SUPPLEMENTAL MATERIAL

Table S1 Strains used in this study

Strain (Published name)	Description*	Source or reference
UA159	Reference strain	University of Alabama at Birmingham
manLMN	*manLMN*::Em^r	(1)
fruI fruCD (TW20)	*fruI fruCD* (Em^r Tet^r)	(2)
fruI fruCD levD	TW20 w/ *levD*::Sp^r	(3)
gtfBC (MMZ945)	*gtfBC*::Tet^r	(4)
gtfBC scrA (MMZ1029)	MMZ945 w/ *scrA*::Em^r	This study
levD (TW143)	*levD*::Em^r	(5)
malT (MMC1)	*malT*::Em^r	(6)
ccpA (TW1Em)	TW1 using an Em^r marker	(7);(8)
comS (SAB310)	*comS*::Em^r	(9)
UA159 P$_{comX}$-*lacZ*	Km^r	(10)
gtfBC P$_{comX}$-*lacZ*	MMZ945 harboring P$_{comX}$-*lacZ* (Tet^r Km^r)	This study
fruI fruCD levD P$_{comX}$-*lacZ*	TW20 w/ *levD*::Sp^r harboring P$_{comX}$-*lacZ* (Em^r Tet^r Sp^r Km^r)	This study
fruI fruCD P$_{comX}$-*lacZ*	TW 20 harboring P$_{comX}$-*lacZ* (Em^r Tet^r Km^r)	This study
levD P$_{comX}$-*lacZ*	TW143 harboring P$_{comX}$-*lacZ* (Em^r Km^r)	This study
treB P$_{comX}$-*lacZ*	*treB*::Sp^r harboring P$_{comX}$-*lacZ* (Sp^r Km^r)	This study
malT P$_{comX}$-*lacZ*	MMC1 harboring P$_{comX}$-*lacZ* (Em^r Km^r)	This study
gtfBC scrA P$_{comX}$-*lacZ*	MMZ1029 harboring P$_{comX}$-*lacZ* (Tet^r Em^r Km^r)	This study
manLMN P$_{comX}$-*lacZ*	*manLMN* harboring P$_{comX}$-*lacZ* (Em^r Km^r)	This study
ccpA P$_{comX}$-*lacZ*	TW1Em harboring P$_{comX}$-*lacZ* (Em^r Km^r)	This study
UA159 P$_{comS}$-*lacZ* (SQ01)	Km^r	(9)
UA159 P$_{comYA}$-*lacZ*	Km^r	This study
UA159 P$_{cipB}$-*lacZ*	Km^r	(11)
UA159 P$_{comX}$-*gfp* (SJ380)	UA159 harboring pDL278-P$_{comX}$-*gfp* (Km^r)	(10)
UA159 P$_{comS}$-*gfp*	UA159 harboring pDL278-P$_{comS}$-*gfp* (Km^r)	This study
UA159 P$_{comR}$-*gfp*	UA159 harboring pDL278-P$_{comR}$-*gfp* (Km^r)	This study

comS P_{comS}-*gfp*	SAB310 harboring pDL278-P_{comS}-*gfp* (Em^r Km^r)	This study

*Antibiotic resistance of strains indicated by superscript r. Em^r, erythromycin; Tet^r, tetracycline; Sp^r, spectinomycin; Km^r, kanamycin.

FIGURE S1. Effect of growth in BHI supplemented with various carbohydrates on *comX* activation. *S. mutans* UA159 containing P_{comX}-*lacZ* was grown in BHI media or BHI supplemented with 20 mM glucose (glc), 20 mM fructose (fru), 10 mM maltose, or 10 mM trehalose (tre). When cultures reached an OD_{600} of 0.1, 1 μM CSP was added, or cells were left untreated. Cultures were grown an additional two hours, and LacZ assays were performed to determine *comX* expression. The data represent the mean of three independent replicates with error bars indicating the standard deviation. ns, not significant (by the Student *t* test).

