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Prebiotics and Probiotic in Dentistry

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Submitted by

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Declaration

This is to certify that the organization and preparation of this dissertation have been made by the student **Yousif Fouad M.Ali,** under my supervision in the Collage of Dentistry, University of Baghdad in partial fulfillment of the requirement of B.D.S. degree in Preventive Dentistry.

Signature

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Dedication

First off, I want to thank God because that's who I look up. He's graced my life with opportunities that I know are not of my hand or of any other hand. He's shown me that it's a scientific fact that gratitude reciprocates.

To my beloved parents, who educated me, made me reach this level and who taught me and my brothers and sister how to respect ourselves and in turn we learned we were better able to learn how to respect others.

To my sister and my two brothers, who gave me continuous support so I can move forward without afraid of being alone.

To my friends, especially Hasan and Riyam who made me recognize that life is amazing when you have loyal friends and friendship never ends.

With my love and respect

Acknowledgement

Firstly, I want to send my gratefulness and thanks to our god **(Allah)** for his inspiration and blessing to make me finish this project.

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I will not forget to thank my **college of Dentistry and university of Baghdad** for this opportunity to make me a good dentist in future.

Finally, I want to tell my family that without you I couldn't do this, so I dedicate this project to my father, mother, sister, and brothers.

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abbreviation	Full word
AIDS	acquired immune deficiency syndrome
C (gtfC)	Glucosyltransferase-SI of *Streptococcus mutans serotype C*
CD4	co-receptor of the T cell receptor (TCR)
DNA	Deoxyribonucleic acid
F.	*Fusobacterium*
GCF	gingival crevicular fluid
HIV	Human immunodeficiency virus
IgA	Immunoglobulin A
IL	Interleukin
L.	Lactobacillus
LGG	*Lactobacillus rhamnosus gg*
MMPs	Matrix metalloproteinases
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PGE2	Prostaglandin E2
PH	potential of hydrogen
S.	Streptococcus
Vit B	Vitamin B complex
Vit K	Vitamin K
VSC	Volatile sulphur compounds
WHO	World Health Organization

Introduction

Prebiotics could promote the growth of beneficial micro-organisms that comprise part of the resident microbiota. The evidence for the use of pro or prebiotics for the prevention of caries or periodontal diseases is reviewed, and issues that could arise from their use, as well as questions that still need to be answered, are raised. A complete understanding of the broad ecological changes induced in the mouth by probiotics or prebiotics will be essential to assess their long-term consequences for oral health and disease. (Devine & Marsh, 2009).

Probiotics are live bacteria and yeasts that are good for health, mainly in digestive system. The body is full of bacteria good and bad, but the probiotics is not germs that cause diseases they are often called "good or helpful" bacteria because they help to keep the gut healthy. The term probiotic came from Greek words (pro=for, bios=life). The probiotics are found in supplements and some foods, like yogurt. Doctors often suggest them to help with digestive problems (Haukiojaa, 2010).

Probiotics aid in the treatment or prevention of disease by modifying the balance of gastrointestinal (GI) microflora or modulating the host's immune response (Guarner et al., 2010).

Probiotics have both direct and indirect interactions. The mechanism of adhesion to oral surfaces is an issue of importance for the long-term probiotic effect of the microorganisms. Probiotics have many positive influences in creating better oral health. (El-Nezami *et al.*, 2006).

The first probiotic species introduced into research were *Lactobacillus acidophilus* by Hull *et al,.* in 1984 and *Bifidobacterium bifidum* by Holcombh *et al.,* in 1991.

Probiotic technology represents a breakthrough approach to maintaining oral health by utilizing natural beneficial bacteria commonly found in healthy mouth to provide a natural defense against those bacteria though to be harmful to periodontal tissue (Thaer & Omran, 2012).

Very encouraging studies exploring probiotics in the fields of caries, periodontal diseases and few other areas have come up in the recent past and the results tend to suggest beneficial effects of probiotics on oral health and on the whole body in general (Bhardwaj & Bhardwaj, 2012).

1. Literature review

1.1 Definition

A. Prebiotic:

The term ‘prebiotic’ was introduced by Gibson and Roberfroid . It is a non-digestible food ingredient that confers benefits on the host by selectively stimulating the growth and/or activity of one bacterium or a group of bacteria in the colon, and thus improves the host health. Prebiotics are dietary carbohydrates that escape digestion in the upper gastrointestinal tract and alter the bacterial composition of the gut by changing the type of the substrate provided to the existing microbial population in the gut. (Gibson, *et al.* 2004)

Prebiotics (e.g. inulin-type fructans, maltodextrin, fructooligosaccharides and galactooligosaccharides) have been defined as non-digestible oligosaccharides that affect the proliferation of resident commensal bacteria that may then exert probiotic effects.

Studies of prebiotics have mainly been focused on gastrointestinal microbiota and health benefits; there has been little work in the oral cavity. (Forchielli & Walker, 2005)

Prebiotics are non-digestible food ingredients such as fructo-oligosaccharides (FOS), Lactulose and inulin that beneficially affect the host by selectively stimulating growth and / or increase activity of a limited number of probiotic like bacteria in a colon (Suvarna & Boby, 2005).

B. Probiotics:

Probiotics can be defined as living microbes, or as food ingredients containing living microbes, that beneficially influence the health of the host when used in adequate numbers (Meurman & Stamatova, 2007).

