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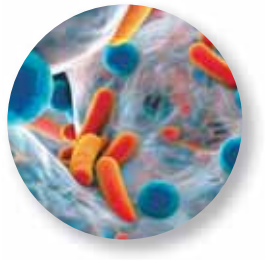

Fifteen Years of Probiotic Therapy in the Dental Context: What Has Been Achieved?

Svante Twetman, DDS, Odont Dr; Mette Rose Jørgensen, DDS, PhD; and Mette Kirstine Keller, DDS, PhD

ABSTRACT Many oral diseases are driven by an ecological shift from a balanced microbial consortium to dysbiotic communities with reduced diversity. Probiotic bacteria offer an opportunity to prevent and manage conditions such as dental caries, periodontal conditions and candidiasis. Regular intakes may support a healthy microbiome via direct interference with the biofilm and systemically through modulation of the host's immune response. Placebo-controlled trials have shown substantial beneficial effects but further research is needed for general treatment recommendations.

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More than 15 years have passed since the first clinical study on the effect of probiotic bacteria on caries risk and caries development in preschool children was published.[1] Milk supplemented with *L. rhamnosus* GG was served in day care settings over a period of seven months, and the results indicated clear beneficial effects on selected caries risk factors. The interest generated around these findings was the virtual starting point for a novel avenue of research in clinical dentistry, widening the outcome measures to gingivitis, periodontal disease, implantitis, peri-implantitis, mucositis, candidiasis and halitosis. In fact, the probiotic concept became a hot topic and soon there were far more review publications available than original studies. So, one may ask if another review really is needed? The answer could be yes in light of the rapid advances in the human microbiome and microbial ecology. Molecular and functional studies have provided insights that bacterial biofilms have co-evolved with humans and play an important role in health and well-being.[2] Consequently, the composition and function of the oral microbiota plays an active role in the oral cavity. A diverse and balanced microbiota is associated with oral health while dysbiosis, commonly driven by ecological stress, is linked to a variety of oral conditions and opportunistic infections.[2] In this context, probiotic therapy may offer an additional measure to established and evidence-based interventions. The background principle behind the use of probiotic bacteria (or bacteriotherapy) is quite simple: to modulate or replace unwanted microbes with the aid of harmless or friendly bacteria instead of using antibiotics or antimicrobial agents. Probiotic bacteria

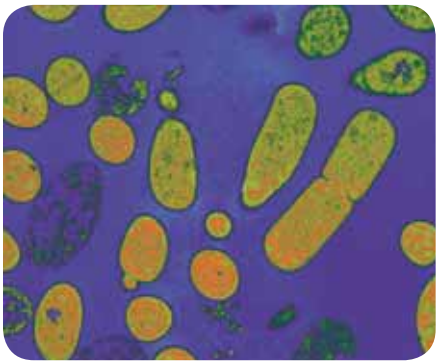

FIGURE 1. *Lactobacillus reuteri* "swimming" in salt solution. This probiotic strain is commonly used in clinical trials and has shown to compete with pathogens and reduce plaque and gingivitis (with permission from BioGaia AB, Stockholm).

are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host."[3] The aim of this narrative and subjective review is to briefly provide the general dental practitioner with an update on recent advances concerning the clinical use of probiotics for oral health. The paper is based mainly on systematic reviews and human trials with clinical endpoints of importance for the individual patient.

Genera, Strains and Dose

The main probiotic strains used for oral bacteriotherapy belong to the *Lactobacillus* (**FIGURE 1**) and *Bifidobacterium* genera but also some *Streptococcus* species may express probiotic properties. Generally, the effects of probiotic bacteria are strain specific and properties of one strain cannot necessarily be applied to others.[4] The very same strain may also display different effects in different individuals. A general clinical recommendation is that the probiotic bacteria must be ingested on a regular basis, which in clinical practice means at least four days per week. There is currently no evidence to support a permanent colonization of probiotic bacteria in the oral cavity although exceptions following early-in-life exposures seem to exist.[5] It should, however, be stressed that a permanent colonization is not a prerequisite for probiotic action in the oral biofilm.[6] The common vehicles for administration are dairy products (milk, yogurt, sour cream) or tablets, capsules, lozenges and drops. Any "optimal" dose for oral care is unfortunately not established. The common recommendations of 1–2 deciliter of yogurt/milk per day with each milliliter containing $1 \times 10^{8,9}$ live cells or 1–2 tablets per day ($1 \times 10^{8,9}$ live cells in each tablet) are derived from gastrointestinal health. For infants, the recommended regime is five probiotics drops per day, sometimes in combination with vitamin D. It is possible, and perhaps even likely, that there is dose-response relationship for oral effects but this is yet to be explored.