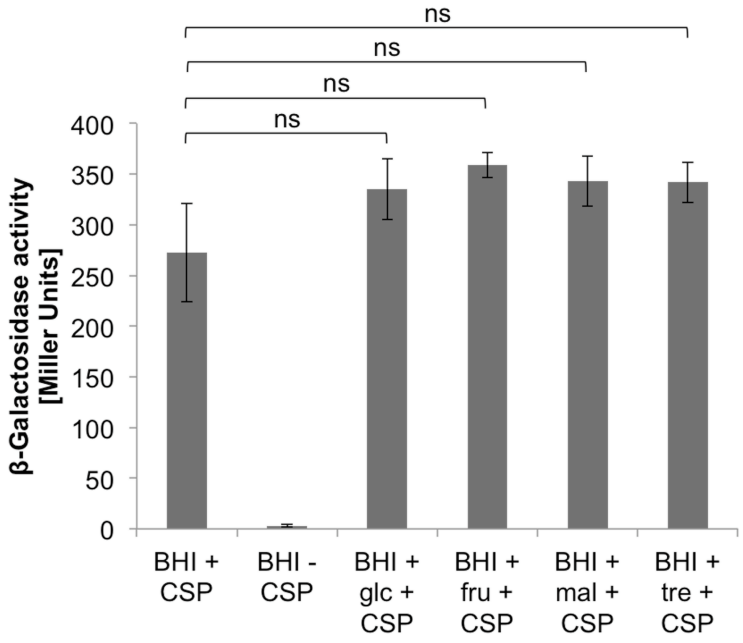


FIGURE S2. Activation of *comX* and growth of *S. mutans* in TVB and TV media. (A) *S. mutans* UA159 containing P_{comX}-*lacZ* was grown in TVB or TV base media supplemented with 20 mM glucose (glc). When cultures reached an OD_{600} of 0.1, 1 μM CSP was added, or cells were left untreated. After two additional hours of growth, *comX* expression