IRTA
RESEARCH & TECHNOLOGY
FOOD & AGRICULTURE

Application of melanized fungi for the removal of complex mixtures of volatile organic compounds in totally confined indoor environments

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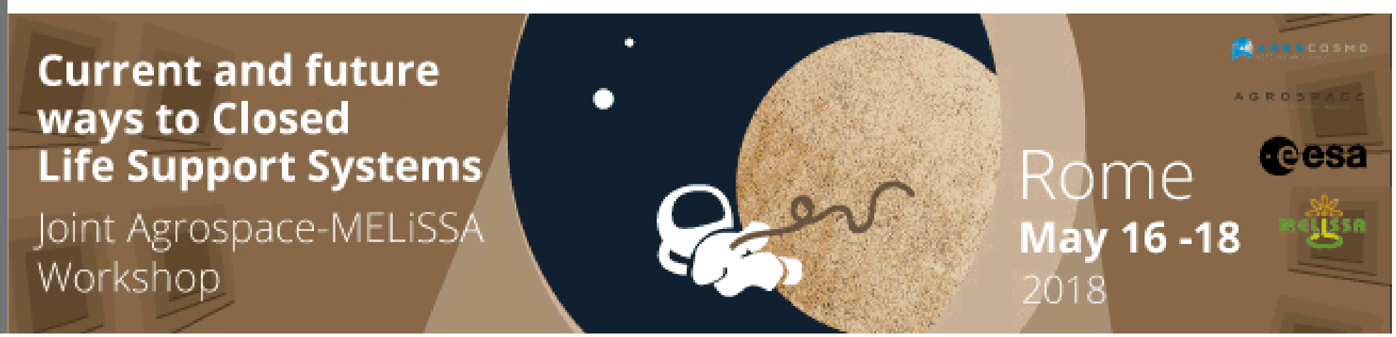
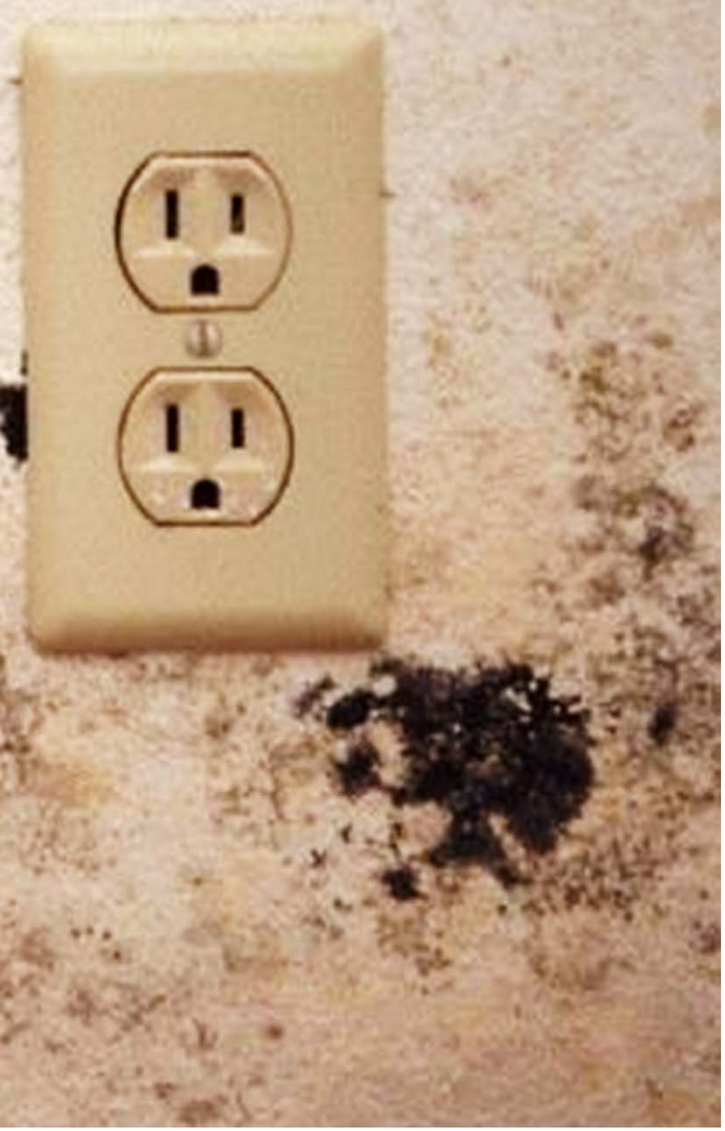


The ‘sick building syndrome’ (SBS)

‘Are energy efficient homes making us ILL?’

