SUPPORTING INFORMATION

Supporting Figure Legends

Figure S1: Cell morphology of suppressor mutants was analyzed by electron microscopy. *A. oris* cells of various strains (**A**-**P**) were immobilized on nickel-coated carbon grids and stained with 1% uranyl acetated prior to microscopic examination. Scale bars indicate 0.5 μm.

Figure S2: Transposon insertion at ANA_1289 caused a polar effect on downstream genes *acaC* and *lcp.* Expression levels of ANA_1289 (**A**), *acaC* (ANA_1291) (**B**), *lcp* (ANA_1292) (**C**) and *srtC2* (**D**) in wild-type MG-1 and two suppressor mutants Tn5-2 and Tn5-12 were analyzed by real-time PCR (RT-PCR). The data were presented here as average of three independent experiments and expressed relative to the transcript abundance of MG1, which was arbitrarily assigned a value of 1. All measured transcripts were normalized by using the 16S rRNA gene as a housekeeping control. Asterisk (*) indicates significance with $P < 0.001$ by Student's *t* test.

Figure S3: Expression of AcaC in representative strains in each suppressor group was analyzed by immunoblotting. Cells of various strains grown to mid-log phase were normalized by optical density. Culture supernatant (S), cell wall (W), membrane (M), and cytoplasmic (C) fractions were obtained by cell fractionation. Equivalent protein samples were separated on 4-12% Tris-Glycine gradient gels and detected by immunoblotting with antibodies against AcaC (α-AcaC) and SrtC2 (α-SrtC2). Molecular mass markers are shown in kDa.

Supporting Table S1: Bacterial strains and plasmids used

Strain & Plasmid	Description	Reference
Strain		
A. oris MG-1	Parental strain	(Mishra *et al.*, 2007)
A. oris CW1	Δ*galK*; an isogenic derivative of MG-1	(Mishra *et al.*, 2010)
A. oris AR4	Δ*fimA*; an isogenic derivative of CW1	(Mishra et al., 2010)
A.oris WU36	Conditional *srtA* deletion mutant (Δ*srtA*), containing pTetR-Ω-SrtA	This study
A.oris WU19	Δ*srtA* containing P*rpsJ*-SrtA	This study
A.oris WU51	Deletion of *1291* (Δ*acaC*); an isogenic derivative of CW1	This study
A.oris WU70	Deletion of *1289;* an isogenic derivative of CW1	This study
A.oris WU72	Deletion of *1292;* an isogenic derivative of CW1	This study
A.oris WU73	Deletion of *acaC* and *srtA*; Δ*acaC*/Δ*srtA*	This study
A.oris WU74	Deletion of *1292* and *srtA;* Δ*1292*/Δ*srtA*	This study
A.oris WU51c	WU51 containing pAcaC$_{H6\text{-}TEV}$	This study
S. oralis 34	RPS positive	(Yoshida *et al.*, 2006)
S. oralis OC1	RPS negative	(Yoshida et al., 2006)
Plasmid		
pJRD215	*Actinomyces*/*E. coli* shuttle vector, Kan^R, Str^R	(Yeung & Kozelsky, 1994)
pRMC2	a tetracycline-inducible expression vector	(Corrigan & Foster, 2009)
pJRD-Sm	pJRD215 lacking the kanamycin-resistance cassette	This study
pTetR-SrtA	pJRD-Sm expressing SrtA under the control of the tetracycline-inducible system	This study
pTetR-R*-SrtA	A derivative of pTetR-SrtA with a riboswitch element incorporated upstream of the start codon ATG	This study
pTetR-Ω-SrtA	a derivative of pTetR-R*-SrtA with the fimQ promoter inserted upstream of the P$_{xylR}$ promoter	This study
P*rpsJ*-SrtA	pJRD215 expressing SrtA under the control of the *rpsJ* promoter	This study
P*rpsJ*-SrtA-C216A	P*rpsJ*-SrtA with C216A mutation in SrtA	This study
P*ribo*-SrtA-C216A	a riboswitch element incorporated into P*rpsJ*-SrtA-C216A	This study
pAcaC	pJRD215 expressing AcaC	This study
pAcaC$_{\Delta LPXTG}$	pJRD215 expressing AcaC lacking the LPXTG motif	This study
pAcaC$_{\Delta cws}$	pJRD215 expressing AcaC lacking the cell wall sorting signal (CWS)	This study
pAcaC$_{H6\text{-}TEV}$	pJRD215 expressing AcaC with a TEV cleavage site, followed by a 6xHis-tag and the CWS	This study

