Supplementary Information to "SPONTANEOUS MUTANTS OF *STREPTOCOCCUS SANGUINIS* WITH DEFECTS IN THE GLUCOSE-PTS SHOW ENHANCED POST-EXPONENTIAL PHASE FITNESS"

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Includes Tables S1&S2, and Figures S1 to S7.

Table S1. Single nucleotide polymorphisms (SNPs) in three SK36 stocks in comparison to SK36 genome published at the GenBank.

Position	Mutation	Annotation	Gene	Description
137,500	Δ1 bp	pseudogene (164/604 nt)	SSA_RS00875 →	hypothetical protein
138,032	T→A	intergenic (+92/+51)	SSA_RS00875 → / ← SSA_RS00880	hypothetical protein/TetR/AcrR family transcriptional regulator
138,086	T→G	S190S (TCA→TCC)	SSA_RS00880 ←	TetR/AcrR family transcriptional regulator
157,709	T→G	intergenic (+593/+691)	SSA_RS00950 → / ← SSA_RS00955	beta-carotene 15,15'-monooxygenase/hypothetical protein
358,487	T→G	H13Q (CAT→CAG)	SSA_RS01945 →	DUF2268 domain-containing protein
458,657	+A	intergenic (-117/-78)	SSA_RS02370 ← / → SSA_RS02375	sucrose-6-phosphate hydrolase/PTS beta-glucoside transporter subunit IIBCA
492,271	C→T	P182S (CCA→TCA)	SSA_RS02555 →	NADH-dependent flavin oxidoreductase
551,499	+A	pseudogene (1745/1830 nt)	SSA_RS02850 →	hypothetical protein
551,507	Δ1 bp	pseudogene (1753/1830 nt)	SSA_RS02850 →	hypothetical protein
551,525	T→A	pseudogene (1771/1830 nt)	SSA_RS02850 →	hypothetical protein
551,528	C→A	pseudogene (1774/1830 nt)	SSA_RS02850 →	hypothetical protein
551,601	C→A	intergenic (+17/-4)	SSA_RS02850 → / → SSA_RS02855	hypothetical protein/hypothetical protein
551,616	C→A	S4R (AGC→AGA)	SSA_RS02855 →	hypothetical protein
577,069	(G)5→4	pseudogene (90/367 nt)	SSA_RS02995 →	hypothetical protein
648,433	+T	coding (2/417 nt)	SSA_RS03360 →	GNAT family N-acetyltransferase
648,657	C→G	R76G (CGA→GGA)	SSA_RS03360 →	GNAT family N-acetyltransferase
685,509	T→A	intergenic (+93/-119)	SSA_RS03540 → / → acnA	magnesium transporter CorA family protein/aconitate hydratase AcnA

685,551	2 bp→A	intergenic (+135/-76)	SSA_RS03540 → / → acnA	magnesium transporter CorA family protein/aconitate hydratase AcnA
685,554	C→T	intergenic (+138/-74)	SSA_RS03540 → / → acnA	magnesium transporter CorA family protein/aconitate hydratase AcnA
685,556	C→A	intergenic (+140/-72)	SSA_RS03540 → / → acnA	magnesium transporter CorA family protein/aconitate hydratase AcnA
685,558	1 bp→AA	intergenic (+142/-70)	SSA_RS03540 → / → acnA	magnesium transporter CorA family protein/aconitate hydratase AcnA
714,548	Δ1 bp	pseudogene (721/910 nt)	SSA_RS03665 →	CPBP family intramembrane metalloprotease
714,550	G→T	pseudogene (723/910 nt)	SSA_RS03665 →	CPBP family intramembrane metalloprotease
784,486	A→T	Y16F (TAT→TTT)	SSA_RS04010 →	TIGR00159 family protein
784,489	A→C	Y17S (TAT→TCT)	SSA_RS04010 →	TIGR00159 family protein
902,998	+A	coding (8953/8973 nt)	SSA_RS04485 →	YSIRK-type signal peptide-containing protein
920,303	A→G	H155R (CAC→CGC)	SSA_RS04505 →	ABC transporter substrate-binding protein
1,079,878	+G	coding (911/1353 nt)	SSA_RS05245 →	VWA domain-containing protein
1,079,886	C→A	P307T (CCC→ACC)	SSA_RS05245 →	VWA domain-containing protein
1,079,899	C→A	P311Q (CCA→CAA)	SSA_RS05245 →	VWA domain-containing protein
1,079,904	Δ1 bp	coding (937/1353 nt)	SSA_RS05245 →	VWA domain-containing protein
1,079,917	+T	coding (950/1353 nt)	SSA_RS05245 →	VWA domain-containing protein
1,079,922	Δ1 bp	coding (955/1353 nt)	SSA_RS05245 →	VWA domain-containing protein
1,205,479	Δ1 bp	coding (95/1047 nt)	SSA_RS05775 ←	alpha-ketoacid dehydrogenase subunit beta
1,222,228	G→T	P188T (CCC→ACC)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,253	C→G	G179G (GGG→GGC)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,270	A→T	F174I (TTT→ATT)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,282	G→T	P170T (CCA→ACA)	SSA_RS05865 ←	DNA alkylation repair protein

1,222,287	G→C	A168G (GCC→GGC)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,312	T→C	K160E (AAG→GAG)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,363	Δ1 bp	coding (427/651 nt)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,375[A]	+A	coding (415/651 nt)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,378[B]	C→A	G138W (GGG→TGG)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,384	T→C	N136D (AAT→GAT)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,387	T→C	K135E (AAG→GAG)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,393	+A	coding (397/651 nt)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,396	T→C	K132E (AAG→GAG)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,399	A→G	L131L (TTG→CTG)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,405	+A	coding (385/651 nt)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,419	+T	coding (371/651 nt)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,422	T→G	Q123P (CAA→CCA)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,428	2 bp→CG	coding (361-362/651 nt)	SSA_RS05865 ←	DNA alkylation repair protein
1,222,433	C→A	M119I (ATG→ATT)	SSA_RS05865 ←	DNA alkylation repair protein
1,245,886	+T	pseudogene (1077/1202 nt)	rpsA ←	30S ribosomal protein S1
1,247,787	G→A	F304F (TTC→TTT)	SSA_RS06020 ←	branched-chain amino acid aminotransferase
1,299,320	C→A	intergenic (-14/+333)	SSA_RS06265 ← / ← SSA_RS06270	M15 family metallopeptidase/rhodanese-like domain-containing protein
1,332,042	A→G	L34P (CTA→CCA)	SSA_RS06460 ←	hypothetical protein
1,355,483	A→G	V11A (GTT→GCT)	SSA_RS06585 ←	pneumococcal-type histidine triad protein
1,543,365	G→A	intergenic (-87/-384)	SSA_RS07505 ← / → SSA_RS07510	cyclase family protein/biotin transporter BioY

1,543,598	C→T	intergenic (-320/-151)	SSA_RS07505 ← / → SSA_RS07510	cyclase family protein/biotin transporter BioY
1,543,690	G→A	intergenic (-412/-59)	SSA_RS07505 ← / → SSA_RS07510	cyclase family protein/biotin transporter BioY
1,543,703	+A	intergenic (-425/-46)	SSA_RS07505 ← / → SSA_RS07510	cyclase family protein/biotin transporter BioY
1,572,746	A→T	intergenic (+56/-53)	SSA_RS07660 → / → SSA_RS07665	amino acid ABC transporter permease/hypothetical protein
1,572,759	2 bp→TG	intergenic (+69/-39)	SSA_RS07660 → / → SSA_RS07665	amino acid ABC transporter permease/hypothetical protein
1,572,767	G→A	intergenic (+77/-32)	SSA_RS07660 → / → SSA_RS07665	amino acid ABC transporter permease/hypothetical protein
1,572,770	G→A	intergenic (+80/-29)	SSA_RS07660 → / → SSA_RS07665	amino acid ABC transporter permease/hypothetical protein
1,572,785	C→A	intergenic (+95/-14)	SSA_RS07660 → / → SSA_RS07665	amino acid ABC transporter permease/hypothetical protein
1,572,793	G→A	intergenic (+103/-6)	SSA_RS07660 → / → SSA_RS07665	amino acid ABC transporter permease/hypothetical protein
1,632,654	+G	intergenic (-26/+42)	SSA_RS07975 ← / ← SSA_RS07980	class C sortase/isopeptide-forming domain-containing fimbrial protein
1,668,222	C→A	C104F (TGT→TTT)	SSA_RS08140 ←	hypothetical protein
1,670,834	Δ1 bp	pseudogene (104/418 nt)	SSA_RS08155 ←	DUF3021 domain-containing protein
1,759,713	T→A	Y157F (TAT→TTT)	SSA_RS08615 ←	ECF transporter S component
1,760,293	T→A	intergenic (-111/+286)	SSA_RS08615 ← / ← SSA_RS08620	ECF transporter S component/tRNA (cytidine(34)-2'-O)-methyltransferase
1,760,397	G→T	intergenic (-215/+182)	SSA_RS08615 ← / ← SSA_RS08620	ECF transporter S component/tRNA (cytidine(34)-2'-O)-methyltransferase
1,772,677	+C	coding (270/492 nt)	SSA_RS08695 ←	HD domain-containing protein
1,772,682	Δ1 bp	coding (265/492 nt)	SSA_RS08695 ←	HD domain-containing protein
1,821,025	2 bp→GC	coding (551-552/777 nt)	SSA_RS08900 →	aminoglycoside 3'-phosphotransferase

