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Probiotics and the Reduction of Dental Caries Risk

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1. Introduction

Dental caries and periodontal disease are major public health problems that bother all countries in the world. Dental carie is an infectious, communicable disease that acid-forming bacteria of dental plaque can destroy tooth structure in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. So, the infection results in loss of tooth minerals from the outer surface of the tooth and can progress through the dentin to the pulp, finally compromising the tooth vitality. Industrialized nations have controlled the problem with fluoride enriched water and personal hygiene products since early in the 1960s, but cariogenicity remains a crisis that economically burdens the health care system. Dental disease remains a "silent epidemic" in the world that threatens children and adults. The oral streptococci especially mutans Streptococci are related with the development of caries in humans and animals (Caglar et al., 2001; Natcher, 2001; Kargul, 2003). For the past 150 years, the predominant mode of caries management has been the surgical approach, predating our current understanding and reliable with the original concept that dental caries was a gangrenous process resulting in extraction of carious teeth. Later, just the demineralized portions of the tooth were removed and replaced with an inert restorative material. This mechanical solution for a biological problem prevailed.

Today, dental practitioners still teach the removal of diseased tooth structure which suggests we should expect a "cure". The insight however, is that it has repeatedly been shown not to remove the causative infection. There is a paradigm shift in the management of dental caries. Research in cariology is sky-rocketing, bringing out hidden facts of this age-old disease, but education and clinical practice are adopting them at a snail's pace. In clinical practice dental caries is still being treated symptomatically, just like the common cold. Clinicians have adopted a comfort level from many years of practicing 'restorative' dentistry, but unlike the common cold that does not have a cure, dental caries has abundant options to be cured and eradicated (Anderson and Shi, 2006; Carounanidy, 2010).

Throughout the past few decades, changes have been observed not only in the incidence of dental caries, but also in the distribution and pattern of the disease in the population. These changes have main hints for diagnosis and management of early lesions, predicting caries risk, and conducting effective disease prevention and management programs for

populations. In order to make continued progress in eliminating this, new strategies will be required (Natcher, 2001).The broad management of dental caries should involve the management of disease as well as the lesion. There is now an intense focus on preventive strategies. Essentially, all preventive treatment strategies either alter or modify the causative factors in dental caries etiology, such as diet, host, salivary, and microbial factors.

Numerous anti-plaque agents available in the market have been tested for their ability to interfere dental biofilm formation or metabolism. However, due to several undesirable side effects associated with these agents, going along with the increasing global problem with antimicrobial drug resistance, the search for alternate agents is necessary (Tahmourespour, 2011). Targeted agents are so expected to be highly specific, to pose an insignificant resistance development problem, and to have minimal effects on vital human cell functions. A suggested approach to overcome the limitations of the traditional disease management strategies is using inexpensive, effective, stable, novel and natural products as anti biofouling agent. Whole bacteria replacement therapy or using natural products of some bacteria such as the secondary metabolites of them for decreasing of oral cavity pathogens must be investigate.

2. Biofilm formation, a pioneer step of dental caries

Dental plaque has been discussed as a biofilm (figure 1). Donlan and Costerton (2002) presented the most relevant description of a biofilm.

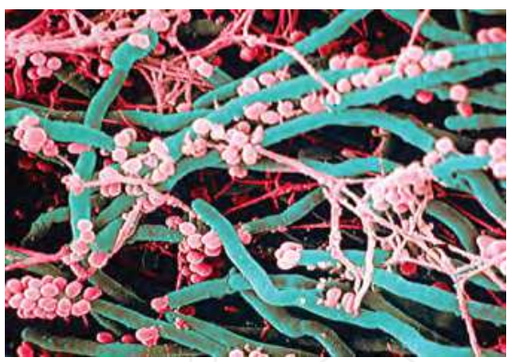

Fig. 1. Colored scanning electron micrograph of dental plaque (*Streptococcus mutans* bacteria are pink).

They declared that a biofilm is "a microbially derived sessile community characterized by cells that are attached to a substrate or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription."

A biofilm is structured to maximize energy. Degree of organization and multispecies organization characterize the four stages of biofilm growth (Figure 2). There are four stages in the lifecycle whether the organism is planktonic or as member of a biofilm. Stage I is the inactive or least metabolically active state. Transformation from Stage I to Stage II needs significant genetic up-regulation. Stage III involves maturity of the biomass, and total organism concentration can come near 10^{11} or 10^{12} colony-forming units per milliliter. At this phase, new antigens may be expressed, genetic exchange enhanced and membrane transport

maximized. Stage IV (apoptosis or death) signals detachment or sloughing from the biofilm (Donlan & Costerton, 2002; Thomas et al., 2006).

