academicJournals

Vol. 9(5), pp. 275-279, 4 February, 2015
DOI: 10.5897/AJMR2014.7170
Article Number: 17CF1DE50452
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Expression of adherence genes of *Streptococcus mutans* in the presence of *Lactobacillus acidophilus* and glucose

Karina Gomez-Garcia[1], Luis J. Galan-Wong[1], Katiushka Arevalo-Niño[1], Andrea Guadalupe Alcazar-Pizaña[2], Jaime de Jesus Covarrubias-Martinez[1] and Myriam Angelica De La Garza-Ramos[2]*

[1]Facultad de Ciencias Biologicas, Instituto de Biotecnologia, Universidad Autónoma de Nuevo Leon, Monterrey, Mexico.
[2]Facultad de Odontología, Universidad Autónoma de Nuevo Leon, Monterrey, Mexico.

Received 2 October, 2014; Accepted 26 January, 2015

Streptococcus mutans **is the bacteria most frequently associated with dental caries. One of its virulence factors is its ability to form biofilm. *gtf*, *ftf* and *spaP* are genes of *S. mutans* that are involved in adhesion and colonization of teeth surfaces and therefore biofilm formation. Exopolysaccharides are catalyzed by a group of bacterial enzymes termed glycosyltransferases (GTF) and fructosyltransferases (FTF), encoded by the genes *gtf* and *ftf*, respectively, which under certain conditions can be strongly associated with cell surfaces. *Lactobacillus acidophilus* is one of the pathogens associated with *S. mutans*. We carried out research to determine whether the presence of glucose and *L. acidophilus* promote adhesion mechanisms that increase the expression of *gtf*, *ftf* and *spaP* of *S. mutans*.**

Key words: Exopolysaccharides, dental caries, *Streptococcus mutans*, *Lactobacillus acidophilus*, glucose, *gtf*, *ftf*, *spaP*.

INTRODUCTION

Streptococcus mutans is strongly associated with the onset and progression of dental caries, one of the most frequent public health problems in the world that can compromise overall health through the years (Allukian, 2000). *Streptococcus mutans* is considered the main etiological agent responsible for dental caries. It is a Gram-positive, acidogenic microorganism that ferments glucose, lactose, raffinose and mannitol; it is non mobile, catalase-positive and can metabolize sucrose (Hamada and Slade, 1980).

One of the virulence factors of *S. mutans* is its ability to form biofilm. Biofilm formation consists of three steps: 1) surface adhesion of microorganisms, 2) the formation of highly structured clusters (microcolonies), and 3) the development and stabilization of microcolonies. This process is carried out in response to environmental, physical or chemical signals that regulate certain physiological processes that depend on bacterial density (quorum sensing) and regulate the expression of genes involved in biofilm formation. Adhesion is mediated by exopolysaccharides (glucans and fructans) that are produced by the bacteria that grow in the presence of

*Corresponding author. E-mail: myriam.garzarm@uanl.edu.mx. Tel: +52 (81) 83 29 40 00. Ext: 1781. Fax: +52 (81) 83 29 40 00 Ext 1781.

sucrose and are considered virulence factors (Paes Leme et al., 2006).

Exopolysaccharide synthesis is catalyzed by a group of bacterial enzymes termed glycosyltransferases (GTF) and fructosyltransferases (FTF), encoded by the genes *gtf* and *ftf*, respectively, which under certain conditions can be strongly associated with cell surfaces and which apparently mediate glucan induced agglutination (Li and Burne, 2001).

S. mutans produces a surface antigen protein of the Ag I/II family. One of these is SpaP encoded by the *spaP* gene, which contributes to initial adherence and development of the microbial community, since it can interact with a large number of substrates (Ono et al., 1994).

Lactobacillus acidophilus is one of the pathogens associated with *S. mutans*, which is part of the diverse microorganisms termed non-cariogenic mutans (Marsh, 2003). *Lactobacilli* can produce organic acids that decalcify the dentinal matrix and have been found in both superficial and deep caries (Byun et al., 2004). *Lactobacilli* represent approximately 1% of the culturable oral flora. Some studies have associated their presence with oral health (Haukioja, 2010). In a study by Martin et al. (2002), *Lactobacillus* sp. were found to be a numerically dominant species in human carious dentine. However, the presence of *Lactobacilli* delays the expression of adherence genes and the accumulation of glucose (Rooj et al., 2010). They possibly contend with *S. mutans* for the substrate and reduce the risk of caries (Baca-Castañón, 2014).

