Microbiology of Cheese Rinds

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Microbiology of Cheese Rinds

What is the microbial diversity of cheese rinds?

What are the design principles for cheese rinds?

How can we use this knowledge to improve cheese quality?
Background: surface-ripened cheeses
Background: surface-ripened cheeses
Background: How cheese cheese rinds develop

Production of cheese from milk
- Obtain milk
- Make curds
- Drain curds and form into shape

Salting
- Brine or dry salt wheels

Aging (affinage)
- Age for weeks to years

Raw milk microbes
- Starter Cultures
- Cheese house microbes
- Salt microbes
- Cave microbes
Background: Three styles of microbial cheese rinds

- **Inoculate w/ mold**
  - **Bloomy Rind**

- **Wash with salt**
  - **Washed Rind**

- **Do very little**
  - **Natural Rind**
Background: Three styles of microbial cheese rinds

- **Bloomy Rind**: Inoculate with mold
- **Washed Rind**: Wash with salt
- **Natural Rind**: Do very little

*Background image of cheese varieties.*
Background: basic microbiology of cheese rinds
Background: basic microbiology of cheese rinds

Bacteria vs. Yeast vs. Mold

“tubes” are hyphae of molds

larger circles are yeast

smaller circles are bacteria

400X magnification
Mold  |  yeast  |  bacteria

*Period at end of sentence is 1000 µm*
Background: basic microbiology of cheese rinds

Bacteria vs. Yeast vs. Mold
**Background:** basic microbiology of cheese rinds

**Bacteria vs. Yeast vs. Mold**

<table>
<thead>
<tr>
<th></th>
<th>Size</th>
<th>Cell Structure</th>
<th>Appearance in Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td>0.5 - 5µm</td>
<td>Single - celled</td>
<td>Smooth blobs</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td>3 – 5 µm</td>
<td>Single - celled</td>
<td>Smooth blobs</td>
</tr>
<tr>
<td><strong>Mold</strong></td>
<td>up to 50 µm long</td>
<td>Multicellular</td>
<td>Fuzzy/ diffuse</td>
</tr>
</tbody>
</table>
Background: basic microbiology of cheese rinds

Bacteria

Yeast

Mold
Microbiology of Cheese Rinds

What is the microbial diversity of cheese rinds?

What are the design principles for cheese rinds?

How can we use this knowledge to improve cheese quality?
Large-scale survey of rind microbial diversity

137 different cheeses  362 wheels of cheese

Geography

Rind Type

Bloomy (24)  Washed (52)  Natural (61)

137 different cheeses  362 wheels of cheese

Geography

Rind Type

Bloomy (24)  Washed (52)  Natural (61)
How do we measure cheese rind microbial diversity?

**EXTRACT** DNA from cheese rind sample

**AMPLIFY** “fingerprint” genes

**SEQUENCE** DNA

**MATCH** DNA to sequences in databases

Staphylococcus
Brevibacterium
Brachybacterium
.....
Microbial diversity of 137 different cheese rinds

Rind communities are relatively simple:

- 7 bacterial genera/cheese, 14 dominant genera across samples
- 3 fungal genera/cheese, 10 dominant genera across samples

For closer look see Wolfe et al. 2014, Cell, 158: 422-433
OR
MicrobialFoods.org
Bloomy rind cheeses are dominated by the fungi *Galactomyces* and *Penicillium* and various *Proteobacteria*.
Penicillium camemberti
Galactomyces geotrichum (aka “Geo”)

Yeast like (arthrospore forming)

Filamentous (few arthropores)
Mucor
Rhodotorula and Rhodosporidium

Common on washed rind cheeses

Common contaminant on bloomy rind cheeses

Rhodotorula mucilaginosa

Rhodosporidium
Proteobacteria

Marine Bacteria

*Pseudoalteromonas*

*Vibrio*

*Halomonas*

Starter culture *Hafnia* and other Proteobacteria make many volatile sulfur compounds that we perceive as cooked cabbage.
Proteobacteria

Motile bacteria that love to ‘swim’ around cheese

*Serratia proteamaculans* (bacterium) on *Mucor lanceolatus* (fungus) hyphae
Natural rind cheeses are dominated by *Staphylococcus*, Actinobacteria, notably *Brevibacterium*, the yeast *Debaryomyces*, and various filamentous fungi (especially *Scopulariopsis*).
Staphylococcus in my cheese?!

