Microbial growth – a potentially harmful source of microbial agents

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Microbes and microbial products are everywhere

- Microbes are always present – where do they come from?
- Outdoor sources
- Indoor sources – normal and harmful
- It is obvious that some sources are more harmful than others
- What could be the explanation?
- Epi evidence on associations between mold growth and adverse health effects (not between microbial counts and health effects)
Sources of indoor fungi

- Normal sources
  - Outdoor air
  - Foodstuff, firewood, pets’ food, bedding
  - House dust (reservoir)
  - Users of the building; transport on peoples’ clothes, on pets’ fur
  - Colonies on wet surfaces
    - bath, kitchen, toilet, sinks, plumbing, plants, food, fruit, vegetables, any organic material
Sources of indoor fungi

- Non-normal source
  - Microbial growth on damp or wet surfaces
  - One source among others, but has more health relevance than others
Microbes in indoor air – normal situation

- Act as any particles while airborne
  - Depending on particle size, density
- Microbial material may occur in size range 10 nm-100 μm
- Continuous traffic from outdoors, occupants and indoor sources
- Settling on floor and surfaces, resuspension
- Removal by cleaning and ventilation
Normal microbes - friendly companions?

- Normal flora of skin is necessary for health
  - become air pollutants when released to air
- "normal" mycobiota of indoor air similar as outdoors
- Possible protection from allergy
  - Microbes of farming environment
Harmful pollution?

- Infectious pathogens
  - source: humans
  - Alertness a necessary policy
  - Role of ventilation
  - Importance of cleaning
- Mold growth on moistened surfaces
  - Risk for many adverse health effects
  - Toxin production a potential risk
  - Renovation and clean-up a necessary policy
It matters where they come from

- Microbes are present everywhere;
- People have symptoms in certain indoor environments but get better when elsewhere;
- In the context in question, no one gets sick from outdoor microbes, or microbes from normal sources;
- It is the dampness, moisture and consequent mold growth that is the critical issue.
A harmful source should be eliminated

- In case of building mold, is not enough to ventilate airborne microbial agents away,
- one should eliminate the source

- Learning more about the harmfulness of microbial growth:
  - It is the source we should focus on: what grows there, what are the metabolites produced
Indoor environments as habitats of microbes...

• Typically dry habitats compared to most natural environments;
• Nutrition available in buildings
  – Biomaterial from humans, animals, plants, house dust, nutrients of tapwater, sewage water...
• No extreme temperatures – not too cold, not too warm
• No extreme pH
  – except concrete; high pH limits the diversity of microbes growing on it
When does harmful growth start?

- All the other necessary factors for growth are there… just add the water
- Excess water may come from
  - condensation, leakage, rain, snow, flooding, capillary raise of soil water…
- Spores present everywhere, those microbes start to grow for which the niche is most optimal
- Growth will start within hours or days
- The longer the growth may continue – often months or years – the more diverse the growth becomes and the more potential for toxin production
General rules of microbial growth

- Wherever and whenever excess moisture, some microbial growth will take place
- The more water, the more mold
- Moisture sometimes fluctuating; microbial growth follows the fluctuation
- Drying the substrate (and mycelium) does not destroy the microbes; spores are there to wait conditions "improve"
What will grow there?

- Microbial growth involves various species
- Never a pure growth of a single fungus
- Communities of species, an ecosystem develops
- Usually start with molds, yeasts and bacteria
- Later amoebae and nematodes, ants, insects...
- Microbial interactions are important for toxin production
**Examples of fungal genera found in infested building materials**

