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A galactose-specific sugar:phosphotransferase permease is prevalent in the non-core genome of *Streptococcus mutans*

Lin Zeng¹, Peng Xue^{1,#}, Michael J. Stanhope², and Robert A. Burne^{1,*}

¹Department of Oral Biology, College of Dentistry, University of Florida, Gainesville, Florida 32610

²Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA

SUMMARY

Three genes predicted to encode the A, B and C domains of a sugar:phosphotransferase system (PTS) permease specific for galactose (EII^{Gal}) were identified in the genomes of 35 of 57 recently-sequenced isolates of *Streptococcus mutans*, the primary etiological agent of human dental caries. Mutants defective in the EII^{Gal} complex were constructed in 6 of the isolates and showed markedly reduced growth rates on galactose-based medium relative to the parental strains. An EII^{Gal}-deficient strain constructed using the invasive serotype *f* strain OMZ175 (OMZ/IIGal) expressed significantly lower PTS activity when galactose was present as the substrate. Galactose was shown to be an effective inducer of catabolite repression in OMZ175, but not in the EII^{Gal}-deficient strain. In a mixed-species competition assay with galactose as the sole carbohydrate source, OMZ/IIGal was less effective than the parental strain at competing with the oral commensal bacterium *Streptococcus gordonii*, which has a high-affinity galactose transporter. Thus, a significant proportion of *S. mutans* strains encode a galactose PTS permease that could enhance the ability of these isolates to compete more effectively with commensal streptococci for galactose in salivary constituents and the diet.

Sugar

phosphotransferase system; galactose-PTS; tagatose pathway; biofilm; dental caries

INTRODUCTION

Galactose is one of the major constituents of the glycoconjugates produced by eukaryotes (Caldwell & Pigman, 1966), and is abundant in the glycoproteins and other secretions that bathe the surfaces of the oral cavity. When liberated via bacterial glycosidases or through other processes, galactose can be catabolized by many oral bacteria, so this carbohydrate helps to shape the composition and function of the oral microbiome in health and disease (Hojo *et al.*, 2009, Van der Hoeven & Camp, 1991). As the abundant streptococcal species that colonize the mouth and nasopharynx, including the viridans and mitis streptococci, as well as group A and B streptococci, rely on fermentation of carbohydrates for generation of

*Corresponding author: Mailing address: Department of Oral Biology, University of Florida, College of Dentistry, P.O. Box 100424, Gainesville, FL 32610. Phone: (352) 273-8850, Fax: (352) 273-8829, rburne@dental.ufl.edu.

#Present address: University of Pittsburgh School of Medicine, Pittsburgh, PA.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The nucleic acid and amino acid sequences of the EII^{Gal} locus in strain OMZ175.

Table S1. Prevalence of non-core genes in association with EII^{Gal} complex.

energy, the ability to efficiently uptake and metabolize galactose is believed to enhance the persistence and ability of these organisms to compete with other members of the microbial flora.

Our present knowledge about galactose utilization in the dental caries pathogen *Streptococcus mutans* comes primarily from the reference strain UA159 and indicates that this organism does not utilize galactose as efficiently as many other oral streptococci, in part due to the lack of a high affinity galactose transporter (Zeng *et al.*, 2010). Like many bacteria, *S. mutans* depends mainly on the PEP-energized sugar:phosphotransferase systems (PTS) to internalize and concomitantly phosphorylate a variety of carbohydrates that exist in the oral cavity. The PTS is usually composed of two general enzymes (Enzyme I and the phospho-carrier protein HPr) and a series of substrate-specific, membrane-associated Enzyme II (EII) permeases that are directly responsible for sugar transport (Postma *et al.*, 1993). *S. mutans* strain UA159 has been reported to harbor as many as 14 PTS permeases in its genome (Ajdic *et al.*, 2002). However, previous studies have indicated an overall low affinity in substrate acquisition and slower growth on galactose compared with PTS sugars, such as glucose or fructose (Abranches *et al.*, 2004, Ajdic & Ferretti, 1997, Ajdic *et al.*, 1996). Furthermore, a recent publication from our group has suggested that this deficiency is likely due to the absence of a high-affinity galactose-specific PTS permease (Zeng *et al.*, 2010), which is often present in related Gram-positive bacteria, including *S. pneumoniae* (Kaufman & Yother, 2007), *S. pyogenes* (Loughman & Caparon, 2007) and *S. gordonii* (Zeng *et al.*, 2012). Instead, the major glucose-PTS permease EII^{Man} and a lactose-PTS (EII^{Lac}) permease are the primary transporters for galactose in UA159, but they require relatively high concentrations of galactose to allow for optimal growth (Zeng *et al.*, 2010).

