The LicT protein acts as both a positive and a negative regulator of loci within the bgl regulon of Streptococcus mutans

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An open reading frame (ORF) that would encode a putative antiterminator protein (LicT) of the BglG family was identified in the genomic DNA sequence of Streptococcus mutans. A DNA sequence that would encode a potential ribonucleic antiterminator (RAT) site in the mRNA at which the putative antitermination protein LicT would bind was located immediately downstream from this ORF. These putative antitermination components are upstream of a glucose-independent β-glucoside-utilization system that is responsible for aesculin utilization by S. mutans NG8 in the presence of glucose. It was hypothesized that these putative regulatory components were an important mechanism that was involved with the controlled expression of the S. mutans bglP locus. A strain of S. mutans containing a licT::Ω-Kan2 insertional mutation was created. This strain could not hydrolyse aesculin in the presence of glucose. The transcriptional activity associated with other genes from the bgl regulon was determined in the licT::Ω-Kan2 genetic background using lacZ transcriptional fusions and β-galactosidase assays to determine the effect of LicT on these loci. The LicT protein had no significant effect on the expression of the bglC promoter, a regulator of the bglA locus. However, it is essential for the optimal expression of bglP. These data correlate with the phenotype observed on aesculin plates for the S. mutans wild-type strain NG8 and the licT::Ω-Kan2 strain. Thus, the glucose-independent β-glucoside-specific phosphotransferase system (PTS) regulon in S. mutans relies on LicT for BglP expression and, in turn, aesculin transport in the presence of glucose. Interestingly, LicT also seems to negatively regulate the expression of the bglA promoter region. In addition, the presence of the S. mutans licT gene has been shown to be able to activate a cryptic β-glucoside-specific operon found in Escherichia coli.

INTRODUCTION

β-Glucosides are carbohydrates that are largely derived from plant sources and include salicin, arbutin, cellobiose and aesculin. β-Glucoside-utilization systems have been described in several bacteria, including Escherichia coli (Ausubel et al., 2001), Erwinia chrysanthemi (el Hassouni et al., 1990), Clostridium longisporum (Brown & Thomson, 1998), Lactobacillus plantarum (Marasco et al., 2000), Bacillus subtilis (Le Coq et al., 1995; Tobisch et al., 1997) and Streptococcus mutans (Cote et al., 2000). These organisms rely on the phosphoenolpyruvate-dependent phosphotransferase system (PTS) (Postma & Lengeler, 1985) for the transport and the subsequent utilization of various β-glucosides. The structural features of the genes encoding these β-glucoside-utilization systems are markedly similar.

In most bacteria, these genes are organized in a simple operon structure (Cote et al., 2000). However, in S. mutans, these genes are organized as a regulon (Cote et al., 2000). It has been previously shown that the S. mutans bgl regulon encodes a β-glucoside-specific enzyme II of the PTS (bglP), a transcriptional regulator of the AraC/XylS family (bglC), and a phospho-β-glucosidase (bglA) (Cote et al., 2000). There are two additional ORFs within this region (bglB and ORF4) that would potentially encode proteins whose function is unknown at this time (Fig. 1). The β-glucoside-specific locus isolated from S. mutans is unique because of its regulon gene arrangement and because of the BglC protein, which acts as a positive regulator of the bglA gene (Cote & Honeyman, 2002).

Recently, an additional ORF has been identified (Cote & Honeyman, 2002) from the S. mutans genomic DNA sequence database (http://www.genome.ou.edu/smutans.shtml), isolated by PCR amplification, and examined for possible regulatory functions that affect the bgl regulon. This ORF (the licT gene) would encode a putative protein
belonging to the BglG family of transcriptional antitermini-
tors (Schnetz et al., 1987). Antiterminator proteins are
believed to be involved in the transcriptional regulation of
\( \beta \)-glucoside-specific genes from
*Escherichia coli* (Hall & Xu, 1992),
*Erwinia chrysanthemi* (el Hassouni et al., 1990),
*Lactococcus lactis* (Bardowski et al., 1994),
*Lactobacillus plantarum* (Marasco et al., 2000),
*B. subtilis* (Le Coq et al., 1995; Tobisch et al., 1997) and
*C. longisporum* (Brown & Thomson, 1998). It is believed that these antiterminator
proteins would bind to a ribonucleic antiterminator (RAT)
site present in a specific mRNA secondary structure and
would prevent the formation of a hairpin terminator
structure that terminates transcription (Rutberg, 1997).

