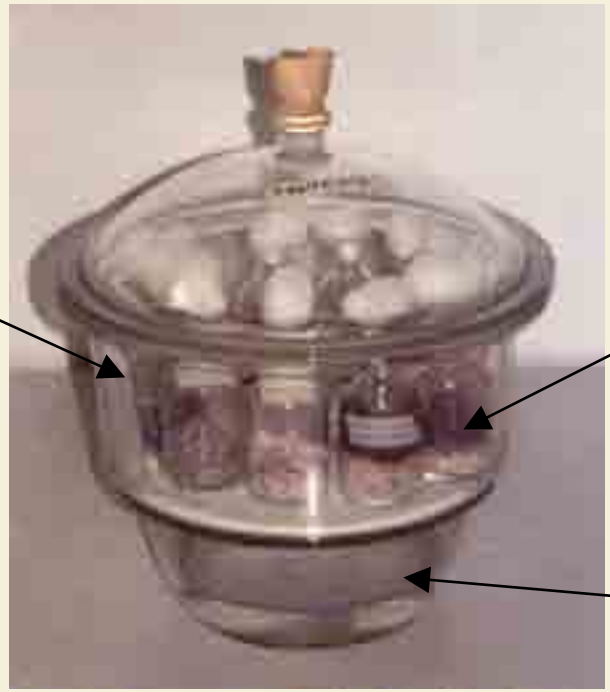
Gries, G. *et al.* (1990) *Experientia*

R. Holzinger *et al*. (2000) *J. Geophys. Res. Atmosph.*
P.Vrkocova et al. (2000) *Biochem. System. Ecol.*

Selective isolation of VOC-degrading black yeasts

Solid state-like enrichment on BTEX hydrocarbons

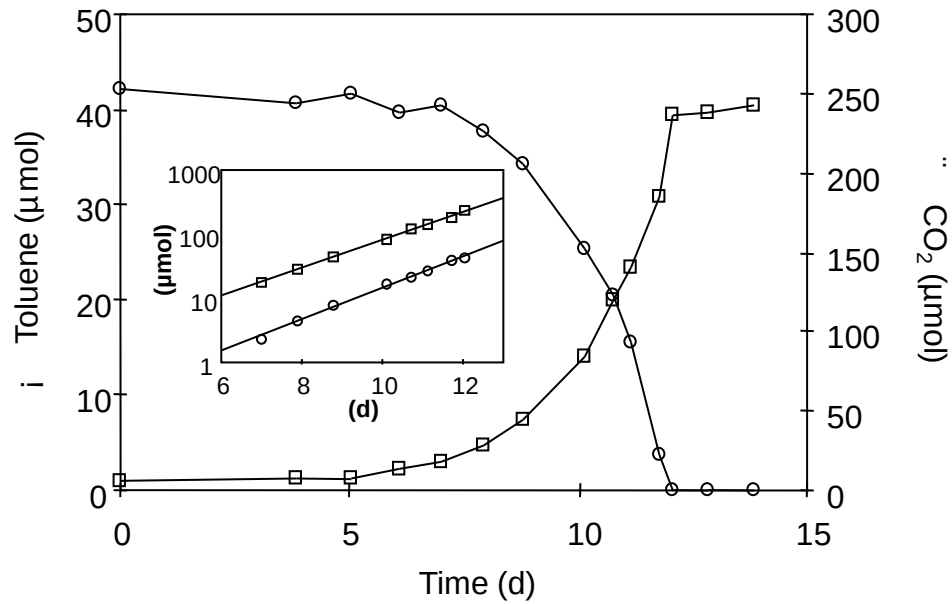
Prenafeta et al. *Mycol Res* 105 (2001)



Isolation of *C. psammophila* CBS 110553 on toluene from a BTEX polluted soil treated by bioventing

