Malassezia – host-fungal interactions

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Malassezia (basidiomycetes)

- Previously, *Pityrosporum*
- Lipophilic yeast that belongs to the normal skin microflora

1-8 μm

Host-microbe interactions +/-?
**Malassezia - cell wall**

**Cell wall**

a thick bilayered lipid rich structure, 0.12 μm

70% sugars
10% proteins
15-20% lipids

(1→6)-β-D-glucan is the major carbohydrate component of the *M. sympodialis* cell wall. 
(*Kruppa MD et al Carbohydrate Research 2009*)

Surrounded by a lipid-rich capsular-like structure
(Mittag H. Mycoses 1995)

Bar: 1.0 μm

*Marek, D. et al 2007*
### 13 *Malassezia* strains and their hosts

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>M. caprae</em></td>
<td>Goat, horse</td>
</tr>
<tr>
<td>2. <em>M. dermatis</em></td>
<td>Human</td>
</tr>
<tr>
<td>3. <em>M. furfur</em></td>
<td>Human</td>
</tr>
<tr>
<td>4. <em>M. equina</em></td>
<td>Horse, cow</td>
</tr>
<tr>
<td>5. <em>M. globosa</em></td>
<td>Human</td>
</tr>
<tr>
<td>6. <em>M. japonica</em></td>
<td>Human</td>
</tr>
<tr>
<td>7. <em>M. nana</em></td>
<td>Cat, cow</td>
</tr>
<tr>
<td>8. <em>M. obtusa</em></td>
<td>Human</td>
</tr>
<tr>
<td>9. <em>M. pachydermatis</em></td>
<td>Dog</td>
</tr>
<tr>
<td>10. <em>M. restricta</em></td>
<td>Human</td>
</tr>
<tr>
<td>11. <em>M. slooffiae</em></td>
<td>Dog</td>
</tr>
<tr>
<td>12. <em>M. sympodialis</em></td>
<td>Human, sheep, horse</td>
</tr>
<tr>
<td>13. <em>M. yamatoensis</em></td>
<td>Human</td>
</tr>
</tbody>
</table>

Malassezia

- All *Malassezia* species, except *M. pachydermatis*, require long chain fatty acids to grow (*Gueho* et al., 1996).

- *Malassezia* has been found to preferentially colonize sebum-rich areas of the head, neck, back, and chest (*Faergemann* et al., 1983).

- The colonisation increases during puberty when the sebaceous glands become active (*Faergemann* et al., 1980).
**Malassezia**

Can cause skin and systemic infections

- pityriasis versicolor
- seborrheic dermatitis/dandruff
- pityrosporum folliculitis
- stratum corneum

PAS staining
Atopic eczema

- chronic inflammatory skin disease
- characterized by severely itchy and dry skin
- genetic predisposition and dysfunctional skin barrier
- 80% increased levels of serum IgE
- lifestyle and environmental factors, microorganisms

- prevalence of 10-20% in children, 1-3% in adults

Malassezia and atopic eczema (AE)

- Ketoconazole treatment improves the eczema and decreases serum IgE
- Specific serum IgE and/or positive skin prick test and atopy patch test (APT) to Malassezia (30-80%)
- Elevated levels of serum IgG and IgG₄ to Malassezia
- Significantly higher T-cell response to Malassezia in vitro
- Malassezia-reactive T-cell clones derived from lesional skin have a Th2-like profile.
- Association between circulating Malassezia-specific Th2 cells and positive APT reactions.

Reviews: Scheynius et al 2010; Schmid-Grendelmeier et al 2006
Host-microbe interactions

Skin damage/antigen/allergen entry

Sensitization – where, when, how?

Epidermis

Dermis

LCs

I DEC

DCs

NK cells

Cytokines

Migration

Maturation

Lymph nodes

MDDC

M. furfur

Buentke E. et al 2000, 2001
Positive atopy patch test reaction to *M. sympodialis* extract (strain ATCC 42132)

After 48, 72 or 96 hours

IgE⁺ cells
Global expression profiling in AE skin reveals reciprocal expression of inflammatory and lipid metabolism genes

DNA microarray
24,500 genes

Red highest level and green lowest level of expression

The global transcriptional response in positive APT reactions to *M. sympodialis* is very similar to the gene-signature in lesional AE skin (*Sääf AM* et al 2008).
Higher pH of the skin surface in patients with atopic eczema

<table>
<thead>
<tr>
<th>Normal skin</th>
<th>Atopic eczema skin</th>
</tr>
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<tbody>
<tr>
<td>• is thicker</td>
<td>• is more fragile</td>
</tr>
<tr>
<td>• the lipid composition protects the skin from antigen penetration</td>
<td>• has altered lipid composition</td>
</tr>
<tr>
<td>• pH 5 - 5.5</td>
<td>• increased permeability</td>
</tr>
<tr>
<td></td>
<td>• pH 6 (Rippke F. et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>• higher pH values correspond to more intense itching (Sparavigna A. et al., 1999)</td>
</tr>
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Release of allergens from *M. sympodialis* is enhanced with higher pH.

Cell growth and viability similar.