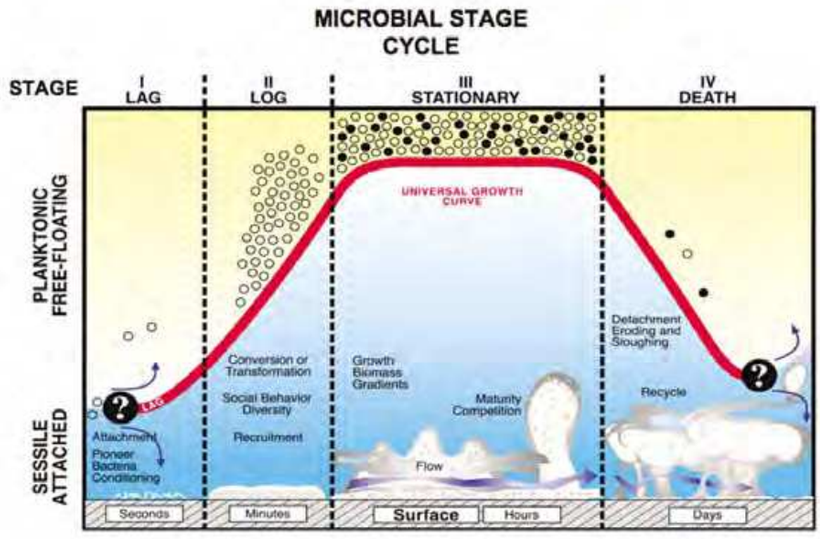

Fig. 2. Four stages of dental plaque biofilm growth: Stage I attachment (lag [not inert, but metabolically reduced]), Stage II growth (log [exponential growth]), Stage III maturity (stationary) and Stage IV dispersal (death) (Thomas et al 2006).

3. Dental plaque biofilm management

For therapeutic purposes, it is necessary to attack the formed biofilm. For prophylactic purposes, it seems reasonable to target processes involved in the actual biofilm formation of single- or mixed-bacterial communities that have the potential to cause or support disease, without disturbing the balance of the normal flora. It is known that the mature oral biofilm is the result of a well regulated series of processes, which begins by adhesion of planktonic cells to the surfaces and could represent potential targets for biofilm control.

The shift in the treatment paradigm incorporates the ecological plaque hypothesis, which states that prevention of disease should not only focus on the putative pathogens inhibition, but also on interference with environmental factors that drive selection and enrichment for these bacteria as reported by Marsh(2005). One of the key characteristics of biofilm that could be targets for dental plaque management includes its behavior as an adhesive mass.

The environmental key factors in concerned with biofilm formation are the fermentable dietary carbohydrates and Streptococci, as pioneer strains, depend on them as an energy source (Tahmourespour et al., 2010). The cariogenicity of sugar-containing foods can be modified by many factors including the amount and type of carbohydrates, protective components (proteins, fats, calcium, phosphate, fluoride) and physical and chemical properties (liquid vs. solid retentiveness, solubility, pH, buffering capacity). The fact that sugars are readily metabolized by oral bacteria, leading to the production of organic acids and extra cellular polysaccharides such as glucan and fructan was shown repeatedly in clinical studies (Zero, 2004; Touger et al., 2003). Numerous studies have established the role

of sugars in caries etiology and the importance of sugars as the principal dietary substrate that drives the caries process (Caglar et al., 2005; Touger et al., 2003; Loo et al., 2003).

In a study, the ability of Mutans Streptococci to form biofilm measured in the presence of some sugars. The biofilm formation (percentage of strongely adherent strains in Fig. 3) in the presence of sucrose was higher than other carbohydrates significantly ($p < 0.05$). It is also revealed that the number of attached bacteria increased with the increase of sucrose concentration. The results corresponded to a non linear increase of attached bacteria (Tahmourespour et al., 2010). Therefore, among the various tested carbohydrates in this study and other different researches, sucrose is considered the most cariogenic dietary carbohydrate, because it is fermentable, and also serves as a best substrate for the synthesis of extracellular and intracellular polysaccharides and dental plaque formation (Brown et al., 2005; Bowen, 2002; Cury et al., 2000; Pecharki et al., 2005; Ribeiro et al., 2005; Leme et al., 2006).

Evidences show that expression of required genes for glucan and fructan synthesis, such as gtfB, gtfC and ftf, is well-regulated after initial adhesion and results in forming dental plaque, caries and other periodontal disease (Zero, 2004)

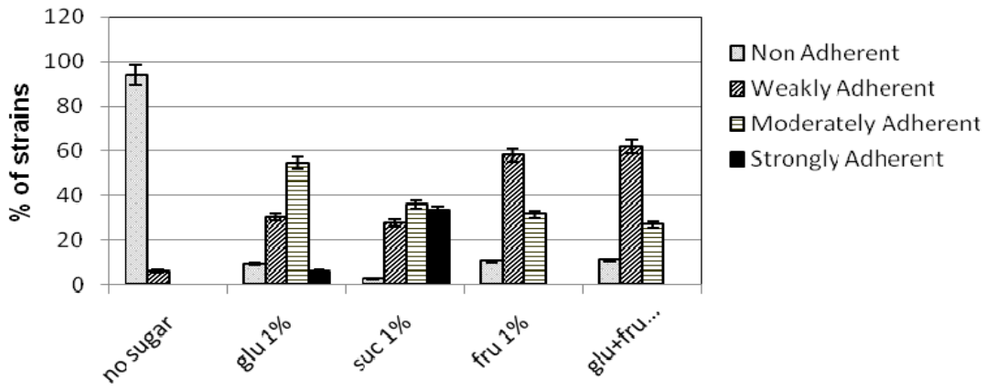

Fig. 3. The effect of different substrates in the adherence potential of streptococcal isolates. All isolates were classified into four groups. Data are expressed as means and standard deviations of triplicate experiments (Tahmourespour et al., 2010)

The ability of mutans Streptococci to adhere to teeth surfaces is vital for the progression of the disease. The bacterial adhesion mechanism is mediated by synthesis of both extracellular enzymes, glucosyltransferase (GTF) and fructosyltransferase (FTF). These extracellular enzymes identified in *Streptococcus mutans* are responsible for the synthesis of extracellular polysacharides such as glucans and fructans. These polymers are fundamental factors in dental biofilm formation. *a*-(1-3) - and *a*-(1-6)-linked glucan polymers through the concerted action of three secreted GTFs are encoded by the genes *gtfB*, *gtfC* and *gtfD*. In vitro studies have indicated that *gtfB* and *gtfC* are essential for the sucrose-dependent attachment of *S. mutans* cells to hard surfaces but *gtfD* is dispensable. The glucan polymers are involved in the colonization of cariogenic Streptococci and therefore have become a potential target for protection against dental caries.