The binding of the antiterminator protein to the mRNA
would allow transcription through the disrupted termina-
tor structure into the \( \beta \)-glucoside-specific genes that are
not normally transcribed. Thus, the antitermination mecha-
nism of transcriptional regulation allows for the expression
of \( \beta \)-glucoside-specific loci in the absence of a metabolically
preferred carbon source (Rutberg, 1997).

In this report, we determine that the LicT protein regulates
the *S. mutans* \( \beta \)-glucoside-specific PTS regulon in the pre-
sence of glucose via a putative antitermination mechanism.
Additional evidence that LicT acts as an antiterminator is
provided by the fact that this protein activates a cryptic
*Escherichia coli* operon. Based upon protein similarities and
potential RAT structures, we believe that the *E. coli* bglGFB
operon is induced by the *S. mutans* LicT protein. LicT is
shown to be essential for the efficient expression of bglP and
asculin hydrolysis by *S. mutans* in the presence of glucose.
LicT also acts as a negative regulator of the bglA locus.

**METHODS**

**Bacterial strains and media.** All strains used in this report are
listed in Table 1. *S. mutans* NG8 was used as the wild-type strain, as
the source of chromosomal DNA and as the recipient strain for all
*S. mutans* transformations. *E. coli* CC118 \([\Delta(ara-leu)7697 araD139
\Delta lacX74 galE galK ΔphoA20 thi-1 rpsE rpoB argE(Am) recA1]
\(\text{(Manoil & Beckwith, 1985)\) was used for all recombinant DNA pro-
cedures. *S. mutans* strains were grown on brain–heart infusion
(BHI) agar plates or in BHI broth (Difco). *S. mutans* strains were
grown under antibiotic selection, when appropriate, with kana-
mycin (500 \(\mu\)g ml\(^{-1}\)), erythromycin (20 \(\mu\)g ml\(^{-1}\)) or a combination

![Fig. 1. Diagram of \( \beta \)-glucoside PTS operons. (a) The simple operon structure of \( \beta \)-glucoside PTSs described in other organ-
isms. (b) The unique regulon structure displayed by the *bgl* regulon from *S. mutans*.](image)

Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Host cell</th>
<th>Description/genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. mutans</em> NG8</td>
<td><em>E. coli</em> CC118</td>
<td>Wild-type, ALH76</td>
<td></td>
</tr>
<tr>
<td>ALH199</td>
<td><em>E. coli</em> CC118</td>
<td>pALH185</td>
<td>Cote et al. (2000)</td>
</tr>
<tr>
<td>ALH201</td>
<td>NG8</td>
<td>NG8 (licT:::\Omega)-Kan2</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH205</td>
<td>NG8</td>
<td>pALH187</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH206</td>
<td>NG8</td>
<td>pALH188</td>
<td>Cote &amp; Honeyman (2002)</td>
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<td>ALH207</td>
<td>NG8</td>
<td>pALH189</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH213</td>
<td>NG8</td>
<td>pALH122</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH231</td>
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<td>pALH188</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH233</td>
<td>ALH201</td>
<td>pALH189</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH234</td>
<td>ALH201</td>
<td>pALH122</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH376</td>
<td>NG8</td>
<td>pALH189</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>ALH378</td>
<td>ALH376</td>
<td>pALH189</td>
<td></td>
</tr>
<tr>
<td><strong>Plasmid</strong></td>
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<td></td>
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<tr>
<td>pALH122</td>
<td></td>
<td>lacZ transcriptional fusion vector</td>
<td>Honeyman et al. (2002)</td>
</tr>
<tr>
<td>pALH163</td>
<td></td>
<td>Entire bgl region cloned into pACKS30</td>
<td>Cote et al. (2000)</td>
</tr>
<tr>
<td>pALH185</td>
<td></td>
<td>HindIII PCR fragment containing (licT) in pACKS30</td>
<td>Cote et al. (2000)</td>
</tr>
<tr>
<td>pALH186</td>
<td></td>
<td>pALH185 (licT:::\Omega)-Kan2</td>
<td></td>
</tr>
<tr>
<td>pALH187</td>
<td></td>
<td>pALH122 with bglP promoter region at Smal</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>pALH188</td>
<td></td>
<td>pALH122 with bglC promoter region at Smal</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>pALH189</td>
<td></td>
<td>pALH122 with bglA promoter region at Smal</td>
<td>Cote &amp; Honeyman (2002)</td>
</tr>
<tr>
<td>pALH198</td>
<td></td>
<td>pALH163 bglP::\Omega-Kan2 bglBCA</td>
<td></td>
</tr>
</tbody>
</table>
of both antibiotics. All *E. coli* strains were grown on Lennox Broth (LB) agar plates or in LB broth (Gibco-BRL). *E. coli* strains were grown under antibiotic selection with ampicillin (100 μg ml⁻¹) or chloramphenicol (20 μg ml⁻¹), when appropriate. *S. mutans* cultures were grown statically at 37 °C, while *E. coli* cultures were grown at 37 °C with shaking aeration. Aesculin plates, with and without supplemental glucose, were made as described previously (Cote et al., 2000). Arbutin assay plates were made in a similar manner with arbutin as the sole carbon source rather than aesculin. Unless otherwise noted, all chemicals and reagents were purchased from Sigma-Aldrich.

