COST WG3-Workshop from 16th-18th December in Brussels

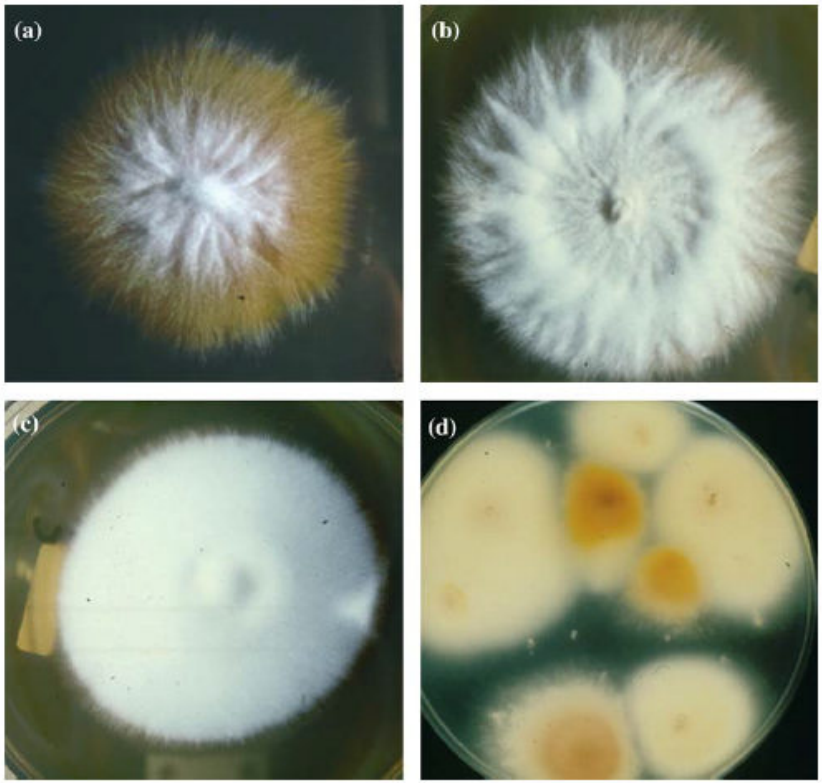
„New concepts and strategies for longterm cultivation and conservation of competent endophytes for plant growth and plant protection”

- Some thoughts -

Dr. Matthias Döring (INOQ GmbH)

Problems of fungal and bacterial cultures on artifical media for cultivation/conservation

Changing of micro/macromorphology



Microsporum canis (a, b, c)

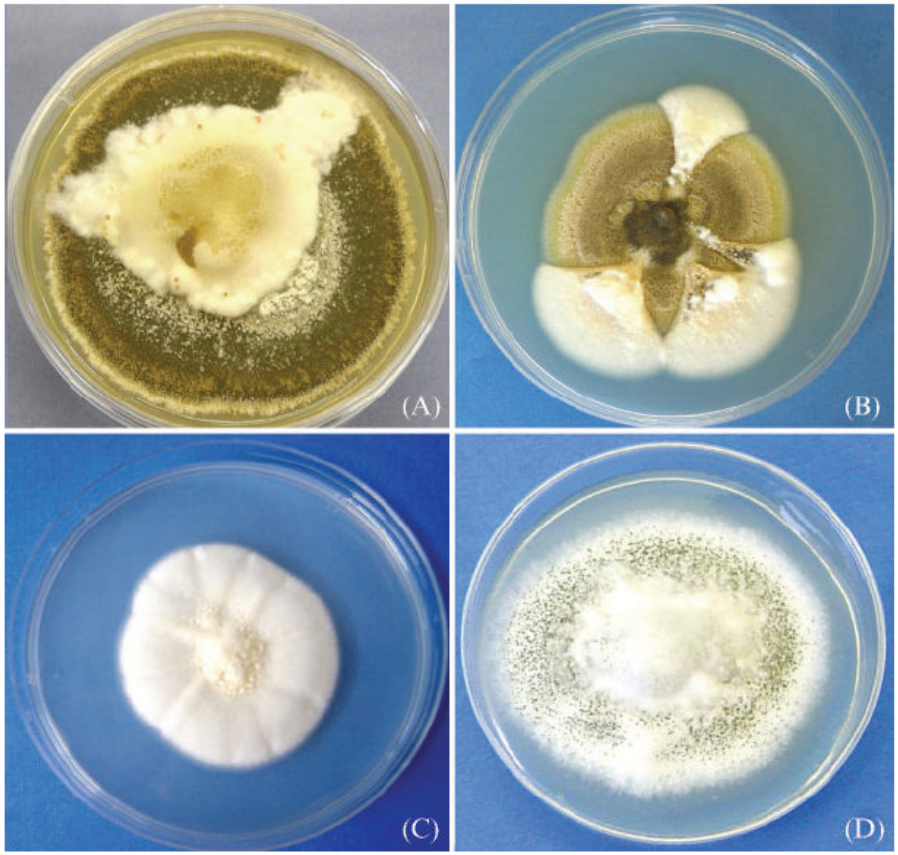
Trichophyton interdigitale (d)

