A Forbidden Kingdom: The Fungal Microbiome in Skin Health. Why it’s Important, What Needs To Be Done, and How to Intervene

Thomas L. Dawson, Jr  
Senior Principal Investigator  
Agency for Science, Technology, and Research (A*STAR)  
Institute of Medical Biology (IMB)  
Director, Healthy Hair and Skin Program  
Director, Malassezia Research Consortium
A Forbidden Kingdom: Fungal Microbiome in Skin Health

Agenda:

• Asia and the world
• Skin and fungi – the “Forbidden Kingdom”
  • The skin – an underexplored niche
  • Fungi – a crucial knowledge gap
  • Economic impact
• Example: dandruff and seborrheic dermatitis
  • What it is
  • Learning from Koch
  • Early skin mycobiome
  • Role of microbiome – Malassezia
  • Current Malassezia genomics
• Key next steps for skin
• Summary
A Forbidden Kingdom: Fungal Microbiome in Skin Health

Agenda:

• Asia and the world
• Skin and fungi – the “Forbidden Kingdom”
  • The skin – an underexplored niche
  • Fungi – a crucial knowledge gap
  • Economic impact
• Example: dandruff and seborrheic dermatitis
  • What it is
  • Learning from Koch
  • Early skin mycobiome
  • Role of microbiome – *Malassezia*
  • Current *Malassezia* genomics
• Key next steps for skin
• Summary
The Middle Class: Consumers

Percentage Of World Middle Class

Data from Gapminder.com
A Forbidden Kingdom: Fungal Microbiome in Skin Health

Agenda:

• Asia and the world
• Skin and fungi – the “Forbidden Kingdom”
  • The skin – an underexplored niche
  • Fungi – a crucial knowledge gap
  • Economic impact
• Example: dandruff and seborrheic dermatitis
  • What it is
  • Learning from Koch
  • Early skin mycobiome
  • Role of microbiome – Malassezia
  • Current Malassezia genomics
• Key next steps for skin
• Summary
New mushrooms found in porcini packet

DNA sequencing of mushrooms in a shop-bought packet of porcini results in the description of three species new to science.

Contents of a commercial packet of dried porcini containing three species new to science (Photo: B. Dentinger)

This is from 2014!
Microbiome – defining the cutaneous microbiome in health and disease

The Skin Microbiome

Microbiome publications by year

μBiome publications with skin and fungi

Microbiome publications by year
Microbiome – defining the cutaneous microbiome in health and disease

Presentations last winter in KL

Today:
7/42
17%
Cutaneous microbiome in health and disease

✧ Increasingly implicated in common diseases, global direct/indirect costs >$20B USD.
✧ So far, investigations primarily gut/bacteria, leaving skin eukaryotes poorly understood.
✧ A crucial and exploitable knowledge gap.
✧ The microbiome is influenced by or influences everything human.
**Size of the prize – Skin Microbiome**

*example:* Dandruff and seborrheic dermatitis

- Dandruff and seborrheic dermatitis account for a substantial percentage of dermatologist visits
- The anti-dandruff shampoo market is roughly $10 Billion USD/year
- In the anti-dandruff business efficacy is point of entry
- Highly competitive market
- Current technology modulates the microbiome by killing fungi - *Malassezia*
Why Study Dandruff, anyway?

- Incidence is high globally – huge market
- About 50% of all people are affected
- May vary by ethnicity

![Bar chart showing dandruff prevalence by gender and ethnicity.](chart.png)
Dandruff and seborrheic dermatitis

What is it?

- cosmetic manifestations

<table>
<thead>
<tr>
<th>Normal Scalp</th>
<th>Dandruff Scalp</th>
<th>Seborrheic Dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Normal Scalp Image" /></td>
<td><img src="image2" alt="Dandruff Scalp Image" /></td>
<td><img src="image3" alt="Seborrheic Dermatitis Image" /></td>
</tr>
</tbody>
</table>

- Flaking
- Itching
- Flaking
- Itching
- Visible irritation

Do we know the “cause”?
1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

2. The microorganism must be isolated from a diseased organism and grown in pure culture.

3. The cultured microorganism should cause disease when introduced into a healthy organism.

4. The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

“Koch’s Postulates” (1890):

Heinrich Hermann Robert Koch (11 Dec 1843 – 27 May 1910)

wikipedia
“Koch’s Postulates” (modern issues):

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms (susceptibility).
2. The microorganism must be isolated from a diseased organism and grown in pure culture (unculturables).
3. The cultured microorganism should cause disease when introduced into a healthy organism (ethics).
4. The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent (see above).

Some difficult examples: viruses, aspergillosis, cholera
Important published historical data

1. *Malassezia* is on everyone (and every warm blooded animal)

2. In **susceptible** individuals
   - Remove all fungi, dandruff goes away
   - Remove bacteria, it does not
   - Remove both, benefit is the same
   - Add back resistant *Malassezia*, dandruff returns

*Malassezia* meet 3 of 4 of Koch's postulates

A three factor causal model
The first skin *mycobioime* - tFLP

The key gene cluster

18S Forward

18s Ribosomal gene → ITS1 → 5.8s gene → ITS2 → 28s Ribosomal gene

28S Reverse

Amplification

ITS 1 Forward

Middle Reverse

Middle Forward

ITS 2 Reverse

Fragment Analysis by Size

All Known *Malassezia* Genotypes in One Sample (and others)

*M. furfur* (7982),
*M. globosa* (7966),
*M. globosa* (7874),
*M. globosa* (7990),
*M. obtusa* (7968),
*M. restricta* (7991),
*M. restricta* (7877),
*M. restricta* (8742),
*M. slooffiae* (7971),
*M. slooffiae* (7956),
*M. sympodialis* (7977) and
*M. pachydermatis* (74522)

(newly identified *M. nana*, *M. yamatoensis*, and *M. dermatis* also uniquely identified with many control non- *Malassezia* fungi)
The first skin microbiome - tFLP

Example scalp sample

all fungi (almost?) assessed – only 2 major players

Dandruff and seborrheic dermatitis

Early Microbiome: *Malassezia*

Is there a causal species?