1,821,030	T→C	S186P (TCC→CCC)	SSA_RS08900 →	aminoglycoside 3'-phosphotransferase
1,884,838	A→T	pseudogene (353/2085 nt)	SSA_RS09235 ←	helicase
1,884,840	C→A	pseudogene (351/2085 nt)	SSA_RS09235 ←	helicase
1,884,842	T→C	pseudogene (349/2085 nt)	SSA_RS09235 ←	helicase
1,884,845	G→A	pseudogene (346/2085 nt)	SSA_RS09235 ←	helicase
1,884,850	Δ1 bp	pseudogene (341/2085 nt)	SSA_RS09235 ←	helicase
1,884,852	2 bp→TT	pseudogene (338-339/2085 nt)	SSA_RS09235 ←	helicase
1,884,858	Δ1 bp	pseudogene (333/2085 nt)	SSA_RS09235 ←	helicase
1,884,874	C→T	pseudogene (317/2085 nt)	SSA_RS09235 ←	helicase
1,884,876	C→T	pseudogene (315/2085 nt)	SSA_RS09235 ←	helicase
1,884,878	2 bp→TT	pseudogene (312-313/2085 nt)	SSA_RS09235 ←	helicase
1,884,881	C→T	pseudogene (310/2085 nt)	SSA_RS09235 ←	helicase
1,884,888	Δ1 bp	pseudogene (303/2085 nt)	SSA_RS09235 ←	helicase
1,884,893	2 bp→TT	pseudogene (297-298/2085 nt)	SSA_RS09235 ←	helicase
1,884,897	C→T	pseudogene (294/2085 nt)	SSA_RS09235 ←	helicase
1,884,899	A→C	pseudogene (292/2085 nt)	SSA_RS09235 ←	helicase
1,884,901	C→A	pseudogene (290/2085 nt)	SSA_RS09235 ←	helicase
1,929,294	(18-bp)2→1	coding (458-475/1383 nt)	SSA_RS09485 →	aspartate kinase
1,976,025	Δ1 bp	intergenic (-62/+866)	SSA_RS09690 ← / ← SSA_RS09695	LytTR family transcriptional regulator/hypothetical protein
1,976,027	Δ1 bp	intergenic (-64/+864)	SSA_RS09690 ← / ← SSA_RS09695	LytTR family transcriptional regulator/hypothetical protein
1,989,366	A→T	S233T (TCT→ACT)	SSA_RS09750 ←	fructose-bisphosphate aldolase

1,989,622	T→G	I147I (ATA→ATC)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,637	2 bp→AC	coding (425-426/882 nt)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,640	3 bp→ACC	coding (421-423/882 nt)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,646	G→A	A139T (GCC→ACT)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,648	C→T	A139T (GCC→ACT)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,650	G→C	A138G (GCT→GGT)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,652	2 bp→AA	coding (410-411/882 nt)	SSA_RS09750 ←	fructose-bisphosphate aldolase
1,989,670	T→G	I131I (ATA→ATC)	SSA_RS09750 ←	fructose-bisphosphate aldolase
2,053,043	Δ1 bp	intergenic (-59/-352)	pnp ← / → SSA_RS10055	polyribonucleotide nucleotidyltransferase/MFS transporter
2,239,359	A→T	S25T (TCG→ACG)	SSA_RS10965 ←	hypothetical protein
2,327,141	2 bp→TTT	intergenic (-126/-42)	SSA_RS11370 ← / → SSA_RS11375	cation transporter/TetR/AcrR family transcriptional regulator
2,327,146	A→T	intergenic (-131/-38)	SSA_RS11370 ← / → SSA_RS11375	cation transporter/TetR/AcrR family transcriptional regulator
2,351,801	G→C	intergenic (-3/+200)	SSA_RS11505 ← / ← rpsD	NAD(P)-dependent oxidoreductase/30S ribosomal protein S4
2,351,885	Δ1 bp	intergenic (-87/+116)	SSA_RS11505 ← / ← rpsD	NAD(P)-dependent oxidoreductase/30S ribosomal protein S4
2,351,889	A→C	intergenic (-91/+112)	SSA_RS11505 ← / ← rpsD	NAD(P)-dependent oxidoreductase/30S ribosomal protein S4
2,351,892	C→A	intergenic (-94/+109)	SSA_RS11505 ← / ← rpsD	NAD(P)-dependent oxidoreductase/30S ribosomal protein S4
2,355,559	A→T	intergenic (-167/+136)	SSA_RS11525 ← / ← SSA_RS11530	ABC transporter permease/HAD family phosphatase
2,362,836	C→G	G345A (GGA→GCA)	mnmA ←	tRNA 2-thiouridine(34) synthase MnmA

[A] Present only in strain MMZ1922.
[B] Present in strains MMZ1612 and MMZ1922.

Table S2. Primers used in this study.

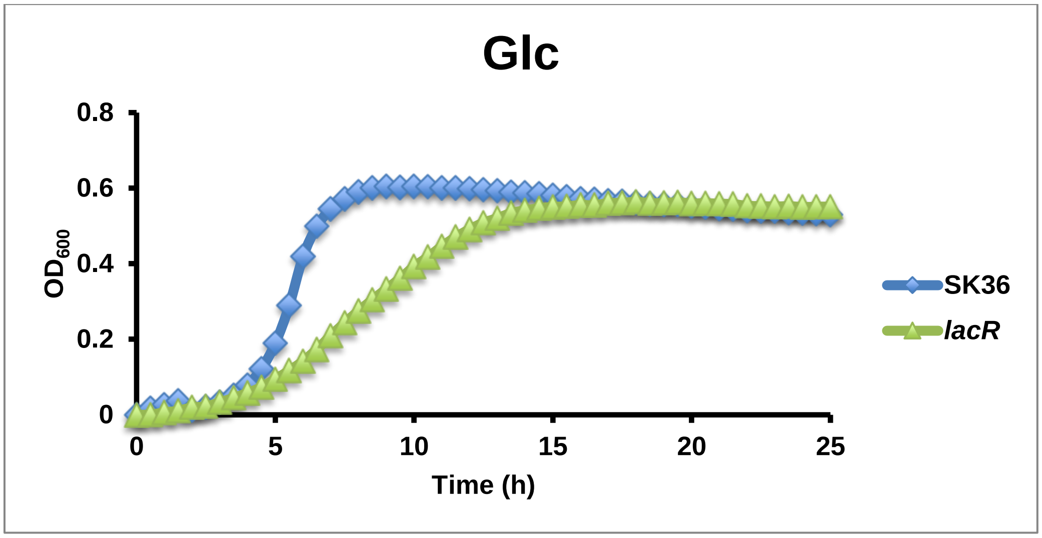
Primers	Sequence	Purpose
Ssa_lacR-1	5’- CTT TAT CTG ATG GTG ATT TAA AGC ATG A -3’	Deleting *lacR*
Ssa_lacR-2GA	5’- GCC ATT TAT TAT TTC CTT CCT CTT TTA CAT CCT GTC TCT GAT TTT TAC CCA -3’	Deleting *lacR*
Ssa_lacR-3GA	5’- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GCT CAG GAA GAA CTG GAA GT -3’	Deleting *lacR*
Ssa_lacR-4	5’- CAG ATG AGG AGC TCA CCA GTA A -3	Deleting *lacR*
Ssa1918-1	5’- CCA GGG CAA TGG ACA GTT ATC T -3’	Deleting *manL*
Ssa1918-2GA	5’- GCC ATT TAT TAT TTC CTT CCT CTT TTA CCG ATA CTC ATT ATT GAC ATT CCT C -3’	Deleting *manL*
Ssa1918-3GA	5’- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GCC AAC GTG CAG TAG AAT CA -3’	Deleting *manL*
Ssa1918-4	5’- GCA TAC GTT CGT AGT TCC AAG AA -3’	Deleting *manL*
Ssa_manL_Comp-2GA	5’- GCC ATT TAT TAT TTC CTT CCT CTT TTA TGA TTC TAC TGC ACG TTG GCT -3’	Complementing *manL*
GA-Km-5’	5’- TAA AAG AGG AAG GAA ATA ATA AAT GGC TAA -3’	Marker for GA
GA-Km-3’	5’- TCT ACT AAA ACA ATT CAT CCA GTA AAA TAT AAT ATT TT -3’	Marker for GA
GA-Em-5’	5’- TAA AAG AGG AAG GAA ATA ATA AAT GGC TAA CTA GAG GAT CTA GTC TTA TAA CTA TAC TG -3’	Marker for GA
GA-Em-3’	5’- TCT ACT AAA ACA ATT CAT CCA GTA AAA TAT GGA CGA CGA TGA CAA GTG AT -3’	Marker for GA
GA-Sp-5’	5’- TAA AAG AGG AAG GAA ATA ATA AAT GGC TGG GAG CTC TCC GGA TCC AA -3’	Marker for GA
GA-Sp-3’	5’- TCT ACT AAA ACA ATT CAT CCA GTA AAA TAT ATG CTC CTC TAG ACT CGA GGA A -3’	Marker for GA

