Full Length Research Paper

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

Karina Gomez-Garcia¹, Luis J. Galan-Wong¹, Katiushka Arevalo-Niño¹, Andrea Guadalupe Alcazar-Pizaña², Jaime de Jesus Covarrubias-Martinez¹ and Myriam Angelica De La Garza-Ramos²*

¹Facultad de Ciencias Biologicas, Instituto de Biotecnologia, Universidad Autónoma de Nuevo Leon, Monterrey, Mexico.  
²Facultad de Odontologia, 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...
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 mutants (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-Castañon, 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 Actinomyces (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 virulence 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.

**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.

<table>
<thead>
<tr>
<th>Strand</th>
<th>5'-3 Sequence</th>
<th>ΔG Selfdimer</th>
<th>ΔG hairping</th>
<th>ΔG heterodimer</th>
<th>Tm</th>
<th>CG %</th>
<th>Length</th>
<th>Prod size</th>
<th>nMoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>ftf – NCBI: AE014133.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fwd Primer</td>
<td>GCCGTCATTAACAGGTATCAGA</td>
<td>-4.85</td>
<td>0.04</td>
<td>-11.16</td>
<td>56.5</td>
<td>47.8</td>
<td>23</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Rvs Primer</td>
<td>TGGCGAACGCGCCTTATCA</td>
<td>-3.61</td>
<td>-1.42</td>
<td>-11.16</td>
<td>57.1</td>
<td>58.8</td>
<td>17</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>FAM/TACTGGAAC/ZEN/AGCATAATAA/31ABkFQ/</td>
<td>-3.55</td>
<td>-2.3</td>
<td>-4.38/-3.14</td>
<td>45.3</td>
<td>31.6</td>
<td>19</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>gtf – NCBI: AE014133.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primer</td>
<td>GTTATGATTTTGCCTGCTATG</td>
<td>-3.14</td>
<td>0.31</td>
<td>-3.9</td>
<td>54.6</td>
<td>41.7</td>
<td>24</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Rvs Primer</td>
<td>ACGGTCACCTTGCTGAAT</td>
<td>-6.76</td>
<td>-0.56</td>
<td>57</td>
<td>50</td>
<td>20</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>TGACGCTCTACAGCCTA</td>
<td>-4.74</td>
<td>-0.81</td>
<td>-3.55/-3.14</td>
<td>45.3</td>
<td>31.6</td>
<td>19</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>spaP – NCBI: AE014133.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fwd Primer</td>
<td>AAGTCAGTGCCACGTTTATCC</td>
<td>-3.61</td>
<td>0.04</td>
<td>-8.38</td>
<td>55.4</td>
<td>43.5</td>
<td>23</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Rvs Primer</td>
<td>TTATCTTATAAGTGCGCCTCATT</td>
<td>-10.48</td>
<td>0.31</td>
<td>-3.55/-3.14</td>
<td>51.4</td>
<td>56.3</td>
<td>16</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>FAM/CAGTGGTCG/ZEN/GACAAGT/31ABkFQ/</td>
<td>-3.61</td>
<td>-0.81</td>
<td>-5.19/-5.02</td>
<td>54.6</td>
<td>36</td>
<td>25</td>
<td>390</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fwd Primer</td>
<td>TTGGAACTGGAACACGTTGTG</td>
<td>-6.3</td>
<td>-0.42</td>
<td>-9.88</td>
<td>55.8</td>
<td>47.6</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rvs Primer</td>
<td>TAAAGCTATTGGTCTTGCTGAAT</td>
<td>-6.34</td>
<td>0.13</td>
<td></td>
<td>54.6</td>
<td>36</td>
<td>25</td>
<td>390</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S. mutans : L. acidophilus ratio</th>
<th>ftf</th>
<th>gtf</th>
<th>spaP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm</td>
<td>6.12</td>
<td>1.63</td>
<td>0.4492</td>
</tr>
<tr>
<td>1:1</td>
<td>2.400</td>
<td>4.20</td>
<td>3.428</td>
</tr>
<tr>
<td>1:10</td>
<td>3.830</td>
<td>7.10</td>
<td>4.43</td>
</tr>
<tr>
<td>10:1</td>
<td>11.84</td>
<td>2.77</td>
<td>1.54</td>
</tr>
</tbody>
</table>

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

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, spaP 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
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 hygiene, the risk of caries increases in the presence of a diet rich in carbohydrates.

*ttf, 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 two-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 signalases 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. acidophilus promote adhesion mechanisms that increase the expression of gtf, fft, and spaP. However, bacteria were harvested during their exponential phase to investigate gene expression.

Dietary carbohydrates and L. acidophilus 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


