Supplemental Methods

Construction of deletion mutants and complemented strains. Gene deletion mutants of *S. mutans* UA159 were constructed according to a method described elsewhere (1). Briefly, erythromycin (Em^r) or spectinomycin (Spc^r) resistance gene without the terminator was amplified by PCR from pResEmNot (2) or pSPC2310 (3) with specific primers and cloned into pBluescript SK II (+) (yielding pBSSKEm^r and pBSSKSpc^r, respectively). The 5' and 3'-flanking regions of the target *S. mutans* gene were then PCR-amplified from *S. mutans* genomic DNA with specific primers, and each fragment was cloned into both ends of the Em^r/Spc^r gene to generate a gene cassette comprising the Em^r/Spc^r gene with the flanking region of the target gene. After PCR amplification of the whole gene, the PCR fragment was transformed into *S. mutans*. Mutants were isolated by selection for erythromycin/spectinomycin resistance. The primers used are listed in Table S1.

For genetic complementation, we constructed a DNA fragment in which the gene containing the Em^r/Spc^r gene and the target gene was inserted into the *ftf* gene, which encodes fructosyltransferase. First, the target gene and the 3'-terminal region of the *ftf*

gene (ftf-2) were amplified with specific primers, which resulted in the addition of an extra eight or nine nucleotides for annealing. The target gene and ftf-2 were then fused. The 5'-terminal region of the *ftf* gene (ftf-1) was cloned into pBSSKEm^r/pBSSKSpc^r, yielding pBSSKEm^r::ftf-1 and pBSSKSpc^r::ftf-1, respectively. Then, the fusion fragment of the target gene and ftf-2 were cloned into pBSSKEm^r::ftf-1/ pBSSKSpc^r::ftf-1 downstream of the Em^r/Spc^r gene. Finally, the fragment for complementation was amplified with specific primers and transformed into each mutant, permitting insertion of the fragment into *S. mutans* chromosomal DNA by homologous recombination. The complemented strains were isolated by selection for erythromycin and spectinomycin resistance. Finally, the insertion of the Em^r/Spc^r and target genes into the *ftf* gene was verified by PCR.

Microarray analysis. Overnight cultures of *S. mutans* (10^8 cells) were inoculated into 10 ml of fresh TSB, which was, then cultured at 37°C with 5% CO_2. When the optical density (OD) at 660 nm reached 0.3, nisin A (2 μg/ml) or nukacin ISK-1 (4 μg/ml) was added to the medium. When the OD_{660} reached 0.5, the bacterial cells were pelleted by centrifugation at 5,000 x g and 4°C for 5 min and stored at -80°C until needed. Total

RNA was extracted from the bacterial cells using a FastRNA Pro Blue Kit (MP Biomedicals, Cleveland, OH, USA) according to the manufacturer's protocol. For microarray analysis, cDNA was synthesised from 10 μg of total RNA using a FairPlay III Microarray Labeling Kit (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions. The Agilent eArray platform was used to design a microarray; 14,028 probes (60-mers) were designed for the 2,012 protein-coding genes of *S. mutans* UA159 (up to seven probes per gene). For microarray analyses, test and control cDNAs were labeled with Alexa Fluor® 555 and Alexa Fluor® 647 (Molecular Probes Inc., OR, USA), respectively. The fluorescently labeled cDNA was purified using the QIAquick PCR Purification Kit (QIAGEN Inc., CA, USA). The Alexa Fluor® 555-labeled and Alexa Fluor® 647-labeled DNAs were mixed and hybridised on an array using a Hi-RPM Gene Expression Hybridization Kit (Agilent Technologies). The arrays were then scanned with an Agilent scanner (Agilent Technologies), and data extraction, filtering and normalisation were conducted using Feature Extraction Software (Agilent Technologies), according to the manufacturer's instructions. The experiments were performed as two biological replicates, and the expression data were deposited in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under

accession No. GSE44602. We considered greater than 3-fold changes with *P*-values of < 0.05 to be significant.

