Probiotics: Where are we and where are we going?

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University of Alberta
Disclosures

• I have no disclosures
CDDW/CASL Meeting Session: *(Faculty Template Slide)*

CanMEDS Roles Covered in this Session:

<table>
<thead>
<tr>
<th>Role</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical Expert</td>
<td>(as <em>Medical Experts</em>, physicians integrate all of the CanMEDS Roles, applying medical knowledge, clinical skills, and professional attitudes in their provision of patient-centered care. <em>Medical Expert</em> is the central physician Role in the CanMEDS framework.)</td>
</tr>
<tr>
<td>Communicator</td>
<td>(as Communicators, physicians effectively facilitate the doctor-patient relationship and the dynamic exchanges that occur before, during, and after the medical encounter.)</td>
</tr>
<tr>
<td>Collaborator</td>
<td>(as <em>Collaborators</em>, physicians effectively work within a healthcare team to achieve optimal patient care.)</td>
</tr>
<tr>
<td>Manager</td>
<td>(as <em>Managers</em>, physicians are integral participants in healthcare organizations, organizing sustainable practices, making decisions about allocating resources, and contributing to the effectiveness of the healthcare system.)</td>
</tr>
<tr>
<td>Health Advocate</td>
<td>(as <em>Health Advocates</em>, physicians responsibly use their expertise and influence to advance the health and well-being of individual patients, communities, and populations.)</td>
</tr>
<tr>
<td>Scholar</td>
<td>(as <em>Scholars</em>, physicians demonstrate a lifelong commitment to reflective learning, as well as the creation, dissemination, application and translation of medical knowledge.)</td>
</tr>
<tr>
<td>Professional</td>
<td>(as <em>Professionals</em>, physicians are committed to the health and well-being of individuals and society through ethical practice, profession-led regulation, and high personal standards of behaviour.)</td>
</tr>
</tbody>
</table>
Learning Objectives

At the end of this session participants will be able to:

• Describe new advances in the field of microbiome and probiotics research.

• Recognize some of the many benefits that commensal microorganisms provide to their hosts.
What is a Probiotic?

- Probiotic comes from the Greek “pro bios” which means “for life”
- 1970’s …. term introduced to describe microbial feed supplements for animals
- 2014 …live microorganisms that, when administered in adequate amounts, confer a health benefit on the host

# Common Probiotics

<table>
<thead>
<tr>
<th>Lactobacillus (Firmicute)</th>
<th>Bifidobacteria (Actinobacteria)</th>
<th>Streptococcus (Firmicute)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td><em>B. bifidum</em></td>
<td><em>S. thermophilus</em></td>
<td><em>E. Coli Nissle 1917</em></td>
</tr>
<tr>
<td><em>L. casei GG</em></td>
<td><em>B. infantis</em></td>
<td><em>S. lactis</em></td>
<td><em>Serotype O6:K5:H1</em></td>
</tr>
<tr>
<td><em>L. rhamnosom</em></td>
<td><em>B. longum</em></td>
<td><em>S. salivarius</em></td>
<td><em>Saccharomyces boulardi</em></td>
</tr>
<tr>
<td><em>L. salavarius</em></td>
<td><em>B. thermophilum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. delbruecki</em></td>
<td><em>B. adolescents</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. reuteri</em></td>
<td><em>B. Lactis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td><em>B. breve</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. bulgaricus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Names of Probiotics

**Genus**

Lactobacillus plantarum 299v

**Species**

Bifidobacterium animalis lactis DN-173 010

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Probiotic Strain</th>
<th>Commercial Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activia</td>
<td><em>Bifidobacterium (animalis) lactis</em> DN-173 010</td>
<td><em>Bifidus regularis</em></td>
</tr>
</tbody>
</table>
Global sales of probiotic products steadily increasing worldwide 2010-2015
Some examples of food with probiotics….

