Probiotics: A Double-edged Sword in Caries Prevention

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http://www.taamirplus.com/probiotics-benefits/

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http://myphilippinedentist.com/tooth-decay.htm
Probiotics and Oral Health

- Probiotics: “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (WHO, 2002)
- Proven safety and gastrointestinal health benefits (Butel, 2014)
- Increasing interest in oral health applications with focus on dental caries prevention (Devine and Marsh, 2009)

Overview of oral health and disease:
Ecological Plaque Hypothesis in Caries Etiology

- Caries: localized destruction of dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates (Selwitz et al., 2007)
- Dynamic relationship - environmental change produces a shift in the balance of resident microflora, thereby favoring demineralization (Marsh, 1994)
- Mutans streptococci (MS) and Lactobacilli (LB) as major groups of cariogenic bacteria
Extended Caries Ecological Hypothesis

- Incorporation of the microbiological, biochemical, ecological and clinical perspectives
- **Environmental acidification** as main determinant of *phenotypic and genotypic changes* in microflora during caries
- **Non-MS bacteria** associated with caries include *Lactobacillus spp.*, *Bifidobacterium spp.*, Prevotella spp. and Propionibacterium spp. (Takahashi and Nyvad, 2011)
Probiotics and Dental Caries

- Lactic acid bacteria (LAB) \textit{Lactobacillus} \textit{spp.} and \textit{Bifidobacterium} \textit{spp.} most commonly studied
- Natural inhabitants of oral cavity; generally safe (Chouraqui et al., 2008)
- In particular, ability of \textit{Lactobacilli (LB)} to reduce \textit{S. mutans} has attracted lots of attention (Russell and Ahmed, 1978)
- HOWEVER, LAB are \textit{acidogenic and aciduric} themselves

**Current obstacles:**
- Limited clinical studies
- Widely different study designs and target populations
- Incongruous findings
- Lack of knowledge on sustainability of effects
Research on Probiotics Use Against Dental Caries

- Randomised controlled trial (in vivo) studies into LAB effect on S. mutans
- Mixed results
- Multiple multifactorial LB cariostatic mechanisms??

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Oral Outcome</th>
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<tbody>
<tr>
<td>Nase et al. (2001)</td>
<td>594, 1-6 years, DB</td>
<td>Milk, 7 months</td>
<td><em>L. rhamnosus GG</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Ahola et al. (2002)</td>
<td>74, 18-35 years, DB</td>
<td>Cheese, 3 weeks</td>
<td><em>L. rhamnosus</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Montalto et al. (2004)</td>
<td>35, 24-33 years, DB</td>
<td>Liquid 45 days, Capsules 45 days</td>
<td><em>Lactobacillus spp.</em></td>
<td>S. mutans count was not significantly modified and salivary counts of LB in saliva was increased**</td>
</tr>
<tr>
<td>Nikawa et al. (2004)</td>
<td>40, 20 years, DB</td>
<td>Yoghurt, 2 weeks</td>
<td><em>L. reuteri</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Caglar et al. (2005)</td>
<td>26, 21-24 years, DB</td>
<td>Yoghurt, 4 weeks</td>
<td><em>Bifidobacterium DN - 173 010</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Caglar et al. (2006)</td>
<td>120, 21-24 years, DB</td>
<td>Straw, tablet 3 weeks</td>
<td><em>L. reuteri ATCC 53730</em></td>
<td>Decreased SMC with straw* and tablet*</td>
</tr>
<tr>
<td>Caglar et al. (2007)</td>
<td>80, 21-24 years</td>
<td>chewing gum, 3 weeks</td>
<td><em>L. reuteri</em></td>
<td>Decreased SMC</td>
</tr>
<tr>
<td>Caglar et al. (2008b)</td>
<td>24, 20 years, DB</td>
<td>Ice-cream, 40 days</td>
<td><em>Bifidobacterium lactis Bb-12</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Caglar et al. (2008a)</td>
<td>20, 20 years, DB</td>
<td>Lozenges, 10 days</td>
<td><em>L. reuteri ATCC</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Lexner et al. (2010)</td>
<td>18, adolescent, DB</td>
<td>Milk, 2 weeks</td>
<td><em>L. rhamnosus LB21</em></td>
<td>No Effect</td>
</tr>
<tr>
<td>Lodi et al. (2010)</td>
<td>10, 21-34 years</td>
<td>Yakult® ®®, 20% sucrose solution (control), Batavito®, 42 days</td>
<td><em>Lactobacillus sp.</em></td>
<td>EPS was significantly lower in Batavito® as compared to Yakult® ®® and sucrose*</td>
</tr>
<tr>
<td>Singh et al. (2011)</td>
<td>40, 12-14 years, DB, crossover, placebo</td>
<td>Ice-cream, 10 days</td>
<td><em>B. lactis Bb12 ATCC27536 and L. acidophilus La5</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Jindal et al. (2011)</td>
<td>150, 7-14 years,</td>
<td>Powder, 14 days</td>
<td><em>L. rhamnosus, Bifidobacterium sp.</em></td>
<td>Decreased SMC*</td>
</tr>
<tr>
<td>Chuang et al. (2011)</td>
<td>78, 20-26 years, DB</td>
<td>Tablet, 2 weeks</td>
<td><em>Lactobacillus GMNL-33</em></td>
<td>Decreased SMC **</td>
</tr>
</tbody>
</table>