As adopted by the International Scientific Association for probiotics and prebiotics, “Live microorganisms, which when administered in adequate amounts, confer beneficial effect on the health of the host.” (Meurman, 2005) An International Life Science Institute Europe consensus document proposed a simple and widely accepted definition of probiotics as “Viable microbial food supplements which beneficially influence the health of human.” These bacteria should belong to the natural flora in order to resist gastric secretion and survive during intestinal transit. They should also adhere to the intestinal mucosa and finally should have the ability to inhibit gut pathogens (Izumita, 2001; Salminen *et al*. 2002; Patil & Reddy 2006).

C. Synbiotic:

The term ‘synbiotic’ is used when a product contains both probiotics and prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound as shown in Figure 1 (Schrezenmeir & de Vrese, 2001).

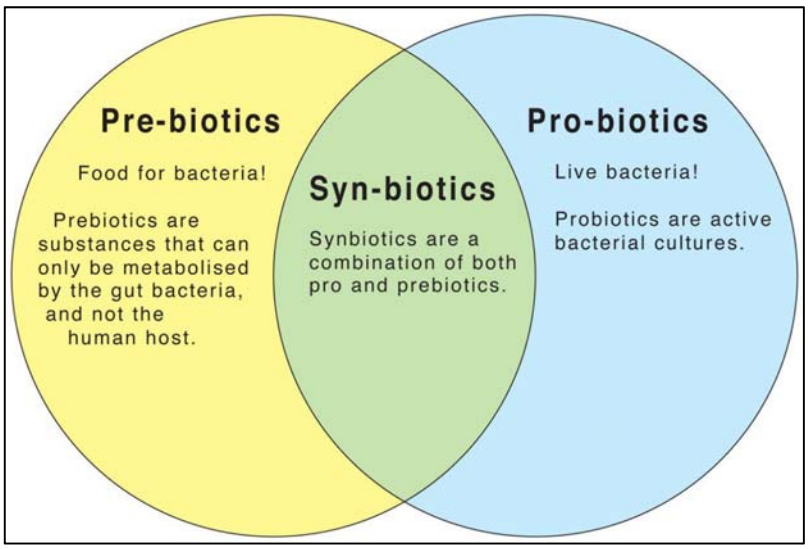

Figure (1): Show the combination of prebiotic and probiotic (Schrezenmeir & de Vrese, 2001).

1.2 History

The idea of probiotics dates back to the first decade of 1900s when the Ukranian bacteriologist and Nobel Laureate Metchnikof (1908) studying the flora of the human intestine developed a theory that senility in humans is caused by poisioning of body by the products of some of thses bacteria. To prevent the multiplication of these organisms he proposed a diet containing milk fermented by lactobacilli, which produce large amounts of lactic acid that could increase the life span of humans. The concept of probiotics was thus born and a new field of bacteriology was opened (Meurman & Stamatova, 2007).

Lilley and Stillwell (1965) introduced the term probiotics. Mann and Spooering in 1974 discovered that the fermented yogurt reduced blood serum cholesterol. In 1984 Hull identified the first probiotic species, the lactobacillus acidophilus. Later in 1991, Holcombh identified *Bifidobacterium bifidum*. WHO in 1994 described the probiotics as next

most important in immune defense system following antibiotic resistance. These incidences paved way for a new concept of probiotics in medicine and dentistry (Manisha, *et al.* 2001; Elisa & Scott, 2008).

The human gut contains 10 times more bacteria than cells elsewhere in human body. The enormous biomass consists of over 400 known bacterial species that generate intense metabolic activity and are of key importance for human health. This ecosystem gets disrupted when exposed to toxics in the form of polluted water and food as well as injudicious use of antibiotics (Salminen *et al.*, 2002; Suvarna & Boby; 2005).

Antibiotic resistance, with the emergence of multiple resistant strains, is an increasingly important global problem (Meurman, 2005).

This causes destruction of beneficial bacteria leaving resistant ones, pathogenic. Of late it has been realized by health care professionals and prompted them to seek alternative therapeutic options. One such alternative is the use of beneficial bacteria, the probiotics, which stimulate health – promoting indigenous flora and reverting back the change (Izumita, 2001; Suvarna & Boby, 2005; Patil & Reddy, 2006).

1.3 Mechanisms of action of Prebiotics and Probiotics

a) Mechanisms of action of Probiotics:

The general mechanisms of probiotics can be divided into three main categories (Parvez, *et al.* 2006):

A. Normalization of intestinal microbiota

B. Modulation of immune response

C. Metabolic effects

The diversity of conditions that may benefit from ingestion of probiotics illustrates the variety of mechanisms that may be involved in their actions and that some effects are systemic rather than only local. It is likely that these mechanisms vary according to the specific strain or combinations of strains used, the presence of probiotics and the condition that is being treated, as well as the stage of the disease process in which the probiotic is administered (Geier, *et al.* 2007).

There are common themes emerging in studies of the modes of action of probiotics and numerous mechanisms have been proposed (Tagg & Dierksen, 2003; Picard, *et al.* 2005; Marco, *et al.* 2006) including:

- Prevention of adhesion of pathogens to host tissues.
- Stimulation and modulation of the mucosal immune system, e.g. by reducing production of pro-inflammatory cytokines through actions on NFkB pathways, increasing production of anti-inflammatory cytokines such as IL-10 and host defence peptides such as b-defensin 2, enhancing IgA defences and influencing dendritic cell maturation.
- Modulation of cell proliferation and apoptosis through cell responses to, for example, microbially produced short chain fatty acids.
- Improvement of intestinal barrier integrity and up-regulation of mucin production.
- Killing or inhibition of growth of pathogens through production of bacteriocins or other products, such as acid or peroxide, which are antagonistic towards pathogenic bacteria.