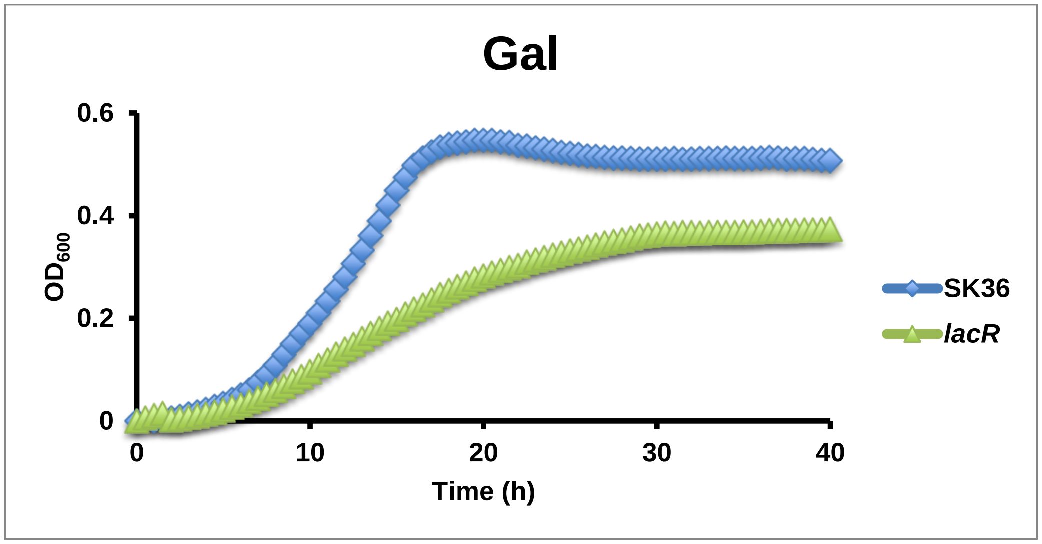
GA-Seq-5’	5’- TAG CCA TTT ATT ATT TCC TTC CTC TT -3’	Sanger sequencing
GA-Seq-3’	5’- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA -3’	Sanger sequencing
Ssa_1927-5'	5’- GGA GTT GCT TCG TGA ACA GCA A -3’	Sanger sequencing
Ssa_1927-3'	5’- CCG AGT TCA ACT CAG AGA AAA TCA A -3’	Sanger sequencing
Ssa_1918-500-5'	5’- CGT TGA AGC TGT TGT TGC TAA CA -3’	Sanger sequencing
Ssa_1918-840-3'	5’- CAG TTG AGT GAG CCA TGG AAC CAA -3’	Sanger sequencing
Ssa_alkD-5'	5’- GAC GGC AAG CGT CTC GT -3’	Sanger sequencing
Ssa_alkD-3'	5’- CCT TCC AGC CAA ACC GAG AA -3’	Sanger sequencing
Ssa_1885-5'	5’- ATC CAG GCA CTA CAC AAT AAG TTA A -3’	Sanger sequencing
Ssa_1885-3'	5’- AAG GCA TTT TTT ATC CTC CAT TTT ATA AA -3’	Sanger sequencing
Ssa_1885-607	5’- GCT AAT AGT AAA AAA GAT GAT AGA GTG AGT CAT -3’	Sanger sequencing
Ssa_1885-1485Rv	5’- GAA TAA CGA TTT CTG CTT TTC CAC ATT ATA GTA -3’	Sanger sequencing
Ssa_rpsD-492	5’- GAC GGC AAA CGC GTT GA -3’	Sanger sequencing
Ssa_galE1-300Rv	5’- CTC CCA GGG TCC CCA AA -3’	Sanger sequencing
Ssa_citB-5'	5’- CAC GGG TTT CTT TGG TAT GAA TGT -3’	Sanger sequencing
Ssa_citB-70Rv	5’- AGG CTT TTT CAA GGT CTA CAT ATT GG -3’	Sanger sequencing

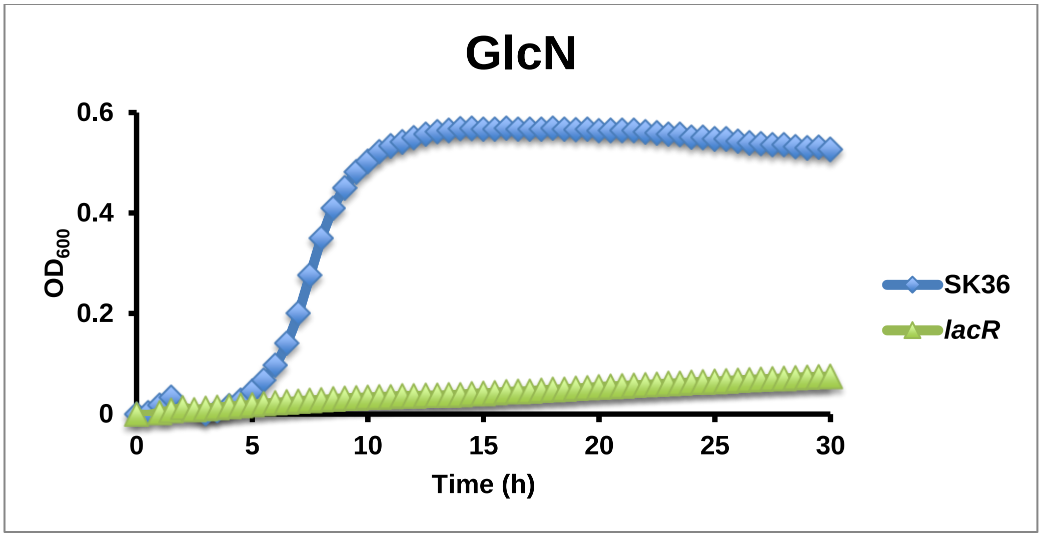
Ssa_ccpA-1	5’- GCG GTC TAC TTC ATG AGC T -3’	Deleting *ccpA*
Ssa_ccpA-2GA	5’- GCC ATT TAT TAT TTC CTT CCT CTT TTA CTG TGT CGT CTG TGT TCA TAT AGT -3’	Deleting *ccpA*
Ssa_ccpA-3GA	5’- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GCA TTA GCG AGC GTA AAT CAA CT -3’	Deleting *ccpA*
Ssa_ccpA-4	5’- TCC TCA TAG GAC GGG AAA AAG AA -3’	Deleting *ccpA*
Ssa_arcA-1	5’- ATC ATG ATT ACC AAG GAG CAT TAT CAA T -3’	Deleting *arcA*
Ssa_arcA-2GA	5’- GCC ATT TAT TAT TTC CTT CCT CTT TTA CAG TAC ATG GTT AAT ATT AGA ATA TTT TGA AT -3’	Deleting *arcA*
SK36_arcA-3GA	5’- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GTC TAT GCC ATT TGA ACG TGA AGA -3’	Deleting *arcA*
Ssa_arcA-4	5’- CTG CTT GGT CAA AAT GAC GAG CA -3’	Deleting *arcA*
Ssa_arcA-S	5’- TGG CAA TAT CGT AGC TGC TG -3’	RT-qPCR
Ssa_arcA-AS	5’- TGT TGC GGT CAT ACA CCA CT -3’	RT-qPCR
Ssa_1176-S	5’- AAC CAG CGG TTT TAT TGG TG -3’	RT-qPCR
Ssa_1176-AS	5’- CAT TGG CAC ATC TTC ACC TG -3’	RT-qPCR
Ssa_1221-S	5’- CTT CCT CGT TGC TGC TAA CC -3’	RT-qPCR
Ssa_1221-AS	5’- GGA AGC GAG CTG AGT CAA GT -3’	RT-qPCR
Ssa_1137-S	5’- CGG AGA GCT GAA GAA GCA GT -3’	RT-qPCR
Ssa_1137-AS	5’- CCT TCT CAG CCT CAT CCT TG -3’	RT-qPCR
Ssa_0342-S	5’- CTT GGC AGG TCC TAC TGA GC -3’	RT-qPCR

Ssa_0342-AS	5’- AGT GTC CAT TGG GAA ACG AG -3’	RT-qPCR
Ssa_spxB-S	5’- ATC ACT CAA CAC CGT CCA CTT CCA -3’	RT-qPCR
Ssa_spxB-AS	5’- TCT TCC AAG AAG AGG CGG AAT GGT -3’	RT-qPCR
Ssa_nox-S	5’- GAT ACT TGC TTG GCG GGT TA -3’	RT-qPCR
Ssa_nox-AS	5’- GGC CAA AGG CTA ATT GAA CA -3’	RT-qPCR
Ssa_ackA-S	5’- GAC CCA GCC ATC ATT CCT TA -3’	RT-qPCR
Ssa_ackA-AS	5’- AAG CAA GCC AGA TTC ACG AT -3’	RT-qPCR
Ssa_pta-S	5’- GCT CAG CTA TTC GAC CAA GG -3’	RT-qPCR
Ssa_pta-AS	5’- CAA ACT GCA ATT CAC CAT CG -3’	RT-qPCR
Ssa_pykF-S	5’- AAC GGC CAT GTT CAA TTG TT -3’	RT-qPCR
Ssa_pykF-AS	5’- TAC CGT CAG CAG CTT CAA TG -3’	RT-qPCR

Fig. S1. Growth curves of the *lacR* mutant with a deficiency in glucose-PTS (*manL*Q217*). The wild type SK36 (MMZ1896) and the *lacR* mutant strain were cultured in BHI till exponential phase (OD_{600} = 0.5), diluted 100-fold into FMC media supplemented with 10 mM glucose (Glc), galactose (Gal), GlcN or GlcNAc, covered with 60 µL of mineral oil, and then monitored for growth on a Bioscreen C system maintained at 37°C.



