Sticking it to the gut: Promoting probiotic biofilm development

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Probiotics

- Live microorganisms that when ingested in adequate amounts confer an advantage to the host - *World Health Organization, 2002*

- Probiotic candidates must be a taxonomically defined microbe or combination of microbes (genus, species and strain level)

- Commonly, effects of probiotics are often strain-specific and cannot be extended to other probiotics of the same genus or species

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em></td>
<td><em>rhamnosus</em></td>
<td>GG</td>
</tr>
</tbody>
</table>
Common Uses for Probiotics

Animal nutrition

• Stabilize intestinal microflora
• Prevent disease

Human health

• Irritable bowel syndrome
• Inflammatory bowel disease
• Antibiotic-related/traveller’s diarrhea
Probiotics - Mechanisms of Action

Probiotics modulate the gut microbiota and/or interact with host intestinal epithelium

Mechanisms of action

1. Enhanced barrier function
2. Increased adhesion to host
3. Inhibition of pathogen adhesion
4. Competitive exclusion of pathogenic microorganisms
5. Production of antimicrobial substances
6. Modulation of host immune response
Making Probiotics Stick

Effects of probiotics are often transient and therefore require large numbers of organisms to be ingested at regular intervals:

Can we prolong the probiotic effect?

Bermudez-Brito et al., Nutrition and Metabolism 2012
Gastrointestinal Biofilms

Bollinger et al., J Thoe Biol. 2007
Bacterial Biofilm Development

Majority of microorganisms found in natural environments are biofilm-associated

Adapted from Mikkelsen et al., J Bacteriol. 2005
Probiotic Biofilms

Determine the culture-specific conditions that favour probiotic biofilm formation

**Hypothesis** - Probiotics form biofilms under culture-specific conditions (e.g. environmental factors such as growth medium, pH and nutrient availability)

Emily Vis, MSc

Dr. Thomas Tompkins
# Probiotic Biofilm Screen

<table>
<thead>
<tr>
<th>Species</th>
<th>Preferred Growth Media</th>
<th>Aeration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus helveticus</em> (R0052)</td>
<td>MRS</td>
<td>Anaerobic</td>
</tr>
<tr>
<td><em>Lactobacillus helveticus</em> (R0389)</td>
<td>MRS</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em> (R0215)</td>
<td>MRS</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> (R1012)</td>
<td>MRS</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> (R0011)</td>
<td>MRS</td>
<td>Anaerobic</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em> (R0071)</td>
<td>RCM</td>
<td>Anaerobic (strict)</td>
</tr>
<tr>
<td><em>Bifidobacterium infantis</em> (R0033)</td>
<td>RCM</td>
<td>Anaerobic (strict)</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em> (R0175)</td>
<td>RCM</td>
<td>Anaerobic (strict)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (R0179)</td>
<td>TSB</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> (R0026)</td>
<td>MRS</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> (R1058)</td>
<td>Elliker</td>
<td>Anaerobic</td>
</tr>
<tr>
<td><em>Pediococcus acidilactici</em> (R1001)</td>
<td>MRS</td>
<td>Anaerobic</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> (R0049)</td>
<td>MRS</td>
<td>Anaerobic</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> (R0343)</td>
<td>MRS</td>
<td>Anaerobic</td>
</tr>
</tbody>
</table>

MRS - Mann, Rogosa and Sharpe; RCM - Reinforced Clostridial Medium; TSB - Tryptic Soy Broth.
Probiotic Biofilm Screen

- 14 strains
- 2 aeration states
- 8 media
- 4 time intervals
- 4 pH values

256 culture conditions \times 14 strains = 3584 total samples (in triplicate)
72 h

Visualization
Imaging *P. acidilactici*

- Hallmark features of bacterial biofilms
Future Directions

- Continue to screen other libraries for biofilm-forming probiotics using ‘gut simulating’ conditions
- Identify colonization factors that promote probiotic retention in the gut
- Characterize probiotic retention in relevant in vitro, animal and human models