**DNA isolation, manipulation and PCR amplification.** Streptococcal DNA was isolated as described previously (Cote et al., 2000). Plasmid DNA mini-preparations were performed using the QiAprep 8 Miniprep kits or the QiAprep Spin Miniprep kits (Qiagen). Recombinant plasmid constructs were electroporated into *E. coli* as described by Dower (1990). *S. mutans* was transformed as described by Murchison et al. (1986) or Cvitkovich et al. (1998). All DNA manipulations and enzyme procedures were done using standard protocols (Sambrook et al., 1989) and according to the manufacturer’s instructions. All experiments were conducted under the National Institutes of Health recombinant DNA guidelines.

The *licT* gene was PCR-amplified from chromosomal DNA of *S. mutans* NG8 by primers designed using DNA sequence data obtained from the *S. mutans* genome database at the University of Oklahoma (http://www.genome.ou.edu/smutans.html). The primers were bglP1 (5’-GGCAAGCGGATACACAGTG-3’) and licT (5’-GATTAGCAAATGAAAGCAG-3’). The annealing temperature used for this primer pair was 45 °C. The polymerase used was KlenTaq-LA (Clontech) and was used according to the manufacturer’s recommendations for elongation times and temperatures.

**Aesculin transport and hydrolysis in *S. mutans*.** To measure the relative amount of aesculin transported and hydrolysed by the cell, the hydrolysis product of aesculin, 6,7-dihydroxycoumarin, was measured in the following manner. *S. mutans* cells were grown overnight as 10 ml cultures in CDM (van de Rijn & Kessler, 1980) supplemented with 0·5 % glucose and 0·5 % aesculin. The cells were collected by centrifugation and washed twice with TES buffer (10 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 8·0). The cells were resuspended in 250 ml TES buffer and transferred to a 1·5 ml screw-cap microfuge tube (Sarstedt). A small amount of 0·1 mm zirconia/silica beads (Biospec Products) was added to each tube (approx. 1/6 of the total tube volume). The cells were lysed by shaking the tubes in a Mini-BeadBeater-8 (Biospec Products) for 80 s. Cleared cell lysates were formed by centrifugation in a microcentrifuge for 10 min at 13 000 r.p.m. The protein concentration of the lysate was determined using the Coomassie Plus Protein Assay reagent (Pierce). Thirty micrograms of protein from the lysate was diluted with TES to a final volume of 100 μl, and 200 μl of 0·002 M ferric citrate was added to each sample. The samples were mixed briefly and added to wells of a clear, flat-bottomed microtitre plate. The absorbance of the samples was read on a Molecular Devices Max I microplate reader set at 595 nm. TES buffer served as the blank, while lysates of *S. mutans* cells grown in the absence of aesculin served as the negative control. In this assay, the breakdown product of aesculin, 6,7-dihydroxycoumarin, reacts with the ferric citrate to form a black product. The relative amount of product formed was assayed by the absorbance at 595 nm. The obtained optical density (OD) values were divided by the OD value of the lysate from wild-type *S. mutans* NG8 cells. The ODs were plotted as percentage of the wild-type and the data presented are a mean of at least three independent experiments.