Co-culture of *S. mutans* with *S. warneri* or *L. lactis*. Aliquots of overnight *S. mutans*, *S. warneri* ISK-1, *S. warneri* ISK-1^{-}, *L. lactis* ATCC 11454 and *L. lactis* NZ9000 cultures were inoculated into TSB and grown to an OD_{660} of 0.5. For co-culture assays, appropriate numbers of *S. mutans* (UA159 and the mutants: 5×10^6 cells) and *L. lactis* (ATCC11454 or NZ9000: 5×10^6) or *S. mutans* (UA159 and the mutants: 2.5×10^5 cells) and *S. warneri* (ISK-1 or ISK-1ΔpPI-1: 5×10^6) cells were added to 5 ml of TSB and grown at 37°C with 5% CO_2 for 8 h. The optimal ratios for the co-culture assays were determined through preliminary experiments investigating the effects of various ratios of *L. lactis* or *S. warneri* on *S. mutans* growth. The appropriate dilutions were plated on TSA with or without 64 μg/ml of bacitracin for the selection of *S. mutans*. After 2 days, the numbers of CFUs on TSA plates with and without bacitracin were determined, and the percentage of the population represented by the *S. mutans* strain was calculated.

Supplemental Tables

Table S1. Strains used in this study

Strain names	Inactivated Gene ID[1]	Gene name	Features	References
UA159	-	-	laboratry strain	(4)
TCS45	SMU.45-6	unassigned	hk[2]-rr[3] deletion mutant, Em[r4]	This study
TCS1	SMU.486-7	*liaSR*	hk-rr deletion mutant, Em[r]	This study
TCS2	SMU.577-6	unassigned	hk-rr deletion mutant, Em[r]	This study
TCS3	SMU.659-60	*nsrRS*[5]	hk-rr deletion mutant, Em[r]	This study
TCS4	SMU.928-7	*relSR*	hk-rr deletion mutant, Em[r]	This study
TCS5	SMU.1009-8	unassigned	hk-rr deletion mutant, Em[r]	This study
TCS6	SMU.1037-8	unassigned	hk-rr deletion mutant, Em[r]	This study
TCS7	SMU.1128-9	*ciaRH*	hk-rr deletion mutant, Em[r]	(5)
TCS8	SMU.1146-5	*lcrRS*[5]	hk-rr deletion mutant, Em[r]	This study
TCS9	SMU.1516-7	*vicK*	hk deletion mutant, Em[r]	This study
TCS10	SMU.1548-9	unassigned	hk-rr deletion mutant, Em[r]	This study
TCS11	SMU.1815-4	unassigned	hk-rr deletion mutant, Em[r]	This study
TCS12	SMU.1916-7	*comDE*	hk-rr deletion mutant, Em[r]	This study
TCS13	SMU.1965-4	*levRS*	hk-rr deletion mutant, Em[r]	This study
TCS14	SMU.1924	*gcrR*	rr deletion mutant, Em[r]	This study
MM3105	SMU.659	*nsrS*	hk deletion mutant, Em[r]	This study
MM3098	SMU.1145	*lcrS*	hk deletion mutant, Em[r]	This study
MM3015	SMU.654	unassigned	ABC transporter deletion mutant, Em[r]	This study
MM3014	SMU.654-7	unassigned	ABC transporter deletion mutant, Em[r]	This study
MM3008	SMU.654+656	unassigned	ABC transporter deletion mutant, Em[r], Spc[r]	This study
MM3019	SMU.658	*nsrX*[5]	SMU.658 deletion mutant, Em[r]	This study
MM3081	SMU.658-660	*nsrXRS*	*658-nsrRS* deletion mutant, Em[r]	This study
MM3021	SMU.1148-50	*lctFEG*	ABC transporter deletion mutant, Em[r]	This study

Strain names	complement Gene ID	Gene name	Features	References
MM3106	SMU.659	*nsrS*	*nsrS* complement strain in MM3105, Em^r, Spc^{r6}	This study
MM3104	SMU.1145	*lcrS*	*lcrS* complement strain in MM3098, Em^r, Spc^r	This study
MM3055	SMU.658	*nsrX*	*nsrX* complement strain in MM3019, Em^r, Spc^r	This study
MM3074	SMU.658	*nsrX*	*nsrX* mplement strain in TCS3, Em^r, Spc^r	This study
MM3083	SMU.658	*nsrX*	*nsrX* complement strain in MM3081, Em^r, Spc^r	This study

