

Are we ready for caries prevention through bacteriotherapy?

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Abstract: Recent insights in medical science indicate that human biofilms play an important role in health and well-being, and have put microbiota modulation through bacteriotherapy into focus. In dentistry, bacterial interference with probiotic bacteria to support the stability and diversity of oral biofilms has gained similar interest. Investigations *in vitro* into metabolic activity, co-aggregation, growth inhibition, bacteriocin production, and adhesion have collectively suggested a potential role for probiotic lactobacilli and bifidobacteria to modulate the oral microbial ecology. Likewise, short-term clinical studies with intermediate microbial endpoints indicate that interference with caries-associated bacteria seems possible through probiotic dairy products, tablets, lozenges and chewing gum in various dose regimens. Few randomized controlled clinical trials with caries outcomes are available, but three studies with preschool children and the elderly have demonstrated preventive fractions between 21% and 75%, following regular intake of milk supplemented with probiotic lactobacilli. However, further large-scale trials with orally derived anti-caries candidates are needed before we can say that we are ready for bacteriotherapy as an adjunct to complement the existing evidence-based methods for preventing and controlling caries in daily practice.

Descriptors: Bacteria; Biofilms; Dental Caries; Probiotics.

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Introduction

The interest in bacteriotherapy to prevent and control medical and oral conditions has grown remarkably in recent years. The Human Microbiome Project has provided insights that the biofilms inside and outside our bodies have co-evolved with mankind, and play an important role in maintaining health. This is also true of the oral cavity in which the microbiota does not play a passive role but actively contributes to maintaining oral health.¹ In other words, the balance between beneficial and pathogenic bacteria is essential for stability and well-being. However, certain ecological shifts in the microbiome allow pathogens to manifest themselves and cause disease. Regarding caries, a frequent consumption of fermentable carbohydrates can lead to an environment that favors aciduric species (e.g. mutans streptococci and lactobacilli), reducing the biofilm diversity and resulting in enamel demineralization. Bacteriotherapy is the term used when a harmless effector strain is implanted in the host's microflora to maintain or restore a natural microbiome by interference and/or inhibition of other microorganisms, especially patho-

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gens. This could lead to alternative ways of fighting infectious diseases with less harmful side effects, and may also help in the treatment of disorders that seem to have nothing to do with bacteria, such as asthma, obesity and diabetes. Probiotic bacteria, defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (WHO), are commonly suggested candidates for bacteriotherapy. Whether or not such bacterial interference applies to dental caries is still an open question. Clinical evaluations of probiotic bacteria that are naturally specific to the mouth are uncommon. So far, strains isolated and developed mostly for gastrointestinal health have been adopted to conduct dental research. These strains could be suboptimal, considering the unique ecological environment that exists in the oral biofilm. The aim of this conference paper was to summarize the current literature on the use of probiotic bacteria as a potential anti-caries measure. For a more comprehensive review, readers may refer to De Vrese and Schrezenmeir.²

Mechanisms of action

Probiotics means “for life” and, in principle, either naturally occurring or genetically engineered strains are used for intervention. The most common probiotic bacteria belong to the lactobacilli and bifidobacteria genera, but certain strains of streptococci have also been investigated.³ Lactobacilli are highly acidogenic and aciduric, and grow optimally under slightly acidic conditions. They are considered a part of the normal oral flora and comprise about 1% of the cultivable species. Bifidobacteria, on the other hand, occur in only minute amounts in the normal oral biofilm. It is important to stress, however, that not all lactic acid bacteria (or bifidobacteria) are probiotic and possess the ability to confer health benefits for the host.

Although the theory that certain bacteria may have beneficial effects on health was presented in early 1900 by Nobel Prize laureate Ilya Metchnikof, the mechanisms of action are still not fully understood. It seems clear, however, that there are local (direct) as well as systemic (indirect) events that occur by regulation of the immune response. Accord-

ing to Reid,⁴ the potential avenues are

- (i) co-aggregation and growth inhibition,
- (ii) bacteriocin and hydrogen peroxide production,
- (iii) competitive exclusion through antagonistic activities on adhesion and nutrition, and,
- (iv) immunomodulation.

