

Supplementary Material

Supplementary Table S1. Minimum Inhibitory Concentrations (MIC) for wild type and transposon mutants of genes mutated in EFC3Py.

Gene Tag	Locus	Gene Product	Position	Insertion sites	MIC of DAP (μM)
<i>E. faecalis</i> OG1RF	-	-	-	-	1.23
OG1RF_11765		MerR family transcriptional regulator	1853415	295 nt (Position) /768 nt (Gene length)	1.23
OG1RF_11766		Multidrug ABC superfamily ATP binding cassette transporter, ABC protein	1855237	1414 nt /1728 nt	1.23
OG1RF_11767		ABC superfamily ATP binding cassette transporter, membrane protein	1855713	110 nt/1779 nt	1.23
OG1RF_11462		Membrane protein of hypothetical function, adjacent to PspC	1523233	133 nt/357 nt	1.23

Supplementary Table S2. List of genes differentially expressed in *E. faecalis* OG1RF wild type upon treatment with COE1-3C.

Locus	Description	Fold change
OG1RF_10301	RND transporter	-5.0
OG1RF_10302	ABC transporter ATP-binding protein	-5.0
OG1RF_10303	ABC transporter permease	-5.0
OG1RF_10878	adhesin	5.4
OG1RF_11260	membrane protein	7.5
OG1RF_11384	permease	-5.5
OG1RF_11385	peptide ABC transporter ATP-binding protein	-6.2
OG1RF_11462	membrane protein	-4.8
OG1RF_11463	PspC family transcriptional regulator	-4.9
OG1RF_12269	RND transporter	-9.9
OG1RF_11185	molybdenum cofactor biosynthesis protein MoaB	-8.5
OG1RF_10527	peptide ABC transporter ATP-binding protein	-7.5
OG1RF_12267	permease	-6.2
OG1RF_12268	ABC transporter ATP-binding protein	-5.8
Signal Transduction		
OG1RF_11656	peptide ABC transporter permease	-7.3
OG1RF_11657	ABC transporter ATP-binding protein	-5.4
Hypothetical Proteins		
OG1RF_10304	hypothetical protein	-15.3
OG1RF_10353	hypothetical protein	-7.0
OG1RF_10354	hypothetical protein	-8.1
OG1RF_10531	hypothetical protein	-7.6
OG1RF_10532	hypothetical protein	-7.1
OG1RF_11751	hypothetical protein	-4.3
OG1RF_10659	hypothetical protein	-4.2
OG1RF_11252	hypothetical protein	-4.7
OG1RF_11464	hypothetical protein	-4.0

Supplementary Table S3. List of genes differentially expressed in *E. faecalis* OG1RF wild type on treatment with COE1-3Py.

Locus	Name	Fold change
OG1RF_10288	sodium:proton antiporter	4.7
OG1RF_10367	decarboxylase	14.2
OG1RF_10369	sodium:proton antiporter	8.1
OG1RF_10526	permease	-6.1
OG1RF_11227	mandelate racemase	-6.3
OG1RF_11810	transporter	7.9
Overview		
OG1RF_11314	katA; catalase	6.7
Hypothetical protein		
OG1RF_10075	hypothetical protein	-4.0
OG1RF_12152	hypothetical protein	4.4
OG1RF_10659	hypothetical protein	-4.2
OG1RF_11252	hypothetical protein	-4.7
OG1RF_11464	hypothetical protein	-4.0
Nucleotide metabolism		
OG1RF_12279	allantoinase	-6.5
Cellular communication		
OG1RF_10368	amino acid permease	20.4
Carbohydrate metabolism		
OG1RF_10682	beta-phosphoglucomutase	-5.6
Signal transduction		
OG1RF_11938	fumarate reductase flavoprotein subunit	4.2
OG1RF_11185	molybdenum cofactor biosynthesis protein MoaB	-8.5
OG1RF_10527	peptide ABC transporter ATP-binding protein	-7.5
OG1RF_12267	permease	-6.2
OG1RF_12268	ABC transporter ATP-binding protein	-5.8

Supplementary Table S4. List of genes differentially expressed that were common to both EFC3C and EFC3Py in comparison to the wild type in the absence of COEs.

