SUPPLEMENTAL INFORMATION

SUPPLEMENTAL TABLES

Table S1. Strains used in this study.

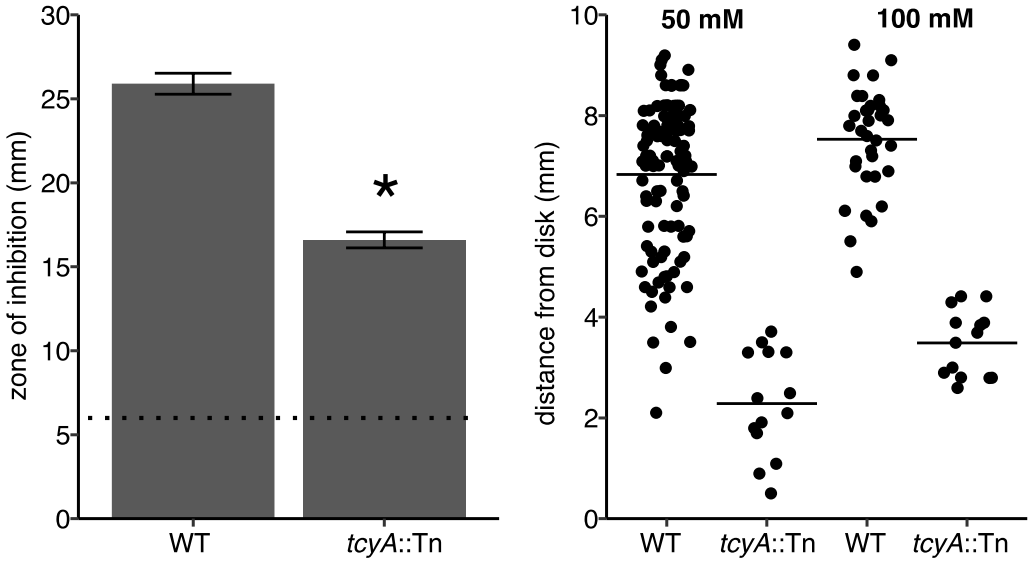
Strain	Description	Reference/Source
methicillin resistant strains		
wild type	USA300 JE2	(1)
tcyA::Tn	*tcyA::*erm	(1)
tcyP::Tn	*tcyP*::erm	(1)
tcyP::Tn	*tcyP::*tet	This study
tcyAP::Tn	*tcyA*::erm *tcyP*::tet	This study
methicillin sensitive strains		
wild type	Newman	(2)
tcyA::Tn	*tcyA*::erm	This study
tcyP::Tn	*tcyP::erm*	This study
tcyP::Tn	*tcyP*::tet	This study
tcyAP::Tn	*tcyA*::erm *tcyP*::tet	This study

Table S2. Primers used in this study.

Primers	Description	Sequence
tcyP ORF F	sequencing *tcyP* ORF	AAGTTCAACATATTGACTTATCCGGC
tcyP ORF R	sequencing *tcyP* ORF	TAGGAATTGAATATTTGACCAAACC
P_{tcyP} F	sequencing tcyP promoter	GCGAGCCATCATGTGCAATATTACG
P_{tcyP} R	sequencing *tcyP* promoter	CGAATCGCACAAGTGCACACTC
pKK22 F	amplification for pKK22 for Gibson assembly	GCGGCCGCTAGCCTAGGAGC
pKK22 R	amplification for pKK22 for Gibson assembly	ATCGCCTGTCACTTTGCTTGATATATGA
P*tcyP* *tcyP* F	amplification of P*tcyP tcyP* for cloning into pKK22	AGCAAAGTGACAGGCGATGCGGCCGCA GAATTTTTTACAACGTGTTTG
P*tcyP* *tcyP R*	amplification of P*tcyP tcyP* for cloning into pKK22	GAGCTCCTAGGCTAGCGGCCTTAGTGTG AAGTTAATGCAG
P_{tcyABC} *tcyABC*	amplification of P_{tcyABC} *tcyABC* for cloning into pKK22	AGC AAA GTG ACA GGC GAT GCT GTT GGC AAC AGT TTA TG
P_{tcyABC} *tcyABC*	amplification of P_{tcyABC} *tcyABC* for cloning into pKK22	TAC CGA GCT CCT AGG CTA GCT TAT TCT TCA TTT ATA ACA TTT AAG AAA C
rho F	qRT-PCR	AAACGTCCGCATTTCCAAGC
rho R	qRT-PCR	TGGCGCCACTATTAAACCAC
tcyA F	qRT-PCR	TATTGGCTGCATGCGGAAAC
tcyA R	qRT-PCR	AATGGTGCATAAGTCCCCTCAG
tcyP F	qRT-PCR	TGCTGCGATTGTTGGTGTTG
tcyP R	qRT-PCR	ATTTCGCTTCCACGTGCTTG