The comparison between the mRNA level of *gtfB* in planktonic, biofilm and unattached cells of *S. mutans* by real time RT PCR also showed that, the level of *gtfB* gene expression in the biofilm condition was significantly higher than the planktonic condition (Fig4).

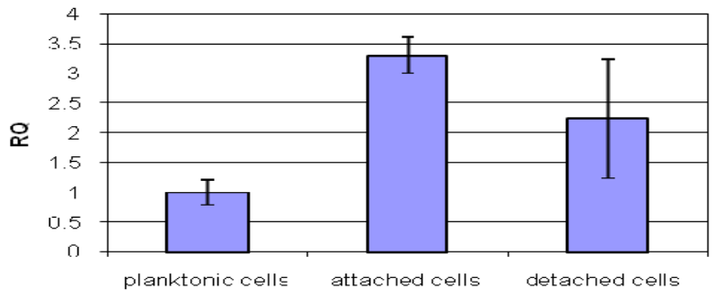

Fig. 4. The comparison of *gtfB* gene expression level in different condition (planktonic cells in the absence of sucrose, attached cells in the present of 1% sucrose and unattached or detached cells from biofilm in the presence of 1% sucrose) (Tahmourespour et al., 2010).

So, despite the fact that, the relationship between sugar consumption and caries is so strong; sugar consumption restriction still has an important role in prevention of caries going along with other new strategies.

4. Probiotics

In general, a probiotic, is a live microorganism which beneficially affects the host animal by improving its intestinal microbial balance. The concept of probiotic evolved from Elie Metchnikoff's ideas that the bacteria in fermented products could compete with microbes that are harmful to host and are hence injurious to health. The term probiotic, meaning "for life," is derived from the Greek language. It is the antonym of the term antibiotics, was introduced in 1965 by Lilly and Stillwell as substances produced by microorganisms which promote the growth of other microorganisms. Since then several definitions for probiotics have been proposed (Table 1).

Year with reference	Definition
1965 Lilly & Stillwell	Substances produced by microorganisms that promote the growth of other microorganisms
2001 Schrezemeir & de Vrese	A preparation of, or a product containing, viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and as such exert beneficial health effects in this host
2001 WHO/FAO report	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host
International Life Science Institute (ILSI) Europe	a live microbial food ingredient that, when ingested in sufficient quantities, exerts health benefits on the consumer

Table 1. Some definitions of probiotic bacteria.

The idea in the beneficial effects of probiotics is based on the knowledge that the intestinal flora can protect humans against infection and interruption of this flora can enhance

susceptibility to infection. The most important sources of probiotics for humans are the bacteria in yogurt and fermented milk products.

The valuable effects of probiotics may be mediated by direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers or by an effect on their metabolism or by stimulation of immunity (Ouwehand et al.,2001; Teugheles et al., 2008; Millette et al., 2008; Tahmourespour & Kermanshahi, 2011).

Probiotics have been suggested to have the following properties and functions:

- adherence to host epithelial tissue,
- acid resistance and bile tolerance,
- elimination of pathogens or reduction in pathogenic adherence,
- production of acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth,
- safety, non-pathogenic and non-carcinogenic, and
- Improvement of intestinal microflora (Kaur *et al*. 2002; Ouwehand *et al*. 2002).

Lactic Acid Bacteria or LAB, as the main probiotic species, are thought to be safe that have been ingested from foods without any problems for many years and are known as GRAS (Generally Recognized As Safe) bacteria that are important for animal health(Saito, 2004). The proposed mechanisms of the actions of probiotics are summarized in Fig. 5.