Several pharmacologic/metabolic effects have been attributed to probiotics (Figure 2). These include increased disaccharide activity, the production of antibacterial substances, competition for bacterial adhesion, stimulation of various immune defense mechanisms and in case of saccharomyces, antisecretory/protease effects against toxins as well trophic effects on the mucosa (Wingate, *et al.* 2001).

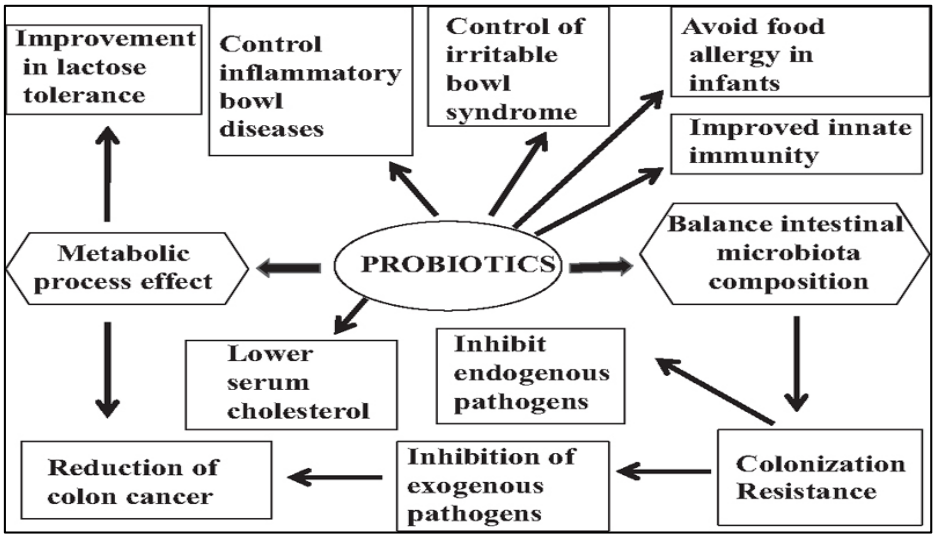

Figure (2): Several pharmacologic/metabolic effects have been attributed to probiotics (Wingate, *et al.* 2001).

b) Mechanisms of action of Prebiotics:

The ability of certain oligosaccharides to enhance the growth of resident commensal gut bacteria, particularly *Bifidobacteria* and *Lactobacilli*, is well documented (Gibson, *et al.* 2005). Thus, the major mechanism of action of prebiotics is assumed to be indirect, i.e. facilitating the proliferation of beneficial components of the resident microflora, with probiotic effects resulting from the actions of these bacteria as described above. Cellobiose has the additional property of

down regulating virulence factors of *Listeria monocytogenes* (Park & Kroll, 1993).

There is evidence that some prebiotics also exert direct effects on the host, independent of their effects on resident bacterial populations. These include stimulation of expression of IL-10 and interferon g, enhancement of IgA secretion, modulation of inflammatory responses to pathogens and stabilization of the gut mucosal barrier (Kleessen & Blaut, 2005; Forchielli & Walker, 2005).

Additionally, prebiotics with enhanced function have been designed. These oligosaccharide derivatives contain sugars that are specific epithelial cell receptors to which pathogens adhere and they, therefore, provide 'decoy' adhesion sites and cause pathogens to adhere to luminal contents rather than to epithelial cells (Gibson, *et al.* 2005).

1.4 Potential Mechanisms of Probiotic Effects in Oral Cavity

The mechanisms of probiotic action in the oral cavity could be analogous to those described for the intestine. Thus far oral colonization by probiotic bacteria has often been considered essential for them to exert oral effects; however, the possibility of systemic effects cannot be excluded, although the total IgA levels in saliva seem unaffected by probiotic use (Kekkonen, *et al.* 2008; Paineau *et al.* 2008).

Normalization of intestinal/oral microbiota is supported by the ecological plaque hypothesis which suggests that selective pressure in

environmental conditions can change the balance between oral health and disease (Marsh, 2003) As bacteria can influence their environment, and both synergistic and antagonistic interactions are suggested for bacteria in dental plaque, the environmental pressure described in the ecological plaque hypothesis could be introduced partly by bacteria. As there are bacterial species associated with oral diseases, there are also species that seem to be associated with oral health; however, it is questionable whether bacteria administered in food could influence relatively stable oral microbiota, in particular in adults (Haukioja, 2010).

Such friendly bacteria can be used as probiotics to normalize oral microbiota. Immunomodulation - Rather than directly inhibiting the growth or viability of the pathogen, probiotics may compete for an ecological niche or, otherwise, create conditions that are unfavorable for the pathogen to take hold in the intestinal tract. There are many possible mechanisms for how pathogen exclusion may take place (Marsh and Martin, 2009).

First, several probiotics have been demonstrated to alter the ability of pathogens to adhere to or invade colonic epithelial cells in vitro. Second, probiotics could sequester essential nutrients from invading pathogens and impair their colonization ability. Third, probiotics may alter the gene expression program of pathogens in such a way as to inhibit the expression of virulence functions. Lastly, probiotics may create an unfavorable environment for pathogen colonization by altering pH, the mucus layer, and other factors in the local surroundings. It is important to note that although many of these possible effects have been demonstrated in vitro, the ability of

probiotics to exclude pathogens in vivo remains to be proven as shown in Figure 3 (Britton & Versalovic, 2008).

