

# Supplementary Materials

## Genetic mutations that confer fluoride resistance modify gene expression and virulence traits of *Streptococcus mutans*

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**Table S1.** Oligonucleotides used in this study.

Target gene	Orientation	Sequence (5' to 3')
16S rRNA	Forward	CTTACCAGGTCTTGACATCCCG
	Reverse	ACCCAACATCTCACGACACGAG
<i>glpF</i>	Forward	CTACTGGACCTGCCCTTAATC
	Reverse	GAATACCACCACTTTGAGTTACC
<i>hsdS</i>	Forward	TGGGAAGTGACAATTTGGGATA
	Reverse	CTGAGACCACCATTGAGAGAG
<i>pykF</i>	Forward	GGTAGAAATCCGTGGTGGTAAA
	Reverse	ACATTGGCTCCTTCTGTAATCA
<i>SMU.1289c</i>	Forward	GCCCATGGCAGCTATACTATT
	Reverse	TGAGAGGTCAAACCTAGCCGTA
<i>SMU.1290c</i>	Forward	AGGGCTGAGTACTGATTTGGTT
	Reverse	CGCCTTGATAACCTGCTGATA
<i>murC2</i>	Forward	CTGTCCTTCTCAATGCCAATTATG
	Reverse	CGGACACCACCTGTAAAGATT
<i>SMU 2059c</i>	Forward	CATCCTTACCCATTATCGTAGCA
	Reverse	AGAACCGACTCCAACCAAAG

**Table S2.** Up-regulated genes in the FR strain ( $P < 0.01$ ).

Gene name	Fold change (Log2 value)	Description
<i>SMU.1367c (ubiE)</i>	6.657	conserved hypothetical protein
<i>SMU.932</i>	4.154	hypothetical protein
<i>SMU.935</i>	4.138	putative amino acid ABC transporter, permease protein
<i>SMU.934</i>	4.011	putative amino acid ABC transporter, permease protein
<i>SMU.933</i>	3.916	putative amino acid ABC transporter, periplasmic amino acid-binding protein
<i>SMU.936</i>	3.869	putative amino acid ABC transporter, ATP-binding protein
<i>SMU.1341c, SMU.1340 (bacA2)</i>	2.930	putative gramicidin S synthetase / putative surfactin synthetase
<i>SMU.1339 (bacD)</i>	2.918	putative bacitracin synthetase
<i>SMU.1342 (bacA1)</i>	2.846	putative bacitracin synthetase 1
<i>SMU.1343c</i>	2.714	putative polyketide synthase
<i>SMU.1568 (malX)</i>	2.659	putative maltose/maltodextrin ABC transporter, sugar-binding protein
<i>SMU.1898, SMU.1899</i>	2.594	putative ABC transporter, ATP-binding and permease protein
<i>SMU.1345c</i>	2.455	putative peptide synthetase similar to MycA
<i>SMU.503c</i>	2.353	hypothetical protein
<i>SMU.651c</i>	2.351	putative ABC transporter, substrate-binding protein
<i>SMU.1344c</i>	2.302	putative malonyl-CoA-acyl-carrier-protein transacylase
<i>SMU.1289c (permease_B, eriC1b)</i>	2.299	putative permease, chloride channel
<i>SMU.1336 (pksD)</i>	2.261	conserved hypothetical protein, involved in polyketide synthesis
<i>SMU.962</i>	2.260	putative dehydrogenase
<i>SMU.961</i>	2.232	conserved hypothetical protein
<i>SMU.609</i>	2.199	putative 40K cell wall protein precursor
<i>SMU.1564 (glgP), smu.1565 (malQ)</i>	2.147	putative glycogen phosphorylase / putative 4-alpha-glucanotransferase
<i>SMU.1293c</i>	2.134	conserved hypothetical protein
<i>SMU.1290c (permease_A, eriC1a), SMU.1291c (mut)</i>	1.984	putative permease, chloride channel / putative chorismate mutase
<i>SMU.1292c</i>	1.967	conserved hypothetical protein
<i>SMU.1184c</i>	1.939	putative transcriptional regulator, antiterminator