Not *S. aureus*!

Coagulase-negative *Staphylococcus* (sometimes CNS)

‘Good staph’ that play roles in rind development and aromas

Also very common on salami surface
**Brevibacterium**

Usually thought of as important in washed rinds, but also very common on natural rinds.

Likes higher pH and needs *Staph* and yeast to grow.
Scopulariopsis
Sporendonema casei

Murray’s cave (NY)
Chrysosporium sulfureum
Acremonium
- Mycotoxin production is significant in many of these species

- Aflatoxins are especially dangerous

- Few reports of mycotoxins in cheese, but probably best to be precautionary and keep this mold in low abundance on rinds

- Especially abundant on cheeses that are covered in plant material
Natural rinds are a wonderland for cheese mites!
Cheese mites mold preferences
Cheese mites mold preferences

*Penicillium* pads had highest number of mites
Washed rind cheeses have a mix of both bloomy and natural rind microbes, as well as unique genera: *Vibrio*, *Pseudoalteromonas*, *Corynebacterium*, and *Fusarium*.
Fusarium domesticum

Common on washed rind cheeses

Important for cheese rind stability
Fusarium domesticum

Cardo (goat, UK)

Twig Washed Wheel (goat, USA)

Manigodine (Cow, France)
Marine-associated bacteria are common in cheese rinds

*Pseudoalteromonas, Vibrio, and Halomonas are associated with the marine environment*
Two new rind-associated bacteria

- Nocardiopsis
- Yaniella

Nocardiopsis and Yaniella have never been reported on cheese before.

Yaniella is mostly found on Swiss alpine style cheeses.
‘Wild’ microbes make up a large portion of rind communities

Production of cheese from milk

Obtain milk
Make curds
Drain curds and form into shape

Salting
Brine or dry salt wheels

Aging (Affinage)
Age for weeks to years

Raw milk microbes
Starter Cultures
Cheese house microbes
Salt microbes
Cave microbes
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Cheese rind design principles

migration to environment + ability to tolerate environment + access to resources for growth + interactions between species = rind community

'mild' microbes vs. starter cultures

pH, salt, temperature, moisture

iron, free amino acids

antibiotic production, metabolic cooperation

ability to tolerate environment

access to resources for growth

interactions between species

rind community

Cheese rind design principles

migration to environment + ability to tolerate environment + access to resources for growth + interactions between species = rind community

'mild' microbes vs. starter cultures

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antibiotic production, metabolic cooperation

rind community
Discovering design principles from our cheese survey

Bacteria
- Firmicutes
  - Staphylococcus
  - Brevibacterium
  - Corynebacterium
  - Brachybacterium
  - Arthrobacter
  - Nocardiosis
  - Yaniella
  - Halomonas
c  - Psychrobacter
  - Pseudomonas
  - Pseudoalteromonas
  - Vibrio
  - Hafnia/Serratia
  - Sphingobacterium
  - Taxa < 1% ave. abund.