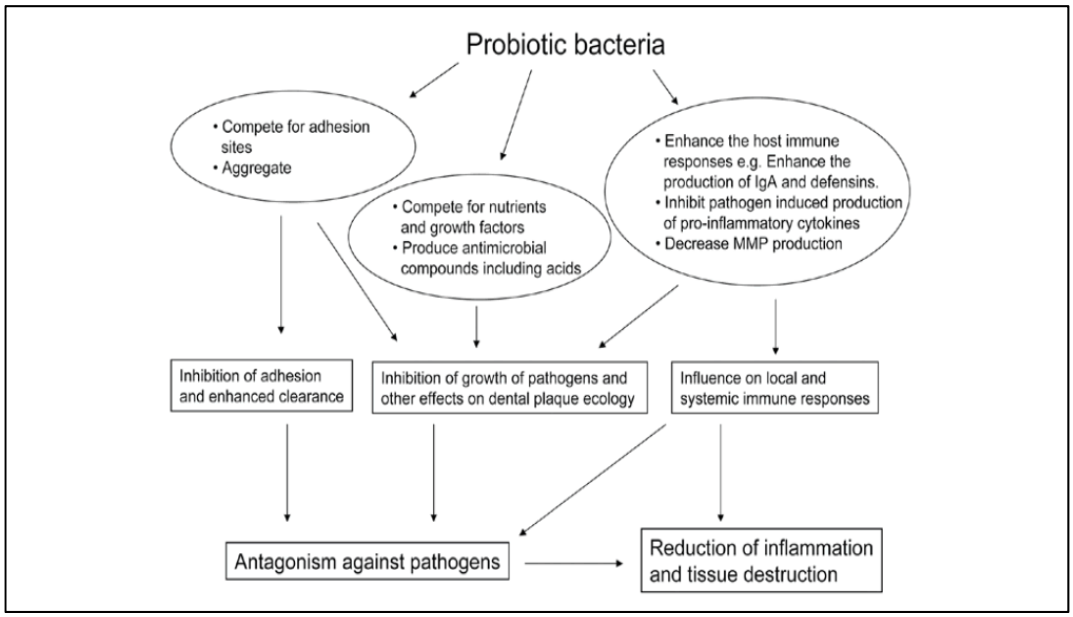

Figure (3): Potential mechanism of probiotic bacteria affected on oral health (Britton & Versalovic, 2008).

1.5 Species of Probiotics

Probiotics can be varied. They can be yeast, bacteria or moulds. But most commonly, bacterial species are predominant. Some of these species are:

a) Lactic acid producing bacteria: *Lactobacillus, Bifidobacterium, Streptococcus.*

b) Non lactic acid producing bacterial species: *Bacillus, Propionibacterium.*

c) Nonpathogenic yeasts: *Saccharomyces.*

d) Non spore forming and non-flagellated rod or *coccobacilli.*

The *Lactobacillus* species help in production of enzymes to digest and metabolize proteins and carbohydrates. They aid in synthesis of Vit B and Vit K and facilitates breakdown of bile salts. More than 100 species such as: *L. acidophilus, L.brevis, L.casei, L. rhamnous, L. salivarius* has been identified. They are usually dispensed in gel, paste, power and liquid forms. They enhance innate and acquired immunity as well as help in inhibition of pro-inflammatory mediators (Meurman, 2005, Patil & Reddy, 2006).

Bifidobacterium species are strictly anaerobic and predominate the large intestines. Over 30 species had been identified. The benefits from these include metabolization of lactose, generate lactic acid and synthesize vitamins. They also ferment indigestible carbohydrates and produce beneficial short chain fatty acids,

Streptococcus thermophillus and *lactobacillus bulgaricus* are primary cultures used in yogurt production. Most noted benefits are to metabolize lactose, improve lactose intolerance and antimicrobial activity,

Saccharomyces boulardii is a non colonizing lactic acid producing yeast. It prevents or treats antibiotic associated diarrhoea. It secretes proteases and other substances that breakdown bacterial enterotoxins and inhibits their binding to intestinal receptors. It also helps in immune function enhancement. It also enhances vitamin production and reduces serum-cholestrol level and in anticarcinogenic activity (de Roos & Katan, 2000).

1.6 Properties of Probiotics

(Narwal, 2011).

- Should be non-toxic and non-pathogenic preparation.
- Produce beneficial effect.
- Should withstand gastrointestinal juice.
- Should have good shelf life.
- Should replace and reinstate the intestinal microflora.

1.7 Role of probiotics in dental caries

Dental caries can be defined as one of the most common infections and communicable disease afecting human of all age (Hurlbutt *et al.,* 2010). It is a localized beakdown of susceptible tooth surface by acids produced by bacterial fermentation of dietary carbohydrates (Marsh and Martin, 2009; Sil, 2014).

Dental caries is one of the most prevalent chronic disease of people worldwide; individuals are susceptible to this disease throughout their lifetime (Pitts, 2004). It is involving a complex interaction among the plaque microorganisims, diet and a susceptible tooth surface in the presence of time factor (Selwitz *et al,*. 2007; Karpinski and Szkaradkiewicz, 2013). However, it is a preventable disease and can be arrested and potentially reversed in its early stages (Mitchell and Mitchell, 2009; Garg and Garg, 2013). It is act as a major public health problem globally due to its great social effect and high prevalence (Petersen *et al.,* 2005).