Toxic mould caused by poor air circulation could trigger 'sick building syndrome‘

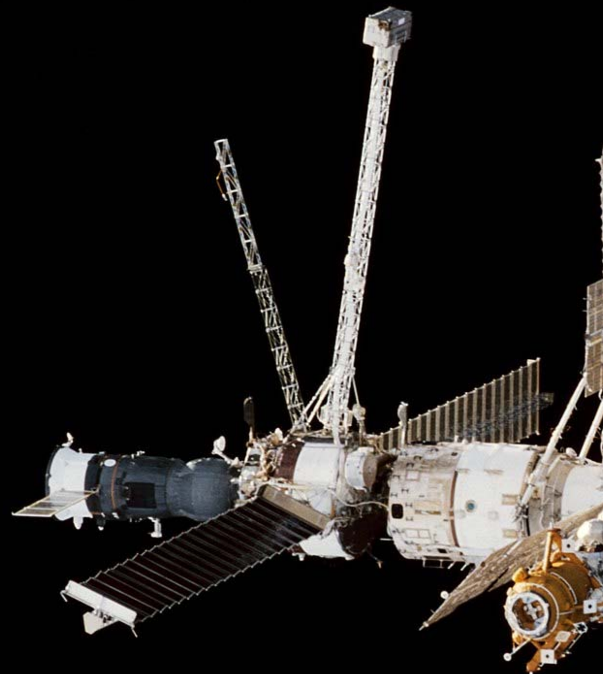


- **It is thought to be present in 30-50 per cent of new or refurbished buildings**
- **The most common symptoms are headaches, lethargy, poor concentration, skin irritation, dry itchy eyes and a congested nose**
- **In rare cases, mould spores can damage lung tissue and affect breathing**

Ellie Zolfagharifard (2014), Dailymail, 18 February 2014

The 'sick building syndrome' (SBS)

MIR space station



(Bell 2007)

The 'sick building syndrome' (SBS)

Medical condition where people in a building suffer from symptoms of illness or feel unwell for no apparent reason. The main identifying observation is an increased incidence of complaints of symptoms such as headache, eye, nose, and throat irritation, fatigue, and dizziness and nausea

(WHO 1986)

Causes:

- Poor ventilation and/or bad humidity control (damp air)
- Volatilization of VOCs from anthropogenic (synthetic materials) and biogenic sources (plants, animals, and microorganisms)
- Aerosolization of toxins, spores, and microbes

Consequences:

- Negative wealth effects
- Health impairment
- Biodeterioration of materials
- Alteration of biological systems (e.g. accumulation of phytohormones)

The 'sick building syndrome' (SBS)

Prevention:

- Enhance ventilation and control humidity
- Use of adequate materials
- Control microbial development

Air treatment technologies:

- Filtration/separation
- Catalytic oxidation
- Ad/absorption (active carbon)
- Biodegradation (biofiltration)

To this day, there are no satisfactory treatment technologies available for indoor air!

(Guieysse et al. 2008; Luengas et al. 2015)

Black yeast-like fungi: Dr Jekyll or Mr Hyde?

(Gueidan et al. 2011)