Utilizing next-generation high-throughput sequencing technology, an international collection of 57 clinical isolates of *S. mutans* has been subjected to whole genome shotgun sequencing (Cornejo *et al.*, 2012, Do *et al.*, 2010). This effort has resulted in the identification of a core genome of approximately 1490-ORFs for the species *S. mutans*. Also identified were over 1900 genes that are present in only a subset of the strains, highlighting considerable genomic heterogeneity in the *S. mutans* population. Among the non-core genes, we identified a PTS permease complex bearing homology to galactose-PTS permeases previously identified in some Gram-positive bacteria. In this report we initiated the characterization of this new PTS permease and began to explore whether it could potentially impact the ecology of the oral microbiome.

METHODS

Bacterial strains and culture conditions

Wild-type strains of *S. mutans*, including strains UA159 (serotype *c*) and OMZ175 serotype *f*, 10 clinical isolates (Table 1), and *Streptococcus gordonii* DL1 were routinely maintained on BHI (Difco Laboratories, Detroit, MI) agar plates and grown in BHI liquid medium. Antibiotic-resistant mutants were kept on BHI agar plates supplemented with erythromycin (Em) 10 µg/mL or kanamycin (Km) 1 mg/mL (Sigma-Aldrich, St. Louis, MO). Tryptone-vitamin (TV) base medium (Burne *et al.*, 1999) supplemented with various carbohydrates was used to test bacterial growth phenotypes and to grow cells for enzymatic assays. All bacterial cultures were incubated without agitation in a 5% CO₂ aerobic atmosphere at 37°C, unless specified otherwise.

DNA manipulation and construction of mutants

Ligation transformation—The deletion mutant of OMZ175 defective in the putative EII^{Gal} complex was constructed via ligation transformation procedure described previously

(Lau *et al.*, 2002). Primers specific for the regions flanking the EII^{Gal} complex were designed to include restriction enzyme sites that facilitated fusion with an antibiotic-resistance cassette: for the region 5' of EII^{Gal}, OMZ-gal-1 (5'-GGT GAT GAA GAA GTT AAC CAG CAA-3') and OMZ-gal-2 *Hind*III (5'-GCA ATC TCT TTG AAA GCT TCT TCT TTT TCT T-3'); and for region 3' of EII^{Gal}, OMZ-gal-3 *Eco*RI (5'-TGC TGT GAA TTC ACT TGC TAT TTA TAC TCT-3') and OMZ-gal-4 (5'-GTT CCC ATA AAG GCA CTC AAG AAA-3'). The PCR products were treated with the respective restriction enzymes and ligated with an erythromycin (*em*) cassette released from plasmid NPEM1 (Zeng & Burne, 2009) via digestion using *Hind*III and *Eco*RI, and subsequently used to transform the wild-type strain OMZ175. The transformation was carried out in BHI medium in the presence of 10% horse serum and 100 nM competence-stimulating peptide (Petersen & Scheie, 2010). Em-resistant transformants were further confirmed by PCR and sequencing, including sequencing the flanking regions to ensure no unwanted mutations were introduced.