Fungi
- Saccharomyazales
  - Debaryomyces
  - Galactomyces
  - Candida
- Microascales
  - Scopulariopsis
- Hypocreales
  - Fusarium
  - Acremonium
- Eurotiales
  - Penicillium
  - Aspergillus
- Onygenales
  - Sporodonema
  - Chrysosporium
  - Taxa < 1% ave. abund.
Geography doesn’t matter… but, big caveats

<table>
<thead>
<tr>
<th>Mantel Correlation</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic Distance</td>
<td>0.0353</td>
</tr>
</tbody>
</table>
Environment (moisture) matters

PC1 (20%)  
PC2 (12%)

- Blue: Bloomy rind
- Green: Natural rind
- Orange: Washed rind

$R^2 = 0.348$  
P < 0.0001

% moisture vs. PC1

pH vs. PC1

[NaCl] (M) vs. PC1
Watching the assembly of a cheese rind

These data represent final communities

Can we create lab models that mimic finer-scale patterns of community assembly?

Bayley Hazen Blue natural rind

Cellars at Jasper Hill
0 days
21 days
42 days
63 days
**In vitro communities:** community development over time

![Image showing the development of in vitro communities over 63 days across three batches. The graphs display the percentage of different bacterial and fungal taxa over time.](image-url)
Discovering cheese rind design principles in the lab
Quantifying microbial war and peace
Candida catenulata, a yeast commonly found on the surface of blue cheeses with a natural rind, kills other yeast species.
Microbial Interactions: Killer Yeasts

*Debaryomyces* (DH)
Microbial Interactions: Altered aesthetics of Geo
Microbial Interactions: Case of the missing Geo rind…

Geo alone
Geo + Pseudomonas
Geo + Staph
Microbial Interactions: Pigments from bacterial warfare

**Arthrobacter**
- alone
- + *Penicillium*

*Penicillium*
- alone
Microbial interactions in cheese rinds

- Dispersal facilitation

- Direct chemical interactions

- Indirect chemical interactions

- Alter abiotic environment

- Alter resource availability
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How can we use this knowledge to improve cheese quality?
Identifying the causes of rind defects
Finding novel species/strains to create new cultures

Geotrichum candidum
Industrial cultures limit diversity of cheese flavors

**CHOOZIT™ Cheese Cultures**
A comprehensive range of products for controlled acidification and for emphasizing and diversifying flavor profiles.

**Key benefits**
- Easy to use cultures for direct inoculation
- Provide controlled acidification profiles
- Offer comprehensive range of textures, flavors and colors

<table>
<thead>
<tr>
<th>Product</th>
<th>Benefit</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOOSIT DM lactococcus</td>
<td>Texture and flavor development</td>
<td>All cheese types</td>
</tr>
<tr>
<td>CHOOSIT FLAV</td>
<td>Flavor development</td>
<td>Hard and semi-hard cheese</td>
</tr>
<tr>
<td>CHOOSIT PC</td>
<td>Flavor formation Color and appearance</td>
<td>White mold cheese, soft blue cheese and whey cheese</td>
</tr>
<tr>
<td>CHOOSIT P. Roqueforti</td>
<td>Flavor formation Color and appearance</td>
<td>Blue cheese</td>
</tr>
<tr>
<td>CHOOSIT Geotrichum</td>
<td>Flavor formation Color and appearance</td>
<td>Red smear cheese, soft cheese</td>
</tr>
<tr>
<td>CHOOSIT Yeasts</td>
<td>Flavor formation Color and appearance</td>
<td>Mix and smear cheese, soft cheese</td>
</tr>
<tr>
<td>CHOOSIT Brovibacteria</td>
<td>Colour formation from bright red to cream orange</td>
<td>Red smear cheese</td>
</tr>
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Jasper Hill “Geo”
Small Cheese Makers Invest in a Stinky Science

By LARISSA ZIMBEROFF  FEB. 6, 2017
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Learn more about cheese rind microbiology at...

MicrobialFoods.org

What causes cheese to turn pink?

Various attributes of a cheese, including both flavor and appearance, contribute to the final quality of the product. During the production of some cheeses, microbial processes can cause strange quality defects, often with colorful outcomes. Researchers in University College in Cork, Ireland identified the microbial culprit behind a notorious pink cheese defect. In this Science Digested, Adam Shutes from the Boston Cheese Cellar explains what they found.