The total number of bacteria and the composition of the oral flora associated with cavities can give indications of the individual risk and incidence of the disease. The bacteria involved in the initiation and development of dental caries are mainly *Streptococci*, *Lactobacilli* and *Actinomycetes* (Marsh, 2003). However, environmental factors such as the presence of sugar and non-mutans carbohydrate fermenting bacteria that promote a pH homeostasis that demineralizes enamel can change the biochemical composition of the biofilm or plaque (Takahashi and Nyvad, 2011). In these conditions, *S. mutans* is able to grow by increasing their adhesion factors due to their metabolic ability to decompose carbohydrate from the diet, from which they produce a large amount of organic acids (acidogenesis), and by its ability to withstand environmental stress, specifically a low pH (aciduricity) (Lemos and Burne, 2008).

The aim of this study is to assess if *Lactobacilli* can positively or negatively influence the virulance of bacteria; therefore, the objective of this research is to determine whether the presence of glucose and *L. acidophilus* promote adhesion mechanisms that increase the expression of *gtf*, *ftf* and *spaP* of *S. mutans*.

MATERIALS AND METHODS

The gene expression of *gtf*, *ftf* and *spaP* in *S. mutans* was studied. *S. mutans* strain AU159 (ATCC 700610) and *L. acidophilus* strain LA3 (Lyofast LA3) were lyophilized and stored at −80°C. The lyophilized strains were activated in the medium appropriate for each bacterial culture: brain-heart infusion (BHI) broth (Becton, Dickinson and Company, Franklin Lakes, NJ) was used for *S. mutans* and Rogosa broth (Oxoid Ltd., Hampshire, UK) for *L. acidophilus*.

Culture tubes were inoculated with 50 mL of BHI broth for *S. mutans* and incubated at 37°C for 12 h. *L. acidophilus* (50 mL) was inoculated in Rogosa broth and incubated at 37°C for 24 h. Subsequently, tubes containing 20 mL of BHI alone and BHI supplemented with 40% glucose (BHIG) were inoculated with a *S. mutans* : *L. acidophilus* culture ratio of 1:1, 1:10 and 10:1. These cultures were then incubated at 37°C. The exponential phase of *S. mutans* is 12 h and *L. acidophilus* 6 h. *S. mutans* was placed in a brain heart infusion with glucose. *Lactobacillus* was placed at 6 h in MRS (OD 600 nm, 0.5) in the same tube with *S. mutans* at 12 h. After six hours, the experiment was stopped to extract RNA. Both species fermented glucose, but *Lactobacillus* increased acidification. We wanted to observe the effect of *Lactobacillus* on *S. mutans* since *L. acidophilus* is not currently considered cariogenic but probiotic. This can be promoted together with other *Lactobacilli* as a controller of caries. *S. mutans* fermented the sugar and *L. acidophillus* had strong resistance to acid pH. This can be protective since it regulates the metabolism of other cells.

A resulting biomass of 3 mL was taken from each culture and washed twice with Tris-HCl Buffer (10 mM, pH 8.0); lysozime 500 µL (10 mg ml^{-1}) was added and the mixture incubated at 37°C for 2 h. Total RNA extraction was carried out using TriPure Isolation Reagent (Roche Diagnostics, Basel, Switzerland) according to the manufacture's recommendations. Total RNA was read at 260 nm in a SmartSpec Spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA). cDNA was generated using the Improm II Reverse Transcription System (Promega Corporation, Madison, WI). The pellet that resulted from the extraction process was resuspended in 50 µL of DEPC water and was stored at -80°C until use.

In order to confirm the identity of bacterium strains, primers based on the rRNA 16S of *S. mutans* and *L. acidophilus* were designed using specific sequences reported in GenBank (http://www.ncbi.nlm.nih.gov/). Oligonucleotides were designed based on DNA gene sequences in GenBank (Table 1).