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0.2
0
OD600
0
5
10
15
20
25
30
Time (h)
SK36
lacR

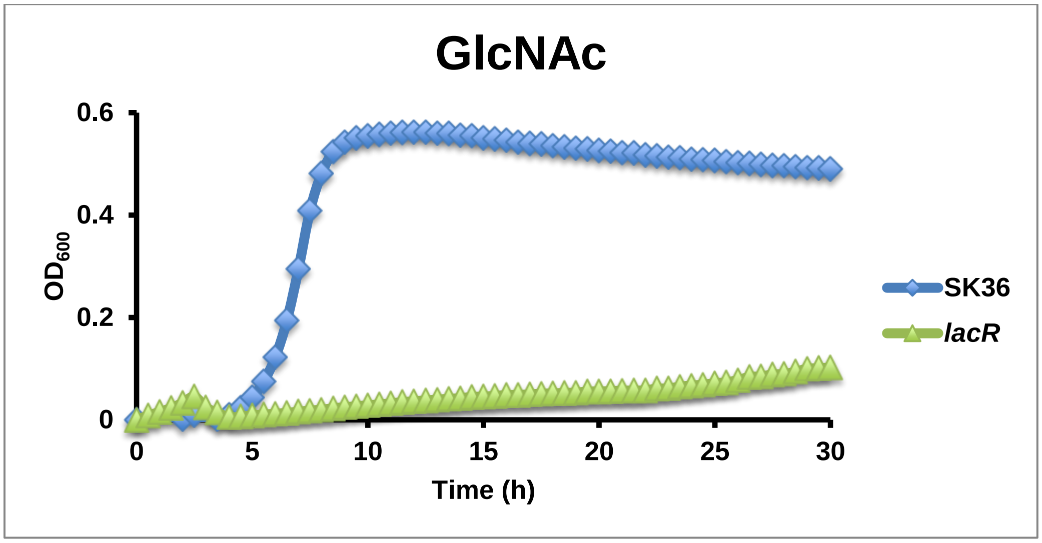
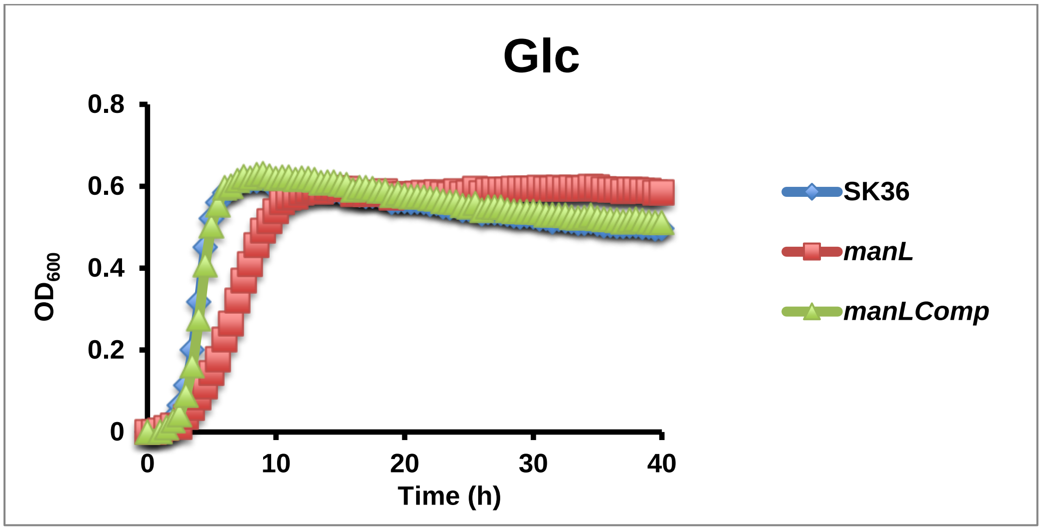
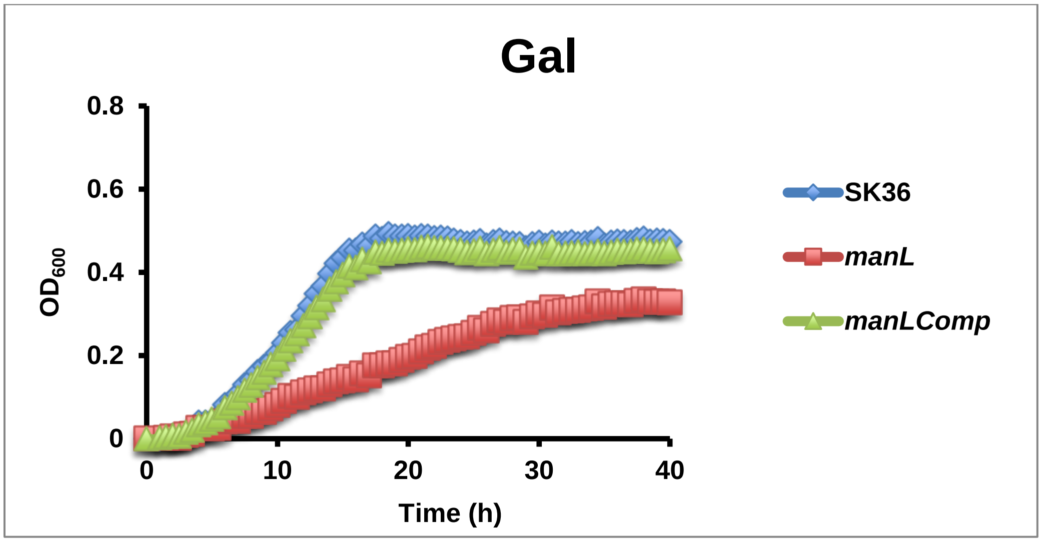
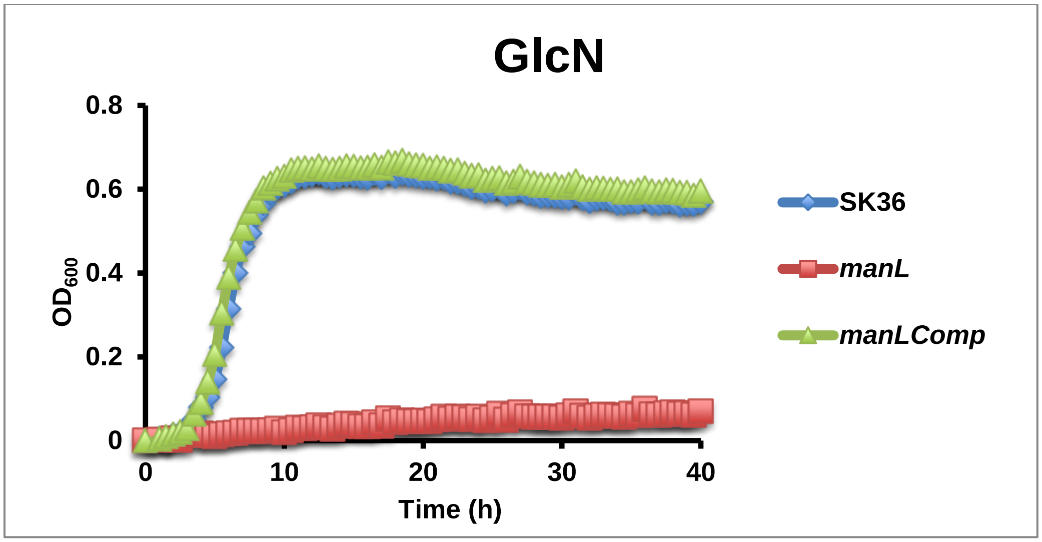

GlcNAc
0.6
0.4
0.2
0
OD600
0
5
10
15
20
25
30
Time (h)
SK36
lacR

Fig. S2. Growth curves of SK36, the *manL* mutant and the *manLComp* strain. Strains were each cultured in BHI till exponential phase (OD_{600} = 0.5), diluted 100-fold into FMC media supplemented with 10 mM glucose (Glc), galactose (Gal), GlcN or GlcNAc for growth monitoring.






GlcN
0.8
0.6
0.4
0.2
0
OD600
0
10
20
30
40
Time (h)
SK36
manL
manLComp

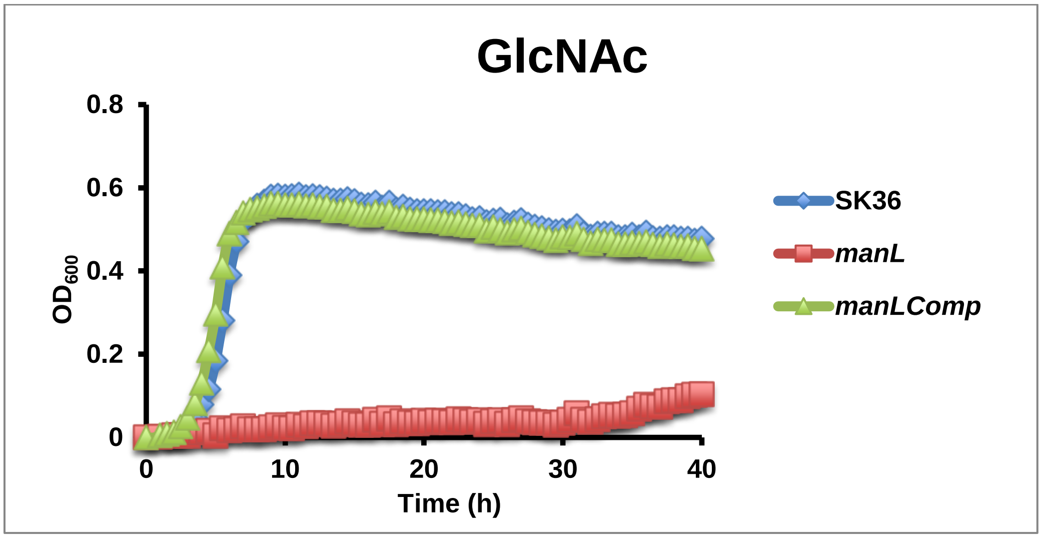

GlcNAc
0.8
0.6
0.4
0.2
0
OD600
0
10
20
30
40
Time (h)
SK36
manL
manLComp

Fig. S3. Antagonism of *S. mutans* (top) and H_2O_2 production (below) by strains SK36, *manL* mutants and *manLComp*.

