PREBIOTIC MECHANISMS OF ACTION

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Institute of Medicine Workshop on the Human Microbiome, Diet, and Health
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Ingredients/novel compounds being examined for activities other than providing nutrients
- Health promotion
- Disease reduction

Area of study of great practical importance to the food and feed industries, and to suppliers to these industries

Non-digestible oligosaccharides (NDOs)
- Nutritional attributes:
  - Low caloric value, enhance mineral absorption
- Health related attributes:
  - May lower risk of infections and diarrhea; may modulate immune system; microbiota modulation (prebiotic activity)
Many NDOs have ability to alter the composition of the colonic microbiota in a positive manner:
- ↑ bifidobacteria and lactobacilli populations
- ↓ pathogenic populations

Concept of prebiotics:
- Introduced in 1995 (Gibson and Roberfroid)
- Attracted much academic and private sector attention
  - Positive effects on health outcomes
BACKGROUND

Definition of a prebiotic

1. Resistant to gastric acidity, to enzymatic hydrolysis, and to gastrointestinal absorption (i.e., not hydrolytically digestible)

2. Fermented by cecal/colonic microflora

3. Selectively stimulates growth and/or activity of those bacteria that contribute to colonic and host health

Gibson et al., 2004
What are the major dietary sources of prebiotics?
PREBIOTICS

- Well established
  - Fructans
    - Chicory root extract
    - Inulin
    - Oligofructose
    - Short-chain fructooligosaccharides (DP 3-5)
  - Galactooligosaccharides
  - Lactulose
POTENTIAL PREBIOTIC CANDIDATES

- Soybean oligosaccharides
- Glucoooligosaccharides
- Cyclodextrins
- Gentioooligosaccharides
- Oligodextrans
- Glucuronic acid
- Pectic oligosaccharides
- Isomaltoooligosaccharides
- Lactosucrose
- Xyloooligosaccharides
- Human milk oligosaccharides
- Mannanooligosaccharides (Yeast cell wall)
- Lactose
- Resistant starch and derivatives
- Oligosaccharides from melobiose
- N-Acetylchitooligosaccharides
- Polydextrose
- Sugar alcohols
- Konjac glucomannan
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WHY CATEGORIZED AS “POTENTIAL” PREBIOTIC CANDIDATES?

- Do not meet all of the specifications of definition to date
  - Selective stimulation of bacterial growth may be limited
  - Research is not yet complete
Adapted from Mussatto and Mancilha, 2007.
How do prebiotics modify composition of the gut microbiota?
PREBIOTICS

- Depending on type and dietary concentration provided, potential effects on:
  - Gastric emptying
  - Intestinal transit rate
  - Nutrient digestibility
  - Fecal bulking/frequency of defecation
  - SCFA production
  - Intestinal morphology
  - Gut immune modulation
  - GI microbiota
IMPACT OF GalOS ON THE INTESTINAL MICROBIOTA OF HEALTHY ADULTS

- 18 healthy human volunteers (19-50 years old)
- Study conducted over a 16 week period

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Testing</th>
<th>Washout</th>
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<tbody>
<tr>
<td>2 weeks</td>
<td>3 weeks/treatment</td>
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- Treatments – chocolate-flavored chewable candies containing 0.0, 2.5, 5.0, or 10.0 g GalOS/d
- Fresh fecal samples collected within 1 h of defecation

Davis et al., 2010
### Enumeration Through Culturing

#### Log 10 CFU/g feces (Mean ± SD)

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Baseline</th>
<th>0.0 g</th>
<th>2.5 g</th>
<th>5.0 g</th>
<th>10.0 g</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose-fermenting enterobacteria</td>
<td>5.60</td>
<td>5.68</td>
<td>5.64</td>
<td>5.18</td>
<td>5.59</td>
<td>5.78</td>
</tr>
<tr>
<td>Enterococci</td>
<td>5.02</td>
<td>5.02</td>
<td>4.95</td>
<td>4.67</td>
<td>4.70</td>
<td>5.13</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>10.19</td>
<td>10.19</td>
<td>10.11</td>
<td>10.24</td>
<td>10.35*§</td>
<td>10.19</td>
</tr>
</tbody>
</table>

Significantly different vs 0.0 g: * (p < 0.05).
Significantly different vs 2.5 g: § (p < 0.05).
BIFIDOGENIC EFFECT OF GalOS AS DETERMINED BY qRT-PCR

For all 18 subjects

Dosage of GalOS

Davis et al., 2010

For the 9 responders

Dosage of GalOS
IMPACT OF GalOS ON THE INTESTINAL MICROBIOTA

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- Pyrosequencing of V1-V3 region of 16S rDNA