was determined by performing a LacZ assay. The data represent the mean of three independent replicates with error bars indicating the standard deviation. (B and C) *S. mutans* UA159 was grown in TVB or TV with or without the addition of 20 mM glucose (glc). *, $P < 0.05$ (by the Student *t* test).

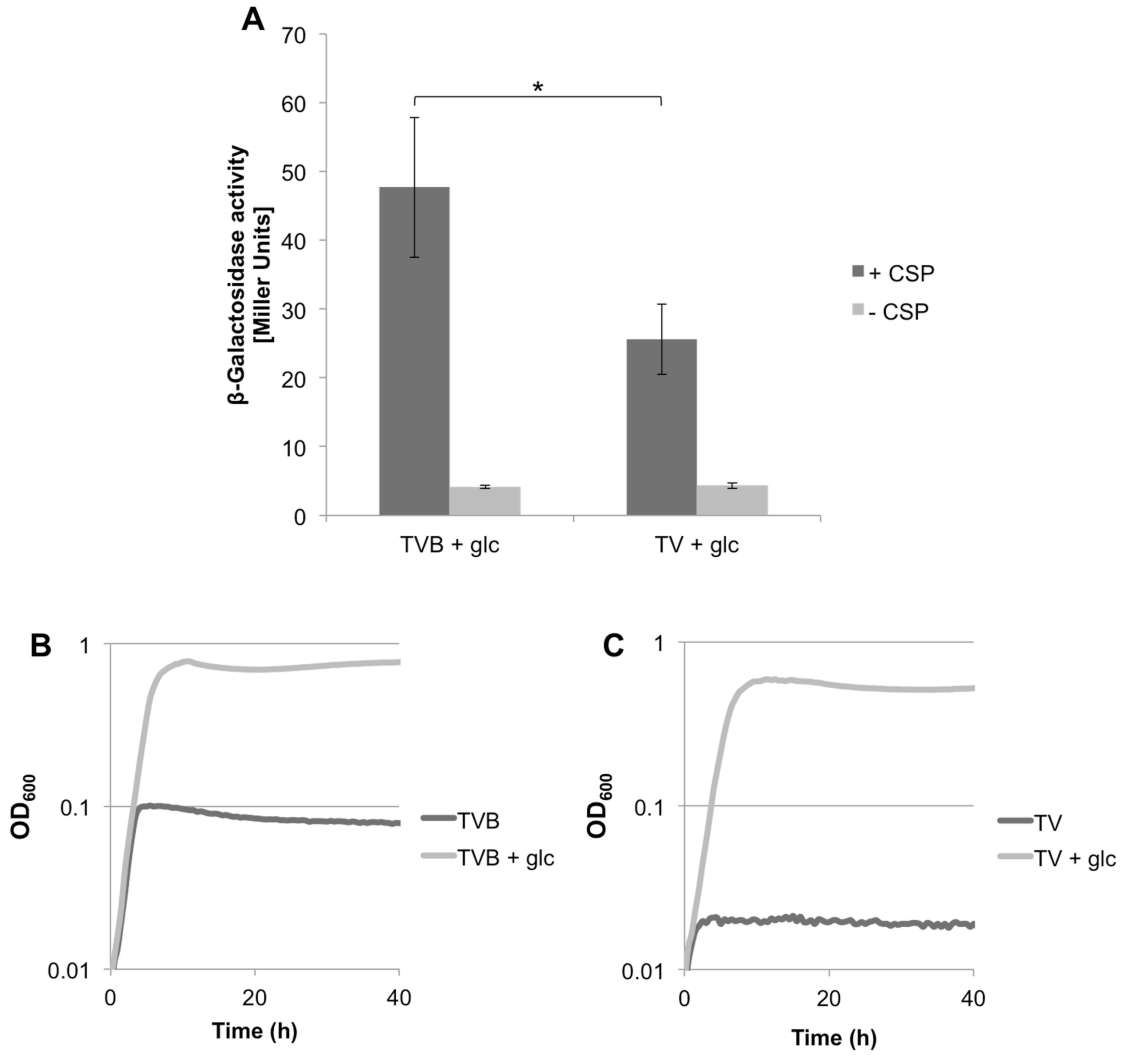
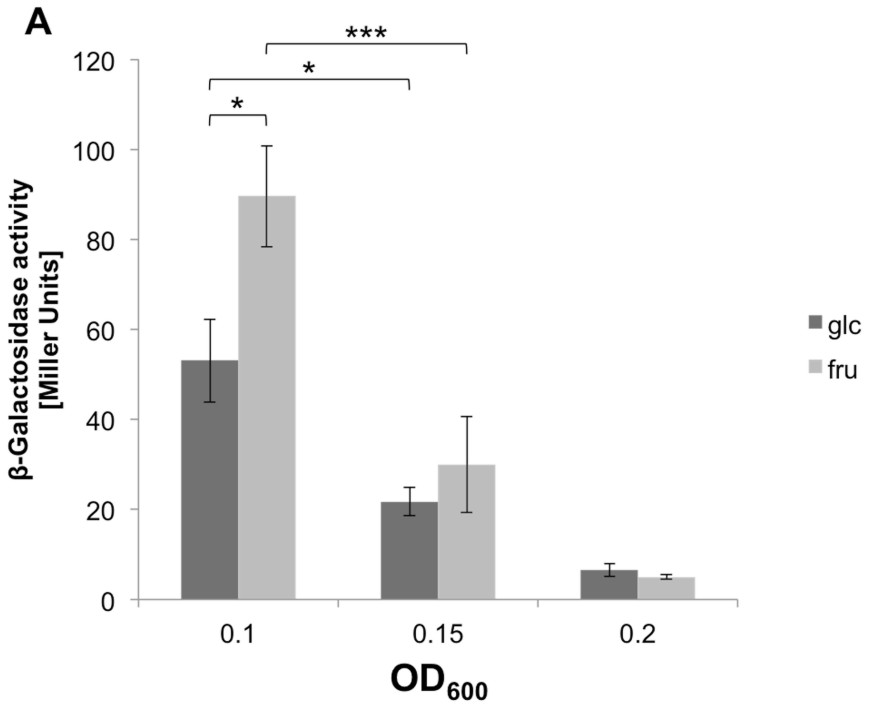


