

Do probiotics offer opportunities to manipulate the periodontal oral microbiota?

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Abstract

Background: As in other fields of healthcare, probiotics have been introduced for prevention and treatment of periodontal diseases.

Objective: This review was initiated to explore whether the use of probiotics can influence the periodontal microbiota and periodontal health.

Materials and Methods: Literature on the mode of action of oral probiotics was reviewed and a systematic review was performed on the microbiological and clinical effects of oral probiotics on periodontal health.

Results: Three animal and 11 in vivo human studies were retrieved. Six studies reported on microbiological effects whereas eight studies report on clinical effects. Seven studies were performed on healthy or gingivitis patients and four studies on periodontitis patients. Many of the retrieved studies are pilot in nature and with low quality. The high degree of heterogeneity between studies hampered analysis.

Conclusion: Taking into consideration all limitations, the currently available data indicate an effect of probiotics on the oral microbiota and a more limited effect on clinical periodontal outcome measures. However, there is an urgent need for properly conducted clinical trials where probiotics are used as adjuncts to standard periodontal care, similar to antibiotics, using probiotic strains with, at least at an in vitro level, proven periodontal probiotic effects.

Key words: gingivitis; microbiology; oral; periodontitis; periodontology; probiotics; review

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The interest in probiotics and the modulation of the microbiota for restoring and maintaining health have gained a lot of attention over the past decade. The term “probiotic” is a relatively new word and is currently used to name bacteria with beneficial effects for humans and animals. As an antonym of the term “antibiotics”, it was intro-

duced by Lilly & Stillwell (1965) as “Substances produced by micro-organisms which promote the growth of other micro-organisms”. However, 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 [Plinius Secundus (maior) 77 AD]. Since 1965, several definitions for probiotics have been proposed (Parker 1974, Fuller 1989, Havenaar & Huis In’t Veld 1992, Schaafsma 1996, Naidu et al. 1999, Schrezenmeir & de Vrese 2001). The currently used consensus definition of probiotics was put forward by the World Health Organization, and the Food and Agriculture Organization of the United States. They defined probiotics as “Live

micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” (http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf). The changing definition 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 scientists rediscover events occurring at the interface between mucosal surfaces and the microbiota, and interactions of probiotic microorganisms with the host (Böhm & Kruis 2006).

There are a number of reasons why probiotic research has become a hot topic in medicine. Despite over 50 years of antibiotics, infectious diseases remain a major health problem, with gastroen-

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teritis killing a child every 15 s. Hospital infection rates are not declining, multi-drug-resistant bacteria continue to emerge as the antibiotic pipeline dries up and pathogenic microorganisms are being linked with induction or worsening of many chronic diseases. Added to this the alarming spread of infectious diseases, plus the pending threat of a deadly flu pandemic, and worried consumers, government, scientists and industries are looking for new approaches to health restoration and retention. Science itself is playing a major role, with an ever-growing number of studies providing tangible evidence that probiotics can alleviate some disease processes (Reid et al. 2006).

Currently, well-established probiotic effects are: (1) Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhoea as well as alleviation of complaints due to lactose intolerance (Majamaa et al. 1995, Buydens & Debeuckelaere 1996, Cremonini et al. 2002, Hawrelak et al. 2005). (2) Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut (Goldin & Gorbach 1984, Haskard et al. 2001, Ouwehand et al. 2002). (3) Prevention and alleviation of unspecific and irregular complaints of the gastrointestinal tracts in healthy people (de Vrese & Schrezenmeir 2008). (4) Beneficial effects on microbial aberrancies, inflammation and other complaints in connection with: inflammatory diseases of the gastrointestinal tract, *Helicobacter pylori* infection or bacterial overgrowth (Vanderhoof et al. 1998, Gionchetti et al. 2000, Felley et al. 2001, Gaon et al. 2002, Ishikawa et al. 2003b). (5) Normalization of passing stool and stool consistency in subjects suffering from constipation or an irritable colon (Niedzielin et al. 2001, Ouwehand et al. 2002, Koebnick et al. 2003). (6) Prevention or alleviation of allergies and atopic diseases in infants (Isolauri et al. 2000, Kalliomaki et al. 2001, Isolauri 2003, Ogawa et al. 2006, Forsythe et al. 2007). (7) Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections. Insufficient or at most preliminary evidence exists with respect to cancer prevention, the so-called hypocholesterolaemic effect, prevention or therapy of ischaemic heart diseases or amelioration of autoimmune diseases (e.g. arthritis)

(Agerbaek et al. 1995, Rafter 1995, Reid et al. 1995, Reid 2001, Naruszewicz et al. 2002, Baharav et al. 2004, de Vrese et al. 2005, de Vrese et al. 2006).

In contrast to the beliefs of some physicians, the oral cavity is not a confined compartment within the human body. Anatomically, the oral cavity is connected to the nasopharynx, the larynx, the tonsils, the middle ear through the Eustachian tube and the gastrointestinal tract. Physiologically it is connected to the whole body and by this, the oral cavity is influenced by and influences general health. Consequently, dentists are confronted with similar healthcare problems as physicians. Because the oral microbiota is at least as complex as the gastro-intestinal or vaginal microbiota and dental biofilms are considered to be difficult therapeutic targets (Socransky & Haffajee 2002), the encouraging effects of probiotics in different fields of healthcare have resulted recently in the introduction of probiotics for oral healthcare (Meurman 2005, Teughels et al. 2008). Today, several clinical studies on the effects of probiotics in different fields of oral healthcare have been published such as: halitosis (Henker et al. 2001, Burton et al. 2006, Kang et al. 2006b), oral candidiasis (Ahola et al. 2002, Hatakka et al. 2007) and tooth decay (Nase et al. 2001, Montalto et al. 2004, Nikawa et al. 2004, Caglar et al. 2005b, Caglar et al. 2006, Caglar et al. 2008, Caglar et al. 2009, Cildir et al. 2009, Stecksens-Blicks et al. 2009).

Probiotics have also been introduced in the field of periodontal healthcare. The reason why probiotics might provide opportunities for periodontal healthcare can be related to the current view on the aetiology of plaque-related periodontal inflammation. This aetiological view considers three factors that determine whether disease will develop in a subject (Slots & Rams 1991, Socransky & Haffajee 1992, Wolff et al. 1994): a susceptible host, the presence of pathogenic species and the reduction or absence of the so-called 'beneficial bacteria'. Because it is difficult to influence the host response, traditional periodontal therapies focused on the reduction of the bacterial threat (Salvi & Lang 2005). This globally applied treatment strategy is based on a mechanical subgingival debridement (eventually including periodontal surgery to reduce the depth of the periodontal pocket), in combination with

improved oral hygiene (Haffajee et al. 2003). This shifts the subgingival microbiota to a less pathogenic composition, which is characterized by high proportions of Gram-positive aerobic species and low proportions or preferably an absence of periodontopathogens (Ximenez-Fyvie et al. 2000b, Roberts & Darveau 2002). Unfortunately, it is currently unclear to which proportions pathogens need to decrease or Gram-positive aerobic species need to increase to consider a subgingival biofilm as being not pathogenic. Although reductions in the total subgingival microbiota of up to 2-log values can easily be achieved, a re-colonization primarily by less pathogenic bacteria towards baseline numbers occurs within weeks (Harper & Robinson 1987, Goodson et al. 1991, Maiden et al. 1991). The shift towards a less pathogenic microbiota is only temporary, with a re-establishment of more aggressive microbiota within weeks to months (Mousques et al. 1980, Magnusson et al. 1984b, van Winkelhoff et al. 1988b, Wade et al. 1992, Quirynen et al. 2005). The dynamics of this re-colonization process depends on the level of oral hygiene, the efficacy of the subgingival debridement and the residual probing pocket depths (PPD) (Magnusson et al. 1984a, van Winkelhoff et al. 1988a, Sbordone et al. 1990, Pedrazzoli et al. 1991, Petersilka et al. 2002). The temporary use of antibiotics or antiseptics, either locally or systemically, does not really improve the long-term effect of periodontal therapy (Quirynen et al. 2002). Therefore, some authors have started to focus on the third aetiological factor for plaque-related periodontal inflammation: 'the reduction or absence of the 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 prevention and treatment of plaque-related periodontal diseases.