Davis et al., 2011
RESULTS

- Consumption of GalOS did not alter bacterial diversity of fecal microbes

- Predominant phyla
  - Firmicutes 64%
  - Bacteroidetes 28%
  - Actinobacteria 3%
  - Verrucomicrobia 1%
  - Proteobacteria 1%
RESULTS

- **Predominant families**
  - Lachnospiraceae 31%
  - Ruminococcaceae 18%
  - Bacteroidaceae 12%
  - Bifidobacteriaceae 2%

- **Most common genera**
  - Bacteroides 12.2%
  - Faecalibacterium 7.7%
  - Blautia 7.4%
  - Ruminococcus 3.7%
  - Roseburia 2.2%
  - Bifidobacterium 1.5%
# Proportion of Bacterial Taxa Affected by GalOS Consumption

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10.0 g§</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actinobacteria (Phyla)</strong></td>
<td>2.52</td>
<td>7.19</td>
</tr>
<tr>
<td>Bifidobacteriaceae (Family)</td>
<td>1.56</td>
<td>6.14*</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> (Genus)</td>
<td>1.28</td>
<td>5.20*</td>
</tr>
<tr>
<td>B. adolescentis</td>
<td>0.37</td>
<td>1.03</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp I</td>
<td>0.15</td>
<td>0.77*</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp II</td>
<td>0.46</td>
<td>2.00*</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp III</td>
<td>0.62</td>
<td>2.50*</td>
</tr>
<tr>
<td>B. longum</td>
<td>0.09</td>
<td>0.33</td>
</tr>
<tr>
<td>B. catenulatum</td>
<td>0.15</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* Significantly different from 2.5 g GalOS treatment (P < 0.05).
§ Significantly different from 0 g GalOS treatment (P < 0.05).

Davis et al., 2011
Responses vary among individuals
EFFECT OF RESISTANT STARCH ON THE COMPOSITION OF FECAL MICROBIOTA

- 10 healthy human volunteers (23-38 years old)
- Three types of crackers (33 g of RS per 100 g of crackers) containing:
  - RS2 (Hi-Maize 260)
  - RS4 (Fibersym® RW)-chemically modified phosphorylated cross-linked type 4 RS from wheat starch
  - Native wheat starch
- Subjects consumed 33 g of RS per day
- Double-blind, crossover study

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Treatment 1</td>
<td>Washout</td>
<td>Treatment 2</td>
<td>Washout</td>
<td>Treatment 3</td>
<td>Washout</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

Martinez et al., 2010
<table>
<thead>
<tr>
<th>Phylum</th>
<th>Baseline</th>
<th>Control</th>
<th>RS2</th>
<th>RS4</th>
<th>Washout</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Firmicutes</strong></td>
<td>78.2</td>
<td>79.6</td>
<td>75.9</td>
<td>65.6</td>
<td>78.1</td>
<td>0.0010</td>
</tr>
<tr>
<td><strong>Bacteroidetes</strong></td>
<td>12.7</td>
<td>10.4</td>
<td>10.1</td>
<td>16.3</td>
<td>12.2</td>
<td>0.0028</td>
</tr>
<tr>
<td><strong>Actinobacteria</strong></td>
<td>3.1</td>
<td>4.1</td>
<td>6.1</td>
<td>11.4</td>
<td>4.1</td>
<td>0.0334</td>
</tr>
</tbody>
</table>

Numbers in red represent proportions that were significantly higher than numbers in blue.
## PROPORTION OF BACTERIAL FAMILIES

<table>
<thead>
<tr>
<th>Family</th>
<th>Baseline</th>
<th>Control</th>
<th>RS2</th>
<th>RS4</th>
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<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacteriaceae</td>
<td>2.1</td>
<td>3.0</td>
<td>5.8</td>
<td>11.1</td>
<td>2.8</td>
<td>0.0262</td>
</tr>
<tr>
<td>Porphyromonadaceae</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>3.4</td>
<td>0.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ruminococcaceae</td>
<td>19.3</td>
<td>23.2</td>
<td>24.8</td>
<td>16.7</td>
<td>20.7</td>
<td>0.0031</td>
</tr>
<tr>
<td>Erysipelotrichaceae</td>
<td>4.7</td>
<td>3.9</td>
<td>3.1</td>
<td>2.6</td>
<td>3.9</td>
<td>0.0279</td>
</tr>
</tbody>
</table>
# Proportion of Bacterial Genera