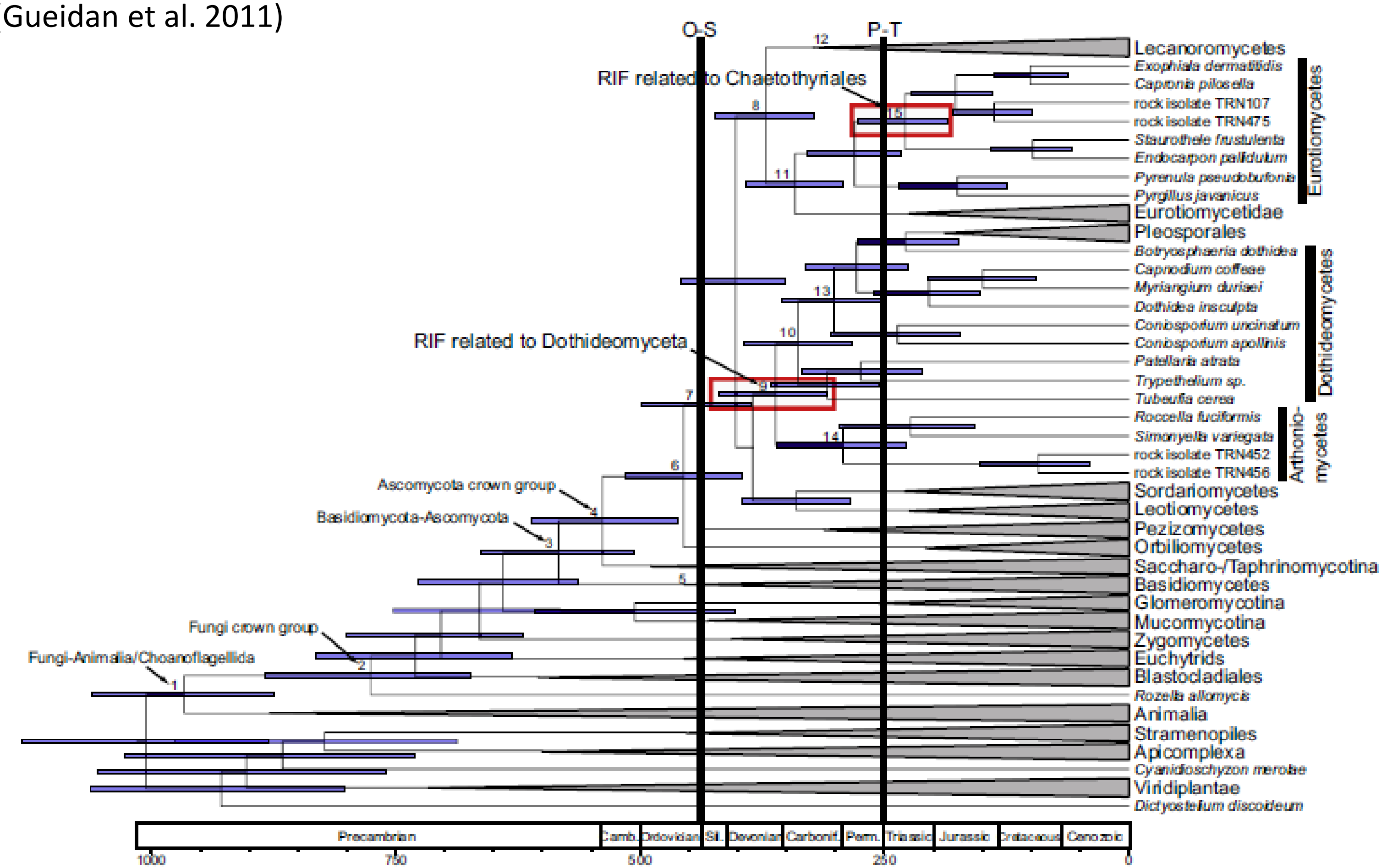


Fig 2 – Chronogram picturing divergence time estimates for main groups of fungi. Bars correspond to 95% confidence intervals and values for the 15 numbered nodes are available in Table 2. Rock-inhabiting fungi and lichens are highlighted in red, as well as the two time periods in which RIF originated. The following abbreviations were used for geological periods: Camb. = Cambrian; Carbonif. = Carboniferous; Perm. = Permian; Sil. = Silurian. The Ordovician-Silurian and Permian-Triassic mass extinctions are indicated by O-S and P-T, respectively.

Black yeast-like fungi: Dr Jekyll or Mr Hyde?

Black yeasts are polyextremophilic fungi that stand for extreme cold/heat, desiccation, salinity, acidity, radiation, exposure to toxic chemicals, etc.)

(Teixeira et al. 2017)

Black yeast-like fungi: Dr Jekyll or Mr Hyde?

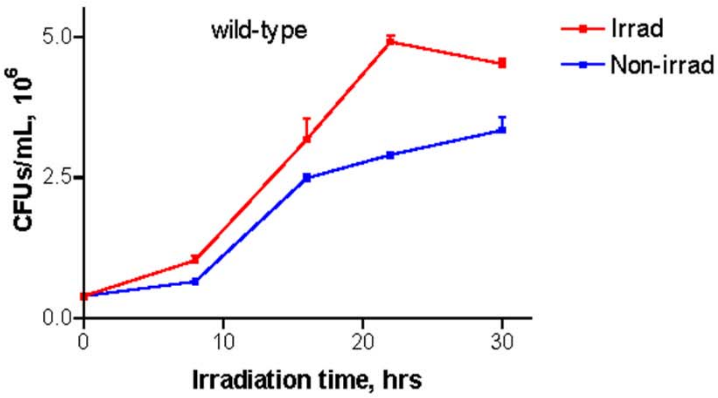
An astronaut fixes the EXPOSE-E platform onto the International Space Station. Credit: ESA

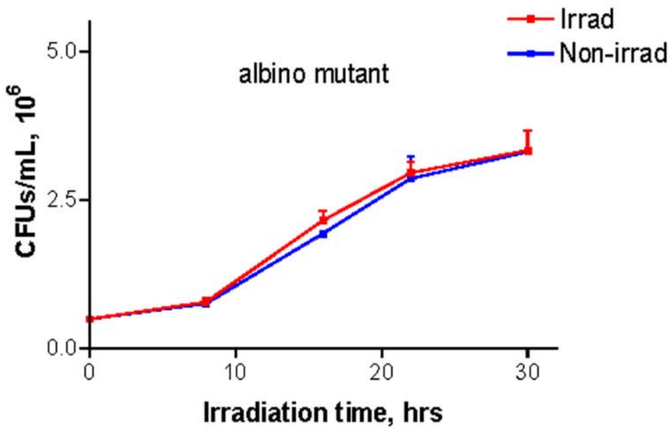
Black yeast-like fungi: Dr Jekyll or Mr Hyde?