It is, however, important to realize, as outlined below, that probiotic microorganisms do not act exclusively by affecting the microbiota. They can also exert effects either by modulating immunological parameters, epithelial permeability and bacterial translocation, or by providing bioactive or regulatory metabolites (de Vrese & Schrezenmeir 2008). The latter effects are appealing for periodontal healthcare because current evidence shows that the destruction of the periodontium is substantially

mediated by the host and driven by the bacterial challenge (Sanz et al. 2005). Therefore, probiotics might not only suppress the emergence of endogenous pathogens or prevent the superinfection with exogenous pathogens but also they might also protect us through the promotion of a beneficial host response (Roberts & Darveau 2002).

Surprisingly, back in 1954, although not called probiotics at that time, a beneficial effect of lactic acid bacteria on inflammatory infections of the oral mucosa was reported (Kragen 1954). Noteworthy are also some anecdotal Russian reports from the 1990s, on the use of probiotics in the treatment of periodontitis (Pozharitskaia et al. 1994, Grudianov et al. 2002, Volozhin et al. 2004). Next to the scientific introduction of probiotics, commercial exploitation almost immediately followed with claimed beneficial effects on periodontal health. Given the potent paradigm shift that this phenomenon of oral probiotics can give rise to in the field of periodontal healthcare, it should therefore be based on solid clinical evidence.

Purpose

The purpose of this review was to analyse whether the use of probiotics can influence the periodontal microbiota. In order to answer the focused question "Can probiotics offer opportunities to manipulate the periodontal microbiota?", the possible mechanisms of action of probiotics specifically focusing on the periodontal environment were addressed narratively. Additionally, the clinical effects of oral probiotics on periodontal health were reviewed systematically.

Possible Mechanisms of Probiotic Action (Narrative Review)

Search strategy

In order to review the possible mechanisms of action of oral probiotics on the periodontal environment, a Medline (PubMed) search was performed to identify articles investigating the addressed question. The search was restricted till 30 June 2010. A similar search was conducted on the ISI Web of KnowledgeSM database. The literature was searched specifically focusing on the general working concepts of probiotics where we specifically looked for literature related to the oral environment and oral

bacteria. Owing to the low number of papers that came out of the initial searches, the searches were not limited to searches where the word "probiotic" was one of the search terms. A variety of search terms were used based on the known mechanisms of probiotic interaction in other fields of healthcare (de Vrese & Schrezenmeir 2008).

Additional hand searches were performed and included: (1) bibliographies of previous reviews on the topic of oral probiotics (Caglar et al. 2005a, Meurman 2005, Meurman & Stamatova 2007, de Vrese & Schrezenmeir 2008, Teughels et al. 2008, Bonifait et al. 2009, Stamatova & Meurman 2009a, Stamatova & Meurman 2009b) (2) bibliographies of all publications considered in this review and (3) cited reference searches of all publications considered in this review using the ISI Web of KnowledgeSM database.

A priori, this review was restricted to full-text peer-reviewed publications dealing with oral microbial interactions, which could constitute the basis for probiotic periodontal healthcare in the English language. Data from in vitro, human and animal studies were evaluated.

Results

The mechanisms of probiotic action in the mouth are expected to be similar to those observed in other parts of the body. However, it has been suggested that gastrointestinal tract probiotics may need some additional properties when used as oral probiotics. For instance, oral probiotic bacteria should adhere to and colonize periodontal tissue including hard non-shedding surfaces and should become part of the biofilm. They should not ferment sugars, which subsequently lowers the pH and can be detrimental, resulting in caries (Caglar et al. 2005a, Meurman 2005). Although from a theoretical standpoint, this might be plausible, currently there is no evidence to support these suggestions. Moreover, many of the gastro-intestinal probiotics exert their effect without colonizing or with only a temporary colonization of the host. As soon as their intake stops, the probiotic bacteria are excreted. Even without a permanent colonization, it may be anticipated that the repeated daily use of probiotic products over a long period of time will support an increased level of the probiotic in the oral cavity. The observation that probiotic bacteria do not need to

permanently colonize their host in order to exert their effects can be attributed to their mechanisms of action.

The effects of probiotics can originate from three main modes of action: (1) modulation of host defenses including the innate as well as the acquired immune system, (2) production of anti-microbial substances against periodontopathogens and (3) competitive exclusion mechanisms. In all likelihood, there exists not a single probiotic bacterium exhibiting all three principles, at least not to the extent that it could be a remedy for prevention or therapy of all types of diseases (Bonifait et al. 2009, Oelschlaeger 2010). Therefore, probiotic strains are often used in combination with each other in order to increase the number of beneficial effects. Also extrapolating effects exerted on the gastro-intestinal microbiota to effects on the oral microbiota is cumbersome. Additionally, it is important to realize that probiotic bacterial strains can behave differently or induce completely opposite effects, which make generalizations of strain effects to species effects difficult.

Ultimately, evidence must emerge from clinical studies. While certain modes of actions shown in vitro suggest a means, these action mechanisms might be altered or degraded within the oral cavity and thereby have little chance of conferring oral health benefits (Reid et al. 2006).

Immune modulation

Despite the obvious anti-microbial actions of probiotics, they can also act on a wide variety of cells to modulate the immune system towards anti-inflammatory action. Probiotic bacteria or their products (e.g. metabolites, cell wall components and DNA) can be recognized by host cells such as epithelial cells and immune cells (Delcenserie et al. 2008, Oelschlaeger 2010). Increased phagocytic capacity of macrophages when challenged with *Lactobacillus acidophilus* and *Lactobacillus casei* has been reported (Perdigon et al. 2002). It is known that probiotics can regulate the expression of phagocytosis receptors in the neutrophils of healthy individuals (Pelto et al. 1998) and enhance natural killer cell activity (Takeda et al. 2006). They have also been shown to modulate the immune response via the adaptive immunity (Link-Amster et al. 1994, Braat et al. 2004).

Only few studies have been conducted to determine whether immunomodulation by so-called beneficial bacteria also applies to the oral environment. In this aspect, several publications have shown that certain streptococci, such as *Streptococcus cristatus*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguinis* can attenuate the IL-8 response induced by periodontopathogens such as *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* on epithelial cells (Cosseau et al. 2008, Zhang et al. 2008, Sliepen et al. 2009a). The exact regulatory systems are still unclear, although there are indications that these streptococci can inhibit the nuclear factor κ B-pathway (Cosseau et al. 2008, Zhang et al. 2008). Recently, Della Riccia et al. (2007) tested in vivo the immunomodulatory effects of *Lactobacillus brevis* on periodontal disease. The in vivo use of this probiotic led to a significant decrease in inflammatory markers in the saliva, such as metalloproteinase and nitric oxide synthase activity, prostaglandin E₂ (PGE₂) and interferon γ (IFN- γ) levels. No effect was observed on IgA levels.

Antimicrobial substances produced by probiotics

Probiotic bacteria can produce a diverse range of compounds that act as antimicrobial agents such as lactic acid, hydrogen peroxide, bacteriocins and bacteriocin-like inhibitory substances. (Gillor et al. 2008, Gordon 2009, Oelschlaeger 2010)

Short-chain fatty acids such as lactic acids can pass across bacterial cell membranes and acidify the cytoplasm, which in turn can inhibit bacterial proliferation. In this respect, Sookkhee et al. (2001) were able to isolate lactic acid bacteria from healthy oral cavities of Thai volunteers and showed that they had an antimicrobial activity against *Porphyromonas gingivalis* and *Streptococcus mutans*. This activity was higher at an acidic pH, indicating that the antimicrobial effect was partly mediated by organic acids like lactic acid. This observation was largely confirmed by Koll-Klais et al. (2005) who showed higher prevalence of obligatory homofermentative lactobacilli, especially *Lactobacillus gasseri*, among healthy persons when compared with periodontitis persons. Homofermentative lactobacilli produce higher concentra-

tions of lactic acid in comparison with heterofermentative lactobacilli and induced therefore a more pronounced inhibition of *P. gingivalis* or *Prevotella intermedia*.

Various in vitro and in vivo studies have shown that production of hydrogen peroxide by probiotic bacterial strains can inhibit the growth of pathogenic bacterial species (Mashimo et al. 1985, Tompkins & Tagg 1986, Makras & De Vuyst 2006, Falagas et al. 2007). In this aspect, Hillman & Shivers (1988) showed in a gnotobiotic rat model that the level of *A. actinomycetemcomitans* colonization in these rats was 45-fold lower in animals infected with a hydrogen peroxide-producing *S. sanguinis* strain when compared with rats infected with a hydrogen peroxide-deficient mutant of this *S. sanguinis* strain. Vanderhoeven & Camp (1993) also showed that *S. mutans*, in co-culture with *S. sanguinis*, was more inhibited when hydrogen peroxide was added to the mixture.