Dental caries is an infectious disease that affects most of the population. This multifactorial and complex disease process occurs along the interface between the dental biofilm and enamel surface.

Several methods may be used to alter the cariogenicity of the biofilm which is responsible for dental caries. Probiotic and molecular genetics techniques have been used to replace cariogenic organisms such as *mutans streptococci* and *Lactobacillus* species with strains of bacteria that are not cariogenic (Marsh and Martin, 2009).

Several mutated strains of *S. mutans* that lack the machinery to efficiently meatbolize fermentable carbohydrates to organic acids have been developed. One example is *S. mutans* with a glucosyltransferase C (gtfC) gene mutation. The pathogenicity of both *S. mutans* and *S. sorbinus* is related to their acidogenic potential and ability to form water insoluble extracellular and enzymatically undegradable polysaccharides from sucrose. These extracellular polysaccharides (glucans) promote adhesion and colonization of cariogenic organisms and mediate protection against antimicrobial agents and resistance to toxic compounds. Synthesis of these glucans is via glucosyltransferase B, glucosyltransferase C and glycosyltransferase D genes. The introduction of mutated gtfC gene that affects the ability of *S. mutans* to produce extracellular glucans has resulted in a decrease in extracellular matrix component of mixed oral biofilms from 51 to 33% of the biofilm volume (Ahola, *et al.* 2002).

Several studies suggest that consumption of products containing probiotic lactobacilli or bifidobacteria could reduce the number of mutans streptococci in saliva (Nase, *et al.* 2001). Using randomized controlled trials, Meurman and colleagues demonstrated that long term

consumption of milk containing the probiotic *Lactobacillus rhamuosus* GG strain reduced initial caries in kindergarten children. Nase *et al.* (2001), Caglar *et al.* (2006) also showed that administration of probiotic bacterium *Lactobacillus reuteri* ATCC 55739 or *Bifidobacterium* DN-173 010 induced significant reduction of cariogenic *S. mutans* in saliva. (Caglar *et al.* 2006)

In addition to the classical probiotic strains, other oral residents or genetically modified strains have also been tested for their ability to inhibit cariogenic microbes. Hillman and his colleagues introduced a non-acid producing S. mutans strain that produces a bacteriocin active against other S. mutans strains into the oral cavity to replace the naturally occurring cariogenic strains (Hillman, 2002).

1.8 Some of the studies involving probiotics for decreasing dental caries

Comelli *et al.* (2002) studied 23 dairy bacterial strains for the prevention of dental caries and reported that only two strains, namely *Streptococcus thermophilus* and *Lactococcus lactis*, were able to adhere to saliva-coated hydroxyapatite and were further successfully incorporated into a biofilm similar to the dental plaque.

Furthermore, they could grow together with five strains of oral bacterial species commonly found in supra-gingival plaque. In this system, *Lactococcus lactis* was able to modulate the growth of the oral bacteria, and was particularly able to diminish the colonization of *Streptococcus oralis, Veillonella dispar, Actinomyces naeslundii* and *Streptococcus sobrinus* (Comelli, *et al.* 2002).

L. rhamnosus is one of the most extensively studied probiotics in oral biology, since it does not readily ferment sucrose and is safer for teeth than lactic acid-producing bacteria. Controlled studies have shown the effectiveness of *L. rhamnosus* in reducing caries (Meurman, 2009).

L. rhamnosus was found to inhibit cariogenic *S. mutans*, but colonization of the oral cavity by *L. rhamnosus* seems improbable (Yli-Knuuttila, *et al.* 2006). In a seven-month study on a kindergarten by Nase *et al.* (2001), children received the probiotic *L. rhamnosus* and the caries risk was subsequently calculated according to clinical and microbiological data (*S. mutans* level in saliva and plaque). Results showed less dental caries and lower levels of *S. mutans* in the probiotic milk-consuming group (Näse, *et al.* 2001).

A study aimed at showing the benefit of cheese-containing *Lactobacillus rhamnosus* showed that probiotic intervention helped in reducing the highest level of *Streptococcus mutans* (Ahola, *et al.* 2002).

In order to assess whether naturally occurring oral *Lactobacilli* have probiotic properties, *Lactobacilli* were isolated from saliva and plaque in children and adolescents, with or without caries lesions. Twenty-three *Lactobacillus spp*. completely inhibited the growth of all *mutans streptococci* tested. The species with maximum interference capacity against *mutans streptococci* included *Lactobacillus paracasei*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* (Simark-Mattsson, *et al.* 2007).

Few studies have reported a reduction in mutans streptococci levels in saliva following the use of probiotic-containing yogurts, but it

is not clear whether this decrease is due to the bactericidal activity of yogurt or other mechanisms.

Petti investigated the differences in susceptibility of strains of viridians streptococci. In vitro, yogurt with live bacteria showed selective anti-mutans activity suggesting that the overall decrease in mutans streptococci in vivo could be due to a bactericidal effect on S. mutans (Petti, *et al.* 2008).

Yogurt products containing L.reuteri showed a significant growth inhibitory effect against S. mutans, while yogurts with lactobaccilli other than L. reuteri did not show such inhibition. Moreover, a double-blind, placebo-controlled trial demonstrated that consuming yogurt with L. reuteri significantly reduced the oral carriage of mutans streptococci, compared to the placebo yogurt (Nikawa, *et al.* 2004).