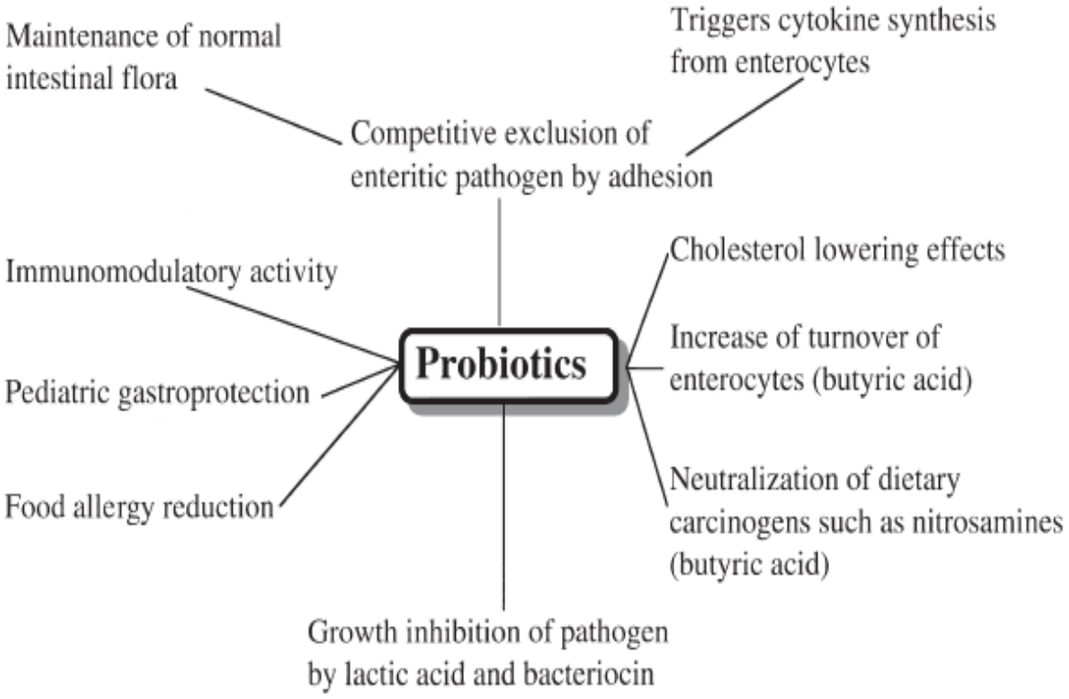

Fig. 5. Mechanisms of the actions of probiotics (Saito, 2004).

Promising probiotic strains include members of the genera Lactobacillus, Bifidobacterium and Enterococcus. The most commonly used probiotics mainly come from two genera Lactobacillus and Bifidobacterium (table2).

lactobacillus species	Bifidobacterium species	others
L. acidophilus *L. rhamnosus* *L. gasseri* *L. casei* *L. reuteri* *L. bulgaricus* *L. plantarum* *L. johnsonii* *L. lactis*	*B bifidum lanimalisl* *B. longum* *B. breve* *B. infantis* *B. lactis* *B. adolescentis*	*Bacillus cereus* *Clostridium butyricum* *Escherichia coli* *Proprionibacterium freundendsreichii* *Saccheromyces boulardii* *Enterococcus faecalis* *Streptococcus thermophilus*

Table 2. The most commonly used probiotics.

4.1 Oral microbiota as a source of probiotics

The oral cavity is a complex habitat of a great diversity of microbial species.

Recently, it has been estimated that over 1000 bacterial species are present in it. The most commonly used probiotic bacterial strains belong to the genera Lactobacillus and Bifidobacteria. So, there is of special interest to realize whether such microbes naturally inhabit the oral cavity. In the oral cavity, lactobacilli usually comprise 1% of the total cultivable bacteria; commonly isolated species include *L .paracaseie*, *L.plantarum, L.rhamnosus, L.salivarius*. Bifidobacterial species isolated from oral samples include *B.bifidum, B.dentium and B.longum*.

A promising finding was that lactobacilli population differed in healthy and individuals with periodontal disease. In another study it is observed that healthy persons are populated by *L. gasseri*and *L. fermentum*, whereas the predominant species in periodontitis patients was *L. plantarum* while the first two were undetectable (Koll Kalis et al., 2005). Observations also showed that microorganisms with probiotic properties may really exist and inhabit in the oral cavity. Though, the complexity of biofilm development and interspecies interactions require more detailed investigations in order to state true probiotic candidates with activity in the oral cavity (Stamatova & Meurman, 2009).

4.2 Probiotics and resistance to oral defense mechanisms

At first, ingested probiotics are exposed to saliva. During this first step of contact, survival and resistance to oral environmental factors are very important. Salivary proteins such as lysozyme, lactoferrine, salivary peroxidase, and secretory IgA can collectively affect viability or cell surface morphology of probiotic species. The adhesion and metabolic activity of them is then affected. Saliva role on microbial establishment can be contradictory. In one hand, saliva can inhibit colonization of probiotics (by growth inhibition, killing, or prevention of adherence to host tissues), and on the other hand, it can promote microbial colonization. It has been observed that, Lysozyme pretreatment could significantly reduce the adhesion of *L. rhamnosus* GG, *L. rhamnosus* Lc705 and *L. casei* Shirota. However, the adhesive properties of *L. johnsonii* La1 and *B. lactis* Bb12 remained unaffected. These results highlight the strain-specific response to proteolytic enzymes and this feature needs to be considered when selecting probiotics for the oral cavity.

Other studies have also shown that lysozyme pretreatment of lactobacilli can slightly increase their adhesiveness to saliva coated surfaces. Lysozyme pretreatment could not significantly reduce the viability of lactobacilli but cell surface alterations might have contributed to the increased adhesion. Further studies on the mechanism whereby lysozyme affects adhesion are necessitated (Stamatova & Meurman, 2009).

Another aspect in oral establishment of probiotics is saliva-mediated aggregation.This ability is related to cell adherence properties. The adhesion mechanisms of lactobacilli involve hydrophobicity and surface charge, as well as specific carbohydrate and/or proteinaceous components. Organisms able to co-aggregate with other bacteria may have superior advantages over non-coaggregating organisms which are easily removed from the oral cavity. Recently, results have shown that *L. salivarius* was not able to form a biofilm in monoculture (in a microplate model), whereas when the species was added simultaneously with the inoculum of other commensal oral microorganisms, it established itself irrespective of pH. Similar findings were observed with *L. plantarum* SA-1 and *L. rhamnosus* that failed to form substantial biofilms in mono-culture but biofilm mass increased when cocultured with *A. naeslundii* (Filoche et al., 2004).