Deletion of the EII^{Gal} complex in 5 additional isolates of *S. mutans*—Five clinical isolates of *S. mutans* (3SN1, U2A, 11SSST2, SM6, ST1) (Cornejo *et al.*, 2012) were incubated for 3 h with 1 µg of chromosomal DNA extracted from strain OMZ/IIGal, and then plated on BHI agar plates containing Em. Em-resistant transformants were subsequently confirmed by PCR reactions using primers that target the *lacD-lacF* region of the genome.

Growth tests

Growth phenotypes of the wild-type strain UA159, OMZ175 and its isogenic EII^{Gal} mutant OMZ/IIGal were studied at 37°C in a 5%CO₂ aerobic environment in TV base medium supplemented with various carbohydrates. The optical density (OD₆₀₀) of the cultures was monitored hourly using a spectrophotometer. Conversely, a Bioscreen C automated growth monitor (Oy Growth CurvesAb, Ltd., Helsinki, Finland) was employed to assess the growth characteristics of 10 clinical isolates and their isogenic EII^{Gal} mutants (when applicable) in TV-galactose medium at 37°C. When the Bioscreen C was employed, 40 µL of sterile mineral oil was overlaid on top of the cultures to minimize the inhibitory impact of air on the growth of *S. mutans* (Ahn *et al.*, 2007).

Mixed-species liquid culture competition assay

Following a protocol employed previously (Zeng *et al.*, 2012), *S. mutans* and *S. gordonii* strains were cultured in BHI medium to mid-exponential phase, mixed in a 1:1 ratio, and diluted 100-fold into TV medium supplemented with 0.5% galactose. *S. mutans* strains each contained a kanamycin-resistance marker to facilitate enumeration. The CFU of each bacterial strain was immediately measured by serial dilution and plating on both BHI and BHI-Km plates. The mixed cultures were then incubated at 37°C in a 5%CO₂ atmosphere for 24 h, before the pH was measured and CFU of each bacterial species were again determined by plating and incubation for 48 h. CFU from BHI plates represented the total of the two bacterial species, while CFU on BHI-Km plates represented that of *S. mutans* alone. Data were derived from three biological replicates.

Enzymatic assays

CAT (chloramphenicol acetyltransferase) (Shaw, 1975) and PEP (phosphoenolpyruvate)-dependent PTS sugar transport assays (LeBlanc *et al.*, 1979) were carried out according to previously published protocols (Zeng *et al.*, 2006).

RESULTS AND DISCUSSION

Presence of genes for the EII^{Gal} complex in clinical isolates of *S. mutans*

From a total of 57 strains sequenced (Cornejo et al., 2012), the coding sequences of a putative galactose-PTS EII complex, with open reading frames for EIIGal, EIIBGal and EIICGal, were identified in the genomes of 35 isolates, but not in the completely-sequenced reference strain UA159. Using the same query protein sequences, a BLAST search (<http://blast.ncbi.nlm.nih.gov/>) identified only one *S. mutans* strain (JL23), out of the 4 strains for which complete genome sequence information is available, that contained the same EII^{Gal} complex. Searches using nucleic acid sequences resulted in no additional hits in *S. mutans* (data not shown). Among these EII^{Gal}-positive strains was OMZ175, a Bratthall serotype *f* strain that was chosen for further study on the basis of our previous demonstration that it is capable of invading human coronary artery endothelial cells (HCAEC) (Abranches et al., 2009) and has markedly enhanced virulence in the greater waxworm model than *S. mutans* UA159 (Abranches et al., 2011). The sequences of the genes and proteins from OMZ175 are presented in the Supporting Information and the sequences can be accessed at <http://strep-genome.bscb.cornell.edu/cgi-bin/hgBlat> using the *S. mutans* pull-down menu under "Genomes". Further, the genome sequences of all isolates examined in this study have been deposited with GenBank (Cornejo et al., 2012).