**Construction of *lacZ* fusions.** The *lacZ* transcriptional fusions with the bglP, bglC and bglA promoter regions were created using the gene reporter vector pALH122 (Honeyman et al., 2002). This plasmid is a derivative of the shuttle vector pVA838 (Macrina et al., 1982) and contains the *lacZ* gene from *E. coli* for use as a reporter of transcriptional activity (Honeyman et al., 2002).

Construction of the plasmids pALH187, pALH188 and pALH189 has been reported previously (Cote & Honeyman, 2002). Briefly, a 490 bp Xhol–HindIII fragment, a 260 bp PCR fragment and a 233 bp PCR fragment were inserted into the unique *Sma*I site of pALH122, resulting in the reporter constructs pALH187, pALH188 and pALH189, respectively. These plasmids contained the promoter regions of the *bglP*, *bglC* and *bglA* genes, respectively. *S. mutans* strains NG8 and ALH201 containing these plasmids were used to monitor the transcriptional activity of the indicated genes.

**Construction of the *licT*::Ω-Kan2 mutant strain.** The PCR fragment containing the *licT* gene was isolated following amplification by agarose gel electrophoresis. A PCR fragment of approximately 2·15 kb was isolated from the agarose, digested with the restriction endonucleases HindIII and cloned into the HindIII site of the vector pACKS30, a derivative of pBluescript II (Alting-Mees & Short, 1989) that has a pACYC184 (Chang & Cohen, 1978) origin of replication (A. L. Honeyman, unpublished data). This resulted in the creation of pALH185. A *licT* gene interruption was created by inserting the Ω-Kan2 cassette (Perez-Casal et al., 1991) into the unique *Sty*I site located 293 bp 3’ to the *LicT* initiation codon within the cloned *licT* gene contained on pALH185. This resulted in the creation of pALH186. Plasmid pALH186 was then linearized by restriction endonuclease digestion with the enzyme Xhol and the linear restriction fragment transformed into *S. mutans*. Integration of the gene interruption into the *S. mutans* NG8 chromosome was selected for by kanamycin resistance. This resulted in the *S. mutans* strain ALH201. The interruption of the *licT* gene in this strain was confirmed by Southern blot analysis of chromosomal DNA isolated from the kanamycin-resistant transformant (data not shown). The described *lacZ* transcriptional fusions containing the various promoter regions from the *bgl* region were then transformed into this *licT*::Ω-Kan2 strain, ALH201, to generate the strains listed in Table 1.

**Construction of the *bglP*::Ω-Kan2 mutant strain.** To create a *S. mutans* strain that has an insertional mutation in the *bglP* gene, plasmid pALH163 (Cote et al., 2000) was altered. Plasmid pALH163, which contains the *S. mutans* bglPBCA gene, was digested with the restriction endonucleases ClaI and EcoRI to remove a HindIII site located in the multiple cloning region of the vector. The linearized plasmid was treated with the Klenow fragment to generate blunt ends and then re-ligated to itself. The resulting plasmid contains a unique HindIII site located within the cloned *bglP* gene. This plasmid was digested with HindIII and the 5’ overhanging ends were converted to blunt ends using the Klenow fragment. The Ω-Kan2 cassette (a *Sma*I restriction fragment) was ligated to the linearized plasmid (Perez-Casal et al., 1991). The insertion of the Ω-Kan2 cassette into the unique HindIII site located within the *bglP* gene resulted in plasmid pALH198. This plasmid was then linearized with the restriction endonuclease Xbal and the resulting DNA fragment was transformed into *S. mutans* NG8. Growth on kanamycin selected for the integration of the *bglP*::Ω-Kan2 gene interruption into the chromosome of *S. mutans* NG8 to generate strain ALH376.