Strong melanization:

- Induces radiotrophism (absorb high energy radiation for metabolic functions)

Exophiala dermatitidis



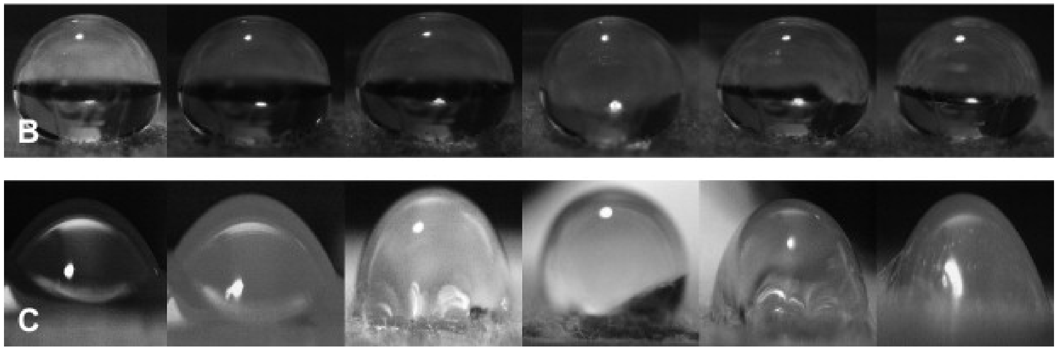


(Dadachova et al. 2007)

- Form extremely hydrophobic biofilms (good for the adsorption of contaminants)

Cladosporium cladosporioides

Suillus tomentosus



(Wuai Chao et al. 2009)

Black yeast-like fungi: Dr Jekyll or Mr Hyde?

Broad biodegradation capacity:

- Assimilation of alkylbenzenes and aliphatics as sole source of carbon and energy
- Excretion of degradative enzymes (laccases)

Other critical traits depending on the species:

- Many are proven non-pathogens (but some are!)
- Poor sporulation
- Slow growth

Use of black yeasts for the cost-effective biofiltration of volatile industrial solvents

Biofiltration of toluene vapors with *Cladophialophora psammophila*

(Prenafeta-Boldú et al. 2009)

Study objectives

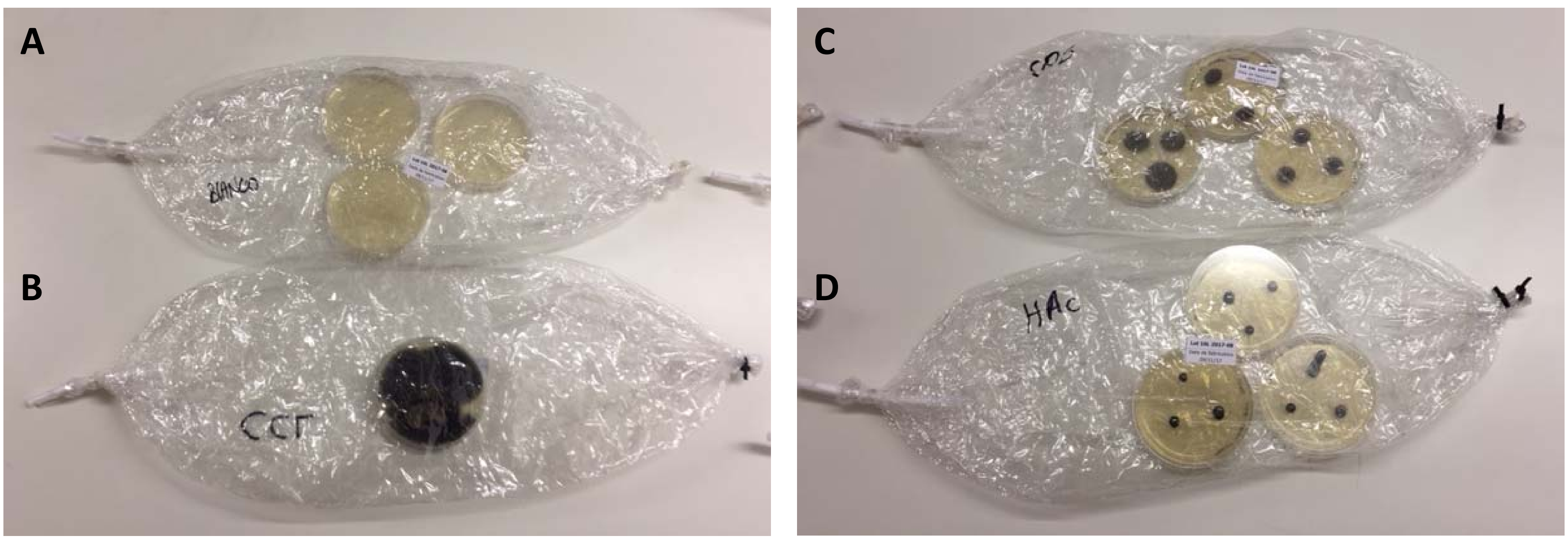
- To test on a few number of suitable black yeast species (that are resistant to space conditions, non-sporulating, slow growth, and non pathogens) for their ability to eliminate complex mixtures of volatile organic compounds (VOCs) from indoor air
- Analyze prospective applications in earth and space biotechnology, for the treatment of contaminated indoor air (i.e. for preventing the ‘sick building syndrome’) with a fully regenerative technology

