Periodontology 2000, Vol. 48, 2008, 111–147
Printed in Singapore. All rights reserved

PERIODONTOLOGY 2000

Probiotics and oral healthcare

WIM TEUGHELS, MARK VAN ESSCHE, ISABELLE SLIEPEN & MARC QUIRYNEN

Chemotherapeutics are widely used to prevent and treat infections caused by indigenous and exogenous microbes. The availability of effective and cheap antibiotics in the latter half of the 20th century revolutionized the treatment of infectious diseases and, for developed countries at least, reduced the death rate. The Nobel laureate in immunology, Macfarlane Burnett, stated in 1962 that 'by the late twentieth century, we can anticipate the virtual elimination of infectious diseases as a significant factor in social life'. However, the development of resistance to a range of antibiotics by some important pathogens has raised the possibility of a return to the pre-antibiotic 'dark-ages'. Also, orally, the widespread use of antibiotics is reflected in the level of resistance in the subgingival microbiota of adult periodontitis patients (202). These developments have encouraged researchers in various fields of healthcare to develop alternative antimicrobial approaches. The application of 'health-promoting' bacteria for therapeutic purposes is one of the strongest emerging fields in this regard. Although the use of such probiotics specifically to improve oral health is still in its infancy, oral healthcare workers are probably confronted with dietary probiotics on a daily basis. The widespread oral intake of probiotics as preventive and therapeutic products for gastrointestinal health makes it of considerable interest for oral healthcare workers. These products usually contain streptococci, lactobacilli or bifidobacteria. Therefore, dietary probiotics can confer an oral health risk. This review focussed on the use of probiotics as preventive and therapeutic products for oral healthcare and the potential risks associated with dietary probiotics.

Semantics and history

The term 'probiotic' is a relatively new word meaning 'for life' and it is currently used when referring to bacteria associated with beneficial effects on humans and animals. The use of microorganisms to promote health is very ancient and can even be traced back to the classical Roman literature where food fermented with microorganisms was used as a therapeutic agent (142). Observations showing that relatively harmless bacteria can be introduced into the indigenous microbiota of humans, either to enhance resistance to or to treat infection, goes back to the very origins of microbiology. Pasteur and his associate, Joubert, noted as early as 1877 that the growth of anthrax bacilli in cocultures with 'common bacilli' (probably *Escherichia coli*) was suppressed. They commented that 'these facts perhaps justify the highest hopes for therapeutics' (138). The original observation of the positive role played by some selected bacteria was scientifically investigated by Eli Metchnikoff, the Ukrainian-born Nobel Prize winner working at the Pasteur Institute at the beginning of the last century. He proposed, in 1907, that the lactic acid-producing strain *Lactobacillus bulgaricus* (contained in Bulgarian yoghurt) is able to displace pathological intestinal microbiota. He suggested that 'the dependence of the intestinal microbes on food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes' (119). Around that time, Henry Tissier, a French pediatrician, observed that children with diarrhea had, in their stools, a low number of bacteria characterized by a peculiar, Y-shaped morphology. These 'bifid' bacteria (later on called *Bifidobacterium*) were, on the contrary, abundant in healthy children (191). Tissier suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora. In the following years, some brave physicians attempted to protect against and treat diseases by dosing patients with putatively innocuous commensal bacteria. Alfred Nissle studied soldiers during World War I and isolated bacteria from the stool of soldiers who remained healthy despite the fact that most of their comrades suffered from diarrhea. He used one isolate (*E. coli* stain Nissle 1907) to treat a 20-year-old woman with chronic active ulcerative colitis. After 5 weeks of treatment

with 200 mg/day of the strain, remission was achieved (132). By the end of World War II, several protective treatments had been developed for tuberculosis, anthrax and diphtheria (37). The observations of Metchnikoff, Tissier and others were so appealing that commercial exploitation immediately followed their scientific works. Unfortunately, the results were not always positive and most of these observations were anecdotal. Additionally, except for the treatment of minor ailments or as a supplemental therapy, the application of so-called 'bacteriotherapy' or 'bacterio-prophylaxis' was largely discontinued upon the spectacular advent of antibiotics. Both physicians and the general public became convinced that all infectious diseases would become treatable by antibiotics. Therefore, the bacteriotherapy concept became regarded as scientifically unproven and it received minor interest for decades. But, within the span of a single human generation, many bacterial species adapted to their antibiotic-laced ecosystems, and mutated bacterial strains have developed that are capable of resisting our most potent designer antimicrobials. The medical community must now face the reality that most chemotherapeutic agents are probably destined for a relatively short half-life of effectiveness. This dilemma might encourage us to reconsider Pasteur's approach, that bacteria themselves could be our most effective allies (184). Therefore, research in the probiotic area has progressed considerably in the last 20 years, and significant advances have been made in the selection and characterization of specific probiotic cultures and substantiation of health claims relating to their consumption.

The term 'probiotics', the antonym of the term 'antibiotics', was introduced in 1965 by Lilly & Stillwell as 'Substances produced by microorganisms which promote the growth of other microorganisms' (105). They showed that several species of protozoa, during their logarithmic phases of growth, produce substances that prolong the logarithmic phase in other species. The effect was not as striking as the inhibition of growth caused by antibiotics, but a consistent 50% increase in growth was obtained with *Tetrahymena pyriformis* in response to a factor produced by *Colpidium campylum*. In 1974, Parker described a dietary supplement for animals and extended the definition of probiotics to 'Organisms and substances which contribute to intestinal microbial balance' (137). The importance of living cells in probiotics was emphasized by Fuller, in 1989, who defined probiotics as 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (40). With the definition 'A viable mono- or mixed-culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microbiota' von Havenaar & Huis In't Veld emphasized the necessity for a beneficial effect in humans (61). Since then, several definitions of probiotics have been proposed, as shown in Table 1 (126, 166, 167). The currently used consensus definition of probiotics was put forward by the World Health Organization and by the Food and Agriculture Organization of the United States. They defined probiotics as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' (http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf). It is clear that this definition restricts the use of the word probiotic to products that contain live microorganisms and points out the need for providing an adequate dose of probiotic bacteria in order to exert the desirable effects.

In contrast, 'prebiotics' are generally defined as 'not digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health' (43). These prebiotics include inuline, fructo-oligosaccharides, galacto-oligosaccharides and lactulose. The concept of prebiotics essentially has the same aim as probiotics, which is to improve host health via modulation of the intestinal flora, although by a different mechanism.

However, there are some cases in which prebiotics may be beneficial for the probiotic, especially with regard to bifidobacteria. This is known as the synbiotic concept. Synbiotics are defined as 'mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host' (6).

The term 'probiotics' is often connected to the term 'functional foods'. This term comprises the knowledge of the relationship between foods and health and the effect of food ingredients on physiological functions.

The history of the term 'probiotics' mirrors the rapid developments in our understanding and use of microorganisms in human conditions and diseases. The definition will surely have to be further adapted as we learn even more about the actions of probiotic microorganisms and their interaction with the host (12).

The term 'replacement therapy' (also called 'bacteriotherapy' or 'bacterial interference' is sometimes

Table 1. Definitions of probiotics

Year	Definition	Reference
1965	Substances produced by microorganisms that promote the growth of other microorganisms	Lilly & Stillwell (105)
1974	Organisms and substances that contribute to intestinal microbial balance	Parker (137)
1989	A live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance	Fuller (40)
1992	A viable monoculture or mixed-culture of microorganisms that, when applied to animal or human, beneficially affects the host by improving the properties of the indigenous microflora	Havenaar & Huis In't Veld (61)
1996	Living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition	Schaafsma (166)
1999	A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as by improving nutritional and microbial balance in the intestinal tract	Naidu et al. (126)
2001	A preparation of, or a product containing, viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and as such exert beneficial health effects in this host	Schrezemeir & de Vrese (167)
2001	Live microorganisms that, when administered in adequate amounts, confer a health benefit to the host	FAO / WHO report

Table 2. Differences between 'replacement' therapy and 'probiotic' therapy

Replacement therapy	Probiotic therapy
Effector strain is not ingested and is applied directly on the site of infection	Probiotics are generally used as dietary supplements
Colonization of the site by the effector strain is essential	Probiotics are able to exert a beneficial effect without permanently colonizing the site
Involves dramatic and long-term change in the indigenous microbiota	Rarely a dramatic and long-term microbiological change
Directed at displacing or preventing colonization of a pathogen	
Has a minimal immunological impact	Exerts beneficial effects by influencing the immune system

used interchangeably with 'probiotics' (for review see 210). Although both approaches use live bacteria for the prevention or treatment of infectious disease, there are some slight differences (Table 2). Because there is much confusion over the terminology, we did not specifically differentiate between probiotic therapies and replacement therapies in this review.

Probiotics and general health

Gastrointestinal

Probiotics have traditionally been used to treat diseases related to the gastrointestinal tract. The most widely used species belong to the genera *Lactobacillus* and *Bifidobacteria*, although these species are not predominant in the gastrointestinal microbial ecology. The focus remains, however, on these species because these organisms are already produced in the dairy industry and because they are very rarely implicated in infections of humans. Therefore, they are categorized by the United States Food and Drug Administration as 'Generally Regarded As Safe (GRAS)'.

Several gastrointestinal health claims have been made for probiotics, such as the relief of enzymatic maldigestion (25). Probiotic bacteria containing β-galactosidase can be added to food to improve lactose maldigestion (92). Similarly, milk fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* is tolerated well by lactose maldigesters (deficient in lactase production) compared with regular milk and helps to relieve symptoms such as loose stools and abdominal pain (44, 93, 113). A similar effect has been observed for sucrase-deficient children, in whom the intake of *Saccharomyces cerevisiae* enhances the digestion of a sucrose

load (56). This is explained by the presence of the deficient enzyme (β-galactosidase or sucrase) in the probiotic bacteria administered. Upon ingestion, these bacteria are lysed in the small intestine, releasing their enzymes, which helps to degrade lactose and sucrose in the host. Additionally, the more viscous properties of fermented milk increases the gastro-cecal transit time, which aids the digestion of lactose (206).

Several attempts have been made to determine whether probiotics prevent antibiotic-associated diarrhea. Antibiotic-associated diarrhea, which occurs in ≤20% of patients who receive antibiotics, results mainly from a microbial imbalance and an overgrowth of *Clostridium difficile* and *Klebsiella oxytoca*. *Saccharomyces boulardii* has been shown to reduce this risk and to shorten the duration of antibiotic-associated diarrhea (118, 182). The mechanisms involved remain unclear, but multiple biological effects of yeasts on the population levels of opportunistic pathogens, toxins and intestinal secretion may contribute to the clinical efficacy (32). The therapeutic efficacy of other probiotics, such as *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* and *Enterococcus faecium* SF68 have been suggested (7, 8, 22, 24, 47, 51, 174, 204, 211).

For rotavirus-associated diarrhea, both curative and preventive effects have been shown for selected probiotics. *L. rhamnosus* GG, *Lactobacillus reuteri*, *Lactobacillus casei* Shirota, *Bifidobacterium lactis* and *E. faecium* SF 68 can shorten the duration of rotavirus-associated diarrhea by approximately 1 day (53, 54, 77, 79, 82, 110, 173, 183). *Bifidobacterium bifidum* and *S. thermophilus* have additionally been shown to prevent diarrhea and rotavirus shedding in infants admitted to a hospital (160). Increased rotavirus-specific IgA production, reduced intestinal mucosa permeability and normalization of the intestinal microbiota induced by these probiotics are thought to be the mechanisms behind this favourable outcome (78, 82, 161).

The protective effect of probiotics against various other intestinal infections have been shown in animal models (32). Mechanisms that may drive the protective effects are production of acids, hydrogen peroxide, antimicrobial substances, competition for nutrients or adhesion receptors, antitoxin actions and stimulation of the immune system (114). Several open-label studies in a limited number of patients suggested that some probiotics may help to eradicate pathogens in chronic carriers of *Salmonella* or *Campylobacter*, or to reduce the recurrence of *C. difficile* infections (3, 11, 50, 193). Although antagonistic actions of some lactobacilli have been shown *in vitro* on *Helicobacter pylori* (2, 23, 81, 122), a demonstration of the efficacy *in vivo* has failed to date (103). Some effects can be expected from selected probiotics on the prevention of travellers' diarrhea (73, 96, 134). However, the selection of the probiotic strain is important to attain the desired effect because several studies showed negative results (27, 89).

There is a continuously growing body of literature for a beneficial effect of probiotics in inflammatory bowel disease (12). These are disorders of unknown cause that are characterized by chronic or recurrent intestinal inflammation. Such disorders include ulcerative colitis, Crohn's disease and pouchitis. The latter is thought to be a recurrence of ulcerative colitis in patients who have undergone an ileal pouch anal anastomosis after a proctocolectomy for ulcerative colitis. The etiology of the diseases is not fully understood, but an overly aggressive cell-mediated immune response to luminal commensal bacteria in genetically susceptible hosts is thought to play an important role (164, 170). The currently available data demonstrate that probiotics are more effective in preventing relapse of inflammatory bowel diseases than in suppressing active disease (12). This is illustrated by the solid evidence for activity of *E. coli* Nissle 1917 in maintaining remission in ulcerative colitis (101, 102, 154) and of VSL#3 (containing four strains of lactobacilli, three strains of bifidobacteria and *Streptococcus salivarius* ssp. *thermophilus*) in preventing relapse of chronic pouchitis (46, 205). These indications have found entry into guidelines developed by gastroenterological societies of various countries. There is also some evidence that VSL#3 may prevent pouchitis when administered immediately after surgery (45). The use of probiotics in active inflammation is intriguing, but data are scant (12). Although one of the pioneer studies in modern probiotic therapy examined the efficacy of *E. coli* Nissle 1917 in maintaining remission in Crohn's disease (111), the use of probiotics in this entity of inflammatory bowel disease is still the least substantiated (12).

There are some suggestions that probiotics might reduce the risk for colorectal cancer (214). This hypothesis is based on the observation that selected lactobacilli reduce the activity of certain fecal enzymes that convert pro-carcinogens into carcinogens (214) and on some epidemiological studies which suggest that regular consumption of fermented diary products are related to a lower risk for certain types of cancer (147). Large-scale randomized placebo-controlled trials are currently in progress to substantiate or contradict this hypothesis (114).

Table 3. Substantiated indications of probiotics for gastrointestinal disturbances

Disease	Indication	Reference
Lactose maldigestion	Replace milk with yoghurt	Marteau et al. (114)
Antibiotic-associated diarrhea	Freeze-dried *S. boulardii* or *E. faecium* SF68	Marteau et al. (114)
Prevention of *C. difficile*-associated diarrhea recurrence	Freeze dried *S. boulardii*	Marteau et al. (114)
Rotavirus-associated diarrhea in children	Fermented milk containing *L. rhamnosus* GG	Marteau et al. (114)
Maintaining remission in ulcerative colitis	*E. coli* Nissle 1917	Böhm & Kruis (12)
Prevention of relapse of chronic pouchitis	Combination of *L. acidophilus, B. longum, L. caseï, B. breve, L. plantarum, B. infantis, L. bulgaricus, S. thermophilus* (VSL#3)	Böhm & Kruis (12)
Prevention of pouchitis	Combination of *L. acidophilus, B. longum, L. caseï, B. breve, L. plantarum, B. infantis, L. bulgaricus, S. thermophilus* (VSL#3) when administered immediately after surgery	Böhm & Kruis (12)

B. breve, Bifidobacterium breve.

In general, accumulating evidence suggests that probiotics may have a role in gastrointestinal health. The well-substantiated indications of probiotics for gastrointestinal disturbances are summarized in Table 3.

Urogenital infections

The dominant presence of lactobacilli in the urogenital microbiota of healthy women, and the obliteration of lactobacilli in patients who develop urinary tract infections, bacterial vaginosis and other genital infections, has led to a focus on lactobacilli as potential probiotics for the prevention of urogenital disease. Although a number of so-called probiotic products claim to be useful for treating and preventing urinary tract infections, their marketing is not supported by properly performed human studies (150). In a small-scale study, vaginal application of *L. rhamnosus* GR1 in combination with *L. reuteri* strains appeared to prevent urogenital tract infections at a level similar to that of prebiotic milk or daily antibiotic therapy (72, 148, 149, 151, 152). Several other studies using oral administration of, for example, *L. rhamnosus* GG, *L. rhamnosus* GR1 and *L. reuteri* RC-14 either did not show, or showed only indirectly, a possible reduction of the risk for infection (9, 97, 153). There is no clear evidence that probiotics can help to treat urogenital tract infections (150). However, a recent Cochrane review focussed on the effect of probiotics in preventing urogenital tract infections in pregnant women or in women planning pregnancy (135). Although the studies were too small to draw firm conclusions, pooled results showed an 81% reduction in the risk of genital infection with the use of probiotics (128, 131).

Atopic disease

The prevalence of atopic diseases has been progressively increasing in western societies. The hygiene hypothesis conceives the rapid increase in atopy to be related to reduced exposure to microbes at an early age as a result of constant and thorough hygienic practices, almost sterile food, vaccination, *etc* (181). To date, clinical effects have been seen as a significant improvement in the course of atopic eczema in infants given *L. rhamnosus* GG or *B. lactis* BB-12 (76, 109). The preventive potential of *L. rhamnosus* GG in atopic disease has been demonstrated recently (83, 84). Probiotics administered prenatally and postnatally for 6 months to children at high risk of atopic eczema succeeded in reducing the prevalence of atopic eczema by half compared with that in infants receiving placebo. The precise mechanisms have not been elucidated, but the premise is based upon the ability of lactobacilli to reverse increased intestinal permeability, enhance gut-specific IgA responses, promote gut barrier function through the restoration of normal levels of microbes, and enhance transforming growth factor-β and interleukin-10 production as well as cytokines that promote the production of IgE antibodies (75, 83). Whether the levels of T-helper-1 cells are enhanced and/or T-helper-2 cell dominance is reduced remains to be determined, as do the time-points of these types of events. Certain microorganisms can contribute to the generation of counter-regulatory T-helper cell

immune responses, indicating that the use of specific probiotic microorganisms could redirect the polarized immunological memory to a healthy one (117).

Other types of disease

Although the concept of probiotics may occasionally favour the overestimation of effects, accumulating evidence suggests that probiotics may have a role in human therapies (114). Many other potential applications exist in addition to those summarized above, but more controlled studies are required.