*, p ≤ 0.05; **, p ≤ 0.016
Research on Probiotics Use Against Dental Caries

- Studies done in vitro on effects of LB and their products on S. mutans
- Mixed results
- LB cariostatic mechanisms highly dependent on specific probiotic strains and target oral pathogens?

<table>
<thead>
<tr>
<th>References</th>
<th>Aim to Study/characterize</th>
<th>Species/product</th>
<th>Outcome</th>
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<tr>
<td>O'Connor et al. (2006)</td>
<td>To explore the inhibition activity of lactacin 3147 on S. mutans</td>
<td>Lactacin 3147</td>
<td>Decreased SMC</td>
</tr>
<tr>
<td>Yang et al. (2008)</td>
<td>Probiotics characteristics of L. plantarum HO-69 applied in oral cavity</td>
<td>L. plantarum</td>
<td>≠ S. mutans activity</td>
</tr>
<tr>
<td>Russell and Ahmed (1978)</td>
<td>To assess the interrelationship between LAB and S. mutans in plaque formation</td>
<td>L. casei, L. fermentum, L. acidophilus and S. mutans</td>
<td>L. casei ≠ growth of S. mutans at pH 4</td>
</tr>
<tr>
<td>Hillman et al. (2009)</td>
<td>To study the ability of daily applications of S. rattus JH145 to affect the numbers of an implanted S. mutans strain in rats model.</td>
<td>S. rattus JH145</td>
<td>Decreased SMC</td>
</tr>
<tr>
<td>Zahradnik et al. (2009)</td>
<td>Preliminary assessment of safety and effectiveness of ProBiora3 a probiotics mouthwash</td>
<td>S. oralis, S. suberis, lactic acid deficient variant of S. rattus</td>
<td>Decreased SMC</td>
</tr>
<tr>
<td>Lang et al. (2010)</td>
<td>Specific Lactobacillus/S. mutans co-aggregation</td>
<td>L. paracasei DSMZ16671, heat stable and can be delivered in variety of vehicles</td>
<td>L. paracasei DSMZ16671 has no affinity for hydroxyapatite. Coaggregation of S. mutans.</td>
</tr>
<tr>
<td>Westbroek et al. (2010)</td>
<td>Interaction of Lactobacilli with pathogenic S. pyogenes</td>
<td>L. crispatus and L. jensenii</td>
<td>Lactobacilli did not inhibit the growth of S. pyogenes and vice versa</td>
</tr>
<tr>
<td>Wei et al. (2002)</td>
<td>Effect of LGG on immune product antibodies at ultra-high temperature and increase storage time</td>
<td>L. GG</td>
<td>≠ the adherence of S. mutans *</td>
</tr>
<tr>
<td>Soderling et al. (2011)</td>
<td>Probiotics lactobacilli interfere with Streptococcus mutans biofilm formation in vitro</td>
<td>Lactobacillus rhamnosus GG, L. plantarum 299v, and L. reuteri strains PTA 5289 and SD2112</td>
<td>L. reuteri SD2112 and L. rhamnosus GG strongly ≠ biofilm formation by S.mutans</td>
</tr>
<tr>
<td>Teanpaisan et al. (2011)</td>
<td>Inhibitory effect of oral Lactobacillus against oral pathogens</td>
<td>L. fermentum, L. salivarius, L. casei, L. gasseri, L. rhamnosus, L. paracasei, L. mucosae, L. oris, L. vaginalis</td>
<td>lactobacillus showed a strong inhibitory effect against S.mutans</td>
</tr>
</tbody>
</table>