Bacteriocins are ribosomally synthesized cationic peptides with a narrow spectrum of antimicrobial activity, whereas bacteriocin-like inhibitory substances have a broader spectrum (Silva et al. 1987, Cintas et al. 2001). Several bacteriocins derived from indigenous oral bacteria have been described (Oliveira et al. 1998, Teanpaisan et al. 1998, Hillman et al. 2000, Hillman 2002, Lima et al. 2002, Hillman et al. 2007). *S. salivarius* produces even two potent bacteriocins, salivaricin types A and B. This strain has been used to prevent dental caries caused by *Streptococcus sobrinus* and *S. mutans*. Salivaricin B was effectively used to treat halitosis caused by *Prevotella* spp. and *Micromonas micra* (Balakrishnan et al. 2000, Burton et al. 2005, Burton et al. 2006). Additionally, a bacteriocin from *Lactobacillus paracasei* HL32 was shown to be able to kill *P. gingivalis* by changing the cell envelope of the pathogen (Pangsomboon et al. 2006).

Competitive exclusion

The competitive exclusion principle, also referred to as Gause's law, states that two species that compete for the same resources cannot stably co-exist. One of the two competitors will always have a slight advantage over the other that leads to extinction of the second competitor or a shift of this species to another niche. The competitive exclu-

sion mechanism used by beneficial bacteria can occur on two levels: (1) hindering the adhesion of pathogenic bacteria or (2) competing for the same nutrients.

Hindering the adhesion of pathogenic bacteria

The literature points out that antagonistic strains are better adapted to their niche than potential pathogens, and can therefore interfere in disease by passively occupying the niche or actively restricting the adhesion capability of pathogens to surfaces. However, definitive proof that any of these mechanisms occur in vivo, has seldom been given. It has been shown that several bacterial strains, mainly streptococci can hinder colonization of periodontopathogens to hard and soft tissue surfaces in vitro (Teughels et al. 2007a, Sliepen et al. 2008, Van Hoogmoed et al. 2008a, Sliepen et al. 2009b).

An alternative way for probiotics to hinder pathogens is the production of biosurfactants that prevent adhesion. Van Hoogmoed et al. (2000) observed that a biosurfactant generated by *S. mitis* BA and BMS cells was able to decrease the adhesion of not only *S. mutans* but also from several periodontopathogens.

Interestingly, probiotics have been shown to inhibit adhesion by modifying the protein composition of the binding site. In this aspect, Haukioja et al. (2008) have shown that certain probiotic strains modify the salivary pellicle protein composition by removing an important adhesion protein, salivary agglutinin gp340, which is necessary for adhesion of *S. mutans*. The latter resulted in a lower colonization efficiency of *S. mutans*.

Competition for essential nutrients

Bacteria can compete for certain essential nutrients or chemicals required for growth and in doing so can inhibit the growth of a pathogen (Elli et al. 2000). As an example, *P. intermedia* utilizes vitamin K to grow. However, this resource may be replaced by progesterone or oestrogen. The levels of progesterone and oestrogen in gingival crevicular fluid are greatly increased during pregnancy. This may explain the transition from a healthy microbiota to the pathogenic one seen during pregnancy gingivitis. Probiotic bacteria, able

to outcompete periodontopathogens for uptake of these nutrients, could improve oral health. More studies have yet to be performed in this field (Wang et al. 1990, Smith & Pippin 1998).

Other mechanisms of probiotic action

The above outlined mechanisms of probiotic action are numerous; however, other modes of action exist. These are either not applicable for the oral situation or studies have not yet been conducted in these areas. One example of a probiotic mechanism that is relevant for improving gastro-intestinal health is the enhancement of the mucosal barrier function. Probiotics can influence mucosal cell-cell interactions by the enhancement of the intestinal barrier function. Disruption of this epithelial barrier is encountered in several conditions including inflammatory bowel disease and autoimmune diseases such as Type 1 diabetes. Enhancement of the barrier by probiotics can benefit the host in such diseases (Ng et al. 2009). Additionally, invasion of epithelial cells is an important mode by which bacteria exert their pathogenicity. For gut epithelial cells, anti-invasive properties of probiotic bacteria have already been established. Secreted factors of *Bifidobacterium bifidum* strain Bb12 interfere with the invasion of epithelial cells by *Salmonella typhimurium* (Botes et al. 2008). These domains have not yet been explored in relation to the oral cavity.

Adverse effects and safety

Whereas it is important to understand the mode of action of oral probiotics, systemic safety of probiotics is even more important. It is obvious that, when probiotics are applied orally, at least a part of them will be ingested and can interact with a patient's systemic health. When ingested orally, probiotics are generally considered safe and well tolerated with bloating and flatulence occurring most frequently (Kligler & Cohnssen 2008). One theoretical concern associated with probiotics includes the potential for these viable organisms to move into the blood stream and cause systemic infections. Although rare, probiotic-related bacteraemia has been reported (Snydman 2008). It is estimated that the risk of developing bacteraemia from ingested lactobacilli probiotics is <1 per 1 million users

(Borriello et al. 2003). Although no serious adverse events have been described in clinical trials, systemic infections associated with specific probiotics have been noted in isolated reports. These include sepsis or endocarditis and liver abscess (Snydman 2008). Bacteraemia due to lactobacilli rarely occurs, but predisposing factors include immunosuppression, prior hospitalization, severe underlying comorbidities, previous antibiotic therapy and prior surgical interventions (Salminen et al. 2004). To date, there have been no reports of bifidobacterial sepsis associated with the use of a probiotic, supporting the low pathogenicity of bifidobacteria species (Boyle et al. 2006). Fortunately, most cases of probiotic bacteraemia have responded well to appropriate antibiotic therapy. Recently, major and minor risk factors for probiotic-associated sepsis have been identified. Major risk factors include immunosuppression (including a debilitated state or malignancy) and prematurity in infants. Minor risk factors are the presence of a central venous catheter, impairment of the intestinal epithelial barrier (such as with diarrhoeal illness), cardiac valvular disease (*Lactobacillus* probiotics only), concurrent administration with broad-spectrum antibiotics to which the probiotic is resistant and administration of probiotics via a jejunostomy tube (this method of delivery could increase the number of viable probiotic organisms reaching the intestine by bypassing the acidic contents of the stomach). Therefore, Boyle and colleagues recommend that probiotics should be used cautiously in patients with one major risk factor or more than one minor risk factor. Probiotics should also be used cautiously in patients taking immunosuppressants, such as cyclosporine, tacrolimus, azathioprine and chemotherapeutic agents, because probiotics could cause an infection or pathogenic colonization in immunocompromised patients. Additionally, probiotic strains of *Lactobacillus* have also been reported to cause bacteraemia in patients with short-bowel syndrome, possibly due to altered gut integrity (Kligler & Cohnssen 2008) and *Lactobacillus* preparations are contraindicated in persons with a hypersensitivity to lactose or milk. No contraindications are currently listed for bifidobacteria, because most species are considered non-pathogenic and non-toxicogenic (Kligler & Cohnssen 2008).

Clinical Effects of Oral Probiotics on Periodontal Health (Systematic Review)

Materials and methods

Focused question

Do probiotics alter the periodontal condition or the outcome of periodontal therapy?

Search strategy

A Medline (PubMed) search was performed to identify all articles investigating the addressed question. The search was restricted till June 30, 2010. A similar search was conducted on the Cochrane and the ISI Web of KnowledgeSM databases.

Additional hand searches were performed and included: (1) bibliographies of previous reviews on the topic of oral probiotics (Caglar et al. 2005a, Meurman 2005, Meurman & Stamatova 2007, de Vrese & Schrezenmeier 2008, Teughels et al. 2008, Bonifait et al. 2009, Stamatova & Meurman 2009a, Stamatova & Meurman 2009b) (2) bibliographies of all publications considered in this review and (3) cited reference searches of all publications considered in this review using the ISI Web of KnowledgeSM database.