Bacteriocin-producing strains	class	bacteriocins	References
Lactococcus lactis ATCC11454	I	nisin A	(6)
Lactococcus lactis NZ9000	-	nisin A non-producing	(7)
Staphylococcus warneri ISK-1	I	nukacin ISK-1	(8)
S. warneri ISK-1ΔpPI-1	-	pPI-1 cured strain, nukacin ISK-1	(9)
Lactococcus lactis CNRZ481	I	lacticin 481	(10)
Enterococcus mundtii QU 2	IIa	munditicin	(11)
Streptococcus mutans UA159	IIb	mutacin IV	(12)
Lactococcus sp. QU 12	IIc	lactocyclicin Q	(13)
Lactococcus lactis QU 5	IId	lacticin Q	(14)

[1] Gene IDs are from the GEO of the NCBI Database (http://www.ncbi.nlm.nhi.gov/geo/)

[2] histidine kinase

[3] response regulator

[4] erythromycin resistance

[5] designated in this study

[6] spectinomycin resistance

81 Table S2. Primers used in this study.

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name of primers	purpose	forward	reverse
tcs45-46-up	SMU.45-6 deletion	aataccaagagtctgagc	cagtcgaggatcccatctaagactaacata
tcs45-46-dw	SMU.45-6 deletion	gctgacctagt aattattgatgaatcaggag	gagatgtttgcacatagt
tcs1-up	*liaSR* deletion	atccttatacaattgctgc	cagtcgaggatccgcgtataaaaaaatcatt
tcs1-dw	*liaSR* deletion	gctgacctagttttagtgccacaggacg	cctcatgatttttataaaaat
tcs2-up	SMU.577-6 deletion	ttaaatatttggttaaagagg	cagtcgaggatccaagtctctgaaataataa
tcs2-dw	SMU.577-6 deletion	gctgacctagtagaattgaatgctcatttg	ccttcagctttgagaatat
tcs3-up	*nsrRS* deletion	ttttccgattattcttccc	cagtcgaggat ttaccaaaatacgggtcat
tcs3-dw	*nsrRS* deletion	gctgacctagtttaccactggctaaaatgt	ctccacaggttcaccaa
tcs4-up	*relSR* deletion	ttgcacgtataaagaccaa	cagtcgaggatctgctcttcgtcttctgct
tcs4-dw	*relSR* deletion	gctgacctagtgatgccattacttttaagg	ccctcaaacttcttaataa
tcs5-up	SMU.1009-8 deletion	tttactcaactgctgcg	cagtcgaggatcaaccaagtaaatttttct
tcs5-dw	SMU.1009-8 deletion	gctgacctagtattgatcaaggaacacag	cgcattgatctagctatt
tcs6-up	SMU.1038-7 deletion	atttagaacaatgccggc	cagtcgaggattcttcaatttgtaaaatacgt
tcs6-dw	SMU.1038-7 deletion	gctgacctagttttattttaactgtaacgcta	gcgaaccggttatttctt
tcs7-up	*ciaRH* deletion	gtgagaattgggcttgga	cagtcgaggatctattatagcatgacttgg
tcs7-dw	*ciaRH* deletion	gctgacctagtctcgcctgcttgctaata	aaaatggccagtctgc
tcs8-up	*lcrRS* deletion	aatttaagcaaacgttga	cagtcgaggatcatgctgttacctcgata
tcs8-dw	*lcrRS* deletion	gctgacctagtttgaaagtgacgattgtc	attgactttgacggctga
tcs9-up	*vicK* deletion	cagccatgttccaattattatg	cagtcgaggataagggacttgattcaaaca
tcs9-dw	*vicK* deletion	gctgacctagt caatagtgaggaaggcga	aactagtatgagtaaaggc
tcs10-up	SMU.1548-9 deletion	tttctattattgtgacttttat	cagtcgaggat tccacataggataatagttt
tcs10-dw	SMU.1548-9 deletion	gctgacctagtacctgttttgcagaactg	caaggcgacatataaggc
tcs11-up	SMU.1815-4 deletion	gtcgtccgcatcaagatagtg	cagtcgaggatggatatgctttttcaataatt
tcs11-dw	SMU.1815-4 deletion	gctgacctagttacttctggttcagacaat	acacgagagaaatcaatga
tcs12-up	*comDE* deletion	ggtgtcgtcattcttcct	cagtcgaggataggttagctgattaacac
tcs12-dw	*comDE* deletion	gctgacctagttagaggaggcctattctc	ctatcagctgcgctgtta
tcs13-up	*levRS* deletion	tctcaatgctgaagtgga	cagtcgaggatctgccaccctgttaaatc
tcs13-dw	*levRS* deletion	gctgacctagtacctattggtcttgttgg	agcaaccttaccctcatc
tcs14-up	*gcrR* deletion	cacggacaagtcaagaga	cagtcgaggatggaaactccttacgttac
tcs14-dw	*gcrR* deletion	gctgacctagttaggtctgttgaagtggt	agcagcatcactgccaat

