Identification of Penicillium

- **Penicillium**
  - A common genus with more than 250 species
  - Two ascomycetous teleomorphs (Talaromyces and Eupenicillium)
  - Has preference for moderate and colder climates
  - Spoilage agents, industrial application, mycotoxins
  - Only a few species are pathogenic

Overview of presentation

- **Macro-morphology:**
  - Colony colour (obverse/reverse)
  - Colony size
  - Exudate
  - Soluble pigment
- **Micro-morphology:**
  - Branching pattern
  - Shape phialide
- **Ehrlich test**
Exudate production in *Penicillium*

Soluble pigment production in *Penicillium*

**Summery of presentation I**

- Macro-morphology:
  - Colony colour (obverse/reverse)
  - Colony size
  - Exudate
  - Soluble pigment
- Micro-morphology:
  - Branching pattern
  - Shape phialide
- Ehrlich test
Flask-shaped
Lanceolate = acerose

Penicillium conidiophore = Penicillus

Phialides
Conidia
BRANCHING PATTERNS IN *Penicillium*

- Monoverticillate (unbranched, simple)
- Biverticillate (one-stage branched)
- Terverticillate

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*Penicillium glabrum*

**Monoverticillate**

(= unbranched, simple)

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*Penicillium citrinum*

**Biverticillate**

(=one-stage branched)
Penicillium corylophilum
Biverticillate
(=one-stage branched)

Penicillium brevicaespactum
Terverticillate
(=Two-staged branched)

Penicillium expansum
Terverticillate
(=Two-staged branched)
Paecilomyces

- Phialides with broad base and long narrow neck
- Conidiophore branching irregularly
- Cultures mostly not green
- Thermophilic

Paecilomyces variotii
**Penicillium Biverticillium**

- *Penicillus biverticillate* = one-staged branched
- *Phialides acerose* = lanceolate

**Flask-shaped phialides**  
**Lanceolate phialides**
Colony surfaces in Penicillium

Cultivation for identification

- Czapek Yeast agar (CYA): Macro-morphology
- Czapek Yeast agar (CYA) incubated at 30°C Colony diameter (and ratio with 25°C)
- Malt Extract Agar (MEA): macro- and micromorphology
- Creatine agar (CREA), used for identification of terverticillate *Penicillia*
- Yeast Extract Agar (YES), only macro-morphology
- Incubation 7 days at 25°C
CREA: Creatine Sucrose agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Creatine (H₂O)</td>
<td>3 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>30 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.5 g</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.5 g</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>0.01 g</td>
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<tr>
<td>K₂HPO₄·3H₂O</td>
<td>1.3 g</td>
</tr>
<tr>
<td>Bromocresol purple</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Final pH 8.0 ± 0.2 (adjust after medium is autoclaved).
NOTE: a modification of CREA (CRE) with 1.6 g K₃PO₄·7H₂O can also be used.

Examples growth on CREA

Acid / Base production on CREA
Ehrlich reaction

- Filter paper test; detection of presence of indole metabolites
- Plug for 7 days old CYA culture
- Place filterpaper with Ehrlich reagens on the mycelium side of the plug (propeller)
- Read after 5 and 10 minutes
- No reaction / violet (yellow / brown)

Ehrlich reaction patterns

Violet reactions

No reaction

Summary of presentation II

- Macro-morphology:
  Colony color (obverse/reverse)
  Colony size
  Exudate
  Soluble pigment
- Micro-morphology:
  Branching pattern
  Shape phialide
- Ehrlich test
Macroscopical features

- CYA, obverse - 30°C
- MEA obverse
- CYA obverse
- CYA reverse
- YES obverse
- YES reverse
- CREA obverse

Microscopic preparation of *Aspergillus* and *Penicillium*

- Lactic acid with analine blue
- Drop of alcohol
- Preparations from Malt Extract Agar

On-line database about *Penicillium* subg. *Penicillium*:

http://www.cbs.knaw.nl/databases/index.htm (select *Penicillium* database)