Protein profiling: a promizing tool to study stress adaptation of black fungi in extreme environments.

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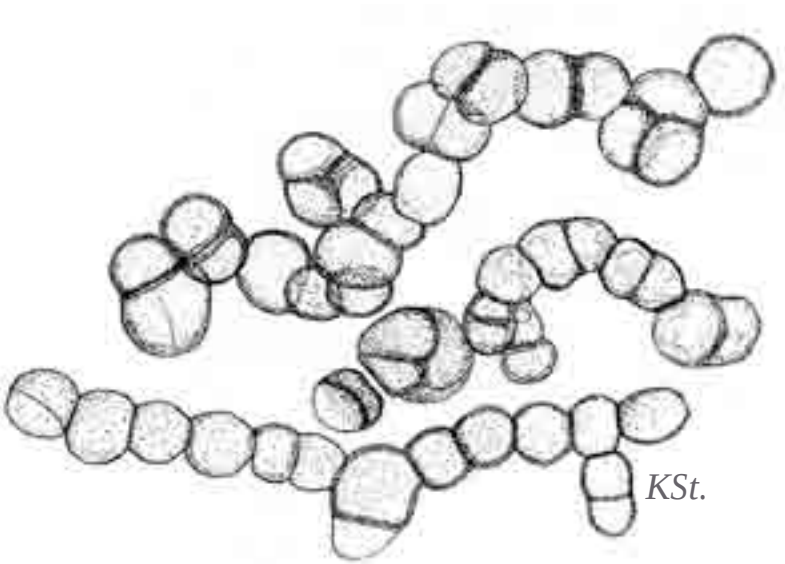


Environmental conditions of rock fungi are characterized by

- low a_W
- extreme temperatures: hot and cold
- low nutrient availability: lack of autotrophs, lack of bacterial biofilms
- extreme changes of conditions
- high UV radiation

The metabolism of MCF is supposed to be low in the cold and hot environments.

Example: no production of toxins in black fungi

(Vishwanath V, Sulyok M, Sterflinger K et al., 2010, unpublished data)

HPLC screening for 171 toxins:

- 20 Trichothecenes
- 4 Fumonisins
- 6 Aflatoxins
- 8 Fusarium metabolites
- 10 Alternaria toxins
- 7 Cytochalasins
- 4 Cyclosporins
- 25 Ergot alkaloids
- 16 exotic metabolites
- 21 Aspergillus toxins
- 22 Penicillium toxins
- 28 bacterial metabolites

HA 1291	*Coniosporium apollinis*	no tox
HA 1293	*Hortaea werneckii*	no tox
MA 2853	*Exophiala jeanselmei*	no tox
MA 3320	*Exophilala salmonis*	no tox
MA 3375	*Sarcinomyces petricola*	no tox
MA 3478	*Trimmatostroma abietis*	no tox
MA 4597	*Coniosporium sümbülii*	Chloramphenicol ?
MA 4642	*Capnobotryella renispora*	no tox
MA 4659	*Capnobotryella antalyensis*	no tox
MA 4760	*Sarcinomyces sidedicae*	no tox
MA 4790	*Mycocalicium victoriae*	Chloramphenicol ?
MA 4968	*Exophilala spinifera*	no tox
MA 5682	*Cryomyces antarcticus*	no tox
MA 5683	*Cryomyces minteri*	no tox
MA 5723	*Coniosporium perforans*	no tox
MA 5727	asexual clade from Antarcica	no tox

Protein profiling: why?

- A protein profile can reflect the metabolic status of a cell: quantity and quality.
- The quantity of different proteins (diversity) can be a measure for ecological plasticity.
- The up- and downregulation of proteins can be a measure for stress response.
- The identification of „stress response proteins“ can give new insights in the ecology of MCF.
- New extremotolerant proteins have high potentials for new biotechnological applications (e.g. cold adapted or acitiviy at low a_w values).

What is 2D-protein profiling?