Experimental setup

- Incubation of fungal cultures (pre-grown on PDA medium) inside Nalophan™ bags containing a model **anthropogenic indoor air** (1.5 liters)

- Treatments: A: Abiotic control
  B: an **SBS** fungus (*Cladosporium cladosporioides*)
  C: an **VOCs degrading** fungus (*Cladophialophora psammophila*)
  D: an **acidophilic** fungus (*Neohortaea acidophila*)

- **Characterization of VOCs** profiles at the end of incubations (48 hours)



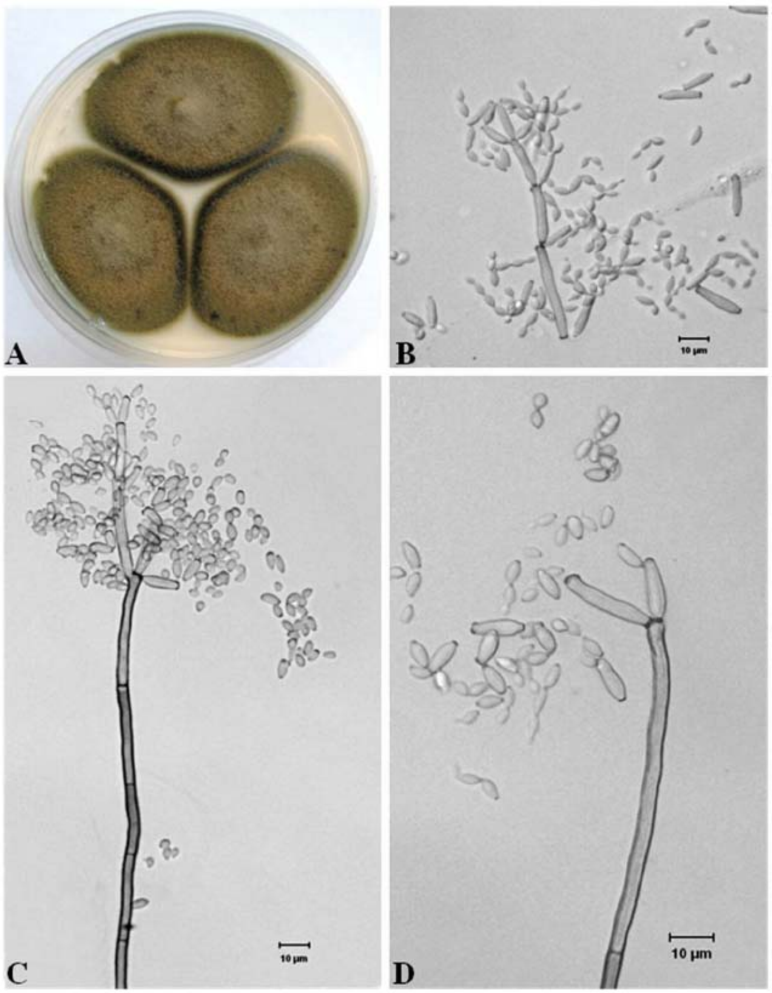
Model anthropogenic indoor air

Our own laboratories!

Gas Chromatography – Time of Fly – Mass Spectrometry (GC-ToF-MS)