Fungal metabolism of toluene

Growth on toluene of *C. psammophila* CBS 110553

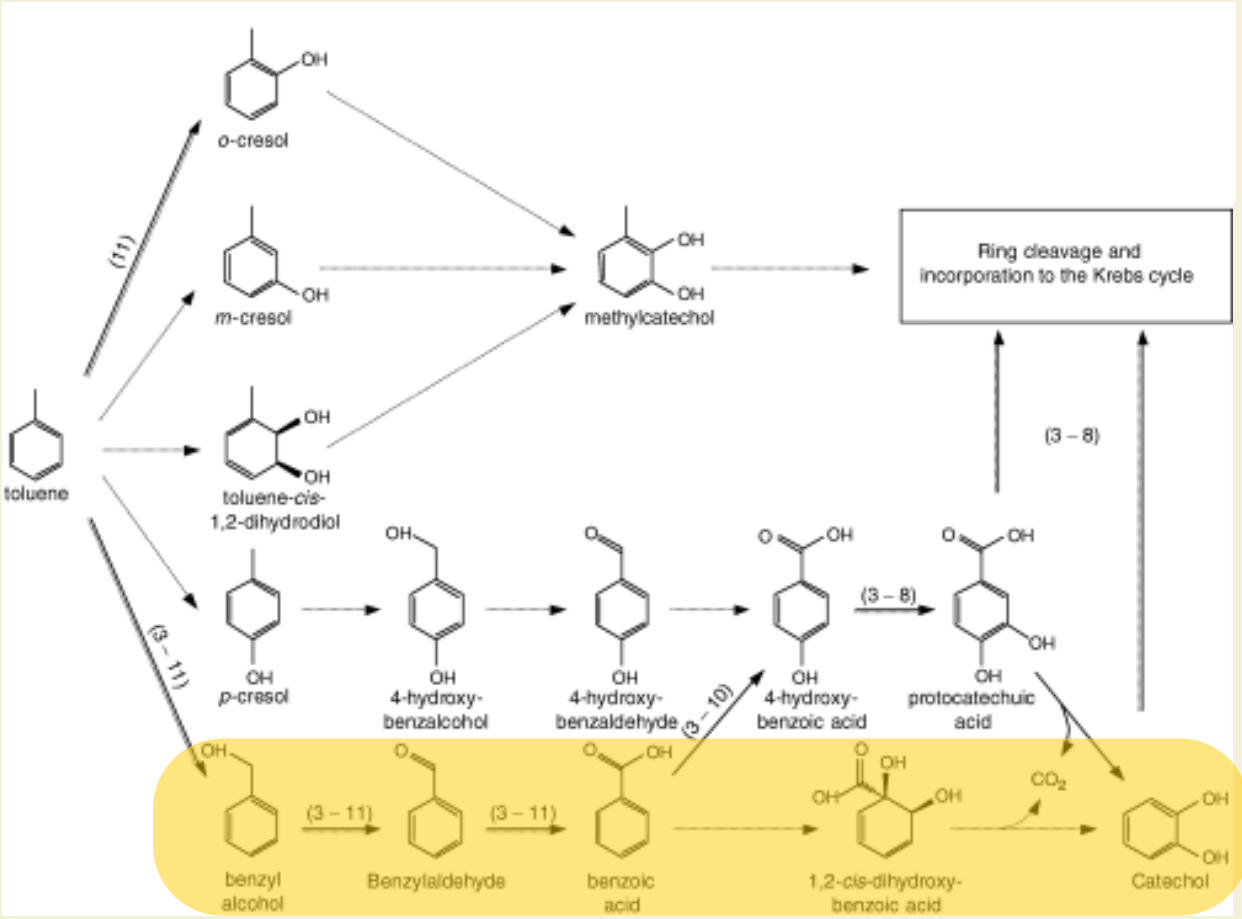


C-toluene recovery as CO_2 > 80 %

Fungal metabolism of toluene

Metabolic pathway for the fungal assimilation of toluene

Prenafeta et al. *Appl Environ Microbiol* 67 (2001)



Fungal metabolism of benzene

Sources of inocula:

-Soil polluted with PAH’s

-BTEX Biofilter biomass

-Creosoted wood

Substrate:

-Benzene

Fungal metabolism of benzene

Solid state-like incubations of railway ties (cresoted wood) under atmospheres of **benzene** yielded several strains of two black yeast species:

-Exophiala xenobiotica

-Exophiala bergeri

Mineral medium | Mineral medium + benzene | Mineral medium + benzene + glucose

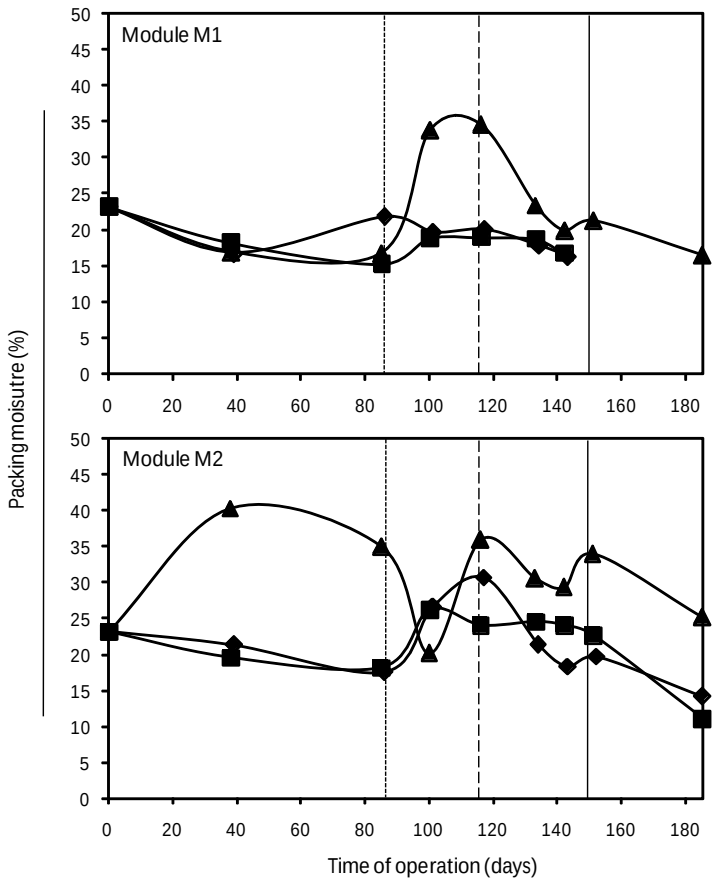
Fungal-bacterial interactions in the biofiltration of BTEX

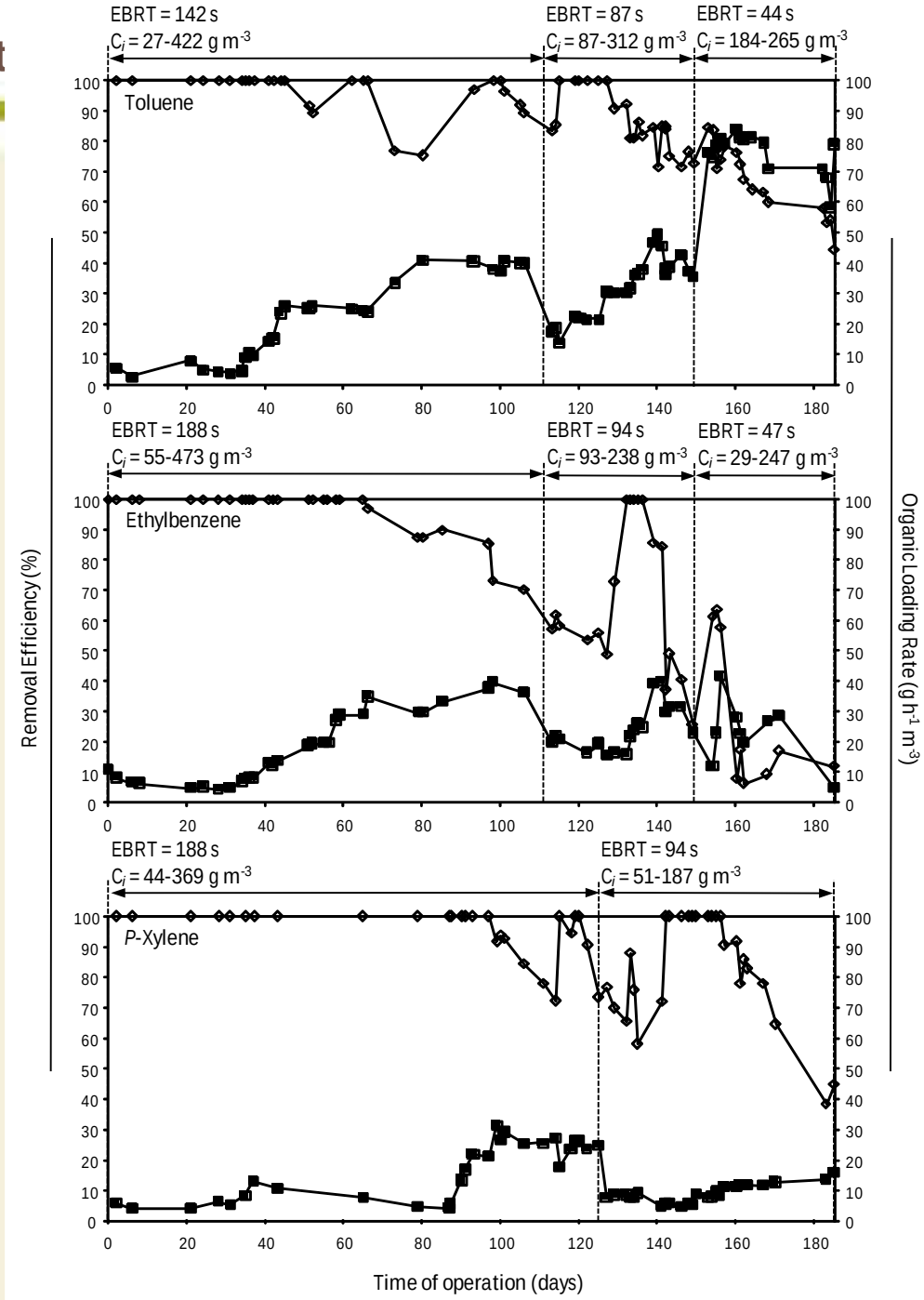
Hydrocarbon degrading black yeasts in biofilters