SMU.652c, SMU.653c	1.930	putative ABC transporter, ATP-binding protein, possible nitrate transport system / putative ABC transporter, permease protein
SMU.1569 ( <i>malF</i> ), SMU.1570 ( <i>malG</i> )	1.927	putative maltose/maltodextrin ABC transporter, permease protein
SMU.1335c	1.926	putative enoyl-(acyl-carrier-protein) reductase
SMU.1575c, SMU.1576c	1.923	hypothetical protein
SMU.1183 ( <i>mtlA2</i> )	1.900	PTS system, mannitol-specific enzyme IIA
SMU.1157c	1.865	conserved hypothetical protein
SMU.984	1.827	hypothetical protein
SMU.1337c	1.820	putative alpha/beta superfamily hydrolase
SMU.1347c, SMU.1346 ( <i>bacT</i> )	1.800	conserved hypothetical protein, possible permease / putative thioesterase
SMU.840c	1.789	hypothetical protein
SMU.1160c	1.725	hypothetical protein
SMU.877 ( <i>agaL</i> )	1.720	alpha-galactosidase
SMU.43	1.715	putative site-specific DNA-methyltransferase restriction-modification protein
SMU.1650 ( <i>end3</i> )	1.704	putative endonuclease III (DNA repair)
SMU.1365c	1.685	hypothetical protein, possible permease
SMU.1048	1.680	conserved hypothetical protein
SMU.930c	1.661	putative transcriptional regulator
SMU.1287	1.645	putative transcriptional regulator
SMU.1295 ( <i>add</i> )	1.645	putative adenosine deaminase
SMU.1182 ( <i>mtlD</i> )	1.610	mannitol-1-phosphate dehydrogenase
SMU.1571	1.586	putative ABC transporter, ATP-binding protein
SMU.1577c	1.579	conserved hypothetical protein
SMU.1334 ( <i>sfp</i> )	1.569	putative phosphopantetheinyl transferase
SMU.878 ( <i>msmE</i> )	1.566	multiple sugar-binding ABC transporter, sugar- binding protein precursor
SMU.605	1.564	hypothetical protein
SMU.1771c, SMU.1772c, SMU.1773c	1.519	hypothetical protein
SMU.895, SMU.896	1.505	possible DNA-damage-inducible protein / conserved hypothetical protein
SMU.44	1.490	possible DNA mismatch repair protein
SMU.431	1.450	putative ABC transporter, ATP-binding protein
SMU.1069c, SMU.1070c	1.445	hypothetical protein / conserved hypothecial protein

<i>SMU.879 (msmF)</i>	1.429	multiple sugar-binding ABC transporter, permease protein
<i>SMU.684, SMU.685</i>	1.423	hypothetical protein
<i>SMU.1338c</i>	1.403	putative permease, possible multidrug-efflux transporter
<i>SMU.1067c, SMU.1068c</i>	1.380	putative ABC transporter, permease protein / putative ABC transporter, ATP-binding protein
<i>SMU.432</i>	1.372	putative ABC transporter, integral membrane protein
<i>SMU.985 (bglA)</i>	1.368	putative beta-glucosidase
<i>SMU.45</i>	1.351	hypothetical protein
<i>SMU.986c</i>	1.350	hypothetical protein
<i>SMU.880 (msmG)</i>	1.335	multiple sugar-binding ABC transporter, permease protein
<i>SMU.510c</i>	1.335	hypothetical protein
<i>SMU.876 (msmR)</i>	1.327	putative MSM operon regulatory protein
<i>SMU.1185 (mtlA1)</i>	1.322	PTS system, mannitol-specific enzyme IIBC component
<i>SMU.1294 (flaW)</i>	1.317	putative flabodoxin
<i>SMU.1288 (rl19)</i>	1.306	50S ribosomal protein L19
<i>SMU.1052</i>	1.293	conserved hypothetical protein
<i>SMU.1574c</i>	1.218	conserved hypothetical protein
<i>SMU.560c</i>	1.204	conserved hypothetical protein
<i>SMU.897</i>	1.200	putative type I restriction-modification system, helicase subunit
<i>SMU.998</i>	1.200	putative ABC transporter, periplasmic ferrichrome-binding protein
<i>SMU.2027</i>	1.199	putative transcriptional regulator
<i>SMU.1000, SMU.999</i>	1.191	hypothetical protein
<i>SMU.561c</i>	1.183	putative hydrolase
<i>SMU.136c</i>	1.183	putative transcriptional regulator
<i>SMU.1366c</i>	1.177	putative ABC transporter, ATP-binding protein
<i>SMU.1126 (coaA)</i>	1.175	putative pantothenate kinase
<i>SMU.46, SMU.47</i>	1.172	hypothetical protein
<i>SMU.1161c</i>	1.171	hypothetical protein
<i>SMU.1348c</i>	1.171	putative ABC transporter, ATP-binding protein
<i>SMU.2165</i>	1.164	putative SpoJ
<i>SMU.704c</i>	1.154	putative autolysin, amidase
<i>SMU.1402c</i>	1.150	conserved hypothetical protein
<i>SMU.1404c,</i>	1.138	conserved hypothetical protein