Detection threshold for individual VOCs: 0.1 µg·m^{-3}

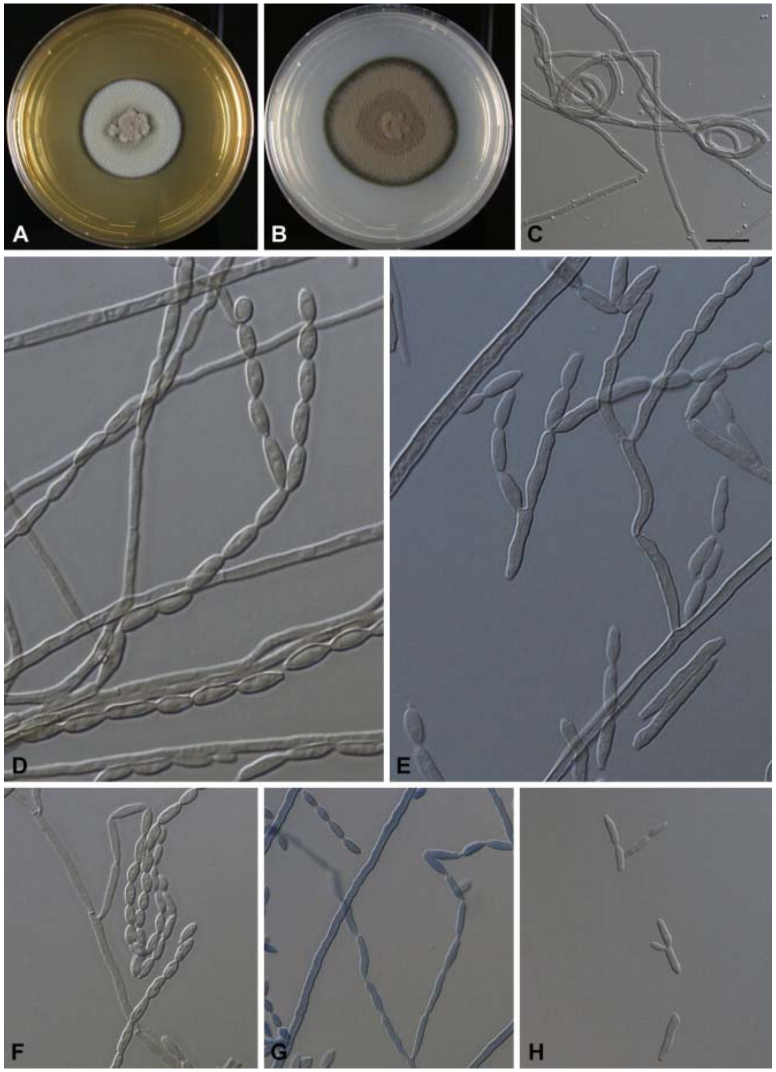
Cladosporium cladosporioides



(Zhan et al. 2014)

- *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (CBS 101367)
- Order *Capnodiales*, family *Cladosporiaceae*
- Very cosmopolitan, growing relatively fast, sporulates rather profusely
- Typically found in relation to the sick building syndrome, well-known pattern of biogenic VOCs
- Not a human pathogen (BSL 1), but it might trigger allergic reactions

Cladophialophora psammophila



(Badali et al. 2011)

- *Cladophialophora psammophila* Badali, Prefaneta-Boldú, Guarro & de Hoog (CBS 110553, type strain)
- Order *Chaetothyriales*, family *Herpotrichiellaceae*
- Very specialized, grows rather slowly
- Single-strain species. Isolated from a gasoline polluted soil, is able to grow on volatile hydrocarbons
- Used in the biofiltration of air polluted with toluene and other alkylbenzenes
- Non-pathogenic to human (BSL 1), but is related phylogenetically to a virulent species (BSL 3)

Neohortaea acidophila

(Hölker et al. 2004)

- *Neohortaea acidophila* (Hölker, Bend, Pracht, Tetsch, Tob.Müll., M. Höfer & de Hoog) Quaedvlieg & Crous, (CBS 113389, type strain)
- Order *Capnodiales*, family *Teratosphaeriaceae*
- Very specialized, very slow growth as a yeast, non-sporulating
- Single-strain species. Isolated from lignite, grows at a pH = 1 (good for NH_3 absorption)
- Non-pathogen to human (BSL 1)