Probiotics and oro-pharyngeal infections

In contrast to what some physicians might think, the oral cavity is not an isolated region within the human body. Anatomically, the oral cavity is connected to the nasopharynx, the larynx, the tonsils and the middle ear through the Eustachian tube. Because the oral cavity is an ecological open growth system, it is conceivable that these anatomically related regions can influence or can be influenced by the oral microbial ecology. As the aim of this article was to review the effect of probiotics on oral health, the use of probiotics to prevent or treat infections of these anatomically neighbouring regions deserves attention. The most relevant studies are summarized in Table 4.

Acute otitis media

Acute otitis media is the most common bacterial infection in young children. The causative bacteria typically translocate from the oro-naso-pharyngeal cavity to the middle ear via the Eustachian tube. The principal strategies to provide protection against repeat infections are antibiotic prophylaxis and fitting tympanostomy tubes. However, the increasing numbers of antibiotic-resistant pathogens, the risk of affecting the balance of the indigenous oro-naso-pharyngeal microbiota (facilitating colonization with pathogens), as well as the costs and risks associated with tympanostomy tube placement, has led researchers to explore the possibilities of using probiotics. The rationale for such an approach lies within the observation that children who are prone to acute otitis media harbor fewer α-hemolytic streptococci in the nasopharynx than children who are more resistant to acute otitis media (10, 13, 38). Additionally, some α-hemolytic streptococci have an interfering activity against pathogens that cause acute otitis media (187). Roos et al. (157) recently reported their experience of spraying α-hemolytic streptococci with interfering activity into the nose of 108 otitis-prone children. The application was initiated immediately after antibiotic therapy for an acute episode of otitis media. The spray consisted of two *Streptococcus sanguinis* strains, two *Streptococcus mitis* strains and one *Streptococcus oralis* strain, in equal proportions. The application continued for 10 days and a 'booster dose' of the spray was given 2 months later. Forty-two per cent (22 of 53) of the children who received the α-hemolytic streptococci spray remained healthy during the follow-up period and had a normal tympanic membrane compared with 22% (12 of 55) of the children in the placebo group. Furthermore, a significantly lower number of children who were treated with active spray had secretory otitis media after 3 months of follow-up. In a similar study (186), 43 children under 4 years of age were sprayed once daily for 4 months with the streptococcal spray and were monitored for 6 months. Sixteen children in the active group and 20 children in the placebo group were evaluated. No significant differences were observed in the number of episodes of acute otitis media and no significant changes of the nasopharyngeal flora occurred. Although both studies seem to contradict each other, it is important to underline that in the latter study, no antibiotic pretreatment was given. Such pretreatment might be of utmost importance for increasing the efficacy of this ecological treatment. Next to pretreatment with antibiotics, the selected effector strains, or the means by which effector strains are introduced into their ecological niche, might be of importance. Hatakka and coworkers examined whether probiotics would reduce the occurrence or duration of acute otitis media, or the nasopharyngeal carriage of otitis pathogens, in otitis-prone children, using probiotic capsules containing two *L. rhamnosus* strains, one *Bifidobacterium breve* strain and one *Propionibacterium freudenreichii* strain (59). Three-hundred and nine otitis-prone children consumed either one probiotic capsule or a placebo capsule, daily, for 24 weeks. The hypothesis of Hatakka et al. was based on positive reports of the beneficial effect of probiotic milk containing *L. rhamnosus* GG on the nasal colonization of *Staphylococcus aureus*, *Streptococcus pneumoniae* and ß-hemolytic streptococci (48) and on respiratory tract infections in children attending day care centers (60). In the study of Hatakka et al. the probiotic treatment showed a tendency to decrease, but did not significantly reduce, the occurrence or

Table 4. Probiotics and oro-pharyngeal infections

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pretreatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Roos et al. (157)	Recurrent acute otitis media	Otitis media prone children (0.5–6 years)	Double blind Randomized Placebo controlled	3	Placebo	55	10 days of antibiotics	Sodium chloride nasal spray	Twice daily for 10 days after pretreatment and after 60 days			(1) Recurrence of otitis media (2) Clinically normal tympanic membrane	Significantly less recurrence of otitis media and more healthy tympanic membranes in the probiotic group
					Probiotic	53	10 days of antibiotics	Sodium chloride nasal spray	Twice daily for 10 days after pretreatment and after 60 days	2 *S. sanguinis* 2 *S. mitis* 1 *S. oralis*	5×10^8		
Tano et al. (186)	Recurrent acute otitis media	Otitis media-prone children (0.4–3.9 years)	Double blind Randomized Placebo controlled	6	Placebo	20	None	Sodium chloride nasal spray	Once daily for 4 months			(1) Recurrence of otitis media (2) Nasal detection of otitis media pathogens (3) Upper respiratory infection episodes	No significant differences between groups
					Probiotic	16	None	Sodium chloride nasal spray	Once daily for 4 months	2 *S. sanguinis* 2 *S. mitis* 1 *S. oralis*	>10 (7)		
Hatakka et al. (59)	Healthy	Otitis media-prone children (0.8–6 years)	Double blind Randomized Placebo controlled	6	Placebo	154	None	Gelatine capsule	Once daily for 6 months	Cellulose microcrystalline		(1) Acute otitis media episodes (2) Upper respiratory infection episodes (3) Nasal detection of otitis media pathogens	No significant differences between groups. Tendency to less upper respiratory tract infections in probiotic group. Significantly increased *M. catarrhalis* prevalence in probiotic group.
					Probiotic	155	None	Gelatine capsule	Once daily for 6 months	*L. rhamnosus* GG *L. rhamnosus* LC705 *B. breve* 99 *P. freudenreichii* JS	8×10^9		

Table 4. *Continued*

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pretreatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Sprunt et al. (180)	'Abnormal' colonization of the nasopharynx	Neonates in an intensive care unit (7–64 days)	Case series	Up to 14	Probiotic	22	Antibiotics	Naso-pharyngeal rinse	Single application	α-Hemolytic streptococcus strain 215	$>1 \times 10^4$	(1) Implantation of the probiotic strain in the nasopharynx (2) Conversion of 'abnormal' colonization to 'normal' colonization of nasopharynx	Implantation can be achieved and the treatment results in a conversion to a 'normal' colonization of the nasopharynx
Roos et al. (156)	Recurrent acute streptococcal pharyngotonsillitis and acute primary pharyngotonsilitis (control group)	Pharyngotonsillitis prone and not prone (control group) patients (2–34 years)	Open label Parallel Prospective	3	Control	149	10 days of antibiotics	None				Recurrence of streptococcal tonsillitis	Less recurrence of streptococcal tonsillitis in probiotic group
					Probiotic	31	10 days of antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment	3 *S. sanguinis* 1 *S. mitis*	1×10^7		
Roos et al. (158)	Recurrent acute streptococcal tonsillitis	Tonsillitis-prone patients (5–40 years)	Double blind Randomized Placebo controlled	3	Placebo	19	10 days of antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment			Recurrence of streptococcal tonsillitis	Significantly less recurrence of tonsillitis in probiotic group vs. placebo group
					Probiotic	17	10 days antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment	1 *S. mitis* 3 *S. sanguinis*	1×10^6		
Roos et al. (159)	Recurrent acute streptococcal pharyngotonsillitis	Pharyngotonsillitis-prone patients (3–59 years)	Double blind Randomized Placebo controlled Multicentre	2	Placebo	61	10 days of antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment	Ethyl cellulose		Recurrence of streptococcal pharyngo-tonsillitis	Significantly less recurrence of tonsillitis in probiotic group vs. placebo group
					Probiotic	51	10 days of antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment	1 *S. mitis* 3 *S. sanguinis*	1×10^6		

Table 4. *Continued*

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pretreatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Falck et al. (34)	Acute streptococcal pharyngotonsillitis	Patients with acute streptococcal pharyngotonsillitis (3–65 years)	Double blind Randomized Placebo controlled	1.5–2.5	Placebo	93	10 days of antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment	Ethyl cellulose		(1) Recurrence of pharyngo-tonsillitis (2) Presence of group A streptococci in throat culture	Significantly less recurrences and healthy group A streptococci carriers in probiotic group vs. placebo group
					Probiotic	189	10 days of antibiotics	Sodium chloride throat spray	Twice daily for 10 days after pretreatment	1 *S. mitis* 3 *S. sanguinis*	7×10^6		
Schwandt et al. (169)	Need for replacement of voice prostheses	Patients with mean prosthesis lifetime <75 days (51–83 years)	Open label Randomized Parallel	6	Buttermilk	8	Replacement of voice prosthesis	Milk drink	125 ml after each meal for 6 months	*L. lactis* *L. lactis cremoris*	?	(1) Lifetime of prosthesis (2) Number of bacteria and yeasts on prosthesis	Significantly increased prosthesis lifetime, significantly lower number of yeasts and tendency for lower bacterial counts in fermented milk group vs. buttermilk group
					Fermented milk	10	Replacement of voice prosthesis	Milk drink	65 ml after each meal for 6 months	*L. casei* Shirota	?		

B. breve, Bifidobacterium breve.

the recurrence of acute otitis media episodes. The probiotics did not affect the carriage of *S. pneumoniae* or *Haemophilus influenzae*, but increased the prevalence of *Moraxella catarrhalis*. It should also be noted that, in this study, no antibiotic pretreatment was given prior to attempting to install the effector strains in their ecological niche and the biofilm was not destroyed. Such pretreatment obviously will reduce the indigenous microbiota. It can be hypothesized that this facilitates a probiotic effect because the probiotic strains do not have to displace bacteria to become installed in the established microbiota.

Streptococcal pharyngotonsillitis

Acute streptococcal pharyngitis infections present a specific danger for susceptible populations, especially young children, the elderly and people living under stressful crowded conditions. The only effective treatment strategy for acute streptococcal pharyngitis infections is the administration of therapeutic doses of a broad-spectrum antibiotic, such as penicillin. The failure of penicillin therapy to eradicate pharyngotonsillitis caused by group A β-hemolytic streptococci is of great clinical concern as it can lead to streptococcal toxic shock syndrome. In more than 35% of patients treated with oral penicillin V and in 37% of patients treated with benzathine penicillin G, the treatment failed from a microbiological point of view at either 10–14 or 29–31 days after therapy (87). Bacterial replacement therapy appears to offer an ecologically sound alternative for the control of streptococcal pharyngotonsillitis. For example, the predominant microorganisms in the pharynx of healthy neonates are one or more species of the α-hemolytic streptococci. The absence of these species was shown to correlate with a significantly increased risk of infections, including sepsis, meningitis, pneumonia and cystitis (for review see 179). Additionally, throat cultures from children who develop *Streptococcus pyogenes* pharyngitis contain a lower proportion of bacteria that are inhibitory or bacteriocidal for *S. pyogenes* than cultures from children who do not become infected (163). Also, in older individuals, natural antibiotic-induced low levels of α-hemolytic streptococci in the pharynx have been shown to correlate with increased susceptibility to *S. pyogenes* infections (39). This suggests the potential for replacement therapy in the prevention of streptococcal pharyngitis in susceptible subjects.

In 1980, Sprunt and coworkers investigated the feasibility of implanting a carefully selected, naturally occurring strain of α-hemolytic streptococcus in the nasopharynx of neonates considered to be at high risk of infection because of the abnormal colonization of their pharynx with potential pathogens (180). The selection of the naturally occurring α-hemolytic streptococcus strain was based on its *in vitro* ability to inhibit a variety of common pathogens that initially colonize the pharynx. Twenty-two infants in the neonatal intensive care unit received nasopharyngeal implantation with this α-hemolytic streptococcus strain. In 16 infants, α-hemolytic streptococci, including the implant strain in pure or mixed α-hemolytic streptococcal populations, constituted the predominant pharyngeal flora within 48–72 h of implantation. The implant strain was not recovered from the remaining six infants. The number of potential pathogens declined to low or undetectable levels and the infants suffered a significantly lower incidence of infections than uninoculated controls. It is interesting to note that although no antibiotic pretreatment was given in this study, the probiotic α-hemolytic streptococcus strain could be successfully implanted. This can be attributed to the study population – neonates – who may not yet have a fully mature nasopharyngeal microbial ecology and therefore are more easily affected by the probiotic effector strain than are adults. This agrees with the hypothesis that a mature endogenous ecological microbiota might impede the installation of an exogenous probiotic strain, and therefore antibiotic pretreatment improves the chance of installation.

Roos et al. (156) selected, in an open and non-randomized study, 31 patients with recurrent streptococcal tonsillitis. They were given antibiotics for 10 days. At the end of this treatment, the patients were sprayed in their mouths with four selected α-hemolytic streptococcal strains known to have strong growth-inhibiting activity *in vitro* against most β-hemolytic streptococci of group A. The follow-up period after this colonization was 3 months. After α-hemolytic streptococcal treatment, none of the patients contracted a new tonsillitis during the follow-up period, in contrast to 8% of the controls who contracted a second tonsillitis.

The second study carried out by the same author was a double-blinded, randomized, placebo-controlled study (158). Thirty-six patients with recurrent streptococcal group A tonsillitis were treated with antibiotics followed by either placebo (19 patients) or a pool of four selected α-hemolytic streptococcal strains (17 patients) with good interfering activity against clinical isolates of β-hemolytic streptococci. No patient in the group treated with α-hemolytic streptococci experienced recurrent streptococcal

group A tonsillitis during the first 2 months of follow-up, in contrast to seven patients treated with antibiotics and placebo. After 3 months, one patient in the group treated with antibiotics and α-streptococci and 11 patients in the placebo-treated group experienced recurrent streptococcal group A tonsillitis.

The third study carried out by this author was a randomized, placebo-controlled, double-blind, multicentre trial (159). A total of 111 patients with recurrence of group A β-hemolytic streptococci and clinical signs of pharyngotonsillitis were analysed. The patients received antibiotics for 10 days, followed by 10 days of test (α-hemolytic streptococci inhibitory to group A β-hemolytic streptococci) or placebo spray therapy. The clinical recurrences (bacteriologically verified) in the test and placebo-treated patient groups were 2% and 23% respectively.

The fourth study was randomized, placebo-controlled and multicentre, and included 342 patients with tonsillitis but did not focus on individuals with recurrent episodes of infection (34). The patients received antibiotic treatment for 10 days, followed by 10 days of test (α-streptococcal) or placebo spray treatment. A significantly lower number of recurrences of tonsillitis were also found in patients treated with the test spray. The recurrence rates in this study were 19% and 30% in patients given α-hemolytic streptococcal spray and placebo, respectively.

In older individuals, natural antibiotic-induced low levels of α-hemolytic streptococci in the pharynx have been shown to correlate with increased susceptibility to bacterial infections (39). This suggests the potential for replacement therapy in the prevention of streptococcal pharyngitis in susceptible subjects but also in the prevention of nosocomial infections. The prevalence of methicillin-resistant *S. aureus* (MRSA) has increased in many hospitals. For this type of infection, alternative, nonantimicrobial methods for reducing MRSA carriage would be more than welcome. Uehara et al. (196) artificially implanted Corynebacterium Co304 into the nares of 17 *S. aureus* carriers. *S. aureus* was completely eradicated in 71% of the carriers by up to 15 inoculations. However, similar doses of 0.9% NaCl or *Streptococcus epidermidis* into the nares of 10 volunteers did not eradicate *S. aureus*. A replacement therapy using a naturally occurring bacteriocin-producing *S. aureus* strain has been shown to be successful in curtailing various diseases caused by this species (4). However, therapeutic use was not possible because the strain was demonstrated to be pathogenic (28).

Voice prostheses

Tracheoesophageal speech with the use of valved prostheses gives patients with laryngectomies the immediate ability to restore their voice after surgery. Tracheoesophageal speech is known to be superior to alternative methods, such as esophageal speech and artificial laryngeal speech. Colonization of the esophageal side of the prosthesis by bacteria and yeasts causes either leakage or increased airflow resistance, which impedes fluent speech, respiration and swallowing (80, 107, 129). Therefore, these prostheses generally need to be replaced every 3–4 months. However, in some individuals they need to be replaced every 1–2 weeks. Anecdotes within the community of patients within the Medical Centre of the University of Groningen (the Netherlands), inspired a group of researchers at this institution to evaluate the effect of probiotics on voice prostheses. In several *in vitro* studies, these authors observed that buttermilk containing *Lactobacillus lactis* and *Lactococcus lactis* ssp. *cremoris*, and a fermented milk drink containing *L. casei* Shirota, decreased the amount of both bacteria and yeasts on voice prostheses. Recently, these positive *in vitro* results were confirmed in an *in vivo* study (169). Eighteen patients with a mean implantation period of fewer than 75 days in the past 6 months and at least 12 months of experience with voice prostheses were enrolled in this prospective study. The mean *in situ* lifetime of the voice prosthesis in the 6 months before entering this study was calculated for each patient and served as an individual control lifetime. After these 6 months, the voice prostheses were replaced and patients were divided into two groups using either buttermilk containing *L. lactis* and *L. lactis* ssp. *cremoris* or a fermented milk drink containing *L. casei* Shirota. A significant reduction in bacterial and yeast prevalence, both *in vitro* and *in vivo*, correlated with a significant increase of the *in situ* lifetime of voice prostheses through the use of a fermented milk drink containing *L. casei* Shirota. Consumption of the fermented milk drink significantly increased the mean *in situ* lifetime of voice prostheses by almost fourfold. This increase in the lifetime of voice prostheses was concurrent with a significant decrease in the number of prosthesis replacements, from 64 in the 6 months preceding the experimental period to 39 in the 6 months during which patients used the fermented milk drink. By contrast, buttermilk containing *L. lactis* and *L. lactis* ssp. *cremoris* did not change the prevalence of bacteria and yeast in biofilms on the explanted

prostheses or the *in situ* lifetime of voice prostheses. Although the beneficial effect of *L. casei*̈ Shirota can be based on multiple pathways, including competitive adhesion or displacement of pathogens and the release of anti-adhesive and antimycotic biosurfactants, it is important to realize that each time a voice prosthesis is replaced, a pristine surface is introduced. This implies that the applied probiotic does not have to establish itself in a biofilm that already exists and therefore the likelihood of success is better. This concurs with the previous elaborated hypothesis on the importance of a microbiological depletion of the ecological niche before attempting to install a probiotic strain. However, the significant clinical difference in this study between buttermilk containing *L. lactis* and *L. lactis* ssp. *cremoris*, and a fermented milk drink containing *L. casei*̈ Shirota, compared with the *in vitro*-determined effect implies that next to providing a microbiologically depleted niche, other factors are at least equally as important for a desirable probiotic effect.

