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Probiotics and oral health effects in children

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Background. Probiotics are living micro-organisms added to food which beneficially affect the host by improving its intestinal microbial balance.
Objective. This paper aims to present a general background on probiotics and its health effects in children, and to examine the evidence for oral colonization and the possible impact on oral health in children and young adults.
Methods. For delivery and general health effects, recent systematic reviews, meta-analyses, and other relevant papers were used. Concerning oral installation and oral effects, a broad search for publications in English was conducted through February 2007 in PubMed. Studies describing an installation or intervention trial in humans with a controlled design and an oral endpoint measure were considered. Fourteen papers with dental focus were identified, of which two were narrative reviews.
Results. Only one study of dental interest was conducted in children. Four papers dealt with oral installation of probiotic bacteria, and although detectable levels were found in saliva shortly after intake, the studies failed to demonstrate a long-term installation. Seven papers evaluated the effect of lactobacilli- or bifidobacteria-derived probiotics on the salivary levels of caries-associated bacteria in placebo-controlled designs. All but one reported a hampering effect on mutans streptococci and/or yeast. The single study carried out in early childhood reported a significant caries reduction in 3- to 4-year-old children after 7 months of daily consumption of probiotic milk.
Conclusion. Bacteriotheraphy in the form of probiotic bacteria with an inhibitory effect on oral pathogens is a promising concept, especially in childhood, but this may not necessarily lead to improved oral health. Further placebo controlled trials that assess carefully selected and defined probiotic strains using standardized outcomes are needed before any clinical recommendations can be made.

Introduction

The belief that probiotic bacteria can influence health dates back to the beginning of the 20th century when the Ukrainian-born Nobel Prize laureate Elie Metchnikoff reported that Bulgarians lived longer than other populations and supposed that this was due to their consumption of fermented milk products containing viable bacteria. The idea was that the harmless bacteria in the fermented products competed with pathogenes injurious to health[1]. By definition, probiotics are live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance as documented in clinical trials[2]. These bacteria must belong to the natural flora in order to survive the acid environment during transit to the intestines. Probiotic bacteria can act through several paths: they prevent cellular adhesion and invasion of pathogenic bacteria, modify the intestinal environment by a reduction in pH as a result of fermentation products, and they interact and modulate the local and systemic inflammatory immune response[3,4].

The current knowledge of the important role of the intestinal microflora has led to strategies to promote health by manipulation of its microbial community[4]. Strategies to influence the microflora include dietary modification, increased intake of unabsorbable carbohydrates and intake of live bacteria of human origin. This latter concept is also commonly termed bacteriotherapy or replacement therapy. The background thinking is that harmless microorganism, such as species of lactobacilli

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and bifidobacteria, can occupy a space in a biofilm that otherwise would be colonized by a pathogen. The interest in such an alternative way to combat infections is rapidly growing, especially in the light of the frequent use of antibiotics with a subsequent risk for development of resistant strains. With the competitive event in the gastrointestinal tract in mind, it seems reasonable to question whether probiotics may be active also in the oral cavity by maintaining health. Furthermore, it has become increasingly clear that strategies directed at eliminating specific caries-associated microorganisms, which are members of the endogenous microflora, have not only been proven to be difficult but maybe also unwise[5]. The first aim of this review was to present a general background on probiotic administration and its health effects in children, and secondly to examine the present evidence for oral colonization of probiotic bacteria and its possible effect on oral health in children and young adults.

Methodology

It was decided to base this paper on recent systematic reviews, meta-analysis, and other relevant papers concerning vehicles of delivery and effects on general health. For the possible impact on oral health, a broad search of the PubMed database was conducted through February 2007, using 'probiotics', 'lactobacilli', 'bifidobacteria', 'oral health', 'caries prevention', 'salivary bacteria', and 'dental plaque' as index terms. Relevant papers published in English were identified after a review of the abstracts. To be considered for inclusion, the studies should describe a human installation or intervention trial with a prospective controlled design. As endpoint measure, some kind of oral outcome was required, such as salivary microbial composition or caries incidence. The initial search revealed 69 abstracts describing oral intervention with probiotics but the vast majority had non-oral endpoint measures. A total of 14 papers with dental focus were identified, of which two were narrative reviews[6,7]. The reference lists of these publications were hand searched for additional papers but no further study was identified. Four investigations dealt with oral colonization of probiotic strains and seven articles described an intervention protocol. One study was excluded since it was a safety assessment with no control group[8]. The results of the remaining papers were compiled in Tables 1 and 2 and further discussed below. The initial intention to focus this review on children's oral health had to be abandoned and reconsidered due to the fact that only one single clinical trial conducted in children was identified.