How could his be?
Malassezia convert sebum triglycerides into irritating free fatty acids in vitro.
Malassezia convert sebum triglycerides into irritating free fatty acids \textit{in vivo}.
The model: individual susceptibility

What if some people can’t get it?

• Recruit 2 populations, with and without dandruff
• Treat them – remove *Malassezia*, return to baseline
• Challenge with a “toxic metabolite” – oleic acid
The model: individual susceptibility

Some People Get Dandruff

Flaking Difference from Baseline vs. Days Post Initial Treatment

- 7.5% Oleic Acid
- Placebo vehicle
Dandruff and Susceptibility

The model: individual susceptibility

Some People don’t.
The model:

How *Malassezia* Cause Flaking (in susceptible individuals)
Individual susceptibility and commensalism influence investigation:

- Affected vs unaffected comparisons confuse interpretation.
- Cannot assume “unaffected” microbiome is “benign”.
- In non-susceptible: entire causal apparatus could be present – subjects simply cannot respond

Why studies comparing affected to unaffected remain confusing?

Demonstrates need - longitudinal studies in susceptible individuals

---

**Susceptibility – important considerations**

---

*Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare*

Kern Rei Chng, Angeline Su Ling Tay, Chenhao Li, Amanda Hui Qi Ng, Jingjing Wang, Bani Kaur Suri, Sri Arusha Matta, Naomi McGovern, Baptiste Janela, Xuan Fei Colin C. Wong, Yang Yie Sio, Bijin Veonice Au, Andreas Wilm, Paola Florez De Sessions, Thiam Chye Lim, Mark Boon Yang Tang, Florent Ginhoux, John E. Connolly, E. Birgitte Lane, Fook Tim Chew, John E. A. Common & Niranjan Nagarajan

---

*nature microbiology*
PLOS Genetics, 2015

- Sequenced 24 strains representing all known species
- Clarified the phylogeny
- Defined the gene repertoire
- Identified a set of HTGs which define the genus
- Established lipid dependence in all species
- Identified numerous potential therapeutic targets
- Provided a database for all future skin microbiome studies

New genomes:
- Define 3 main groups:
  - furfur clade (A)
  - common human skin residents (B)
  - uncommon outgroup (C)
- Indicate HTGs
  - define the genus ★
  - define the human skin group ★

Malassezia genomics
Reanalysis of NIH data confirms *Malassezia* as dominant skin eukaryotes.
A Forbidden Kingdom: Fungal Microbiome in Skin Health

Agenda:

- Asia and the world
- Skin and fungi – the “Forbidden Kingdom”
  - The skin – an underexplored niche
  - Fungi – a crucial knowledge gap
  - Economic impact
- Example: dandruff and seborrheic dermatitis
  - What it is
  - Learning from Koch
  - Early skin mycobiome
  - Role of microbiome – Malassezia
  - Current Malassezia genomics
- Key next steps for skin
- Summary
Define “Healthy Skin”
  • Most current data only western Caucasians
  • Superseded methods
  • Different locations, disease states

Things to do first, AKA, the hard parts:
  • Define sampling paradigms
  • Define reproducible protocols
  • Define disorder-specific sampling
  • Build in more replicates
  • “neutrality, stability, reproducibility, accuracy, precision”
  • Initiate longitudinal cohorts
How A*STAR IMB and SICS are approaching it:

- Core focus area
- Well placed for Asia – skin health
- Divergent pan-Singapore teams
- Clinical/Basic science collaboration (IMB/NTU/SSC)

Hypothesis:
Intervention in *eukaryotic* microbiome (mycobiome) can improve skin health
Asia Regional Team for Microbiome Investigations in Skin

Maurice van Steensel (IMB), Acne, Sebum, Seb Derm
Mark Tang (NSC), Clinician, Seb Derm, Psoriasis, Acne
Hazel Oon (NSC), Clinician, Seb Derm, Psoriasis, Acne
Kimberly Kline (NUS), bacterial virulence
Andrea Camattari (BTI), fungal engineering

Tom Dawson (IMB)
Malassezia
Fungal virulence, Seb Derm

John Common (IMB)
Atopic Dermatitis
Skin Health

Niranjan Nagarajan (GIS)
Bioinformatics

Fook Tim Chew (NUS), clinical cohort management
Markus Wenk, SLING, (NUS), lipidomics
YY Teo (NUS, GIS), statistics, cohort management
Chong Yap Seng, (SICS), S-PRESTO cohort
Giulia Rancati (IMB), genomics instability, evolution
Summary:

- Asia is the key developing market for middle class consumers
- Skin mycobiome is an exploitable knowledge gap
- Dandruff is one good example
  - We know mycobiome is involved.
  - Suitable economic opportunity
- Individual susceptibility must be included in experimental design
- New genomics data empowers exploration
- We need to sharpen the tools, specific to skin
- A*STAR IMB is well placed and looking for collaboration
Presented at the 4\textsuperscript{th} Microbiome R&D and Business Collaboration Forum: USA.

To find out more, visit:  

\url{www.global-engage.com}