Caries management

The fact that caries is a bacterially mediated process has been known for more than 115 years (123). Since then, research has refined the process of caries development to a multifaceted disease process. Currently, we know that the host, bacteria and nutrients are required to foment the production of organic acids and the subsequent demineralization activity (91). Because, according to this model, all three elements must be present for disease initiation and progression, the removal of any one element leads to the interception of the disease process (5). To overcome the limitations of the traditional disease-management strategies, a number of researchers are developing 'probiotic' methods to treat the caries-causing infection by interfering with the oral colonization of cariogenic pathogens. Although, to date, the number of studies that have been conducted are limited, the results are encouraging and predict major advances in this field. Different treatment strategies are currently under development (Table 5).

Näse et al. (127) were the first to test a dietary *Lactobacillus* strain, *L. rhamnosus* GG, on its caries-inhibiting ability *in vivo*. Their hypothesis was based on the *in vitro* inhibition of a caries pathogen, *Streptococcus sobrinus* (120), and its well-documented effects on the gastrointestinal microbiota. Additionally, *L. rhamnosus* GG belongs to the homofermentative lactobacilli that cannot ferment sucrose or lactose, and is therefore not considered to be cariogenic. The study was a part of a larger investigation conducted to examine the effects of long-term *L. rhamnosus* GG consumption on children's health (60). Five-hundred and ninety-four children, 1–6 years old, from 18 municipal Finnish day care centres, were included in this randomized, double-blind, placebo-controlled intervention study. The children drank either milk containing rather low concentrations ($5–10 \times 10^5$ colony-forming units / ml) of live *L. rhamnosus* GG, or control milk without *L. rhamnosus* GG, in the day care centres 5 days a week for 7 months. The probiotic milk showed just a very moderate tendency to reduce *Streptococcus mutans* levels, which were semiquantitatively detected in a pooled saliva-plaque sample using a diagnostic test. The study did not show a significant reduction in caries prevalence between test and control milk. Interestingly, a tendency for less caries development in the probiotic milk-drinking group was observed for 3–4-year-old children. This might reflect a 'window for infectivity' for *L. rhamnosus* GG, although the oral colonization of this species was not determined. Only a 'risk index' developed by the authors, based on clinical and microbiological data, was significantly reduced in the probiotic milk-using groups. Whether this reduction is also clinically significant is unclear.

In Finland, Ahola et al. (1) examined whether the short-term consumption of cheese containing *L. rhamnosus GG* and *L. rhamnosus* LC 705 would beneficially affect the oral cariogenic microbial flora of young adults when compared with the consumption of regular cheese. Additionally, Ahola et al. questioned whether the potential beneficial effects of the probiotics would persist during the post-treatment period. Their hypothesis was based on the above-mentioned study (127) and on the positive effect of cheese on dental health (100). The study was a randomized, double-blind, controlled study with two parallel groups. During the 3-week intervention period, the 74 adult subjects (age 18–35 years) ate either the probiotic cheese containing *L. rhamnosus* GG (1.9×10^7 colony-forming units / g) and *L. rhamnosus* LC 705 (1.2×10^7 colony-forming units / g), or the control cheese without these bacteria. The authors chose cheese as the vehicle, which is cleared more slowly from the oral cavity than milk. The daily dose was 15 g five times a day. *S. mutans* counts in saliva were determined using a semiquantitative diagnostic kit at baseline, after the 3-week intervention period and at 3 weeks post-treatment. The results showed that cheese *per se* was beneficial, even

Table 5. Probiotics and caries management

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pre-treatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Näse et al. (127)	Healthy Caries Gingivitis	Daycare children (1.3–6.8 years)	Double blind Randomized Placebo controlled	7	Placebo	220	None	Milk	±250 ml, 5 days/week for 7 months			(1) Pooled plaque and salivary Mutans streptococcus scores (2) Caries prevalence (3) Calculated caries-risk score (defined by authors)	No significant differences in *S. mutans* scores and caries prevalence. Significantly reduced calculated caries-risk score in probiotic group vs. placebo group
					Probiotic	231	None	Milk	±250 ml, 5 days/week for 7 months	*L. rhamnosus* GG	$>5 \times 10^5$		
Ahola et al. (1)	Healthy Caries Gingivitis	Young adults (18–35 years)	Double blind Randomized Placebo controlled	1.5	Placebo	36	None	Cheese	15 g, five times/day for 3 weeks			(1) Salivary *S. mutans* counts (2) Salivary yeast counts (3) Salivary lactobacilli counts	Significantly more patients with decreased *S. mutans* counts after 6 weeks in probiotic group. No significant differences in yeast counts. Tendency of fewer patients with high lactobacilli counts after 6 weeks in probiotic group
					Probiotic	38	None	Cheese	15 g, five times/day for 3 weeks	*L. rhamnosus* GG *L. rhamnosus* LC 705	$>1 \times 10^7$ /g		
Montalto et al. (124)	Healthy	Young adults (23–37 years)	Double blind Randomized Placebo controlled	1.5	Placebo	5	None	Capsule and liquid	45 days, no further info.			(1) Salivary *S. mutans* counts (2) Salivary lactobacilli counts	Significant increase in lactobacilli in both probiotic groups. No change in *S. mutans* counts.
					Probiotic A	14	None	Capsule and placebo liquid	45 days, no further info.	*L. sporogens* *L. bifidum* *L. bulgaricus* *L. termophilus* *L. acidophilus* *L. caseï* *L. rhamnosus*	$>1 \times 10^9$ /day		
					Probiotic B	16	None	Liquid and placebo capsule	45 days, no further info.	*L. sporogens* *L. bifidum* *L. bulgaricus* *L. termophilus* *L. acidophilus* *L. caseï* *L. rhamnosus*	$>1 \times 10^9$ /day		

Table 5. *Continued*

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pre-treatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Nikawa et al. (130)	Healthy	Female dental hygienist students (20 years)	Double-blind two-way crossover	1	Control	40 in total	None	Yogurt	Once daily for 2 weeks	*S. thermophilus* *L. bulgaris*	?	Salivary *S. mutans* counts	Probiotic yogurt significantly decreased *S. mutans* counts in contrast to control yogurt
					Probiotic	40 in total	None	Yogurt	Once daily for 2 weeks	*S. thermophilus* *L. reuteri*	?		
Caglar et al. (18)	Healthy Gingivitis	Young adults (21–24 years)	Double-blind crossover with 2-week washout period Randomized	1.5	Control	21 in total	None	Yogurt	Once daily for 2 weeks			(1) Salivary *S. mutans* counts (2) Salivary lactobacillus counts	Probiotic yogurt significantly decreased *S. mutans* counts in contrast to control yoghurt. No effect on lactobacillus counts
					Probiotic	21 in total	None	Yogurt	Once daily for 2 weeks	*Bifidobacterium* DN-173 010	7×10^7 /g		
Caglar et al. (17)	Healthy	Young adults (21–24 years)	Randomized Placebo controlled	0.75	Placebo A	30	None	Straw containing oil drop	Once daily for 3 weeks by using straw to drink 200 ml of tap water			(1) Salivary *S. mutans* counts (2) Salivary lactobacilli counts	Significant reduction in salivary *S. mutants* counts in both probiotic groups vs. placebo groups. No change in lactobacilli counts
					Probiotic A	30	None	Straw containing oil drop	Once daily for 3 weeks by using straw to drink 200 ml of tap water	*L. reuteri* ATCC 55730	1×10^8		
					Placebo B	30	None	Tablet	Once daily				
					Probiotic B	30	None	Tablet	Once daily	*L. reuteri* ATCC 55730	1×10^8		

L. acidophilus, Lactobacillus acidophilus.

during such a short intervention, in reducing *S. mutans*. However, no statistically significant differences in *S. mutans* counts were found between the study groups during the intervention. By contrast, there were significantly more subjects in the probiotic group than in the control group whose *S. mutans* count decreased during the 3-week post-treatment period compared with the samples taken after the intervention. When comparing between groups, *S. mutans* counts were reduced significantly more in the intervention group, but only during the post-treatment period. This might imply that the intervention time was too short to show differences between the study groups. The results of the present study show that this type of probiotic intervention might be beneficial to those with the high *S. mutans* counts.

Montalto et al. (124) evaluated whether there was any difference between taking probiotic lactobacilli in liquid form or in capsules on *S. mutans* counts in a 45-day double-blind, randomized, placebo-controlled intervention study. Thirty-five healthy adult volunteers (age 23–37 years) were randomly divided into three different treatment groups: placebo; probiotic administration in liquid form; or probiotic administration in capsule form. The probiotic bacteria used were *Lactobacillus sporogens*, *Lactobacillus bifidum*, *L. bulgaricus*, *Lactobacillus termophilus*, *L. acidophilus*, *L. casei* and *L. rhamnosus* at a dosage of 1.9×10^9 live cells / day. *S. mutans* counts and the number of lactobacilli in saliva were determined using a semiquantitative diagnostic kit at baseline and after the 45-day intervention period. The oral administration of these *Lactobacillus* spp. significantly increased the salivary counts of lactobacilli. The effect occurred irrespective whether the lactobacilli were administered in liquid or in capsule form, indicating that probiotics ingested in capsular form might result in a temporary increase in oral lactobacilli. The *S. mutans* counts were not significantly modified by the intervention.

Based on the caries-preventive effects of *L. rhamnosus* GG (127), Nikawa et al. (130) examined the effects of *L. reuteri*-containing yogurt on the oral carriage of mutans streptococci. *L. reuteri* is an obligate heterofermentative resident in the gastrointestinal tracts of humans, and it is reported to produce compounds that exhibit antagonistic activity, such as reuterin (185) and reutericyclin (41), which are water-soluble, broad-spectrum antimicrobials, effective over a wide pH range, and resistant to proteolytic and lipolytic enzymes (30). A group of 40 dental hygienists (age 20 years) were divided into two groups in this double-blind, crossover study. Subjects in the first group were given placebo yogurt, daily for a period of 2 weeks, and then *L. reuteri*-containing yogurt, daily for another 2 weeks. Subjects in the second group were given *L. reuteri*-containing yogurt, daily for 2 weeks, and then placebo yogurt, daily for another 2 weeks. The levels of *S. mutans* in unstimulated saliva were determined by microbial culture. Eating *L. reuteri*-containing yogurt daily for 2 weeks significantly reduced the *S. mutans* levels in saliva by 0.5 $\log_{10}$ colony-forming units. Such an effect was not observed when placebo yogurt was consumed. However, the reduced *S. mutans* levels were maintained when the placebo yogurt was consumed after consuming the *L. reuteri*-containing yogurt (group 2). Taken together, these results suggest that *L. reuteri* in yogurt reduces the *S. mutans* levels in saliva for at least up to 2 weeks after discontinuing the consumption.

The effect of *L. reuteri* on salivary *S. mutants* and lactobacilli counts was also investigated by Caglar et al. (17). Based on the observation that most studies mentioned above used dairy vehicles to deliver probiotic bacteria in the oral cavity, they questioned whether similar effects can be achieved using non-dietary consumer products intended for oral use. They investigated the effect of the probiotic bacterium, *L. reuteri* ATCC 55730, on the levels of salivary mutans streptococci and lactobacilli in young adults when ingested by two different delivery systems. One-hundred and twenty healthy young adults (21–24 years of age) were enrolled in a randomized placebo-controlled study design with parallel arms. No pretreatment was given to the patients. The patients were randomly assigned to four groups: group A drank 200 ml of water through a prepared straw containing *L. reuteri* ATCC 55730 once daily for 3 weeks, while group B drank 200 ml of water through a placebo straw once daily for 3 weeks. Group C was given one tablet containing *L. reuteri* ATCC 55730 once daily for 3 weeks, while group D received placebo tablets without bacteria. Salivary mutans streptococci and lactobacilli were enumerated with chair-side semiquantitative kits at baseline and 1 day after the final ingestion. A statistically significant reduction of the mutans streptococci levels was recorded after ingestion of the probiotic bacteria via either the straw or the tablets, in contrast to the placebo controls.

Next to lactobacilli, bifidobacteria are probiotics commonly used for improving the intestinal microbial balance. Caglar et al. (18) were the first to report the effect of bifidobacteria-derived probiotics on the

oral microbiota. The aim of the study was to examine whether short-term consumption of yogurt containing bifidobacteria affected the levels of salivary mutans streptococci and lactobacilli in young adults. Their hypothesis, of a beneficial effect, was based on the in-general claimed health-promoting effects of bifidobacteria. The study group comprised 21 subjects (age 21–24 years). The study had a double-blind, randomized crossover design and the experimental period consisted of four consecutive time-periods. During periods 2 and 4 the subjects consumed 200 g of yogurt, containing either *Bifidobacterium* DN-173 010 (7×10^7 colony-forming units / g) or no bifidobacteria (control), each day for 2 weeks. Periods 1 and 3 were run-ins and washout periods of 1 and 4 weeks, respectively. *S. mutans* and lactobacilli counts in saliva were determined using a semiquantitative diagnostic kit. The data showed that when bifidobacterium-containing yogurt was consumed, a small, but significant, decrease in salivary *S. mutans* counts occured, in contrast to the lactobacillus counts, which remained unaffected.

Most of the probiotic intervention studies for caries prevention use dietary probiotics that are often employed for the prevention of gastrointestinal pathologies. This line of research has primarily focussed on the potential usefulness of dietary lactobacillus and bifidobacterium strains. This is, however, rather surprising after evaluating the results of replacement therapies for the prevention and treatment of oto-oro-pharyngeal infections, such as acute otitis media or pharyngotonsillitis. The most pronounced successes in these studies were made by using α-hemolytic streptococci after an antibiotic pretreatment (34, 156–159, 179).

Lactic acid bacteria are of considerable interest for oral healthcare as a result of their cariogenic potential. They are highly acidogenic, owing to the production of short-chain carboxylic acids, by fermenting sucrose and consequently lowering the pH, which all dissolve hard tissues such as enamel and dentine (192). However, Fitzgerald et al. (36) showed that only three of 50 lactobacillus strains isolated from the dental plaque of schoolchildren induced significant caries activity in conventional hamsters. Additionally, in an artificial caries model, lactobacilli produced significantly shallower caries lesions than *S. mutans* and *Actinomyces israelii*, although a synergistic effect on the growth of *S. mutans* and *A. israelii* was observed in mixed cultures where *L. acidophilus* was present (172). Therefore, it can be concluded that, in contrast to mutans streptococci, lactobacilli are more related to caries progression than to the initiation of a caries lesion (29, 112). By no means does this imply that lactobacilli are not capable of inducing caries under favorable environmental conditions. Out of 32 lactobacillus strains, comprising eight species and obtained from human dental plaque or other sources, 17 were moderately to highly cariogenic in rats receiving a cariogenic diet (35). Only one, an *L. lactis* strain, was scored as noncariogenic. A similar observation was recently made for *Lactobacillus salivarius* (115). These data suggest that, for patients undergoing long-term probiotic treatment with lactobacilli (e.g. for gastrointestinal disorders), dental health should be monitored closely during the treatment and patients should be caries free prior to the initiation of the probiotic treatment.

Lactobacillus strains are, however, also found in orally healthy persons. Therefore, one might speculate that *Lactobacillus* strains from the oral cavity of caries-free persons possess inhibitory properties towards cariogenic bacteria. Such inhibitory properties are, however, rather uncommon. Sookkhee and coworkers isolated 3790 lactic acid bacteria from 130 orally healthy volunteers. These strains were all screened for their ability to inhibit the growth on agar of several oral pathogens, such as *S. mutans* and *Actinomyces viscosus*, but also *Porphyromonas gingivalis* and *Candida albicans*. Only five oral *Lactobacillus* isolates were good antimicrobial producers that could inhibit a number of oral pathogens. The strains with the most omnipotent antimicrobial activity were *L. paracasei* and *L. rhamnosus*. The latter is a well-known gastrointestinal probiotic.

The vehicle by which probiotics are ingested or delivered in the oral cavity can, however, influence the cariogenic potential and the oral colonization of a probiotic. Fortunately, the most commonly used dietary lactobacilli are consumed in milk products (e.g. fermented milk drink, yoghurt, or cheese). When lactic acid bacteria are being consumed in milk products, the buffer capacity of the milk will decrease the production of acid. The presence of calcium, calcium lactate and other organic and inorganic compounds in milk are anticariogenic (42, 88) and reduce the colonization of pathogens (168). Lactobacilli, in general, are weakly adhesive to surfaces, although they are frequently isolated from retention sites in the oral cavity (200). As the daily consumption of lactobacilli might lead to a transient (albeit permanent) colonization of these bacteria, there might be a potential dental health risk with the daily consumption of probiotic lactobacilli. Busscher and coworkers investigated whether the daily consumption of yoghurt containing *L. acidophilus, L. caseï* and

a *Bifidobacterium bifidum*, by a group of test persons selected on the basis of absence of demonstrable lactobacilli in the oral cavity, would lead to the installation of lactobacilli (16). After 1 week of consumption of the yoghurt, salivary and interproximal plaque samples were still free of lactobacilli. Similarly to the results of Busscher and coworkers, Petti and coworkers were unable to detect an oral colonization of lactobacilli after yoghurt consumption (141). By contrast, volunteers who consumed a daily dose of 250 g of yoghurt containing *L. rhamnosus* GG harbored this lactobacillus in their saliva for up to 2 weeks after discontinuing consumption of yoghurt (121). The same group of researchers recently repeated this experiment using a fruit juice containing *L. rhamnosus* GG for 2 weeks (219). *L. rhamnosus* GG was detected only temporarily, for up to 1 week after discontinuation of the fruit juice. In one female subject, however, whose medical history revealed use of *L. rhamnosus* GG in childhood, the bacterium was detected in all saliva samples taken up to 5 months after discontinuation of the fruit juice. Similarly, Montalto and coworkers reported increased salivary lactobacilli counts after 45 days of use of either a liquid or capsules containing *L. sporogens*, *L. bifidum*, *L. bulgaricus*, *L. termophilus*, *L. acidophilus*, *L. caseï* and *L. rhamnosus* (124). Apparently, probiotic lactobacilli do not colonize the oral cavity permanently. Once the probiotic treatment has been stopped, the probiotic bacteria are eradicated from the oral cavity within weeks. This observation is comforting regarding the safety of dietary probiotics, although it underlines the necessity for repeated application in order to maintain the probiotic effects. However, because in none of the studies were the indigenous microbiota suppressed prior to the initiation of the probiotic therapy (e.g. antibiotics, antiseptics and professional plaque removal), it is possible that the maturation of the indigenous biofilm may be responsible for the only temporary colonization of the probiotics used. This can be derived from the fact that in patients without demonstrable lactobacilli in the oral cavity, even a temporary colonization could not be achieved (16). Additionally, a subject who had received *L. rhamnosus* GG milk at 10 years of age for 1 year as a supportive treatment for atopic dermatitis and thereafter did not use *L. rhamnosus* GG-containing products, was apparently permanently colonized with this strain. This is in line with studies using probiotics in the prevention of acute otitis media and streptococcal pharyngotonsilitis (34, 156–159, 180), which show that an antibiotic pretreatment to inhibit the indigenous microbiota facilitates a probiotic effect in adults with a mature microbiota and that a nasopharyngeal microbial ecology (e.g. neonates) which is not yet fully matured can be more easily affected by a probiotic effector strain than the mature nasopharyngeal microbial ecology of adults. The latter can be corroborated in the oral cavity by the so-called 'window of infectivity' that exists for the acquisition of *S. mutans* and *S. sanguinis* (20, 21). It seems that if a permanent oral colonization of an effector strain is desired in adults, a new 'window of infectivity' should be created by pretreatment of the oral microbiota (e.g. disinfection) to reduce the indigenous microbiota.