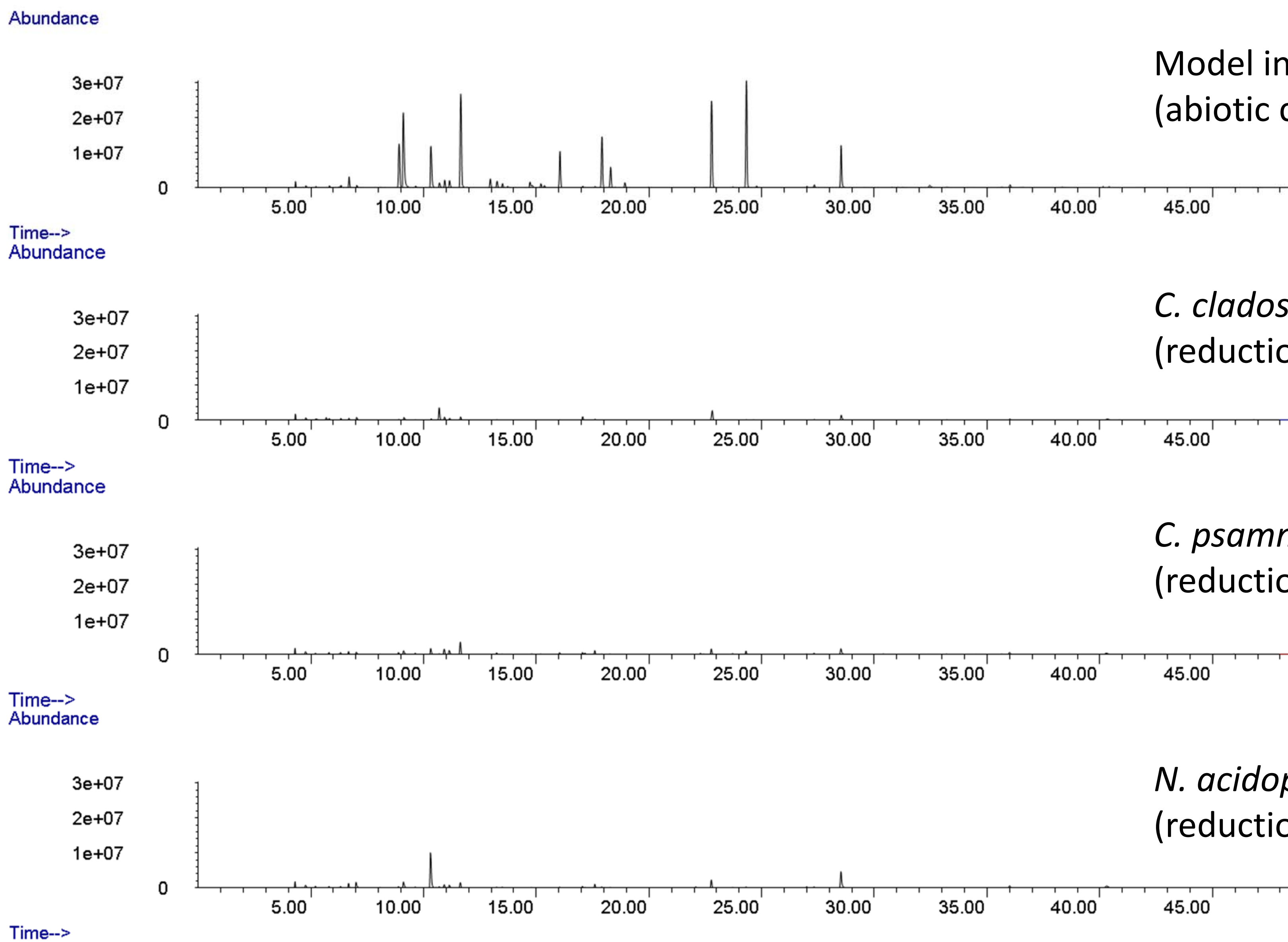
Indoor air VOCs profile

Abiotic control (A):

- 71 VOCs were identified and quantified (total concentration 1.4 mg·m^{-3})
- VOCs belonged to 13 chemical families, predominantly to alcohols, aldehydes, hydrocarbons, esters, ethers, ketones, organohalogens, N- and S-containing compounds
- Most abundant VOCs were common laboratory organic solvents, and other reagents (microbiology media)
- Other contaminants were related to fuel additives, propellants, and building/forniture materials

Compound	CAS No.	µg·m^{-3}
Tetrachloroethylene	127-18-4	233
Toluene	108-88-3	203
Methylene chloride	75-09-2	185
Ethanol	64-17-5	151
Butanal, 3-methyl-	590-86-3	110
Pentane	109-66-0	108
Styrene	100-42-5	84
Trichloromethane	67-66-3	74
Acetone	67-64-1	65
Butanal, 2-methyl-	96-17-3	39
Propanal, 2-methyl-	78-84-2	17
Acetonitrile	75-05-8	15
Carbon disulfide	75-15-0	14
n-Hexane	110-54-3	12
Butanal	123-72-8	10
1-Butanol	71-36-3	10
Acetaldehyde	75-07-0	9
Heptane	142-82-5	9
Benzoyl bromide	618-32-6	9
2-Butanone	78-93-3	6
Methacrolein	78-85-3	5
Methyl Alcohol	67-56-1	4
m,p-Xylene	108-38-3/106-42-3	3
Ethylbenzene	100-41-4	3
Propane, 2-ethoxy-2-methyl-	637-92-3	3
Ethyl Acetate	141-78-6	3
Butane	106-97-8	3
Furan	110-00-9	2
Isobutane	75-28-5	2
Benzene	71-43-2	2

VOCs CG-ToFMS profiles



Model indoor air (abiotic control)

C. cladosporioides (reduction: 96.8%)

C. psammophila (reduction: 98.2%)

N. acidophila (reduction: 97.1%)

VOCs CG-ToFMS profiles

Fungal volatile metabolites:

- *C. cladosporioides*: iodomethane, 1-octene, 3-methylfuran, 3-pentanone, caryophyllene, ß-pinene, D-limonene, and o-cymene

  (49.5 µg·m^{-3}) (In agreement with Sunesson et al. 1995, and Lloyd et al. 2005)