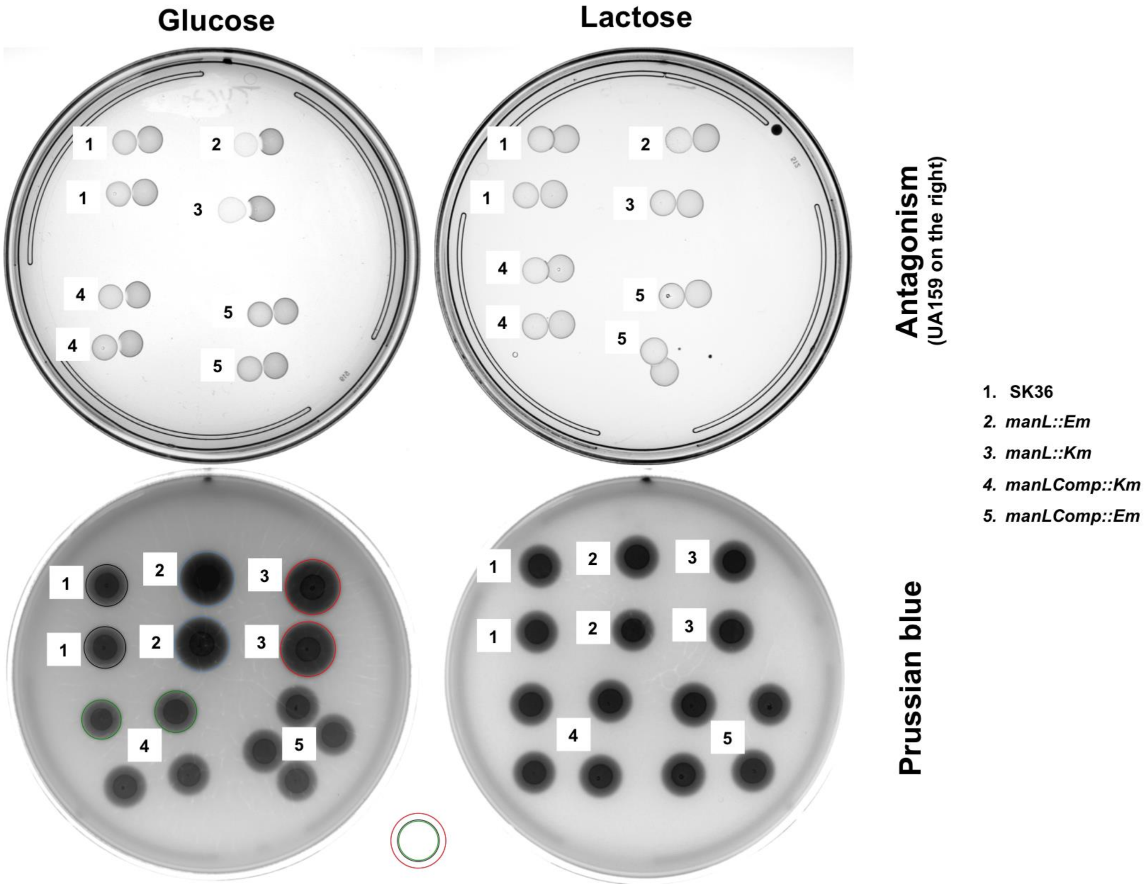


Fig. S4. RT-qPCR showing levels of transcripts of genes belonging to the central carbon metabolism. Strains SK36, *manL* mutant, and *manLComp* were cultivated in TY supplemented with 20 mM glucose (G) or 10 mM lactose (Lac) till exponential phase (OD_{600} = 0.5) before harvesting. Total RNAs were subjected to reverse transcription with gene-specific primers, followed by quantitative PCR (qPCR). Transcript levels are calculated on the basis of ΔCq relative to that of the housekeeping gene *gyrA*. Each sample was represented by at least three biological replicates and assayed at least twice in qPCR. The bars represent the means and error bars the standard deviations.

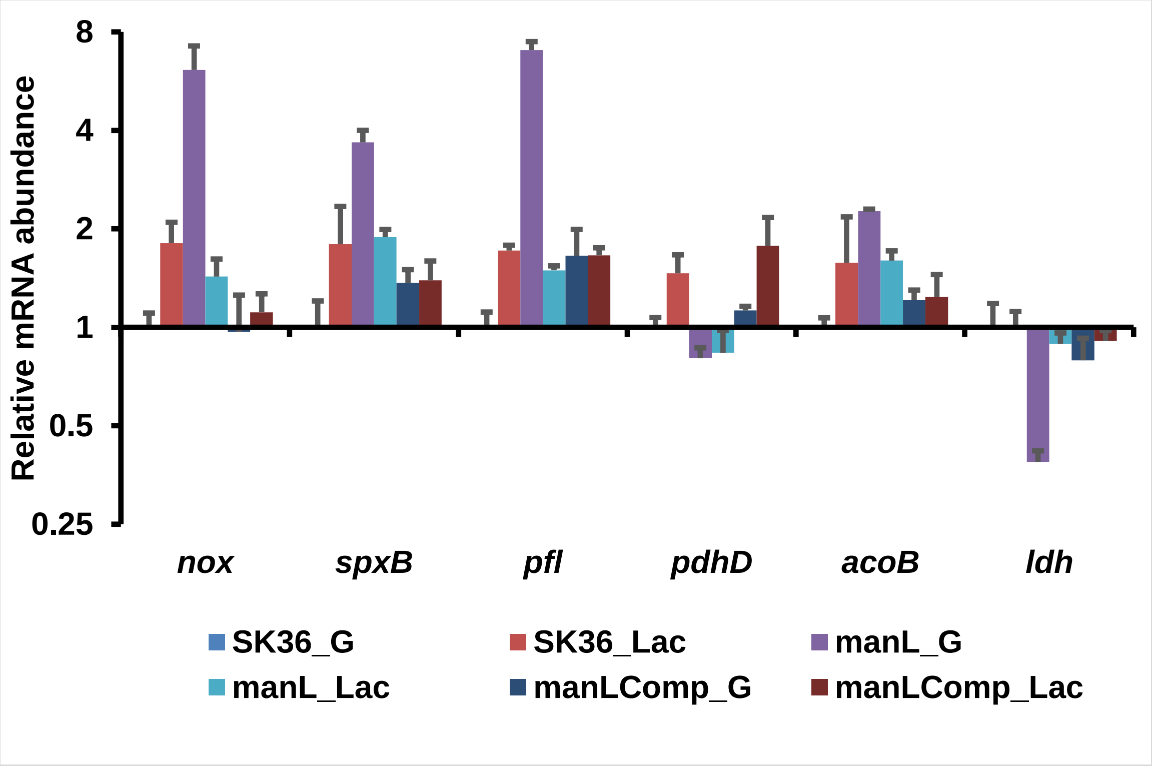
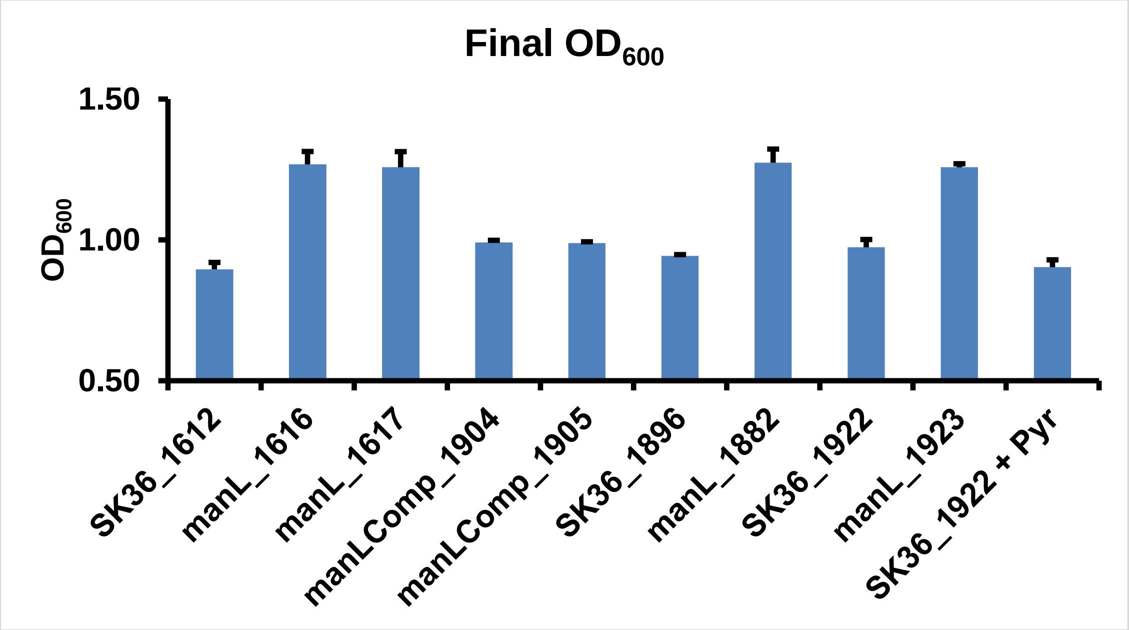
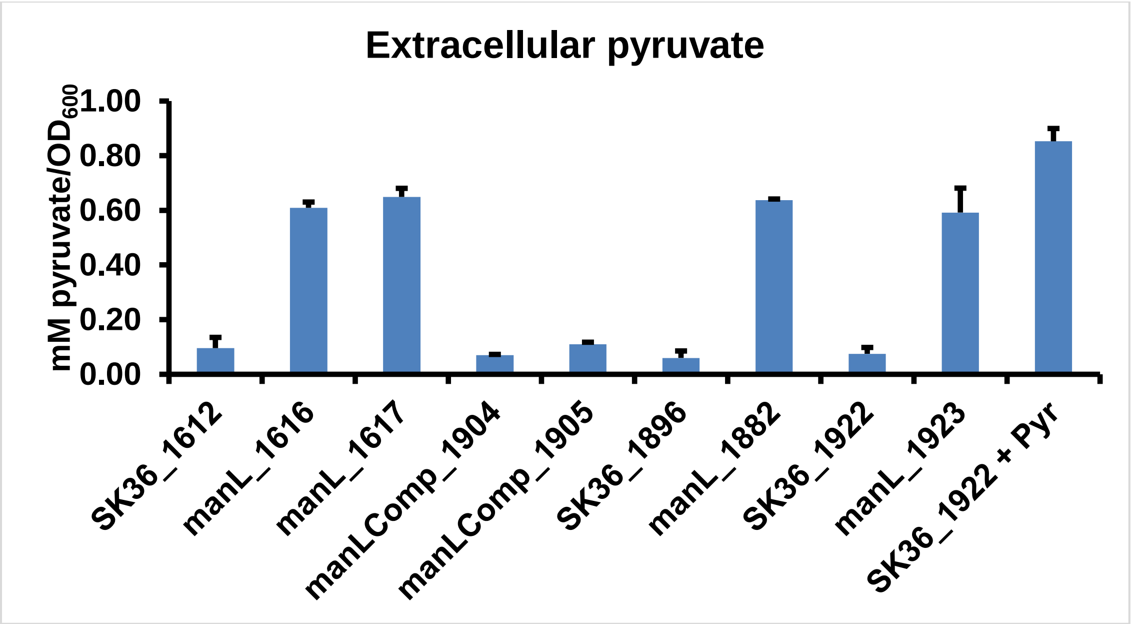