Separation of proteins on a

2-dimentional gel:

(1) Protein extraction

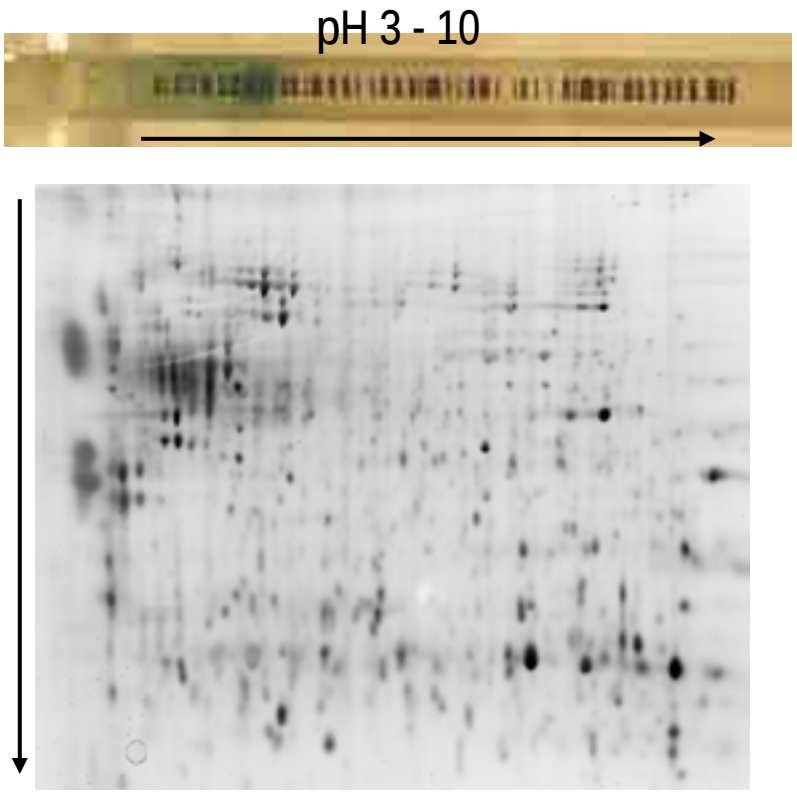
(2) Protein quantification (Bradford)

(3) Isoelectic focussing (first dimention electrophoresis, pH range)

(4) Acrylamid gel (second dimention elecrophoresis)

(5) Data analysis, interpretation

(6) Protein identification: LCMS



Model organisms

Thermotolerant MCF:

- *Coniosporium perforans*
- *Coniosporium apollinis*

Cold adapted MCF:

- *Cryomyces antarcticus*
- *Cryomyces minteri*

Black yeast with high affinity to human pathogens:

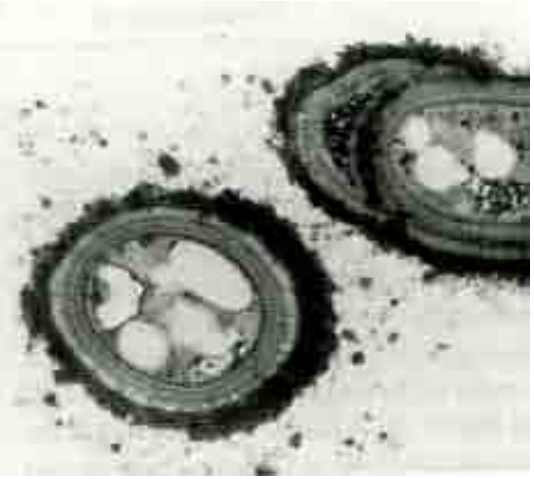
- *Exophiala jeanselmei*

Optimization of extraction methods for MCF:

Isola D, Marzban G, Selbmann L, Onofri S & Sterflinger K (2010): unpublished data.