- **Bigelow Lemon Ginger Tea**: 10 billion/100 ml of *L. casei*.
- **Activia**: 1 billion/100 gm of *B. lactis*.
- **DanActive**: 10 billion/100 ml of *L. casei*.
- **Seedlicious**: Whole grain bread with probiotics.
Some Probiotic Products and Supplements

1 billion CFU

*Bifidobacterium infantis 35624.*

30 billion CFU

*S. Thermophilus KB19*
*L. Acidophilus KB27*
*B. Longum KN31*

450 billion CFU

*B. breve*
*B. longum*
*B. infantis*
*L. acidophilus*
*L. plantarum*
*L. paracasei*
*L. bulgaricus*
*S. thermophilus*
MECHANISMS OF ACTION
Probiotics

Affects barrier function, membrane permeability, mucin production, HSP induction, IgA and β defensin production

Epithelial Cell Layer

β defensins

Pathogens

Dendritic Cell

plgR

Influences signaling pathways

Macrophage

NF-κB

MAPKs

STATs

Enteric Nervous System

Changes in motility and pain perception

Naive T Cell

TH1

TH2

Treg

Influences antibody production

Proliferation/survival, changes in cytokine production

Enlarged IECs

Probiotics

Proliferation/survival, changes in cytokine production
Distribution of mechanisms among probiotics

**Strain-specific effects**
- Neurological effects
- Immunological effects
- Endocrinological effects
- Production of specific bioactives

**Frequent Species-level effects**
- Vitamin synthesis
- Direct antagonism
- Gut barrier reinforcement
- Bile salt metabolism
- Enzymatic activity
- Neutralization of carcinogens

**Widespread**
- Colonization resistance
- SCFA production
- Regulation of intestinal transit
- Stabilization of perturbed microbiota
- Increase turnover of enterocytes
- Competitive exclusion of pathogens

CLINICAL APPLICATIONS
DIARRHEAL DISEASES
Probiotics effective for infectious diarrhea

- Meta-analysis based on 23 controlled studies with 1917 patients
  - mean duration by 30.5 hrs
  - risk of diarrhea (relative risk 0.66)
- especially effective for rotavirus diarrhea
- Dose dependent  $>10^{10}$ CFU
- Strain dependent
  - *Lactobacillus GG*, *Lactobacillus reuteri* DSM 17938

Probiotics are effective for preventing AAD

Probiotics would prevent 84 AAD episodes per 1000 patients treated
Probiotics are effective in preventing *C. Difficile* associated diarrhea