FIGURE S3. Growth phase-dependent activation of *comX* by CSP in various carbohydrates. (A) *S. mutans* UA159 containing P_{comX}-*lacZ* was grown in TVB supplemented with 20 mM glucose (glc) or 20 mM fructose (fru) to an OD_{600} of 0.1, 0.15, or 0.2 and 1 μM CSP was added. Cultures were grown an additional 2 hours, and LacZ assays were performed to measure *comX* promoter activity. (B) A *gtfBC* deletion mutant containing P_{comX}-*lacZ* was grown in TVB supplemented with 20 mM glucose (glc) or 10 mM sucrose (suc) to an OD_{600} of 0.1, 0.2, or 0.4 and treated with 1 μM CSP. After two additional hours of incubation, *comX* expression was monitored by LacZ assays. The data represent the mean of three independent replicates with error bars indicating the standard deviation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ (by the Student *t* test).



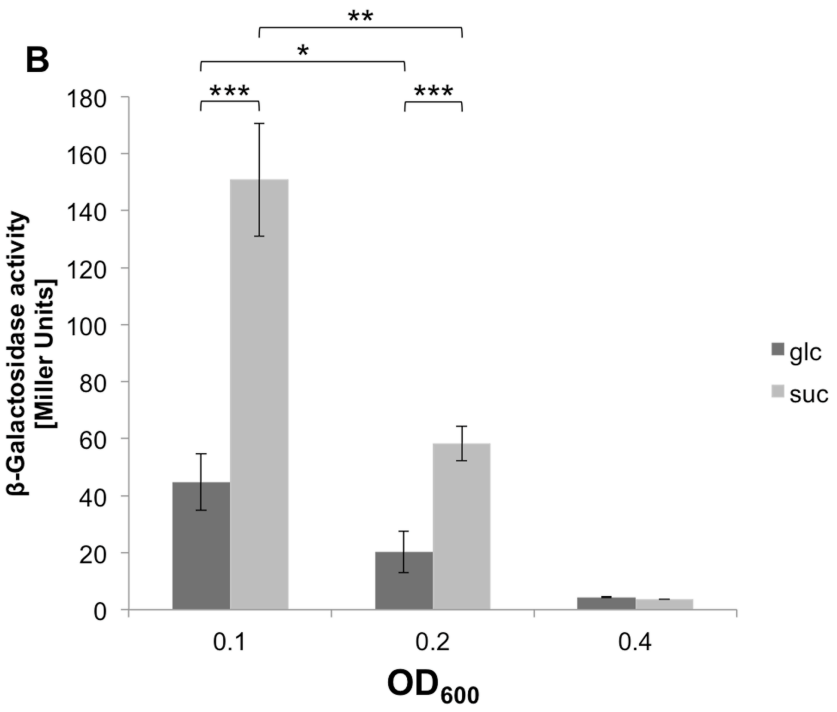

B
**
*
***
***
180
160
140
120
100
80
60
40
20
0
β-Galactosidase activity
[Miller Units]
glc
suc
0.1
0.2
0.4
OD600

FIGURE S4. Growth curves showing the effect of CSP on the wild-type strain grown in various carbohydrates. *S. mutans* UA159 was grown in TVB supplemented with 20 mM glucose (glc) (A), 20 mM fructose (fru) (B), 10 mM trehalose (tre) (C), or 10 mM maltose (mal) with or without the addition of 1 μM CSP.

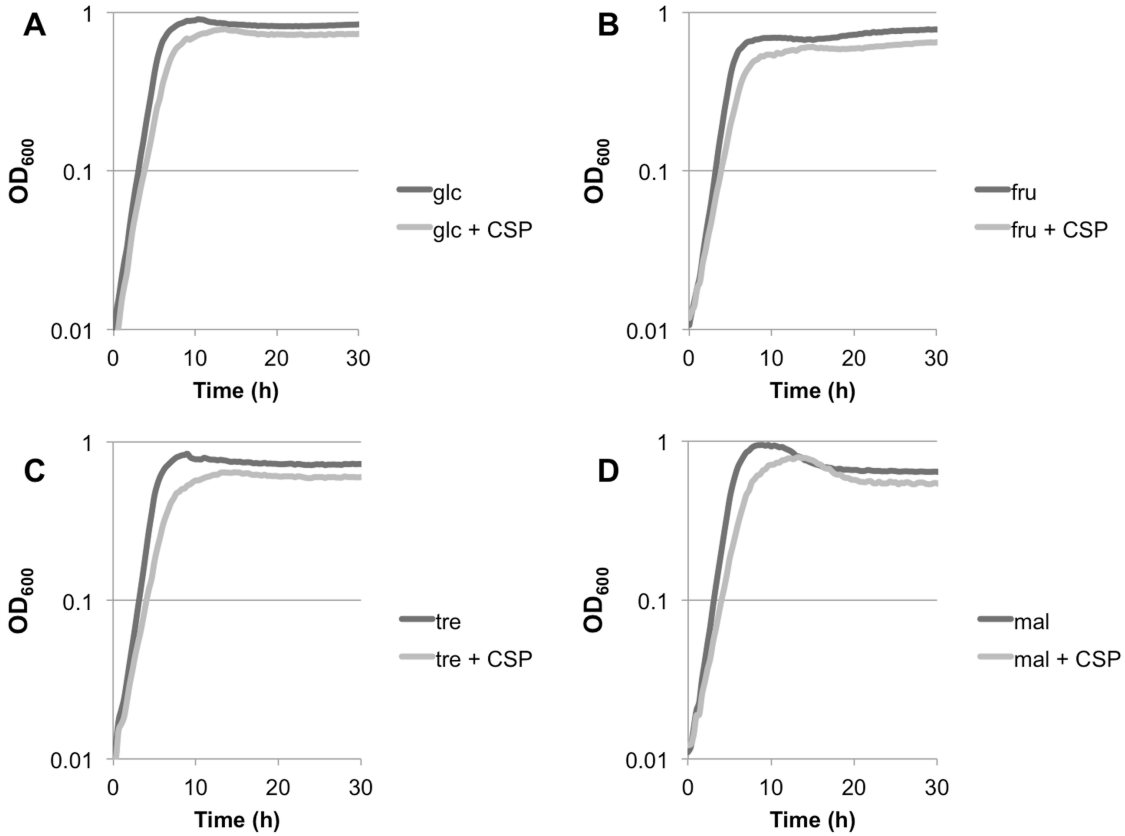


FIGURE S5. Growth curves showing the effect of CSP on a strain unable to produce insoluble glucans. A *gtfBC* deletion mutant of *S. mutans* UA159 was grown in TVB supplemented with 20 mM glucose (glc) (A) or 10 mM sucrose (suc) (B) with or without the addition of 1 μM CSP.

