Supplemental Table 5. Strains and plasmids used in this study.

Strain	Genotype or description	Source or reference
Strains		
E. coli		
Top10	Routine cloning host	Lab stock
DH5α	Routine cloning host	Lab stock
E. faecalis		
OG1	Wild-type laboratory strain isolate (MLST 1)	1
SB23	OG1 Δ*croR*	2
SB35	OG1 Δ(*croR croS)*	3
ST4	OG1 *pbp4(5)* ATAA promoter mutant (4 nucleotide substitutions: ATAA)	This work
JL339	OG1 Δ*pbp4(5)*	4
V583	Vancomycin-resistant clinical isolate (MLST 6)	5
CK221	V583 Δ*ermB* (Erm^S)	6
SB45	CK221 Δ*croR*	7
ST8	CK221 *pbp4(5)* ATAA promoter mutant (4 nucleotide substitutions: ATAA)	This work
JL640	CK221 Δ*pbp4(5)*	8
T1	Wild-type (MLST 21), CDC reference strain	9
SB29	T1 Δ*croR*	6
JL632	OG1 Δ*pbpA(2b)*	8
SB77	OG1 Δ*OG1RF_RS05215*	This work
SK129	OG1 Δ*OG1RF_RS12755*	This work
SB93	OG1 Δ*OG1RF_RS02555*	This work
8N14	OG1RF *OG1RF_RS03660::Tn*	10
13D12	OG1RF *OG1RF_RS05550::Tn*	10
2M10	OG1RF *OG1RF_RS05565::Tn*	10
7B1	OG1RF *OG1RF_RS05385::Tn*	10
25M20	OG1RF *OG1RF_RS05555::Tn*	10
Plasmids		
pCI3340	*E. coli-E. faecalis* shuttle vector (Cm^r)	11
pSLK234	Promoterless *lacZ* in pCI3340	12
pJLL170	P_{croR}'-*lacZ* in pCI3340 (includes 240bp upstream of the translational start site)	12
pSBT3	P_{pbpZ}'-*lacZ* in pCI3340 (includes 400bp upstream of the translational start site)	This work
pSBT8	P_{pbp5}'-*lacZ* in pCI3340 (includes 154bp upstream of the translational start site)	This work
pJRG8	*E. faecalis* expression vector (Erm^r)	13
pJLL59	P_{croR}-*croR croS* in pJRG8	3
pSLB1	P_{croR}-*croR* D52A *croS* in pJRG8	3
pSBT23	P_{pbp5}'-*lacZ* in pCI3340 (includes 130bp upstream of the translational start site)	This work
pSBT24	P_{pbp5}'-*lacZ* in pCI3340 (includes 120bp upstream of the translational start site)	This work
pSBT25	P_{pbp5}'-*lacZ* in pCI3340 (includes 105bp upstream of the translational start site)	This work
pSBT26	P_{pbp5}'-*lacZ* in pCI3340 (includes 90bp upstream of the translational start site)	This work
pSBT29	pSBT25 with 8 nucleotide substitutions **AAATAATT** in CroR-dependent regulatory motif TTTATTAA	This work
pSBT30	pSBT25 with 6 nucleotide substitutions T**AATAAT**A in CroR-dependent regulatory motif TTTATTAA	This work
pSBT31	pSBT25 with 4 nucleotide substitutions TT**ATAA**AA in CroR-dependent regulatory motif TTTATTAA	This work
pSBT38	pJLL170 with 8 nucleotide substitutions **AAATAATT** in CroR-dependent regulatory motif TTTATTAA	This work
pJH086	*E. faecalis* allelic exchange vector (Cm^r); *pheS** counterselection	14
pSBT40	*pbp4(5)* promoter from OG1 with 4 nucleotide substitutions (ATAA) in pJH086	This work

pSBT64	*pbp4(5)* promoter from CK221 with 4 nucleotide substitutions (ATAA) in pJH086	This work
pJRG9	*E. faecalis* expression vector with constitutive P23s promoter (Cm^r)	2
pJLL255	*pbp4(5)* from OG1 in pJRG9	This work
pCPN1	*OG1RF_RS05560* in pJRG9	This work
pJH123	*E. faecalis* expression vector with constitutive P23s promoter (Cm^r)	13
pSLK245	*OG1RF_RS05565-HA* with C-terminal HA tag in pJH123	This work
pEAW9	*pbpA(2b)* from OG1 in pJRG9	8
pSLK239	*OG1RF_RS11270-HA* with C-terminal HA tag in pJH123	This work
pSLK238	*OG1RF_RS05340-HA* with C-terminal HA tag in pJH123	This work
pSLK237	*OG1RF_RS03825-HA* with C-terminal HA tag in pJH123	This work
pSLK241	*OG1RF_RS07205-HA* with C-terminal HA tag in pJH123	This work
pSLK243	*OG1RF_RS03660-HA* with C-terminal HA tag in pJH123	This work
pSLK244	*OG1RF_RS05215-HA* with C-terminal HA tag in pJH123	This work
pSLK250	*OG1RF_RS12755-HA* with C-terminal HA tag in pJH123	This work
pCJK245	*E. faecalis* allelic exchange vector (Cm^r)	15
pSLB72	Δ*OG1RF_RS05215* (ΔL7-T268, 95% deletion) in pCJK245	This work
pSLB90	Δ*OG1RF_RS02555* (ΔK7-D380, 96% deletion) in pCJK245	This work
pSLK233	Δ*OG1RF_RS12755* (ΔK7-K532, 97% deletion) in pJH086	This work
pJLL286	*E. faecalis* expression vector with P_{nisA} nitrate-inducible promoter (Em^r)	16
pJLL310	*pbpA(2b)* from OG1 in pJLL286	This work