Origin and vehicles for probiotic delivery

Probiotic bacteria are natural inhabitants of the intestinal flora and the vast majority of the strains and species that are examined in research for their probiotic properties are isolated from healthy humans although there are some that originate from fermented food. Probiotics are often considered in the context of functional foods and this segment is rapidly growing within the European Union. The increasing interest for replacement therapy has, however, opened a market for other consumer products such as lozenges, sucking tablets and

Table 1. Summary of clinical studies on installation and colonization in the oral cavity with lactobacilli- and bifidobacteria-derived probiotics.

Reference	*N*/age	Vehicle, time	Species	Outcome in oral cavity
Meurman *et al.*[9]	9/25 years	Yoghurt, 1 week	*L. rhamnosus GG*	Harboured lactobacilli up to 2 weeks after discontinuation
Busscher *et al.*[10]	14/17–35 years	Yoghurt, 1 week	*L. acidophilus*, *L. casei*, *B. bifidum*	No installation of lactobacilli
Yli-Knuttila *et al.*[11]	56/25 years	Juice, 2 weeks	*L. rhamnosus* GG	No installation of lactobacilli but possible in some cases?
Krasse *et al.*[12]	59/adults	Gums, 2 weeks	*L. reuteri* 'LR-1; LR-2'	65–95% colonized immediately after 14 days intake

Table 2. Summary of controlled clinical intervention studies in the oral cavity with lactobacilli- and bifidobacteria-derived probiotics.

Reference	*N*/age, design	Vehicle, time, control	Species	Oral outcome
Näse *et al.*[13]	594/1–6 years, RCT, DB	Milk, 7 months, placebo	*L. rhamnosus* GG	Decrease counts of MS in saliva, reduced caries
Ahola *et al.*[14]	74/18–35 years, RCT, DB	Cheese, 3 weeks, placebo	*L. rhamnosus*	Decreased counts of yeast and MS in saliva
Nikawa *et al.*[15]	40/20 years, RCT, cross-over	Yoghurt, 2 weeks, placebo	*L. reuteri*	Decrease counts of MS in saliva
Montalto *et al.*[16]	35, 24–33 years, RCT, DB	Liquid, 45 days	*Lactobacillus* spp.	Increased salivary counts of LB, MS unchanged
		Capsules, 45 days placebo	*Lactobacillus* spp.	Increased salivary counts of LB, MS unchanged
Caglar *et al.*[17]	26/21–24 years, RCT, cross-over	Yoghurt, placebo	*Bifidobacteria*	Decreased counts of MS in saliva
Caglar *et al.*[18]	120/21–24 years, RCT, DB	Water/straw, 3 weeks	*L. reuteri*	Decreased counts of MS in saliva
		Lozenges, 3 weeks placebo	*L. reuteri*	Decreased counts of MS in saliva
Krasse *et al.*[12]	58/adults, RCT, DB	Gums, 2 weeks, placebo	*L. reuteri*	Decreased gingivitis
Hatakka *et al.*[19]	294/70–100 years, RCT, DB	Cheese, 16 weeks, placebo	*L. rhamnosus*	Decreased prevalence of oral candida

DB, double blind; LB, lactobacilli; MS, mutans streptococci; RCT, randomized controlled trial.