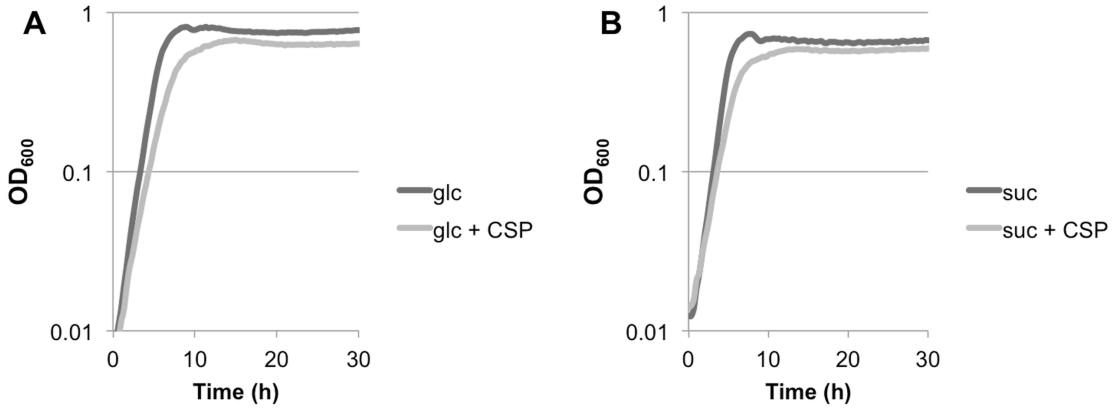


Table S2 Effect of carbohydrates on CSP-mediated growth inhibition.

Growth carbohydrate	Average difference in OD_{600} of cultures ± CSP after 30 h	Standard deviation	*P* value comparing difference in OD_{600} for growth carbohydrate to glc*
UA159 (FIG S4)			
glc	0.11	0.04	
fru	0.13	0.04	0.37
tre	0.13	0.03	0.47
mal	0.10	0.05	0.80
gtfBC (FIG S5)			
glc	0.14	0.07	
suc	0.07	0.04	0.12

*By the Student *t* test

FIGURE S6. Expression of *comX* in the fructose PTS transporter mutants *levD* and *fruI fruCD* is comparable to the parental strain. *S. mutans* UA159, *levD*, or *fruI fruCD* containing P_{comX}-*lacZ* was grown in TVB base medium supplemented with 20 mM glucose (glc) or 17.5 mM glucose (glc) and 2.5 mM fructose (fru) to an OD_{600} of 0.1. Cultures were treated with 1 μM CSP and incubated an additional two hours. Expression of *comX* was determined by performing LacZ assays. Data represent the mean of three independent replicates with error bars indicating the standard deviation. ns, not significant; *, $P < 0.05$; **, $P < 0.01$ (by the Student *t* test).