Borman et al. 2006

Figure 1. Alterations in colonial morphology observed with dermatophytes stored in water. Panel a: Colony of *Microsporum canis* (NCPF 179) from a freeze-dried stock and showing original morphology. Panels b and c: The same isolate after storage in water and showing different degrees of aerial mycelium pleomorphism. Panel d: *T. interdigitale* after long-term storage in water showing a slower growing yellow variant in addition to the original white colonial form (photograph of colony reverse).

Problems of fungal and bacterial cultures on artifical media for cultivation/conservation

„Sectoring“ of fungal mycelia – phenotypic degeneration



Wang et al. 2005

Fig. 1. Different types of culture colonies of *Metarhizium anisopliae* indicating the occurrence of sterile sectorization and the restoration of conidiation. (A) V275 colony with sector; (B) V245 colony with sector; (C) sterile subculture transferred from the sector of either V245 or V275; (D) partial restoration of conidiation of V245sec on 10 mM cAMP agar.

Problems of fungal and bacterial cultures on artifical media for cultivation/conservation

LOSS of VIRULENCE after subculturing on artificial media

- ***Beauveria bassiana*** **strains (Samsinankova et al. 1981)**

- ***Metarhizium anisoplae*** strains– **Decline in sporulation** (Shah et al. 2005)

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Loss of Production of Metabolites

Acremonium coenophialum- endophyte from *Festuca arundinacea*

TABLE 2. Endophyte stability for in vitro ergot alkaloid production during subculture