Quantitative PCR was performed in a LightCycler 480 (Roche) using the Light Cycler 480 Control Kit (Roche), according to the manufacturer's recommendations. Gene amplification was done in a final volume of 50 µL, as follows: one denaturalization cycle at 95°C for 5 min with a ramp rate of 4°C/s; 45 cycles at 95°C for 10 s with a 4°C/s ramp rate; 55°C for 15 s with a 2°C/s ramp rate; 72°C for 10 s with a 4°C/s quantification analysis ramp rate; finally, 1 freezing cycle at 40°C for 30 s with a 20°C/s ramp rate.

RESULTS

An increase in gene expression was observed, especially with *spaP* with and without glucose and with a *Sm : La* ratio of 1:10. In order to quantify gene expression, cDNA samples from three different *S. mutans* and *L. acidophilus* (*Sm : Lm*) ratios (1:0/*Sm* alone) were diluted 10-1, 10-2 and 10-3 and gene amplification was run. Representative results obtained with the 10-1 dilution are shown in Table 2. These results show that in pure culture (*Sm*), the level of gene expression of *Sm gtf* was approximately the same as that of GAPDH. The gene *Sm ftf* is downregulated as well as *spaP*. When *S. mutans* was cultured with *L. acidophilus* in different ratio

Table 1. Primers and probes used to identify bacteria strains.

Strand	5´-3 Sequence	ΔG Selfdimer	ΔG hairping	ΔG heterodimer	Tm	CG %	Length	Prod size	nMoles
***ftf* – NCBI: AE014133.2**									
Fwd Primer	GCCGTCATTAACAGGGTATCAGA	-4.85	0.04	-11.16	56.5	47.8	23		5.0
Rvs Primer	TGGCGAACGGCGACTTA	-3.61	-1.42		57.1	58.8	17	71	5.0
Probe	/56-FAM/TACTGGAAC/ZEN/AGCATAATAA/31ABkFQ/	-3.55	-2.3	-4.38/-3.14	45.3	31.6	19		2.5
***gtf* – NCBI: AE014133.2**									
Primer Fwd	GTTTATGATTTTGCCCTGCCTATG	-3.14	0.31	-3.9	54.6	41.7	24	63	5.0
Primer Rvs	ACGGTCAACCTTGCTCGAAT	-6.76	-0.56		57	50	20		5.0
Probe	TGACGCTCTACAGCCTA	-4.74	-0.81	-3.55/-3.14	45.3	31.	19		2.5
***spaP* – NCBI: AE014133.2**									
Fwd Primer	AAGTCAGTGGCAACGATTTATCC	-3.61	0.04	-8.38	55.4	43.5	23	71	5.0
Rvs Primer	TTATTCTTATAAGTTGCGCCATCATT	-10.48	0.31		53.3	30.8	26		5.0
Probe	/56-FAM/CAGTGGTCG/ZEN/GACAAGT/31ABkFQ/	-3.61	0.6	-5.19/-5.02	51.4	56.3	16		2.5
GAPDH									
Fwd Primer	TTGGAACTGGAACACGTTGTG	-6.3	-0.42	-9.88	55.8	47.6	21	390	
Rvs Primer	TAAAGCTATTGGTCTTGTTCCTGAA	-6.34	0.13		54.6	36	25		

Table 2. Expression levels of *ftf, gt,* and *spaP* in *S. Mutans* culture with glucose.

***S. mutans* : *L. acidophilus* ratio**	***ftf***	***gtf***	***spaP***
Sm	6.12	1.63	0.4492
1:1	2.400	4.20	3.428
1:10	3.830	7.10	4.43
10:1	11.84	2.77	1.54

Sm = *S. mutans*; Units represent the number of PCR product per cycle as compared to the housekeeping gene.

conditions (1:1, 1:10 and 10:1), all genes were upregulated.

S. mutans supressed adherence gene expression, mainly *ftf* in the presence of 40% glucose; however, its behavior was different in the presence of *L. acidophilus* since expression was 11.84 times less than when *L. acidophilus* and glucose were present. Something similar occured with *gtf* since glucose and a greater number of *L. acidophilus* increased *spaP* even more. *spaP* is a gene that codifies for adherence, which can also be supressed in the presence of glucose and when *L. acidophilus* is present in the environment.