Consequently, the composition and metabolic activity of the oral biofilm may be temporarily modified. The effects of probiotic bacteria seem strain-specific, and cannot be applied directly to other strains. Moreover, the same strains may have a different effect on different individuals.⁵ Probiotic bacteria do not colonize the host permanently, but permanent colonization is not required for probiotic action to occur. Studies in feces, plaque and saliva clearly show that ingested probiotic bacteria are recovered only up to one week after termination of an intake.^{6,7}

Laboratory and animal studies

Several experimental studies have recently been performed with the main purpose of investigating various biochemical properties of probiotic bacteria and their interaction with oral bacteria in planktonic cultures or in biofilm models. For example, studies on metabolic activity,⁸ co-aggregation,⁹ growth inhibition,¹⁰ bacteriocin production,³ binding to oral structures, saliva and biofilm formation,¹¹ have collectively suggested a potential role for probiotic bacteria in modulating the oral microbial ecology. Consequently, the search has started for optimal candidates for the major oral diseases. Nonetheless, the proposed strains have not yet been tested in human clinical trials.^{12,13} One selected candidate, however, has been evaluated in an animal model. Pathogen-free rats were inoculated with *S. mutans* and fed with a cariogenic diet supplemented with a heat-killed probiotic strain (*L. paracasei* DSMZ16671) or placebo.¹⁴ The animals were sacrificed after 42 days and a significant caries reduction (preventive fraction of 27%) was displayed in the test group compared with the controls. The authors concluded that the intervention appeared efficacious and safe.¹⁴

Clinical trials with microbial endpoints

There are several short-term studies describing human interventions with probiotic bacteria with caries-related microbiological endpoints in saliva or plaque, as shown in Table 1.¹⁵⁻³⁰ All used parallel arms with intervention versus placebo/control, or a crossover design. The sample sizes were generally small and the interventions were shorter than 1 month. A variety of vehicles for the delivery of the live bacteria were used, such as dairy products, ice cream, tablets and lozenges, and the daily dose ranged between 10⁷ and 10⁹ bacteria. With few exceptions, conventional cultivation on selective agar plates or simple chairside methods were employed; DNA-based methods were reported in only three papers.^{24,25,28} Statistically significant reductions of mutans streptococci in saliva or plaque were reported in two thirds of all studies. However, it was not possible to quantify the magnitude of bacterial decrease, due to the frequent use of categorical scores from chairside bacterial tests rather than bacterial

counts. The follow-up samplings were generally conducted at the termination of probiotic intervention, and any information on the post-intervention re-growth of the suppressed target bacteria afterwards was minimal. Interestingly, only three papers found an increase of lactobacilli after the daily intake of probiotic lactobacilli,^{17,25,28} and no study showed a simultaneous reduction of mutans streptococci and increase of lactobacilli. Another observation was that the vehicle or mode of delivery was of minor importance. Matching results were obtained with dairy products such as milk, yogurt, cheese and ice cream, as well as with conventional preparations like tablets, lozenges, powder and drops. Likewise, similar effects on mutans streptococci were obtained with probiotics derived from lactobacilli and bifidobacteria, using various doses. Studies with single strain probiotics have predominated in the literature and there has been insufficient information on multistrain interventions. No negative side effects following the probiotic interventions have been reported.

Table 1 - Short-term clinical trials with microbial endpoints.

First author, yr	design ^a	n; age (yr)	vehicle	time ^b	strain	major outcome ^c
Ahola, 2002 ¹⁵	RCT	74; 18-35	cheese	3 w	Lactobac. mix	MS↘
Nikawa, 2004 ¹⁶	crossover	40; 20	milk	2 w	<i>L. reuteri</i>	MS↘
Montalto, 2004 ¹⁷	RCT	35; 23-37	liquid/caps	45 d	Lactobac. mix	MS→, LB↗
Caglar, 2005 ¹⁸	crossover	21; 21-24	yogurt	2 w	Bifidobacteria	MS↘
Caglar, 2006 ¹⁹	RCT	120; 21-25	tablet	2 w	<i>L. reuteri</i>	MS↘
Caglar, 2007 ²⁰	RCT	80; 21-24	gum	3 w	<i>L. reuteri</i> (2 strains) ^d	MS↘
Caglar, 2008 ²¹	crossover	40; 20-24	ice cream	10 d	Bifidobacteria	MS↘
Caglar, 2008 ²²	RCT	20; 20	lozenge	10 d	<i>L. reuteri</i> (2 strains) ^d	MS↘
Cildir, 2009 ²³	crossover	24; 12-16	yogurt	2 w	Bifidobacteria	MS↘
Lexner, 2010 ²⁴	RCT	20; 12-15	milk	2 w	<i>L. rhamnosus</i> LB21	MS→
Sinkiewicz, 2010 ²⁵	RCT	23; 18+	diet	12 w	<i>L. reuteri</i> (2 strains) ^d	LB↗, PI↘
Singh, 2011 ²⁶	crossover	40; 12-14	ice cream	10 d	Bifido. / <i>L. acidophilus</i>	MS↘
Jindal, 2011 ²⁷	RCT	150; 7-14	powder	14 d	<i>L. rhamnosus</i> / Bifido.	MS↘
Marttinen, 2012 ²⁸	crossover	13; 20-30	tablet	2 w	<i>L. rhamnosus</i> / <i>reuteri</i>	MS→, LB↗
Chuang, 2011 ²⁹	RCT	78; 20-26	tablet	2 w	<i>L. paracasei</i> GMNL-33	MS, LB→
Cildir, 2012 ³⁰	RCT	19; 4-12	drops	25 d	<i>L. reuteri</i> (2 strains) ^d	MS, LB→