chewing gums. Probiotics are provided into the food items in one of four basic ways: (i) as a culture concentrate added to beverages (e.g. fruit juice); (ii) inoculated into prebiotic fibres which promote the growth of probiotic bacteria; (iii) inoculated into milk and milk-based foods (e.g. milk drinks, yoghurt, cheese, kefir, biodrinks); and (iv) as lyophilized, dried cells packaged as dietary supplements (tablets, chewing gums, straws). The archetypical probiotic food is yoghurt and daily consumption of dairy products seems to be the most natural way to ingest probiotic bacteria[7]. Another advantage is that milk products contain basic nutrients for the growing child; they are also considered safe for the teeth with possible beneficial effects on the salivary microbial composition and inhibition of caries development, due to their natural content of casein, calcium, and phosphorous[20,21]. A formulation of approximately 10^8 probiotic bacteria per gram or millilitre with an intake of 1.5–2 dL per day is recommended and the dairy products should preferably be nonsweetened and contain only natural sugar. It has been pointed out that the dosage provided via routine consumption may be inadequate for therapeutic benefit[22] and food processing may further compromise bacterial viability. Viable counts may fall below recommended levels, especially at the end of shelf life, and some strains of bifidobacteria used in commercial probiotic food may not survive gastric transit. Most often, a brand name contains a single probiotic strain, but it is important to underline that the efficacy of one lactobacilli strain does not necessarily imply that other strains will be equally efficacious, while a combination of strains can enhance adherence in a synergistic manner[6]. Differences between different strains of the same species are probably the reason for the conflicting results on probiotic efficacy that were reported in the early studies. Today most research is carried out with well-defined dairy-based live lactobacilli strains, but during the last years, reports on gums and tablets have started to appear.

Installation of probiotic bacteria

Before birth, the gastrointestinal tract is sterile but as the newborn child is exposed to bacteria

in the environment and in the diet, the colonization process begins[23]. The intestinal microflora is the major driving force in maturation of the immune system after birth[24]. The colonization of intestine is a rather complex process influenced by microbial exposure, host interactions, and by external and internal factors[25]. An important determinant for the initial composition of the flora is the mode of delivery and children born via cesarean section are less often colonized with bifidobacteria than vaginally born children[26]. During the first month of life, the importance of diet on bacterial colonization of the gut have been explored and breastfed children are dominated by bifidobacteria and lactobacilli while formula-fed children have more bacteroides, clostridia, and enterobacteriaceae[26,27]. This difference might be due to the presence of immunological factors such as immunoglobulin A and lysosyme secreted in human milk that prevents the growth of some bacteria[24].

The probiotic action in the gastrointestinal tract is based on adherence to the intestinal mucosa and thereby inhibition of gut pathogens. Similarly in the oral cavity, probiotics should adhere to dental tissues as a part of the biofilm (or plaque) and compete with the growth of cariogenic bacteria or periodontal pathogens[28]. During and shortly after birth, the epithelial surfaces in the oral cavity become colonized by various species of the indigenous microflora that tend to persist in the mouth, and it is possible that they play a role in the competition with other bacteria and prevent the growth of those that may colonize later[29,30]. Interestingly, Meurman *et al.*[31] showed *in vitro* that a competition between *Lactobacillus rhamnosus* and *Streptococcus sobrinus* occurs. Vaginally born children, who are exposed to more maternal and environmental bacteria at birth than children born via caesarean section, have been shown to be colonized later in life by cariogenic bacteria[32], which may be a result of such a competition between lactobacilli and streptococci.

An early installation and colonization of probiotics in the oral environment would be the first step for an anticipated long-term effect, but there are limited data available to support this event. The main findings of the four identified human studies on oral colonization are summarized in Table 1. The probiotic bacteria were recovered in most subjects during the intermediate days after intake but collectively, the results did not suggest that a permanent installation can take place. It should, however, be kept in mind that the trials were conducted in adults and it may be questioned if a permanent installation readily can occur in persons with an already established microflora[6]. The relatively short contact time between the probiotic product and the plaque is probably not enhancing this event. Since it seems unlikely that probiotics have any significant residual effect after discontinuation of intake[9,10], daily intakes seem to be a prerequisite for potential action. An individual response is, however, evident, underlining the host-dependant factors determining colonization in general. For example, a recent investigation displayed that the probiotic strain *L. rhamnus GG* was not permanently installed but temporarily detected in saliva during a period after three times daily consumption of a probiotic juice[11]. A considerable variation was, however, demonstrated and in some individuals, the probiotic bacteria were detected up to 10–12 days after the last ingestion. Another point to consider is that saliva samples may underestimate the true situation in the oral biofilm and that combinations of probiotic strains may act synergistic and enhance the possibilities for installation[6]. Further research, including cultivation of plaque samples, is therefore needed and it seems especially important to carry out studies on infants because it is very likely that the chance of a permanent colonization of probiotics increases with a regular exposure from early childhood.