Mechanisms of Action

It is generally thought that the intake of probiotic bacteria can trigger a chain of direct (local) and systemic (indirect) effects. The direct events in the oral biofilm include co-aggregation, competitive exclusion, bacteriocin (hydrogen peroxide) production and competition for nutrients.[7,8] The ability to produce toxins, particularly H_2O_2-like agents, is perhaps the most powerful local property and probiotic bacteria can thereby modify the composition of the oral biofilm and/or its metabolic activity. The systemic effects rely on immunomodulation of the host's innate and adaptive inflammatory response through activation of T-cells.[9] Consequently, significant effects on IgA and cytokine expression in the guts and the gingival crevicular fluid (GCF) have been displayed.[10] It is however important to emphasize that the detailed mechanisms of action are not fully known and that there are conflicting reports on the probiotic-induced effects on the host response in the oral environment. For example, one recent study failed to show the effects on salivary immunoglobulins and inflammatory mediators[11] while others found increased levels of s-IgA and human neutrophil peptides 1-3 in saliva immediately after probiotic exposure.[12,13] It has also been demonstrated that the presence of *Lactobacillus reuteri* in saliva coincide with higher salivary IgA in young adults after the intake of probiotic lozenges.[14] Contradictory data are also present for periodontal conditions. Studies have shown that *Lactobacillus brevis* CD2 can delay gingivitis development and inhibit periodontitis by downregulating the inflammatory cascade in GCF.[15,16] These anti-inflammatory effects have been attributed to the presence of arginine deaminase which prevented nitric oxide generation.[17] Similar findings reported by Ince et al.[18] show that the GCF levels of the matrix metallopeptidase inhibitor TIMP-1 increased and the MMP-8 levels decreased in patients with chronic periodontitis when probiotic *L. reuteri* was added to traditional nonsurgical therapy. On the other hand, Hallström et al.[19] found no effects on the cytokine levels using the same strains in subjects with healthy periodontal conditions, indicating a therapeutic role of the probiotic supplements rather than a preventive. Another interesting but open question is whether the intake of probiotics can influence the composition of the oral bacterial community. Two studies were unable to demonstrate a shift after two to three weeks of probiotic exposure.[6,19] However, a prolonged study over 12 weeks with *L. reuteri* displayed an

TABLE 1

Randomized Placebo-Controlled Clinical Trials With Caries as Endpoint

First author, year	n/age	Vehicle	Strain	Follow-up	Outcome,[a] comment
Intervention during infancy					
Taipale, 2013[26]	106/newborn	pacifier/spoon	*B. animalis*	4 yr.	NS, low-risk population
Hasslöf, 2013[27]	180/4–13 mo.	gruel	*L. paracasei*	9 yr.	NS
Stensson, 2013[28]	188/newborn	drops	*L. reuteri*	9 yr.	S, primary teeth only
Intervention to preschool children					
Näse, 2001[1]	594/1–6 yr.	milk	*L. rhamnosus*	after 7 mo.	NS/S = 3–4-year-old subgroup
Stecksén-Blicks, 2009[29]	248/1–5 yr.	milk	*L rhamnosus*	after 21 mo.	S, milk contained 2.5 ppm F
Hedayati-Hajikand, 2015[30]	138/2–3 yr.	lozenges	*Streptococcus*	after 12 mo.	S, high-risk population
Rodriguez, 2016[31]	261/2–3 yr.	milk	*L. rhamnosus*	after 12 mo.	S, high-risk population
Intervention to schoolchildren					
Keller, 2014[32]	36/12–17 yr.	lozenges	*L. reuteri*	after 3 mo.	S, assessed with QLF[b]
Teanpaisan, 2015[33]	122/12–14 yr.	milk-powder	*L. paracasei*	after 6 mo.	S, high-risk group
Intervention to adults					
Petersson, 2011[34]	200/56–84 yr.	milk	*L. rhamnosus*	after 15 mo.	S, root caries arrest

[a] S = significant difference in caries prevalence/increment compared with placebo; NS = no significant difference
[b] QLF = quantitative light fluorescence

altered biofilm composition on teeth although the richness of species seemed to be unaffected.[20] The shift was, however, of a transient nature and was "normalized" within one month after termination of the exposure. This may indicate that there is a "colonization memory" in the oral biofilm similar to that of the guts. Clearly, more studies are needed to elucidate both the local and systemic avenues of action.