Fig. S5. Characterization of three SK36 stocks and their respective mutant derivatives: SK36_1612, manL_1616 (Em), manL_1617 (Km), manLComp_1904 (Km), manLComp_1905 (Em); SK36_1896, manL_1882 (Km); SK36_1922, manL_1923 (Km). Strains were each cultured in TY-Glc (A to E) or BHI (C, E, F, G) overnight (>20 h) and measured for final OD_{600} (A), extracellular pyruvate (B), final pH (C), eDNA release as relative fluorescence units (D), growth curves (E) after dilution of BHI cultures (at 1:100) into fresh TY-Glc medium, CFU enumeration of BHI cultures (F), and H_2O_2 production on TY-Glc/Prussian blue plates (G). 5 mM of pyruvate was added to one set of SK36_1922 TY-Glc cultures (A to D).

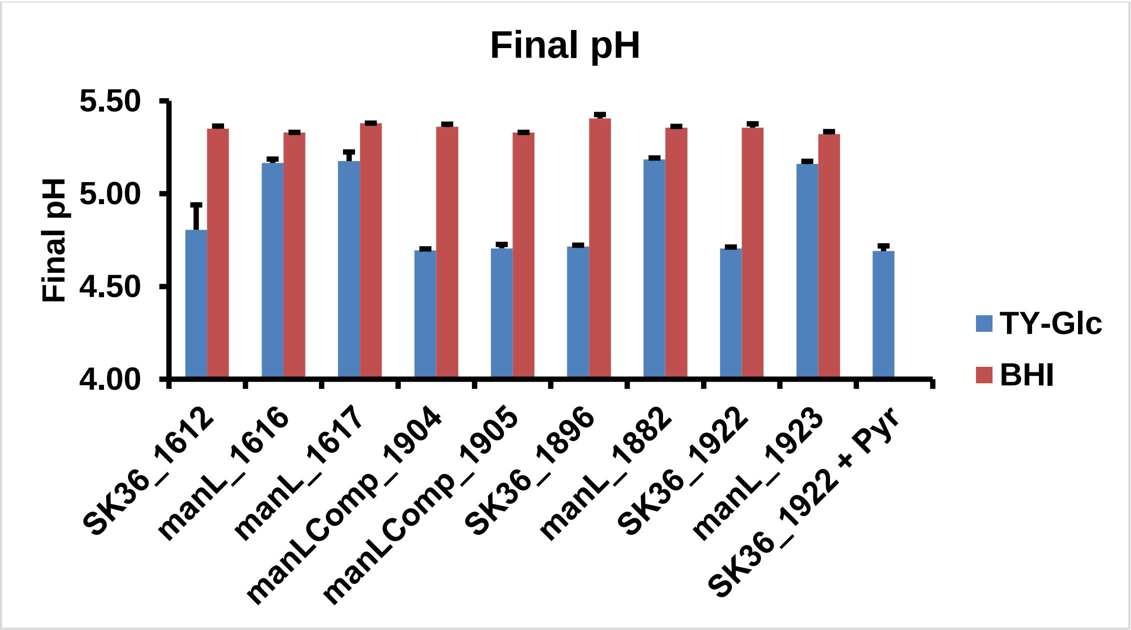
A



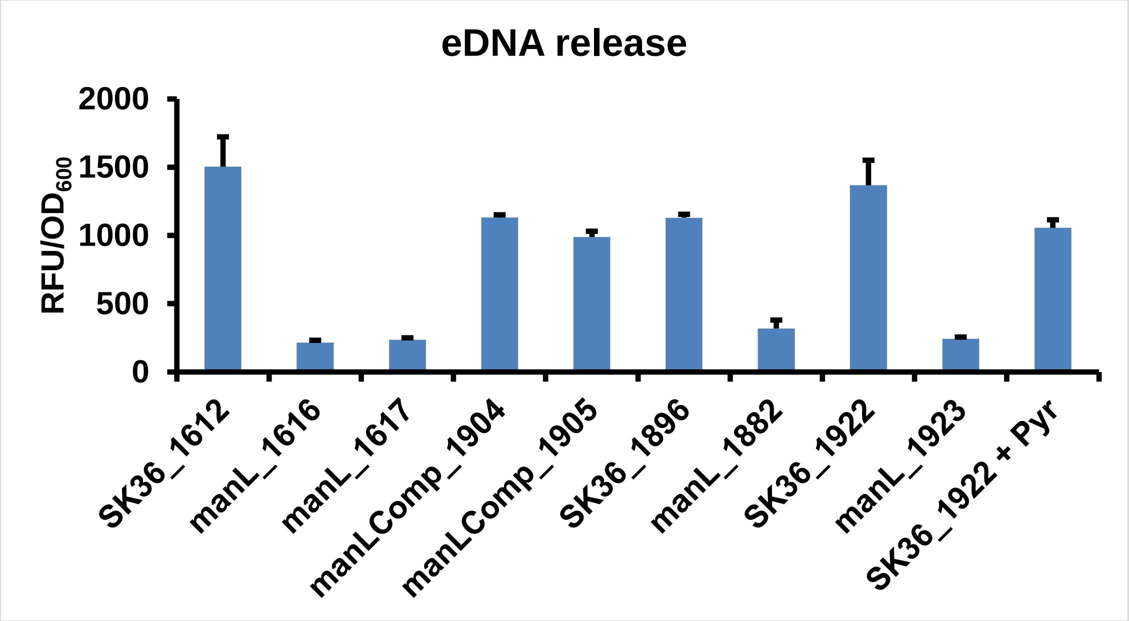
B


Extracellular pyruvate
mM pyruvate/OD600
1.00
0.80
0.60
0.40
0.20
0.00
SK36_1612
manL_1616
manL_1617
manLComp_1904
manLComp_1905
SK36_1896
manL_1882
SK36_1922
manL_1923
SK36_1922 + Pyr

