BAM
Federal Institute for Materials Research and Testing

Freie Universität Berlin

Characterization of a Rock-Inhabiting Microcolonial (Black) Fungus with the Biolog

Restrictions, Solutions, Results

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Introduction: Our research (and why it is relevant)

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Black fungi

(a.k.a. black yeasts, a.k.a. microcolonial fungi, a.k.a. dematiaceous fungi, a.k.a. meristematic fungi)

Krumbein & Jens (1981) *Oecologia*; Friedmann (1982), *Science*; Staley *et al.* (1982), *Science*

Extremotolerance

(UV, salinity, oligotrophy, ionizing radiation,…)

Gostincar *et al.* (2010), *FEMS Microbiol Ecol*

Biofiltration

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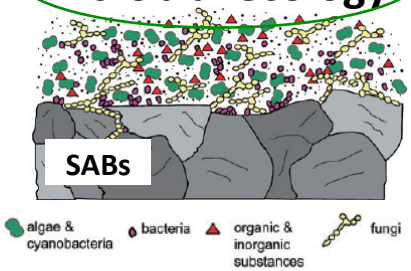
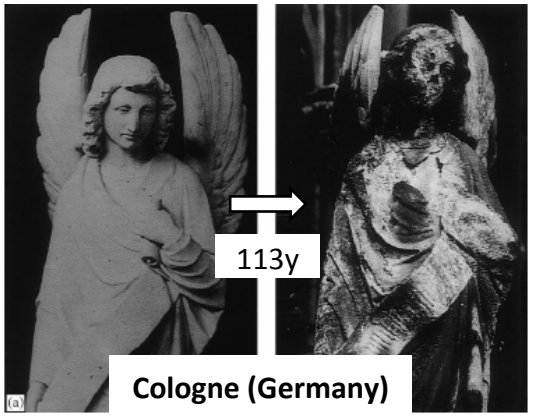
Prenafeta-Boldú *et al.* (2006), *FEMS Microbiol Lett*

Microbial ecology



Gorbushina (2007), *Environm Microbiol*

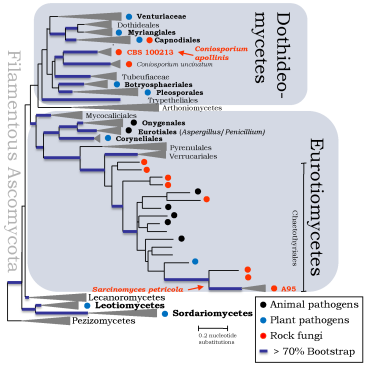
Material colonization



Warscheid & Braams (2000), *Int Biodet Biodegr*

Fungal genetics

(biodiversity, evolution, phylogenetics)



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Gueidan *et al.* (2008), *Stud Mycol*; Ruibal *et al.* (2009), *Stud Mycol*

Fungal pathogenesis

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Astrobiology

e.g. Onofri *et al.* (2008), *Stud Mycol*

Black Yeasts Database at the **Broad Institute**

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Introduction: Our research (how we contribute)

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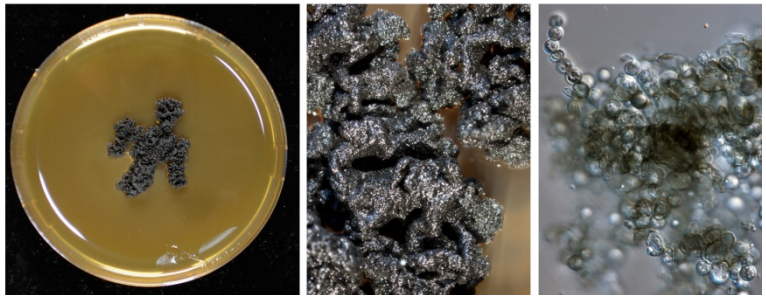
Black fungi

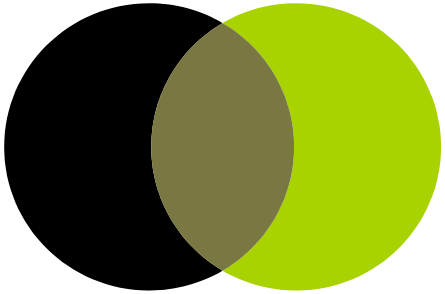
(a.k.a. black yeasts, a.k.a. microcolonial fungi, a.k.a. dematiaceous fungi, a.k.a. meristematic fungi)