4.3 Probiotics and oral health

Several authors have suggested that probiotic bacteria could also be beneficial to oral health. Species of Lactobacillus and Bifidobacteria may exert beneficial effects in the oral cavity by inhibiting cariogenic Streptococci and Candida spp (Bhardwaj, 2010).

The mechanisms of probiotic action in the oral cavity could be similar to those described for the intestine. The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites, stimulating immune modulatory cells and producing lactase. It is also showed that they have influence to the immune system through several molecular mechanisms (Bhushan & Chachra, 2010).

To have a beneficial effect in oral cavity, a probiotic should have a tendency to form a biofilm that acts as a protective lining for oral tissues against oral diseases. Probiotics strains have been shown to vary broadly in their adhesiveness to saliva-coated HA and so in biofilm formation ability. Among probiotics strains *L. rhamnosus* GG exhibited the maximum values of adhesion, comparable to those of the early tooth colonizer *S. sanguinis*. Dairy starter *L. bulgaricus* strains adhered poorly to sHA.

Probiotic bacteria adhesion to oral soft tissues is another aspect that promotes their health effect to the host. Cell adhesion is a complex process involving contact between the bacterial cell and interaction with surfaces. The epithelial lining of the oral cavity despite its function as a physical barrier, actively participates in immune response. It has been shown that probiotic bacteria can stimulate local immunity and modulate the inflammatory response. Lactobacilli as well as other gram positive bacteria express ligands for toll-like receptors (TLRs) which initiate immune responses enabling detection of both pathogens and indigenous microbiota by epithelial cells. Recognition of commensal bacteria by these receptors (TLRs) is necessary for homeostasis, epithelial cells protection from injury and repair stimulation (Stamatova & Meurman, 2009).

Production of antimicrobial substances • Organic acids • Hydrogen peroxide • carbon peroxide, • diacetyl • Biosurfactants • Bacteriocins
Binding in Oral Cavity • Compete with pathogens for adhesion sites • Involvement in metabolism of substrates (competing with oral micro organisms for substrates available)
Immuno modulatory • Stimulate non specific immunity • Modulate humoral and cellular immune response
Modify oral conditions • Modification of oxidation reduction potential • Modulating pH

Table 3. Possible mechanisms of a probiotic in oral health

4.4 Probiotics and dental caries

From a view point, probiotics (lactobacilli) could hydrolyse proteins, stimulate growth of streptococci: the streptococci are acidogenic bacteria and produce low pH conditions in the oral environment (Robinson and Tamine, 1981). Also untreated caries cavities should also be questioned at this point. On the other hand, in recent studies, it was stated that probiotic might decrease the risk of the highest level of Streptococcus mutans (Ahola et al, 2002) or might increases salivary counts of lactobacilli while *S. mutans* levels were not modified (Montalto et al, 2004).

To have a beneficial effect in limiting or preventing dental caries, a probiotic must be able to adhere to dental surfaces and integrate into the bacterial communities making up the dental biofilm. Such a biofilm holds pathogens off oral tissues by filling a space which in future, could have served as a niche for pathogens, and it should also compete with and antagonize the cariogenic bacteria and thus prevent their proliferation (Caglar et al., 2005; Sheikh et al., 2011). According to our researches, it is cleared that the presence of Lactobacillus Sp. Such as *L. acidophilus DSM 20079, L. fermentum ATCC 9338* and *L.rhamnosus ATCC 7469* can cause reduction in the adherence of Streptococcal strains that it is probably related to interaction between bacteria. The mutans streptococci adherence reduction was significantly stronger in the case of *L. acidophilus* and *L. rhamnosus* while in the other study showed that *L. fermentum* reduced the adherence of non mutans Streptococci more than mutans Streptococci (figure 6).

In general, Inoculation of probiotic strain before Streptoccocal isolates to in vitro system showed more effect on adherence reduction (about 25% reduction in adherence) with significant difference (Pvalue< 0.05) especially in the case of *L. rhamnosus*. It is thought that adhesion reduction is likely due to bacterial interactions and colonization of adhesion sites

with probiotic strain before the presence of streptococci. Also, the probiotic strains were able to modify the proportion of the oral species within the biofilm (Tahmourespour & Kermanshahi, 2011).

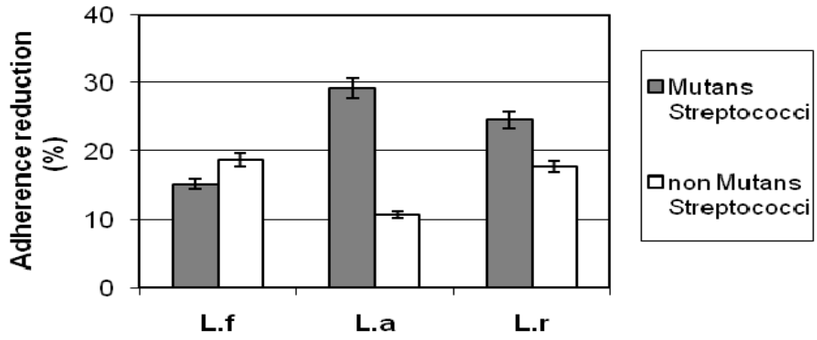

Fig. 6. The percentage of streptococcal adherence reduction in the presence of probiotic strains

