Session: Evaluating next generation ingredients to support digestive health
Wednesday 23\textsuperscript{rd} November 2016

Next generation of probiotics

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Outline

- Emergence of Probiotics & ‘Current Generation’ of Probiotics
- Advances in Scientific Technologies
  - Knowledge of gut microbiota and microbiome
  - Increased interest in gut microbiota and probiotics/therapies
- Next Generation of Probiotics
  - New applications/therapeutic targets for probiotics?
  - New strains of bacteria?
  - Moving away from strain-specific?
  - Do we need to consider more than just adding bacteria?

Raising more questions than answers about the next generation of probiotics.
Emergence of the Probiotic Concept

- E. Metchnikoff in 1900s
- Postulated that supplementation of diet with lactic acid bacteria, an early probiotic intervention, has health benefits including promoting longevity.
- "The dependence of the intestinal microbes on food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes"
Current Generation of Probiotics

- Definition of Probiotic: Live microorganisms that, when administered in adequate amounts confer a health benefit on the host. ¹⁻²

- The active bacteria are mostly *Lactobacillus* and *Bifidobacteria*.

Scientific Advancement

- Advances in sequencing and bioinformatics technologies has fuelled scientific progress, and increased rationale and opportunities for probiotic concepts
Human Microbiome

- Microbiome: *Totality of microbes (microbiota) and their genomes in and on the human body*

- Complex microbial communities
- Large diversity across body sites

Cho and Blaser (2012) *Nat Rev Genet* 13(4); 260-270
The Human Gut Microbiome

Key Findings: high complexity and (interpersonal) diversity

Numbers

- In the population
  - 1100 species
  - 3.3 mil → 10 mil genes

- In each individual
  - At least 160 species
  - Approx 540k genes – we share almost 40% of these genes with any other person

No longer just of scientific interest..
Everyone can get their microbiome profiled..
‘Faecal transplants’ for *C. difficile* have been boosting the enthusiasm

- Gut microbiota transfer via duodenal infusion of donor faeces for recurrent *Clostridium difficile*

### Rates of Cure without Relapse for Recurrent C. diff Infection

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Percentage Cured without Relapse</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Infusion of Donor Feces (N=16)</td>
<td>81.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infusion of Donor Feces Overall (N=16)</td>
<td>93.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vancomycin (N=13)</td>
<td>30.8</td>
<td>0.008</td>
</tr>
<tr>
<td>Vancomycin with Bowel Lavage (N=13)</td>
<td>23.1</td>
<td>0.003</td>
</tr>
</tbody>
</table>

### Microbiota ‘diversity’ in patients before and after infusion of donor faeces, as compared with diversity in healthy donors

‘Faecal transplants’ for *C. difficile* have been boosting the enthusiasm

- Gut microbiota transfer via duodenal infusion of donor faeces for recurrent *Clostridium difficile*

Rates of Cure without Relapse for Recurrent *C. diff* Infection

Microbiota ‘diversity’ in patients before and after infusion of donor faeces, as compared with diversity in healthy donors

Can this be generalised?

Evidence is only currently there to support use in *C. difficile*

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Raising more questions than answers about the next generation of probiotics..
A double-blind placebo-controlled trial: 3-week intervention with either a probiotic (*Lactobacillus casei* Shirota) containing milk drink or a placebo

- N=132 participants, mean age of 61.8 yrs

- Consumption of probiotic-containing yoghurt improved the mood of those whose mood was initially poor.

Effects of Probiotics on Depression: Age Related

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>probiotic</th>
<th>Placebo</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age under 60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akkasheh 2015</td>
<td>-5.7</td>
<td>4</td>
<td>-0.73 [-1.37, -0.09]</td>
</tr>
<tr>
<td>Messaoudi 2011</td>
<td>3.5</td>
<td>4</td>
<td>-0.15 [-0.68, 0.38]</td>
</tr>
<tr>
<td>Mohammadi 2015</td>
<td>9.4</td>
<td>20</td>
<td>-0.60 [-1.20, 0.01]</td>
</tr>
<tr>
<td>Steenbergen 2015</td>
<td>7.25</td>
<td>20</td>
<td>-0.34 [-0.97, 0.28]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>81</td>
<td>89</td>
<td>-0.43 [-0.72, -0.13]</td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 2.28, df = 3 (P = 0.52); I² = 0%
Test for overall effect: Z = 2.60 (P = 0.005)

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<tr>
<td>Age over 65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shinkai 2013</td>
<td>50</td>
<td>52</td>
<td>-0.18 [-0.47, 0.11]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>92</td>
<td>93</td>
<td>-0.18 [-0.47, 0.11]</td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: Z = 1.22 (P = 0.22)