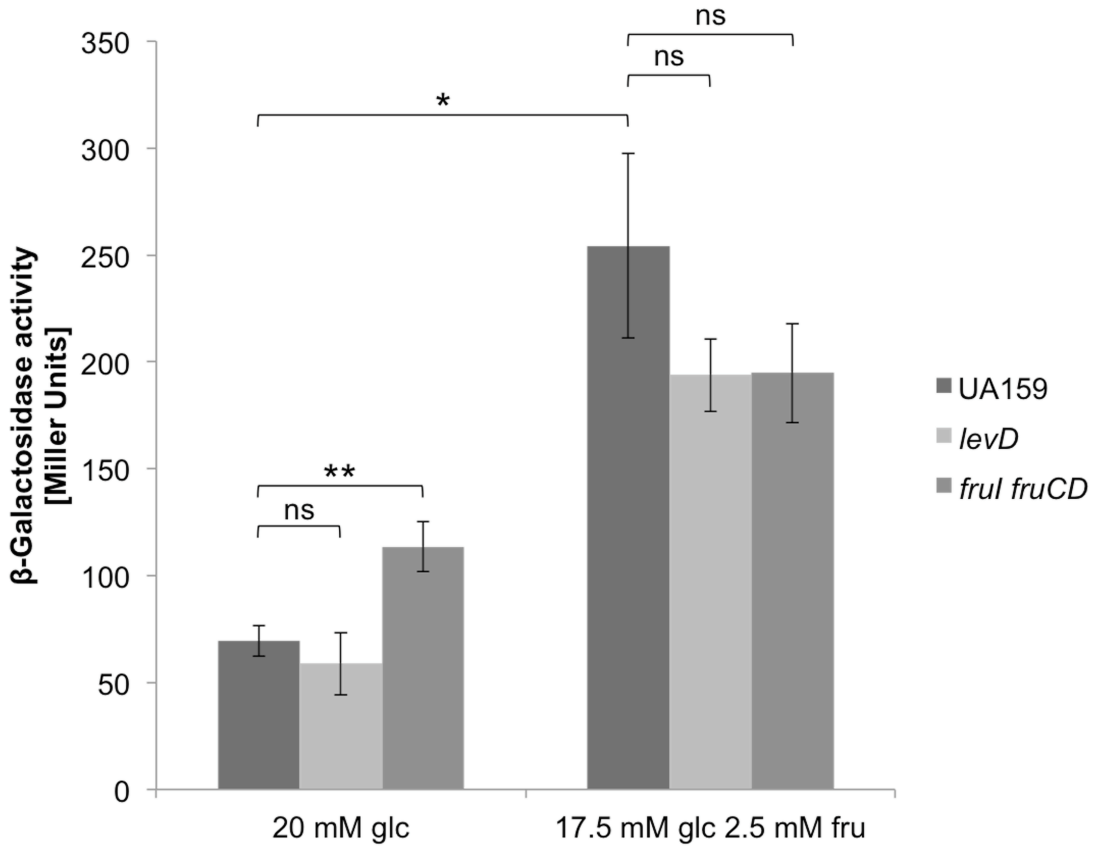


FIGURE S7. Effect of deleting regulators of catabolite repression on the CSP-mediated transformability of *S. mutans*. (A and C) *S. mutans* UA159 and *manLMN* were grown in TVB supplemented with 20 mM glucose (glc), 20 mM fructose (fru), or 10 mM trehalose (tre). (B) *S. mutans* UA159 and *ccpA* were grown in TVB supplemented with 20 mM glucose (glc) or 10 mM trehalose (tre). When cultures reached an OD_{600} of 0.1, they were treated with 1 μM CSP and 100 ng pBGS and incubated an additional three hours. Cultures were diluted and plated on BHI agar plates with or without 1 mg ml^{-1} spectinomycin. After plates had incubated 24-48 hours, colony-forming units were enumerated. Data represent the mean of three (A and C) or six (B) independent replicates with error bars indicating the standard deviation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ (by the Student *t* test).

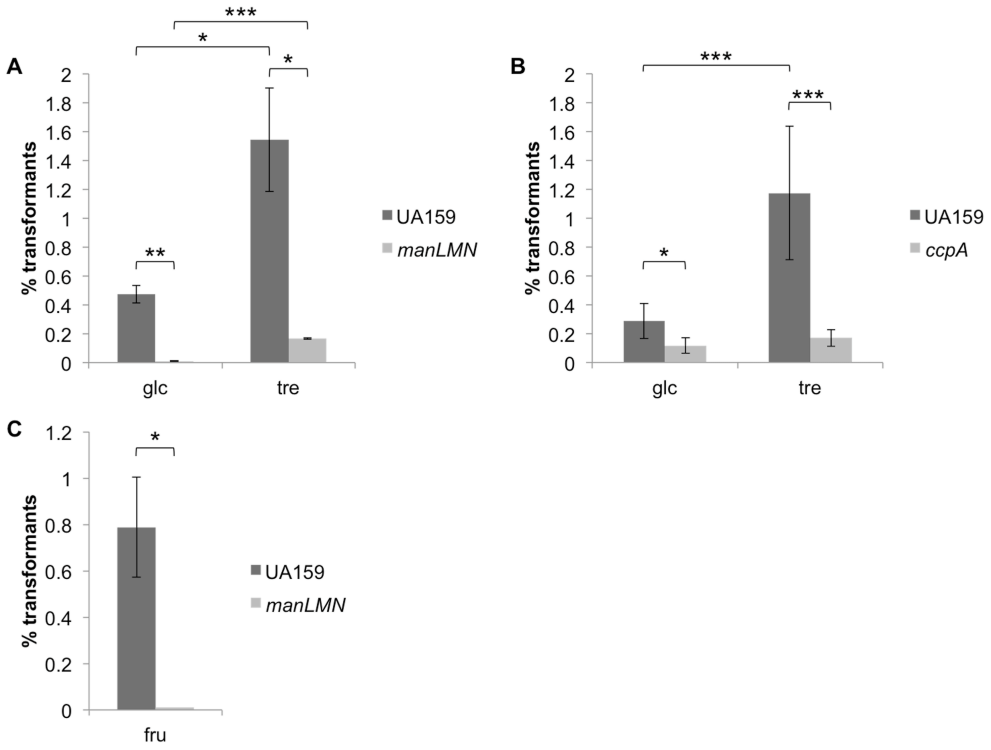


FIGURE S8. Impact of carbohydrate type on the basal level of *comS* expression. A *comS* deletion of *S. mutans* UA159 containing P_{comS}-*gfp* was grown in TVB supplemented with 20 mM glucose (glc), 20 mM fructose (fru), or 10 mM trehalose (tre), BHI, or FMC containing 20 mM glucose (glc). When cultures reached an OD_{600} of 0.1, they were divided in two, and half were treated with 1 μM CSP or 500 nM XIP (red), with the other half left undisturbed (blue). Cultures were incubated an additional two hours, and the expression of *comS* was determined by measuring the florescence of GFP. Microscopy and image analysis are described in the Methods section. An arrow represents the mean GFP florescence for cells incubated with CSP (red) or left untreated (blue).