<table>
<thead>
<tr>
<th>Acremonium</th>
<th>Gliocladium</th>
<th>Scopulariopsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria</td>
<td>Humicola</td>
<td>Sphaeropsidales</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Mucor</td>
<td>Stachybotrys</td>
</tr>
<tr>
<td>Aureobasidium</td>
<td>Oidiodendron</td>
<td>Torula</td>
</tr>
<tr>
<td>Botrytis</td>
<td>Paecilomyces</td>
<td>Trichoderma</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>Penicillium</td>
<td>Tritirachium</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>Phialophora</td>
<td>Ulocladium</td>
</tr>
<tr>
<td>Doratomyces</td>
<td>Phoma</td>
<td>Verticillium</td>
</tr>
<tr>
<td>Eurotium</td>
<td>Rhinocladiella</td>
<td>Wallemia</td>
</tr>
<tr>
<td>Fusarium</td>
<td>Rhizopus</td>
<td>Yeasts</td>
</tr>
<tr>
<td>Geomyces</td>
<td>Rhodotorula</td>
<td></td>
</tr>
</tbody>
</table>

*National Institute for Health and Welfare*
Actinomycetes and other actinobacteria

- Actinomycetes (mainly *Streptomyces* spp.) occur commonly in mouldy materials
- Produce earthy odour, "potato cellar" odour
- Most commonly (48%) on ceramic materials (Hyvärinen et al. 2002)
  - Occur together with *A. versicolor*, *Acremonium*
- Mycobacteria in 23% of samples (Torvinen et al. AEM 2006;72:6822-6824)
  - Occur together with actinomycetes, *Aspergillus* spp., *Fusarium* spp. and yeasts

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Indoor environments are very diverse in their microbial communities

- 200-300 fungal genera cultured
- With sequence analyses of the fungal communities, 5-10 times more genera found than with culturing
- Indoor bacteria less characterized so far
  - Analyses show a rich diversity, too
  - Dominated by gram positive species
Microbial growth on a building material may also include amoebae

- Amoebae occurred in appr. 20% of samples of moldy materials
- Amoebae may protect bacteria growing inside them: *Chlamydia*, mycobacteria etc.
- Presence of amoebae influenced the growth and toxicity of other microbes
Role of material on microbial growth

- Type and content of substrate is one factor regulating microbial growth
- Substrate has also a role in regulating secondary metabolism, e.g. toxin production
Study on the distributions of fungi in various building materials (Hyvärinen et al. 2002)

- Fungi were analyzed from damaged samples
- Quantitative counts and qualitative analyses on genus level
- Statistical analyses on occurrence of fungi on certain materials
- Materials differed for their fungal content
Substrate/material also affects the toxicity of microbial growth

- The same streptomycete strain (*S. anulatus*) was cultured on wetted pieces of building materials in small chambers for 2 months
- Spores were collected to a suspension which was used to expose cell culture
- Cytotoxicity was assessed as percentage of dead cells from total (MTT test)
- *S. anulatus* was most toxic after growth on gypsum board
Importance of material to microbe's toxicity

Cytotoxicity induced by *Streptomyces anulatus* grown on different building materials. Roponen *et al.* Indoor Air 2001;11:179-184

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### Occurrence of toxic bacterial and fungal metabolites on mold damaged building materials

<table>
<thead>
<tr>
<th>Microbial metabolites (positive/total samples)</th>
<th>Detection of metabolites in 9 building material samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4105  4106  4107  4108  4109  4110  4111  4112  4113</td>
</tr>
<tr>
<td><strong>Fungal</strong></td>
<td></td>
</tr>
<tr>
<td>Chaetoglobosin A (8/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Emodin (5/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Meleagrin (9/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Roquefortine C (3/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Stachybotrylactam (4/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Sterigmatocystin (6/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Trichodermol (1/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td><strong>Bacterial</strong></td>
<td></td>
</tr>
<tr>
<td>Monactin (3/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td>Valinomycin (6/9)</td>
<td>+          +          +          +          +      +      +      +      +</td>
</tr>
<tr>
<td><strong>Number of different metabolites per sample:</strong></td>
<td>4          5          4          5          4      6      3      8      6</td>
</tr>
</tbody>
</table>
**Intervention studies**