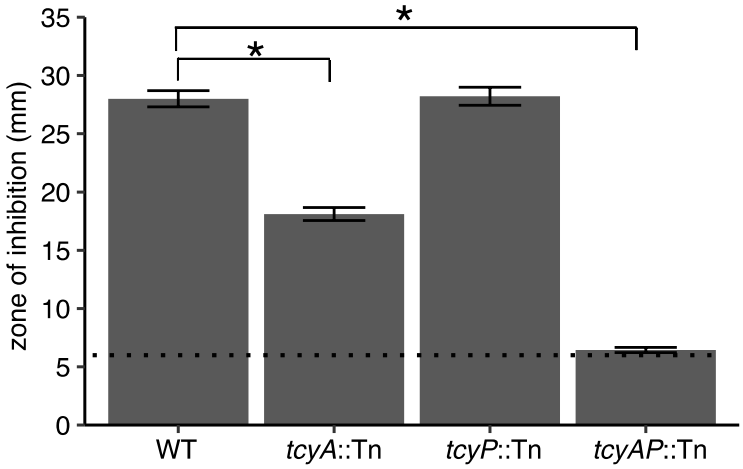
SUPPLEMENTAL FIGURES

Figure S1.



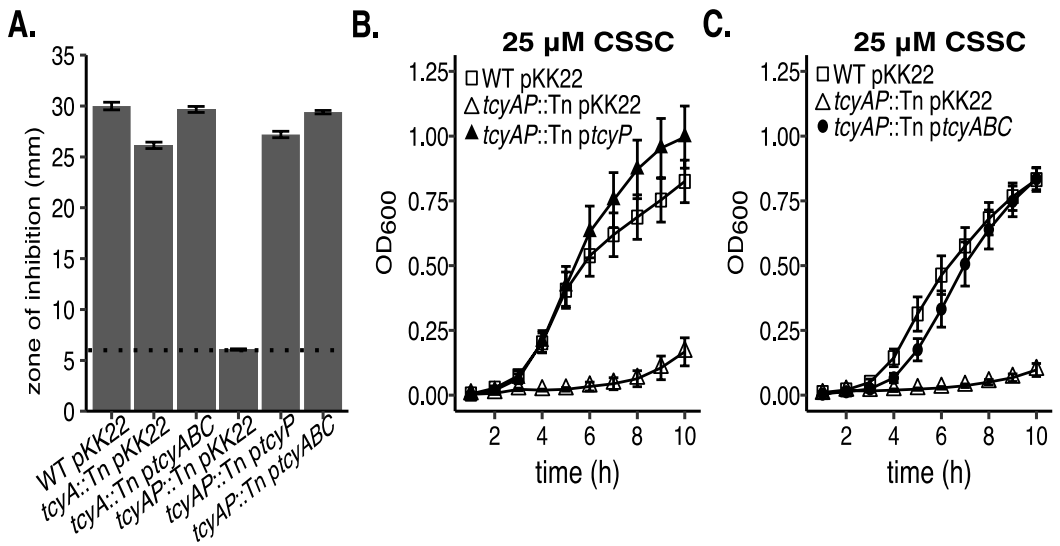
Strain Newman *tcyA* mutants demonstrate enhanced selenocystine resistance. (A) WT and *tcyA*::Tn were plated as a lawn on TSA and a disk supplemented with 100 mM selenocystine was added to the plate. The dotted line represents the disk diameter (6 mm). The mean zone of inhibition of at least three independent trials is presented. Error bars represent ± 1 standard error of the mean. **(B)** WT Newman or *tcyA*::Tn selenocystine resistant mutant colonies grew the indicated distance from a sterile Whatman paper disk containing 50 mM or 100 mM selenocystine. The bar represents the mean distance from the disk. * indicates $P<0.05$ determined from student's t-test.

Figure S2



Newman TcyABC and TcyP are required for selenocystine sensitivity. The zone of inhibition in the presence of 100 mM selenocystine was measured for WT, *tcyA*::Tn, *tcyP*::Tn, and *tcyAP*::Tn. The mean zone of inhibition of at least three independent trials is presented. The dotted line represents the disk diameter (6 mm). The error bars represent ± 1 standard error of the mean. * indicates $P<0.05$ determined from student's t-test.

Figure S3.