Primer specificity was also tested with pure cultures of *S. mutans*. The results of amplification of *ftf, gtf, spsP* and GAPDH in different repetitions are shown in Figure 1. In cycle 23 without glucose, *gtf* shows a positive signal, while in cycle 42 with glucose, *gtf* reduces its positive signal. The positive signal begins for the *ftf* initiators without glucose starting expression at cycle 33, and in the presence of glucose at cycle 44. Later, *SpaP* expression starts at cycle 34, and with glucose at cycle 49. Finally, GAPDH showed a positive signal in cycle 39 without glucose and at cycle 49 with glucose Figure 2.

DISCUSSION

In this study, *S. mutans* was grown in a culture medium with and without glucose. In addition, *S. mutans* was grown with *Lactobacillus acidophilus*, an important member of the oral microbiota. The presence of glucose and *L. acidophilus* increased expression of the genes studied, especially *ftf*. This is noteworthy since it has

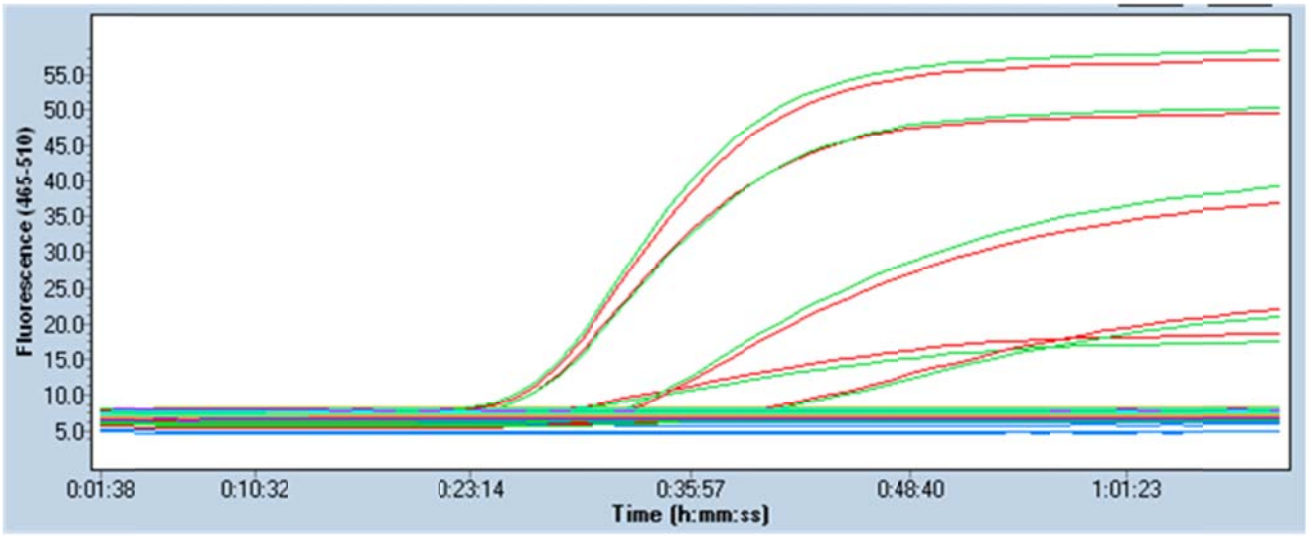

Figure 1. Gene expression by real time RT-PCR without glucose.