pCWU2	Derivative of pHTT177, expressing GalK under the control of the *rpsJ* promoter	(Mishra et al., 2010)
pCWU3	Derivative of pHTT177, expressing mCherry under the control of the *rpsJ* promoter	(Wu & Ton-That, 2010)
pCWU2-Δ*srtA*	An allelic replacement vector of *srtA* using pCWU2	This study
pCWU3-Δ*srtA*	An allelic replacement vector of *srtA* using pCWU3	This study
pCWU2-Δ*1289*	An allelic replacement vector of *1289* using pCWU2	This study
pCWU2-Δ*1292*	An allelic replacement vector of *1292* using pCWU2	This study
pCWU2-Δ*acaC*	An allelic replacement vector of *acaC* using pCWU2	This study

Supplementary Table S2: Bacterial strains and plasmids used

Primer	Sequence[(a)]	Used for
srtA-up-F	GGCGGAATTCATCGTCTCGGCGATCTACGC	pCWU2-Δ*srtA*
srtA-up-R	GGCGGGTACCTCGCACAAGACCTCCTCTAGTCA	pCWU2-Δ*srtA*
srtA-dn-F	GGCGGGTACCGGGGGTCAACTGATGTACGGCTTCA	pCWU2-Δ*srtA*
srtA-dn-R	GGCGTCTAGATAGGACTGGCGCAGCCACTTCT	pCWU2-Δ*srtA*
prpsJ-F	GGCGGGATCCCGCCCGAGCGCGGGGACCAGT	P*rpsJ*-SrtA
prpsJ-R	GGCGCATATGGGCGCCTAACCTCTCTTGTACTTG	P*rpsJ*-SrtA
com-SrtA-F	GGCGGATATGATGACTAGAGGAGGTCTTGTGCGAC GGCGGA	P*rpsJ*-SrtA
com-SrtA-R	GGCGGAATTCGCCGGAGGCGCCGTCGGGGAAG	P*rpsJ*-SrtA
kpnI-tetR-R	GGCGGGTACCTTTAAGACCCACTTTCACATTTAAG	pTetR-SrtA
EcoRI-tetR-F	GGCGGAATTCTCAAGCTTATTTTAATTATACTCTATC	pTetR-SrtA
EcoRI-srtA-F(rbs)	GGCGGAATTCGAGGAGGGGCGATGACTAGAG	pTetR-SrtA
HindIII-srtA-R	GGCGAAGCTTCCGGCAGGTGCCGCCAGATGAAG	pTetR-SrtA
Ribo-srtA-F	ATGCCCTTGGCAGCACCCTGCTAAGGAGGCAACAAGATGACTAGAGGAGGTCTTGTGCGA	pTetR-R*-SrtA
Ribo-tet-R	CAAGACGATGCTGGTATCACCGGTACCTATAGTGAGTCGTATAGAATTGGACATCATCAGGCTAG	pTetR-R*-SrtA
PfimQ-tetR-R	GGCGGAATTCGCCGATGGATTCCGATCATGAG	pTetR-Ω-SrtA
PfimQ-tetR-F	GGCGAGATCTGATTCCTGCGCCCAGGAAAGTG	pTetR-Ω-SrtA
pJRD215-str-F	GTGCTTGCGGCAGCGTGAAGCTAGCTTGG	pJRD-Sm
pJRD215-str-R	CGAGTT CTTCTGAGCG GGACTCTGG	pJRD-Sm
srtA(C216A)-F	GCTCACGGATCGACCGCCGGTG	P*rpsJ*-SrtA-C216A
srtA(C216A)-R	GGTGGTCAGCGTGATGTACCGGTC	P*rpsJ*-SrtA-C216A
Ribo-srtA-F	ATGCCCTTGGCAGCACCCTGCTAAGGAGGCAACAAGATGACTAGAGGAGGTCTTGTGCGA	P*rpsJ*-SrtA-C216A
Ribo-srtA-R	CAAGACGATGCTGGTATCACCGGTACCTATAGTGAGTCGTATTTGTACTTGCTTCTGGCCGGTCCAC	P*rpsJ*-SrtA-C216A
AcaC-up-F	GGCGGAATTCCCTGGGACTGCCGGACGAGTCCTA	pCWU2-Δ*acaC*
AcaC-up-R	GGCGGGTACCTCCAAGACGCATGAGTGCTCCTAG	pCWU2-Δ*acaC*
AcaC-dn-F	GGCGGGTACCACCGACGCCACCCCGGCGGCCTCCA	pCWU2-Δ*acaC*
AcaC-dn-R	GGCGTCTAGATTCTCCTGGGTGATCTTGAAGCTG	pCWU2-Δ*acaC*
1289upF	GGCGGAATTCTCTCACGGGCGTAGACGGTGATGGAC	pCWU2-Δ*1289*
1289upR	GGCGGGTACCCGGTGGCGAGCCACTGGCCGTTGAC	pCWU2-Δ*1289*
1289dnF	GGCGGGTACCATCGTGCGCAATGTGGACGCCTTCT	pCWU2-Δ*1289*
1289dnR	GGCGTCTAGATCCAGCTCCATGCCCTTGCTGTTGAT	pCWU2-Δ*1289*

