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

*Restrictions, Solutions, Results*

Corrado Nai
PhD candidate at the Free University of Berlin
BAM Federal Institute of Material Research and Testing
Department 4 (Materials & Environment)
Prof. Dr. Anna A. Gorbushina
Introduction: Our research
(and why it is relevant)

**Extremotolerance**
(UV, salinity, oligotrophy, ionizing radiation, ...)

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

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


**Biofiltration**

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

**Material colonization**

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Guiedan et al. (2008), *Stud Mycol*

Fungal pathogenesis

© Sybren de Hoog
CBS-KNAW

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

Cologne (Germany)

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

**Microbial ecology**

Gorbushina (2007), *Environm Microbiol*

**Fungal genetics**
(biodiversity, evolution, phylogenetics)

© Cécile Guiedan
Guiedan et al. (2008), *Stud Mycol*
Ruibal et al. (2009), *Stud Mycol*

Black Yeasts Database at the Broad Institute
Introduction: Our research
(how we contribute)

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
A prerequisite: Knowledge of A95

**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
**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)

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**Redox dye mix E (RME)**

- 0.125x 0.25x 0.5x 1x 2x 4x 8x

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

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

Growth in **PM10** (7 d at 25 °C)

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**pH 3.5 4.0 4.5 5.0 5.5 6.0 7.0**

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Growth in PM3 (A1, no N)
(B) PM4 (A1, no P) and
(C) PM4 (F1, no S)
(from Nai C et al., in revision)
A95 & Biolog System - Solutions

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

Growth in PM10 (7 d at 25 °C)

... not solved for S metabolism!
A95 & Biolog System - (Some) Results

- pH optimum at around pH 5 (PM10)
- Halotolerant (PM9)
- Prototrophic (no special nutritional requirements), but thiamine stimulates growth (PM5)

- 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 xylitol (no uptake)

Nai C et al., in revision at Fungal Genetics and Biology
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
Celllobiose [glucose $\beta(1\rightarrow4)$ 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)
Questions?

Thank you for your attention!