Locus	Description	Fold change in EFC3Py	Fold change in EFC3C
OG1RF_12235	protease	12.8	8.0
OG1RF_11260	membrane protein	4.7	5.4
OG1RF_11377	conjugal transfer protein TraX	-11.9	-4.4
OG1RF_10654	two-component system sensor histidine kinase	-10.4	-4.4
OG1RF_10707	DNA repair exonuclease	-12.8	-4.5
OG1RF_11074	magnesium-translocating P-type ATPase	-17.3	-4.7
OG1RF_11072	transcriptional regulator	-16.3	-5.1
OG1RF_10328	peptidase M23	-10.1	-5.2
OG1RF_11073	LysR family transcriptional regulator	-15.5	-5.3
OG1RF_12496	DNA-binding protein	-12.9	-5.4
OG1RF_12131	membrane protein	-10.5	-5.8
OG1RF_11569	membrane protein	-11.6	-5.8
OG1RF_10481	serine hydrolase	-12.8	-6.7
OG1RF_12298	DNA-binding protein	-8.4	-7.4
OG1RF_11094	membrane protein	-7.4	-8.0
OG1RF_12067	tellurite resistance protein	-19.2	-8.0
OG1RF_11522	multidrug MFS transporter	-28.9	-9.2
OG1RF_12500	LytR family transcriptional regulator	-33.0	-10.8
OG1RF_10301	RND transporter	-11.1	-14.1
OG1RF_10302	ABC transporter ATP-binding protein	-11.4	-14.2
OG1RF_10303	ABC transporter permease	-11.0	-14.7
OG1RF_11385	peptide ABC transporter ATP-binding protein	-79.5	-24.3
OG1RF_11384	permease	-67.6	-24.3
OG1RF_12269	RND transporter	-94.3	-27.8
OG1RF_11462	membrane protein	-92.2	-28.5
OG1RF_12268	ABC transporter ATP-binding protein	-115.3	-31.4
OG1RF_11463	PspC family transcriptional regulator	-102.7	-32.3
OG1RF_12267	permease	-139.9	-39.9
	Amino acid metabolism		
OG1RF_10473	amidase	-16.4	-6.3
	Carbohydrate metabolism		
OG1RF_11199	diacetylchitobiose-6-phosphate hydrolase	-12.8	-5.2
	Glycan biosynthesis and metabolism		
OG1RF_10417	penicillin-binding protein 1A	-15.0	-4.4

Lipid metabolism			
OG1RF_11521	alkaline phosphatase	-18.9	-4.4
OG1RF_11570	glycerophosphoryl diester phosphodiesterase	-12.5	-7.1
Membrane transport			
OG1RF_12351	iron ABC transporter substrate-binding protein	4.9	4.9
Metabolism of other amino acids			
OG1RF_12112	D-alanine--poly(phosphoribitol) ligase subunit 1	-13.2	-6.0
OG1RF_12110	D-alanine--poly(phosphoribitol) ligase subunit 2	-13.9	-6.4
Signal transduction			
OG1RF_12212	two-component system sensor histidine kinase	-10.3	-4.9
OG1RF_12211	LuxR family transcriptional regulator	-9.4	-5.3
OG1RF_12111	D-alanyl-lipoteichoic acid biosynthesis protein DltB	-13.9	-6.1
OG1RF_12109	D-alanyl transfer protein DltD	-14.7	-6.6
OG1RF_11657	ABC transporter ATP-binding protein	-24.2	-10.8
OG1RF_11656	peptide ABC transporter permease	-33.6	-12.8
Translation			
OG1RF_10057	peptide ABC transporter substrate-binding protein	12.0	6.7
Hypothetical Protein			
OG1RF_10492	hypothetical protein	8.1	4.0
OG1RF_11239	hypothetical protein	-8.9	-4.6
OG1RF_12328	hypothetical protein	-8.2	-4.7
OG1RF_10305	hypothetical protein	-7.6	-5.7
OG1RF_11093	hypothetical protein	-7.2	-7.4
OG1RF_11031	hypothetical protein	-14.2	-7.7
OG1RF_11032	hypothetical protein	-13.9	-8.0
OG1RF_10354	hypothetical protein	-8.6	-8.7
OG1RF_11751	hypothetical protein	-18.0	-8.9
OG1RF_10353	hypothetical protein	-11.1	-10.3
OG1RF_11200	hypothetical protein	-32.9	-12.7
OG1RF_12066	hypothetical protein	-38.9	-13.8
OG1RF_10659	hypothetical protein	-56.6	-24.0
OG1RF_10304	hypothetical protein	-33.0	-25.0
OG1RF_10532	hypothetical protein	-71.9	-53.7
OG1RF_10531	hypothetical protein	-73.9	-56.2
OG1RF_11464	hypothetical protein	-215.7	-64.2
OG1RF_11252	hypothetical protein	-590.3	-195.0