<i>SMU.1405c</i>		
<i>SMU.2047 (ptsG)</i>	1.129	putative PTS system, glucose-specific IIABC component
<i>SMU.1037c</i> , <i>SMU.1038c</i>	1.129	putative histidine kinase / putative response regulator
<i>SMU.2046c</i>	1.128	conserved hypothetical protein
<i>SMU.606</i>	1.126	hypothetical protein
<i>SMU.287</i>	1.100	putative ComB
<i>SMU.1942c</i>	1.092	putative amino acid binding protein
<i>SMU.1761c</i> , <i>SMU.1762c</i> , <i>SMU.1763c</i>	1.079	conserved hypothetical protein
<i>SMU.1442c</i>	1.056	conserved hypothetical protein
<i>SMU.1941 (atmB)</i>	1.048	putative membrane lipoprotein
<i>SMU.768c</i>	1.046	conserved hypothetical protein
<i>SMU.881 (gtfA)</i>	1.044	sucrose phosphorylase
<i>SMU.2153c</i>	1.034	putative peptidase
<i>SMU.1107c</i>	1.024	conserved hypothetical protein
<i>SMU.804</i>	1.015	hypothetical protein
<i>SMU.1125c</i>	1.011	conserved hypothetical protein
<i>SMU.1116c</i>	1.006	hypothetical protein
<i>SMU.1764c</i>	1.002	conserved hypothetical protein
<i>SMU.682</i>	1.000	hypothetical protein

**Table S3.** Down-regulated genes in the FR strain ( $P < 0.01$ ).

Gene name	Fold change (Log2 value)	Description
<i>SMU.182 (sloA), SMU.183 (sloB)</i>	-1.078	putative ABC transporter, ATP-binding protein / putative Mn/Zn ABC transporter
<i>SMU.564</i>	-1.151	conserved hypothetical protein
<i>SMU.423</i>	-1.288	hypothetical protein
<i>SMU.1774c</i>	-1.296	conserved hypothetical protein
<i>SMU.278</i>	-1.321	hypothetical protein
<i>SMU.1877 (ptnA, manL)</i>	-1.399	putative PTS system, mannose-specific component IIB
<i>SMU.1967 (ssb2)</i>	-1.409	putative single-stranded DNA-binding protein
<i>SMU.1879 (manN)</i>	-1.427	putative PTS system, mannose-specific component IID
<i>SMU.1878 (ptnC, manM)</i>	-1.445	putative PTS system, mannose-specific component IIC
<i>SMU.1397c</i>	-1.593	conserved hypothetical protein
<i>SMU.870, SMU.872, SMU.871 (pfkB)</i>	-1.724	putative transcriptional regulator of sugar metabolism / putative PTS system, fructose-specific enzyme IIAABC component / putative fructose-1- phosphate kinase
<i>SMU.2146c</i>	-1.777	hypothetical protein
<i>SMU.1960c</i>	-1.839	putative PTS system, mannose-specific IIB component
<i>SMU.1961c (manX)</i>	-1.880	putative PTS system, sugar-specific enzyme IIA component
<i>SMU.1958c (manY)</i>	-2.000	putative PTS system, mannose-specific IIC component
<i>SMU.1956c</i>	-2.115	hypothetical protein
<i>SMU.1957 (manZ)</i>	-2.182	putative PTS system, mannose-specific IID component
<i>SMU.141</i>	-3.567	conserved hypothetical protein
<i>SMU.140</i>	-3.779	putative glutathion reductase
<i>SMU.138</i>	-3.797	putative malate permease
<i>SMU.139</i>	-3.896	conserved hypothetical protein
<i>SMU.137 (mleS)</i>	-4.084	malolactic enzyme