Ectopic expression of *tcyP* and *tcyABC* restores selenocystine sensitivity and growth in cystine-supplemented medium of the *tcyAP* double mutant. (A) Selenocystine resistance of WT harboring a pKK22 empty vector (WT pKK22), the *tcyA::*Tn mutant strain harboring a pKK22 empty vector (*tcyA*::Tn pKK22), the *tcyA*::Tn mutant harboring pKK22 vector containing *tcyABC* under the control of its native promoter (*tcyA*::Tn p*tcyABC*), the *tcyA*::Tn *tcyP*::Tn double mutant harboring a pKK22 empty vector (*tcyAP*::Tn pKK22), the *tcyA*::Tn *tcyP*::Tn mutant strain harboring a pKK22 vector containing *tcyP* under the control of its native promoter (*tcyAP*::Tn p*tcyP*), or the *tcyA*::Tn *tcyP*::Tn mutant harboring pKK22 vector containing *tcyABC* under the control of its native promoter (*tcyAP*::Tn p*tcyABC*) was determined in the presence of 100 mM selenocystine. The dotted line represents the disk diameter (6 mm). **(B-C)** Growth of the indicated strains was monitored in medium supplemented with 25 µM cystine. The mean of at least three independent trials is presented, error bars represent ± 1 standard error of the mean.

Figure S4

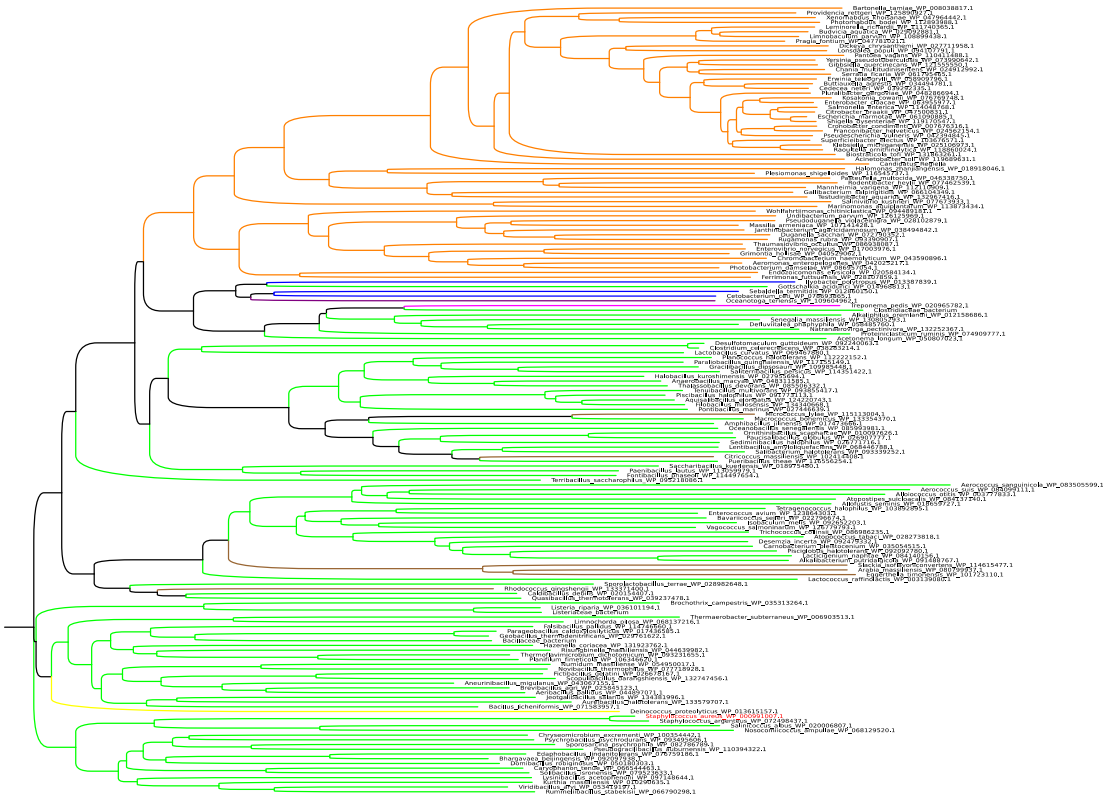


Figure S4. TcyP is conserved across many bacterial phyla. Homologues of TcyP are present in proteobacteria (orange), firmicutes (green), fusobacteria (blue), thermotogae (purple), spirochaetes (magenta), actinobacteria (brown), and deinococcus (yellow). The query, TcyP protein from *S. aureus*, is highlighted in red.

REFERENCES

1. **Fey PD**, **Endres JL**, **Yajjala VK**, **Widhelm TJ**, **Boissy RJ**, **Bose JL**, **Bayles KW**. 2013. A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. MBio **4**:e00537-12.
2. **Duthie ES**. 1952. Variation in the antigenic composition of staphylococcal coagulase. J. Gen. Microbiol. **7**:320–326.