Calgar investigated the effect of the probiotic bacterium Lactobacillus reuteri on levels of mutans strepto- cocci and lactobacilli, which was introduced by two different straws containing L. reuteri and lozenges containing L. reuteri; they concluded that short-term daily ingestion of lactobacilli-derived probiotics delivered by prepared straws or lozenges reduced the levels of salivary mutans streptococci in young adults (Calgar *et al.* 2006)

Calgar evaluated the effect of xylitol and probiotic chewing gums on salivary mutans streptococci and lactobacilli and concluded that daily chewing of gums containing probiotic bacteria or xylitol reduced the levels of salivary mutans streptococci in a significant way. However, a combination of probiotic and xylitol gums did not seem to enhance this effect (Calgar *et al.* 2007).

In a similar study they showed that sucking a medical device containing the probiotic lozenge with L. reuteri once daily for 10 days reduced the levels of salivary mutans (Calgar *et al.* 2008).

1.9 Probiotics in prevention of caries

The oral health applications of either probiotics or 'replacement therapy' with Streptococcus mutans strains of attenuated virulence and increased competitiveness were first suggested for prevention of dental caries more than 20 years ago (Hillman, 2002). Despite this, and the fact that some products have reached the market, there remains a paucity of clinical evidence to support the effectiveness of probiotics to prevent or treat caries (Meurman, 2005; Meurman & Stamatova, 2007).

Many early studies concentrated on utilising bacteria that expressed bacteriocins or bacteriocin-like inhibitory substances that specifically prevented the growth of cariogenic bacteria (Tagg & Dierksen, 2003)

Another approach has been to identify food grade and probiotic bacteria that may have potential in caries prevention. These have been selected because of their likely ability to colonize teeth and influence the supragingival plaque; in vitro models for this selection have included adhesion to hydroxyl-apatite, as a surrogate for colonisation of teeth, and mixed species biofilm models. Also, strains have been screened for suitable antagonistic activity against relevant oral bacteria (Comelli, *et al.* 2002; Haukioja, *et al.* 2006).

In vitro studies of the antibacterial activity of live yoghurts showed inhibition of *S. mutans* but not some other oral *Streptococci*, including *Streptococcus sobrinus*; this activity was heat sensitive implying that the effect was not simply due to acid (Petti, *et al.* 2008).

Recently, oral *Lactobacilli* have also been screened for their utility as potential probiotic strains and strains of oral *Lactobacilli* have been isolated that are inhibitory against *S. mutans, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia*, as well as being tolerant of relevant environmental stresses (Koll, *et al.* 2008).

Another approach utilised a recombinant strain of *S. mutans* expressing urease, which was shown to reduce the cariogenicity of plaque in an animal model (Clancy, *et al.* 2000).

Similarly, genetically modified probiotics with enhanced properties can be developed ('designer probiotics'). For example, a recombinant strain of *Lactobacillus* that expressed antibodies targeting one of the major adhesions of *S. mutans* (antigen I/II) was able to reduce both the viable counts of *S. mutans* and the caries score in a rat model (Krüger, *et al.* 2002).

Clinical studies have indicated that bacteria with established probiotic effects (*Lactobacilli and Bifidobacteria*) have some promise for prevention of caries. LGG ingested in dairy products (milk, cheese) reduced salivary *mutans streptococcal* counts in adults and protected against caries in children (Näse, *et al.* 2001; Ahola, *et al.* 2002). Other lactobacilli have also been shown to reduce *mutans streptococcal* counts in saliva. *Lactobacillus reuteri*, when delivered by yoghurt (Nikawa, *et al.* 2004), straw or tablet (Caglar, *et al.* 2006), by chewing

gum or as a lozenge, significantly reduced the counts of *mutans streptococci* in saliva (p 0.05). The short-term consumption of yoghurt or ice cream containing *Bifidobacterium spp.* resulted in a significant reduction in salivary *mutans streptococci* (p 0.05) but not in *Lactobacilli*. Other studies have reported reductions in *mutans streptococci* levels in saliva following use of probiotic containing yoghurts (Petti, *et al.* 2001; Çaglar, *et al.* 2005; Caglar, *et al.* 2007; Çaglar, *et al.* 2008).

1.10 Role of probiotics in periodontitis

Riccia and colleagues in 2007 studied the anti-inflammatory effects of *Lactobaillus brevis* in a group of patients with chronic periodontitis. Anti-inflammmatory effects of *L.brevis* could be attributed to its capacity to prevent the production of nitric oxide and consequently the release of PGE2 and activation of MMPs induced by nitric oxide. The use of probiotic chewing gum containing *L. reuteri* ATCC55730 and ATCCPTA5289 (American type collection culture) also decreased levels of pro-inflammatory cytokines in GCF (Twetman, *et al.* 2009) and the use of *L.brevis* decreased MMP (collagenase) activity and other inflammatory markers in saliva (Riccia, *et al.* 2007).

Another species, *Streptococcus salivarius* is detected most frequently among people without halitosis and is therefore considered a commensal bacterium of the oral cavity. *S.salivarius* is known to produce bacteriocins, which contribute in reducing the number of bacteria that produce Volatile sulphur compounds (VSC). The use of

gum or lozenges containing *S. salivarius* K12 reduce levels of VSC among diagnosed with halitosis (Burton, *et al.* 2006).