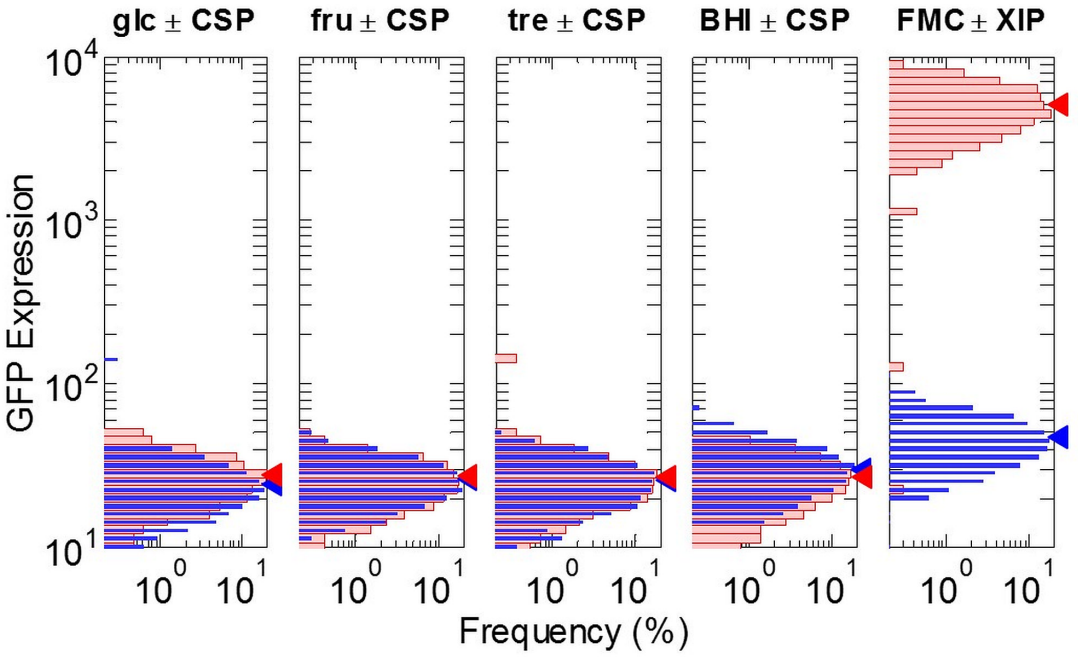


FIGURE S9. Models for sucrose and trehalose impacting *com* gene expression in oral microbial biofilms. *S. mutans* produces multiple enzymes capable of acting on sucrose, a common dietary sugar. The majority of sucrose is internalized by the PTS and metabolized for energy generation and anabolic processes. The fermentation of sucrose by *S. mutans* rapidly drives down the pH and inhibits the growth of more acid sensitive species. *S. mutans* also produces several extracellular enzymes that act on sucrose. In particular, the glycosyltransferases (GTFs) use sucrose as a substrate in the production of an adherent polysaccharide matrix composed predominantly of homopolymers of glucose (glucans), which serve as the architectural matrix for heterogeneous microbial biofilms. Over time, the expansion of this thick polysaccharide matrix can result in limited diffusion, allowing for the accumulation of soluble signaling molecules, such as CSP. High levels of extracellular CSP stimulate the production of bacteriocins by *S. mutans*, which can lyse nearby cells. The liberated extracellular DNA (eDNA) can then be internalized by *S. mutans* and utilized as a nutrient source or incorporated into the chromosome. The mass transport limitations associated with growth in a biofilm matrix may trigger osmotic stress response pathways in bacteria. In some cases, organisms synthesize and sequester trehalose as a compatible solute. Lysis of these cells (represented by the dashed line) could release stored trehalose, as well as DNA, and thus, trehalose may be perceived by *S. mutans* as a signal that nearby cells have lysed.