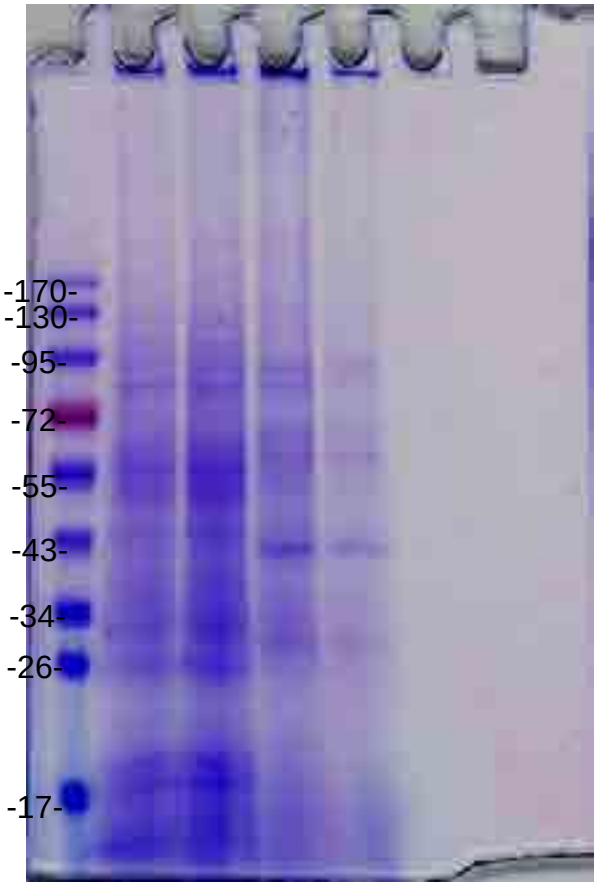
- Extraction protocols might separate for: cell wall proteins, membrane bound proteins, cytoplasm proteins or WHOLE CELL PROTEINS.

- In black fungi, extraction and purification steps are always the most difficult because of the cell walls: this concerns DNA, RNA, chromosomes, protoplasting and proteins.



Cruical steps (to mention just a few):

- Extraction: precipitation by TCA, acetone or phenol?
- Separation: by filtration or centrifugation?
- Rehydration of the strips for IEF.
- Washing.



Stress and proteins: hypothesis 1

There are two main ways to react to stress conditions:

- Downregulation: underexpression of many proteins (can this help to define „dormancy“?)
- Expression of certain proteins (e.g. HSP) necessary to withstand the stress. More proteins are expressed but the household proteins might even be downregulated (can this help to define „stress“?)

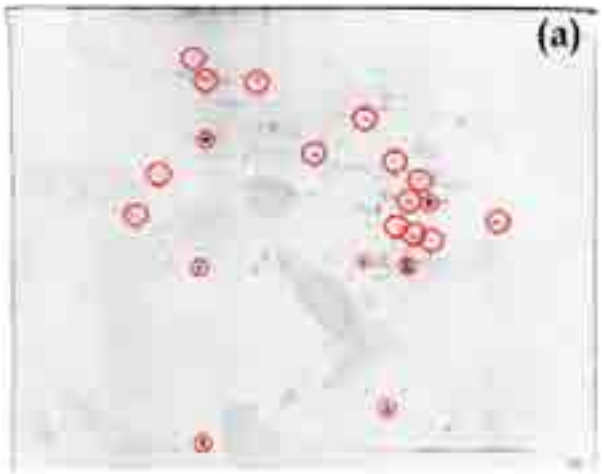
- Both happens in black fungi…

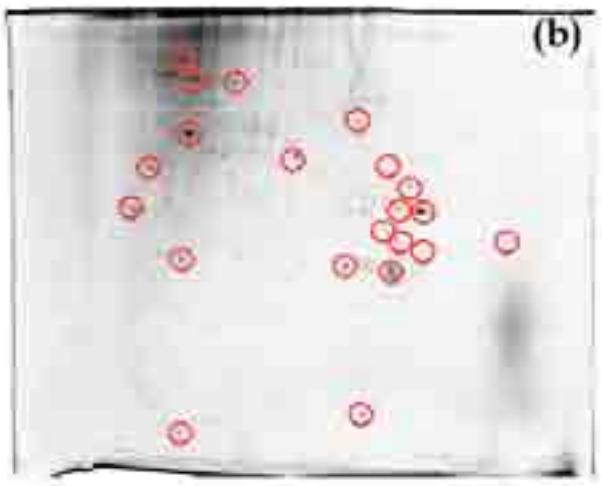
Protein profile: *Cryomyces antarcticus*