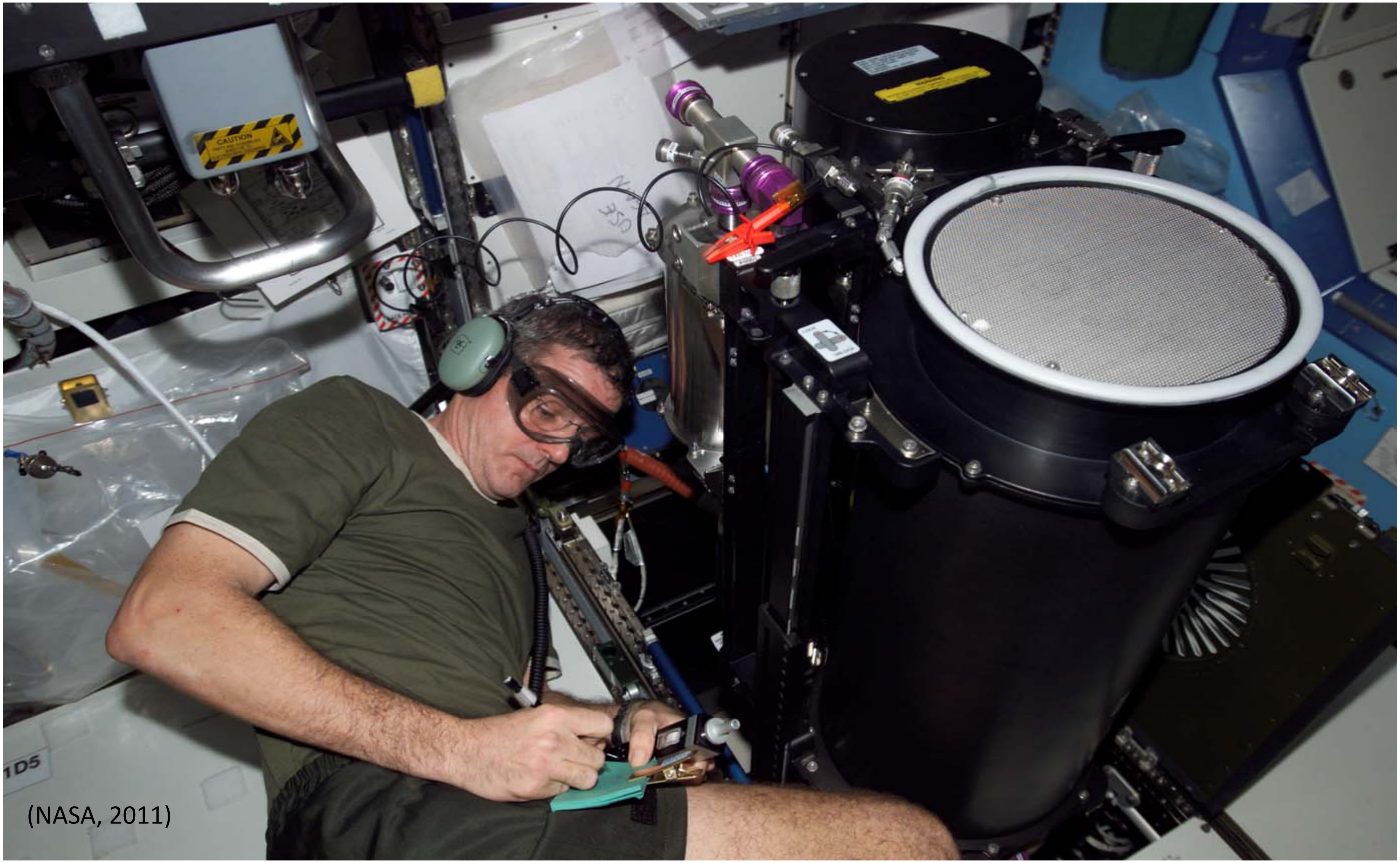
- *C. psammophila*: 3-methyl-1-butanol, methylamine, 1-pentene, 1-heptene, nitroethane, and cyclofenchene, several aliphatic and alkylbenzene hydrocarbons

  (8.6 µg m^{-3})

- *N. acidophila*: 1-(dimethylamino)-2-propanone, decanal, 2-methyl-1-pentene, octane, nonane, acetophenone, dimethyl sulfide, methanethiol, diisobutyl phthalate, 2-methyl-2-oxazoline, (dimethylamino)acetone, 1-pentene, 1-heptene, nitroethane, and cyclofenchene

  (15.8 µg m^{-3})

Can black yeasts contribute to improve the current adsorption / catalytic oxidation approach of the Trace Contaminant Control Subassembly in the ISS?



(NASA, 2011)

Preliminary conclusions

- Black yeasts reduced the total VOCs concentration from a complex indoor air by more than 96% in a lab-scale air passive system
- Fungal hydrophobicity and biodegradation capacity might play a major role in VOCs removal
- The emission of fungal volatile metabolites is rather low and species-specific
- Results on the emitted fungal VOCs match the known profile of the SBS fungus *C. cladosporioides*
- Black yeasts might be used advantageously in the biofiltration of indoor air in confined environments

Future work:

- Verify the observations with additional fungi (including non-melanized species)
- Assure the biosafety of selected species and of the generated metabolites
- Test the elimination capacity on a lab-scale prototypes
- Focus on the combined VOCs and NH_3 removal by cultures of *N. acidophila*
- Investigate the potential radiotrophic and radioprotective capacity of melanized fungi

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DOURNET sensory experts since 1980

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