**Fluorescent β-galactosidase assays.** *S. mutans* strains containing the *lacZ* transcriptional fusions were grown in CDM media (van de Rijn & Kessler, 1980) supplemented with glucose, aesculin or a combination of glucose and aesculin. Each carbohydrate was at a concentration of 0·5 %. The cells were physically lysed with a Mini-BeadBeater-8 and fluorescent β-galactosidase assays were performed.
as described previously (Cote & Honeyman, 2002). All data presented are the result of at least five independent assays and the standard deviations are indicated.

RESULTS AND DISCUSSION

Cloning of the licT gene

The gene encoding the putative antiterminator LicT was identified from DNA sequence data obtained from the University of Oklahoma S. mutans genome database. This ORF was located 5’ to the bgl regulon described previously (Cote et al., 2000) and is shown in Fig. 1. The LicT ORF is located at SMU.889 on the S. mutans chromosome (http://www.oralgen.lanl.gov/). PCR primers that flank two native chromosomal HindIII restriction endonuclease sites were designed using the genomic DNA sequence data and a fragment of 2-15 kb was amplified from chromosomal DNA of S. mutans NG8. The PCR fragment was isolated from an agarose gel following electrophoresis, digested with the restriction endonuclease HindIII, and cloned into the vector pACKS30 at the HindIII site. DNA nucleotide sequence analysis was performed on the cloned fragment to confirm its identity. The resulting construct, pALH185, contained a DNA fragment that contained 839 bp 5’ to the start of the licT gene, the entire coding region of the licT gene and 476 bp 3’ to licT. The gene encoding the putative antiterminator protein was named licT because of the similarity between the putative gene product and the LicT protein from B. subtilis (Schnetz et al., 1996; Yoshida et al., 1996). The putative LicT protein also displays extensive similarity with other antiterminator proteins (Table 2). The LicT ORF consists of 840 nt that would encode a putative protein of 280 aa in length. There is a putative ribosome-binding site, AAGGAA, that is seven bases upstream from the initiation codon (ATG). This sequence further downstream from the termination codon (TAA) of the licT gene and 127 bp upstream of the initiation codon for the bglP gene. This sequence was located 243 bp downstream from the termination codon (TAA) of the licT gene and 127 bp upstream of the initiation codon for the bglP gene (Fig. 1b). This putative RAT sequence, GGATTGTTACTGGTCATGCAGGCAAAACCTA, matches the proposed consensus sequence of RATs (Brown & Thomson, 1998). An alignment of several known and putative RAT sites is shown in Fig. 2, and a high degree of conservation is displayed between the sequences.

Table 2. Proteins with similarity to the S. mutans LicT protein

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Identity (%)</th>
<th>Similarity (%)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis LicT</td>
<td>Antiterminator</td>
<td>40</td>
<td>65</td>
<td>Schnetz et al. (1996); Yoshida et al. (1996)</td>
</tr>
<tr>
<td>Enterococcus faecium BglR</td>
<td>Antiterminator</td>
<td>37</td>
<td>63</td>
<td>Franz et al. (1999)</td>
</tr>
<tr>
<td>Erwinia chrysanthemi ArbG</td>
<td>Antiterminator</td>
<td>36</td>
<td>62</td>
<td>el Hassouni et al. (1992)</td>
</tr>
<tr>
<td>Clostridium longisporum</td>
<td>Antiterminator</td>
<td>37</td>
<td>61</td>
<td>Brown &amp; Thomson (1998)</td>
</tr>
<tr>
<td>Clostridium acetobutylicum ScrT</td>
<td>Antiterminator</td>
<td>34</td>
<td>60</td>
<td>Tangney &amp; Mitchell (2000)</td>
</tr>
<tr>
<td>Escherichia coli bglG</td>
<td>Antiterminator</td>
<td>32</td>
<td>59</td>
<td>Schnetz et al. (1987)</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Antiterminator</td>
<td>33</td>
<td>57</td>
<td>Brehm et al. (1999)</td>
</tr>
<tr>
<td>Bacillus stearothermophilus</td>
<td>Antiterminator</td>
<td>31</td>
<td>55</td>
<td>Li &amp; Ferenci (1996)</td>
</tr>
<tr>
<td>Bacillus subtilis SacY</td>
<td>Antiterminator</td>
<td>29</td>
<td>54</td>
<td>Zukowski et al. (1990)</td>
</tr>
<tr>
<td>Bacillus subtilis SacT</td>
<td>Antiterminator</td>
<td>28</td>
<td>54</td>
<td>Debarbouille et al. (1990)</td>
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<td>Lactococcus lactis BglR</td>
<td>Antiterminator</td>
<td>28</td>
<td>58</td>
<td>Bardowski et al. (1994)</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>Antiterminator</td>
<td>28</td>
<td>54</td>
<td>Marasco et al. (2000)</td>
</tr>
</tbody>
</table>