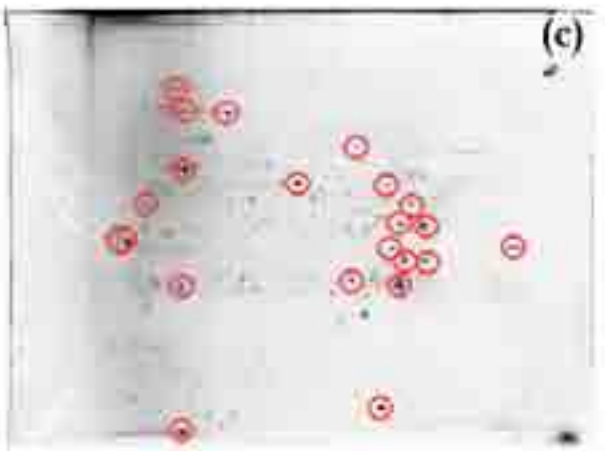
Isola D, Marzban G, Selbmann L, Onofri, S, Sterflinger K (2010): unpublished data.

Dormancy?

Stress: expression of small HSP?







15°C: optimal conditons, 196 spots.

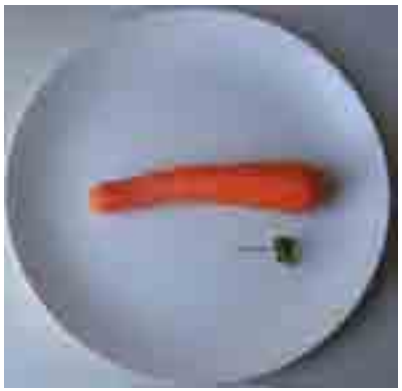
1±1°C: 82 spots.

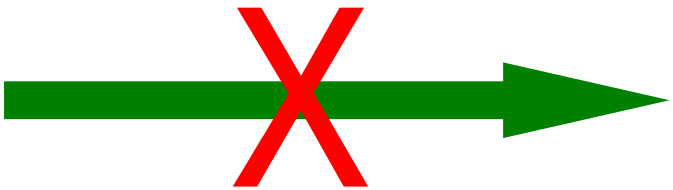
28°C: 187 spots, expression of low molecular weight proteins.

Hypothesis 2:

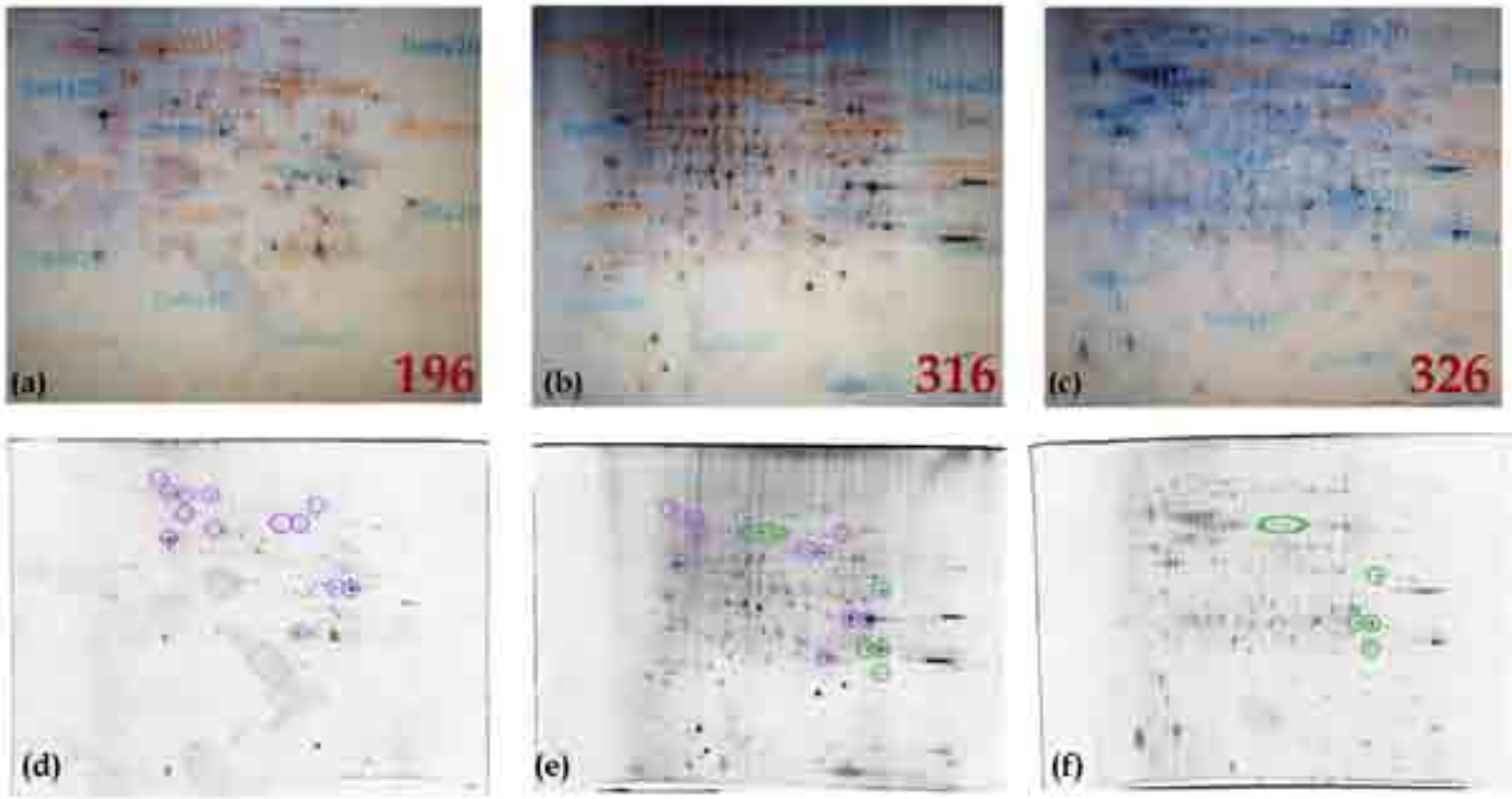
- **Simple living**: metabolism is generally reduced to what is required to sustain life: a limited number of number of proteins is expressed also under optimal conditions.

- **Evolutionary downshifting:** Some fungi have lost the ecological plasticity (and the genomic) to adapt to mesophilic conditions.





Protein profile: comparison of species.