mm3105-up	*nsrS* deletion	tttttgtcgggacattcg	cagtcgaggat ctaactgtcgcaaaatact
mm3105-dw	*nsrS* deletion	gctgacctagttggtctaactattgcagaca	ttgggtctgattaataccat
mm3098-up	*lcrS* deletion	aacttctactataccagta	cagtcgaggatccaagccataaacaaaaat
mm3098-dw	*lcrS* deletion	gctgacctagtttgaaagtgacgattgtc	attgactttgacggctga
mm3015-up	SMU.654 deletion	taaacggccaatgccaag	cagtcgaggatcgctgtcctccttataga
mm3015-dw	SMU.654 deletion	gctgacctagtttcgttccttccctacac	ccaattataggcatccgt
mm3014-up	SMU.654-7deletion	taaacggccaatgccaag	cagtcgaggatcgctgtcctccttataga
mm3014-dw	SMU.654-7deletion	gctgacctagtgaatagaatttagaactgc	acttgagtaaaccataggg
mm3019-up	*nsrX* deletion	cgcagtcccactaacttt	cagtcgaggaagctgaaatcccgttagt
mm3019-dw	*nsrX* deletion	gctgacctagtgctttataccttgtcagta	tcatactcagacttggtc
mm3008-up	SMU.656 deletion	ttcaggagttgcgtaat	cagtcgaggatgccaaagtcagcacttta
mm3008-dw	SMU.656 deletion	gctgacctagttggtccattatgctcag	taatcccgcataaccgt
mm3081-up	*nsrXRS* deletion	cgcagtcccactaacttt	cagtcgaggaagctgaaatcccgttagt
mm3081-dw	*nsrXRS* deletion	gctgacctagttggtctaactattgcagaca	ttgggtctgattaataccat
mm3021-up	*lctFEG* deletion	gtttgacgatgtagtcgt	cagtcgaggattagccgtgcttttgcca
mm3021-dw	*lctFEG* deletion	gctgacctagtttagcagtcagtcagctt	aagcagcagcagatcgta
mm3050-up	*levDFEG* deletion	tcaaaagctttgactgtt	cagtcgaggatgaatacaccctactttctttt
mm3050-dw	*levDFEG* deletion	gctgacctagttcgttgatgattttgaag	ttaatcgtttacaactgct
Emr	erythromycin resistance gene	atcctcgactggaagcaaacttaagagtgtgttgaca	actaggtcagcttatttcctcccgttaaa
Spcr	spectinomycin resistance gene	atcctcgactgatcgattttcgttcgtga	actaggtcagcttccaccatttttttcaattt
ftf-comp	complementation	aagaaacaaagaaagctcatcatgtttcaac	cggccgcggttcgtcttgtttctctca
mm3106	*nsrS* complementation	taaggatccaactattgggcttgagcc	tctttgtttcttaacggaaatcattttatttcc
mm3104	*lcrS* complementation	ggatcctgacagttaagggcttg	tctttgtttcttacttgattataaacacttct
mm3055	*nsrX* complementation	tgggatccttatgatatgtgagggtc	tctttgtttcttattaccaaaatacgggtc

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