1292upF	AAAGGTACCGGTCAACGCCTACTACAACCCGACCA	pCWU2-Δ*1292*
1292upR	AAAGAATTCCGCTGTGCTCGTCGTGGGAGGTGGA	pCWU2-Δ*1292*
1292dnF	AAAGAATTCTAGTCACTCGCCGGAGAGACCCCGA	pCWU2-Δ*1292*
1292dnR	AAATCTAGAACACGTCATGTGTCGTCCCCCGGAGC	pCWU2-Δ*1292*
com-AcaC-F	GGCGCATATGCAAGCACCGGGACGCGGGTACGGAGC	pAcaC
com-AcaC-R	GGCGGAATTCAGTGTTTCCAGTCAGACTGGAAGGA	pAcaC
$AcaC_{\Delta LPXTG}$-F	GCCAACGGCCTGCTCTTCGGCG	$pAcaC_{\Delta LPXTG}$
$AcaC_{\Delta LPXTG}$-R	GGGCTTGCCGGAGGTGGAGGCCGC	$pAcaC_{\Delta LPXTG}$
$AcaC_{\Delta cws}$-F	GGGCTTGCCGGAGGTGGAGGCCGCCGG	$pAcaC_{\Delta CWS}$
$AcaC_{\Delta cws}$-R	TGATGATCGCCTCCTTCCAGT	$pAcaC_{\Delta CWS}$
$AcaC_{6H\text{-}TEV}$-F	CACCACCACCACCACCACGGCAAGCCCCTGGCCCAGACCG	$pAcaC_{H6\text{-}TEV}$
$AcaC_{6H\text{-}TEV}$-R	CGATTGGAAGTAGAGGTTCTCGGAGGTGGAGGCCGCCGGGGTGGCGTC	$pAcaC_{H6\text{-}TEV}$
RT-16s-F	GTCGCTAGTAATCGCAGATCAG	16S rRNA, RT-PCR
RT-16s-R	GGTGTTGCCGACTTTCATG	16S rRNA RT-PCR
RT-srtC2-F	CCAGTACAACCAGTCGAAGG	*srtC2*, RT-PCR
RT-srtC2-R	GAGAGCGCGTCATTGTAGG	*srtC2*, RT-PCR
RT-1289F	GAGATCCCGCACGTCAAC	ANA_1289, RT-PCR
RT-1289R	CTTGAGAGCGATGCCCAG	ANA_1289, RT-PCR
RT-1291F	GACGGCACCTACAAGATCAC	*acaC*, RT-PCR
RT-1291R	AGGAGTCGGTCTTGCTGA	*acaC*, RT-PCR
RT-1292F	GCTCAGTTCGCCACTATCAT	*lcp*, RT-PCR
RT-1292R	AGAGGTTCAGTCCGGTGTAG	*lcp*, RT-PCR

[a] Underlined are the restriction sites in the primers.

Supporting Experimental Procedures

Plasmid construction

P*rpsJ*-SrtA, P*rpsJ*-SrtA-C216A, and P*ribo*-SrtA-C216A – The promoter region of the *rpsJ* gene and the *srtA* coding region were amplified from the MG-1 chromosome by PCR with the primer pairs rpsL-F/R and com-srtA-F/R, respectively (Table S2). PCR products were digested with appropriate restriction enzymes and ligated into the BamHI and EcoRI sites of *E. coli*/*Actinomyces* shuttle vector pJRD215 (Yeung & Kozelsky, 1994) to yield P*rpsJ*-SrtA.

To generate P*rpsJ*-SrtA-C216A, the BamHI and EcoRI fragment above was subcloned into pUC19. The generated plasmid was used as a template for site-directed mutagenesis by inverse PCR using primers srtA(C216A)-F/R as previously described (Wu *et al.*, 2012). The resulting mutagenized *srtA* fragment was cloned into pJRD215 between BamHI and EcoRI sites. The desired mutation was confirmed by DNA sequencing.