Phenotype of the S. mutans licT::Ω-Kan2 strain

The licT::Ω-Kan2 mutant strain, ALH201, was spotted onto aesculin plates with and without supplemental glucose to determine the phenotype of the licT mutation. While the
wild-type *S. mutans* strain NG8 could hydrolyse aesculin in the presence or absence of glucose, strain ALH201 could not hydrolyse aesculin in the presence of glucose (Fig. 3). It has previously been shown that the *bgl* regulon is not totally repressed by glucose and that a second β-glucoside-utilization system, which is repressed by glucose, exists in *S. mutans* (Cote et al., 2000). While the second system may be involved in the absence of glucose, the *bglP* regulon is responsible for the hydrolysis of aesculin in the presence of glucose and the phenotypic data presented here support the concept that the *licT* gene from this regulon is intimately involved with the utilization of β-glucosides.

**LicT activation of the cryptic *E. coli* β-glucoside operon**

*E. coli* contains all of the genes essential for the utilization of β-glucosides. However, these genes remain cryptic and are not expressed unless they are artificially activated by a mutation upstream of the *bglG* gene that enhances the transcription of the *bglG* gene (Reynolds et al., 1981). The BglG protein is an antiterminator that acts at a RAT site to control expression of the *bglGFB* operon. It was observed that the *E. coli* CC118 strain harbouring plasmid pALH185, which contains the *S. mutans licT* gene, could hydrolyse the β-glucosides arabinit and aesculin. However, neither the host strain nor the host strain harbouring the parental vector pACKS30 could efficiently break down either of these β-glucosides (data not shown). The breakdown of these β-glucosides by *E. coli* CC118 harbouring pALH185 is thought to be the result of the expression of the native *E. coli* *bglGFB* operon. It is hypothesized that the *licT* gene from *S. mutans* is expressed in *E. coli* from pALH185 and that LicT interacts with the *E. coli* RAT to mediate antitermination of the *bglGFB* operon, thus allowing expression of the PTS machinery necessary for *E. coli* to transport and utilize these β-glucosides. This potential interaction is plausible due to the high similarity between the various antiterminator proteins (Table 2) and the high conservation of the RAT sites between species (Fig. 2) (Brown & Thomson, 1998). This observation also lends support to the proposed role of LicT as an antiterminator that regulates the *bgl* regulon in *S. mutans*.

**Transport of aesculin into specific *S. mutans* strains**

The phenotype observed when *S. mutans* hydrolyses aesculin on an agar plate, a blackening of the agar, is the result of the interaction of the aesculin hydrolysis product, 6,7-dihydroxy coumarin, and the ferric citrate present in the agar medium. Experiments were performed to further characterize the transport of aesculin into the *S. mutans* cell. Cell lysates from *S. mutans* cultures grown in the presence of glucose and aesculin were able to change a ferric citrate solution from colourless or pale-orange to black, indicating the presence of hydrolysed aesculin within the cell lysate. The *bglP::Ω-Kan2* mutant strain ALH1736 and the *licT::Ω-Kan2* mutant strain ALH201 both exhibited significantly lower levels of intracellular hydrolysed aesculin (approx. 20–50% of wild-type) when compared to the levels observed in the wild-type *S. mutans* NG8 (data not shown). These results support the hypothesis that the *licT* and *bglP* gene products are involved with the transport of aesculin into the *S. mutans* cell in the presence of glucose. The existence of a second transport system for β-glucosides in *S. mutans* is also supported by the fact that aesculin transport is not totally eliminated in the mutant strains. However, it is apparent that the *licT* and *bglP* gene products are necessary for optimal levels of aesculin translocation into *S. mutans*.