Although it seems difficult, albeit impossible, to displace pathogens by dietary probiotics in a mature oral microbial ecology, the 'replacement therapy' of Hillman and coworkers could overcome this obstacle. The ability of an effector strain to colonize preemptively the human oral cavity and aggressively displace indigenous wild-type strains is complex. However, Hillman and coworkers isolated, from a human subject, a strain of *S. mutans* that produces a bacteriocin called mutacin 1140 which was capable of killing virtually all other strains of mutans streptococci against which it was tested (65). Mutants were isolated that produced threefold elevated amounts of mutacin 1140. Apparently, these mutant strains persistently colonize the oral cavities of human subjects and aggressively displace indigenous mutans streptococci (66, 71). Three years following a single, 3-min infection regimen involving brushing and flossing of a concentrated cell suspension onto and between the teeth, all of the subjects remained colonized. No other strains of mutans streptococci were observed in saliva and plaque samples of these colonized volunteers. The same results were found recently, 14 years after colonization, for at least two of three subjects who were still available for testing. Consequently, *S. mutans* strain JH1140, which has a spontaneous mutation resulting in a threefold elevated production of mutacin 1140, served as the starting strain for the construction of a clinically applicable effector strain for replacement therapy against caries. In accordance with the acidogenic theory of dental caries, lactic acid production by *S. mutans* has long been considered to be the main pathogenic mechanism for the production of caries lesions. Consequently, recombinant DNA methods were used to delete essentially the entire open reading frame for lactic acid dehydrogenase. This mutation created a metabolic blockade that was lethal when exchanged for the wild-type allele, but it was

found that replacing the open reading frame for lactic acid dehydrogenase with the open reading frame for alcohol dehydrogenase B from *Zymomonas mobilis* overcame this blockade to yield a viable strain called BCS3-L1. The resulting BCS3-L1 effector strain has no measurable lactic acid dehydrogenase activity and approximately 10-fold elevated levels of alcohol dehydrogenase activity relative to its parent. Fermentation end-product analysis showed that BCS3-L1 produced no detectable lactic acid. Therefore, this strain might act as a worthy effector strain for replacement therapy for caries. The ability of BCS3-L1 to serve as an effector strain in the replacement therapy of dental caries was extensively tested in the laboratory and in animal models (64). The strain was found to have significantly reduced pathogenic potential: it persistently and pre-emptively colonized the niche on the tooth surface normally occupied by wild-type strains of *S. mutans*; it was genetically stable; and it showed no ill effects in acute or chronic toxicity studies. If BCS3-L1 could be shown to have similar properties in humans, it would serve as an idealized effector strain. Unfortunately, although the data look promising, no human trials have as yet been conducted. This is a result of the fact that the use of a persistently implanted, genetically modified bacterium has not been attempted for any purpose. However, as Hillman et al. (67) have recently constructed a BCS3-L1 mutant to test the safety of BCS3-L1 in human trials, the start of a phase 1 clinical trial might be expected shortly.

Bacterial and fungal periodontal infections

The limited knowledge regarding the effect of probiotics on plaque-related periodontitis is even more striking. The oral microbiota is at least equally as complex as the gastrointestinal or vaginal microbiota. Moreover, dental biofilms are considered to be difficult therapeutic targets (177). The current view on the etiology of plaque-related periodontal inflammation considers three factors that determine whether disease will develop in a subject (175, 176, 212): a susceptible host; the presence of pathogenic species; and the reduction or absence of so-called 'beneficial bacteria'. Because it is difficult to influence the host response without the risk of serious side-effects (e.g. as a result of the use of cyclooxygenase-2 inhibitors), periodontal therapy especially envisages the reduction of the bacterial threat (162). The worldwide treatment strategy applied for periodontal disease is based on mechanical subgingival debridement (eventually including periodontal surgery to reduce the depth of the periodontal pocket), in combination with improved oral hygiene (55). This shifts the subgingival flora to a less pathogenic composition, characterized by high proportions of gram-positive aerobic species (155, 217). Although reductions in the total subgingival microbiota of up to two-log values can easily be achieved, a recolonization, primarily by less pathogenic bacteria, towards baseline numbers occurs within 1–2 weeks (49, 57, 108). The shift towards a less pathogenic microbiota is only temporary, with the re-establishment of a more aggressive microbiota within weeks to months (106, 125, 146, 203, 208). The dynamics of this recolonization depends on the level of oral hygiene, the efficacy of the subgingival debridement and the residual probing depth (106, 139, 140, 165, 203). The use of antibiotics or antiseptics, either locally or systemically, does not really improve the long-term effect of periodontal therapy (145). Therefore, some authors start to focus on the third etiological factor for plaque-related periodontal inflammation, namely 'the reduction or absence of so-called beneficial bacteria'. From a theoretical point of view, restoring these reduced numbers of beneficial bacteria via probiotics might be of considerable interest in the treatment of plaque-related periodontal diseases. Probiotics might not only suppress the emergence of endogenous pathogens or prevent the superinfection with exogenous pathogens, they might also protect us through the promotion of a beneficial host response (155). The most relevant studies are summarized in Table 6.

In 1954, a beneficial effect of lactic acid bacteria on inflammatory infections of the oral mucosa was reported (98). Noteworthy also are some Russian anecdotal reports on the use of probiotics in the treatment of periodontitis. The use of a Russian probiotic preparation called Acilact, a complex of five live lyophilized lactic acid bacteria, with or without '*Bifidumbacterin*' (probably *Bifidobacterium*) is claimed to improve both clinical and microbiological parameters in patients with gingivitis and mild periodontitis (52, 143). Recently, a periodontal dressing consisting of collagen and *L. caseï* 37 was reported to exert a beneficial effect on the subgingival microbiota of periodontal pockets (207). Unfortunately, difficulties in obtaining and translating these original papers have made it impossible to include these studies in this review.

The first well-substantiated and large-scale research effort on the applicability of probiotics in periodontitis, in this case replacement therapy, was

Table 6. Probiotics and bacterial periodontal infections

Study	Type of infection present at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pre-treatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Hillman & Shivers (68)	Artificial oral *A. actinomycetemcomitans* infection	Gnotobiotic rats (21 days)	Parallel Open label Placebo controlled	1.25	Placebo A	6	None	Sterile broth applied with swap	Single application at baseline			(1) *A. actinomycetemcomitans* counts (2) *S. sanguinis* counts	Significantly lower *A. actinomycetemcomitans* counts in probiotic A and B groups vs. placebo A and B groups. No significant differences in *S. sanguinis* counts among placebo B, probiotic A and probiotic B groups
					Placebo B	6	None	Overnight broth culture applied with swap	Single application at baseline	*S. sanguinis* KJ3sm hydrogen peroxidase mutant			
					Probiotic A	6	None	Overnight broth culture applied with swap	Single application at baseline	*S. sanguinis* KJ3sm			
					Probiotic B	6	None	Overnight broth culture applied with swap	Single application at baseline	Revertant of *S. sanguinis* KJ3sm hydrogen peroxidase mutant			
Ishikawa et al. (74)	Not mentioned	Adult (22–62 years)	Parallel Open label	1	Control	21	None					Bacterial numbers in saliva	No significant effects of change in total number of bacteria, number of mutans streptococci, or number of lactobacilli. Significant reduction in number of black-pigmented anerobic rods for both probiotic groups
					Probiotic A	27	None	Tablets	Five tablets, five times / day for 8 weeks	*L. salivarius* TI 2711	2×10^7 / day		
					Probiotic B	28	None	Tablets	Five tablets, five times / day for 8 weeks	*L. salivarius* TI 2711	1×10^8 / day		
Krasse et al. (99)	Gingivitis	Adult (age unknown)	Parallel Double blind Placebo controlled Randomized	0.5	Placebo	18	Plaque removal	Chewing gum	Twice daily for 14 days			(1) Gingivitis scores (2) Plaque scores	Significantly higher gingivitis reduction in probiotic group 1 vs. placebo group. Significant reduction in plaque scores for both probiotic groups vs. no reduction in placebo group
					Probiotic A	20	Plaque removal	Chewing gum	Twice daily for 14 days	*L. reuteri* strain 1	1×10^8		
					Probiotic B	21	Plaque removal	Chewing gum	Twice daily for 14 days	*L. reuteri* strain 2	1×10^8		

Table 6. *Continued*

Study	Type of infection present at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pre-treatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Kang et al. (85)	Healthy Gingivitis	Young adult (20–30 years)	One-way crossover Open label Placebo controlled	1 day	Placebo	72	None	Oral rinse	15 ml, three times/day for 2 min			Plaque scores	Significant reduction in plaque score for the probiotic group
					Probiotic	72	None	Oral rinse	15 ml, three times/day for 2 min	*W. cibaria* CMS1	1×10^9 /ml		
Matsuoka et al. (116)	Perio-dontitis	Adult	Double blind Placebo controlled Parallel	4	Placebo	45	None	Tablets	5 tablets, five times/day for 12 weeks			(1) Pocket depth (2) Bleeding upon probing (3) Plaque index (4) Sub-gingival micro-biological changes	Significant changes in bleeding upon probing, pocket depth and total subgingival bacteria in both probiotic A and placebo groups. No significant intergroup differences for these assessment criteria. Significant reduction during active treatment for *P. gingivalis* in probiotic A group only. Increased *L. salivarius* counts in probiotic A group
					Probiotic A	50	None	Tablets	Five tablets, five times/day for 12 weeks	*L. salivarius* TI 2711	2×10^8		
					Probiotic B	13	None	Tablets	Five tablets, five times/day for 12 weeks	*L. salivarius* TI 2711	2×10^7		
Teughels et al. (190)	Artificially created periodontal pockets	Beagle dogs	Split mouth Double blind	3	No treatment	8, two pockets/dog	None	None				(1) Sub-gingival micro-biological changes (2) Bleeding upon probing (3) Probing pocket depth (4) Clinical attachment level	Probiotic group 2 showed significantly more pronounced reductions in total anerobic bacteria, black-pigmented bacteria and *Campylobacter rectus* and a retarded bacterial recolonization of pockets compared with the control group. Significantly more reduction in bleeding upon probing in probiotic group 2 when compared with the control group
					Control	8, two pockets/dog	Scaling and root planing	None					
					Probiotic A	8, two pockets/dog	Scaling and root planing	Pure bacterial pellets	Subgingivally applied once (baseline)	*S. salivarius* *S. mitis* *S. sanguinis*	$>1 \times 10^9$ /ml		
					Probiotic B	8, two pockets/dog	Scaling and root planing	Pure bacterial pellets	Subgingivally applied four times (baseline, and weeks 1, 2 and 4)	*S. salivarius* *S. mitis* *S. sanguinis*	$>1 \times 10^9$ /ml		

A. actinomycetemcomitans, Actinobacillus actinomycetemcomitans.

initiated in the late 1970s by Socransky's group at the Forsyth Institute in Boston (USA). This group of researchers found that subgingival plaque samples of healthy patients contained organisms that could inhibit the growth of *Actinobacillus actinomycetemcomitans* [recently reclassified as *Aggregatibacter actinomycetemcomitans* (133)] and other (at that time presumed) periodontopathogens (69, 70). Subgingival plaque samples from diseased sites of patients with localized juvenile periodontitis and patients with refractory periodontitis almost invariably lacked such inhibitory bacteria. Interestingly, subgingival plaque samples from clinically healthy sites in these periodontitis patients contained inhibitory bacteria in proportions similar to those in subgingival plaque taken from healthy control patients. These microorganisms that inhibited the growth of periodontopathogens were almost invariably identified, at that time, as *Streptococcus sanguis* [later on renamed as *S. sanguinis* (195)] and *Streptococcus uberis*. The basis for their inhibition of *A. actinomycetemcomitans* lay in the production of hydrogen peroxide. These findings, together with the strong negative association between *A. actinomycetemcomitans* and *S. sanguinis* in studies of the predominant culturable microbiota in plaque from healthy and diseased periodontal pockets, encouraged these researchers to proceed to *in vivo* studies using *S. sanguinis* as an effector strain (70, 213). Four groups of six gnotobiotic rats at the age of 21 days were infected with *A. actinomycetemcomitans* Y4 by swabbing their mouths with an overnight broth culture (68). Two weeks after infection, animals allocated to one of the three groups were challenged with an *S. sanguinis* KJ3sm parent strain, a hydrogen-peroxide deficient mutant of *S. sanguinis* KJ3sm, or a revertant of the latter mutant. After 5 weeks, the numbers of *A. actinomycetemcomitans* and *S. sanguinis* colonizing the teeth were determined by selective bacterial culture. The level of *A. actinomycetemcomitans* colonization was approximately 70-fold lower in the animals infected with *S. sanguinis* KJ3sm and the revertant of the hydrogen peroxide-deficient mutant of KJ3sm. A surprisingly nonsignificant 25-fold lower colonization with *A. actinomycetemcomitans* was detected in the animals infected with the hydrogen peroxide-deficient mutant. All *S. sanguinis* strains colonized the oral cavity of the rats to a similar degree. This led the authors to conclude that hydrogen peroxide production serves as the mechanism behind the interaction between *S. sanguinis* and *A. actinomycetemcomitans*. However, because the hydrogen peroxide-deficient mutant could also decrease *A. actinomycetemcomitans* colonization, albeit not statistically significantly, other mechanisms of interaction seem to play a role. Although the experiments were repeated in humans, they were never published because the levels of the *S. sanguinis* effector strain decreased continuously following infection until they were undetectable in saliva and plaque samples, usually within 5 weeks (A. D. Haffajee, personal communication). Unfortunately, this group of researchers abandoned this approach by the end of the 1980s and continued their work in different fields, including caries management.

Since the beginning of the 21st century, the appreciation of the beneficial oral microbiota and their use in the prevention and treatment of plaque-related periodontal inflammation has undergone a revival. In Japan, an *L. salivarius* strain is currently being investigated regarding its potential to suppress periodontopathogens and improve periodontal health. *L. salivarius* is an obligatory homofermentative lactobacillus that is far less aciduric than *L. acidophilus*, which has a possible role in caries development. *L. salivarius* TI 2711 was isolated from the saliva of a healthy human volunteer. The researchers observed *in vitro* that *L. salivarius* TI 2711 starts to kill *P. gingivalis*, *Prevotella intermedia* and *Prevotella nigrescens* after 6–12 h in coculture (74). The researchers tested these observations in two *in vivo* human studies. In the first, a parallel, open-label study, 76 adult volunteers (age 22–62 years) were evaluated. No pretreatment (to improve the likelihood of a persistent colonization of the probiotic bacterium) was performed. The control group did not take any probiotic. The other two groups took *L. salivarius* TI 2711 in tablets (either 2×10^7 colony-forming units / day or 1×10^8 colony-forming units / day) by letting five tablets dissolve in their mouth five times a day for a period of 8 weeks. Salivary bacterial counts were determined at baseline and after 4 weeks by bacterial culture. Significant 1-$\log_{10}$ reductions in salivary black-pigmented anerobic rods were recorded for both probiotic groups. Such a reduction was not observed in the control group. The total bacterial count, the number of mutans streptococci and the number of lactobacilli were not changed in any of the groups. The number of patients with undetectable levels of black-pigmented anerobic rods increased from 8 to 30% for both probiotic groups together. Interestingly, at baseline, the patients showed a large variability in salivary pH, ranging from 5.4 to 8.5. However, after 4 and 8 weeks of treatment, their pH range fell within a small neutral range, around 7.3. The unchanged levels of mutans

streptococci and lactobacilli, together with the absence of the acidification of the salivary pH, led the authors to conclude that this approach can be considered as safe in relation to caries induction by the probiotic strain, which may be a potentially useful probiotic agent against periodontopathogens. However, great caution is still warranted as the safety *L. salivarius* was recently questioned (115).

In a second study, the effect of the same *L. salivarius* strain on periodontopathogens in subgingival plaque was evaluated. In this double-blind, placebo-controlled, parallel study, 108 periodontitis patients were divided into three groups. Again, no pretreatment was given. The patients in both probiotic groups took either 2×10^8 or 2×10^7 colony-forming units of *L. salivarius* TI 2711 for 12 weeks by dissolving five tablets, five times a day, on their tongue. During treatment (4 weeks), the day after the last intake (week 12) and 4 weeks after the last intake (week 16), the subgingival bacterial load was determined by quantitative polymerase chain reaction, together with a clinical examination of the patient. There was a significant decrease in the total amount of subgingival bacteria during the study that persisted for up to 4 weeks after discontinuing the intake of the tablets. However, this significant 1-$\log_{10}$ decrease was also present in the placebo group. Although *S. salivarius* TI 2711 did not alter the levels of subgingival *Tannerella forsythensis*, a significant 1-$\log_{10}$ decrease in *P. gingivalis* was recorded immediately after stopping the probiotic treatment (week 12). The latter change was, however, not persistent because at week 16 baseline levels were reached again. The authors did notice a persistent and significant increase in *L. salivarius* levels in the probiotic group. From a clinical point of view, significant reductions in bleeding upon probing and pocket depth were recorded but these were similar in both the probiotic and placebo groups.