To construct P*ribo*-SrtA-C216A, the pUC19 vector harboring the *rpsJ*-SrtA-C216A fragment was used as a template for introduction of the riboswitch E* sequence(Topp *et al.*, 2010) upstream of the *srtA* coding sequence by inverse PCR using the primer pair Ribo-srtA-F and Ribo-PrpsJ-R. The generated plasmid was confirmed by DNA sequencing before it was digested with BamHI and EcoRI to release the *srtA*-containing fragment, which was then cloned into pJRD215.

pCWU2-Δ*srtA* and pCWU3-Δ*srtA* – One-kb fragments upstream and downstream of *srtA* were amplified from the MG-1 chromosome by PCR with primer sets srtAupF/srtAupR and srtAdnF/srtAdnR, respectively (Table S2). Generated fragments were digested with EcoRI/KpnI and KpnI/XbaI, respectively, and ligated into the *Actinomyces* deletion vector pCWU2 (Mishra et

al., 2010) or pCWU3 (Wu & Ton-That, 2010) precut with EcoRI and XbaI. The resulting plasmids were confirmed by colony PCR and restrictive enzyme digestion.

pAcaC, pAcaC$_{\Delta LPXTG}$ and pAcaC$_{\Delta CWS}$ – A fragment encompassing the promoter region of the *acaC* gene and its open reading frame was PCR-amplified with primers com-AcaC-F/R. The PCR product treated with NdeI and EcoRI was cloned into pJRD215 pre-treated with the same enzymes to create pAcaC. To generate pAcaC$_{\Delta LPXTG}$ *and pAcaC*$_{\Delta CWS}$, the treated *acaC* fragment above was cloned into pCWU2. The generated plasmid was used as a template for inverse PCR amplification using primers AcaC$_{\Delta LPXTG}$-F/R and AcaC$_{\Delta cws}$-F/R, respectively (Table S2). The resulting mutations were verified by DNA sequencing.

pAcaC$_{H6\text{-}TEV}$ – pAcaC was used as a template for inverse PCR amplification using primers Rev-AcaC(H6)-F (5'-CACCACCACCACCACCACGGCAAGCCCCTGGCCCAGACCG-3') and Rev-AcaC(TEV)-R(5'-CGATTGGAAGTAGAGGTTCTCGGAGGTGGAGGCCGCCGGGGTGGCGTC-3'). This strategy allows insertion of a TEV-protease cleavage site (ENLYFQS), followed by a 6xHis-Tag upstream of the AcaC LPXTG motif. The obtained PCR product was circulated to generate pAacC$_{H6\text{-}TEV}$, which was further verified by DNA sequencing.

Generation of antibodies against AcaC

For antibody production, an expression vector for a recombinant AcaC protein lacking its signal peptide and cell wall sorting signal was generated as the following. The primers AcaC$_{Ecoli}$-F (5'-aaaggatccaagatcgccgatgaccagc-3') and AcaC$_{Ecoli}$-R (5'-aaactgcagttacttgccggaggtggagg-3') were used for PCR amplification of the *acaC* coding sequence from the chromosomal MG-1 DNA. The generated amplicon was treated with BamHI and BglII and cloned into the expression vector pQE30 (QIAGEN). The recombinant plasmid was transformed into *E. coli* XL1-Blue. Purification of the recombinant AcaC protein was carried out by affinity chromatography and the purified protein was used for antibody production accordingly (Mishra et al., 2007).