≠, inhibits; *, p ≤ 0.05
Research on Probiotics Use Against Dental Caries

- Effects of LB on caries development
- Promising results but limited validation studies done
- Is early intervention crucial??
- Are effects sustainable??

### Table 2-4 Summary of studies done to characterize the effect of lactobacilli on caries

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<tr>
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<td>594, 1-6 years, RCT DB</td>
<td>Milk, 7 months</td>
<td><em>L. rhamnosus</em> GG</td>
<td>Decreased Caries</td>
</tr>
<tr>
<td>Steeksen-Blicks et al.</td>
<td>248, 1-5 years, RCT</td>
<td>Capsules in milk, 21 mths</td>
<td><em>L. rhamnosus LB21 and fluoride</em></td>
<td>Decreased Caries*</td>
</tr>
<tr>
<td>Tanzer et al. (2010)</td>
<td>20, 21 day old rats, RCT DB</td>
<td>Supplements</td>
<td>*L. paracasei DSMZ16671, S. mutans strain 10449S</td>
<td>Inhibits the colonization of S. mutans and inhibits caries in rats.</td>
</tr>
<tr>
<td>Petersson et al. (2011)</td>
<td>160, 58 – 84 years, RCT</td>
<td>Milk, 15 months</td>
<td><em>L. rhamnosus LB21</em></td>
<td>Reverse primary root carious lesion *</td>
</tr>
</tbody>
</table>

*, p ≤ 0.05
Research on Probiotics Use Against Dental Caries

- Ability of probiotics to adhere to epithelium an Important factor
- Different LAB strains show different adhesion properties

Table 2-6 Summary of studies investigating the adhesion properties of lactobacilli in oral cavity.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Aims of the study</th>
<th>Species</th>
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<tr>
<td>Busscher et al. (1999)</td>
<td>In vitro adhesion to enamel and in vivo colonization of tooth surfaces by Lactobacilli from a bio-yoghurt</td>
<td><em>L. casei</em>, <em>L. acidophilus</em></td>
<td>Adhesion of <em>L. acidophilus</em> is much stronger than <em>L. casei</em>.</td>
</tr>
<tr>
<td>Lima et al. (2005)</td>
<td>To compare the adhesion of <em>L. casei</em> shirota and <em>L. acidophilus</em> to an artificial caries model</td>
<td><em>L. casei</em> shirota, <em>L. acidophilus</em></td>
<td>Inferior adhesion of <em>L. casei</em> shirota as compared to <em>L. acidophilus</em>.</td>
</tr>
<tr>
<td>Stamatova et al. (2009)</td>
<td>In vitro evaluation of yoghurt starter lactobacilli and L.GG adhesion to saliva-coated surfaces</td>
<td><em>L. delbrueckii</em> subsp. bulgaricus strains and <em>L. rhamnosus</em> GG</td>
<td>Adhesion of the <em>L. delbrueckii</em> subsp. bulgaricus strains remained lower in comparison to L. GG.</td>
</tr>
<tr>
<td>Bosch et al., (2012)</td>
<td>Isolation and characterization of probiotics strains for improving oral health</td>
<td><em>S. salivarius</em>, <em>L. reuteri</em>, AB1 to AB46</td>
<td>LAB exhibits antimicrobial activity against oral pathogens, ability to aggregate and to adhere to oral tissues or high tolerance to oral environmental stress factors.</td>
</tr>
<tr>
<td>Tahmourespour and Kermanshahi (2011)</td>
<td>To assess the ability of biofilm formation among mutans and non-oral <em>S. mutans</em> and to determine the effect of <em>L. acidophilus</em> DSM 20079 on the adhesion of streptococcal strains</td>
<td><em>L. acidophilus</em> DSM 20079</td>
<td>The <em>L. acidophilus</em> had more effect on adherence of <em>S. mutans</em> than non-<em>S. mutans</em> significantly *</td>
</tr>
<tr>
<td>Samot et al. (2011)</td>
<td>Adherence capacities of oral lactobacilli for potential probiotics purposes</td>
<td>64 <em>Lactobacillus</em> strains</td>
<td>Lactobacillus <em>rhamnosus</em> GG can adhere to saliva-coated HA</td>
</tr>
</tbody>
</table>