**Effect of the LicT protein on the *bgl* regulon**

The *S. mutans* *licT::Ω-Kan2* strain, ALH201, was transformed with the reporter constructs containing the *lacZ* transcriptional fusions with the promoters from the *bglP*, *bglC* and *bglA* genes. The specific activity associated with each reporter construct, pALH187, pALH188 and pALH189, was determined by β-galactosidase assays following growth in various media. The specific activity of the *bglC* promoter region was not significantly influenced by the absence of LicT (Fig. 4) when compared to its expression level in the wild-type host. However, the transcriptional activity of the *bglP* promoter is significantly lowered in the absence of LicT regardless of the carbohydrate examined (Fig. 4). When glucose is present as the sole carbon source, a fourfold decrease in activity of the *bglP* promoter occurs. In the presence of both glucose and aesculin, the *bglP* promoter is approximately eight times less active in the absence of LicT. When aesculin is the only carbohydrate available, the *bglP* promoter is approximately fourfold less active in the absence of LicT. In all cases, LicT appears to act as a positive regulator of the *bglP* locus. It is our assumption that LicT acts as an antiterminator, which is a positive regulator of the *bglP* operon.
transcription. While this locus is somewhat insensitive to glucose repression, it is interesting to note that the largest decrease in bglP transcriptional activity is in the licT-negative strain in the presence of both glucose and aesculin. Interestingly, LicT apparently also regulates the expression of the bglA locus of S. mutans in a negative manner. As shown in Fig. 4, the expression of lacZ under the direction of the bglA promoter is significantly induced in the absence of LicT. This phenomenon is particularly evident when examining the transcriptional activity following growth of these strains in glucose alone. In the absence of LicT, the bglA promoter region is approximately 10 times more active than in the wild-type host strain. In the presence of glucose/aesculin or aesculin alone, the bglA gene is expressed at approximately three times the level of the wild-type strain. These results were totally unexpected. There is not a RAT site 5' to the bglA gene at which LicT would act. Thus, LicT would presumably regulate bglA by a mechanism other than antitermination. The molecular mechanism mediating the observed negative regulation is unknown at this time.

**Effect of the BglP protein on the bglA gene**

It was unclear if the increased transcriptional activity of the bglA promoter observed in the absence of LicT was due directly to the lack of LicT or due to a decrease in the level of BglP mediated by the licT::Ω-Kan2 mutation. The bglA::lacZ reporter construct (pALH189) was transformed into the bglP::Ω-Kan2 strain ALH376 to generate strain ALH378. The data generated from β-galactosidase assays performed on strain ALH378 following growth on various carbohydrate sources suggested that the absence of BglP does not induce the expression of the bglA promoter (Fig. 5) to above the wild-type expression level in a manner similar to the induction seen in the licT mutant strain. Thus, LicT must act as a negative regulator of the bglA locus. In the absence of BglP, the bglA gene is expressed less than in the wild-type host strain. However, it is uncertain if this slight reduction in transcriptional activity of the bglA promoter is due to the absence of BglP or due to the decreased levels of intracellular aesculin that are mediated by the bglP::Ω-Kan2 mutation. Regardless of the effect of BglP on bglA, these data suggest that LicT negatively regulates the expression of the bglA promoter while positively affecting the expression of the bglP promoter. It is unclear at this time if the effect of LicT on the bglA locus is direct or is mediated by another protein.