As depicted in Figure 1, the EII^{Gal} complex in OMZ175 is organized as part of the *lac* operon, which also includes the tagatose-6-phosphate pathway genes (*lacABCD*), a lactose-PTS EII complex (*lacFE*) and a phospho- β -galactosidase (*lacG*) (Rosey & Stewart, 1992, Zeng et al., 2010). Similarly organized *lac* operons have been detected in all EII^{Gal}-positive strains analyzed thus far (data not shown). When the EII^{Gal} gene products were aligned against known galactose-PTS EII genes of *S. pneumoniae* (Kaufman & Yother, 2007), significant similarity in primary sequences was evident: EIIGal 60%, EIIBGal 82%, and EIICGal 75%. Comparable levels of similarity were also found between these open reading frames and their apparent homologues in *S. gordonii*, *Streptococcus bovis*, *Streptococcus gallolyticus*, *Enterococcus faecalis*, *Streptococcus mitis*, *Streptococcus agalactiae*, *S. pyogenes* and other Gram-positive bacteria (data not shown). Further comparison indicated largely identical primary sequences in the rest of the operon between the genomes of UA159 and OMZ175, with only LacR, LacD and LacG containing a single amino acid replacement in each protein.

The genetic divergence between these two groups of *S. mutans* strains may be the result of gene acquisition by horizontal transfer or from loss of the EII^{Gal} genes. More importantly, the possession or the lack of a high-affinity galactose transport system could represent a fundamental difference within the species *S. mutans* related to the ability of isolates to adapt to particular niches in the oral cavity. For example, strains with the high-affinity galactose porter may have retained the capacity to better compete with commensal streptococci that generally have the same system, perhaps at the expense of caries-associated traits. In fact, a preliminary analysis of the distribution patterns of 1900 non-core genes within the 57 isolates suggests that there may be a small subset of non-core genes that show positive or negative correlations with the presence of the EII^{Gal} locus (see Table S1). While we are currently exploring the significance of these associations, here we undertook experimentation to begin to characterize the function of the putative permease and it could affect traits of *S. mutans* that could be related to competitive fitness or virulence.

Contribution of EII^{Gal} to utilization of galactose by *S. mutans*

To investigate the function of the putative galactose-PTS permease, a deletion mutant was constructed using OMZ175 as the parental strain by replacing the entire coding region of

EIIABC^{Gal} with a nonpolar erythromycin (*em*)-resistance marker (Zeng & Burne, 2009). Growth of the mutant strain, OMZ/IIGal, in TV-base medium supplemented with 0.5% of glucose, fructose, galactose or lactose was compared with the parental strain OMZ175 and the standard lab strain UA159. When compared to the wild-type strain OMZ175 growing on galactose, OMZ/IIGal displayed a dramatically slower growth rate, with a minimum doubling time of 253 min versus 117 min for OMZ175 (Table 2). The mutant also achieved a lower OD₆₀₀ after 24h of incubation. However OMZ/IIGal maintained largely the same growth phenotypes as that of the parental strain when tested on TV medium containing 0.5% of glucose, fructose or lactose (Table 2). Notably, the growth rate of strain OMZ175 on 0.5% galactose was moderately faster than that of *S. mutans* UA159 (135 min), which lacks the EII^{Gal} complex. Furthermore, when the pH of stationary phase cultures grown in TV with 0.5% galactose was measured, OMZ175 produced a final pH of 4.79 ± 0.06 , while OMZ/IIGal had a pH of 5.25 ± 0.06 . These data add further support to the notion that the EII^{Gal} complex contributes to improved assimilation of galactose by OMZ175.

To further characterize the contribution of the EII^{Gal} complex and other galactose-transporting EII permeases to growth on galactose, the same manual growth tests were repeated using TV containing 2% galactose. As shown in Table 2, both OMZ175 and OMZ/IIGal displayed more rapid growth on 2% galactose compared to 0.5% galactose, although OMZ/IIGal still grew significantly slower than the parental strain. Interestingly, out of three strains tested, UA159 grew the fastest on 2% galactose. These findings are consistent with our previous reports demonstrating the presence of lower-affinity galactose transporters in *S. mutans* (Zeng et al., 2010), including EII^{Man} and EII^{Lac}, which are also present in the genome of OMZ175.