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental Events</th>
<th>Experimental Total</th>
<th>Control Events</th>
<th>Control Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arvola 1999</td>
<td>1</td>
<td>61</td>
<td>1</td>
<td>58</td>
<td>1.6%</td>
<td>0.95 [0.06, 14.85]</td>
<td></td>
</tr>
<tr>
<td>Beausoleil 2007</td>
<td>1</td>
<td>44</td>
<td>7</td>
<td>45</td>
<td>2.9%</td>
<td>0.15 [0.02, 1.14]</td>
<td></td>
</tr>
<tr>
<td>Bravo 2008</td>
<td>0</td>
<td>41</td>
<td>0</td>
<td>45</td>
<td>Not estimable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can 2006</td>
<td>0</td>
<td>73</td>
<td>2</td>
<td>78</td>
<td>1.3%</td>
<td>0.21 [0.01, 4.37]</td>
<td></td>
</tr>
<tr>
<td>Cindoruk 2007</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>62</td>
<td>Not estimable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duman 2005</td>
<td>0</td>
<td>196</td>
<td>1</td>
<td>180</td>
<td>1.2%</td>
<td>0.31 [0.01, 7.47]</td>
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</tr>
<tr>
<td>Gao 2010</td>
<td>9</td>
<td>171</td>
<td>20</td>
<td>84</td>
<td>21.9%</td>
<td>0.22 [0.11, 0.46]</td>
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<tr>
<td>Hickson 2007</td>
<td>0</td>
<td>57</td>
<td>9</td>
<td>56</td>
<td>1.5%</td>
<td>0.05 [0.00, 0.87]</td>
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<tr>
<td>Kotowska 2005</td>
<td>3</td>
<td>119</td>
<td>10</td>
<td>127</td>
<td>7.5%</td>
<td>0.32 [0.09, 1.14]</td>
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<tr>
<td>Lonnermark 2010</td>
<td>1</td>
<td>80</td>
<td>0</td>
<td>83</td>
<td>1.2%</td>
<td>3.11 [0.13, 75.26]</td>
<td></td>
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<tr>
<td>McFarland 1995</td>
<td>3</td>
<td>97</td>
<td>4</td>
<td>96</td>
<td>5.6%</td>
<td>0.74 [0.17, 3.23]</td>
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<tr>
<td>Miller 2008a</td>
<td>4</td>
<td>95</td>
<td>7</td>
<td>94</td>
<td>8.4%</td>
<td>0.57 [0.17, 1.87]</td>
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<tr>
<td>Miller 2008b</td>
<td>2</td>
<td>157</td>
<td>0</td>
<td>159</td>
<td>1.3%</td>
<td>5.06 [0.25, 104.63]</td>
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<tr>
<td>Plummer 2004</td>
<td>2</td>
<td>69</td>
<td>5</td>
<td>69</td>
<td>4.7%</td>
<td>0.40 [0.08, 1.99]</td>
<td></td>
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<tr>
<td>Pozzoni 2012</td>
<td>3</td>
<td>106</td>
<td>2</td>
<td>98</td>
<td>3.9%</td>
<td>1.39 [0.24, 8.13]</td>
<td></td>
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<tr>
<td>Psaaradellis 2010</td>
<td>1</td>
<td>216</td>
<td>4</td>
<td>221</td>
<td>2.5%</td>
<td>0.26 [0.03, 2.27]</td>
<td></td>
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<tr>
<td>Rafiq 2007</td>
<td>5</td>
<td>45</td>
<td>22</td>
<td>55</td>
<td>15.3%</td>
<td>0.28 [0.11, 0.67]</td>
<td></td>
</tr>
<tr>
<td>Ruszczynski 2008</td>
<td>3</td>
<td>120</td>
<td>7</td>
<td>120</td>
<td>6.8%</td>
<td>0.43 [0.11, 1.62]</td>
<td></td>
</tr>
<tr>
<td>Safdar 2008</td>
<td>0</td>
<td>23</td>
<td>1</td>
<td>17</td>
<td>1.2%</td>
<td>0.25 [0.01, 5.79]</td>
<td></td>
</tr>
<tr>
<td>Selinger 2011</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>62</td>
<td>Not estimable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surawicz 1989</td>
<td>3</td>
<td>116</td>
<td>5</td>
<td>64</td>
<td>6.2%</td>
<td>0.33 [0.08, 1.34]</td>
<td></td>
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<tr>
<td>Thomas 2001</td>
<td>2</td>
<td>133</td>
<td>3</td>
<td>134</td>
<td>3.8%</td>
<td>0.67 [0.11, 3.96]</td>
<td></td>
</tr>
<tr>
<td>Wenus 2008</td>
<td>0</td>
<td>34</td>
<td>1</td>
<td>29</td>
<td>1.2%</td>
<td>0.29 [0.01, 6.76]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>2177</td>
<td>2036</td>
<td>100.0%</td>
<td></td>
<td>0.36 [0.26, 0.51]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>43</td>
<td>111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.00; \chi^2 = 14.33, df = 19 (P = 0.76); I^2 = 0$

Test for overall effect: $Z = 5.73 (P < 0.00001)$

Probiotic prophylaxis would prevent 35 CDAD episodes per 1000 patients treated
Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhea and *Clostridium difficile* diarrhea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial

Stephen J Allen, Kathie Wareham, Duolao Wang, Caroline Bradley, Hayley Hutchings, Wyn Harris, Anjan Dhar, Helga Brown, Alwyn Foden, Michael B Gravenor, Dietrich Mack

- Multicenter trial with N=2941
- Probiotic treatment did not reduce the incidence or duration of antibiotic-associated diarrhea or *C. difficile* diarrhea
WHAT ABOUT INFLAMMATORY BOWEL DISEASE?
Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease

• 14 RCT in Crohn’s disease
  – *S. boulardii*, VSL#3, LGG, *L. johnsonii*, *E.coli*
  – Still no evidence to support use

• 21 RCT in Ulcerative colitis
  – VSL#3, *Bifidobacterium longum*, *E. coli* Nissle, LGG, *L. acidophils*, *B. breve*
  – Trends seen in improvement in disease/adjunctive therapy

• 5 RCT in pouchitis
  – VSL#3, *Lactobacillus GG*
  – 4 out of 5 trials found probiotics effective in maintenance of remission

Gut inflammation generates oxidation products that allow for the growth of Enterobacteriaceae – a problem for probiotic strains?