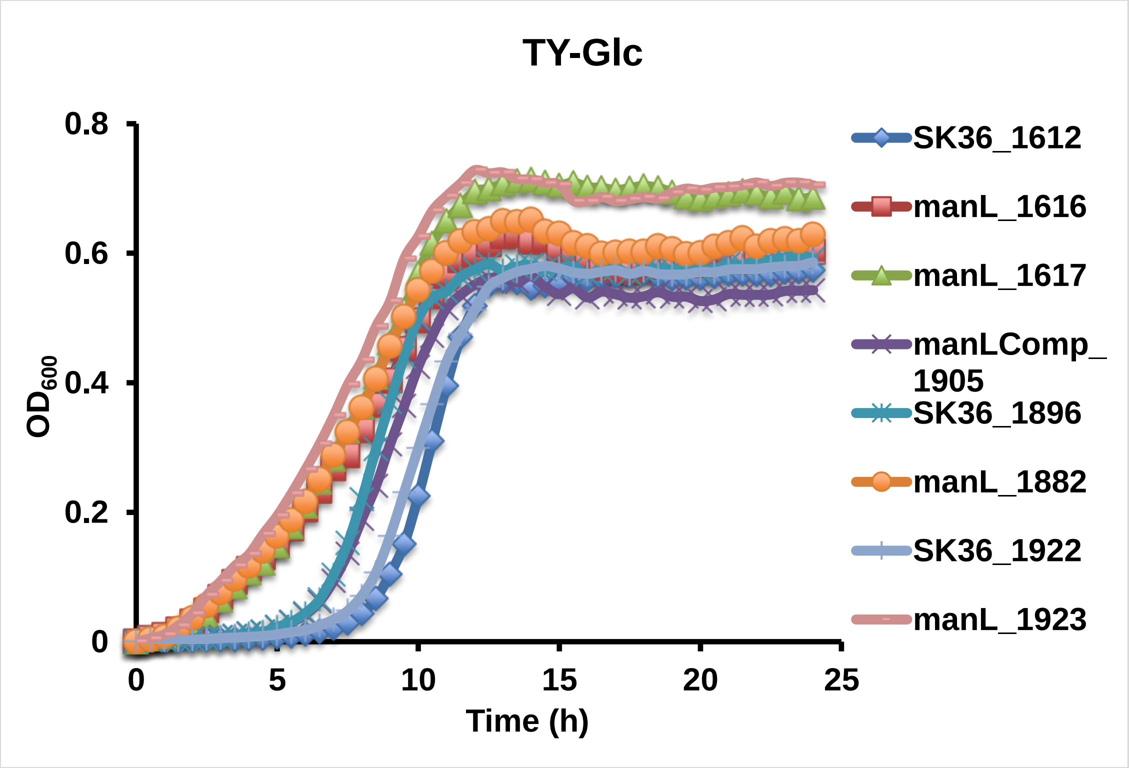
C


Final pH
Final pH
5.50
5.00
4.50
4.00
SK36_1612
manL_1616
manL_1617
manLComp_1904
manLComp_1905
SK36_1896
manL_1882
SK36_1922
manL_1923
SK36_1922 + Pyr
TY-Glc
BHI

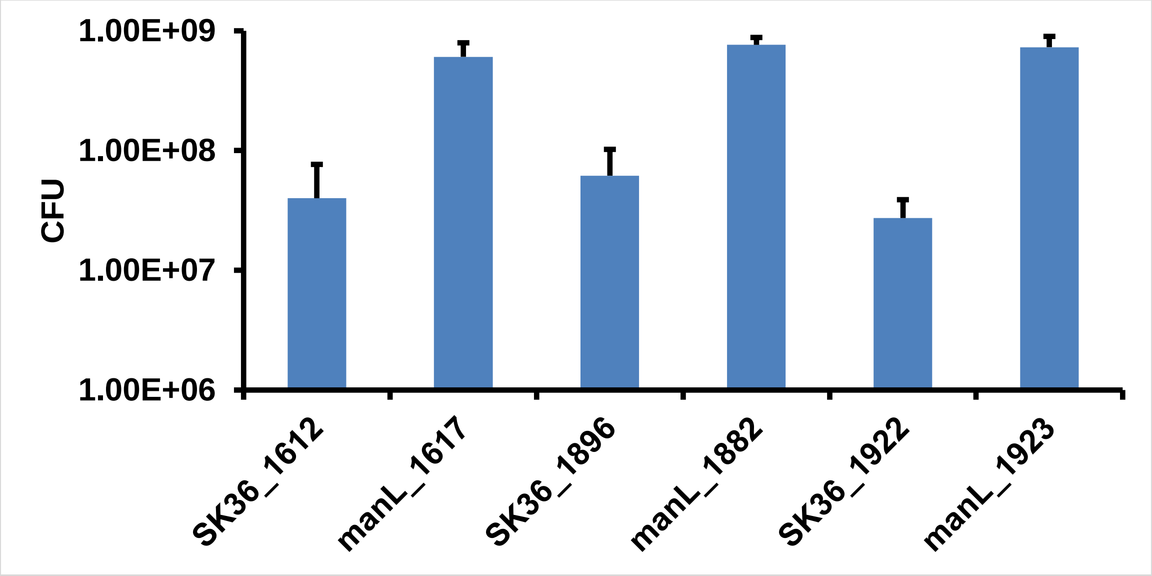
D


eDNA release
RFU/OD600
2000
1500
1000
500
0
SK36_1612
manL_1616
manL_1617
manLComp_1904
manLComp_1905
SK36_1896
manL_1882
SK36_1922
manL_1923
SK36_1922 + Pyr

E


TY-Glc
OD600
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
Time (h)
SK36_1612
manL_1616
manL_1617
manLComp_1905
SK36_1896
manL_1882
SK36_1922
manL_1923

F


1.00E+09
1.00E+08
1.00E+07
1.00E+06
CFU
SK36_1612
manL_1617
SK36_1896
manL_1882
SK36_1922
manL_1923

G

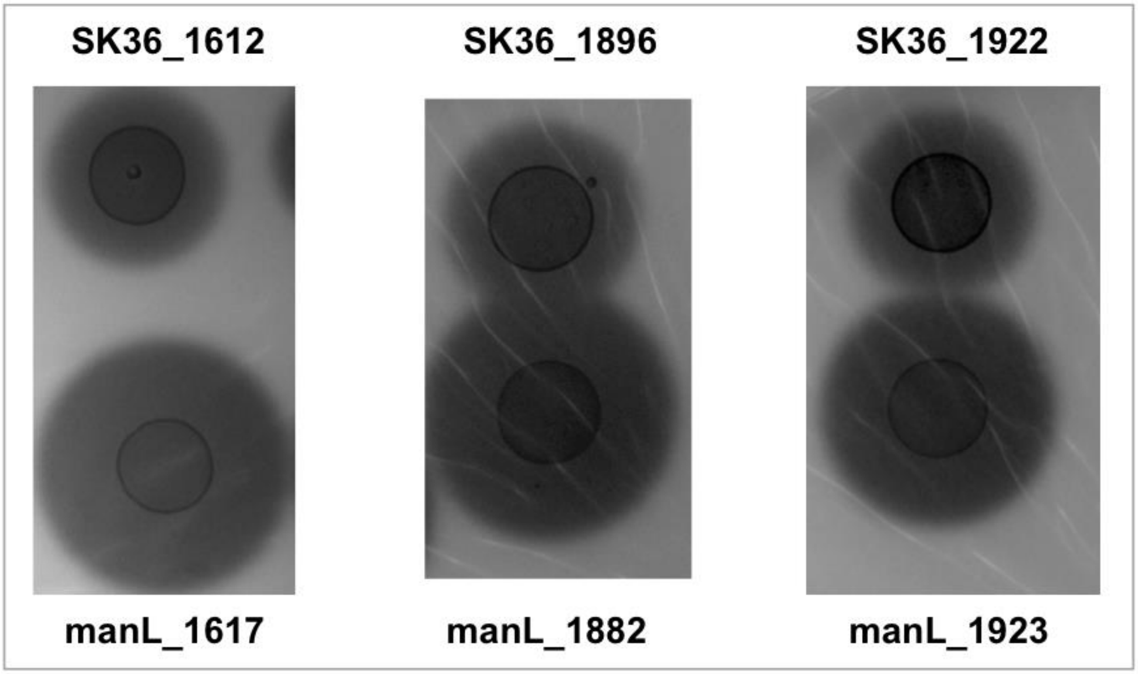

SK36_1612
SK36_1896
SK36_1922
manL_1617
manL_1882
manL_1923

Fig. S6. Results of multi-locus sequence typing (MLST) analysis performed using genome sequences of SK36 from GenBank (SK36), ATCC stock BAA-1455 (MMZ1922), *S. mutans* UA159, and a collection of clinical isolates from the genus of Streptococcus that were recently sequenced (1). The final alignment was visualized using the Ape package in the R environment.