In order to rule out the possibility that the contribution of the EII^{Gal} complex to galactose utilization by OMZ175 was an isolated phenomenon, additional EII^{Gal} mutants were constructed in 5 clinical isolates of *S. mutans*. These strains were selected based on i) the presence of a homologous EII^{Gal} cluster in the genome; ii) their ability to become competent for genetic transformation; and iii) that they were representatives of serotypes *c* and *e* (serotype *c*, 11SSST2, SM6, ST1; and *e* 3SN1, U2A). Chromosomal DNA was extracted from strain OMZ/IIGal and used to transform these isolates, the mutation was verified by PCR and sequencing, and the resultant mutants were compared to their parental strains for their ability to grow on galactose. Five additional isolates that lack a homologous EII^{Gal} locus (serotype *c*, NFSM2, T4, 15JP3; and serotype *e*, 11VS1, NLML4) were also included in this test. Due to the large number of samples to be tested, a Bioscreen C monitor was used to automate the process. Cells were incubated at 37°C in an aerobic environment, but with mineral oil overlaid to mitigate the inhibitory effects of oxygen on *S. mutans* growth (Ahn et al., 2007). As shown in Table 3, various degrees of reduction in growth rate were evident in all 5 mutants lacking the EII^{Gal} complex when growing in TV-galactose. Based on these observations, this novel galactose-PTS could be contributing in significant ways to galactose utilization by a major proportion of the *S. mutans* population colonizing humans.

When tested in 2% of galactose, all the isolates demonstrated improved growth, as compared to that displayed in 0.5% galactose; this was also true in the 5 EII^{Gal} mutants. At the same time, strains that contained the EII^{Gal} complex showed a somewhat modest improvement in growth rate on 2% of galactose, as opposed to strains without the galactose permease. One exception was strain U2A, which had markedly faster growth on 2% galactose, but showed the smallest reduction of growth rate when its EII^{Gal} genes were deleted. Importantly, though, the presence of EII^{Gal} consistently improved growth of strains in lower concentrations of galactose, but when galactose was present in high concentrations, arguably higher than might be encountered in the oral cavity, EII^{Gal} became less important, likely due to the activity of lower-affinity, secondary systems that can internalize galactose (Zeng et

al., 2010). Thus, EII^{Gal} could improve the competitive fitness of *S. mutans* strains *in vivo*, where free galactose would be present in low steady-state levels as it is liberated from glycoconjugates or when it is presented transiently as part of the human diet.

Transport of galactose by EII^{Gal} + isolates

To better assess the role of EII^{Gal} complex in galactose metabolism, PTS assays were employed to compare the rate of galactose transport by both the wild-type strain OMZ175 and its isogenic mutant OMZ/IIGal. Bacterial strains were cultured to the exponential phase in TV medium supplemented with 0.5% galactose before the cells were washed in phosphate buffer, permeabilized with toluene:acetone (1:9) and subjected to PEP-dependent sugar phosphorylation assay. The results (Fig. 2) showed significantly lower PTS activities in the EII^{Gal}-deficient mutant when compared to that of the wild-type strain grown under the same conditions. Given the limited supply of galactose in oral cavity under normal circumstances (Caldwell & Pigman, 1966), a high-affinity EII^{Gal} complex could significantly improve the ability of its host to compete for this carbohydrate against oral commensal bacteria.