Supporting References

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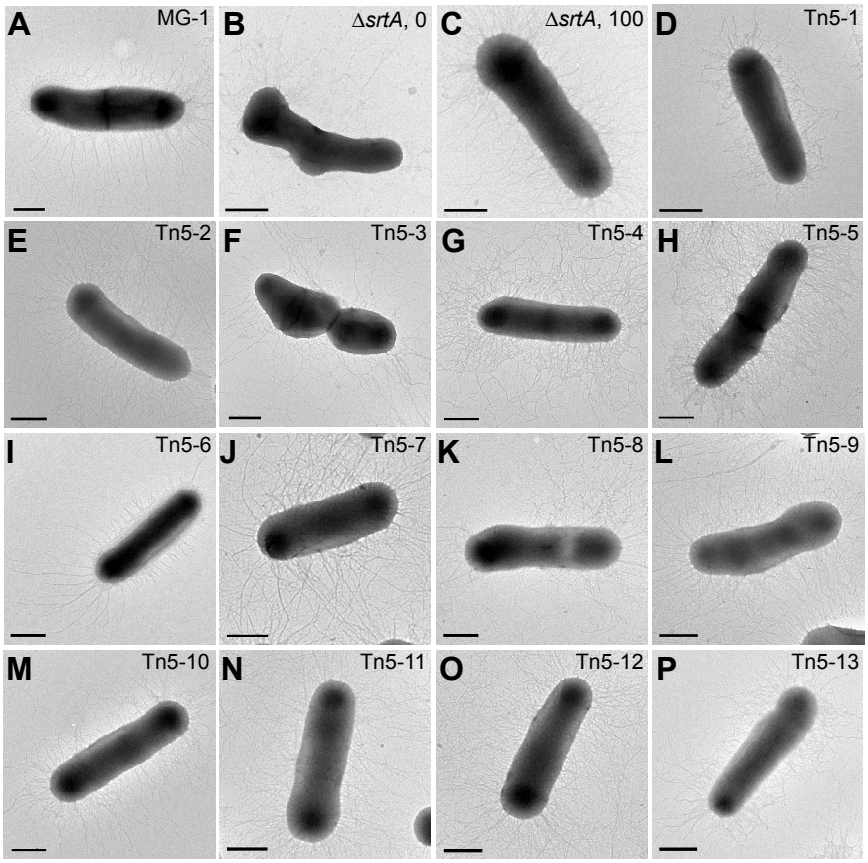

Figure S1: Wu et al.

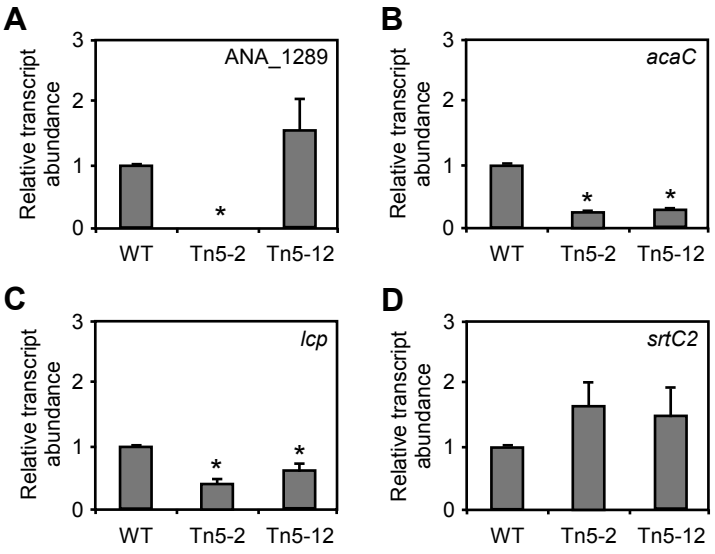

Figure S2: Wu et al.

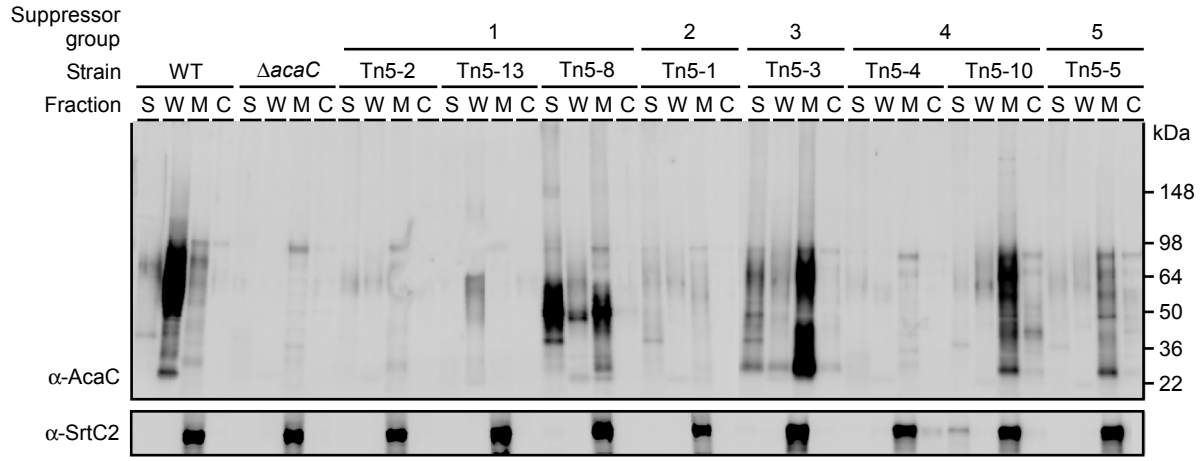

Figure S3: Wu et al.