Search terms

The term "replacement therapy" (also called "bacteriotherapy" or "bacterial interference" is sometimes used interchangeably with "probiotics". Although both approaches use living bacteria for the prevention or treatment of infectious disease, there are some slight differences (Wilson 2005, Teughels et al. 2008). Because there is much confusion over the terminology, we did not specifically differentiate between probiotic therapies and replacement therapies in this review. Therefore, the following MeSH terms and key words were used: "probiotic" AND "periodontal", "probiotic" AND "periodontitis", "probiotic" AND "periodontics", "probiotic" AND "gingivitis", "probiotic" AND "oral health", "replacement therapy" AND "periodontal", "replacement therapy" AND "periodontitis", "replacement therapy" AND "periodontics", "replacement therapy" AND "gingivitis", "replacement therapy" AND "oral health", "bacteriotherapy" AND "periodontal", "bacteriotherapy" AND "periodontitis", "bacteriotherapy" AND "periodontics", "bacteriotherapy"

AND “gingivitis”, “bacteriotherapy” AND “oral health”, “bacterial interference” AND “periodontal”, “bacterial interference” AND “periodontitis”, “bacterial interference” AND “periodontics”, “bacterial interference” AND “gingivitis”, “bacterial interference” AND “oral health”,

Inclusion criteria

A priori, this review was restricted to full-text peer-reviewed publications dealing with probiotics for periodontal healthcare in the English language. Data from both human and animal studies were evaluated. If articles reported on case series, at least five consecutive cases had to be enrolled. All study designs were considered.

Exclusion criteria

Publications not meeting the inclusion criteria were excluded from the review.

Data extraction

Article selection was determined by screening of the titles and the abstracts by two independent reviewers (G. L. & W. T.). In case of disagreement between the reviewers, inclusion/exclusion decision was made by discussion after screening the full-text article. Data were extracted simultaneously by the two reviewers and recorded in a data extraction sheet. If data had to be extracted from graphs, this was properly acknowledged in the corresponding table. The heterogeneity of the studies and outcome variables rendered a meta-analysis impossible.

Results

Out of the 160 studies retrieved by the Medline, Cochrane and ISI Web of KnowledgeSM database searches, nine were selected based on the inclusion and exclusion criteria (Hillman & Shivers 1988, Krasse et al. 2006, Teughels et al. 2007b, Nackaerts et al. 2008, Shimauchi et al. 2008, Mayanagi et al. 2009, Staab et al. 2009, Twetman et al. 2009, Zahradnik et al. 2009). An additional five studies (Ishikawa et al. 2003a, Kang et al. 2006a, Matsuoka et al. 2006, Della Riccia et al. 2007, Sugano et al. 2007) could be retrieved by searching the bibliographies of the selected papers and by cited reference searches. Of these five studies, one

study (Matsuoka et al. 2006) did not meet the inclusion criteria because it was written in Japanese. However, this Japanese paper was identical to an English paper (Sugano et al. 2007) but included additional clinical data. Therefore, the Japanese study was included in this review but only for extracting the clinical data that accompanied the English paper version of the study. This resulted in a retrieval of 14 publications (Hillman & Shivers 1988, Ishikawa et al. 2003a, Kang et al. 2006a, Krasse et al. 2006, Matsuoka et al. 2006, Della Riccia et al. 2007, Sugano et al. 2007, Teughels et al. 2007b, Nackaerts et al. 2008, Shimauchi et al. 2008, Mayanagi et al. 2009, Staab et al. 2009, Zahradnik et al. 2009, Twetman et al. 2009).

The retrieved studies were extremely heterogeneous in the set-up of the study, the used probiotics, the mode of application and outcome measures. This heterogeneity did not allow a meta-analysis.

Only seven of the 14 papers revealed or referenced in vitro experiments showing that the probiotic strains used had the potency to interact with the oral microbiota (Hillman & Shivers 1988, Ishikawa et al. 2003a, Matsuoka et al. 2006, Sugano et al. 2007, Teughels et al. 2007b, Nackaerts et al. 2008, Zahradnik et al. 2009). The other studies either did not reveal any form of interaction or based the selection of the probiotic strain on general assumptions, not specifically relating to periodontal healthcare.

In only four papers, reporting on data from three independent studies, probiotics were administered to a suppressed oral ecology (either as adjunct to scaling and root planing or germ-free animals) (Hillman & Shivers 1988, Krasse et al. 2006, Teughels et al. 2007b, Nackaerts et al. 2008).

Of the 14 retrieved papers, three papers (Hillman & Shivers 1988, Teughels et al. 2007b, Nackaerts et al. 2008) reported on data from two independent animal studies. The general outline of the three papers or two studies is shown in Table 1. Both studies called themselves “replacement therapy” studies rather than “probiotic” studies. Additionally, both studies did not use the rather conventional *Lactobacillus* spp. or *Bifidobacterium* spp. but used streptococci as effector strains. The use of the selected effector strains was supported by a series of in vitro experiments or clinical observations that these strains (1) could inhibit the growth of periodontopathogens (Hillman et al.

1985, Tanzer et al. 1985), (2) were associated clinically with periodontal health (Liljemark et al. 1984, Wolff et al. 1985, Ximenez-Fyvie et al. 2000a) or (3) could inhibit the colonization of periodontopathogens towards hard and soft tissues (Teughels et al. 2007a, Van Hoogmoed et al. 2008b).

Seven of the 14 retrieved papers reported on data from six clinical studies where periodontally healthy or gingivitis subjects used probiotics. The general outline of these seven papers is shown in Table 2. The papers by Krasse et al. (2006) and by Twetman et al. (2009) reported on parallel, double-blind, placebo-controlled, randomized clinical studies in which *Lactobacillus reuteri* strains were administered. However, the study of Krasse and colleagues did not reveal which strains were used. The papers by Shimauchi et al. (2008) and Mayanagi et al. (2009) both reported on one parallel, double-blind, placebo-controlled, randomized clinical study in which *Lactobacillus salivarius* WB21 was used as a probiotic. All subjects who volunteered to participate in the study were company workers of the company that produced the probiotic tablets. Additionally it should be noted that in this study, both the probiotic tablets as the placebo tablets contained xylitol. The additional clinical studies, which were all open label studies, used either *Weissella cibaria* CMS1 (Kang et al. 2006a), *L. casei* Shirota (Staab et al. 2009), or a combination of *Streptococcus oralis* KJ3sm, *Streptococcus uberis* KJ2sm and *Streptococcus rattus* JH145 (Zahradnik et al. 2009).

It should be noted that in only one of the papers reporting on the effects of a probiotic treatment on healthy or gingivitis patients (Krasse et al. 2006), the probiotics were administered as an adjunct to conventional plaque removal.

Of the 14 retrieved papers, four papers reported on data from three independent clinical studies on periodontitis patients. The general outline of these four papers is shown in Table 3. Three of the four papers used the same probiotic strain, *Lactobacillus salivarius* TI 2711 and came from the same group of researchers. The paper of Ishikawa et al. (2003a) did not explicitly mention that the patients that participated in this study were periodontitis patients. However, we assumed that these were periodontitis patients based on the two additional papers (Matsuoka et al. 2006, Sugano et al. 2007) published by co-authors on

Table 1. Animal studies

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 (1988)	Artificial oral <i>A. actinomycetemcomitans</i> infections	Gnotobiotic rats (21 days)	Parallel, open label, placebo-controlled	1, 2.5	Placebo A	6	None	Swap	Single application at baseline		Sterile broth	(1) <i>A. actinomycetemcomitans</i> counts	Significantly lower <i>A. actinomycetemcomitans</i> counts in probiotic A and B groups versus placebo A and B groups. No significant differences in <i>S. sanguinis</i> counts between placebo B, probiotic A and probiotic B groups
					Placebo B	6	None	Swap	Single application at baseline	<i>S. sanguinis</i> KJ3sm hydrogen peroxidase mutant	Overnight broth culture	(2) <i>S. sanguinis</i> counts	
					Probiotic A	6	None	Swap	Single application at baseline	<i>S. sanguinis</i> KJ3sm	Overnight broth culture		
					Probiotic B	6	None	Swap	Single application at baseline	Revertant of <i>S. sanguinis</i> KJ3sm hydrogen peroxidase mutant	Overnight broth culture		
Teughels et al. (2007b)	Artificially created periodontal pockets	Beagle dogs (3.1 years)	Split-mouth, randomized	3	No treatment group	8 dogs, 2 pockets/dog	None	None				(1) Subgingival microbiological changes	Probiotic group 2 showed significantly more pronounced reductions in total anaerobic bacteria, black pigmented bacteria and <i>Campylobacter rectus</i> 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 control group
					Control group	8 dogs, 2 pockets/dog	Scaling and root planing	None				(2) Bleeding upon probing	
					Probiotic group 1	8 dogs, 2 pockets/dog	Scaling and root planing	Pure bacterial pellets	1 time (baseline) subgingivally applied	<i>S. salivarius</i> , <i>S. mitis</i> , <i>S. sanguinis</i>	$> 1 \times 10^9$ /ml	(3) Probing pocket depth	
					Probiotic group 2	8 dogs, 2 pockets/dog	Scaling and root planing	Pure bacterial pellets	4 times (baseline, week 1, 2 and 4) subgingivally applied	<i>S. salivarius</i> , <i>S. mitis</i> , <i>S. sanguinis</i>	$> 1 \times 10^9$ /ml	(4) Clinical attachment level	
Nackaerts et al. (2008)	Artificially created periodontal pockets	Beagle dogs (3.1 years)	Split-mouth, randomized	3	Control group	8 dogs, 2 pockets/dog	Scaling and root planing	None				(1) Bone density	Bone density in probiotic group improved significantly while this was non-significant for the control group.
					Probiotic group	8 dogs, 2 pockets/dog	Scaling and root planing	Pure bacterial pellets	4 times (baseline, week 1, 2 and 4) subgingivally applied	<i>S. salivarius</i> , <i>S. mitis</i> , <i>S. sanguinis</i>	$> 1 \times 10^9$ /ml	(2) Alveolar bone level	Significant increase in bone level in probiotic group while no significant changes were noted in the placebo group