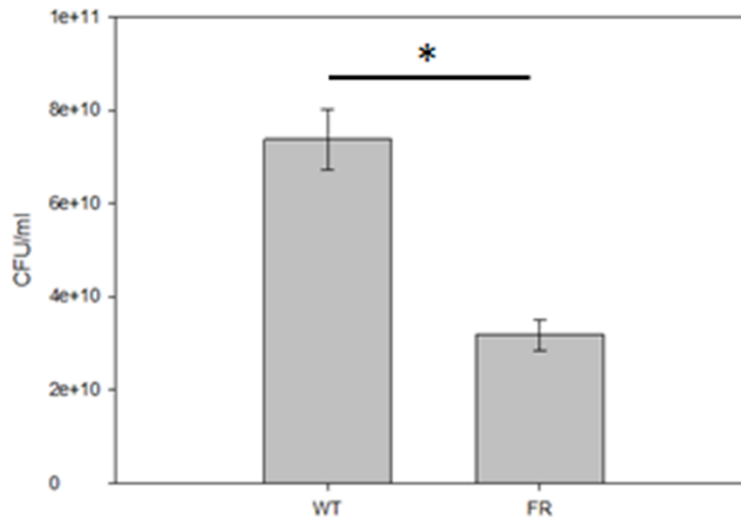


Figure S1. CFU measurement of *S. mutans* wild-type and FR strains. Early exponential cultures grown in BHI medium were diluted 1:100 in a semi-defined biofilm medium (BM). Following 24 h of incubation at 37 °C in a 5% CO<sub>2</sub> aerobic atmosphere, serial dilutions of transformants were plated on BHI medium to determine colony forming units (CFUs). The results are expressed as means of triplicates of three independent isolates. \*, differs from the wild-type strain at  $P < 0.05$ .

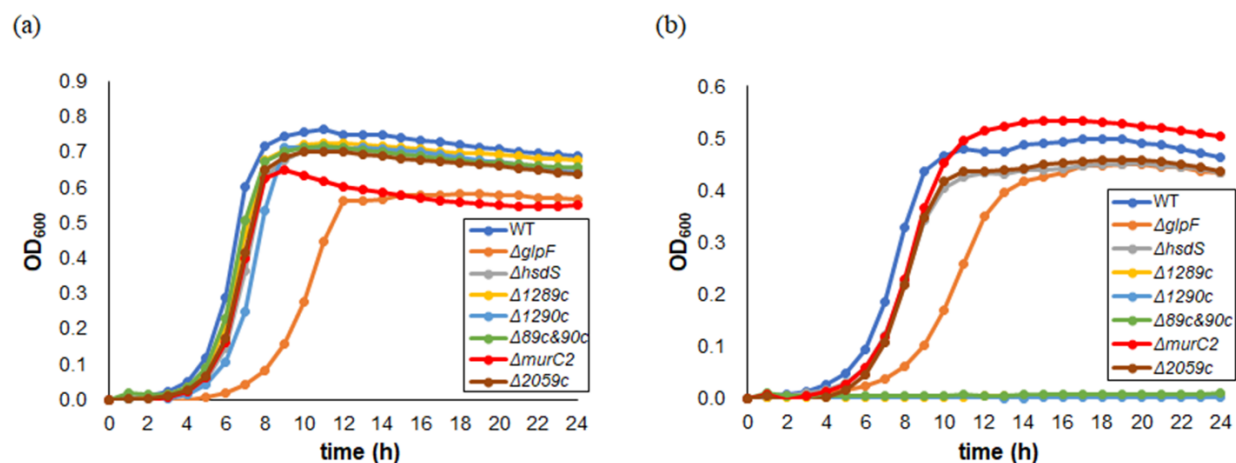


Figure S2. Growth behaviors of *S. mutans* wild-type and mutant strains obtained during growth in BHI medium. The eight strains, wild-type,  $\Delta glpF$ ,  $\Delta hsdS$ ,  $\Delta SMU.1289c$ ,  $\Delta SMU.1290c$ ,  $\Delta SMU.1289c\&1290c$ ,  $\Delta murC2$  and  $\Delta SMU.2059c$  were grown at 37 °C (a) without NaF under 5% CO<sub>2</sub> conditions or (b) with 100 µg mL<sup>-1</sup> NaF. Optical density at 600 nm (OD<sub>600</sub>) was monitored every 1 h at 37 °C using the Bioscreen C Lab System. The results are expressed as means of triplicates of three independent isolates.