Supplemental references

1. Gold, O. G., Jordan, H. V. & van Houte, J. The prevalence of enterococci in the human mouth and their pathogenicity in animal models. *Arch. Oral Biol.* **20**, 473-IN15 (1975).

2. Snyder, H., Kellogg, S. L., Skarda, L. M., Little, J. L. & Kristich, C. J. Nutritional Control of Antibiotic Resistance via an Interface between the Phosphotransferase System and a Two-Component Signaling System. *Antimicrob. Agents Chemother.* **58**, 957–965 (2014).

3. Kellogg, S. L. & Kristich, C. J. Functional Dissection of the CroRS Two-Component System Required for Resistance to Cell Wall Stressors in Enterococcus faecalis. *J. Bacteriol.* **198**, 1326–1336 (2016).

4. Kristich, C. J. & Little, J. L. Mutations in the β Subunit of RNA Polymerase Alter Intrinsic Cephalosporin Resistance in Enterococci. *Antimicrob. Agents Chemother.* **56**, 2022 (2012).

5. Sahm, D. F. *et al.* In vitro susceptibility studies of vancomycin-resistant Enterococcus faecalis. *Antimicrob. Agents Chemother.* **33**, 1588 (1989).

6. Djorić, D. & Kristich, C. J. Extracellular SalB contributes to intrinsic cephalosporin resistance and cell envelope integrity in Enterococcus faecalis. *J. Bacteriol.* (2017) doi:10.1128/JB.00392-17.

7. Djorić, D. & Kristich, C. J. Oxidative Stress Enhances Cephalosporin Resistance of Enterococcus faecalis through Activation of a Two-Component Signaling System. *Antimicrob. Agents Chemother.* (2015) doi:10.1128/aac.03984-14.

8. Djorić, D., Little, J. L. & Kristich, C. J. Multiple low-reactivity class B penicillin-binding proteins are required for cephalosporin resistance in enterococci. *Antimicrob. Agents Chemother.* **64**, (2020).

9. Maekawa, S., Yoshioka, M. & Kumamoto, Y. Proposal of a New Scheme for the Serological Typing of Enterococcus faecalis Strains. *Microbiol. Immunol.* **36**, 671–681 (1992).

10. Kristich, C. J. *et al.* Development and Use of an Efficient System for Random mariner Transposon Mutagenesis To Identify Novel Genetic Determinants of Biofilm Formation in the Core Enterococcus faecalis Genome. *Appl. Environ. Microbiol.* **74**, 3377 (2008).

11. Hayes, F., Daly, C. & Fitzgerald, G. F. Identification of the Minimal Replicon of Lactococcus lactis subsp. lactis UC317 Plasmid pCI305. *Appl. Environ. Microbiol.* **56**, 202 (1990).

12. Kellogg, S. L. & Kristich, C. J. Convergence of PASTA kinase and two component signaling in response to cell wall stress in Enterococcus faecalis. *J. Bacteriol.* (2018) doi:10.1128/JB.00086-18.

13. Kristich, C. J., Little, J. L., Hall, C. L. & Hoff, J. S. Reciprocal Regulation of Cephalosporin Resistance in Enterococcus faecalis. *MBio* **2**, (2011).

14. Kellogg, S. L., Little, J. L., Hoff, J. S. & Kristich, C. J. Requirement of the CroRS two-component system for resistance to cell wall-targeting antimicrobials in Enterococcus faecium. *Antimicrob. Agents Chemother.* (2017) doi:10.1128/AAC.02461-16.

15. Kristich, C. J., Djoric, D. & Little, J. L. Genetic Basis for Vancomycin-Enhanced Cephalosporin Susceptibility in Vancomycin-Resistant Enterococci Revealed Using Counterselection with Dominant-Negative Thymidylate Synthase. (2014) doi:10.1128/AAC.02001-13.

16. Mascari, C. A., Djoric, D., Little, J. L. & Kristich, C. J. Use of an Interspecies Chimeric Receptor for Inducible Gene Expression Reveals that Metabolic Flux through the Peptidoglycan Biosynthesis Pathway is an Important Driver of Cephalosporin Resistance in Enterococcus faecalis. *J. Bacteriol.* **204**, (2022).