Krasse et al. (99) evaluated the effect of another lactobacillus strain, *L. reuteri*, in the treatment of recurrent gingivitis. The selection of this strain was based on anecdotal data, the reported reduction of salivary *S. mutans* levels (130) and on the generally claimed health effects of lactobacilli. For this parallel, double-blind, randomized, placebo-controlled study, 59 patients with moderate-to-severe gingivitis were enrolled. The patients were randomized over a placebo group, or over one of the two probiotic groups. Both probiotic groups received one of two different *L. reuteri* strains delivered via chewing gum at a concentration of 1×10^8 colony-forming units. The placebo group received an identical chewing gum without *L. reuteri*. The patients were instructed to use a chewing gum twice a day for 2 weeks. Gingivitis and plaque scores were recorded at baseline. Afterwards, all dental surfaces were thoroughly cleaned. After 2 weeks, the clinical parameters were re-evaluated. The gingivitis scores were reduced in all three groups. However, the score in the *L. reuteri* strain 1 group, but not in the strain 2 group, was more reduced than in the placebo group. The plaque scores were also reduced for both probiotic groups but not for the placebo group. This led the authors to conclude that *L. reuteri* is efficacious in reducing gingivitis and plaque scores although, when looking closely at the data, the differences are rather small.

Kang et al. (85) isolated lactic acid bacteria from children's saliva. Two bacterial strains, CMS1 and CMS3, exhibited profound inhibitory effects on the formation of *S. mutans* biofilms and on the proliferation of *S. mutans in vitro*. Both strains were identified as *Weissella cibaria* by 16S rDNA sequencing. *Weissella* spp. are lactic acid bacteria and were formerly included among the lactobacilli. *W. cibaria* is a gram-positive, nonspore-forming, nonmotile, heterofermentative and catalase-negative bacillus, which is normally isolated from fermented foods. Although the primary focus of this study seemed to be to isolate and identify lactic acid bacteria that were active against *S. mutans*, these authors tested the ability of *W. cibaria in vivo* to reduce dental plaque. Therefore, can this publication also be considered as of particular importance for periodontal health? The authors enrolled 72 volunteers (20–30 years of age) in a one-way crossover, open-label placebo-controlled study. On the first day, plaque scores were recorded in the morning, before the patients had brushed their teeth. Presumably, after the scoring, the subjects rinsed twice with 15 ml of sterile distilled water. Rinsing was repeated in the afternoon and in the evening, at least 30 min after the patients had brushed their teeth. The next morning, plaque scores were re-assessed. This strategy was repeated with a probiotic oral rinse containing 1×10^9 colony-forming units / ml of *W. cibaria* CMS1. The authors did not mention how much time there was between using the placebo and the probiotic rinse. In contrast to the placebo rinse, there was a significant, 20% reduction in plaque scores when the *W. cibaria* CMS1-containing rinse was used. These results indicate that the *W. cibaria* isolates possess the ability to inhibit biofilm formation, both *in vitro* and *in vivo*.

It is rather surprising that the above-mentioned four studies tested lactobacilli as possible probiotics for bacterial periodontal infections. As in the gastro-

intestinal microbiota, lactobacilli make up only a relatively small percentage of the culturable oral microbiota. The contribution of these lactobacilli to periodontal health is far from being elucidated. However, Kõll-Klais et al. (95) noted that periodontal destruction and inflammation is closely associated with decreased levels of certain lactic acid bacteria. Looking more closely, they showed that the composition of *Lactobacillus* spp. in the oral cavity differs in respect to periodontal health and habitat (94). In their study, using bacterial culture, it seems that lactobacilli rarely colonize subgingival sites, whereas saliva was significantly more colonized. One might question if the subgingival region is a common habitat for lactobacilli, and, if not, this might imply that lactobacilli may not be the bacterial species of choice as a probiotic. There were no differences in the overall counts of salivary lactobacilli between healthy patients and those with chronic periodontitis. However, significantly higher proportions of homofermentative lactobacilli, especially *Lactobacillus gasseri,* were present in the saliva of healthy subjects. The presence of this species was also associated with less dental plaque and inflammation. As a whole, the homofermentative lactobacilli were associated with the absence of subgingival periodontal pathogens. Regardless of these observations, lactobacilli have been shown *in vitro* to possess antimicrobial activities against periodontopathogens (74, 94, 178). With regard to the positive results of using lactic acid bacteria to promote periodontal health, it is unclear whether lactobacilli should be the probiotics of choice for bacterial periodontal infections.

Therefore, in our recent combined multicentre research effort to identify beneficial bacteria that can at least retard and preferably prevent periodontopathogen recolonization after scaling and root planing, no lactobacilli were included. Teughels and coworkers examined seven presumed beneficial oral bacteria for their ability to interfere with the colonization of periodontopathogens. The bacterial strains were selected for their ability to induce, *in vitro* and *in vivo*, growth inhibition of pathogens (70, 188), to downregulate fimbrial expression (215) or biosurfactant production (199), for the absence of co-aggregation or because of their high prevalence in periodontal health (104, 216). In a series of *in vitro* adhesion experiments, the effect of these seven bacterial strains on the colonization of hard surfaces and epithelial cells by *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *Tannerella forsythia* was elucidated (198, unpublished results,189). *S. sanguinis* KTH-4, *S. salivarius* TOVE and *S. mitis* BMS appeared to be the bacterial species that were most effective in inhibiting *in vitro* periodontopathogen colonization. This inhibition was partially caused by direct interbacterial interactions, environmental conditioning and interaction with epithelial cells. Subsequently, Teughels and coworkers tested the hypothesis that the subgingival application of these three selected beneficial bacterial spp. after mechanical debridement would enhance the microbial shift away from periodontopathogens, in an *in vivo* Beagle dog model (190). Eight male beagle dogs with an average age of 3.08 (±0.37) years were enrolled in a split-mouth, double-blind, randomized trial. Bony defects were created surgically 4 months prior to the experiment, even though the dogs already showed a moderate naturally occurring periodontitis. Four pockets in each quadrant were randomized to one of four treatments: a negative-control treatment (no treatment); a positive-control treatment (subgingival scaling and root planing); root planing and a single application of the bacterial mixture at baseline; or root planing and repeated application of the bacterial mixture at baseline, and at weeks 1, 2 and 4. The bacterial mixture consisting of *S. sanguinis* KTH-4, *S. salivarius* TOVE and *S. mitis* BMS was locally applied in the designated periodontal pockets. To challenge the hypothesis of a prolonged beneficial microbial shift after mechanical debridement by deliberate application of beneficial species, a reservoir for recolonization (144) was established, by leaving teeth that were not included in the study unaffected and by incorporating a negative control. To promote recolonization of periodontopathogens, no oral hygiene was performed (106). The dogs were monitored bacteriologically (by bacterial culture) and clinically over 12 weeks. Although application of beneficial bacteria did not exclude pathogen recolonization, it did delay the recolonization process significantly. Inoculation of beneficial bacteria immediately after root planing, and especially with additional inoculations during the recolonization process, resulted in a significant reduction of bacterial counts for all pathogens monitored. The significant differences between root planing alone and root planing with repeated application of the bacterial mixture, at week 12 for anerobic spp., *Porphyromonas gulae* and *P. intermedia*, were identical or exceeded differences reported in similar, human, split-mouth studies using local antiseptics or antibiotics as adjuncts to root planing (145). Although not statistically significant, the post-treatment attachment level was slightly lower for the treatments that included the application of beneficial species. The

limited clinical improvement was attributed to the absence of oral hygiene. Interestingly, this better response also applied to the proportion of sites that exhibited bleeding on probing, a clinical marker for subgingival inflammation. The difference between root planing alone, and root planing with repeated application of the bacterial mixture for this latter variable, was statistically significant. This observation supports the notion that the application of beneficial bacteria can lead to a more host-compatible subgingival microbiota or that they might also protect us through the promotion of a beneficial host response (155). Recently it was shown that *S. salivarius* could reduce the interleukin-8 epithelial response to *Yersinia enterocolitica*. A similar effect has been observed by our group for *S. salivarius*, *S. sanguinis* and *S. mitis* on the interleukin-8 epithelial response to *A. actinomycetemcomitans* (W. Teughels, unpublished results). The questions of whether the applied species really colonized the subgingival habitat, and how the prolongation of the microbial shift was induced, remain unsolved. However, re-application of the beneficial bacteria used seemed to improve the microbiological outcome of the treatment. In addition, it is well established for probiotics in the gastrointestinal tract that they usually colonize for a short time only, or even not at all (for review see 210). Therefore, extrapolating probiotic colonization behaviour on mucosal surfaces, such as in the intestine, to the periodontal pocket, and its close association with the nonshedding, biofilm-prone root surface, might be hard or even impossible. The mechanisms behind the successful inhibition of periodontopathogen (re-)colonization remain hypothetical. Occupation or a physico-chemical alteration of the subgingival niche (189), competition for essential nutrients (163), inhibition of the viability or growth of pathogens (210) and modification of the production or degradation of virulence factors of pathogens or immune responses (218), are possible underlying mechanisms. In summary, these results showed that application of beneficial bacteria as an adjunct to root planing might be a valid, nonantibiotic treatment approach for periodontitis.

Next to bacterial infections, the periodontal tissues are susceptible to fungal infections. Several *Candida* spp., most notably *C. albicans*, cause the most common oral and oropharyngeal fungal infections. Estimates range from 40% to 60% of healthy nonimmunocompromised, nonhospitalized people harboring oral *Candida* spp. (33, 62). Predisposing factors for oral candidiasis (candidosis) include multiple and broad-spectrum antibiotics, immunosuppressive drugs, anticholinergic agents, endocrine dysfunction, bone marrow depression, immunodeficiency disorders, malignancies, nutritional deficiencies, radiation treatment, dentures, xerostomia and extreme old age (62). Fungal infections anywhere in the body are difficult to treat because these infectious agents are ubiquitous in nature and slow to respond to drug therapy. Useful drugs are fungistatic, not lethal and consequently rely heavily on innate immune defenses to rid the body of the infection. Fungi have become major life-threatening pathogens in nosocomial infections as well as in individuals who have become severely immunoincompetent. Similarly to bacteria, fungi have developed sophisticated mechanisms of resistance to current chemotherapeutic agents, bringing into question the use of these agents in trivial or nonlife-threatening clinical disorders (for review see 136). Therefore, some researchers are searching for alternative treatments to control oral candida carriage. The use of probiotics is one of these emerging treatment approaches (Table 7).

Elahi et al. (31) investigated the clearance of *C. albicans* from the oral cavities of mice following the oral administration of *L. acidophilus* LAFTI L10 and *L. fermentum*. Their hypothesis was based on the observation that feeding mice probiotic bacteria can prolong their survival following intestinal challenge with *C. albicans* (209). Additionally, it had been shown for humans that ingestion of yoghurt containing *L. acidophilus* could protect against candidal vaginitis (72). In the first part of the study of Elahi et al. (31), 'infection prone' DBA/2 mice were fed 1×10^9 colony-forming units of lactobacilli, daily for 2 weeks, by gastric intubation using a feeding needle. Control mice were fed phosphate-buffered saline. One day after the last feed, all mice were orally challenged with 1×10^8 colony-forming units of *C. albicans* blastoconidia by topical application. The number of colonizing candida spp. in the oral cavity of the mice was followed over time via culturing procedures. The gastric feeding of probiotics was continued for an additional 14 days after the *C. albicans* challenge. One day after challenge with candida, the *C. albicans* levels in the oral cavity of the mice that were fed with probiotic lactobacilli were similar to the *C. albicans* levels in the control mice. This was followed by a rapid decline in colonization levels on day 2, in the groups of mice fed *L. acidophilus* or *L. fermentum*. By day 6, mice fed *L. acidophilus* had undetectable numbers of yeast in the oral cavity. Colonization persisted up to day 8 in mice fed *L. fermentum*, although at significantly lower levels than found in the control group. In the control

Table 7. Probiotics and periodontal yeast infections

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pre-treatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Elahi et al. (part 1) (31)	Healthy	Infection-prone DBA/2 mice (6–8 weeks)	Open label Parallel Placebo controlled	1	Placebo	35	None	Gastric feeding with phosphate-buffered saline	Once daily for 2 weeks after which topical oral challenge with *C. albicans*			Oral *C. albicans* clearance	Significantly faster *C. albicans* clearence in both probiotic groups vs. placebo group. Significantly faster *C. albicans* clearance in probiotic group A vs. probiotic group B.
					Probiotic A	35	None	Gastric feeding with phosphate buffered saline	Once daily for 2 weeks after which topical oral challenge with *C. albicans*	*L. acidophilus*	1×10^9		
					Probiotic B	35	None	Gastric feeding with phosphate buffered saline	Once daily for 2 weeks after which topical oral challenge with *C. albicans*	*L. fermentum*	1×10^9		
Elahi et al. (part 2) (31)	Healthy	Infection-prone DBA/2 mice (6–8 weeks)	Open label Parallel group Placebo controlled	1.5	Placebo A	15	None	Gastric feeding with phosphate-buffered saline	One/two days for 34 days after which topical oral challenge with *C. albicans*			Oral *C. albicans* clearance	Significantly faster clearance of *C. albicans* for both probiotic groups vs. the placebo groups. The rate of clearance of *C. albicans* was less for probiotic group B vs. probiotic group A
					Probiotic A	15	None	Gastric feeding with phosphate-buffered saline	One/two days for 34 days after which topical oral challenge with *C. albicans*	*L. acidophilus*	1×10^9		
					Placebo B	15	None	Gastric feeding with phosphate-buffered saline	One/two days for 34 days after which topical oral challenge with *C. albicans*				
					Probiotic B	15	None	Gastric feeding with phosphate-buffered saline	One/two days for 20 days followed by 14 days with placebo after which topical oral challenge with *C. albicans*	*L. acidophilus*	1×10^9		

Table 7. *Continued*

Study	Condition at baseline	Type of patient (age)	Study design	Follow-up time (months)	Study group	Number of patients	Pre-treatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Hatakka et al. (58)	Healthy Gingivitis Periodontitis Caries	Elderly (58–95 years)	Double blind Randomized Placebo controlled	4	Placebo	140	None	Cheese	25 g, twice daily for 16 weeks	*L. lactis*		Prevalence of high yeast counts	The prevalence of high yeast counts decreased in probiotic group vs. placebo group.
					Probiotic	136	None	Cheese	25 g, twice daily for 16 weeks	*L. lactis* *L. helveticus* *L. rhamnosus* GG *L. rhamnosus* LC705 *P. freudenreichii* ssp. *shermanii* JS	1×10^7 / g		

C. albicans, *Candida albicans*.

mice, *C. albicans* was detected up to 15 days after the challenge. To elaborate the extent of the protective effect of gastric feeding of *L. acidophilus*, Elahi et al. (31) performed a second study. In this study, mice were fed by gastric intubation, every 2 days for 34 days, with either phosphate-buffered saline (negative control) or 5×10^9 colony-forming units of *L. acidophilus* in phosphate-buffered saline (positive control). The third group of mice was fed 5×10^9 colony-forming units of *L. acidophilus* in phosphate-buffered saline for 20 days, after which they were fed phosphate-buffered saline alone for an additional 14 days. After the 34-day treatment, the mice were challenged with *C. albicans* and, in contrast to the first study, no additional feeding with *L. acidophilus* was performed. The results showed that for both groups of mice that were fed *L. acidophilus*, the oral clearance rate for *C. albicans* was significantly faster than for the mice that were fed phosphate-buffered saline. However, for the group of mice where *L. acidophilus* feeding was ceased 14 days prior to *C. abicans* challenge, the rate of oral clearance was less than that in mice that were fed continuously for 34 days with *L. acidophilus*. By day 6, while higher colonization levels were found in the mice in which oral feeding had ceased, colonization was, similarly to the first study, undetectable in mice continuously fed *L. acidophilus*. The data from both studies show that the probiotic bacteria used can help to protect against an oral candida infection in mice. As direct gastric feeding was used, the protective effect could not be attributed to direct interference of *L. acidophilus* with *C. albicans* colonization. Moreover, oral cultures were used to ensure that the lactobacilli had not colonized the oral cavities of the mice. The results supported the concept that certain probiotic bacteria have the capacity to drive the mucosal immunity to enhance protection at distant mucosal sites. However, the authors reported that in a similar study but using 'infection resistant' BALB/c mice, feeding of lactobacilli did not enhance *C. albicans* clearance. This indicates that the protective effect provoked by feeding lactobacilli is primarily restorative rather than a booster effect associated normally with secondary immunization (31).

Hatakka et al. (58) were the first to perform a randomized, double-blind, placebo-controlled study on the effect of probiotics on the prevalence of oral candida. One-hundred and ninety two elderly people (age 70–100 years), recruited from retirement homes and sheltered housing, completed the study. The elderly were selected for this study because they are vulnerable to candida infection. During the 16-week

intervention, after a 3-week run-in period, the participants consumed daily either 50 g of probiotic cheese or 50 g of control cheese. In the probiotic cheese, *Lactococcus lactis* and *Lactobacillus helveticus* were used as starter cultures, and 10^7 colony-forming units / g of each of the probiotic strains, *L. rhamnosus* GG (ATCC 53103), *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS were added. Control cheese contained only *L. lactis* as a starter culture, and no probiotic strains were added. Microbiological samples were taken by swabbing the oral soft tissues with a cotton swab. A semiquantitative culture technique was used to determine the levels of oral yeast. No pretreatment to enhance the colonization of the probiotic bacteria was given prior to the start of the study and the colonization of the probiotic bacteria used was not monitored. At baseline, 30.4% of the probiotic group and 28.0% of the control group had 'high yeast counts' ($\geq 10^4$ colony-forming units / ml, as defined by the authors). After the intervention, the prevalence of high yeast counts in the probiotic group was reduced by 32%. In the control group, the prevalence of yeast had increased. Surprisingly, the authors did not provide any information on the prevalence of yeasts in patients with 'low yeast counts'. How the effect was achieved is unclear but because the study population was elderly, the immunological restorative theory of Elahi et al. (31) seems plausible. On the other hand, the authors reported an increase of salivation in the probiotic group in comparison to decreased salivation in the control group over the 16-week period. Because reduced salivary flow is a risk factor for candida infection in the elderly (171), it might well be that the reduction in prevalence of high candida levels in the probiotic group is a reflection of the change in the salivation. Whether the latter is a genuine effect of the ingested probiotics needs further investigation.