Supplementary Table S5. Genes up- or downregulated only in the untreated mutant EFC3C compared to untreated *E. faecalis* wild type.

Locus	Description	Fold change in EFC3C
Membrane transport		
OG1RF_12353	iron ABC transporter permease	6.4
OG1RF_10136	ferrichrome ABC transporter substrate-binding protein	4.2
Carbohydrate metabolism		
OG1RF_11753	PTS maltose transporter subunit IIBC	-4.3

Supplementary Table S6. Genes up- or downregulated only in the untreated mutant EFC3Py compared to the untreated *E. faecalis* wild type.

Locus	Description	Fold Change in EFC3Py
OG1RF_12481	choline-binding protein	11.1
OG1RF_10490	cell wall surface anchor protein	6.6
OG1RF_10485	cell wall surface anchor protein	5.0
OG1RF_10486	cell surface protein	4.6
OG1RF_10407	CRISPR-associated protein Csn2	4.3
OG1RF_12572	citrate transporter	4.2
OG1RF_10487	cell surface protein	4.1
OG1RF_10655	glucose transporter GlcU	-4.1
OG1RF_10836	nitrate ABC transporter permease	-4.1
OG1RF_10835	taurine ABC transporter ATP-binding protein	-4.6
OG1RF_11036	ATPase P	-4.8
OG1RF_11077	co-chaperone GrpE	-4.8
OG1RF_11383	NADPH:quinone reductase	-4.8
OG1RF_10516	acyltransferase	-5.3
OG1RF_10761	alpha/beta hydrolase	-5.3
OG1RF_11076	HrcA family transcriptional regulator	-5.3
OG1RF_10996	Cro/CI family transcriptional regulator	-5.5
OG1RF_12210	potassium transporter Trk	-6.1
OG1RF_12536	two-component system sensor histidine kinase	-6.9
OG1RF_12535	two-component system response regulator	-7.5
OG1RF_11452	ABC transporter substrate-binding protein	-7.9
OG1RF_12071	transcriptional regulator	-8.3
OG1RF_12213	membrane protein	-9.0
OG1RF_10653	two-component system response regulator	-9.3
OG1RF_11908	virion core protein	-9.5
OG1RF_11793	chaperone protein ClpB	-10.4
OG1RF_11070	cell division protein FtsW	-11.8
OG1RF_11002	phosphoesterase	-12.6
OG1RF_11071	cell division protein FtsW	-17.8
	Carbohydrate metabolism	
OG1RF_10297	mannitol-specific phosphotransferase enzyme IIA component	-4.6
OG1RF_10432	PTS fructose transporter subunit IIC	-4.6
OG1RF_10434	tagatose 1,6-diphosphate aldolase	-5.5