^aRCT = randomized controlled trial, crossover = randomized crossover trial; ^bd = days, w = weeks; ^cMS↘ = significant decrease in salivary mutans streptococci counts, LB↗ = significant increase in salivary lactobacilli counts, PI↘ = significant decrease in plaque index, → = unchanged salivary counts; ^d*L. reuteri* ATCC 55730 and ATCC PTA 5289.

Clinical trials with caries endpoint

Until now, three randomized controlled trials (RCT) using milk supplemented with *L. rhamnosus* (LB21 or GG), with caries as an endpoint, have been published (Table 2).³¹⁻³³ The first of these studies investigated the effects of probiotic bacteria given to 594 preschool children aged 1–6 years, and showed a significant reduction in caries increment in the probiotic milk group after 7 months, compared to the control group, but only in the subgroup of the 3–4 year-olds.³¹ It was claimed that the 5-day-a-week intake of probiotic milk reduced the risk of caries significantly (OR = 0.56). The second study was a cluster-randomized trial by Stecksén-Blicks *et al.*³² that lasted over 21 months. The results suggested a dramatic reduction in early childhood caries after daily consumption of milk supplemented with *L. rhamnosus* and 2.5 ppm fluoride, but the effect of the two supplements could not be individualized. In the most recent study on root caries in 160 older adults,³³ the two factors, fluoride and probiotic bacteria, were separated into two groups, and then combined in a third group. Compared with the control group, all intervention groups showed statistically significant reversals of existing root caries lesions. The combination of fluoride and probiotic bacteria seemed more effective than the probiotic supplement or fluoride alone.³³ Notably, no statistically significant reductions in the levels of *S. mutans* or lactobacilli were seen in any of the three clinical studies, yet all three showed an obvious effect on caries.

Discussion

Almost all publications concerning probiotic

therapy and oral health were published in the current decade, and there is an obvious risk of publication bias and overrepresentation of positive findings. There is, however, good evidence of probiotic-induced beneficial effects on the gut microbiota and gastro-intestinal health. Insofar as recent studies suggest that there are strong functional similarities between the gut and oral biofilms, it is reasonable to speculate that corresponding health-promoting events may occur in the oral cavity. The observations made in the two major caries trials in children were therefore interesting.^{31,32} Both studies found improvements in general health, along with reductions in caries risk and caries incidence, but the most important findings were probably those related to the use of less prescribed antibiotics.^{32,34} In fact, the findings may support a “metabolic domino effect” in which probiotic therapy is a link between oral and general health; the oral cavity influences and is influenced by general health. Future research will reveal if orally derived probiotic candidates optimized for oral diseases may have a similar dual impact.

A pertinent issue is that the mechanisms of probiotic action are not fully understood. It is generally thought that there are combined local and systemic effects involving adhesion, co-aggregation, competitive inhibition, production of organic acids and bacteriocin-like compounds, and immune-modulation.^{2,3} Although these events have been convincingly demonstrated in the laboratory environment, studies of complex biofilm models or *in vivo* are still scarce. Moreover, the Human Microbiome Project showed that 75 to 100 species of bacteria predominate in each person’s mouth, but different people

Table 2 - Controlled clinical trials with caries as endpoint. Caries development compared with standard milk without supplements.