A. actinomycetemcomitans, *Aggregatibacter actinomycetemcomitans*; *S. sanguinis*, *Streptococcus sanguinis*; *S. salivarius*, *Streptococcus salivarius*; *S. mitis*, *Streptococcus mitis*.

Table 2. Healthy-gingivitis studies

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
<i>Lactobacillus reuteri</i> studies													
Krasse et al. (2006)	Gingivitis	Adult (55.9 years)	Parallel, double-blind, placebo-controlled, randomized	0.5	Placebo group	18	Plaque removal	Chewing gum	2/day for 14 days	<i>L. reuteri</i> formulation 1	1×10^8	(1) Gingivitis scores (2) Plaque scores	Significantly higher gingivitis reduction in probiotic group 1 compared with placebo group. Significant reduction in plaque scores for both probiotic groups in contrast to no reduction in placebo group
					Probiotic group 1	20	Plaque removal	Chewing gum	2/day for 14 days				
					Probiotic group 2	21	Plaque removal	Chewing gum	2/day for 14 days				
Twetman et al. (2009)	Gingivitis	Young adult (24 years)	Parallel, double-blind, randomized, placebo-controlled	1	Placebo	12	None	Chewing gum	2 chewing gums/day for 2 weeks	<i>L. reuteri</i> (ATCC 55730 and ATCC PTA 5289)	1×10^8 CFU/day	(1) BOP (2) Volume GCF (3) Concentration IL-1B, TNF- α , IL-6, IL-8, IL-10	BOP improved and GCF volume decreased in all groups during the 2 first weeks statistically significant in both probiotic groups. The levels of TNF- α and IL-8 decreased significantly in probiotic group B compared with baseline after 1 and 2 weeks
					Probiotic A	13	None	Chewing gum	2 chewing gums/day for 2 weeks				
					Probiotic B	13	None	Chewing gum	2 chewing gums/day for 2 weeks				
<i>Lactobacillus salivarius</i> studies													
Shimauchi et al. (2008)	Healthy volunteers without severe periodontitis	Adult (32–61 years)	Randomized, double-blind, placebo-controlled study	2	Control group	32	None	Tablets	3 tablets/day for 8 weeks	Xylitol (840 mg/day) <i>L. salivarius</i> WB21 + xylitol 840 mg/day	2.01×10^9 /day	(1) Probing pocket depth (2) Gingivitis scores (3) BOP (4) Plaque scores (5) Lactoferrin levels in saliva	Periodontal parameters except probing pocket depth were improved in both groups after an 8-week intervention. No statistically significant differences were observed between groups. However, when analyzing only the current smokers, the test group showed a significantly greater improvement in plaque scores. Also a significant difference was found in probing pocket depth at week 8 between current smokers in the placebo group versus the probiotic group, in favour of the probiotic group
					Probiotic group	34	None	Tablets	3 tablets/day for 8 weeks				
Mayanagi et al. (2009)	Healthy volunteers without severe periodontitis	Adult (32–61)	Randomized, double-blind, placebo-controlled	2	Control group	32	None	Tablets	3 tablets/day for 8 weeks	Xylitol (840 mg/day) <i>L. salivarius</i> WB21 + xylitol 840 mg/day	2.01×10^9 /day	Bacterial numbers (total, <i>P. gingivalis</i> , <i>P. intermedia</i> , <i>T. forsythia</i> , <i>T. denticola</i>)	The numerical sum of five selected periodontopathogenic bacteria in the test was significantly decreased in subgingival plaque after 4 weeks and tended to be lower up to 8 weeks as compared with placebo group
					Probiotic group	34	None	Tablets	3 tablets/day for 8 weeks				

Other studies	Healthy gingivitis	Young adult (20–30 years)	1-period crossover, open label, placebo-controlled	1 day	Placebo group	72	None	Oral rinse	15 ml, 2/day for 2 min.	15 ml, 2/day for 2 min.	1 × 10 ⁹ /ml	Plaque scores	Significant reduction in plaque score for the probiotic group
Kang et al. (2006a)	Healthy gingivitis	Young adult (20–30 years)	1-period crossover, open label, placebo-controlled	1 day	Placebo group	72	None	Oral rinse	15 ml, 2/day for 2 min.	15 ml, 2/day for 2 min.	1 × 10 ⁹ /ml	(1) Papilla bleeding index (2) Interproximal plaque (3) Plaque index (4) GCF tested for polymorphonuclear elastase, MPO and MMP-3	At the end of the study, elastase activity was significant lower in the probiotic group when compared with the control group. When compared with baseline, the plaque index and papilla bleeding index increased and amount of the MMP-3 decreased in the probiotic group
Staab et al. (2009)	Healthy	Young adults (24 years)	Randomized, parallel, open label	2	Control group Probiotic group	25 25	None None	None Probiotic milk drink	None 1/day (65 ml) for 8 weeks				Decrease by 2 orders of magnitude for <i>C. rectus</i> and <i>P. gingivalis</i> in subgingival plaque. No effect on <i>P. intermedia</i> . 0.8 orders of magnitude reduction in <i>S. mutans</i> numbers in saliva. Results were not significant because of small subject number
Zahradnik et al. (2009)	Healthy gingivitis	Young adult colonized by measurable levels of <i>S. mutans</i> and one or more periodontal pathogens (21–35)	Open label follow-up study	1	Probiotic group	11	None	Oral rinse	2/day for 4 weeks		2 × 10 ⁸ of each species/day	Bacterial numbers in subgingival plaque and saliva	

S. mutans, *Streptococcus mutans*; *P. gingivalis*, *Porphyromonas gingivalis*; *P. intermedia*, *Prevotella intermedia*; *T. forsythia*, *Tannerella forsythia*; *T. denticola*, *Treponema denticola*; *W. cibaria*, *Weissella cibaria*; *S. oralis*, *Streptococcus oralis*; *S. rattus*, *Streptococcus rattus*; MMP, matrix metalloproteinase; MPO, myeloperoxidase; BOP, bleeding upon probing; GCF, gingival crevicular fluid; TNF- α ; tumour necrosis factor- α ; IL, interleukin.

the initial Ishikawa paper and based on the use of the same probiotic strains. Only the paper of Matsuoka et al. (2006) provided data on periodontal disease (average PPD of 4.5 mm). The paper by Della Riccia et al. (2007) mentions that these patients were chronic periodontitis patients but does not provide data on periodontal disease. It was a clear limitation that for only one of the three independent studies (or two of the four papers), data were provided that proved that these were real periodontitis patients. It should be noted that in none of the papers reporting on the effects of a probiotic treatment on periodontitis patients, were the probiotics administered as an adjunct to conventional scaling and root planing.