OG1RF_10298	mannitol-1-phosphate 5-dehydrogenase	-5.6
OG1RF_10682	beta-phosphoglucomutase	-6.1
	Cellular communication	
OG1RF_10993	spermidine/putrescine ABC transporter substrate-binding protein	5.6
OG1RF_12366	peptide ABC transporter substrate-binding protein	5.5
OG1RF_10991	spermidine/putrescine ABC transporter permease	5.1
	Folding, sorting and degradation	
OG1RF_10584	signal peptidase I	6.5
OG1RF_11078	molecular chaperone DnaK	-4.7
	Glycan biosynthesis and metabolism	
OG1RF_12158	cell division protein FtsI	-4.1
	Membrane transport	
OG1RF_10897	glutamine ABC transporter substrate-binding protein	11.5
OG1RF_10895	amino acid ABC transporter permease	9.6
OG1RF_10896	glutamine ABC transporter permease	9.0
OG1RF_10898	ABC transporter ATP-binding protein	8.4
	Nucleotide metabolism	
OG1RF_10994	adenine deaminase	4.3
	Signal transduction	
OG1RF_10760	membrane protein	-6.2
	Translation	
OG1RF_10025	RpiR family transcriptional regulator	5.1
	Hypothetical protein	
OG1RF_10796	hypothetical protein	8.7
OG1RF_12152	hypothetical protein	7.9
	hypothetical protein	5.8
OG1RF_10491	hypothetical protein	5.8
OG1RF_10860	hypothetical protein	4.4
OG1RF_10861	hypothetical protein	4.1
OG1RF_10482	hypothetical protein	-4.6
OG1RF_12166	hypothetical protein	-5.1
OG1RF_10445	hypothetical protein	-5.2
OG1RF_11254	hypothetical protein	-5.5
OG1RF_10446	hypothetical protein	-7.4
OG1RF_11910	hypothetical protein	-8.5
OG1RF_11909	hypothetical protein	-9.6
OG1RF_12471	hypothetical protein	-10.4

Locus	Description	Fold change in EFC3Py
OG1RF_10838	manganese transporter	-5.9
OG1RF_10605	aldo/keto reductase	-4.3
OG1RF_11643	universal stress protein UspA	-4.1
OG1RF_12470	cobalamin synthesis protein CobW	4.1
OG1RF_10878	adhesin	4.4
OG1RF_11522	multidrug MFS transporter	4.6
OG1RF_10589	cation transporter	5.6
OG1RF_10328	peptidase M23	6.3
OG1RF_11002	phosphoesterase	6.6
OG1RF_11377	conjugal transfer protein TraX	6.7
OG1RF_10550	polysaccharide lyase	9.5
OG1RF_11767	multidrug ABC transporter ATP-binding protein	699.5
OG1RF_11766	multidrug ABC transporter ATP-binding protein	787.2
Cellular communication		
OG1RF_12368	peptide ABC transporter permease	-5.2
OG1RF_12367	peptide ABC transporter permease	-4.8
OG1RF_12369	peptide ABC transporter ATP-binding protein	-4.6
OG1RF_12366	peptide ABC transporter substrate-binding protein	-4.1
Membrane transport		
OG1RF_11677	ABC transporter ATP-binding protein	-8.6
OG1RF_11678	membrane protein	-7.3
OG1RF_10684	PTS glucose transporter subunit IIABC	-7.1
OG1RF_10297	mannitol-specific phosphotransferase enzyme IIA component	-6.1
OG1RF_11679	manganese ABC transporter substrate-binding protein	-5.6
OG1RF_12479	PTS mannose transporter subunit IID	-4.7
OG1RF_12472	zinc-binding protein	4.8
OG1RF_12353	iron ABC transporter permease	5.9
OG1RF_12354	iron ABC transporter permease	6.0
OG1RF_12351	iron ABC transporter substrate-binding protein	8.0
OG1RF_10137	ferrichrome ABC transporter ATP-binding protein	8.1
OG1RF_12352	iron ABC transporter ATP-binding protein	8.4
OG1RF_10136	ferrichrome ABC transporter substrate-binding protein	13.4
Translation		
OG1RF_10021	PTS mannose transporter subunit IID	-4.4
OG1RF_10020	PTS alpha-glucoside transporter subunit IIBC	-4.3
OG1RF_12469	50S ribosomal protein L33	4.3
OG1RF_10881	Hypothetical protein	20.5

Supplementary Table S7. Differential expression of genes EFC3C treated with COE1-3Py in comparison to the untreated strain.