Halitosis

Halitosis (bad breath) is believed to affect a large proportion of the population, if not the whole population at certain moments. It has a significant socio-economic impact and may reveal, eventually, disease. Halitosis is caused by a number of volatiles, which originate from the oro-pharynx or from expired alveolar air. In oral malodor, the sulphur-containing gases (hydrogen sulfide, methyl mercaptan and dimethyl sulfide), which are derived from the bacterial degradation of sulphur-containing amino acids in the oropharynx, play a significant role. A diverse consortium of bacteria has been found to contribute to the problem, including *Fusobacterium nucleatum*, *P. gingivalis*, *P. intermedia* and *Treponema denticola*. Other gases, such as indole, skatole, putrescine, cadaverine and acetone, are also relevant and sometimes even the dominant cause of halitosis, although their substantively is much lower (194).

Most (85%) of the pathology causing halitosis lies within the oropharynx (tongue coating, gingivitis, periodontitis, tonsillitis) (201). In 10–15% of the patients, however, breath malodor has an extra-oral cause (26). Bad-smelling metabolites can be formed / absorbed at any place in the body and be transported by the bloodstream to the lungs. Exhalation of these volatiles then causes halitosis (197).

Given that oral microorganisms, especially those on the tongue, are the primary cause of halitosis, current treatments focus on the use of chemical or physical antibacterial regimes to reduce the numbers of these bacteria. However, most of these treatments exhibit only a temporary effect or are associated with undesirable side-effects when used over a long period of time (14). The only temporary reduction in malodour can be explained by the re-establishment of halitosis-causing bacteria after treatment is stopped. To prevent the regrowth of odor-causing organisms, pre-emptive colonization of the oral cavity with probiotics might have a potential application as adjuncts for both the treatment and prevention of halitosis.

Given the large number of internet sites dedicated to the sale of probiotic products for people with halitosis, one would anticipate that there are many well-substantiated scientific claims of the efficacy of these products (14). Unfortunately, the opposite is true (Table 8).

Although halitosis is primarily of oro-pharyngeal origin, the first report on the treatment of halitosis via probiotics claims to treat a gut-caused halitosis. In a case report, Henker et al. (63) describe the history of, in 2001, a 9.5-year old girl. She suffered from frequent obstructive bronchitis and later from bronchial asthma in her first years of life. She was therefore treated several times with antibiotics, such as ampicillin. Since the age of 5 years she has suffered from bad breath, which has been the reason for considerable isolation in kindergarten and primary school. The authors ruled out gastrointestinal and oro-laryngeal conditions, including caries, periodontitis, pharyngeal or oesophageal diverticula, chronic sinusitis, abscesses, bronchiectasia, dyspepsia, diabetes, uremia and hepatic encephalopathy. Only a ferritin and IgA deficiency could be detected. It is,

Table 8. Probiotics and halitosis

Study	Condition at baseline	Type of patient (age, years)	Study design	Follow-up time (months)	Study group	Number of patients	Pretreatment	Vehicle	Frequency	Strains	Concentration	Assessment criteria	Results
Henker et al. (63)	Gut-caused halitosis	Child (5)	Case report	48	Control	6	None					Breath gas analysis	Breath gas analysis became comparable to breath gas analysis of healthy subjects. Breath smell remained inconspicuous up to 4 years
					Probiotic	1	None	Suspension	2 ml/day for 3 months	*E. coli* Nissle 1917	?		
Kang et al. (86)	Morning bad breath	Dental students (20–30)	Open label Crossover Placebo controlled	1 day	Placebo	46	None	Gargle solution	15 ml, twice daily, for 2 min			VSC levels in mouth air	Significant reduction in VSC levels in probiotic group C. No changes in other groups
					Probiotic A	10	None	Gargle solution	15 ml, twice daily, for 2 min	*L. casei*	1×10^9 /ml		
					Probiotic B	10	None	Gargle solution	15 ml, twice daily, for 2 min	*W. confusa*	1×10^9 /ml		
					Probiotic C	46	None	Gargle solution	15 ml, twice daily, for 2 min	*W. cibaria* CMU	1×10^9 /ml		
Burton et al. (15)	Halitosis	Adult patients (18–69)	Open label Parallel Placebo controlled	0.25	Placebo	10	Mechanical and chemical oral cleansing using toothbrush, tongue scraper and chlorhexidine (2% gel and 0.2% rinse) for 3 days	Lozenge	Day 1: every 2 h over 8 h. Afterwards: twice daily for 1 week			VSC levels in mouth air	Significantly lower VSC values for the probiotic group vs. the placebo group
					Probiotic	13	Mechanical and chemical oral cleansing using toothbrush, tongue scraper and chlorhexidine (2% gel and 0.2% rinse) for 3 days	Lozenge	Day 1: every 2 h over 8 h. Afterwards: twice daily for 1 week	*S. salivarius* K12	$>1 \times 10^9$		

E. coli, *Escherichia coli*.

VSC, volatile sulphur compound.

however, unclear whether the authors ruled out oral hygiene and the tongue microbiota of the patient as a cause for the halitosis. A gas chromatography mass spectrometer was used to analyze the expired breath of the patient prior to the probiotic treatment. The resulting curve was clearly different from gas chromatography mass spectrometry results of six healthy test subjects. After treatment with a suspension of live nonpathogenic bacteria (*E. coli* strain Nissle 1917), 2 ml daily for almost 3 months, the breath gas analysis showed a result comparable to that of the healthy test subjects. Clinically, the bad breath of our patient had disappeared. It is unclear from the publication whether an antibiotic pretreatment was given prior to starting the probiotic treatment. The authors reported that during a follow-up of 4 years, the breath smell remained inconspicuous.

Kang et al. (86) were the first to use a more scientifically based step-by-step approach in their quest to find a probiotic for the treatment or prevention of halitosis. They collected saliva samples from 460 Korean kindergarten children between the age of 4 and 7 years. None of the children exhibited oral disease, including caries, and all had little supragingival plaque. All lactobacilli were pure cultured and tested for the production of hydrogen peroxide. The three strains that generated the most substantial levels of hydrogen peroxide were identified by 16S rDNA sequence analysis. They were found to be *W. cibaria* strains. In a series of *in vitro* experiments, *W. cibaria* CMU was shown to be the most effective at inhibiting *F. nucleatum* viability and its production of volatile sulfur compounds. Subsequently, the authors evaluated the effect of *W. cibaria* CMU on morning breath in 46 dental students (age 20–30 years). At the start of the experiment (morning of day 1), the level of volatile sulfur compounds in their mouth air was assessed using a portable gas chromatograph. The students were then instructed to gargle 15 ml of test solution or control solution for 2 min, twice a day. The test solution consisted of 1×10^9 colony-forming units of *W. cibaria* CMU in distilled water. Control solutions were distilled water only, distilled water containing 1×10^9 colony-forming units of *L. casei*, or distilled water containing 1×10^9 colony-forming units of *Weissella confusa*. The latter two are both commercial lactic acid bacteria. The next morning (day 2), the level of volatile sulfur compounds was determined again. Gargling with *W. cibaria* CMU significantly reduced the production of both hydrogen sulfide and methyl mercaptan by about 48.2% and 59.4%, respectively. However, when sterile distilled water or commercial lactic acid bacteria were used as a control rinse, there were no statistically significant reductions in the concentrations of volatile sulfur compounds.

Burton et al. (15) investigated the effect of *S. salivarius* on oral malodour parameters. The aim of the study was to alleviate halitosis by pre-emptively colonizing the oral cavity with a competitive commensal bacterium following a short course of mechanical and chemical treatment to reduce the numbers of odor-causing organisms and possibly provide additional attachment sites for the colonizing strain. *S. salivarius* was selected as an oral probiotic because it is an early colonizer of oral surfaces and is amongst the most numerically predominant members of the tongue microbiota of 'healthy' individuals (19, 90). This species also has only a limited ability to produce volatile sulphur compounds (220) and is unlikely to contribute significantly to oral odor. *S. salivarius* has not been implicated either in caries or in other infectious diseases of humans and is most closely related to *S. thermophilus*, a bacterium widely used in the dairy food industry (formerly *S. salivarius* ssp. *thermophilus*). *S. salivarius* K12 is already marketed in several countries and through the internet for the prevention of streptococcal sore throats and halitosis. In order to determine whether strain K12 might also be useful in the treatment of halitosis, a series of bacterial strains, representative of species implicated in halitosis, were tested to see if they were inhibited by the two bacteriocins produced by strain K12 *in vitro*. Inhibition was observed of *Streptococcus anginosus*, *Eubacterium saburreum* and *Peptostreptococcus micros*, but not of *P. gingivalis* and *P. intermedia* (15). On the other hand, when fresh saliva was inoculated onto agar medium impregnated with the bacteriocins produced by strain K12, the inhibition of black-pigmented bacteria, identified as *Prevotella* spp., was observed (14). In a preliminary clinical study, 23 subjects (age 18–69 years) with volatile sulfur compound scores of >200 parts per billion were enrolled (15). All subjects undertook a mechanical and chemical oral cleansing treatment that consisted of brushing their teeth and tongue for 2 min using toothpaste, then using a plastic tongue scraper for 30 s, followed by brushing of their teeth and tongue for 2 min with 2.0% chlorhexidine mouth gel and finally a 30 s chlorhexidine rinse, possibly providing additional attachment sites for the colonizing strain. At 2-h intervals, over the next 8 h, the 13 patients of the experimental group sucked a lozenge containing more than 1×10^9 colony-forming units of *S. salivarius* K12. The 10 patients of the control group sucked a placebo lozenge containing

no bacteria. On days 2 and 3, the patients brushed their teeth and tongue in the morning and rinsed with chlorhexidine, then took the lozenges as on day 1. Subsequently, the subjects refrained from any use of chlorhexidine, but took a lozenge morning and night after normal oral care for 1 week. Assessment of the subjects' volatile sulphur compound levels 1 week after treatment initiation showed a significant reduction in volatile sulphur compound scores in the experimental group when compared with the control group. Eighty-five per cent of the patients in the experimental group, and 30% of the patients in the placebo group, had substantial (>100 parts per billion) reductions in volatile sulphur compound scores. The bacterial composition of the saliva was monitored by culture and polymerase chain reaction-denaturing gradient gel electrophoresis. Changes in the polymerase chain reaction-denaturing gradient gel electrophoresis profiles occurred in most subjects following the treatment with *S. salivarius* K12. As long as the patients continued using the *S. salivarius* K12 lozenge, *S. salivarius* could be detected in the saliva by culture. However, Horz and coworkers followed the colonization of *S. salivarius* K12 in a 40-year-old male patient over 35 days. The set-up of the study was identical to the set-up of the test group, as described by Burton et al. (15). However, after 3 days the patient stopped taking the *S. salivarius* K12 lozenge. *S. salivarius* K12 could not be detected in the supragingival and subgingival plaque at any time-point. By contrast, *S. salivarius* K12 was detected in pharyngeal, mucosal, tongue and saliva samples up to 8 days after initiation of the treatment. After 8 days, *S. salivarius* K12 was only detected in the right pharyngeal samples in very low numbers up to 35 days after initiation of the treatment. Therefore, repeated application of this strain might be required at certain intervals.

Conclusions

From this review, it should be clear that the use of probiotics is an interesting emerging and not to be neglected field in general and oral healthcare. Based on the currently available clinical data, it seems that dietary probiotics do not confer a major risk for oral health. No negative effects of probiotic use on oral health have been reported to date. This can probably be attributed to the only temporary oral colonization and the vehicle (milk, yoghurt) in which most of the probiotics are consumed. However, great care is still warranted because it is uncertain that there is not a 'window of infectivity', either naturally occurring in a growing child or induced by antibiotics, antiseptics, immune suppression or mechanical removal of the indigenous oral microbiota, in which a patient can become permanently colonized. Even without a permanent colonization, it can be anticipated that the repeated daily use of probiotic products over a long period of time will support an increased level of lactic acid bacteria in the oral cavity. Additionally, it should be noted that manufacturers of probiotic foods can sometimes add a lot of sugar to their product to improve the taste. This, as such, can confer an oral health risk. Therefore, well-designed long-term follow-up studies should be conducted to elucidate the oral health risk of the long-term use of dietary probiotics. However, one should realize that probiotic bacterial strains can behave differently or induce completely opposite effects. As there are probably as many different dietary probiotic strains as there are probiotic products, long-term follow-up studies to assess the safety of each dietary probiotic for oral health is utopias. It would therefore be more reasonable to (i) be informed and keep track of patients who are using probiotics on a regular basis (e.g. by asking when obtaining a patient's anamnesis), (ii) monitor the oral health of young children using probiotics more closely, and (iii) monitor more closely the oral health of patients who use or have used probiotics simultaneously with antibiotics, antiseptics or mechanical removal of the indigenous oral microbiota or during a period of immune suppression.

Despite this, the currently available data show that some probiotic bacterial strains might, to a greater or lesser extent, help to improve oral health. Although various 'statistically significant' improvements have been reported, the 'clinical significance', the applied methods, the study set-up, the data presentation and the interpretation are sometimes questionable. Additionally, one can question the rationale for some studies because they are based on statements such as 'this strain is shown to be good for general/gastrointestinal health' rather than on well-substantiated *in vitro* research applied to the oral situation. In this regard it is surprising that so many researchers have focused on dietary probiotics or lactobacilli, whereas the streptococcal population in the oral cavity is more dominant. One can argue that the use of probiotics for oral health is just emerging and that therefore we 'did not know this', but the importance of the streptococcal population could be easily derived from the older work of Roos and coworkers on oto-pharyngeal infections and of the group around Socransky. Several studies that do not use the more

general dietary probiotics show great promise. This does not take away from the fact that some lactobacilli do show important effects. These effects can apparently only be maintained as long as the probiotic strain is applied. The only probiotic approach that might need just a single application is the genetically modified *S. mutans* strain of Hillman and coworkers. We can only hope that this approach can soon be tested *in vivo* in humans. Moreover it was surprising how many studies tried to induce a microbiological shift in a fully matured microbiological environment. Could it not have been anticipated that in such an environment, a probiotic has difficulties in becoming established and exerting beneficial effects? Again, this could have been derived from the work of Roos and coworkers. The fact that we, relatively easily and without major side-effects, can reduce the level of oral indigenous microbiota and thereby provide more sites for colonization by probiotic bacteria, is a major advance that we, as oral healthcare workers, have compared with gastrointestinal or urogenital applications.

In conclusion, probiotics have made their way into oral healthcare and are more likely to be our friend than our enemy. Despite our rapidly increasing knowledge of pathogen–host interactions, the role of beneficial bacteria in preventing the emergence of pathogenic species and oral health remains obscure. There is a great need to elucidate the role of the oral beneficial microbiota, to identify beneficial bacteria and to conduct proper large-scale studies on the usefulness of probiotics to maintain or improve oral health.

Acknowledgments

W. Teughels is supported by the K.U. Leuven Research fund. The authors thank A. Leonov for her help with translating Russian papers.

References

1. Ahola AJ, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlstrom A, Meurman JH, Korpela R. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. *Arch Oral Biol* 2002: **47**: 799–804.
2. Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. *Am J Gastroenterol* 1998: **93**: 2097–2101.
3. Alm L. The effect of *Lactobacillus acidophilus* administration upon the survival of *Salmonella* in randomly selected human carriers. *Prog Food Nutr Sci* 1983: **7**: 13–17.
4. Aly R, Maibach HI, Shinefield HR, Mandel A, Strauss WG. Bacterial interference among strains of *Staphylococcus aureus* in man. *J Infect Dis* 1974: **129**: 720–724.
5. Anderson MH, Shi W. A probiotic approach to caries management. *Pediatr Dent* 2006: **28**: 151–153.
6. Andersson H, Asp NG, Bruce A, Roos S, Wadstrom T, Wold AE. Health effects of probiotics and prebiotics: a literature review on human studies. *Scand J Nutr* 2001: **45**: 58–75.
7. Armuzzi A, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, Cammarota G, Anti M, De Lorenzo A, Pola P, Gasbarrini G, Gasbarrini A. The effect of oral administration of *Lactobacillus* GG on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy. *Aliment Pharmacol Ther* 2001: **15**: 163–169.
8. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, Isolauri E. Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics* 1999: **104**: e64.
9. Baerheim A, Larsen E, Digranes A. Vaginal application of lactobacilli in the prophylaxis of recurrent lower urinary tract infection in women. *Scand J Prim Health Care* 1994: **12**: 239–243.
10. Bernstein JM, Faden HF, Dryja DM, Wactawski-Wende J. Micro-ecology of the nasopharyngeal bacterial flora in otitis-prone and non-otitis-prone children. *Acta Otolaryngol* 1993: **113**: 88–92.
11. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. *J Pediatr Gastroenterol Nutr* 1995: **21**: 224–226.
12. Bohm SK, Kruis W. Probiotics: do they help to control intestinal inflammation? *Ann N Y Acad Sci* 2006: **1072**: 339–350.
13. Brook I, Yocum P. Bacterial interference in the adenoids of otitis media-prone children. *Pediatr Infect Dis J* 1999: **18**: 835–837.
14. Burton J, Chilcott C, Tagg J. The rationale and potential for the reduction of oral malodour using *Streptococcus salivarius* probiotics. *Oral Dis* 2005: **11**: 29–31.
15. Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR. A preliminary study of the effect of probiotic *Streptococcus salivarius* K12 on oral malodour parameters. *J Appl Microbiol* 2006: **100**: 754–764.
16. Busscher HJ, Mulder AF, Van der Mei HC. In vitro adhesion to enamel and in vivo colonization of tooth surfaces by Lactobacilli from a bio-yoghurt. *Caries Res* 1999: **33**: 403–404.
17. Caglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets. *Acta Odontol Scand* 2006: **64**: 314–318.
18. Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with *Bifidobacterium* DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. *Acta Odontol Scand* 2005: **63**: 317–320.