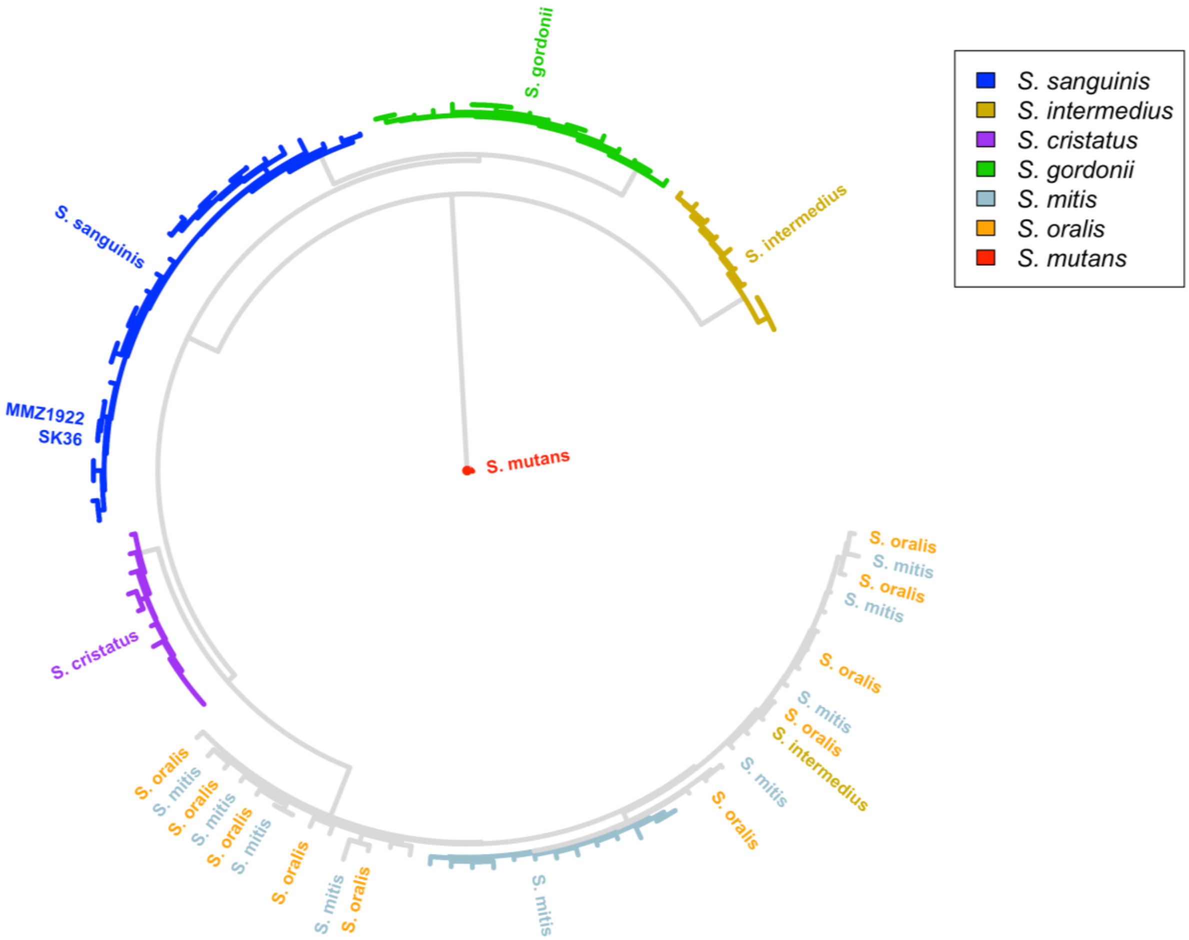
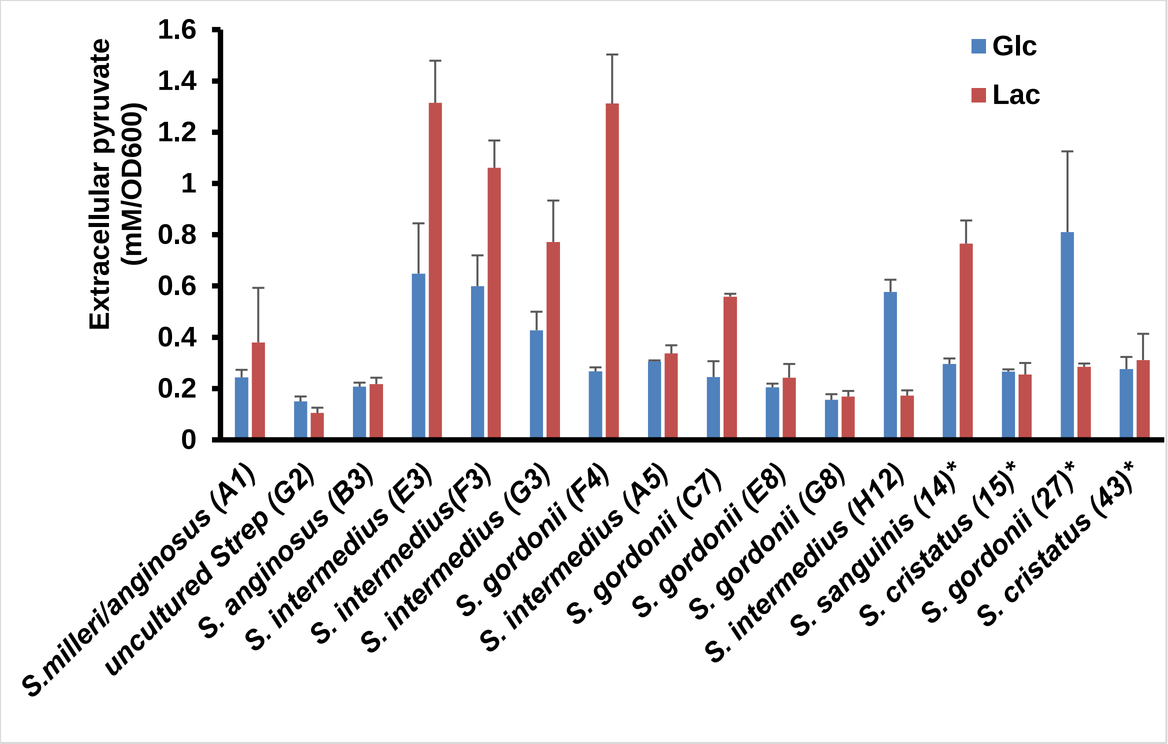
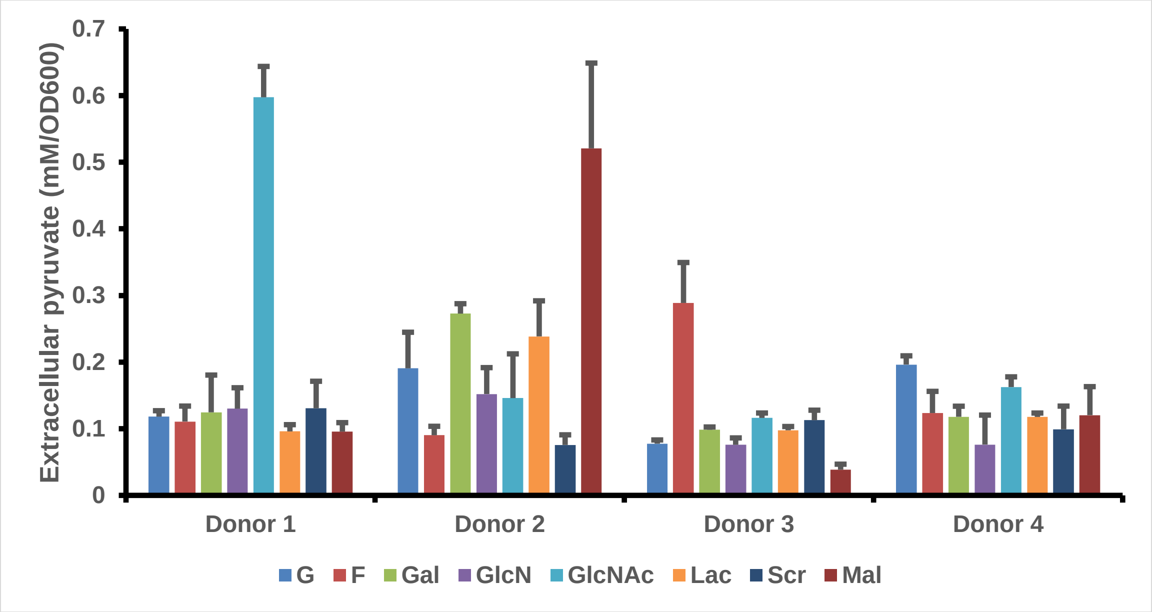


Fig. S7. Pyruvate levels measured in cultures of clinical isolates of commensal bacteria (A) and whole saliva sample (B). Clinical isolates were inoculated into TY media supplemented with 20 mM of glucose (Glc) or 10 mM of lactose (Lac) and incubated for 20 h at 37°C in an aerobic atmosphere maintained with 5% CO_2. Asterisks indicate strains isolated from only caries-free subjects (1), whereas the rest are derived from both caries-free and caries-active patients (2). The donor saliva was similarly cultured in a biofilm medium (3) supplemented with 20 mM of glucose, fructose, galactose, GlcN, GlcNAc, or 10 mM of lactose, sucrose, or maltose. The cultures were harvested by centrifugation (room temperature, 16,000× *g*, 2 min) before the supernates were removed for pyruvate assay. Each sample was represented by three biological repeats and the results (mM/OD_{600}) are each the average and standard deviation (error bars).

A



B



Reference

1. Velsko IM, Chakraborty B, Nascimento MM, Burne RA, Richards VP. 2018. Species designations belie phenotypic and genotypic heterogeneity in oral streptococci. mSystems 3:e00158-18.
2. Garcia BA, Acosta NC, Tomar SL, Roesch LFW, Lemos JA, Mugayar LRF, Abranches J. 2021. Association of *Candida albicans* and Cbp+ *Streptococcus mutans* with early childhood caries recurrence. Scientific reports 11:10802.
3. Loo CY, Corliss DA, Ganeshkumar N. 2000. *Streptococcus gordonii* biofilm formation: identification of genes that code for biofilm phenotypes. J Bacteriol 182:1374-82.