Nikawa et al. (2004) also reported that consumption of yoghurt containing l*actobacillus reuteri (L. reuteri)* over a period of 2 weeks reduced the concentration of *S. mutans* in the saliva by up to 80%. Comparable results were obtained by incorporating probiotics into chewing gum or lozenges. Comelli et al (2002) reported that inoculation of dairy strains before adding the oral bacteria did not increase their colonization. They also found that dairy strains and particularly *L. lactis NCC2211* were able to modify the extent of oral species within the biofilm and also able to reduce cariogenic bacteria levels. They suggest that the reduction of these strains can be explained either by competition for adhesion sites or growth factors. Miller et al., in their study about the effect of microbial interaction on In Vitro plaque formation by *Streptococcus mutans* found that microbial interaction may have the potential to affect the amount and type of plaque formed, depending upon the kinds of organisms involved. They also reported that the addition of the lactobacilli to cultures of *S. sanguis* resulted in more inhibition of plaque formation when compared with pure cultures of *S. sanguis*. A 34% inhibition of plaque formation was observed when *L. casei* interacted with *S. mutans NCTC 10449*. Furthermore Simark-Mattsson et al. (2007) have shown the interference capacities of lactobacilli against strains of *Streptococcus mutans* and *Streptococcus sobrinus*. Meurman (2005) showed the inhibitory activity of *Lactobacillus rhamnosus GG* against *Streptococcus mutans* in low pH and it can be useful for preventing the cariogenic effects of oral streptococci. In vivo studies have also confirmed the effects of probiotic bacteria consumption on decreasing the risk of dental caries and mutans Streptococcus counts. Nase *et al., (2001)* reported long term consumption of milk containing the probiotic *Lactobacillus rhamnosus* CG strain reduced caries in kindergarten children. In one of the earlier studies, Marquis *et al*, demonstrated a potential probiotic approach for reducing dental caries by using oral Streptococci that are able to metabolize arginine or urea to ammonia. Cagler *et al* have showed a reduced *S. mutans* level in patients receiving fluid or tablet probiotic forms. In another study by Cagler *et al* a significantly reduced level was observed for *S.mutans* not for Lactobacillus in an ice-cream containing *Bifidobacterium lactis* (Caglar et al., 2005; Kargul et al., 2003). Lactobacilli have been used to deliver vaccine components for active immunization in vivo. In this way, the vectors, with the ability of the

streptococcal antigen I/II (*S. mutans* adhesion molecules) recognition were constructed and expressed in a strain of Lactobacilli. After the administration of such Lactobacilli to a rat model of dental caries development, *S. mutans* counts and caries scores were reduced obviously (Kruger et al., 2002). The above studies also suggest that consumption of products containing probiotic Lactobacilli or Bifidobacteria could reduce the number of mutans Streptococci in saliva. Oral probiotics may help fight tooth decay, since acid production from sugar is detrimental to teeth, care must be taken not to select strains with high fermentation capacity.

However, according to the researches, it is cleared that, there are some attractive vehicles for probiotic intake such as using fermented dairy products containing probiotic bacteria (milk, cheese, yogurt and ice cream) and also chewing gum, candies, tablets and water containing probiotics.

4.5 Probiotics-derived biosurfactant

Lactobacilli, as a probiotic (because of it`s known probiotic potential and it`s acid resistance and bile salt`s tolerance), are believed to interfere with pathogens by different mechanisms (table 3) and one of their mechanisms is biosurfactant production.

As it is mentioned before, lactobacilli have been recognized for their antimicrobial activity and ability to interfere with the adhesion of pathogens on epithelial cells and for their anti-biofilm production on catheter devices and voice prostheses. The mechanisms of this interfering have been demonstrated to include, among others, the release of biosurfactants. Biosurfactants, a structurally diverse group of surface active molecules synthesized by microorganisms, have recently attracted attentions in biotechnology for industrial and medical applications. Because the reason, they had several advantages on synthetic surfactants, such as low toxicity, inherent good biodegradability and ecological acceptability. Biosurfactants include unique amphipathic properties derived from their complex structures, which include a hydrophilic moiety and a hydrophobic portion (Vater et al. 2002). The use of biosurfactants from probiotic bacteria as antimicrobial and/or anti-adhesive agents has been studied before and their ability to inhibit adhesion of various micro organisms isolated from explanted voice prostheses has been demonstrated (Rodrigues et al. 2004). Biosurfactants adsorption to a surface modifies its hydrophobicity, interfering in the microbial adhesion and desorption processes; so, the release of biosurfactants by probiotic bacteria in vivo can be considered as a defence weapon against other colonizing strains (van Hoogmoed et al., 2004; Rodrigues et al., 2006). Consequently, previous adsorption of biosurfactants can be used as a preventive strategy to delay the onset of pathogenic biofilm growth, reducing the use of synthetic drugs and chemicals.

In a study, we showed that the biosurfactant derived from probiotic bacteria (*L.acidophilus, L. fermentum* and *L. rhamnosus*) could reduce the adhesion of *S. mutans* to the surfaces (fig 7) (Glass slide or Polystyrene micro titer plates). They also could make streptococcal chains shorter.