1.11 Role of probiotics in orthodontic treatment

Fixed orthodontic appliances are considered to jeopardize dental health due to accumulation of microorganisms that may cause enamel demineralization, clinically visible as white spot lesions (Mitchell, 1992).Furthermore, the complex design of orthodontic bands and brackets may create an ecological environment that facilitates the establishment and growth of cariogenic *mutans streptococci* strains (Ahn, *et al.* 2007).

White spot lesion formation can be seen as an imbalance as an imbalance between mineral loss and mineral gain and recent systematic reviews have examined methods to prevent this side effect of orthodontic treatment (Derks, *et al.* 2004).

Cildir *et al.* (2009) conducted a clinical study with probiotics and found out that daily consumption of fruit yogurt with *Bifidobacterium* could reduce the salivary levels of *mutans streptococci* in orthodontic patients with fixed appliances. Further studies are needed to clarify if this approach is an alternative strategy for prevention of demineralization and white spot formation during orthodontic treatment (Cildir, *et al.* 2009).

1.12 Role of probiotics in oral cancer

The anticancer effects of probiotics were long recognized but evidence in literature is minimal. Evidence is cropping up that probiotics can interfere at various stages of cancer process, more so by

interference with chromosomal and DNA damage. However, more research is required to develop specific regulations on their consumption (de Roos & Katan, 2000; Salminen, *et al.* 2002).

1.13 Role of probiotics in infections and oral diseases

There are studies have investigated the effects of probiotic bacteria on oral candida infection in humans (Nase, *et al.* 2001; Riep, *et al.* 2009). When a test group of elderly people consumed cheese containing *L. rhamnosus* strains GG and LC705 *and Propionibacterium freudenreichii ssp.* Shermanii JS for 16 weeks, the number of high oral yeast counts decreased but no changes were observed in mucosal lesions (Nase, *et al.* 2001).In a shorter study with younger subjects, no significant difference was observed between effects of probiotic and those of control cheese on salivary candida counts (Ahola, *et al.* 2002).

Recently it has been postulated that the probiotic bacteria may slow down AIDS progression. Lin Tay and his colleagues screened hundreds of bacteria taken from saliva of volunteers. The results showed that some Lactobacillus strains had produced proteins capable of binding a particular type of sugar found on HIV envelope, called mannose. The binding of the sugar enables the bacteria to stick to the mucosal lining of the mouth and digestive tract, forming colonization. One strain secreted abundant mannose binding protein particles into its surroundings, neutralizing HIV by binding to its sugar coating. They also described that immune cells trapped by *Lactobacilli* formed a clump. This configuration would immobilize any immune cells

harbouring HIV and prevent them from infecting other cells (Lin, 2008).

1.14 Role of probiotic in halitosis

It is not a disease but a discomfort, although some oral diseases including periodontitis may be the underlying cause; however, in approximately 90% of cases, the origin can be found in the oral cavity, (Delanghe, *et al.* 1997) and probiotics are marketed for the treatment of both mouth and gut associated halitosis. Despite that, only a few clinical studies have found different probiotic strains or products to be efficacious. The studied strains include E. coli Nisle (1917), *S. salivarius* K12, three Weissella confusa isolates, and a lactic acid forming bacterial mixture, not specified by the authors of that work. (Henker, *et al.* 2001; Kang, *et al.* 2006)

The common organisms involved in halitosis are *Fusobacterium nucleatum, P. gingivalis, P.intermedia and Treponema denticola.* These organisms degrade amino acids, which are in turn transformed into volatile sulphur compounds which cause halitosis. Kang and colleagues reported that various strains of Weissella cibaria have the capacity to coaggregate with *Fusobacterium nucleatum* and to adhere to epithelial cells and these bacteria produce hydrogen peroxide as well as a *Bacteriocin* which inhibited the proliferation of *F. nucleatum*. These properties could enable W. cibaria to effectively colonize the oral cavity and limit the proliferation of *F. Nucleatum* and thus can prevent halitosis (Kang, et al. 2006).

1.15 Delivery mechanisms of probiotics

Advances in biomedical engineering will prove to be equally important to molecular biology in terms of the developing systems that deliver bacteria and / or nutritional factors to the host. These will include encapsulating probiotics, such that they rehydrate at specific sites, and encasing prebiotics in nano-aggregates that protect against stomach acid and deliver their payload when the pH reaches 7.4.

Potentially, such nano encapsulation will also allow delivery in foods such as biscuits, whereas targeted, water protected macrocapsules containing probiotic organisms may prove useful in animal food pellets and perhaps in liquids, which currently cannot be used because of problems with shelf stability. At the macromolecular level, it will soon be possible to coat capsules with biosensors that detect the optimal conditions for the release of probiotic contents (Reid, 2008).

In summary, molecular, nano, biochemical, microbiological and engineering sciences hold the key to future advances in the clinical applications of probiotic and prebiotic products (Reid, 2008).

1.16 Current applications of probiotics and prebiotics

Most of the applications and research into the mechanisms of action of probiotics and prebiotics concentrate on their roles in influencing intestinal health and function. Although some of the experimental evidence and data from clinical trials is conflicting, there is growing evidence for their efficacy in protecting against acute diarrhoeal disease in children, gastroenteritis and antibiotic-associated

diarrhoea, inflammatory bowel diseases and pouchitis (Reid, *et al.* 2003; Nomoto, 2005; Weng & Walker, 2006; Geier, *et al.* 2007).