Role of EII^{Gal} in competition against *S. gordonii*DL1

To begin to explore the potential role of the EII^{Gal} permease in microbial ecology in the human oral cavity, a liquid culture, mixed-species competition assay was performed using either OMZ175 or OMZ/IIGal competing against *S. gordonii* strain DL1. We previously showed that *S. gordonii* DL-1, which has a high-affinity galactose PTS (Zeng et al., 2012), had a strong competitive advantage over UA159 when galactose was the growth carbohydrate. To facilitate CFU enumeration, both *S. mutans* strains were engineered to contain a kanamycin-resistance marker in the coding sequence of the *gtfA* gene, which is involved only in sucrose metabolism (Russell *et al.*, 1988) and does not affect the competitive fitness of *S. mutans* in an animal model (Yamashita *et al.*, 1993). Both *S. mutans* and *S. gordonii* strains were cultured in BHI medium to mid-exponential phase before being mixed in 1:1 ratios and diluted 1:100 into fresh TV medium supplemented with 0.5% galactose. The mixed cultures were incubated statically for 24 h, then the pH was measured and CFU for both bacterial species were enumerated by plating. After 24 h of co-culturing, the wild type strain OMZ175 made up 24.5% ($\pm 3\%$) of the viable counts of the mixed culture, whereas strain OMZ/IIGal accounted for only 11% ($\pm 2\%$) of the total bacterial population. Measurement of the mixed-species cultures indicated that the OMZ175:DL1 co-cultures had significantly lower pH (4.82 ± 0.02) than those of the OMZ/IIGal:DL1 co-cultures (4.91 ± 0.02). These results indicated that the presence of the EII^{Gal} complex enables *S. mutans* to better compete against an antagonistic commensal species not only by allowing more efficient utilization of galactose, but perhaps by helping the organism to lower the environmental pH to a greater extent, which would be beneficial to the survival of an aciduric bacterium like *S. mutans*.

Ability of galactose to induce CCR in the EII^{Gal} + background

Bacteria have the capacity to repress expression of secondary catabolic genes/pathways when presented with preferred metabolizable sugars through a process known as carbon catabolite repression (CCR). In Gram-positive bacteria, this regulation generally requires the activity of the catabolite control protein CcpA and its cognate binding partner HPr-Ser46-PO₄ (Deutscher, 2008). It has long been observed that galactose is not very effective at eliciting CCR in *S. mutans* UA159. This is partly due to the fact that *S. mutans* metabolizes galactose mainly through the tagatose-6-phosphate pathway (Zeng et al., 2010, de Vos & Vaughan, 1994), which bypasses the production of glucose-6-phosphate and fructose-1,6-bisphosphate that help to trigger the production of HPr-Ser46-PO₄ (Deutscher, 2008). It was demonstrated in our previous studies on regulation of the fructan hydrolase gene *fruA*, which harbors two CcpA-binding catabolite response element (*cre*) in its promoter region,

that CCR was much less efficient in cells growing on galactose compared with cells growing on glucose or fructose (Abranches *et al.*, 2008). Importantly, in *S. mutans*, CCR initiated via CcpA-independent mechanisms exerted by PTS permeases and HPr appear to play dominant roles in controlling carbon metabolism in this bacterium, as opposed to other low-GC Gram-positive bacteria (Zeng & Burne, 2010).