Other researchers demonstrated that, the biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 could interfere in the adhesion and biofilm formation of the *S. mutans*. Also, it is reported that, the release of biosurfactant from *S. mitis BMS* could interfere in the

adhesion of the cariogenic *S. mutans* to glass in the presence and absence of a salivary conditioning film. Others also confirmed that biosurfactants had inhibitory effect on bacterial adhesion and also biofilm formation. However; the precise mechanisms of such effects have not yet been explained. It seems to be highly dependent on biosurfactant type and the properties of the target bacteria. The simplest way to explain biosurfactant antiadhesion and antibiofilm activities would be their direct antimicrobial action. However, the antimicrobial activity of biosurfactants has not been observed in all cases (Tahmourespour et al., 2011 & vater et al., 2002). Thus, it is reported that the way in which surfactants influenced bacterial surface interactions appeared to be more closely related to the changes in surface tension and bacterial cell-wall charge. These factors are very important in overcoming the initial electrostatic repulsion barrier between the microorganism cell surface and its substrate. Surfactants may affect both cell-to-cell and cell-to-surface interactions. Their results support the idea that lactobacilli-derived agents remarkably have an effect on these interactions.

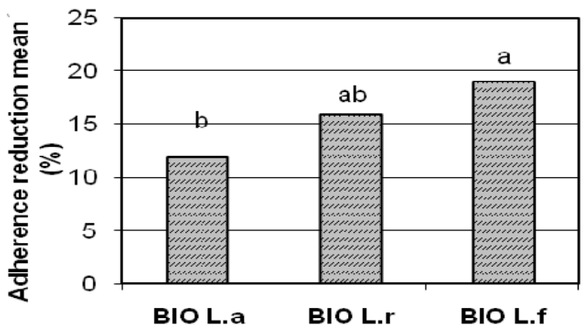

Fig. 7. The mean of adherence reduction percentage of mutans streptococci in presence of biosurfactants derived from *L. acidophilus, L. rhamnosus* and *L.fermentum* (Unpublished data).

As it is clear, colonization of the teeth by mutans streptococci has been associated with the etiology and pathogenesis of dental caries in humans. The ability of these organisms, particularly *Streptococcus mutans*, to synthesize extracellular glucans from sucrose using glucosyltransferases (Gtfs) is a major virulence factor of this bacterium.

The Gtfs secreted by *S. mutans* (particularly GtfB and GtfC) provide specific binding sites for either bacterial colonization of the tooth surface or attachment of bacteria to each other, modulating the formation of tightly adherent biofilms, the precursor of dental caries (Koo et al. 2010; Murata et al. 2010). However, the ability of *S. mutans* to adhere to the tooth surface is vital for the initiation and progression of dental caries. α-(1-3)- and α-(1-6)-linked glucan polymers are encoded by the genes gtfB, gtfC, and gtfD. In vitro studies have indicated that gtfB and gtfC are essential for the sucrose-dependent attachment of *S. mutans* cells to hard surfaces, but gtfD is dispensable (Yoshida et al. 2005). Therefore, these genes have become a potential target for protection against dental caries.

The effect of *L. fermentum* and *L. acidophilus* biosurfactant on gtfB and gtfC gene expression levels was also investigated in our other studies. The expression of these genes and the production of insoluble extracellular glucans mediate the attachment of *S. mutans* not only to surfaces but also to other active types of bacteria that are favorable to the organisms for the persistent colonization of tooth surfaces. Additionally, gtf genes are known virulence

factors associated with the pathogenesis of dental caries and a high content of insoluble glucans in dental plaque, which is related to an elevated risk of biofilm cariogenicity in humans. Several environmental factors can influence the expression and activity of the gtf enzymes. The existence of various enzymes in the process of carbohydrate metabolism and transport, glucan synthesis and secretion and degradation in the oral streptococci, in addition to factors that involve Post-translational modifications of the gtf enzymes, have traditionally complicated the understanding of regulatory studies (Wen et al. 2010).

Our results (figure 8 & 9) suggest that either the *L .fermentum* or *L. acidophilus* derived biosurfactants themselves or a putative signaling molecule in the extract down-regulated the expression level of genes that play an important role in the process of *S. mutans* attachment and biofilm formation. In addition to down regulating gtfB and gtfC (genes involved in insoluble glucan production), it may also have an effect on converting gtf activity from producing insoluble glucans to water-soluble glucans, hence accounting for reduced *S. mutans* biofilm adherence, and this should be studied in the future.

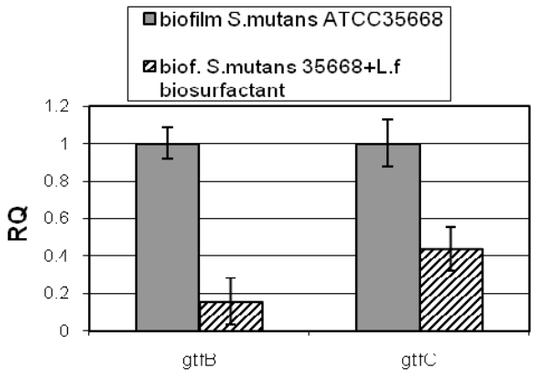

Fig. 8. The effect of *L.fermentum*.-derived biosurfactant on gtfB/C in immobilized biofilm of *S. mutans ATCC 35668;* The mRNA expression levels were calibrated relative to the control group (in the absence of biosurfactant)(Tahmourespour et al.,2011, Biofouling) .