- **Intervention**: removal of the source of microbial contamination
- may involve improvement of ventilation other renovation
- effects of the elimination of source
  - on building condition
  - on IAQ
  - on occupant health
Remediation of two school buildigns

- 2 problem schools, one completely renovated, one partly renovated
- 2 control schools
- microbial measurements and symptom questionnaire before and after remediation

Meklin et al. (2005)
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Airborne fungi before and after school remediation

- Concentrations of airborne fungi decreased to normal
- Note: airborne concentrations low even before remediation;
- Difference significant
Fungal concentrations / partial remediation

Meklin et al. (2005)
Symptom prevalence / complete remediation

- difficulties in concentration
- headache
- fatigue
- eye symptoms
- nocturnal cough
- cough with phlegm
- cough without phlegm
- nasal bleeding
- hoarseness
- sore throat
- rhinitis
- stuffy nose

Meklin et al. (2005)

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Conclusions from the school intervention study

- Fungal concentrations were a marker of harmful exposure; higher than control before renovation
- Decreased to "normal" levels after a thorough remediation, elimination of the source(s)
- Many symptoms decreased as a result of remediation
- After a partial remediation, fungal concentrations higher than before
- Some symptoms decreased; was their cause eliminated?
School study II
(Haverinen et al. 2004)

- The results indicated that the repairs succeeded in the sense that new cases of symptomatic students were no longer identified.
- Reversibility of the symptoms of exposed individuals?¹
- Time period needed for symptom relief?

Effects of moisture remediation on health of adults. Summary of reported intervention studies. (Patovirta 2005)

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<tr>
<th>Place/Number of participants</th>
<th>Methods</th>
<th>Health effects of remediation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rudblad et al 2002</td>
<td>school n=28</td>
<td>increased reactivity to histamine in nasal provocation test</td>
</tr>
<tr>
<td></td>
<td>questionnaire nasal provocation test</td>
<td></td>
</tr>
<tr>
<td>Ebbehoj et al 2002</td>
<td>swimming bath n=25</td>
<td>decrease in symptom levels</td>
</tr>
<tr>
<td></td>
<td>questionnaire clinical examination 2-week peak flow follow-up</td>
<td>decrease in peak-flow variability</td>
</tr>
<tr>
<td>Patovirta et al 2003</td>
<td>school n=26</td>
<td>the connection between elevated IgG-antibodies and sinusitis</td>
</tr>
<tr>
<td></td>
<td>questionnaire IgG-antibodies</td>
<td></td>
</tr>
<tr>
<td>Patovirta et al 2004</td>
<td>school n=44</td>
<td>no new asthma cases respiratory infections decreased</td>
</tr>
<tr>
<td></td>
<td>questionnaire spirometry</td>
<td></td>
</tr>
<tr>
<td>Patovirta et al 2004</td>
<td>school n=56</td>
<td>symptoms of fatigue and headache decreased</td>
</tr>
<tr>
<td></td>
<td>questionnaire</td>
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<tr>
<td>Jarvis and Morey 2001</td>
<td>questionnaire</td>
<td>lower symptom rates no new building related respiratory disease</td>
</tr>
<tr>
<td>11-story structure n=488</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Áhman et al 2000</td>
<td>interview</td>
<td>lower almost “normal” symptom rates</td>
</tr>
<tr>
<td>school n=21 teachers n=224 pupils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigsgaard et al 2000</td>
<td>questionnaire</td>
<td>mucosal and neurological symptoms decreased</td>
</tr>
<tr>
<td>school n=43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudakin 1998</td>
<td>interview</td>
<td>decrease in upper respiratory and neurobehavioral symptoms</td>
</tr>
<tr>
<td>office building n=37</td>
<td></td>
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Growing microbes - bad microbes

- Microbial growth is the source of harmful agents in damp or moisture damaged building
- Microbial growth in the building acts as a source of particle and volatile emissions into indoor air
- It is important to eliminate the source, not to paint on it or encapsulate it

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