Total (95% CI) 183

Heterogeneity: Chi² = 3.63, df = 4 (P = 0.46); I² = 0%
Test for overall effect: Z = 2.83 (P = 0.005)
Test for subgroups differences: Chi² = 1.35. df = 1 (P = 0.25). I² = 25.7%

Cognitive Function in Alzheimer’s Disease

- A 12-week RCT in 60 patients with Alzheimer’s disease (control vs probiotic, n=30 in each group)
- Probiotic intervention: 200 ml/day probiotic milk containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* (2 × 10⁹ CFU/g for each)
- Result: the probiotic treated patients showed a significant improvement in the mini-mental state examination (MMSE) score

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Probiotic group</th>
<th>Difference between the two groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End-of-trial</td>
<td>Baseline</td>
</tr>
<tr>
<td>MMSE (score out of 30)</td>
<td>8.47 ± 1.10</td>
<td>8.00 ± 1.08</td>
<td>8.67 ± 1.44</td>
</tr>
</tbody>
</table>

Next-Generation of Probiotics: Novel Strains

- The genera of microorganisms used in current probiotics (\textit{Lactobacillus}, \textit{Bifidobacterium}, \textit{Saccharomyces} or \textit{Bacillus}) are not the dominant genera in the intestinal microbiota in adults.

- This observation, combined with the increasing knowledge of the human microbiome and its association with health, suggests that a large number of potential novel probiotic candidates can be isolated from the dominant members of the adult microbiota, such as \textit{Faecalibacterium prausnitzii} and \textit{Akkermansia muciniphila}. \textsuperscript{1,2}

- ‘\textit{Other likely next generation probiotics are genetically modified microbes targeting specific therapeutic capabilities and defined consortia designed to cure a disease or restore a depleted microbiota. Targets will include microbiota-impacted physiological functions extending beyond the gut.’} \textsuperscript{1}

\textsuperscript{1} Sanders ME ‘Next Generation of Probiotics’ \textit{California Diary Research Foundation} 5 Jul 2016. Available at \url{http://cdrf.org/2016/07/05/next-generation-probiotics/}

\textsuperscript{2} Dao et al. 2016 \textit{Gut} 65(3): 426-36
Moving Away from Strain-Specific Probiotics

- Over the years, the concept of strain-specific effects of probiotics has been considered unquestionable.

- The consideration of the range of digestive benefits observed among a broad cross-section of well-studied *Lactobacillus* and *Bifidobacterium* species has led to the concept that some core benefits may be expected from adequate doses of such species.

- To the extent that similar mechanisms function among different strains, effects may be assigned to a broader class of microbes (a subspecies, a species or genus, or a group of microbes with specific genes) rather than just a single strain.

Moving Away from Strain-Specific Probiotics

- A meta-analysis\(^1\), which included a broad range of probiotic strains, concluded that, in general, probiotics are beneficial in the treatment and prevention of gastrointestinal diseases.

- A Cochrane review\(^2\) on the use of probiotics in the prevention of necrotising enterocolitis in preterm infants concluded that enteral supplementation of probiotics prevents severe NEC and all cause mortality in preterm infants.

- Consistent benefits despite the differences in strains used in each of the studies – this suggests that some properties are shared between strains.

- Mary Ellen Sanders at Nature Café: The role of microbiota in health and disease. (Japan, 1/11/2016)

  ‘As knowledge develops we will be able to characterise different strains into a class of probiotics that have certain benefits, and even be able to predict functions with reasonable certainty in untested strains.’

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Is simply adding microbes enough?

- Are the microbes not there because conditions were unfavourable?
- Does the next generation of probiotics also need to consider optimising microbiome-host symbiotic relations?
- Or do we need to eat ‘personalised’ diets to optimise health based on our gut microbiota?

Dietary intake, drugs, ingredients impacting pH, inflammation, bile salts

Favourable conditions

Supplementing missing microbes

Adding substrate

Probiotics (next generation)
Therapeutic microbes
Prebiotics (Personalised) diets
Personalised Nutrition – Microbiome & Glycaemic Response

- 800 person cohort - high interpersonal variability in post-meal glucose observed
- Devised an algorithm using personal and microbiome features to accurately predict glucose response
- Validated in an independent 100-person cohort - prediction was accurate and superior to common practice
- Short-term personalised dietary interventions successfully lower post-meal glucose in an RCT

Zeevi et al. 2015 Cell 163, 1079-1094
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Thank you for listening!
Any questions?

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