Winter et al. EMBO 2013
WHAT ABOUT IRRITABLE BOWEL SYNDROME
35 RCTs of probiotics in IBS with 3452 patients

Various combinations of Lactobacillus sp, Bifidobacterium, E. coli, Streptococcus, Saccharomyces have been examined

Significant beneficial effect of probiotics in reducing global symptoms and/or abdominal pain

NNT of 7

Combinations (VSL#3), *L. plantarum*, *L. reuteri* DSM 17938, and *B. infantis* 35624 had the most evidence
PROBIOTICS AND METABOLIC SYNDROME/OBESITY
CAN PROBIOTICS BE USED TO HELP INDIVIDUALS LOSE WEIGHT?

Maybe..............but be careful in strain selection
Lactobacillus sp associated with weight gain

**L. Acidophilus**

**L. fermentum**

**L. ingluvier**
Lactobacillus sp associated with weight loss

### L. Plantarum

<table>
<thead>
<tr>
<th>Study</th>
<th>Weight</th>
<th>SMD (95% CI)</th>
<th>Weight loss</th>
<th>Weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus plantarum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlsson 2011</td>
<td>27.5%</td>
<td>-0.82 [-1.79, 0.15]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee 2007</td>
<td>23.1%</td>
<td>-2.08 [-3.47, -0.70]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takemura 2010</td>
<td>25.2%</td>
<td>0.08 [-1.11, 1.27]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xie 2011</td>
<td>24.3%</td>
<td>-2.65 [-3.91, -1.38]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>100.0%</td>
<td>-1.33 [-2.50, -0.16]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: P = 0.009; I² = 74%</td>
<td></td>
<td>Test for overall effect: Z = 2.22 (P = 0.03)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### L. Gasseri

<table>
<thead>
<tr>
<th>Study</th>
<th>Weight</th>
<th>SMD (95% CI)</th>
<th>Weight loss</th>
<th>Weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harrad 2009</td>
<td>20.8%</td>
<td>0.00 [-0.96, 0.96]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kadooka 2010 (humans)</td>
<td>57.8%</td>
<td>-0.72 [-1.16, -0.29]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kang 2010</td>
<td>21.4%</td>
<td>-1.15 [-2.12, -0.19]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>100.0%</td>
<td>-0.67 [-1.17, -0.16]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: P = 0.24; I² = 29%</td>
<td></td>
<td>Test for overall effect: Z = 2.60 (P = 0.009)</td>
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</tr>
</tbody>
</table>
So why are only modest effects seen in human studies?
Diet of the host can have a large effect on response to probiotics

Effects of *Lactobacillus helveticus* on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome

Christina L. Ohland, Lisa Kish, Haley Bell, Aducio Thiesen, Naomi Hotte, Evelina Pankiv, Karen L. Madsen

Diet alters probiotic *Lactobacillus* persistence and function in the intestine
Probiotics can alter microbial composition quite differently depending upon the diet.

**A**

Chow

- **Firmicutes**
- **Actinobacteria**
- **Bacteroides**
- **Proteobacteria**

**B**

High Fat

- **Firmicutes**
- **Bacteroides**
- **Lactobacilli**
- **Bifidobacteria**

**Graph Legend**

- Blue: Bacteroides
- Green: Firmicutes
- Red: Proteobacteria
- Yellow: Actinobacteria

**Notes**

- **Vehicle**
- **VSL#3**

**Signs**

- *: Statistical significance
- #: Not statistically significant
Effects of probiotic treatment strongly influenced by existing intestinal microbial ecosystem!