Safety

The safety of probiotic administration must of course be considered. Probiotic supplements are from a regulatory point of view classified as food additives and labeled "generally recognized as safe" (GRAS). There have been no reports of adverse effects in healthy humans although interventions for critically ill patients or the very fragile elderly should be considered with some caution. On the other hand, probiotic therapy may be used for cancer patients. Sharma and co-workers[21] have shown that lozenges with *L. brevis* CD2 can reduce the incidence and alleviate the symptoms of radiation- and chemotherapy-induced mucositis in patients with head and neck cancer. Concerns have also been raised for the cariogenic abilities of lactobacilli. Indeed, probiotic lactobacilli are highly acidogenic but there is at this time no data to support that a regular intake of these bacteria would increase the caries risk.[22,23]

Probiotics and Caries

The potential of probiotic bacteria to influence the caries process is commonly addressed with intermediate endpoints rather than caries lesion development. Two systematic reviews, based on 19 and 23 papers respectively, have concluded that probiotic interventions clearly can reduce the mutans streptococci counts in supragingival plaque and saliva, thereby suggesting a positive effect in the prevention of caries.[24,25] With respect to lesion development, seven placebo-controlled studies are currently available in preschool children/primary dentition,[1,26–31] two in adolescents[32,33] and one on root caries arrest in the elderly.[34] The studies are summarized in **TABLE 1**. For the infants and preschool children, the probiotic supplements were typically administrated via drops or gruel from the parents or with milk served in day care settings. The duration of the intervention ranged from six to 21 months. The effectiveness in preventing childhood caries is illustrated in **FIGURE 2**. The probiotic supplements were better than placebo in all seven studies although the difference was statistically nonsignificant in two of them.[26,27] The prevented fraction ranged from 11 to 61 percent with a median of 48 percent. It is important to underline that virtually all families reported regular use of fluoride toothpaste in parallel with the probiotic supplements.

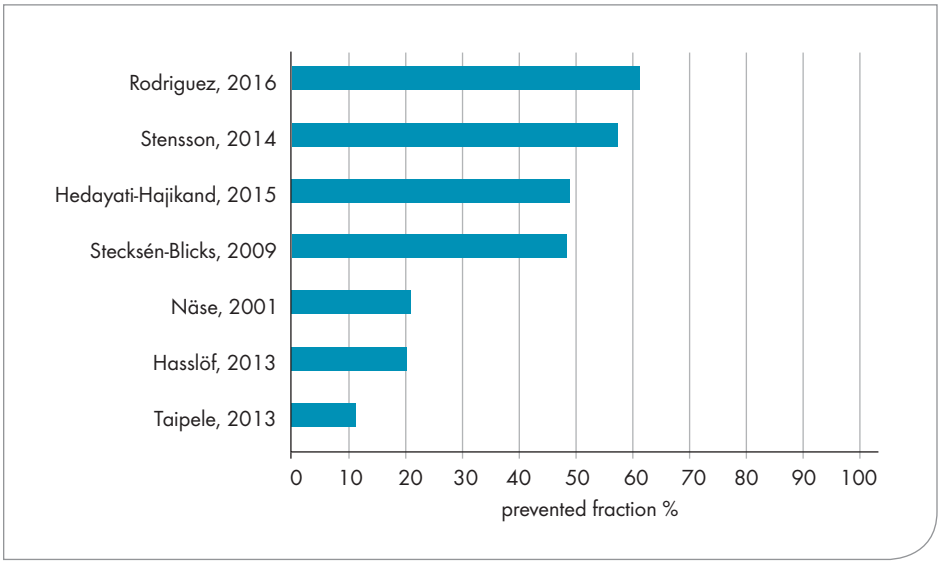

FIGURE 2. Prevention of early childhood caries expressed as prevented fraction (percent) from seven placebo-controlled trials. Modified from Jørgensen et al.[57]

Interestingly, two of the studies reported significant improvements in the general health of the children on top of the dental outcome and a reduced need for antibiotic prescriptions.[29,35] The study by Stensson et al.[28] was of particular interest. Probiotic drops were given to newborn babies during their first year of life and a reduction of caries in the primary dentition was scored eight years later. The results may be interpreted as a "proof of concept" that an early start of probiotic exposure is important in order to support a diverse colonization of the oral biofilm on a "first-come, first-served" basis.[9] The studies carried out in the young permanent dentition point also toward a caries-preventive effect with the best results in schoolchildren with increased caries risk.[32,33] The only adult study focused on root caries over 15 months and both fluoride and probiotic supplements could reverse soft, leathery lesions in a significant way compared to placebo.[34] None of the abovementioned caries studies were, however, free from risk of bias, so further independent studies are required to ascertain efficacy, both from the patient perspective as well as from a public dental health point of view with health-economic analyses.