Operational parameters:

-Modular configuration
-Organic packing
-Poor irrigation (20-30% humidity)
-Increasing organic load (5-70 g h^{-1} m^{-3})

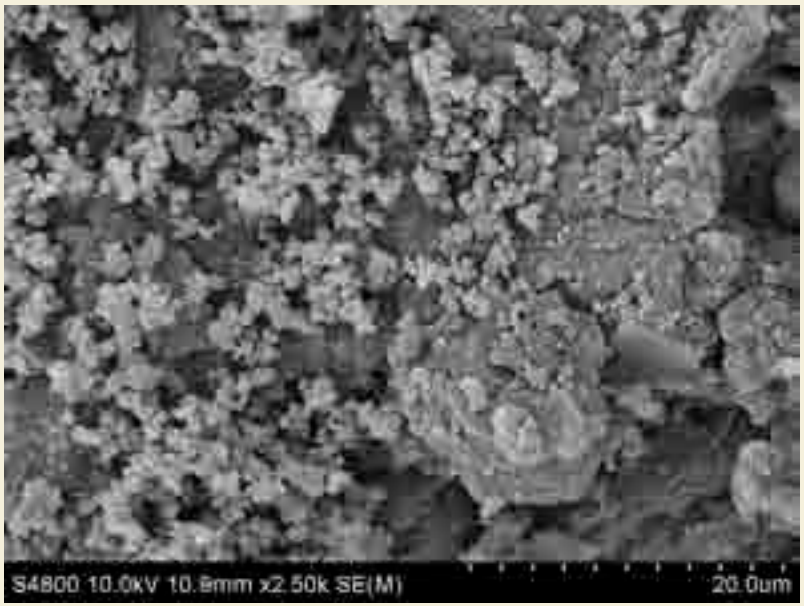
Fungal-bact


Module M1
Module M2
Packing moisutre (%)
Time of operation (days)