Microbiological changes

Six papers reported on microbiological changes induced by probiotic therapies (Table 4). In general, all studies showed at least that probiotic application resulted in microbiological changes, even though they were not always applied as an adjunct to standard periodontal treatment (scaling or root planing). Unfortunately, most of the studies did not perform a statistical inter-group analysis. In the gnotobiotic rat model study of Hillman & Shivers (1988), it was shown that the level oral colonization by *A. actinomycetemcomitans* was approximately 1.8 log lower in animals co-infected with *S. sanguinis* KJ3sm and 1.0 log lower in animals co-infected with the revertant of the hydrogen-peroxide-deficient mutant of KJ3sm than in animals infected only with *A. actinomycetemcomitans*. 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 (Haffajee, personal communication). In the Beagle dog model study of Teughels et al. (2007b), where no oral hygiene was performed, multiple applications of *S. salivarius*, *S. mitis* and *S. sanguinis* resulted in significant microbiological changes in subgingival plaque. Significant decreases in the numbers of anaerobic bacteria, black pigmented bacteria, *Porphyromonas gulae* (canine variant of *P. gingivalis*), *P. intermedia* and *Campylobacter rectus* were noted and a tendency to increasing numbers of aerobic bacteria was

Table 3. Periodontitis studies

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
<i>Lactobacillus salivarius</i> studies Ishikawa et al. (2003a)	Not mentioned	Adult (22–62)	Parallel, open label	2	Control group	21	None	Tablets	5 tablets, 5/day for 8 weeks	<i>L. salivarius</i> TI 2711	2×10^7 /day	Bacterial numbers in saliva	No significant effects change in total number of bacteria, number of mutants streptococci, number of lactobacilli. Significant reduction in number of black pigmented anaerobic rods for both probiotic groups
					Probiotic A	28	None	Tablets	5 tablets, 5/day for 8 weeks	<i>L. salivarius</i> TI 2711	1×10^8 /day		Significant 0.6 log reduction in total bacteria at week 12, and 1.2 log at week 16. Significant 0.5 log reduction in <i>P. gingivalis</i> at week 12. Baseline numbers returned for <i>P. gingivalis</i> 4 weeks after discontinuing probiotic. Significant 4 log increase at week 12 and 3 log at week 16 in subgingival Lactobacilli
					Probiotic B	29	None	Tablets	5 tablets, 5/day for 8 weeks	<i>L. salivarius</i> TI 2711	2×10^7 /day		Significant changes in bleeding upon probing, pocket depth No significant inter-group differences for these assessment criteria.
Sugano (2007)	Periodontitis	Adult	Double-blind, placebo-controlled, parallel	4	Placebo	45	None	Tablets	5 tablets, 5/day for 12 weeks			Subgingival bacterial numbers	All clinical parameters analysed decreased statistically significant between baseline and day 4 with <i>L. brevis</i> containing lozenges. The authors mention that the antibiotic treatment did not significantly modify signs and symptoms** but did not provide the data.
					Probiotic A	50	None	Tablets	5 tablets, 5/day for 12 weeks	<i>L. salivarius</i> TI 2711	2×10^8 /day		Significant decreases were seen in nitrite/nitrate, PGE ₂ , matrix metalloproteinase and IFN- γ levels in saliva after treatment with <i>L. brevis</i> containing lozenges
					Probiotic B	13	None	Tablets	5 tablets, 5/day for 12 weeks	<i>L. salivarius</i> TI 2711	2×10^7 /day		
Matsuoka et al. (2006)	Periodontitis	Adult	Double-blind, placebo-controlled, parallel	4	Placebo	45	None	Tablets	5 tablets, 5/day for 12 weeks			(1) Average pocket depth (2) Bleeding upon probing	
					Probiotic A	50	None	Tablets	5 tablets, 5/day for 12 weeks	<i>L. salivarius</i> TI 2711	2×10^8 /day		
					Probiotic B	13	None	Tablets	5 tablets, 5/day for 12 weeks	<i>L. salivarius</i> TI 2711	2×10^7 /day		
Other studies Della Riccia et al. (2007)	Chronic periodontitis	Adult (30–51)	Double-blind paired-comparison study? 2 arm open label comparative study	4 days	Probiotic group	21	None	Lozenges	4 lozenges/day for 4 days	<i>L. brevis</i> (CD2)	200 mg/lozenge	(1) Plaque scores (2) Gingivitis scores (3) Bleeding on probing (4) Calculus (5) Temperature sensitivity scores (6) Inflammatory markers in saliva (MMP, NOS, IgA, PGE ₂ , IFN- γ)	
					Control group	5 (29–48)	None	Unknown	“Conventional” spiramycin 1×300 mg/day				

NOS, nitric oxide synthase; PGE₂, prostaglandin E₂; IFN- γ , interferon- γ .

Table 4. Microbiological outcomes

Study	Type of infection	Study follow-up time (weeks)	Time of assessment (weeks)	Type of sample	Statistical comparison	Study group	Strains	Microbiological log changes in comparison with baseline						Remarks		
								total anaerobes	total aerobes	total bacterial count	pigmented bacteria	A. actinomycetemcomitans	C. rectus		P. gingivalis/gulae	P. intermedia
Hillman & Shivers et al. (1988) (2007b)	Artificial oral A. actinomycetemcomitans infection of rats	5	5	Oral swab	INTER	(A) No treatment						0 ^{a-d}			Log differences expressed in comparison with Placebo A. In the absence of oral hygiene	
						(B) Placebo	S. sanguinis KJ3sm hydrogen peroxidase mutant					– 0.42 ^{c,d}				
						(C): Probiotic 1	S. sanguinis KJ3sm					– 1.83 ^{ab}				
						(D) Probiotic 2	Revertant of S. sanguinis KJ3sm hydrogen peroxidase mutant					– 1.02 ^{ab}				
Teughels et al. (2007b)	Artificially created periodontal pockets in Beagle dogs	12	12	Subgingival plaque	INTER	(A) No treatment		0.05 ^{b,c,d}	– 0.10		0.34 ^{b,c,d}		– 0.24 ^{c,d}	0.23 ^{b,c,d}	In the absence of oral hygiene. Data calculated from graphs	
						(B) Control		0.00 ^{a,c,d}	– 0.04		0.00 ^{a,d}		– 0.48	– 0.35 ^{a,c,d}		
						(C) Probiotic 1	S. salivarius, S. mitis, S. sanguinis	– 0.35 ^{a,b,d}	0.15		– 0.11 ^{a,d}		– 0.55 ^a	– 0.60 ^{a,b,d}		
						(D) Probiotic 2	S. salivarius, S. mitis, S. sanguinis	– 0.55 ^{a,b,c}	0.25		– 0.59 ^{a,b,c}		– 0.65 ^a	– 0.57 ^{a,b,c}		– 1.01 ^{a,b,c}
Mayanagi et al. (2009)	Healthy volunteers without severe periodontitis	8	8	Supra and subgingival plaque	INTER	(A) Placebo	Xylitol (840mg/day)								Format of data presentation did not allow data extraction. However, the numerical sum of five selected periodontopathogenic bacteria in the probiotic group was significantly decreased in subgingival plaque after 4 weeks and tended to be lower up to 8 weeks when compared with the placebo group	
						(B) Probiotic	L. salivarius WB21 + xylitol 840mg/day									
Ishikawa et al. (2003a)	Unknown	8	8	Saliva	INTRA	Zahradnik (2009)	Healthy gingivitis	4	4	Subgingival plaque	INTRA	Probiotic	S. oralis KJ3sm, S. uberis KJ2sm, and S. rattus JH145	– 2.60*	– 2.10*	
						(A) Control		– 0.30			0.00					
						(B) Probiotic 1	L. salivarius TI 2711	0.00			– 1.20*					
Sugano et al. (2007)	Periodontitis	16	12	Subgingival plaque	INTRA	(C) Probiotic 2	L. salivarius TI 2711	– 0.20			– 1.30*					
						(A) Placebo				– 0.63*					0.35	0.18
						(B) Probiotic 1	L. salivarius TI 2711			– 1.03*					– 0.26	– 0.29
						(C) Probiotic 2	L. salivarius TI 2711			– 0.58*			– 0.58*	– 0.17		

*Statistically difference result from baseline.

Superscript alphabets represent statistically significant difference from each other.

INTRA, intra-group statistical comparison (baseline versus time of assessment); INTER, inter-group statistical comparison at time of assessment study; *L. brevis*, *Lactobacillus brevis*.

observed. In comparison with scaling and root planing alone, multiple subgingival applications of *S. salivarius*, *S. mitis* and *S. sanguinis* resulted in a significant additional microbiological reductions of 0.5 log in anaerobic bacteria, 0.6 log in *P. gulae*, 0.6 log in black pigmented bacteria and 0.7 log in *P. intermedia*, 12 weeks after root planing and without any form of oral hygiene.

The publication by Mayanagi et al. (2009) unfortunately did not allow data extraction. However, the authors reported that the numerical sum of five selected periodontopathogenic bacteria in the probiotic group was significantly decreased in subgingival plaque after 4 weeks of probiotic usage and tended to be lower after 8 weeks when compared with the placebo group. Using a multivariate model adjusting for bacterial counts at baseline, plaque index and smoking status, the authors calculated that the odds ratio for a reduction of *Tannerella forsythia* in the probiotic group was significantly increased over the course of the study compared with the placebo group.