Probiotics and Periodontal Disease

A large number of studies have addressed the use of probiotics for gingivitis and periodontitis in recent years. Typical clinical endpoints are plaque index, gingival bleeding index, bleeding on probing, periodontal probing depth and clinical attachment loss. In addition, a number of periodontal pathogens and the levels of cytokines and chemokines in gingival crevicular fluid are often assessed as biomarkers of the inflammatory activity. A recent systematic review and meta-analysis has summarized that probiotic therapy compared with placebo reduced bleeding on probing and gingival bleeding in a significant way but did not affect the amount of plaque.[36] Likewise, a meta-analysis supported the adjunctive use of *L. reuteri* to scaling and root planing in the treatment of chronic periodontitis at short-term, especially in deep pockets.[37] Similar conclusions were drawn from the systematic review of Matsubara and co-workers.[38] Based on 12 included studies, it was summarized that oral administration of probiotics improved the recognized clinical signs of chronic and aggressive periodontitis such as probing pocket depth, bleeding on probing and attachment loss, with a concomitant reduction in the levels of major periodontal pathogens. The authors highlighted that a continuous probiotic administration was necessary to maintain these benefits and that the adjunctive use to conventional mechanical treatment was likely to reduce the need for antibiotics. The included studies were however disparate and of limited size which may reduce the strength of these conclusions. Probiotic bacteria have also recently been applied for the prevention and treatment of peri-implant mucositis. Flichy-Fernández and co-workers[39] found that a one-month exposure to *L. reuteri* significantly improved clinical parameters around the implants in edentulous patients compared with placebo.

Probiotics and *Candida*

Oral candidiasis is a common problem among the fragile elderly but it may also appear in young individuals. Over the last years, a number of randomized controlled clinical trials investigating the antifungal effects in the oral cavity from probiotic therapy have been published[40-43] (**TABLE 2**). It is known that probiotic bacteria have the ability to co-aggregate with various *Candida* species, interfere with hyphae formation and inhibit growth via production of bacteriocins.[44] Therefore, it was not surprising to find that all clinical studies resulted in reduced salivary counts of *Candida albicans*, the most common fungi in the oral cavity. Interestingly, the significant reductions seemed to be obtained irrespective of probiotic strains, administration mode and frequency. It should however be underlined that also for opportunistic *Candida* infections probiotic therapy should be regarded as a bioecological adjunct rather than an alternative to the conventional pharmaceutical treatment.

TABLE 2

Most Recent Randomized Controlled Clinical Trials on the Antifungal Effect of Probiotic S Supplements in the Oral Cavity

First author, year	Patient group	Probiotic strain	Duration, dose	Outcome according to authors
Li, 2014[40]	stomatitis	probiotic mix[a]	4 weeks, 3/day	reduced candida counts
Ishikawa, 2015[41]	dentures	probiotic mix[b]	5 weeks, 1/day	reduced candida counts
Kraft-Bodi, 2015[42]	frail elderly	*L. reuteri* (2 strains)[c]	12 weeks, 2/day	reduced candida counts
Mishra, 2016[43]	children	Probiora3[d]	1 week, 2/day	equally effective as CHX[e]

[a] *L. bulgaricus, B. longum, S. thermophiles* in tablets after gargling 2% sodium bicarbonate and 2% nystatin paste
[b] *L. rhamnosus HS111, L. acidophilus HS101, B. bifidum* in capsules
[c] DSM1793; ATCC PTA5289 in lozenges
[d] *S. oralis, S uberis, S. rattus* in oral rinse
[e] 0.2% chlorhexidine digluconat rinse

TABLE 3

Recent Randomized Placebo-Controlled Trials on the Effect of Probiotic Supplements on Oral Malodor

First author, year	Age group	Probiotic strain	Duration	Outcome according to authors
Iwamoto, 2010[45]	adults	*L. salivarius* WB21	14 days	reduced OLT[a]
Keller, 2012[46]	adults	*L. reuteri* (2 strains[b])	14 days	slightly reduced OLT
Suzuki, 2014[47]	adults	*L. salivarius* WB21	14 days	reduced VSC[c] and OLT
Marchetti, 2015[48]	adults	*L. brevis* CD2	14 days	no effect on OLT
Jamali, 2016[49]	children	*S. salivarius* K12[d]	1 week	reduced OLT
Penala, 2016[50]	adults	*L. salivarius* + *L. reuteri*[e]	14 days	reduced OLT