*Selander C et al Allergy 2006*
Increased release of a 67 kDa allergen at higher pH

mRNA expression

Expression level ($2^{-\Delta\Delta CT}$)

Culture period (h)

24 h, pH 5
24 h, pH 6.1

Protein expression

culture supernatants

Selander et al Allergy 2006
## Cloned allergens from *M. sympodialis*

*ATCC strain 42132*

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<th>Allergen</th>
<th>Size kDa</th>
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<td>36</td>
<td>no known protein</td>
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<td>Mala s 6</td>
<td>17</td>
<td>cyklophilin from <em>A. fumigatus</em>, <em>S. pombe</em>, <em>H. sapiens</em></td>
<td>- &quot;&quot;, Glaser et al 2006</td>
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<tr>
<td>Mala s 7</td>
<td>16</td>
<td>no known protein</td>
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<tr>
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<td>19</td>
<td>no known protein</td>
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</tr>
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<tr>
<td>Mala s 10</td>
<td>86</td>
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<td>Mala s 11</td>
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<td>manganese superoxide dimutase (MnSOD) from <em>A. fumigatus</em>, <em>S. pombe</em>, <em>H. sapiens</em></td>
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Cross-reactivity, Mala s 11 - hMnSOD and Mala s 13 - hThioredoxin

**IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis.**  

**Malassezia sympodialis thioredoxin-specific T cells are highly cross-reactive to human thioredoxin in atopic dermatitis.**  
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Mala s 1

- no sequence homology to known proteins (BLAST homology search)
- major allergen 58% IgE binding frequency
- expressed on the cell surface
- can be released into the cell medium

**Function?**

confocal microscopy

The crystal structure of Mala s 1 – a novel fold among allergens

A β-propeller consisting of six blades surrounding a central pore.

Vilhelmsson M. et al 2007
Hits corresponding to two hypothetical fungal proteins with unknown functions:

- *Ustilago maydis* (maize parasite) residues in orange
- *Gibberella zeae* (wheat parasite) residues in yellow

The putative active site

Vilhelmsson M. et al 2007
Mala s 1

- The crystal structure of Mala s 1 reveals a six-fold β-propeller fold
- A novel fold among allergens
- Possible fungi homologues

Function?

Knock-outs?

Full genome?

Zargari A. et al 1998
Does *Malassezia* activate mast cells?

**Mast cells**
- are increased in skin lesions in AE (*Järvikallio* et al. 1997).
- are key effector cells in IgE-associated immune responses
- play an important role in the first line of defence against microbial invasion and use Toll-like receptors (TLRs) to respond to products of bacteria and zymosan (*Marshall JS* et al. 2004).
- **Dectin-1** is a fungal receptor on mast cells (*Olynch et al* 2006).
Mast cells from AE patients have an impaired up-regulation of the fungal recognition receptor Dectin-1

P4-37A
Carolina Lunderius Andersson
Exosomes and nanovesicles
Exosomes - nanovesicles

• 1985 first described as waste vesicles during erythrocyte maturation (Pan BT et al 1985).

• 1996 exosomes were detected from immune competent cells (Raposo G et al 1996).


30 - 100 nm in diameter


Found in:
Blood serum
Malignant effusions
Urine
Saliva
Breast milk
Bronchoalveolar lavage
Fungi can release extracellular exosome-like vesicles

The structures identified are similar to vesicles produced by \textit{C. neoformans} and \textit{H. capsulatum}. Bars, 100 nm. (Albuquerque PC et al 2008).

\textbf{S. cerevisiae}

Fungal extracellular vesicles contain:
- Enzymes for protein, sugar and fat metabolism
- Heat shock proteins
- Molecules for cell signaling

\textbf{C. parapsilosis}

Vesicles from \textit{Cryptococcus neoformans} modulate mouse macrophage functions (Oliveira DL et al, \textit{Infect Immun} 2010) and Poster 4-10A

\textbf{S. schenckii}

\textbf{C. albicans}

M. sympodialis releases exosome-like nanovesicles

Scale bar = 100 nm

TEM

IEM

Anti-M. sympodialis IgG

Rabbit IgG

Ulf Gehrmann et al submitted
**M. sympodialis** derived vesicles induce IL-4 production in AE patients and TNF-α in patients and healthy controls (HC).
*M. sympodialis* derived vesicles are immunogenic and carry allergen
Can exosomes formed in antigen-presenting DC carry *M. sympodialis* allergens?

*M. sympodialis*

- Antigen uptake
- Enzymatic degradation
- Peptide loading onto MHC
- Fusion and release

Exosomes

Formed by inward budding of endosomal compartments

*Théry C et al*  *Nat Rev Immunol* 2002
*Admyre C et al*  *Allergy* 2007 (Review)
DCexo Mala carry MHC class II and *M. sympodialis* antigens and induce IL-4 in AE patients and TNF-α in patients and controls.
Host microbe interactions by nanovesicles - a possible model

Skin

γδ-T

MalaExo

M. sympodialis

IL-4

TNF-α

IL-4

IL-4

DCexo Mala

Dendritic Cell

T

NK

T
Summary

- *Malassezia* can be used as a tool to dissect host-microbe interactions and pathogenic mechanisms in atopic eczema:
  - Role of host responses to different fungal components?
  - *M. sympodialis* whole genome
  - Therapeutic targets
  - Knock-outs
Co-workers

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- Eva Buentke
- Susanne Gabrielsson
- Ulf Gehrmann
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- Sara Johansson
- Maria Karlsson
- Qazi Khaleda Rahman
- Lena Lundeberg
- Carolina Lunderius Andersson
- Gunnar Nilsson
- Omid Rasool
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- Adnane Achour

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- Mahmoud Ghanem
- Govianni Gadda