General health effects of probiotics in children

The first species introduced into probiotic research were *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and a number of potential health benefits have been suggested such as reduced susceptibility to infections, reductions in allergies, and lactose intolerance, as well as regulation of blood pressure and serum cholesterol values[6,33]. Lower counts of lactobacilli have been observed in children with infantile colic[34–36] and supplementation with *Lactobacillus reuteri* (ATCC 55730) improved the symptoms of colicky children[37].

One systematic review and two meta-analyses have shown that co-administration of probiotics with standard oral rehydration therapy significantly reduced the duration of acute infectious diarrhoea in infants and children, especially diarrhoea due to rotavirus[38–40]. In a recent review by Michail *et al.*[41], the quality of evidence for the use of probiotics for different paediatric disorders was analysed. They reported that the strongest evidence for the clinical effects in children so far has been obtained for treatment of acute infectous diarhorrea, prevention of antibiotic-associated diarrhoea, and prevention and treatment of allergic manifestations. On the other hand, the scientific evidence was inconclusive or lacking concerning other suggested conditions such as inflammatory bowel disease, irritable bowel syndrome, Crohn's disease, ulcerative colitis and cancer prevention. In general, the compliance with the probiotic interventions are described as good and the reported side or adverse effects are extremely rare.

Effects of probiotic bacteria on oral ecology

Seven clinical studies were identified with the aim to evaluate the effect of a daily intake of probiotic bacteria on the salivary levels of mutans streptococci, lactobacilli, and yeast, and one report examined the effect on plaque levels and gingival inflammation. The findings of these studies are summarized in Table 2. Notably, only one of the investigations was conducted in children of preschool age and none of the papers described that side effects had occurred. The studies are reviewed below based on the origin of the species.

Bifidobacteria

Since the late 1980s, a wide range of dairy products containing bifidobacteria has been marketed in several countries worldwide and studies have been performed to validate the survival and positive effects of *Bifidobacterium* DN-173 010 within the gastrointestinal tract[33]. Bifidobacteria are the predominant anaerobic bacteria naturally occurring within the small intestinal lumen and play a critical role in maintaining the equilibrium among normal intestinal flora. In the oral cavity, bifidobacteria are prevalent in deep caries lesions and may play an important role in the progression of caries[42]. The influence of probiotic bifidobacteria on oral ecology has been reported in one single study[17]. The investigation was a double-blind, randomized, cross-over study in which two groups of young adults consumed for 2 weeks either a probiotic yoghurt containing *Bifidobacterium* DN-173 010 bacteria or a control yoghurt without viable bacteria. It was concluded that the yoghurt with living bacteria had a significantly diminishing effect on salivary mutans streptrococci in general but the effect on individuals with the highest counts was rather scanty. No alteration was reported concerning the salivary lactobacilli. More studies are definitely needed before it is possible to draw any conclusions on bifidobacteria.

Lactobacilli

Lactobacilli have gained a great interest in dental research for several decades and modern molecular techniques have underlined the concept that the bacteria are more associated with carious dentine and the advancing front of caries lesions rather than with the initiation of the dental caries process[42]. Polonskaya[43] first described the phenomena that *L. acidophilus* strains may inhibit the *in vitro* growth of other bacteria and this observation has been confirmed by many investigators since then. This event is explained by the fact that lactobacilli can produce low molecular weight bacteriocins with an inhibitory activity against a wide range of bacterial species, including oral streptococci[31,44,45]. Lactobacilli are extremely aciduric and can withstand a pH as low as 3.5, which is a prerequisite to survive the low-pH transition into the intestines.

Four of the five identified publications that evaluated the effect of lactobacilli-derived probiotics on mutans streptococci reported significant reductions of salivary mutans streptococci immediately after the termination of daily intakes[13–15,18]. The diminished post-treatment levels seemed not directly to be dependant on the daily administration vehicle, which was milk, cheese, yoghurt, lozenges, or prepared straws with freeze-dried strains. For example, in the