*p < 0.05
Research on Probiotics Use Against Dental Caries

- Classic criterion for a successful probiotics agent: ability to colonize in intestine and oral cavity (Michail, 2005)

- Limited studies

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<tr>
<td>Busscher et al. (1999)</td>
<td>In vitro adhesion to enamel and in vivo colonization of tooth surfaces by lactobacilli from a bio-yoghurt</td>
<td><em>L.acidophilus</em>, <em>L.casei</em></td>
<td>Lactobacilli fail to colonize oral cavity after one week consumption of bio-yoghurt</td>
</tr>
<tr>
<td>Tanzer et al. (2010)</td>
<td>Caries inhibition by and safety of <em>L.paracasei</em> DSMZ16671</td>
<td><em>L.paracasei</em> DSMZ16671</td>
<td>Inhibits the colonization of <em>S.mutans</em></td>
</tr>
<tr>
<td>Aminabadi et al. (2011)</td>
<td>Effect of chlorhexidine pretreatment on the stability of salivary lactobacilli probiotics in six- to twelve-year-old children: a randomized controlled trial</td>
<td><em>L.rhamnosus GG</em></td>
<td>Increased colonization of LGG but not steady after 5 weeks*</td>
</tr>
</tbody>
</table>

*p < 0.05
**Yakult® Study Rationale**

**WHAT?**
- Introduced in 1935
- Widespread global popularity
- $10^8$ CFU/mL live L. casei Shirota strain (LcS)
- Documented safety and gastrointestinal health benefits

**Concerns**
- Acidic drink
- High in sugar (~17.9g/100g) → classified “HIGH” by UK Food Standards; ~88% of which is sucrose
- L. casei is itself associated with caries
- Limited research on oral applications
AIM:
To conduct a clinical study to investigate the oral effects of Yakult® and their sustainability in two weeks
Yakult® Study Design

Recruitment of healthy adult volunteers (21-40 years)

Screening Visit 1 (Baseline)
- Day before start of intervention
- Supragingival plaque pH characterization
- Biofilm collection from 5 predetermined sites

Rinsing with commercial probiotic drink
Yakult® daily after dinner for 1 week

Screening Visit 2
- Day after cessation of intervention

Screening Visit 3
- 2 weeks after cessation of intervention

Plotting of Stephan Curves

Parameters of Interest
- Area under curve (AUC)
- Lowest pH reached
- Recovery time

Real-time PCR

Parameters of Interest
- 16S rDNA copy number (CN)
- Normalized CN ratio of LcS
- Normalized CN ratio of S. mutans
Yakult® Study Conclusions

- Short-term probiotic consumption may be transiently cariostatic in individuals with higher baseline plaque acidogenicity, but cariogenic in lower-risk individuals.

- Proper individualized caries risk assessment critical prior to probiotics applications in caries prevention.

- Unconventional LcS cariostatic mechanism involving S. mutans gene regulation.

- Findings corroborate with extended caries ecological hypothesis.

- Balance between probiotic’s cariostatic capabilities and its own acidogenicity.
Probiotics in Caries Prevention - An Enigma

- Further investigations to validate use of probiotics in oral health applications
- Microbiome studies are warranted to study ecology shifts during health and disease and role of probiotic bacteria
- Exploring the oral-systemic-gut link in dental caries
References

References

- Chouraqui JP, Grathwohl D, Labaune JM, Hascoet JM, de Montgolfier I, Leclaire M et al. (2008). Assessment of the safety, tolerance, and protective effect against diarrhea of infant formulas containing mixtures of probiotics or probiotics and prebiotics in a randomized controlled trial. Am J Clin Nutr 87(5):1365-1373.


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  - Dr. Ng Ching Ging
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