***Knufia petricola* A95 (former *Sarcinomyces petricola*)**
+ other black fungi (e.g. ***Coniosporium apollinis***)



A model rock-inhabiting black fungus

A95 + *Nostoc punctiforme* ATCC29133



(Subaerial-)**Biofilm model**

Fungal genetics
(DNA isolation, first-runs of pyrosequencing, generation of cDNA, karyotyping by PFGE)
Collaboration with Dr. Christina Cuomo *et al.* (Broad Institute)

Extremotolerance
(mechanisms of oligotrophy, melanin synthesis, secondary metabolites)

Microbial ecology
(establishment of SABs, interactions with phototrophs, analysis of natural biofilm communities by DGGE)
Dr. Steffi Noack *et al.*

Material colonization
(colonization of solar panels, simulation of degradation of stone material with flow through-chambers)
Dr. Steffi Noack, Franz Seiffert

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A prerequisite: Knowledge of A95

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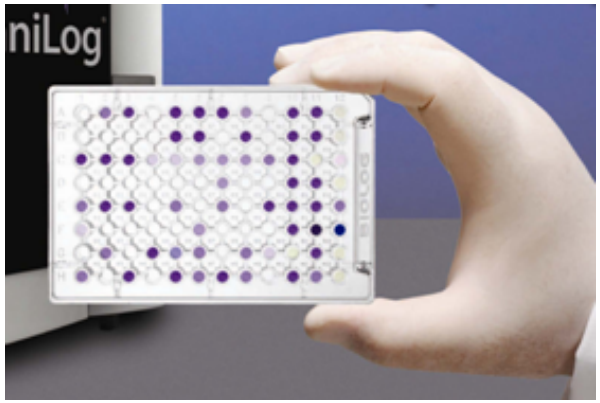
***Knufia petricola* A95**



Use of the **Biolog™ System** to generate a **broad phenotypical profile of A95**

- PM1-2 (C metabolism), PM3 (N metabolism), PM4 (P & S metabolism)
- PM5 (growth factors, vitamins)
- PM9 (salt stress), PM10 (pH)

Total ~ 1,000 different growth conditions

Support genome annotation

Nutritional requirements

Stress resistance

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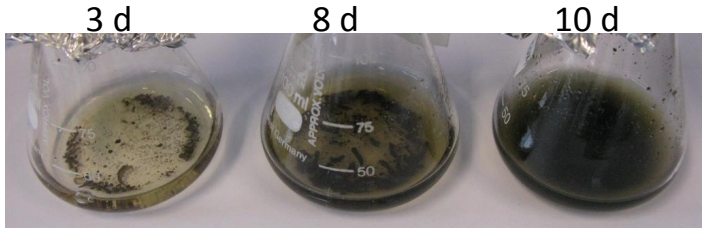
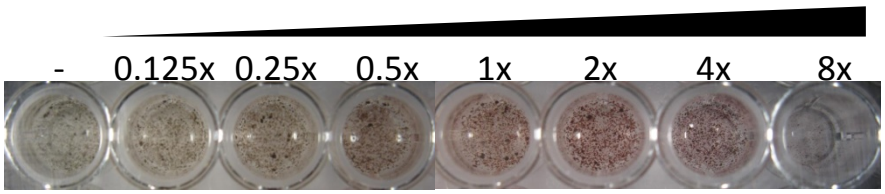
A95 & Biolog System - *Restrictions*

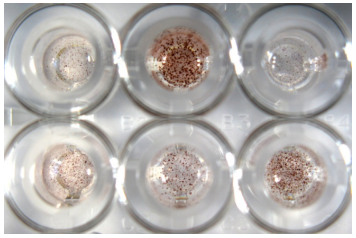
1) **Clumpy growth** → OD measurements not reproducible
2) **Association of dye with cell clumps, melanization** → dito
3) **Extremely oligotrophic** → growth in negative control wells (especially without N, P and S)