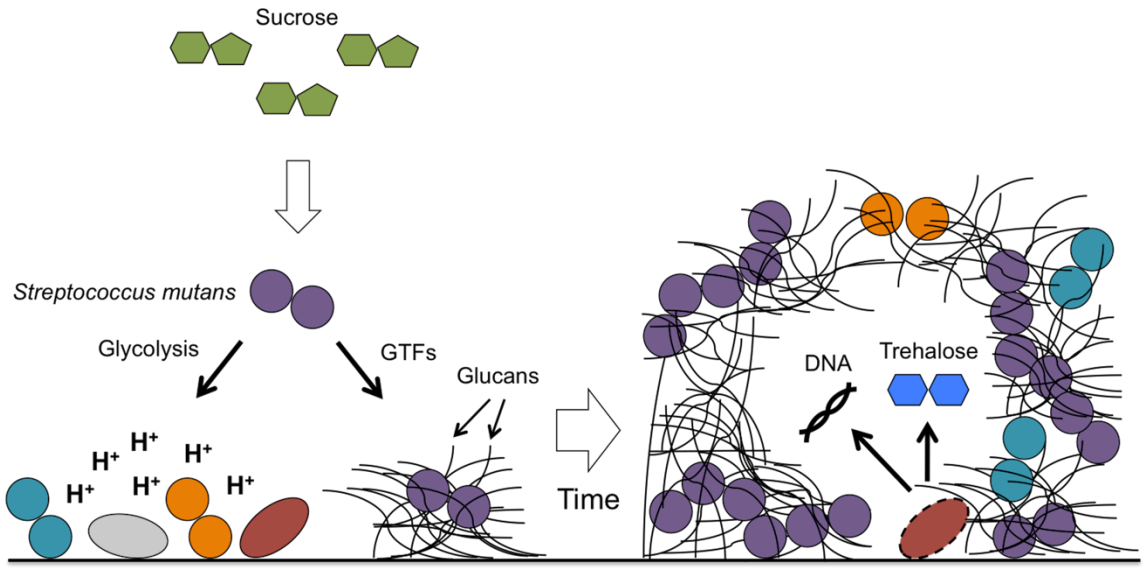

Sucrose
Streptococcus mutans
Glycolysis
GTFs
Glucans
H+
H+
H+
H+
H+
H+
Time
DNA
Trehalose

Table S3. Parameters for ComR/S autofeedback simulation. Since complex medium is used in this study, the importation rate of XIP (or ComS) from the extracellular environment (D_i) is very low (10). Values for other parameters are estimated based on previous work (10) and this study. Estimated value for A_{GFP}/β_{GFP} is high due to the long half-life of GFP.

Parameter	Value	Units	Description
D_i/β_S	0.01	dimensionless	Importation rate of extracellular XIP (or ComS) / degradation rate of XIP (or ComS) inside a cell
D_o/β_S	0.01	dimensionless	Exportation rate of intracellular XIP (or ComS) / degradation rate of XIP (or ComS) inside a cell
a_R/β_R	0.7	dimensionless	Constitutive expression rate of ComR / degradation rate of ComR
a_S/β_S	0.7	dimensionless	Constitutive expression rate of XIP (or ComS) / degradation rate of XIP (or ComS) inside a cell
a_{GFP}/β_{GFP}	1	dimensionless	Constitutive expression rate of GFP by P*comS* / degradation rate of GFP
A_R/β_R	1	dimensionless	Expression rate of ComR by CSP / degradation rate of ComR
A_S/β_S	25	dimensionless	Expression rate of XIP (or ComS) by ComR+XIP multimer / degradation rate of ComR
A_{GFP}/β_{GFP}	50	dimensionless	Expression rate of GFP by M (the ComR+XIP multimer) / degradation rate of GFP
K_{CSP}	100	nM	Dissociation constant for CSP inducing *comR*
K_{RS}	30	nM^3	Dissociation constant for ComR+XIP multimer reaction ($M \rightleftarrows 2\cdot ComR + 2\cdot XIP$ (or ComS))
K_M	15	nM	Dissociation constant for M activating P*comS*
n	1.5	unitless	Cooperativity term for M activating P*comS*

Using these parameters, the ComR/S auto-feedback circuit can be described by:

$$\frac{dR}{dt} = a_R + \frac{A_R \cdot CSP}{CSP + K_{CSP}} - \beta_R \cdot R$$

$$M = \frac{1}{K_{RS}} R^2 \cdot S^2$$

$$\frac{dS}{dt} = a_S + \frac{A_S \cdot M^n}{M^n + K_M} - \beta_S \cdot S + D_i \cdot Z - D_o \cdot S$$

$$\frac{dGFP}{dt} = a_{GFP} + \frac{A_{GFP} \cdot M^n}{M^n + K_M} - \beta_{GFP} \cdot GFP$$

where *R* denotes the concentration of ComR, *M* denotes the concentration of the ComR/XIP (or ComR/ComS) complex, *S* indicates the concentration of XIP (or ComS), and GFP describes the concentration of GFP molecule inside a cell (10). This model assumes the ComS can either bind to ComR or equally mature to XIP inside the cell (10); hence, does not distinguish between ComS and XIP. Simulations based on this model are portrayed in Figure 10.

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