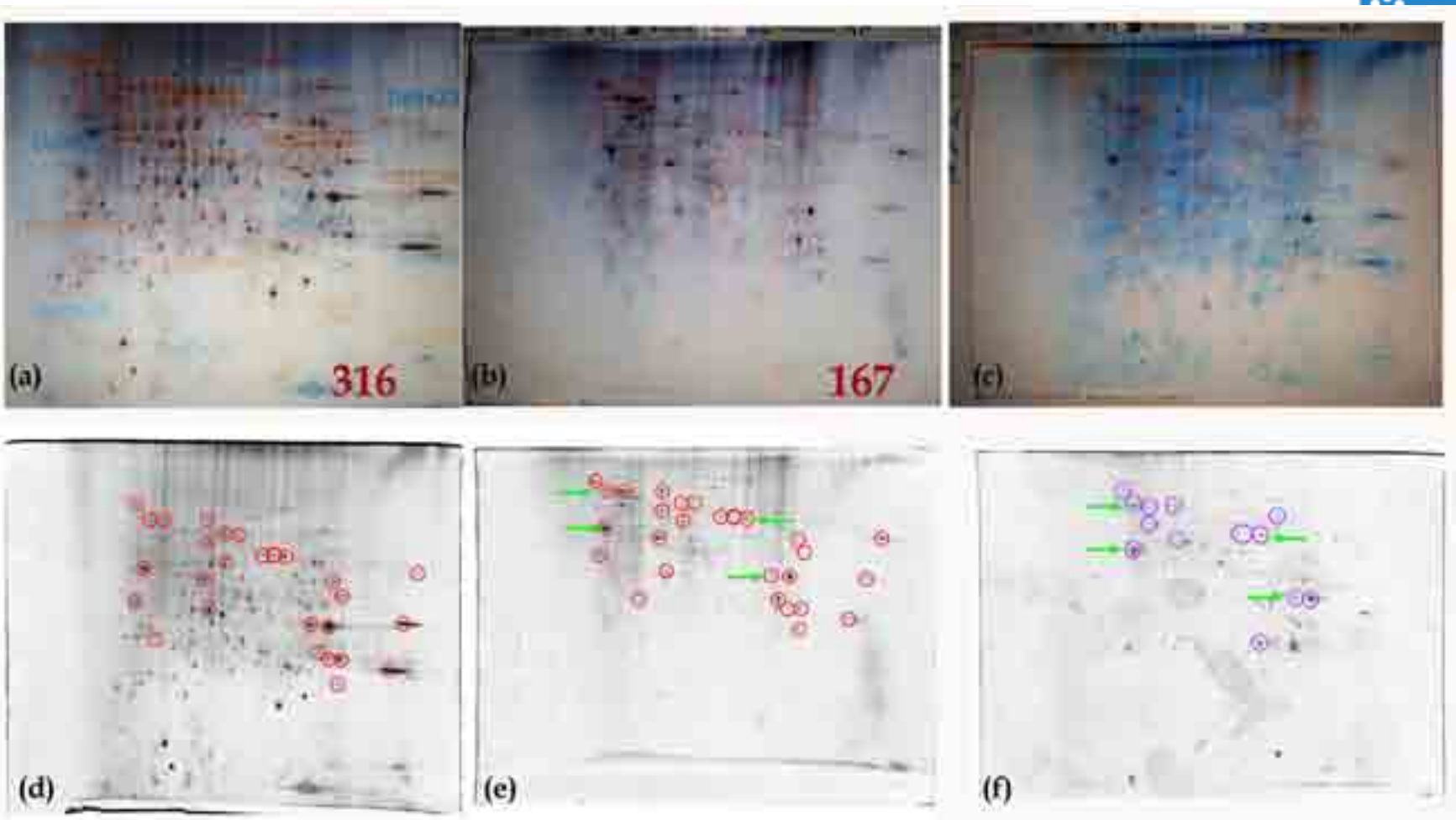
Cryomyces antarcticus:

optimal conditions, 15°C.

Coniosporium perforans:
optimal conditons, 28°C.