<table>
<thead>
<tr>
<th>Genus</th>
<th>Baseline</th>
<th>Control</th>
<th>RS2</th>
<th>RS4</th>
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<th>P value</th>
</tr>
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<tr>
<td><em>Faecalibacterium</em></td>
<td>8.4</td>
<td>10.8</td>
<td>9.7</td>
<td>7.8</td>
<td>8.8</td>
<td>0.0336</td>
</tr>
<tr>
<td><em>Parabacteroides</em></td>
<td>0.5</td>
<td>0.4</td>
<td>0.6</td>
<td>3.4</td>
<td>0.5</td>
<td>0.0002</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>1.5</td>
<td>2.2</td>
<td>4.5</td>
<td>8.9</td>
<td>2.1</td>
<td>0.0342</td>
</tr>
<tr>
<td><em>Dorea</em></td>
<td>2.9</td>
<td>3.0</td>
<td>1.7</td>
<td>1.6</td>
<td>2.7</td>
<td>0.003</td>
</tr>
</tbody>
</table>
NOVEL FIBER STUDY

- 20 healthy men (21-28 years)
- Randomized, double-blind, placebo-controlled crossover study
- Three study periods - 21 d
  - 16 d adaptation followed by 5 d of total fecal collections
- Treatments included- 3 fiber bars per day (7 g fiber/bar)
  - No supplemental fiber (NFC)
  - Polydextrose (PDX)
  - Soluble corn fiber (SCF)

Hooda et al., 2012
## PROPORTION OF BACTERIAL GENERA

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<th>PDX</th>
<th>SCF</th>
<th>P value</th>
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<tr>
<td>Faecalibacterium</td>
<td>20.7(^a)</td>
<td>24.1(^b)</td>
<td>25.5(^b)</td>
<td>0.0022</td>
</tr>
<tr>
<td>Phascolarctobacterium</td>
<td>1.5(^a)</td>
<td>2.3(^b)</td>
<td>2.8(^b)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Dialister</td>
<td>1.0(^a)</td>
<td>2.35(^b)</td>
<td>2.87(^b)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.3(^a)</td>
<td>0.3(^a)</td>
<td>0.5(^b)</td>
<td>0.0241</td>
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Are prebiotics effective in achieving host health benefits?
HEALTH BENEFITS

- Healthy individuals
- Changes in composition of microbes
  - Increase in beneficial microbes
    - Bifidobacteria and lactobacilli – GalOS, Inulin
  - Increase in butyrate producers
    - Eubacterium, Faecalibacterium, Roseburia - Resistant starch, polydextrose, soluble corn fiber
HEALTH BENEFITS

- Microbes that are correlated with disease
  - Faecalibacterium
    - Inflammatory bowel disease-decrease in Faecalibacterium
    - Anti-inflammatory properties
    - Soluble corn fiber, polydextrose, inulin, fructo-oligosaccharides, pea fiber
  - Increased Firmicutes and decreased Bacteroidetes
    - Linked with obesity
HEALTH BENEFITS

- Changes in metabolites
  - Increase in SCFA, especially butyrate
    - Inulin, fructooligosaccharides, GalOS
  - Decrease in ammonia, phenols, and indoles

- Recent approach
  - Correlating shift in microbiota to indices of gut health
    - PCA plots of microbes and fermentative end-products
Lachnospiraceae and Lactobacillaceae- positively linked with SCFA, negatively with phenols, indoles, and ammonia

Veillonellaceae- positively linked with fiber intake, negatively with phenols, indoles, ammonia, and BCFA

Hooda et al., 2012
RESPONSE OF GUT MICROBIOTA TO PREBIOTICS IN GENETICALLY AND DIET-INDUCED OBESE MICE

- Ob/ob mice fed control diet- Ob-CT
- Ob/ob mice fed control diet with prebiotics (oligofructose)- Ob-Pre
- Fed for 5 weeks
- Pyrosequencing of V1-V3 region of 16S rDNA
- Oral glucose tolerance test

Everard et al., 2011
RESULTS

- Effect of prebiotic treatment using 16S rDNA analysis
  - Phylum
    - ↑Bacteroidetes, Actinobacteria, Proteobacteria
    - ↓Firmicutes
  - Family
    - Bifidobacteriaceae- only present in Ob-Pre group
  - Genera
    - Bifidobacterium- only present in Ob-Pre group
RESULTS

- Prebiotic treatment
  - Lowered fasting glycemia
  - Improved glucose tolerance
  - Decreased fat to muscle mass ratio
  - Decreased plasma triglycerides
  - Improved gut barrier function
  - Lowered plasma LPS concentrations
  - Reduced the expression of oxidative stress and inflammatory markers
TO ADVANCE THE FIELD OF PREBIOTICS...

- Compositional analysis of potential prebiotics
  - Monomeric composition
  - Chain length
  - Linkages
  - Branching
  - Side chains

- Prebiotic activity of natural foods
  - Soybean products
  - Beet fiber
  - Whole grains and co-products
TO ADVANCE THE FIELD OF PREBIOTICS...

- Microbiota beyond bifidobacteria
  - Detailed composition – 16S rDNA pyrosequencing
  - Metabolic function – metagenomics approach
- Need to study microbiome-indices of health relationships
- How do prebiotics achieve health benefits in diseased populations?