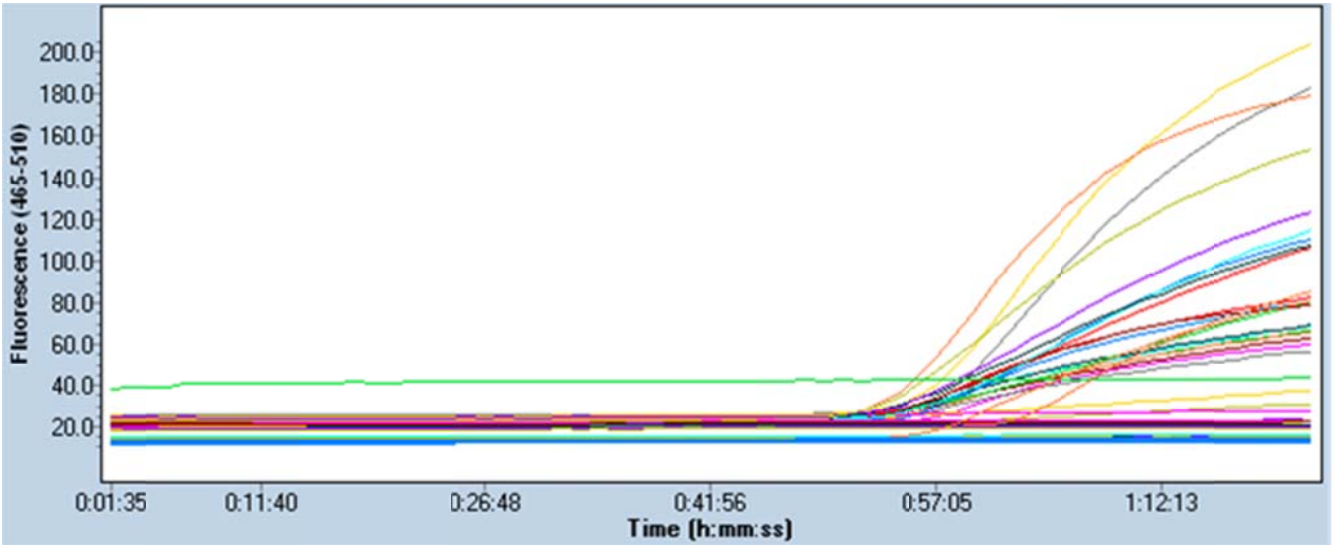

Figure 2. Gene expression by real time RT-PCR with glucose.

been shown in previous studies that the addition of sucrose, glucose or fructose increases expression of these genes (Banas, 2004; Koo et al., 2010). Thus, when there is a lack of oral higiene, the risk of caries increases in the presence of a diet rich in carbohydrates.

ftf, *gtf* and *spaP* are important for *S. mutans* to grow in a community of organisms and establish on the tooth surface. These genes encode the expression of fructans, glucans and adhesins, substances that are considered important virulence factors and that are involved in the development of biofilm. *S. mutans* can adhere to moist teeth surfaces through saliva. It is part of the normal flora of the oral cavity and has the ability to withstand both an abundance and shortage of sugars in food (Carlsson, 1983). This ability makes it persist in an absence of food and in carious lesions, thus making it the predominant bacteria in dental caries. Also, it can easily adapt to conditions of acid pH and oxidative stress, which are present in saliva, making it also the prevalent species in plaque (Banas, 2004). This phenomenon is not an independent response of the genes but a coordinated event that integrates the changing events that affect the environment. In this study, we have found that *S. mutans* in extreme conditions of glucose and in the presence of *L. acidophilus* is capable of expressing adhesion genes and forming biofilms.

Stephenson and Hoch in 2002 suggested the use of bacterial tow-component signal transduction systems (TCSTS) as a good antimicrobial design strategy since it is capable of engaging phosphotransferase events between histidine residues and aspartate transmembrane sinases that are responsible for transcription regulation by binding to DNA and suppressing and/or expressing it (Smith and Spatafora, 2012). Some suggest that cross-regulation between certain histidine kinases can regulate plaque formation (Chong et al., 2008).

Our findings are similar to Shemesh et al. (2006) and Decker et al. (2014) who studied the effect of different carbohydrates. In addition to this, they investigated the expression of glucosyltransferases and other biofilm-associated genes and found that the combined presence of carbohydrates stimulate the upregulation of glucan- and biofilm-associated genes in a different way than glucose alone. Shemesh et al. (2006) also found that gene expression was dependent on the growth phase.

We did not use biofilms in our study since our objective was to determine whether the presence of glucose and *L. acidophillus* promote adhesion mechanisms that increase the expression of *gtf, ftf,* and *spaP*. However, bacteria were harvested during their exponential phase to investigate gene expression.