Modulation of Fecal Clostridiales Bacteria and Butyrate by Probiotic Intervention with *Lactobacillus paracasei* DG Varies among Healthy Adults¹–³

Chiara Ferrario,⁴ Valentina Taverniti,⁴ Christian Milani,⁵ Walter Fiore,⁶ Monica Laureati,⁴ Ivano De Noni,⁴ Milda Stuknyte,⁴ Bessem Chouaia,⁴ Patrizia Riso,⁴ and Simone Guglielmetti⁴⁵

- Randomized, double blind, placebo-controlled crossover trial
- 34 healthy adults consumed daily *Lactobacillus paracasei* DG
- Fecal microbiota analysis

- Subjects with high initial butyrate levels saw a *reduction* in butyrate levels
- Subjects with low initial values saw an *increase* in butyrate levels
Different probiotic strains can have competitive or inhibitory effects on each other

**Lactobacillus acidophilus** NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study

T. Ringel-Kulka*, J. R. Goldsmith†, I. M. Carroll†, S. P. Barros‡, O. Palsson†, C. Jobin†,§ & Y. Ringel†

- **Lactobacillus acidophilus** NCFM alone induced colonic mu-opiod receptor and cannabinoid receptor in women with mild-moderate abdominal pain

- When taken in combination with **Bifidobacterium lactis** Bi07, there was no induction of receptors
Host adaptation to probiotic effects

Would pulsing work better than continuous treatment?
Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults

- Acute randomized controlled cross-over study
- Randomized, double-blind placebo-controlled parallel designed study consuming 10 gm daily for 24 weeks

- **Acute study** showed that propionate increased release of PYY and GLP-1 and reduced energy intake

- **Long-term study** showed no change in PYY or GLP-1 release or energy intake

- **Host adaptation – desensitization of specific receptors?**
ARE WE USING THE WRONG STRAINS?
Why focus on Lactobacillus and Bifidobacteria?

• Lactobacillus a component of fermented foods – easy to isolate and culture
• Bifidobacteria is found in high concentrations in infants
• *Neither one is a high colonizer of the adult human gut*

• What about other gut commensals?
WHERE ARE WE GOING?
Publications 2000-2014

Probiotics

Microbiome
Ecosystem Effects

- Lactate producers (e.g., lactobacilli, bifidobacteria)
- Methanogens (e.g., methanogenic archaea)
- Mucin degraders (e.g., Bacteroidetes)
- Short chain fatty acids producers (e.g., Clostridium)

Fecal transplant (100s of strains, undefined composition)
Consortium (defined composition of more than one strain, which together, perform a function of interest)
Single strain (one strain, pure isolate)
Bioactive (molecule produced by strain that mediates effect on host)

Specificity

BUGS IN THE NEWS
Review Article

Association between *Faecalibacterium prausnitzii* Reduction and Inflammatory Bowel Disease: A Meta-Analysis and Systematic Review of the Literature

Yuan Cao, Jun Shen, and Zhihua Ran

Commentary: is *Faecalibacterium prausnitzii* a potential treatment for maintaining remission in ulcerative colitis?

Y. H. Siaw & A. Hart

St Mark’s Hospital, London, UK.
E-mail: yihan@doctors.org.uk

doi:10.1111/apt.12404
LETTER

doi:10.1038/nature13828

Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile


• “Identify a “probiotic” candidate that can correct a clinically relevant microbiome deficiency”

• Clostridium scindens, a bile acid 7α-dehydroxylating intestinal microbe, increases resistance to infection
Akkermansia muciniphila and obesity

- *A. muciniphila* is a normal commensal that digests mucus

- Obese people (and fat mice) and those with type 2 diabetes have much lower levels

- Mice on a high fat diet had less *A. muciniphila* and when the levels were restored the mice lost weight, had reduced insulin resistance, and reduced metabolic disorders

- Could *A. muciniphila* be used as a probiotic to treat diabetes or help people lose weight?
My bacteria made me do it…

- Microbes in the gut can alter behaviour
  - Norepinephrine
  - Vagus nerve stimulation
  - Release of active biomolecules

- Can probiotic strains be identified that could be used to treat psychiatric disorders?
Questions and Confounding Factors in Human Studies

• Diet of host

• Existing microbiota and luminal environment

• Adaptation by host

• Combinations don’t work together

• Wrong strain – match desired outcome with probiotic
Conclusions

• Effects of current probiotics are modest and best results seen when used as adjunctive therapy

• Microbiome research will, in the near future, provide answers to ongoing debates about *which strains to use* – *timing of treatment* – *and concentrations and duration of treatment*
ANY QUESTIONS?