study by Caglar *et al.*, the use of lozenges and prepared straws were compared with placebo in a randomized design with four parallel arms in young adults[18]. It was anticipated that the slowly melting tablets would allow a more thorough contact between the probiotic bacteria and the oral environment compared with the direct swallowing pattern from the straw, but both regimes reduced the prevalence of salivary mutans streptococci equally after 2 weeks of use. The results may indicate that a direct contact with the oral tissues is not a prerequisite for a beneficial effect. Similar, but at the same time somewhat conflicting, findings were reported by Montalto *et al.*[16]. They evaluated whether an oral and systemic administration of probiotic lactobacilli could change the salivary counts of cariogenic bacteria compared with placebo. The probiotic intervention was given to volunteer subjects in a liquid form and in capsules in order to determine the role of direct contact with the oral tissues. Interestingly, it was found that both ways of administration significantly increased the salivary lactobacilli counts while the levels of mutans streptococci remained unchanged[16]. This result indicates that a pure systemic administration of probiotics could enhance lactobacilli proliferation in the oral cavity[16]. Although there is evidence that oral lactobacilli are influenced by sugar consumption and associated to caries[46], the increased numbers were not suggested to increase the caries risk. First, lactobacilli in general are hardly involved in the initiation of cavities. Second, not all *Lactobacillus* spp. have a caries-inducing effect[47]. And third, a dairy-based vehicle for lactobacilli is favourable because of its buffering effect that may hamper the bacterial acidogenicity. It has been pointed out that *L. rhamnosus* slowly can ferment sucrose and produce lactic acid[13], but Nikawa *et al.*[15] concluded that acids from *L. reuteri* did have a negligible effect on calcium release from the enamel. However, a practical consequence with a 'safty-first' perspective would be not to advocate regular consumption of lactobacilli-derived probiotics to children with open, untreated dental cavities until temporary fillings are placed.

One study tested the hypothesis that cheese containing probiotic bacteria would reduce the prevalence of oral candida[19]. The study was conducted in elderly persons and the daily consumed cheese contained a mixture of lactobacilli and propionibacteria. After 16 weeks, a reduced prevalence of salivary yeast was evident in the intervention group and the probiotic intervention diminshed the risk of harbouring high yeast counts by 75%. However, a general aspect to be rembered is that a reduction of potentially harmful bacteria in the saliva for a shorter or longer period does not necessarily means an improved oral health or fewer cavities.

The lack of probiotic research in odontology is very obvious when it comes to issues that really matters for the patient, and only two randomized controlled trials with caries or gingivitis as endpoint measure were identified[12,13]. The first study was carried out on preschool children, 1–6 years of age, in Finland and the experimental group was served milk containing *L. rhamnosus* GG at their daycare centres 5 days per week for 7 months. Although a significant reduction of the salivary mutans streptococci counts was noted, the outcome on caries development was less pertinent. The best effect was found among the 3- to 4-year-olds in which 6% of the children developed new caries lesions in the experimental group during the study period compared with 15% in the 'normal milk' control group, but it must be remembered that the follow-up period was short. This means that 11 children had to be treated in order to gain one individual that stayed free from the disease (number needed to treat = 11). Despite a relatively high dropout rate, the study was important because it demonstrated that it is possible that a regular intake of probiotic bacteria can prevent dental caries in young children and that the efficiency may vary by age. The second clinical study evaluated the use of probiotic chewing gums on gingival conditions in adults with moderate or severe gingivitis[12]. After 14 days of use of *L. reuteri*, significantly reduced gingival and plaque scores was found compared to baseline as well as to the placebo control group. One single examiner carried out all the clinical examinations but no validation or reproducibility tests of the indices was presented. Further studies with nonsurrogate endpoints and a prolonged duration including health-economic

evaluations are required before any clinical recommendations to combat caries or periodontal pathogens can emerge.

Conclusion

Bacteriotherapy in the form of probiotics seems to be a natural way to maintain health and protect oral tissues from disease, and data suggest that the potential benefits increase with an early childhood start. The research is still in its infancy but a daily intake of probiotic lactobacilli with an inhibitory effect on other bacteria is currently most promising. Milk, milk drinks, or yoghurt containing one or more probiotic strains could be a treatment option in the long-term prevention of childhood caries. However, further double-blind, randomized, placebo-controlled trials that assess carefully selected and defined strains of probiotics using standardized outcomes are needed before any clinical recommendations can be made.

What this paper adds

- There is strong evidence for a beneficial effect of probiotics on diarhorrea and allergies in children.
- There is limited evidence that probiotic bacteria may hamper presence of mutans streptococci in saliva.

Why this paper is important to paediatric dentists

- Probiotic exposure during early childhood when the gastrointestinal microflora is establishing increases the chance of colonization and has a positive life-long influence on health.

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