**Effect of different carbohydrates on the bgl regulon**

It is interesting that the bgl regulon of S. mutans is somewhat resistant to catabolite repression by glucose (not repressed to expression levels similar to those found following growth in glucose alone). To determine if the...
transcriptional activity of the \textit{bgl} regulon was different in the presence of other carbohydrates, \(\beta\)-galactosidase assays were performed on various \textit{S. mutans} strains containing the \textit{bglP::lacZ}, \textit{bglC::lacZ} and \textit{bglA::lacZ} reporter fusion constructs grown in the presence of different carbohydrates. The carbohydrates fructose, lactose, mannitol and sucrose all significantly repressed the transcription of \textit{bglP} to lower levels than following growth in glucose as the sole carbon source (data not shown). The \textit{bglP} promoter activity (pALH187) was approximately one-third to one-half as active in the presence of these carbohydrates as compared to its activity in the presence of glucose. There were no significant changes in the transcriptional activity of the \textit{bglC} (pALH188) and \textit{bglA} (pALH189) promoters when they were tested following growth on these carbohydrates (data not shown).

These observations can potentially be explained by examining the products obtained from the hydrolysis of \(\beta\)-glucosides, specifically aesculin. Hydrolysis of aesculin results in the formation of a glucose 6-phosphate molecule and a 6,7-dihydroxycoumarin molecule. It is hypothesized that if this glucose 6-phosphate, either intra- or extracellularly, or extracellular glucose, which is transported to form glucose 6-phosphate, tightly repressed or regulated the \textit{bglP} gene, the subsequent transport of additional aesculin would be inhibited. This would effectively block further utilization of an available substrate that is present in the environment. Both extra- and intracellular glucose 6-phosphate has been shown in \textit{E. coli} to induce catabolite repression (Hogema \textit{et al}., 1998a, b). We propose that a similar phenomenon occurs in \textit{S. mutans}. Therefore, \textit{bglP} must be somewhat insensitive to catabolite repression by the hydrolysis product of the \(\beta\)-glucoside substrate, glucose 6-phosphate, and must also be insensitive to repression by glucose, which generates glucose 6-phosphate following transport by the PTS.

This \(\beta\)-glucoside-specific region of the \textit{S. mutans} chromosome has been shown to contain several interesting features. These characteristics include a unique regulon structure and the fact that this \textit{bgl} regulon is not totally repressed to non-induced levels by the presence of glucose. This region also contains an additional transcriptional regulator encoded by the \textit{bglC} gene that partially controls the expression level of the \textit{bglA} gene (Cote & Honeyman, 2002). This type of regulatory element is not found in any other \(\beta\)-glucoside PTS described previously (Cote \textit{et al}., 2000).

This report adds transcriptional antitermination to the list of regulatory mechanisms that may govern \(\beta\)-glucoside utilization by \textit{S. mutans}. We have shown that LicT is essential for optimal expression of the \(\beta\)-glucoside-specific enzyme II of the PTS encoded by the \textit{bglP} gene. Based upon analogy with other \(\beta\)-glucoside PTSs, and our data, we believe that LicT positively regulates this locus at the level of transcription. It is proposed that LicT acts on the \textit{bglP} locus via an antitermination mechanism of transcriptional regulation which occurs at the RAT site located upstream of the \textit{bglP} gene. The binding of LicT to the RAT site would result in transcription through the putative terminator structure and allow the expression of the BglP protein, which translocates \(\beta\)-glucosides such as aesculin into the cell. The proposal of an antitermination mechanism to control the expression of \textit{bglP} is supported by the activation of a cryptic operon in \textit{E. coli} by the presence of the \textit{S. mutans licT} gene. Based upon the amino acid sequence similarity between the LicT protein of \textit{S. mutans} and the \textit{E. coli} BglG protein, and their corresponding RAT sites, we believe that the \textit{bglGFB} operon is induced in \textit{E. coli} by the expression of the \textit{S. mutans} gene.

LicT also regulates the transcriptional activity of the \textit{bglA} promoter in a negative manner. This is also a unique characteristic of this regulon. At this time it remains unclear why LicT would negatively regulate the expression of a gene that encodes the catabolic protein responsible for the breakdown of the translocated carbohydrate while positively regulating the gene encoding the protein responsible for transporting the carbohydrate. A possible explanation for this is that the second \(\beta\)-glucosidase enzyme present in \textit{S. mutans} also acts upon the substrates transported by BglP and that LicT does not regulate this locus. This dual regulatory role of LicT is very interesting and is currently under further study.

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\textbf{REFERENCES}