Zahradnik et al. (2009) detected 2.6 and 2.1 log reductions for respectively *C. rectus* and *P. gingivalis* in subgingival plaque when subjects were asked to rinse with a mixture of three streptococci. Although the data were not provided, the authors mention that the probiotic mixture did not influence the subgingival *P. intermedia* numbers.

When combining the microbiological effects for *L. salivarius* TI 2711 on untreated periodontitis patients, Ishikawa et al. (2003a) and Sugano et al. (2007) showed that this probiotic could reduce the salivary black pigmented bacteria levels with 1.3 log. Additionally, when compared with a placebo treatment, additional subgingival reductions of 0.93 log in *P. gingivalis* levels could be achieved.

Changes in plaque index

5 studies (Kang et al. 2006a, Krasse et al. 2006, Della Riccia et al. 2007, Shimauchi et al. 2008, Staab et al. 2009) report on changes in the amount of plaque when probiotics were used (Table 5). Surprisingly, only in the study of Krasse et al. (2006), plaque was removed before starting the probiotic therapy. With the exception of Staab et al. (2009), who even found an increase in plaque index, all studies report significant reductions on plaque index

when compared with baseline values. Only two studies have performed an inter-group comparison. Krasse et al. (2006) found no statistically significant differences between placebo and the probiotic groups whereas Shimauchi et al. (2008) could find a significant difference in favour of the probiotic group but only for current smokers.

Changes in gingivitis index

Of the four studies that reported on changes in gingivitis index, three studies (Krasse et al. 2006, Della Riccia et al. 2007, Shimauchi et al. 2008) report statistically significant decreases in gingivitis index when compared with baseline values (Table 5). In contrast, the study by Staab et al. (2009) shows a statistically significant increase in gingivitis index. Of the two studies that performed an inter-group statistical analysis, only the study by Krasse et al. (2006) showed significant differences between the placebo and one of the probiotic formulations.

Bleeding upon probing

All three human studies that report on bleeding upon probing show significant decreases when compared with baseline values (Table 5) (Della Riccia et al. 2007, Twetman et al. 2009). However, in the study by Twetman et al. (2009), the authors observed that as soon as the probiotic intake was stopped, the percentage of sites that were bleeding upon probing positive, increased again. Only one study looked for significant inter-group differences. This clinical study (Shimauchi et al. 2008) did not detect a statistically significant difference between the probiotic groups and the placebo group. However, in the Beagle dog study of Teughels et al. (2007b) (not incorporated in Table 5), a statistically significant lower bleeding upon probing was observed for pockets that received multiple applications of probiotics, when compared with scaled and root-planed pockets alone (30% versus 45%, respectively). This was considered to be remarkable by the authors because in this 12-week study, no oral hygiene was provided to the dogs.

PPD and clinical attachment level (CAL)

Of the two human studies that reported on changes in PPD ((Matsuoka et al.

2006, Shimauchi et al. 2008), only the study by Shimauchi et al. (2008) could detect statistically significant greater improvements in PPD for the probiotic group, but only for current smokers (Table 5). Also, the Beagle dog study of Teughels et al. (2007b) failed to show any significant inter-group differences in either PPD or in CAL improvements. These results were not surprising to the authors because no oral hygiene was provided to the dogs during the 12-week duration of the study.

Inflammatory markers

In the study of Twetman et al. (2009), the focus was predominantly on gingival inflammation and the production of pro- and anti-inflammatory cytokines [IL-1b, tumour necrosis factor- α (TNF- α), IL-6, IL-8 and IL-10]. During the 2 weeks of intervention, the gingival crevicular fluid volume decreased significantly in the probiotic groups, whereas no significant changes were observed in the placebo group. The levels of TNF- α and IL-8 also decreased significantly after 1 and 2 weeks respectively in the probiotic group, which used the highest dose of probiotics, compared with baseline. However, these effects were only temporary and tended to return to baseline values 2 weeks after discontinuing the probiotics.

Shimauchi et al. (2008) not only reported mainly on the clinical outcome of the study but also analysed salivary lactoferrin levels. The study showed that during the course of the study, for both the placebo as well as the probiotic group, salivary lactoferrin levels decreased significantly from baseline values. However, no significant differences were found between both study groups.

Staab et al. (2009), who investigated the effects of a commercially available probiotic milk containing *L. casei* Shirata on gingival health analysed, next to the amount of interproximal plaque and plaque index, the papilla bleeding index and polymorphonuclear elastase, myeloperoxidase and matrix metalloproteinase-3 in gingival crevicular fluid. At the end of this 8-week study, elastase activity was significantly lower in the probiotic group when compared with the control group. When compared with baseline values, the plaque index and papilla bleeding index increased and amount of the matrix metalloproteinase-3 decreased in the probiotic group

Table 5. Clinical outcomes

Study	Type of infection	Study time (weeks)	Time of follow-up assessment (weeks)	Statistical comparison	Study group	Strains	Microbiological log changes in comparison with baseline										PPD/CAL	Remarks
							plaque index			gingivitis index			bleeding upon probing					
							baseline	end of probiotic treatment	end of follow-up	baseline	end of probiotic treatment	end of follow-up	baseline	end of probiotic treatment	end of follow-up	baseline		
Krasse et al. (2006)	Gingivitis	2	2	INTER INTRA	(A) Placebo		1.5	1.3	2.2	1.8 ^{a,b}							Plaque removal before start probiotic therapy. Data calculated from graphs	
Twetman et al. (2009)	Gingivitis	4	2	INTRA	(B) Probiotic 1	<i>L. reuteri</i> formulation 1	1.3	1.1*	2.3	1.5 ^a							Format of data presentation did not allow data extraction. However, periodontal parameters were improved in both groups after an 8-week intervention. No statistically significant differences were observed between groups. However, when analysing only the current smokers, the test group showed a significantly greater improvement in plaque scores and PPD.	
					(C) Probiotic 2	<i>L. reuteri</i> formulation 2	1.7	1.5*	2.4	1.8*								
					(A) Placebo	<i>L. reuteri</i> (ATCC 55730 and ATCC PTA 5289)					68	45	64					
					(B) Probiotic 1	<i>L. reuteri</i> (ATCC 55730 and ATCC PTA 5289)					54	8*	23					
Shimauchi et al. (2008)	Healthy volunteers without severe periodontitis	8	8	INTER INTRA	(C) Probiotic 2	<i>L. reuteri</i> (ATCC 55730 and ATCC PTA 5289)					58	8*	15					
					(A) Placebo	Xylitol (840mg/day)												
Kang et al. (2006a)	Healthy gingivitis	1 day	1 day	INTRA	(A) Placebo	Distilled water	0.88	0.86								Data for BOP unclear		
					(B) Probiotic	<i>W. cibaria</i> CMS1	0.86	0.68*										
Staab et al. (2009)	Healthy	8	8	INTER	(A) control	<i>Lactobacillus casei</i> Shirota	0.68	0.82	0.80	0.89						Data for BOP unclear		
					(B) Probiotic	<i>Lactobacillus casei</i> Shirota	0.76	0.98*	0.67	0.99*								
Della Riccia et al. (2007)	Chronic periodontitis	4 days	4 days	INTRA	Probiotic	<i>L. brevis</i> (CD2)	2.10	1.10*	2.24	0.14*	1.38	0.05*						

Table 5. (Contd.)

Study	Type of infection	Study time (weeks)	Time of follow-up assessment (weeks)	Statistical comparison	Study group	Strains	Microbiological log changes in comparison with baseline						PPD/CAL		Remarks
							plaque index		gingivitis index		bleeding upon probing		baseline end of probiotic treatment	baseline end of probiotic treatment	
							baseline	end of follow-up	baseline	end of follow-up	baseline	end of follow-up			
Matsuoka et al. (2006)	Periodontitis	16	12	INTRA	(A) Placebo	<i>L. salivarius</i> TI 2711									
					(B) Probiotic 1		33.3	23.0*	21.0*	4.5	3.9*	3.8*			
					(C) Probiotic 2		35.7	27.2	28.3	4.2	3.9	3.6			
						<i>L. salivarius</i> TI 2711	33.7	24.7*	20.9*	4.5	3.7*	3.7*			

*Statistically difference result from baseline.

Superscript letters represents statistically significant difference from each other.

inter-pocket, inter-group statistical comparison at time of assessment; PPD, probing pocket depth; CAL, clinical attachment level; W., Weisella cibaria; L. brevis, Lactobacillus brevis; L. reuteri, Lactobacillus reuteri; BOP, bleeding upon probing.

whereas they did not change in the control group.