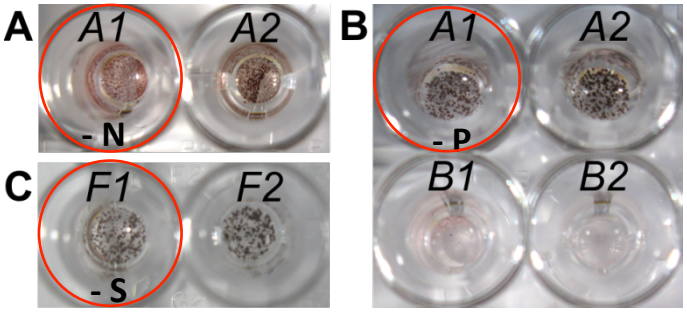
Malt extract media, 25 °C



Pre-experiment: FF-IF pH 5, yeast nitrogen base + 100 mM glc + N source + Redox dye (8 days at 25 °C)



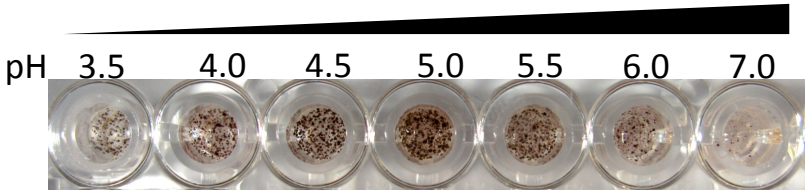
Growth in **PM1** buffered at pH 5 (11 d at 25 °C)



Growth and pink colour development (~3 wks at 25 °C) in negative control well of:

(A) PM3 (A1, no N)
(B) PM4 (A1, no P) and
(C) PM4 (F1, no S)

(from Nai C *et al.*, in revision)



Growth in **PM10** (7 d at 25 °C)

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A95 & Biolog System - *Solutions*

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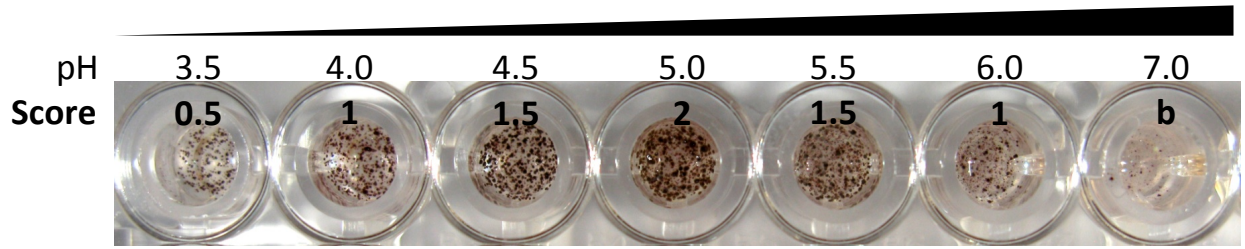
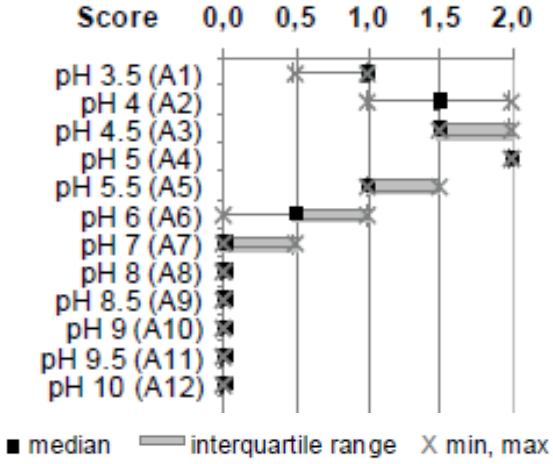
1) **Reduce inoculum and/or incubation time** → reduce growth in negative control
2) **Grow at suboptimal conditions** (e.g. unbuffered plate) → dito
3) **Adopt an ad hoc scoring system (evaluation by eye)** → semi-quantitative method

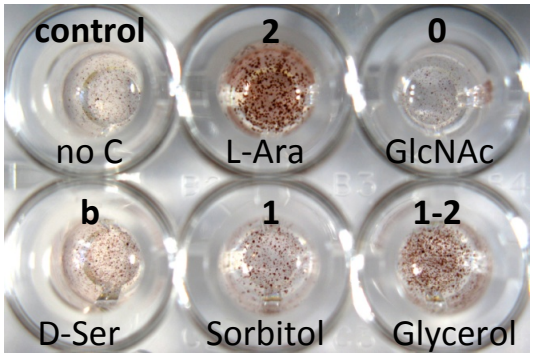
at least 2 unbiased (independent) evaluations
at least 3 biological replicates
calculate median and quartiles

0.5-2 observed growth
0 no growth
b borderline (doubtful growth)



Growth in **PM10** (7 d at 25 °C)





Growth in plate **PM1** buffered at pH 5 (11 d at 25 °C)

… not solved for S metabolism !