Dietary carbohydrates and *L. acidophillus* are important environmental factors in the development of biofilms that can cause oral infections.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Allukian M Jr (2000). The neglected epidemic and the surgeon general's report: a call to action for better oral health. Am. J. Public Health 90(6):843-845.

Baca-Castañón ML, De la Garza-Ramos MA, Alcázar-Pizaña AG, Grondin Y, Coronado-Mendoza A, Sánchez-Najera RI, Cárdenas-Estrada E, Medina-De la Garza CE, Escamilla-García E (2014). Antimicrobial effect of *Lactobacillus reuteri* on cariogenic bacteria *Streptococcus gordonii*, *Streptococcus mutans*, and Periodontal Diseases Actinomyces naeslundii and Tannerella forsythia. Probiotics Antimicrob. Proteins. Nov 25 [Epub ahead of print]

Banas JA (2004). Virulence properties of Streptococcus mutans. Front. Biosci. 9:1267-1277.

Byun R, Nadkarni MA, Chhour K-L, Martin FE, Jacques NA, Hunter N. (2004) Quantitative Analysis of Diverse Lactobacillus Species Present in Advanced Dental Caries. J. Clin. Microbiol. 42(7):3128-3136.

Carlsson J (1983). Regulation of sugar metabolism in relation to feast-and-famine existence of plaque, p. 205-211. In B. Guggenheim (ed.), Cariology today. Karger, Basel, Switzerland. pp. 205-211.

Chong P, Drake L, Biswas I (2008). LiaS regulates virulence factor expression in Streptococcus mutans. Infect. Immun. 76: 3093-3099.

Decker EM, Klein C, Schwindt D, von Ohle C (2014). Metabolic activity of Streptococcus mutans biofilms and gene expression during exposure to xylitol and sucrose. Int. J. Oral Sci. 6(4):195-204.

Hamada S, Slade HD (1980). Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol. Rev. 44(2):331-84.

Haukioja A (2010). Probiotics and oral health. Eur. J. Dent. 4: 348-355.

Koo H, Xiao J, Klein MI, Jeon JG (2010). Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. J. Bacteriol. 192(12):3024-3032.

Lemos JA, Burne RA (2008). A model of efficiency: stress tolerance by *Streptococcus mutans*. Microbiology 154 (Pt 11):3247-3255.

Li Y, Burne RA (2001). Regulation of the gtfBC and ftf genes of *Streptococcus mutans* in biofilms in response to pH and carbohydrate. Microbiology 147(Pt 10):2841-2848.

Marsh PD (2003). Are dental diseases examples of ecological catastrophes? Microbiology 149 (Pt 2): 279-294.

Martin FE, Nadkarni MA, Jacques NA, Hunter N (2002). Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis. J. Clin. Microbiol. 40(5):1698-704.

Ono T, Hirota K, Nemoto K, Fernandez EJ, Ota F, Fukui K (1994). Detection of Streptococcus mutans by PCR amplification of spaP gene. J. Med. Microbiol. 41(4):231-235.

Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA (2006). The role of sucrose in cariogenic dental biofilm formation-new insight. J. Dent. Res. 85(10):878-87.

Rooj AK, Kimura Y, Buddington RK (2010). Metabolites produced by probiotic *Lactobacilli* rapidly increase glucose uptake by Caco-2 cells. BMC Microbiol. 10:16.

Shemesh M, Tam A, Feldman M, Steinberg D (2006). Differential expression profiles of *Streptococcus mutans* ftf, gtf and vicR genes in the presence of dietary carbohydrates at early and late exponential growth phases. Carbohydr. Res. 341(12):2090-2097.

Smith EG, Spatafora GA (2012). Gene regulation in S. mutans: complex control in a complex environment. J. Dent. Res. 91(2):133-141.

Stephenson K, Hoch JA (2002). Two-component and phosphorelay signal-transduction systems as therapeutic targets. Curr. Opin. Pharmacol. 2(5):507-512.

Takahashi N, Nyvad B (2011). The role of bacteria in the caries process: ecological perspectives. J. Dent. Res. 90:294-303.