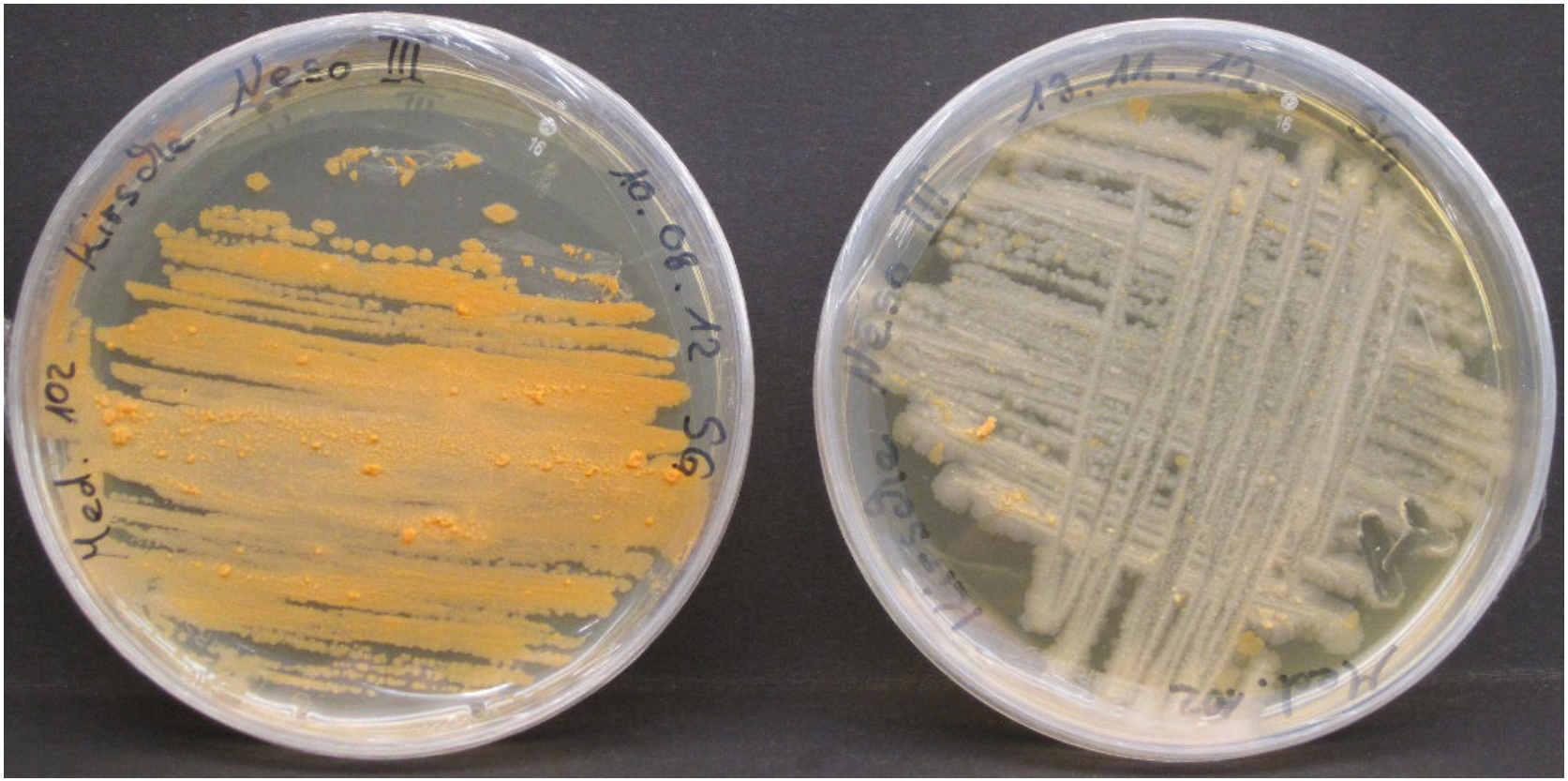
Isolate	Total ergot alkaloid produced (mg/liter) in subculture[a]:				
	0	1	5	10	20
RRC 347	772	578 (0.482)	515	321	356 (0.874)
RRC 358	38	ND (0.573)	ND	ND	ND (1.322)
RRC 321	292	215 (0.382)	ND	ND	ND (1.021)
RRC 241	221	ND (0.521)	ND	ND	ND (1.321)
RRC 360	391	ND (0.422)	ND	ND	ND (1.723)
RRC 320b	362	210 (0.722)	39	ND	ND (2.045)
RRC 347wc	521	409 (0.631)	ND	ND	ND (1.221)

[a] Each subculture was made after 14 days of growth in M102 and was transferred either to fresh M102 for growth (dry weights) or to M104T for ergot alkaloid determination. ND, None detected. Numbers in parentheses refer to the means of three replications of dry mycelial weight (grams per 100 ml) produced after 21 days of growth of subcultures 1 and 20.

Bacon (1988)

Problems of fungal and bacterial cultures on artifical media for cultivation/conservation

Loss of pigments –example endophytic bacterial isolates from Prunus avium



DISCUSSION POINT

What are your experiences with endophytic fungal and bacterial isolates in subcultivation and preservation?

Are my endophytic microbial isolates really monoxenic?

- Background:
- **Mycoviruses** are often distributed in fungal mycelia

- **Endofungal bacteria**

How can we improve long-term cultivation and preservation, esp. for endophytes?

- Inoculation of plant material and reisolation of microbial endophytes could improve ecological fitness and colonization behaviour
- Design of new natural culture media with **plant extracts** (talk of Nabil Hegazi)
- Design of new culture systems/matrix (talk of Colin Ingham)

DISCUSSION POINTS

Plant extracts, pure compounds in culture media?
(talk of N. Hegazi)

Associated microbes?
(talk of B. Murphy)

MICROBIAL ENDOPHYTE

Plant in vitro culture as new place for conservation
(talk of M. Schumacher)

and further ideas…