Penicillium chrysogenum:
optimal conditions, 28°C

Protein profile: Coniosporium perforans



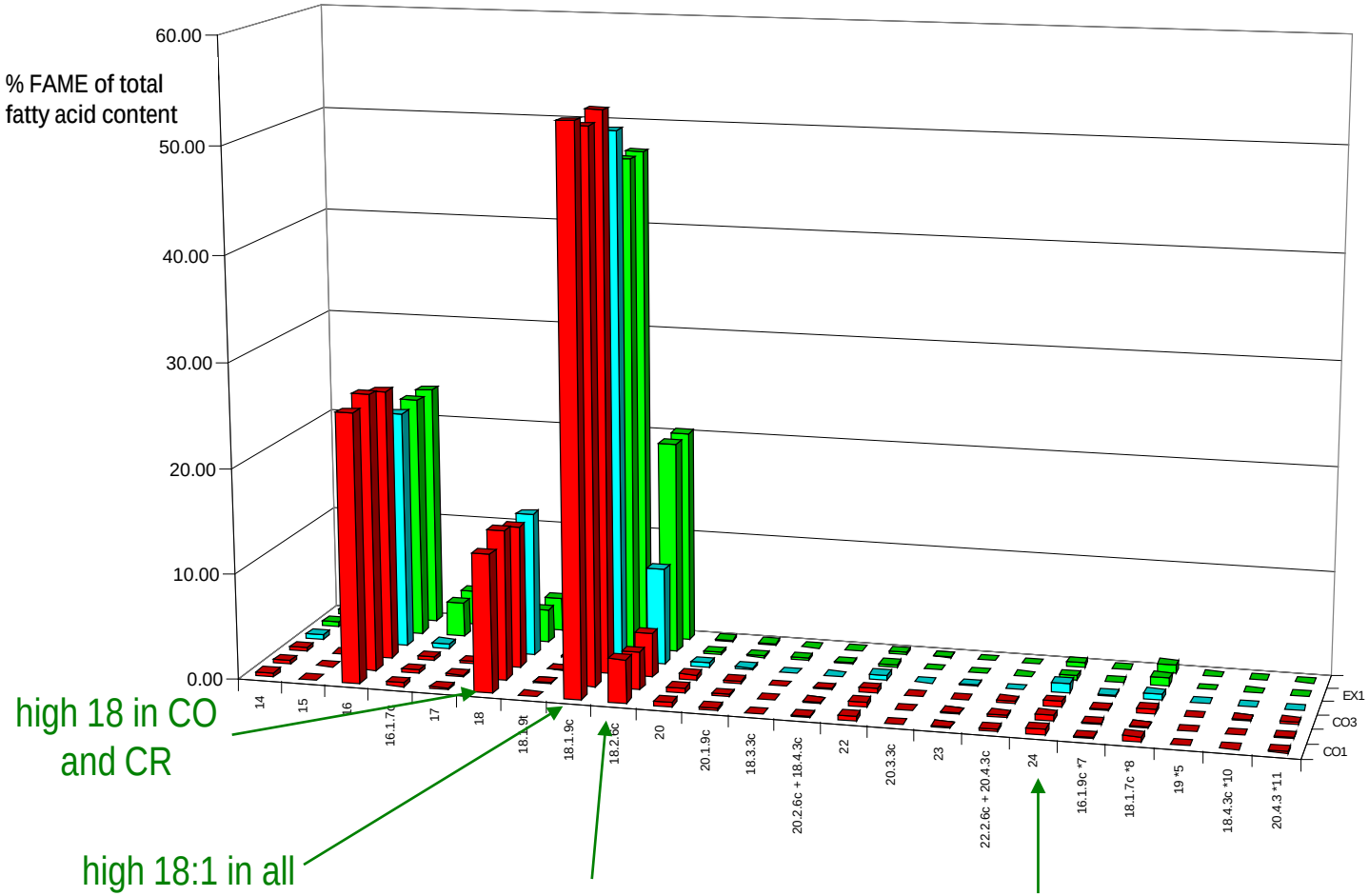
28 °C, optimal conditions

1±1 °C, reduction to housekeeping: dormancy?

In circles: common proteins of *Cryomyces* and *Conosporium*

What is in the cell? Lipids and fatty acids.

(Kainz, Sterflinger, 2010, unpublished data)



high 18 in CO and CR

high 18:1 in all

low 18:2 in all

Presence of 24 is rare in mesophilic fungi!

whole cell lipid content:

CO: 15.4 %

CR: 34.4%

Exo 5.9%

Summary

- First protein profiles gave strong evidence that a **minimized metabolic activity** is one of the major traits for black fungi to live in extreme environments.
- Black fungi show differences in their ecological and physiological plasticity: **evolutionary downshifting** in *Cryomyces*.
- Protein profiles can be used as a measure for **stress** and for **dormancy**.
- First mycotoxin analysis gave evidence for symbiotic bacteria.
- Fatty acid analysis showed remarkable differences between black fungi, for the cold adapted fungus they might play the most important role.

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Thank you for your attention!