There is also evidence to support further investigation of the use of probiotics and prebiotics in the treatment of illnesses affecting sites other than the intestinal tract, e.g. urinary tract infections, vaginal infections, arthritis, atopic eczema, pharyngitis and otitis media (Tagg & Dierksen, 2003; Lenoir-Wijnkoop, *et al.* 2007).

Recently, Lactobacillus rhamnosus GG (LGG) administered in yoghurt was reported to enhance faecal clearance of vancomycin resistant enterococci (Manley, *et al.* 2007).

The possibilities of applying probiotic therapy for other medical conditions are being investigated, including recovery from haemorrhagic shock, recovery from burn injury, cholesterol reduction and protection from coronary heart disease, effects on breast cancer cells, enhancement of tolerance of food allergens, protection from respiratory tract infections, liver conditions, skin infections, enhancement of bone health and reduction of obesity. However, the evidence-base for many of these is relatively under developed (Reid, *et al.* 2005; Walker, *et al.* 2006; Scholz-Ahrens, *et al.* 2007).

The potential applications of probiotic bacteria have been further expanded by the development of strains that have been genetically engineered to produce the anti-inflammatory cytokine IL-10 (Steidler, *et al.* 2000), trefoil factor family proteins to enhance wound healing (Vandenbroucke, *et al.* 2004) or the 2D-CD4 receptor to try to reduce HIV infectivity (Chang, *et al.* 2003).

1.17 Colonization and safety of probiotics in the oral cavity

Some probiotic *Lactobacillus* and *Streptococcus* strains seem able to colonize the oral cavity of some people during the time that products containing them are in active use. However, both in vitro and in vivo evidence indicate that the differences between various probiotic strains, products, and also host individuals are obvious. (Busscher, *et al*. 1999; Haukioja, *et al.* 2006; Krasse, *et al.* 2006)

L. rhamnosus GG and two different *L. reuteri* strains have been reported to colonize the oral cavity of 48–100% of volunteers consuming products containing them. (Yli-Knuuttila, *et al.* 2006; Caglar, *et al.* 2009)

In addition, S. salivarius K12, used for treating oral malodor, temporarily colonizes the oral cavity for a short time after use. (Horz, *et al.* 2007)

Furthermore, consumption of a mixture of seven different Lactobacillus strains increased the number of salivary Lactobacillus counts, although the identities of the strains in the saliva were not determined. (Montalto, *et al.* 2004)

It seems feasible that probiotic bacteria would colonize the oral cavity only when they were used in products in contact with the mouth. Indeed, Maukonen *et al.* (2008) did not detect any of the probiotic bacteria administered in capsules in saliva samples. Surprisingly, consumption of capsules containing a mixture of seven different *Lactobacillus* strains increased the number of salivary *Lactobacillus* counts. (Montalto, *et al.* 2004)

L. reuteri ATCC 55730 (= *L. reuteri* SD2112) does not seem to influence the total number of salivary *lactobacilli*, but *L. rhamnosus* GG may increase it Maybe because long-term colonization by probiotic bacteria is unlikely, albeit possible (Yli-Knuuttila, *et al.* 2006).

Potential adverse effects of probiotic bacteria in the oral cavity have not been a subject of much intensive research; however, probiotic products are used widely; therefore, when dental health is considered, the acidogenicity of lactobacilli and bifidobacteria cannot be overlooked. For example, one L. salivarius strain is able to induce caries in an animal model, and another is able to make a biofilm model more cariogenic. (Matsumoto, *et al.* 2005; Pham, *et al.* 2009)

1.18 Future aspects

Recently, oral lactic acid bacteria and *Bifidobacteria* have been isolated and characterized for various oral health purposes, including caries, periodontal diseases, and halitosis. (Sookkhee, *et al.* 2001; kang, *et al.* 2006; Simark-Mattsson, *et al.* 2007; Koll, *et al.* 2008)

In addition, dairy strains have been studied with the aim of characterizing potential new oral probiotics; (Comelli, *et al.* 2002; Stamatova, *et al.* 2009) thus; the new probiotic products targeted for oral health purposes do not necessarily comprise the same species as products now in market. Furthermore, the species might not necessarily belong only to genera *Lactobacillus* or *Bifidobacteium.* Indeed, *S. salivarius* K12 is used to treat oral malodor (Burton, *et al.* 2006) and preliminary results have been published on the safety and

efficacy of a probiotic mouthwash containing three different oral streptococci for reducing the number of bacteria associated with dental caries and periodontitis. (Zahradnik, *et al.* 2009)

Genetically modified microbes bring a new dimension to the concept of probiotics. One approach is to reduce the harmful properties of pathogenic strains naturally colonizing the oral cavity. The modified strain could then be used to replace the original pathogen. One ambitious and promising example is the generation of an S. mutans strain with a complete deletion of the open reading frame of lactate hydrogenase and thus significantly reduced cariogenicity. (Hillman, *et al.* 2007)

Conclusion

- The oral cavity with a well maintained balance of the species and species interactions may be a potential source for health-promoting probiotic bacteria. On the other hand, daily intake of probiotic supplements may control common oral and dental infections.
- Probiotics are living microorganisms, principally bacteria, which are safe for human consumption and have beneficial effects on human health.
- Probiotics incorporated into dairy products neutralize acidic conditions in the mouth and interfere with cariogenic bacteria.
- Patient with periodontal disease who used chewing gum or lozenges containing probiotics saw their periodontal status improve.

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