First author, yr	design ^a	n; age (yr)	vehicle, time ^b	drop-out	strain	caries test/control	PF ^c
Näse, 2001 ³¹	RCT	594; 1–6 164; 3–4	milk, 7 m milk, 7 m	24% –	<i>L. rhamnosus</i> GG <i>L. rhamnosus</i> GG	15% / 19% 10% / 23%	21% 56%
Stecksén-Blicks, 2009 ³²	CRCT	174; 1–5	milk, 21 m	25%	<i>L. rhamnosus</i> LB21 + 2.5 ppm F	Δdmfs 0.4 / 1.6 ^d	75%
Petersson, 2011 ³³	RCT	160; 58–84	milk, 15 m	38%	<i>L. rhamnosus</i> LB21	rev 54% / 24% ^e	55%

^aRCT = randomized controlled trial, CRCT = cluster randomized controlled trial; ^bm = months; ^cPF = prevented fraction; ^dcaries prevalence, %; ^eroot caries reversals, %.

have different combinations of species, insofar as it was found that some of the species prevailing in one person's mouth were rare in another's mouth. Thus, it seems that there is no "one-size-fits-all" which might call for exposure to a mix of beneficial strains rather than to one single strain. The optimal dose for dental diseases is yet to be explored. Up to now, the adopted dose regimens have been based on gastro-intestinal and pediatric health dosing standards. Genetically engineered strains with targeted properties are also expected to appear,³⁵ albeit emotional barriers for introducing such strains, especially in children, have already been reported.

The fact that probiotic supplements require regular daily intakes (or at least 4–5 days per week) may be a challenge in terms of patient compliance and costs. The use of dairy products as vehicle for probiotic delivery is desirable from a cariological point of view. Non-sweetened milk, cheese and yogurt have high natural contents of calcium and phosphate that readily decrease the "critical pH" for enamel dissolution and enhance remineralization. Dietary supplements with probiotic bacteria are also a more economical alternative for the average consumer than tablets or capsules. Nevertheless, commercial interests come before science; there are already a number of probiotic oral health products available for the informed consumer, such as tablets, rinses and toothpastes, but with very limited clinical documentation, if any.

As found in the short-term trials, it seems likely that probiotic lactobacilli and bifidobacteria may exert an antagonistic effect on mutans streptococci in existing biofilms. The current understanding is that enamel demineralization is a result of a carbohydrate-induced shift in the biofilm to aciduric phenotypes. Accordingly, the use of a single group of bacteria as a biomarker for caries development is only partly relevant. A short-term reduction in salivary mutans streptococci may not necessarily be associated with either less caries or reduced caries risk. Attempts have been made to combine professional tooth cleaning and use of antibacterial agents preceding the bacterial interference, in or-

der to amplify the effect and or delay re-growth of pathogens.³ However, in a recent multicenter study, we failed to demonstrate a lactobacilli-induced effect on re-growth of salivary mutans streptococci after a full-mouth disinfection with chlorhexidine in healthy adults.³⁶ On the other hand, the long-term study on root caries indicated that the probiotic supplement worked well when combined with fluoride,³¹ and it seems reasonable to consider the use of bacteriotherapy as one possible adjunct to complement the existing evidence-based methods to prevent and control caries.

The use of probiotics is generally considered safe, although some probiotic strains have been isolated from infections such as endocarditis.³⁷ No adverse effects to oral health have been reported so far in clinical trials. Most recent trials were unable to demonstrate increased counts of lactobacilli after the daily intake of probiotic bacteria. Acid production from probiotic lactobacilli could theoretically lead to increased caries risk, but clinical investigations of biofilm acidogenicity have not indicated that this would be the case.²⁷ However, long-term studies are needed to firmly establish that the intake of probiotic supplements are more beneficial than harmful. Studies on selected individuals with an ecologically stressed biofilm and proven caries activity would also be helpful to fill the gaps of knowledge.

Conclusions

Bacterial interference with probiotic bacteria to support the stability and diversity of oral biofilms is gaining momentum in dentistry. Investigations *in vitro*, as well as clinical studies with bacterial endpoints, suggest that probiotic interference with the oral biofilm seems possible. Only a few randomized, controlled clinical trials reporting caries outcomes are available, but the connection to general health and the obvious caries preventive fractions are promising. However, further large-scale trials with orally derived anti-caries candidates are needed before we can say that we are ready to adopt bacteriotherapy for preventing and controlling caries in clinical practice.

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