To ascertain whether the presence of the EII^{Gal} permease could affect the response of catabolite repressible operons to the presence of galactose, we examined expression of the CcpA-independent *levD* gene using a *levD-cat* gene fusion (Zeng *et al.*, 2006) integrated in single copy into the chromosome of strain UA159, OMZ175 and OMZ/IIGal via double cross-over recombination. Each strain was grown to exponential phase in TV medium supplemented with galactose, the inducing polysaccharide inulin, or a combination of both inulin and galactose, before being harvested for CAT assays to assess the *levD* promoter activity. When tested in strain UA159, the results (Fig. 3) indicated that the addition of galactose lowered the expression of the *levD* promoter from 2021 ± 98 units when growing in inulin alone to 418 ± 31 units when growing on a combination of galactose and inulin. For comparison, we noted that the ratio of CAT activity on inducer versus inducer + galactose as a CCR index of 4.8. In contrast, when the promoter fusion was tested in the background of strain OMZ175, the CCR index was 75, so galactose was much more effective at triggering CCR in the EII^{Gal}-positive OMZ175 compared with UA159. However, when the same comparison was done in strain OMZ/IIGal, the CCR index for the promoter fusion was a mere 1.7, indicating significant alleviation of CCR. Also of note was the elevated expression of this promoter in strain OMZ/IIGal when growing in TV-galactose or TV-inulin, as compared to OMZ175 growing in the same conditions. Therefore, it appears that the EII^{Gal} complex in strain OMZ175 can contribute to CcpA-independent CCR when galactose is present, indicating that galactose is a preferred carbohydrate in OMZ175, unlike in strain UA159. These new findings add to the support for the notion that CCR in *S. mutans* is mediated directly through multiple components of the PTS, bypassing the requirement of CcpA and certain metabolic intermediates that are essential for CCR in other low G+C Gram-positive bacteria.

CONCLUDING REMARKS

A galactose-PTS was identified in more than half of a collection of 57 newly-sequenced *S. mutans* clinical isolates and its functionality was confirmed by growth and sugar transport assays. Although it is clear that the presence of this EII^{Gal} gene cluster affords its hosts an enhanced capacity for assimilating galactose, especially when the carbohydrate is present in lower concentrations, it remains to be determined if such a trait correlates with altered cariogenicity, or better colonization, persistence or competition with commensals; or perhaps even enhanced survival or virulence when the organism enters the bloodstream. Nevertheless, since galactose is known to be an important carbohydrate utilized as an energy source and structural component of many oral bacterial species, the ability to efficiently uptake this hexose by a well-characterized caries agent could have important implication in our understanding of the microbial ecology that affects the balance between healthy and a cariogenic oral biofilms. It will also be of interest to determine whether the possession of particular non-core genes that are positively or negatively associated with the presence of EII^{Gal} influences the physiology or virulence of *S. mutans* isolates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Diagrams depicting the tagatose-6-phosphate pathway clusters in *S. mutans* strains UA159 and OMZ175. A three-gene cluster encoding a putative galactose PTS Enzyme II, *EIIA^{Gal}*, *EIIB^{Gal}* and *EIIC^{Gal}* exists in strain OMZ175, but not UA159. A single amino acid replacement was identified in each of the LacR, LacD and LacG primary sequences between OMZ175 and UA159. Otherwise 100% sequence identity was observed among all homologous open reading frames. Other *lac* clusters similar to that in OMZ175 have been detected in all other *EII^{Gal}*-containing isolates analyzed so far (data not shown).

Fig. 2.

Results of the PEP-dependent galactose-phosphorylation assay. Strains OMZ175 and OMZ/IIGal were grown to exponential phase in TV with 0.5% galactose, washed with phosphate buffer, permeabilized with toluene:acetone, and then subjected to PTS assays as described previously (Zeng et al., 2006). The data [where units are nmol of NADH oxidized (mg of protein)⁻¹ min⁻¹] were the averaged results of three independent biological replicates each assayed in triplicate. The error bars represent standard deviations. The asterisk represents a *P* value of less than 0.005 as determined by the Student *t*-test.

Fig. 3. Results of the CAT assays representing the expression of a *P_{levD}-cat* fusion. The reporter fusion was tested in the background of OMZ175, OMZ/IIGal and UA159, while growing exponentially in TV media supplemented with 0.5% of galactose, inulin or a combination of both carbohydrates. Specific CAT activities are expressed as nmol of chloramphenicol acetylated (mg of protein)⁻¹ min⁻¹. The error bars represent standard deviations calculated using three independent biological replicates.

Table 1

Bacterial strains used in this study.