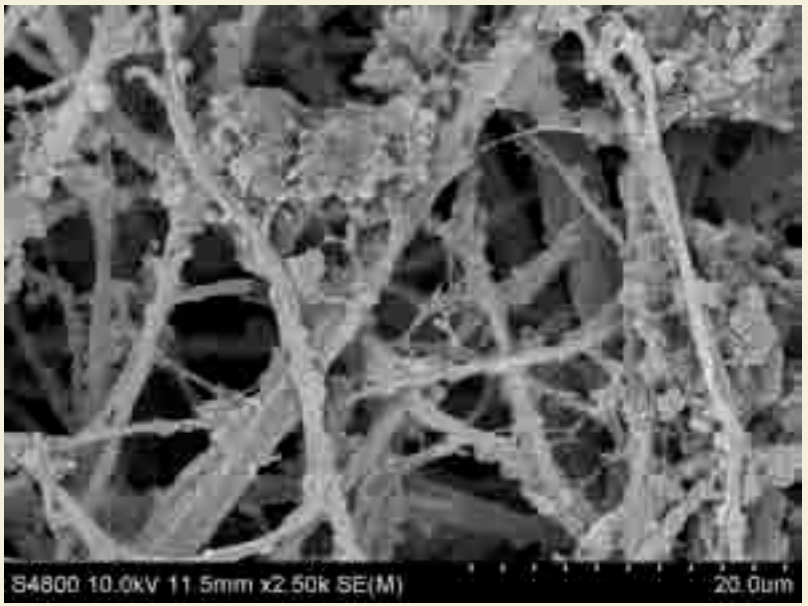

EBRT = 142 s
Ci = 27-422 g m-3
EBRT = 87 s
Ci = 87-312 g m-3
EBRT = 44 s
Ci = 184-265 g m-3
Toluene
EBRT = 188 s
Ci = 55-473 g m-3
EBRT = 94 s
Ci = 93-238 g m-3
EBRT = 47 s
Ci = 29-247 g m-3
Ethylbenzene
EBRT = 188 s
Ci = 44-369 g m-3
EBRT = 94 s
Ci = 51-187 g m-3
P-Xylene
Removal Efficiency (%)
Organic Loading Rate (g h-1 m-3)
Time of operation (days)