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A95 & Biolog System - *(Some) Results*

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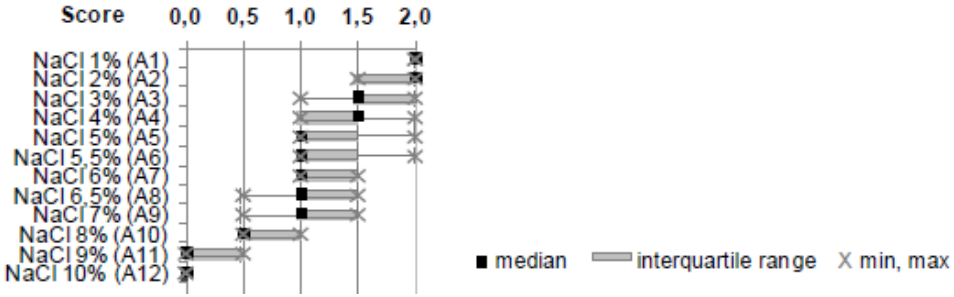
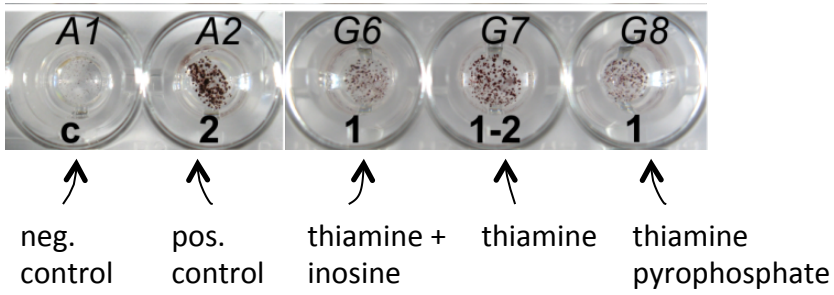
- **pH optimum at around pH 5 (PM10)**
- **Halotolerant (PM9)**



- **Prototrophic (no special nutritional requirements), but thiamine stimulates growth (PM5)**



Growth in **PM5** (11 d at 25 °C)

- **Good growth on monoaromatic compounds**

p-Hydroxybenzoic acid, *p*-/*m*-hydroxyphenylacetic acid

- **Little overlap between sets of nutrient sources**

Amino acids/dipeptides: poor C source, preferred N source

- **Limited ability to grow on metabolic intermediates**

e.g. pentose pathway: good growth on L-ara and D-xylose, but not on L-arabitol and xylotol (no uptake)

Nai C *et al.*, in revision at *Fungal Genetics and Biology*

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A95 & Biolog System - Outlook

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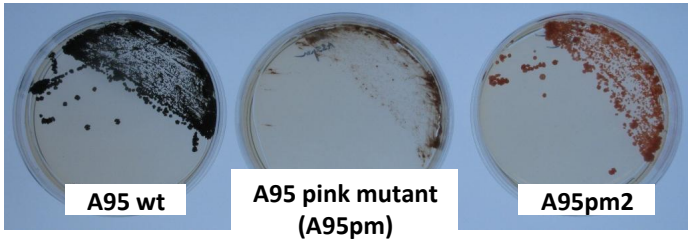
Further characterization of spontaneous mutants and other black fungi

Coniosporium apollinis *CBS100218 (annotated sequence released on 03/2013 by Broad)*

Cell wall biosynthesis mutants



Mechanisms of oligotrophy in A95

Sustained growth under nutrient limitation (especially N, P and S)

Test metabolization of hydrocarbons (benzene, toluene, pentane)

p-*Hydroxybenzoic acid very good C source*

Degradation of cellulose

Cellobiose [glucose β(1→4) glucose] is a good C source

Mutants seem unable to grow on cellulose

Role of trehalose

Trehalose is one of the few very good C sources at suboptimal growth conditions (high pH)

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Questions?

Thank you for your attention !