[a] OLT = organoleptic test
[b] PTA5289 and DSM17938
[c] VSC = volatile sulphurus compounds
[d] adjunct to chlorhexidine rinses
[e] adjunct to scaling and root planing

Probiotics and Halitosis

A number of placebo-controlled studies have focused on the treatment of oral malodor as summarized in **TABLE 3**.[45–50] Although all studies but one reported a short-term reduction of halitosis, the studies were heterogeneous with respect to the intervention. The probiotic strains were administrated alone or as adjunct to mechanical cleaning, scaling and root planing and/or antibacterial rinses. It is also important to stress that the main outcome measure was organoleptic scores, which calls for some subjectivity. Due to the short duration of the studies, another issue is whether the improvements in malodor are stable over time.

Probiotics and the Future

What will happen in the next 15 years? In today's -omics era, it is obvious that the general awareness concerning the co-evolvement and symbiosis between the human host and bacteria is increasing along with a demand for "health-by-nature" instead of an overuse of chemicals and antibiotics. Hopefully, probiotic administration to combat biofilm-mediated diseases will help to reduce the need for antibiotics in the future. Furthermore, the common risk factor approach with close links between oral and general diseases (diabetes, metabolic syndrome, obesity), which calls for a patient-centered holistic view, will unite the efforts of dental and medical professionals in health promotion. In fact, due to evidence supporting the role of probiotics for the prevention of eczema in infants, management of side effects related to antibiotics and alleviation of functional bowel symptoms, five states within the European Union have recognized probiotics in their national dietary guidelines.[51] The interest for prebiotic substances that induce the growth or activity of beneficial microorganisms is also emerging. Recently, arginine was described as a genuine oral prebiotic because of its ability to promote a healthy oral ecology from a caries point of view.[52] It is therefore likely that an

increasing number of consumer oral care products with prebiotics and/or orally optimized probiotic strains will be developed and available over the counter in the coming years.

The next probiotic area to investigate in clinical dentistry could very well be oral wound healing and control of postoperative pain and discomfort, for example after third molar surgery. Research has indicated that lactobacilli-derived probiotics may enhance chronic wound healing, which could also be applicable in the oral cavity.[53–55] In this context, our research group has recently shown that bacterial products secreted from *L. reuteri* are noncytotoxic for human gingival fibroblasts and may stimulate the production of prostaglandin E_2.[56] Thus, probiotic bacteria may play a role in the resolution of inflammation in human gingival fibroblasts, which is an important first step in accelerated oral wound healing.

Clinical Considerations

Will general dental practitioners include probiotic therapy in their toolboxes for the prevention and maintenance of oral health? How much more evidence is needed? With novel technologies, there is always a risk of publication bias; positive findings are more likely to be reported, particularly when studies are sponsored by commercial interests. So far, the evidence is not solid enough for general guidelines on a population level, but it is clear that the potential of a beneficial outcome clearly outweighs the risk of harm for the individual patient. Because there are no documented side effects associated with probiotic intervention, it seems reasonable to initiate adjunctive daily probiotic supplements in compliant patients suffering from periodontal conditions, oral *Candida* and/or halitosis. The outcome should be evaluated within a period of three weeks to three months; most often, the patients have subjectively perceived if the supplements were helpful or not. The caries preventive effect displayed in preschool children with high caries risk is of special interest because early childhood caries is associated with impaired quality of life and high costs for families and the society. In this context, the "metabolic domino effect" of gaining both oral and general health seems especially appealing. Yet, in the rapidly growing interest in self-controlled health, a wide range of probiotic products have been marketed directly to the consumer with none or very limited background research. It is the responsibility of each clinician to advocate for safe products with documented effectiveness from clinical trials.

Will general dental practitioners include probiotic therapy in their toolboxes for the prevention and maintenance of oral health?

Conclusions

The current literature displays without a doubt a growing body of evidence that probiotics might help to improve oral conditions such as dental caries, periodontitis, halitosis, mucositis and oral *Candida* load. Probiotic supplements are safe to use and may very well be added to the general practitioner's preventive and therapeutic toolbox. However, further research is needed to verify and expand the current knowledge base and particularly, long-term randomized clinical trials with a health-economic approach would be welcome. ■

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