Colonization of the biofilter packing with fungi

SEM imaging of the biofilter biomass



Packing material at time 0 days

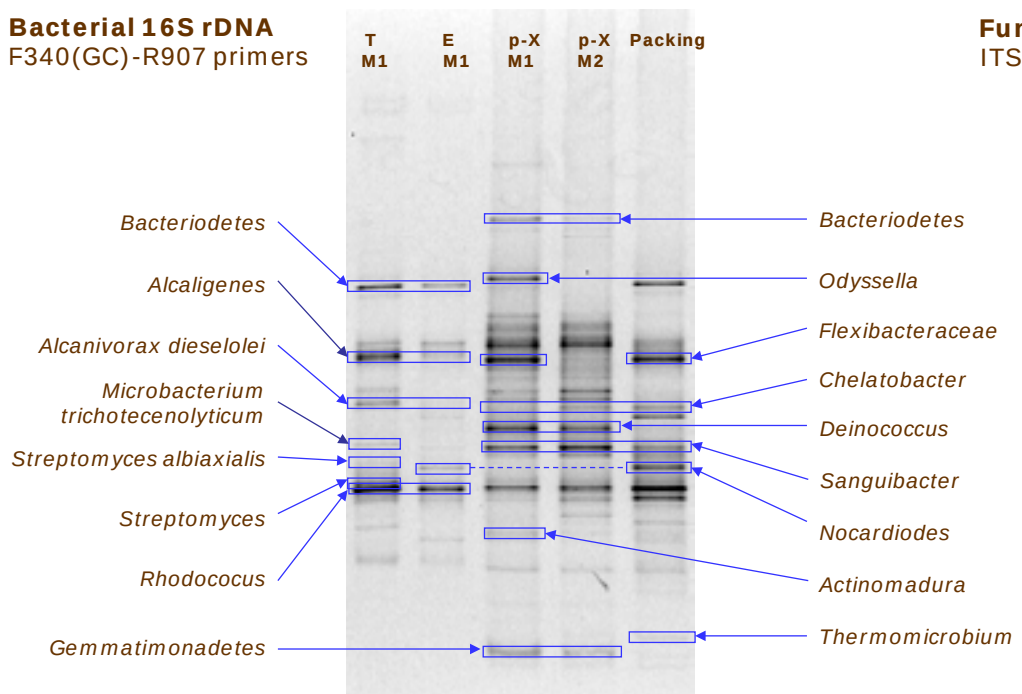


Packing material at time 190 days

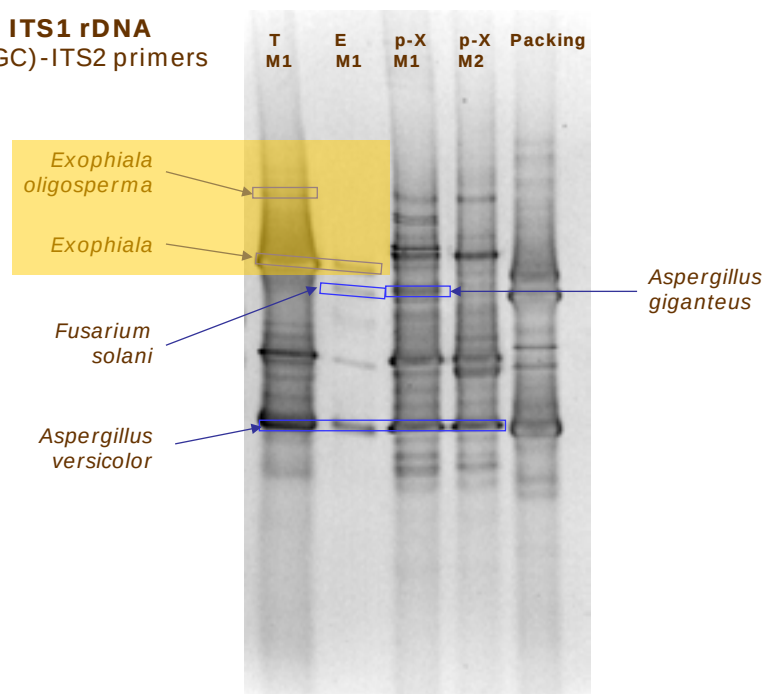
Fungal versus bacterial microbial community structure

Molecular community profiling (PCR-DGGE)

Bacterial 16S rDNA
F340(GC)-R907 primers

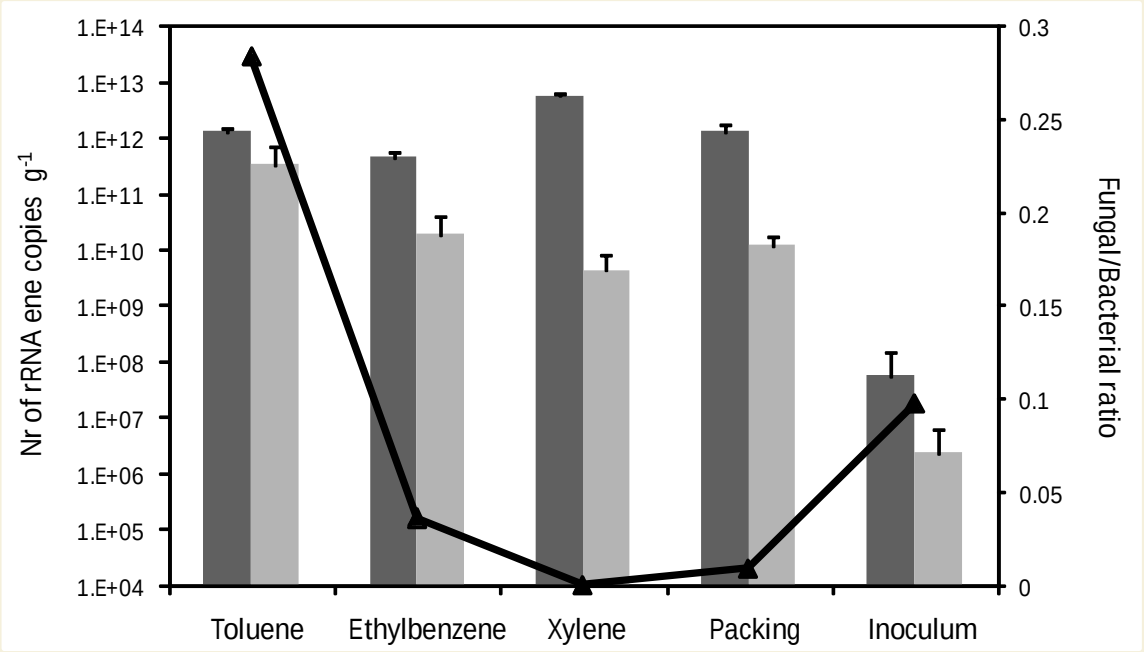


Fungal ITS1 rDNA
ITS1F(GC)-ITS2 primers



Fungal-bacterial biomass ratio

Nr of copies of bacterial 16S and fungal ITS rDNA genes (qPCR)



Conclusions

Virulence and capacity to assimilate aromatic hydrocarbons is predominant within the *Herpotrichiellaceae* family (*Chaetotyriales*), while members of the *Dothideales* lack those characteristics

The ecology presence of sibling species with a very divergent ecology (pathogenic versus xenobiont) suggest that black yeasts are experimenting an intense process of speciation in recent evolution

The amazing capacities of black yeast potentially have several biotechnological applications that are currently investigated: production of extremetolerant enzymes, bioremediation agents (biodegradation of aromatic compounds), etc.

Acknowledgements

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Working Group Black Yeasts

http://www.blackyeast.org/