In the 4-day study of Della Riccia et al. (2007), using a *L. brevis* (CD2) lozenge on untreated periodontitis patients, significant decreases were seen in nitrite/nitrate, PGE₂, matrix metalloproteinase and IFN- γ levels in saliva at the end of the study.

Other effects

Based on the Beagle dog model study of Teughels et al. (2007b), Nackaerts et al. (2008) analysed radiologically the alveolar bone around the teeth that received the positive control treatment and the alveolar bone around the teeth that received root planing and repeated application of the bacterial mixture. These authors observed that the bone density within periodontal pockets treated with beneficial bacteria improved significantly after 12 weeks, while this improvement was not statistically significant for the positive control pockets. There was also a statistically significant increase in the bone level at the end of the study for the pockets receiving beneficial bacteria whereas no statistically significant increase was noted for the control pockets. It should be noted that, as mentioned before, in these Beagle dog studies, no oral hygiene was provided to the dogs during the 12-week study period. Therefore, these data might not be generalized to more conventional human studies.

Additionally, in the 4-day study of Della Riccia et al. (2007), significant reductions in calculus and tooth temperature sensitivity were noted.

Discussion/Conclusions

The present review tried to address the question whether probiotics offer opportunities to manipulate the periodontal oral microbiota, and by this offer opportunities to prevent or treat periodontal infections.

Although there is a clear rationale for using probiotics in periodontal health-care, the possible mechanisms by which probiotics can influence the oral microbiota and periodontal health have been only sparsely investigated. They have been based mainly on mechanisms of action observed in gastrointestinal indications. The variety of mechanisms on which probiotics can act make it difficult to suggest any form of *in vitro* test

to substantiate any probiotic claim before going into clinical testing. Nevertheless, appropriate target specific in vitro tests that correlate with in vivo tests or outcome measures are recommended. In relation to gastro-intestinal probiotics, the World Health Organization, suggested in 2002 a combination of tests to verify the gastro-intestinal survival of probiotics (resistance to gastric acid and bile, adherence to mucus, human cells or cell lines) and the microbiological effect (antimicrobial activity against potentially pathogenic bacteria or the ability to reduce pathogen adhesion). Obviously, not all of these recommendations are applicable to probiotics for periodontal healthcare. Additionally, these recommendations focus on a substantiating an antimicrobial effect whereas it is currently known that the anti-inflammatory/immune modulatory properties of probiotics are at least as important. Before translating these recommendations to the field of periodontal healthcare, it is necessary before clinical testing, to demonstrate that the putative probiotic shows at least a beneficial potential (either antimicrobial, anti-inflammatory, immune modulatory or any other clinically verifiable outcome measure specific to the periodontal field) at an in vitro level. Any additional material supporting the survival of the potential probiotic in the oral cavity would be beneficial. These recommendations do not prioritize local safety regulations and testing with regard to probiotic use. It should be noted that the currently available testing mechanisms are not fully adequate to predict functionality of probiotic microorganisms in the oral cavity. It should also be noted that in vitro data available for particular strains are not sufficient for describing them as probiotic. Probiotics for human use will require substantiation of efficacy with human trials.

Several clinical studies were identified that addressed the focused question. These studies could mainly be divided in studies directly addressing the issue by providing microbiological outcome measures and studies that indirectly addressed the issue by providing clinical outcome measures.

These studies often utilized small sample sizes and often lacked appropriate randomization, blinding, study set-up or control groups. Owing to this low quality of some studies, one needs to be careful in the interpretation of the data.

The number of papers that report on real periodontitis outcome measures is low and there is currently no RCT involving periodontitis patients with an appropriate placebo control that performed an inter-group statistical analysis. Moreover, low number of studies make inter-group comparisons with true placebo's or negative controls. This was rather surprising because this is the only reliable way of accounting for Hawthorne effects.

There is also a lot of heterogeneity among studies because different probiotic doses (2×10^7 – 2×10^9 CFU/day), treatment durations (1 day–12 weeks), models (human, animal), patient populations (healthy, gingivitis, periodontitis), strains and modes of application are being used. Reflecting upon the probiotic strains used, it was surprising that some studies lacked strain specification. Because probiotic effects are specific to a particular strain, this may have important implications for the interpretation of generalizing review data, particularly when strain designations were not provided. With regard to the modes of application, the vehicle by which they are ingested or delivered in the oral cavity can also influence their therapeutic potential and the oral colonization of a probiotic. The currently available data makes it impossible to draw any firm conclusions, given the wide variety of delivery vehicles (chewing gum, mouth-rinse, tablets etc.). Therefore the results should be interpreted cautiously due to all of these methodological limitations.

Taking into account the above mentioned limitations and generalizing the data provided in both animal and human studies reporting on microbiological outcomes of various probiotic treatments, these studies report up to 0.55 log reductions in total anaerobic bacteria, up to 0.25 log increases in total aerobic bacteria, up to 1.3 log reductions in black pigmented bacteria, up to 1.8 log reductions in *A. actinomycetemcomitans* numbers, up to 2.6 log reductions in *C. rectus* numbers, up to 2.1 log reductions in *P. gingivalis* numbers, up to 1 log reductions in *P. intermedia* numbers and up to 0.17 log reductions in *T. forsythia*.

When taking a closer look at the clinical findings of the different human studies concerning probiotics taking again into account the above mentioned limitations of the studies and warning for generalization of the data, it seems

that the effects of probiotic bacteria on the periodontal condition (plaque index, gingivitis index, bleeding upon probing, PPD) are much more limited in magnitude when compared with the studies reporting on microbiological outcomes.

Despite these observations, four additional considerations can be made based on the reviewed studies and with regard to the use of probiotics to improve periodontal health.

- (1) It was surprising for the reviewers that many of the studies tried to induce a microbiological shift or a clinical probiotic effect in an already matured oral microbiological environment. Based on our knowledge of the effect of antiseptics and antibiotics on established biofilms and nicely demonstrated in a recent study by Pham et al. (2009), it seems logical that a probiotic will have difficulty colonizing the mouth and exerting beneficial clinical effects under these circumstances. Pre-treatment to reduce the levels of oral indigenous microbiota, and thereby create more sites for colonization by probiotic bacteria, might be a good option but this approach was limited in most of the studies (Krasse et al. 2006, Teughels et al. 2007b, Tsubura et al. 2009).
- (2) The often limited clinical results may be attributed to the use of dietary lactobacilli as probiotics of choice for a large number of the studies. Indigenous bacteria offer the advantage of being perfectly adjusted to the human oral ecology. Therefore the existence of probiotics in the indigenous microbiota needs exploration and the use of orally derived probiotics can be recommended. Some groups have investigated the potential of the indigenous streptococcal population to act as probiotics. The importance of this population has already been described by Roos et al. (1993) and Roos et al. (1996) for oto-pharyngeal infections.
- (3) Probiotic therapy should not be seen as a treatment that permanently alters the oral microbiota. There are indications that the probiotic effect will only take place as long as the probiotic is applied. As soon as the patient discontinues its use, the effect will likely disappear and therefore does not appear to sustain

a shift to a stable non-pathogenic microbiota.

- (4) Finally, one should realize that probiotics are currently regulated as dietary supplements and not subjected to the same rigorous standards as medications. As a result, individuals may obtain a product that is ineffective or that contains varying quantities of bacteria.

In conclusion, data suggest that probiotics might offer opportunities to manipulate the oral microbiota or, albeit more limited, periodontal health by either direct microbiological interactions or by immunomodulatory interactions. However, due to all the limitations discussed above, it is currently premature to draw any conclusion on the clinical significance of the statistically significant results. Future research needs to encompass better designed clinical trials in larger populations in which we urgently need to address following issues:

- Long-term effects of oral probiotics?
- Specific oral probiotics coming from the oral cavity or general lactobacilli probiotics?
- Adjunct therapy or mono-therapy?
- Statistical inter- as well as intra-group comparisons should be made

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Clinical Relevance

Scientific rationale for the study: Oral healthcare workers are likely to be confronted with the new phenomenon of probiotics. Therefore, the present review aimed to evaluate the available scientific evidence

regarding the effects of probiotics on periodontal health.

Principal findings: Although one can question the selection of certain probiotic strains and the applied methodology of the studies, the currently available literature shows that probiotics could be effective in im-

proving periodontal health by manipulating the periodontal microbiota.

Practical implications: At this moment, practical implications remain difficult due to the lack of long-term studies using probiotics as an adjunct to standard periodontal healthcare.

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