Strains	Serotype	Source or reference
<i>Streptococcus mutans</i>		
UA159	<i>c</i>	ATCC 700610
OMZ175	<i>f</i>	(Scholler <i>et al.</i> , 1981)
OMZ/IIGal	<i>f</i>	This study
11SSST2	<i>c</i>	(Cornejo <i>et al.</i> , 2012)
11SSST2/IIGal	<i>c</i>	This study
SM6	<i>c</i>	(Do <i>et al.</i> , 2010)
SM6/IIGal	<i>c</i>	This study
ST1	<i>c</i>	(Do <i>et al.</i> , 2010)
ST1/IIGal	<i>c</i>	This study
3SN1	<i>e</i>	(Cornejo <i>et al.</i> , 2012)
3SN1/IIGal	<i>e</i>	This study
U2A	<i>e</i>	(Do <i>et al.</i> , 2010)
U2A/IIGal	<i>e</i>	This study
15JP3	<i>c</i>	(Cornejo <i>et al.</i> , 2012)
11VS1	<i>e</i>	(Cornejo <i>et al.</i> , 2012)
NFSM2	<i>c</i>	(Cornejo <i>et al.</i> , 2012)
T4	<i>c</i>	(Do <i>et al.</i> , 2010)
NLML4	<i>e</i>	(Cornejo <i>et al.</i> , 2012)
<i>Streptococcus gordonii</i>		
DL1		(LeBlanc & Hassell, 1976)

Doubling time (T_d in min) and final optical density (OD_{600}) of strains OMZ175, OMZ/IIGal and UA159 growing in various carbohydrate conditions.

Table 2

TV with	OMZ175		OMZ/IIGal		UA159	
	T_d	OD_{600}	T_d	OD_{600}	T_d	OD_{600}
0.5% Glucose	80.7 ± 3.7	0.84	77.3 ± 5.2	0.85	ND	ND
0.5% Fructose	71.5 ± 2.8	0.78	74.9 ± 1.2	0.82	ND	ND
0.5% Galactose	117.1 ± 16	0.83	253.3 ± 19	0.68	135.3 ± 11	0.93
2% Galactose	97.1 ± 14	0.80	106.6 ± 15	0.73	83.5 ± 2.4	0.91
0.5% Lactose	67.2 ± 0.1	0.82	69.7 ± 3.1	0.81	ND	ND

Results (average $\pm$ standard deviation) are based on manual growth curve analyses using TV media containing various carbohydrates, in the presence of 5% of CO₂. ND, not determined.

Table 3

Doubling time ($T_d \pm$ standard deviation in min) of various clinical isolates, and their EII^{Gal} deletion mutants if applicable, obtained using a Bioscreen monitor in TV with 0.5% or 2% of galactose.

Strain	Serotype	T_d (0.5% galactose)	T_d (2% galactose)
U2A	<i>e</i>	272.3 ± 16	167.6 ± 12
U2A/IIGal	<i>e</i>	305.5 ± 3.1	209.5 ± 7.3
3SN1	<i>e</i>	189.3 ± 22	129.3 ± 4.5
3SN1/IIGal	<i>e</i>	403.3 ± 40	212.5 ± 5.3
11SSST2	<i>c</i>	172.5 ± 2.3	144.8 ± 9.5
11SSST2/IIGal	<i>c</i>	312.5 ± 40	175.7 ± 16
SM6	<i>c</i>	202.5 ± 20	127.1 ± 4.4
SM6/IIGal	<i>c</i>	250.5 ± 40	206.1 ± 36
ST1	<i>c</i>	172.0 ± 0.3	156.6 ± 9.3
ST1/IIGal	<i>c</i>	256.0 ± 2.1	172.5 ± 4.1
15JP3	<i>c</i>	331.1 ± 17	181.4 ± 7.2
11VS1	<i>e</i>	228.1 ± 6.3	153.7 ± 6.1
NFSM2	<i>c</i>	348.0 ± 59	202.3 ± 9.8
T4	<i>c</i>	239.1 ± 11	147.2 ± 7.6
NLML4	<i>e</i>	266.4 ± 4.7	151.7 ± 6.4