19. Carlsson J, Grahnen H, Jonsson G, Wikner S. Early establishment of *Streptococcus salivarius* in the mouth of infants. *J Dent Res* 1970: **49**: 415–418.
20. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. *J Dent Res* 1993: **72**: 37–45.
21. Caufield PW, Dasanayake AP, Li Y, Pan Y, Hsu J, Hardin JM. Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. *Infect Immun* 2000: **68**: 4018–4023.
22. Clements ML, Levine MM, Ristaino PA, Daya VE, Hughes TP. Exogenous lactobacilli fed to man – their fate and ability to prevent diarrheal disease. *Prog Food Nutr Sci* 1983: **7**: 29–37.
23. Coconnier MH, Lievin V, Hemery E, Servin AL. Antagonistic activity against *Helicobacter* infection in vitro and in vivo by the human *Lactobacillus acidophilus* strain LB. *Appl Environ Microbiol* 1998: **64**: 4573–4580.
24. Colombel JF, Cortot A, Neut C, Romond C. Yoghurt with *Bifidobacterium longum* reduces erythromycin-induced gastrointestinal effects. *Lancet* 1987: **2**: 43.
25. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. Probiotics: compensation for lactase insufficiency. *Am J Clin Nutr* 2001: **73**: 421S–429S.
26. Delanghe G, Ghyselen J, van Steenberghe D, Feenstra L. Multidisciplinary breath-odour clinic. *Lancet* 1997: **350**: 187.
27. dios Pozo-Olano J, Warram JH Jr, Gomez RG, Cavazos MG. Effect of a lactobacilli preparation on traveler's diarrhea. A randomized, double blind clinical trial. *Gastroenterology* 1978: **74**: 829–830.
28. Drutz DJ, Van Way MH, Schaffner W, Koenig MG. Bacterial interference in the therapy of recurrent staphylococcal infections. Multiple abscesses due to the implantation of the 502A strain of staphylococcus. *N Engl J Med* 1966: **275**: 1161–1165.
29. Edwardsson S. Bacteriological studies on deep areas of carious dentine. *Odontol Revy Suppl* 1974: **32**: 1–143.
30. el Ziney MG, Debevere JM. The effect of reuterin on *Listeria monocytogenes* and *Escherichia coli* O157:H7 in milk and cottage cheese. *J Food Prot* 1998: **61**: 1275–1280.
31. Elahi S, Pang G, Ashman R, Clancy R. Enhanced clearance of *Candida albicans* from the oral cavities of mice following oral administration of *Lactobacillus acidophilus*. *Clin Exp Immunol* 2005: **141**: 29–36.
32. Elmer GW, Surawicz CM, McFarland LV. Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. *J Am Med Assoc* 1996: **275**: 870–876.
33. Eversole LR. Inflammatory diseases of the mucous membranes. Part 1. Viral and fungal infections. *J Calif Dent Assoc* 1994: **22**: 52–57.
34. Falck G, Grahn-Hakansson E, Holm SE, Roos K, Lagergren L. Tolerance and efficacy of interfering alpha-streptococci in recurrence of streptococcal pharyngotonsillitis: a placebo-controlled study. *Acta Otolaryngol* 1999: **119**: 944–948.
35. Fitzgerald RJ, Adams BO, Fitzgerald DB, Knox KW. Cariogenicity of human plaque lactobacilli in gnotobiotic rats. *J Dent Res* 1981: **60**: 919–926.
36. Fitzgerald RJ, Fitzgerald DB, Adams BO, Duany LF. Cariogenicity of human oral lactobacilli in hamsters. *J Dent Res* 1980: **59**: 832–837.
37. Florey HW. The use of micro-organisms for therapeutic purposes. *Yale J Biol Med* 1946: **19**: 101–117.
38. Fujimori I, Kikushima K, Goto R, Hisamatsu K, Murakami Y, Yamada T. Investigation of the nasopharyngeal bacterial flora in children with otitis media with effusion. *ORL J Otorhinolaryngol Relat Spec* 1996: **58**: 147–150.
39. Fujimori I, Kikushima K, Hisamatsu K, Nozawa I, Goto R, Murakami Y. Interaction between oral alpha-streptococci and group A streptococci in patients with tonsillitis. *Ann Otol Rhinol Laryngol* 1997: **106**: 571–574.
40. Fuller R. Probiotics in man and animals. *J Appl Bacteriol* 1989: **66**: 365–378.
41. Ganzle MG, Holtzel A, Walter J, Jung G, Hammes WP. Characterization of reutericyclin produced by *Lactobacillus reuteri* LTH2584. *Appl Environ Microbiol* 2000: **66**: 4325–4333.
42. Gedalia I, Dakuar A, Shapira L, Lewinstein I, Goultschin J, Rahamim E. Enamel softening with Coca-Cola and rehardening with milk or saliva. *Am J Dent* 1991: **4**: 120–122.
43. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995: **125**: 1401–1412.
44. Gilliland SE, Kim HS. Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. *J Dairy Sci* 1984: **67**: 1–6.
45. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003: **124**: 1202–1209.
46. Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000: **119**: 305–309.
47. Gismondo MR, Drago L, Lombardi A. Review of probiotics available to modify gastrointestinal flora. *Int J Antimicrob Agents* 1999: **12**: 287–292.
48. Gluck U, Gebbers JO. Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and beta-hemolytic streptococci). *Am J Clin Nutr* 2003: **77**: 517–520.
49. Goodson JM, Tanner A, McArdle S, Dix K, Watanabe SM. Multicenter evaluation of tetracycline fiber therapy. III. Microbiological response. *J Periodontal Res* 1991: **26**: 440–451.
50. Gorbach SL, Chang TW, Goldin B. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet* 1987: **2**: 1519.
51. Gotz V, Romankiewicz JA, Moss J, Murray HW. Prophylaxis against ampicillin-associated diarrhea with a lactobacillus preparation. *Am J Hosp Pharm* 1979: **36**: 754–757.
52. Grudianov AI, Dmitrieva NA, Fomenko EV. Use of probiotics *Bifidumbacterin* and Acilact in tablets in therapy of periodontal inflammations. *Stomatologiia (Mosk)* 2002: **81**: 39–43.
53. Guandalini S, Pensabene L, Zikri MA, Dias JA, Casali LG, Hoekstra H, Kolacek S, Massar K, Micetic-Turk D, Papadopoulou A, de Sousa JS, Sandhu B, Szajewska H,

Weizman Z. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *J Pediatr Gastroenterol Nutr* 2000: **30**: 54–60.
54. Guarino A, Canani RB, Spagnuolo MI, Albano F, Di Benedetto L. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. *J Pediatr Gastroenterol Nutr* 1997: **25**: 516–519.
55. Haffajee AD, Arguello EI, Ximenez-Fyvie LA, Socransky SS. Controlling the plaque biofilm. *Int Dent J* 2003: **53** (Suppl. 3): 191–199.
56. Harms HK, Bertele-Harms RM, Bruer-Kleis D. Enzyme-substitution therapy with the yeast *Saccharomyces cerevisiae* in congenital sucrase-isomaltase deficiency. *N Engl J Med* 1987: **316**: 1306–1309.
57. Harper DS, Robinson PJ. Correlation of histometric, microbial, and clinical indicators of periodontal disease status before and after root planing. *J Clin Periodontol* 1987: **14**: 190–196.
58. Hatakka K, Ahola AJ, Yli-Knuuttila H, Richardson M, Poussa T, Meurman JH, Korpela R. Probiotics reduce the prevalence of oral candida in the elderly: a randomized controlled trial. *J Dent Res* 2007: **86**: 125–130.
59. Hatakka K, Blomgren K, Pohjavuori S, Kaijalainen T, Poussa T, Leinonen M, Korpela R, Pitkaranta A. Treatment of acute otitis media with probiotics in otitis-prone children: a double-blind, placebo-controlled randomised study. *Clin Nutr* 2007: **26**: 314–321.
60. Hatakka K, Savilahti E, Ponka A, Meurman JH, Poussa T, Nase L, Saxelin M, Korpela R. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *Br Med J* 2001: **322**: 1327.
61. Havenaar R, Huis In't Veld MJH. Probiotics: a general view. In: *Lactic acid bacteria in health and disease*, Vol. 1. Amsterdam: Elsevier Applied Science Publishers, 1992.
62. Hearne K, Cirelli R, Lee P, Tyring SK. Antiviral therapy of acute herpes zoster in older patients. *Drugs Aging* 1996: **8**: 97–112.
63. Henker J, Schuster F, Nissler K. Successful treatment of gut-caused halitosis with a suspension of living non-pathogenic *Escherichia coli* bacteria: a case report. *Eur J Pediatr* 2001: **160**: 592–594.
64. Hillman JD, Brooks TA, Michalek SM, Harmon CC, Snoep JL, Der Weijden CC. Construction and characterization of an effector strain of *Streptococcus mutans* for replacement therapy of dental caries. *Infect Immun* 2000: **68**: 543–549.
65. Hillman JD, Chen A, Duncan M, Lee SW. Evidence that L-(+)-lactate dehydrogenase deficiency is lethal in *Streptococcus mutans*. *Infect Immun* 1994: **62**: 60–64.
66. Hillman JD, Dzuback AL, Andrews SW. Colonization of the human oral cavity by a *Streptococcus mutans* mutant producing increased bacteriocin. *J Dent Res* 1987: **66**: 1092–1094.
67. Hillman JD, Mo J, McDonell E, Cvitkovitch D, Hillman CH. Modification of an effector strain for replacement therapy of dental caries to enable clinical safety trials. *J Appl Microbiol* 2007: **102**: 1209–1219.
68. Hillman JD, Shivers M. Interaction between wild-type, mutant and revertant forms of the bacterium *Streptococcus sanguis* and the bacterium *Actinobacillus actinomycetemcomitans in vitro* and in the gnotobiotic rat. *Arch Oral Biol* 1988: **33**: 395–401.
69. Hillman JD, Socransky SS. Bacterial interference in the oral ecology of *Actinobacillus actinomycetemcomitans* and its relationship to human periodontosis. *Arch Oral Biol* 1982: **27**: 75–77.
70. Hillman JD, Socransky SS, Shivers M. The relationships between streptococcal species and periodontopathic bacteria in human dental plaque. *Arch Oral Biol* 1985: **30**: 791–795.
71. Hillman JD, Yaphe BI, Johnson KP. Colonization of the human oral cavity by a strain of *Streptococcus mutans*. *J Dent Res* 1985: **64**: 1272–1274.
72. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. *Ann Intern Med* 1992: **116**: 353–357.
73. Hilton E, Kolakowski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. *J Travel Med* 1997: **4**: 41–43.
74. Ishikawa H, Aiba Y, Nakanishi M, Oh-hashi Y, Koga Y. Suppression of periodontal pathogenic bacteria in the saliva of humans by the administration of *Lactobacillus salivarius* TI 2711. *J Jpn Soc Periodontol* 2003: **45**: 105–112.
75. Isolauri E. Probiotics in the prevention and treatment of allergic disease. *Pediatr Allergy Immunol* 2001: **12** (Suppl. 14): 56–59.
76. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy* 2000: **30**: 1604–1610.
77. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human lactobacillus strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* 1991: **88**: 90–97.
78. Isolauri E, Kaila M, Arvola T, Majamaa H, Rantala I, Virtanen E, Arvilommi H. Diet during rotavirus enteritis affects jejunal permeability to macromolecules in suckling rats. *Pediatr Res* 1993: **33**: 548–553.
79. Isolauri E, Kaila M, Mykkanen H, Ling WH, Salminen S. Oral bacteriotherapy for viral gastroenteritis. *Dig Dis Sci* 1994: **39**: 2595–2600.
80. Izdebski K, Ross JC, Lee S. Fungal colonization of tracheoesophageal voice prosthesis. *Laryngoscope* 1987: **97**: 594–597.
81. Kabir AM, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. *Gut* 1997: **41**: 49–55.
82. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human lactobacillus strain. *Pediatr Res* 1992: **32**: 141–144.
83. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001: **357**: 1076–1079.
84. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003: **361**: 1869–1871.
85. Kang MS, Chung J, Kim SM, Yang KH, Oh JS. Effect of *Weissella cibaria* isolates on the formation of *Streptococcus mutans* biofilm. *Caries Res* 2006: **40**: 418–425.

86. Kang MS, Kim BG, Chung J, Lee HC, Oh JS. Inhibitory effect of *Weissella cibaria* isolates on the production of volatile sulphur compounds. *J Clin Periodontol* 2006: **33**: 226–232.
87. Kaplan EL, Johnson DR. Unexplained reduced microbiological efficacy of intramuscular benzathine penicillin G and of oral penicillin V in eradication of group a streptococci from children with acute pharyngitis. *Pediatrics* 2001: **108**: 1180–1186.
88. Kashket S, Yaskell T. Effectiveness of calcium lactate added to food in reducing intraoral demineralization of enamel. *Caries Res* 1997: **31**: 429–433.
89. Katelaris PH, Salam I, Farthing MJ. Lactobacilli to prevent traveler's diarrhea? *N Engl J Med* 1995: **333**: 1360–1361.
90. Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, Dewhirst FE, Paster BJ. Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. *J Clin Microbiol* 2003: **41**: 558–563.
91. Keyes PH. Research in dental caries. *J Am Dent Assoc* 1968: **76**: 1357–1373.
92. Kim HS, Gilliland SE. *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. *J Dairy Sci* 1983: **66**: 959–966.
93. Kolars JC, Levitt MD, Aouji M, Savaiano DA. Yogurt: an autodigesting source of lactose. *N Engl J Med* 1984: **310**: 1–3.
94. Koll-Klais P, Mandar R, Leibur E, Marcotte H, Hammarstrom L, Mikelsaar M. Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. *Oral Microbiol Immunol* 2005: **20**: 354–361.
95. Koll-Klais P, Mandar R, Leibur E, Mikelsaar M. Oral microbial ecology in chronic periodontitis and periodontal health. *Microb Ecol Health Dis* 2005: **17**: 146–155.
96. Kollaritsch H, Holst H, Grobara P, Wiedermann G. Prevention of traveler's diarrhea with *Saccharomyces boulardii*. Results of a placebo controlled double-blind study. *Fortschr Med* 1993: **111**: 152–156.
97. Kontiokari T, Sundqvist K, Nuutinen M, Pokka T, Koskela M, Uhari M. Randomised trial of cranberry-lingonberry juice and *Lactobacillus* GG drink for the prevention of urinary tract infections in women. *Br Med J* 2001: **322**: 1571.
98. Kragen H. The treatment of inflammatory affections of the oral mucosa with a lactic acid bacterial culture preparation. *Zahnarztl Welt* 1954: **9**: 306–308.
99. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*. *Swed Dent J* 2005: **30**: 55–60.
100. Krobicka A, Bowen WH, Pearson S, Young DA. The effects of cheese snacks on caries in desalivated rats. *J Dent Res* 1987: **66**: 1116–1119.
101. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004: **53**: 1617–1623.
102. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997: **11**: 853–858.
103. Lesbros-Pantoflickova D, Corthesy-Theulaz I, Blum AL. *Helicobacter pylori* and probiotics. *J Nutr* 2007: **137**: 812S–818S.
104. Liljemark WF, Bloomquist CG, Uhl LA, Schaffer EM, Wolff LF, Pihlstrom BL, Bandt CL. Distribution of oral *Haemophilus* species in dental plaque from a large adult population. *Infect Immun* 1984: **46**: 778–786.
105. Lilly DM, Stillwell RH. Probiotics: growth-promoting factors produced by microorganisms. *Science* 1965: **147**: 747–748.
106. Magnusson I, Lindhe J, Yoneyama T, Liljenberg B. Recolonization of a subgingival microbiota following scaling in deep pockets. *J Clin Periodontol* 1984: **11**: 193–207.
107. Mahieu HF, Van Saene HK, Rosingh HJ, Schutte HK. Candida vegetations on silicone voice prostheses. *Arch Otolaryngol Head Neck Surg* 1986: **112**: 321–325.
108. Maiden MF, Tanner A, McArdle S, Najpauer K, Goodson JM. Tetracycline fiber therapy monitored by DNA probe and cultural methods. *J Periodontal Res* 1991: **26**: 452–459.
109. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997: **99**: 179–185.
110. Majamaa H, Isolauri E, Saxelin M, Vesikari T. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr* 1995: **20**: 333–338.
111. Malchow HA. Crohn's disease and *Escherichia coli*. A new approach in therapy to maintain remission of colonic Crohn's disease? *J Clin Gastroenterol* 1997: **25**: 653–658.
112. Maltz M, de Oliveira EF, Fontanella V, Bianchi R. A clinical, microbiologic, and radiographic study of deep caries lesions after incomplete caries removal. *Quintessence Int* 2002: **33**: 151–159.
113. Marteau P, Flourie B, Pochart P, Chastang C, Desjeux JF, Rambaud JC. Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans. *Br J Nutr* 1990: **64**: 71–79.
114. Marteau PR, de Vrese M, Cellier CJ, Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics. *Am J Clin Nutr* 2001: **73**: 430S–436S.
115. Matsumoto M, Tsuji M, Sasaki H, Fujita K, Nomura R, Nakano K, Shintani S, Ooshima T. Cariogenicity of the probiotic bacterium *Lactobacillus salivarius* in rats. *Caries Res* 2005: **39**: 479–483.
116. Matsuoka T, Sugano N, Takigawa S, Takane M, Yoshinuma N, Ito K, Koga Y. Effect of oral *Lactobacillus salivarius* TI 2711 administration on periodontopathic bacteria in subgingival plaque. *J Jpn Soc Periodontol* 2006: **48**: 315–324.
117. McCracken VJ, Lorenz RG. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* 2001: **3**: 1–11.
118. McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, Melcher SA, Bowen KE, Cox JL. Prevention of beta-lactam-associated diarrhea by *Saccharomyces boulardii* compared with placebo. *Am J Gastroenterol* 1995: **90**: 439–448.
119. Metchnikoff E. Lactic acid as inhibiting intestinal putrefactions. In: Metchnikoff E, Mitchell PC, editors. *The prolongation of life; optimistic studies*. London: W. Heinemann, 1907: 161–183.
120. Meurman JH, Antila H, Korhonen A, Salminen S. Effect of *Lactobacillus rhamnosus* strain GG (ATCC 53103) on the