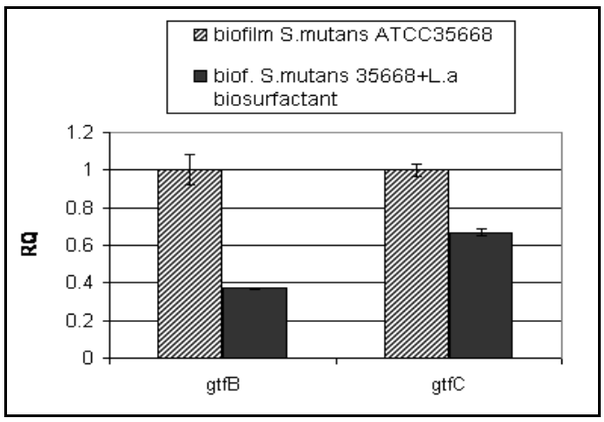

Fig. 9. The effect of *L.acidophilus*-derived biosurfactant on gtfB/C in immobilized biofilm of *S. mutans ATCC 35668;* The mRNA expression levels were calibrated relative to the control group (in the absence of biosurfactant) (Tahmourespour et al.,2011, Brazillian J of Microbiology).

Other studies have focused on the production and gene regulation of virulence factors, such as gtfs, which play an important role in biofilm formation by *S. mutans*, for controlling dental caries (Tamwsada and Kawabata 2004; Huang et al. 2008). The ability of *S. mutans* to produce extracellular polysaccharides from dietary carbohydrates has been demonstrated to significantly enhance its cariogenicity. Thus, the less extracellular polysaccharide produced, the lower the cariogenicity of *S. mutans*. Also it is demonstrated that chemical surfactants exerted different effects on the synthesis of glucosyltransferases in *S. mutans*; Tween 80 sig nificantly increased the level of gtfs, while Triton X-100 decreased gtf levels. So, It is proposed that the secondary metabolite of the probiotic bacteria (*L.fermentum and L.acidophilus*) decreases the expression level of gtf genes and therefore may be useful for the control of *S. mutans* and possibly other species.

4.6 Safty aspects of probiotics

Although probiotics can affect most important caries pathogens, lactobacilli may correlate with caries development. Some strains of *Lactobacillus* spp., together with *S. mutans*, play a key role in development of dental caries. The production of organic acids from dietary carbohydrates is also a main factor in dental caries progression. If lactobacilli taken orally are able to adhere or temporarily establish themselves in the oral environment, their metabolism and acid production should not support caries induction. Studies addressing sugar fermentation has shown a strain dependent

pH drop and the decrease was the fastest with glucose for all tested strains, thus highlighting the acidogenic potential of probiotics. The diversity of *in vitro* results does not allow clear conclusions about which probiotics may add benefit to the oral cavity. More large scale, multicenter clinical investigations are required to support the true effectiveness of probiotics in the prevention of oral and dental diseases.

It has been also observed that caries free subjects are colonized by lactobacilli that possess a significantly increased potential to suppress the growth of mutans streptococci compared with caries active or arrested subjects (Stamatova & Meurman, 2009). Finally, it can be concluded that the lactobacilli effect on caries prevention seems favorable when probiotic strains are well selected.

Furthure more, it should be mentioned that, orally lactobacillus species are well tolerated by about every one. Flatulence or gas is the most common side effect of Lactobacilli supplementation. It is usually very mild and goes away after 2 or 4 days. Immunocompromised people should be careful with the probiotics use as there have been reports of entering the blood stream (sepsis) in these individuals.

Bifidobacteria, is not associated with any side effects. It can occasionally cause mild diarrhea in children.

5. Future directions

According to the researches, probiotic bacteria have been characterized for different oral health purposes, including caries, periodontal diseases, and halitosis.

Genetically modified microbes including probiotics can take a new dimension to the concept of probiotics. Their main aim is the reduction of harmful effects of pathogenic strains

naturally colonizing the oral cavity. The modified strain could then be used to replace the original pathogen. They also could be used to increase the properties of a potentially beneficial strain. In field of oral immunology, probiotics are being used as passive local immunization vehicles against dental caries. Bacteriophages, have also been detected in oral pathogens, such as *Actinobacillus Actinomycetemcomitans* (Sheikh et al., 2011).

The selection of the best probiotic for oral health and investigation the effect of other probiotic's metabolites on virulence genes and other traits of *S. mutans* are also issues that calls for further studies. It is possible that the administration way of probiotics might positively affect the effects observed as related to mutans streptococci reduction. So, further studies regarding the selection of best way for probiotic administration are necessitated.

Furthermore, the dosage of probiotic administration in each indication should be defined. Probiotics should be administered carefully and cautiously, and only on the basis of strong scientific evidence. Such evidence should direct the cautious, deliberate addition of clinically proven probiotics to commonly consumed food products to allow consumers to conveniently benefit from these organisms. Finally, safety issues are very important with any kind of bacteriotherapy.

Consequently, future studies should be conducted to investigate if phage therapy might be applied for oral and dental diseases in the same way as has been attempted for systemic infections.

6. Conclusion

Adhesion reduction can be an effective way on decreasing cariogenic potential of oral streptococci and all of the evidence has shown that probiotic bacteria such as Lactobacillus spp. can affect the oral ecology. In general, the above promising results suggest a potentially beneficial application of probiotics for the prevention of dental caries. These data also suggest that biosurfactant treatment can provide an option for controlling biofilm development and also influence the adhesive ability of bacterial pathogens

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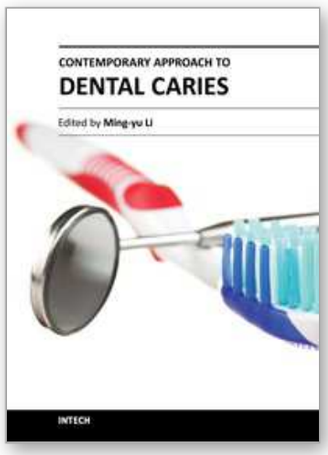

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