growth of *Streptococcus sobrinus* in vitro. *Eur J Oral Sci* 1995: **103**: 253–258.
121. Meurman JH, Antila H, Salminen S. Recovery of *Lactobacillus* strain GG (ATCC 53103) from saliva of healthy volunteers after consumption of yoghurt prepared with the bacterium. *Microb Ecol Health Dis* 1994: **7**: 295–298.
122. Midolo PD, Lambert JR, Hull R, Luo F, Grayson ML. In vitro inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *J Appl Bacteriol* 1995: **79**: 475–479.
123. Miller WD. *Micro-organisms of the human mouth*. Philadelphia: SS White, 1890.
124. Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, Santoro L, Cuoco L, Manna R, Gasbarrini G. Probiotic treatment increases salivary counts of lactobacilli: a double-blind, randomized, controlled study. *Digestion* 2004: **69**: 53–56.
125. Mousques T, Listgarten MA, Phillips RW. Effect of scaling and root planing on the composition of the human subgingival microbial flora. *J Periodontal Res* 1980: **15**: 144–151.
126. Naidu AS, Bidlack WR, Clemens RA. Probiotic spectra of lactic acid bacteria (LAB). *Crit Rev Food Sci Nutr* 1999: **39**: 13–126.
127. Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka A, Poussa T, Korpela R, Meurman JH. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res* 2001: **35**: 412–420.
128. Neri A, Sabah G, Samra Z. Bacterial vaginosis in pregnancy treated with yoghurt. *Acta Obstet Gynecol Scand* 1993: **72**: 17–19.
129. Neu TR, Van der Mei HC, Busscher HJ, Dijk F, Verkerke GJ. Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials* 1993: **14**: 459–464.
130. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, Darmawan S, Hamada T, Hara K, Matsumoto A, Takemoto T, Aimi R. *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol* 2004: **95**: 219–223.
131. Nishijima K, Shukunami K, Kotsuji F. Probiotics affects vaginal flora in pregnant women, suggesting the possibility of preventing preterm labor. *J Clin Gastroenterol* 2005: **39**: 447–448.
132. Nissle A. Die antagonistische behandlung chronischer darmstörungen mit colibakterien. *Med Klein* 1918: **2**: 29–33.
133. Norskov-Lauritsen N, Kilian M. Reclassification of Actinobacillus actinomycetemcomitans, *Haemophilus aphrophilus, Haemophilus paraphrophilus* and *Haemophilus segnis* as Aggregatibacter actinomycetemcomitans gen. nov., comb. nov., Aggregatibacter aphrophilus comb. nov. and Aggregatibacter segnis comb. nov., and emended description of Aggregatibacter aphrophilus to include V factor-dependent and V factor-independent isolates. *Int J Syst Evol Microbiol* 2006: **56**: 2135–2146.
134. Oksanen PJ, Salminen S, Saxelin M, Hamalainen P, Ihantola-Vormisto A, Muurasniemi-Isoviita L, Nikkari S, Oksanen T, Porsti I, Salminen E. Prevention of travellers' diarrhoea by *Lactobacillus* GG. *Ann Med* 1990: **22**: 53–56.
135. Othman M, Neilson JP, Alfirevic Z. Probiotics for preventing preterm labour. *Cochrane Database Syst Rev* 2007: CD005941.
136. Pallasch TJ. Antifungal and antiviral chemotherapy. *Periodontol 2000* 2002: **28**: 240–255.
137. Parker RB. Probiotics, the other half of the antibiotic story. *Anim Nutr Health* 1974: **29**: 4–8.
138. Pasteur L, Joubert JF. Charbon et septicémie. *C R Soc Biol Paris* 1877: **85**: 101–115.
139. Pedrazzoli V, Kilian M, Karring T, Kirkegaard E. Effect of surgical and non-surgical periodontal treatment on periodontal status and subgingival microbiota. *J Clin Periodontol* 1991: **18**: 598–604.
140. Petersilka GJ, Ehmke B, Flemmig TF. Antimicrobial effects of mechanical debridement. *Periodontol 2000* 2002: **28**: 56–71.
141. Petti S, Tarsitani G, D'Arca AS. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. *Arch Oral Biol* 2001: **46**: 705–712.
142. Plinius Secundus Maior G. *Naturalis historiae* 77 AD.
143. Pozharitskaia MM, Morozova LV, Mel'nichuk GM, Mel'nichuk SS. The use of the new bacterial biopreparation Acilact in the combined treatment of periodontitis. *Stomatologiia (Mosk)* 1994: **73**: 17–20.
144. Quirynen M, De Soete M, Dierickx K, van Steenberghe D. The intra-oral translocation of periodontopathogens jeopardises the outcome of periodontal therapy. A review of the literature. *J Clin Periodontol* 2001: **28**: 499–507.
145. Quirynen M, Teughels W, De Soete M, van Steenberghe D. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. *Periodontol 2000* 2002: **28**: 72–90.
146. Quirynen M, Vogels R, Pauwels M, Haffajee AD, Socransky SS, Uzel NG, van Steenberghe D. Initial subgingival colonization of 'pristine' pockets. *J Dent Res* 2005: **84**: 340–344.
147. Rafter JJ. The role of lactic acid bacteria in colon cancer prevention. *Scand J Gastroenterol* 1995: **30**: 497–502.
148. Reid G. Probiotic agents to protect the urogenital tract against infection. *Am J Clin Nutr* 2001: **73**: 437S–443S.
149. Reid G, Bruce A, Taylor M. Installation of *Lactobacillus* and stimmulation of indigenous organisms to prevent recurrence of urinary tract infections. *Microecol Ther* 1995: **23**: 32–45.
150. Reid G, Bruce AW. Probiotics to prevent urinary tract infections: the rationale and evidence. *World J Urol* 2006: **24**: 28–32.
151. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. *FEMS Immunol Med Microbiol* 2001: **30**: 49–52.
152. Reid G, Bruce AW, Taylor M. Influence of three-day antimicrobial therapy and lactobacillus vaginal suppositories on recurrence of urinary tract infections. *Clin Ther* 1992: **14**: 11–16.
153. Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, Bruce AW. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunol Med Microbiol* 2003: **35**: 131–134.
154. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic *Escherichia coli* versus mesal-

azine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999: **354**: 635–639.
155. Roberts FA, Darveau RP. Beneficial bacteria of the periodontium. *Periodontol 2000* 2002: **30**: 40–50.
156. Roos K, Grahn E, Holm SE, Johansson H, Lind L. Interfering alpha-streptococci as a protection against recurrent streptococcal tonsillitis in children. *Int J Pediatr Otorhinolaryngol* 1993: **25**: 141–148.
157. Roos K, Hakansson EG, Holm S. Effect of recolonisation with 'interfering' alpha streptococci on recurrences of acute and secretory otitis media in children: randomised placebo controlled trial. *Br Med J* 2001: **322**: 210–212.
158. Roos K, Holm SE, Grahn E, Lind L. Alpha-streptococci as supplementary treatment of recurrent streptococcal tonsillitis: a randomized placebo-controlled study. *Scand J Infect Dis* 1993: **25**: 31–35.
159. Roos K, Holm SE, Grahn-Hakansson E, Lagergren L. Recolonization with selected alpha-streptococci for prophylaxis of recurrent streptococcal pharyngotonsillitis: a randomized placebo-controlled multicentre study. *Scand J Infect Dis* 1996: **28**: 459–462.
160. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 1994: **344**: 1046–1049.
161. Salminen S, Isolauri E, Salminen E. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. *Antonie Van Leeuwenhoek* 1996: **70**: 347–358.
162. Salvi GE, Lang NP. The effects of non-steroidal anti-inflammatory drugs (selective and non-selective) on the treatment of periodontal diseases. *Curr Pharm Des* 2005: **11**: 1757–1769.
163. Sanders E. Bacterial interference. I. Its occurrence among the respiratory tract flora and characterization of inhibition of group A streptococci by viridans streptococci. *J Infect Dis* 1969: **120**: 698–707.
164. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004: **126**: 1620–1633.
165. Sbordone L, Ramaglia L, Gulletta E, Iacono V. Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. *J Periodontol* 1990: **61**: 579–584.
166. Schaafsma G. State of art concerning probiotic strains in milk products. *IDF Nutr News Lett* 1996: **5**: 23–24.
167. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics: approaching a definition. *Am J Clin Nutr* 2001: **73**: 361S–364S.
168. Schupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. *J Dent Res* 1996: **75**: 1779–1788.
169. Schwandt LQ, van Weissenbruch R, Van der Mei HC, Busscher HJ, Albers FW. Effect of dairy products on the lifetime of Provox2 voice prostheses in vitro and in vivo. *Head Neck* 2005: **27**: 471–477.
170. Shanahan F. Physiological basis for novel drug therapies used to treat the inflammatory bowel diseases I. Pathophysiological basis and prospects for probiotic therapy in inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 2005: **288**: G417–G421.
171. Shay K, Truhlar MR, Renner RP. Oropharyngeal candidosis in the older patient. *J Am Geriatr Soc* 1997: **45**: 863–870.
172. Shen S, Samaranayake LP, Yip HK. In vitro growth, acidogenicity and cariogenicity of predominant human root caries flora. *J Dent* 2004: **32**: 667–678.
173. Shornikova AV, Casas IA, Mykkanen H, Salo E, Vesikari T. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. *Pediatr Infect Dis J* 1997: **16**: 1103–1107.
174. Siitonen S, Vapaatalo H, Salminen S, Gordin A, Saxelin M, Wikberg R, Kirkkola AL. Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhoea. *Ann Med* 1990: **22**: 57–59.
175. Slots J, Rams TE. New views on periodontal microbiota in special patient categories. *J Clin Periodontol* 1991: **18**: 411–420.
176. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol* 1992: **63**: 322–331.
177. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol 2000* 2002: **28**: 12–55.
178. Sookkhee S, Chulasiri M, Prachyabrued W. Lactic acid bacteria from healthy oral cavity of Thai volunteers: inhibition of oral pathogens. *J Appl Microbiol* 2001: **90**: 172–179.
179. Sprunt K, Leidy G. The use of bacterial interference to prevent infection. *Can J Microbiol* 1988: **34**: 332–338.
180. Sprunt K, Leidy G, Redman W. Abnormal colonization of neonates in an ICU: conversion to normal colonization by pharyngeal implantation of alpha hemolytic streptococcus strain 215. *Pediatr Res* 1980: **14**: 308–313.
181. Strachan DP. Hay fever, hygiene, and household size. *Br Med J* 1989: **299**: 1259–1260.
182. Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J, van Belle G. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*: a prospective study. *Gastroenterology* 1989: **96**: 981–988.
183. Szajewska H, Kotowska M, Mrukowicz JZ, Armanska M, Mikolajczyk W. Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants. *J Pediatr* 2001: **138**: 361–365.
184. Tagg JR, Dierksen KP. Bacterial replacement therapy: adapting 'germ warfare' to infection prevention. *Trends Biotechnol* 2003: **21**: 217–223.
185. Talarico TL, Casas IA, Chung TC, Dobrogosz WJ. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. *Antimicrob Agents Chemother* 1988: **32**: 1854–1858.
186. Tano K, Grahn HE, Holm SE, Hellstrom S. A nasal spray with alpha-haemolytic streptococci as long term prophylaxis against recurrent otitis media. *Int J Pediatr Otorhinolaryngol* 2002: **62**: 17–23.
187. Tano K, Olofsson C, Grahn-Hakansson E, Holm SE. In vitro inhibition of *S. pneumoniae*, nontypable *H. influenzae* and *M. catharralis* by alpha-hemolytic streptococci from healthy children. *Int J Pediatr Otorhinolaryngol* 1999: **47**: 49–56.
188. Tanzer JM, Kurasz AB, Clive J. Competitive displacement of mutans streptococci and inhibition of tooth decay by

Streptococcus salivarius TOVE-R. *Infect Immun* 1985: **48**: 44–50.
189. Teughels W, Kinder Haake SA, Sliepen I, Pauwels M, Van Eldere J, Cassiman JJ, Quirynen M. Bacteria interfere with *A. actinomycetemcomitans* colonization. *J Dent Res* 2007: **87**: 611–617.
190. Teughels W, Newman MG, Coucke W, Haffajee AD, Van der Mei HC, Haake SK, Schepers E, Cassiman JJ, Van Eldere J, van Steenberghe D, Quirynen M. Guided periodontal pocket recolonization: a proof of concept. *J Dent Res* 2007: **86**: 1078–1082.
191. Tissier H. Traitement des infections intestinales par la méthode de la flore bactérienne de l'intestin. *C R Soc Biol Paris* 1906: **60**: 359–361.
192. Toi CS, Mogodiri R, Cleaton-Jones PE. Mutans streptococci and lactobacilli on healthy and carious teeth in the same mouth of children with and without dental cariës. *Microb Ecol Health Dis* 2000: **12**: 227–233.
193. Tojo M, Oikawa T, Morikawa Y, Yamashita N, Iwata S, Satoh Y, Hanada J, Tanaka R. The effects of *Bifidobacterium breve* administration on *campylobacter enteritis*. *Acta Paediatr Jpn* 1987: **29**: 160–167.
194. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol* 1977: **48**: 13–20.
195. Truper HG, De Clari L. Taxonomic note: necessary correction of specific epithets formed as substantives (nouns) 'in apposition'. *Int J Syst Bacteriol* 1997: **47**: 908–909.
196. Uehara Y, Nakama H, Agematsu K, Uchida M, Kawakami Y, Abdul Fattah AS, Maruchi N. Bacterial interference among nasal inhabitants: eradication of *Staphylococcus aureus* from nasal cavities by artificial implantation of *Corynebacterium* sp. *J Hosp Infect* 2000: **44**: 127–133.
197. Van Den Velde S, Quirynen M, Van Hee P, van Steenberghe D. Differences between alveolar air and mouth air. *Anal Chem* 2007: **79**: 3425–3429.
198. Van Hoogmoed CG, Geertsema-Doornbusch G, Teughels W, Quirynen M, Busscher HJ, Van der Mei HC. Reduction of periodontal pathogens adhesion by antagonistic strains. *Oral Microbiol Immunol* 2007: **????**: ????–????.
199. Van Hoogmoed CG, Van der Mei HC, Busscher HJ. The influence of biosurfactants released by *S. mitis* BMS on the adhesion of pioneer strains and cariogenic bacteria. *Biofouling* 2004: **20**: 261–267.
200. Van Houte J. Role of micro-organisms in caries etiology. *J Dent Res* 1994: **73**: 672–681.
201. van Steenberghe D, Quirynen M. Breath malodor. In: Lindhe J, Karring T, Lang NP, editors. *Clinical periodontology and implant dentistry*. Oxford: Blackwell Munksgaard, 2003: 512–516.
202. Van Winkelhoff AJ, Herrera GD, Winkel EG, Dellemijn-Kippuw N, Vandenbroucke-Grauls CM, Sanz M. Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *J Clin Periodontol* 2000: **27**: 79–86.
203. Van Winkelhoff AJ, van d V, de Graaff J. Microbial succession in recolonizing deep periodontal pockets after a single course of supra- and subgingival debridement. *J Clin Periodontol* 1988: **15**: 116–122.
204. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. *J Pediatr* 1999: **135**: 564–568.
205. Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther* 1999: **13**: 1103–1108.
206. Vesa TH, Marteau P, Korpela R. Lactose intolerance. *J Am Coll Nutr* 2000: **19**: 165S–175S.
207. Volozhin AI, Il'in VK, Maksimovskii I, Sidorenko AB, Istranov LP, Tsarev VN, Istranova EV, Aboiants RK. Development and use of periodontal dressing of collagen and *Lactobacillus casei* 37 cell suspension in combined treatment of periodontal disease of inflammatory origin (a microbiological study). *Stomatologiia (Mosk)* 2004: **83**: 6–8.
208. Wade WG, Moran J, Morgan JR, Newcombe R, Addy M. The effects of antimicrobial acrylic strips on the subgingival microflora in chronic periodontitis. *J Clin Periodontol* 1992: **19**: 127–134.
209. Wagner RD, Pierson C, Warner T, Dohnalek M, Farmer J, Roberts L, Hilty M, Balish E. Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. *Infect Immun* 1997: **65**: 4165–4172.
210. Wilson M. Manipulation of the indigenous microbiota. In: Wilson M, editors. *Microbial inhabitants of humans*. New York: Cambridge University Press, 2005: 395–416.
211. Witsell DL, Garrett CG, Yarbrough WG, Dorrestein SP, Drake AF, Weissler MC. Effect of *Lactobacillus acidophilus* on antibiotic-associated gastrointestinal morbidity: a prospective randomized trial. *J Otolaryngol* 1995: **24**: 230–233.
212. Wolff L, Dahlen GG, Aeppli DM. Bacteria as risk markers for periodontitis. *J Periodontol* 1994: **65**: 498–510.
213. Wolff LF, Liljemark WF, Bloomquist CG, Pihlstrom BL, Schaffer EM, Bandt CL. The distribution of *Actinobacillus actinomycetemcomitans* in human plaque. *J Periodontal Res* 1985: **20**: 237–250.
214. Wollowski I, Rechkemmer G, Pool-Zobel BL. Protective role of probiotics and prebiotics in colon cancer. *Am J Clin Nutr* 2001: **73**: 451S–455S.
215. Xie H, Cook GS, Costerton JW, Bruce G, Rose TM, Lamont RJ. Intergeneric communication in dental plaque biofilms. *J Bacteriol* 2000: **182**: 7067–7069.
216. Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *J Clin Periodontol* 2000: **27**: 648–657.
217. Ximenez-Fyvie LA, Haffajee AD, Som S, Thompson M, Torresyap G, Socransky SS. The effect of repeated professional supragingival plaque removal on the composition of the supra- and subgingival microbiota. *J Clin Periodontol* 2000: **27**: 637–647.
218. Yasui H, Shida K, Matsuzaki T, Yokokura T. Immunomodulatory function of lactic acid bacteria. *Antonie Van Leeuwenhoek* 1999: **76**: 383–389.
219. Yli-Knuuttila H, Snall J, Kari K, Meurman JH. Colonization of *Lactobacillus rhamnosus* GG in the oral cavity. *Oral Microbiol Immunol* 2006: **21**: 129–131.
220. Yoshida Y, Negishi M, Amano A, Oho T, Nakano Y. Differences in the betaC-S lyase activities of viridans group streptococci. *Biochem Biophys Res Commun* 2003: **300**: 55–60.