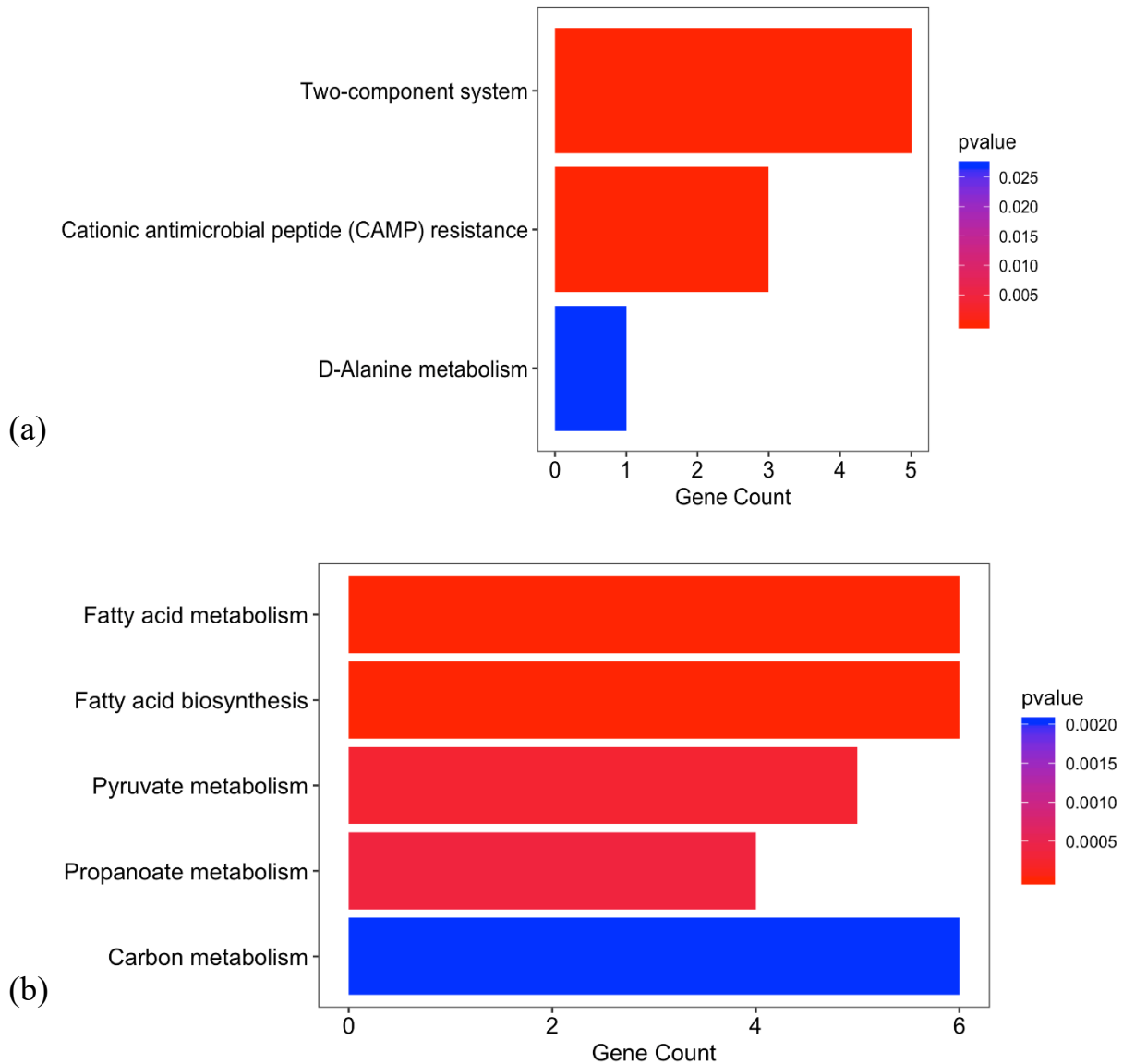
Locus	Description	Fold change in EFC3C
OG1RF_12420	large-conductance mechanosensitive channel	-3.6
Cellular communication		
OG1RF_10993	spermidine/putrescine ABC transporter substrate-binding protein	4.8
OG1RF_10434	tagatose 1,6-diphosphate aldolase	5.1
Membrane transport		
OG1RF_11677	ABC transporter ATP-binding protein	-7.8
OG1RF_10432	PTS fructose transporter subunit IIC	5.5

Supplementary Table S8. Differential expression of genes EFC3Py treated with COE1-3C in comparison to the untreated EFC3Py.

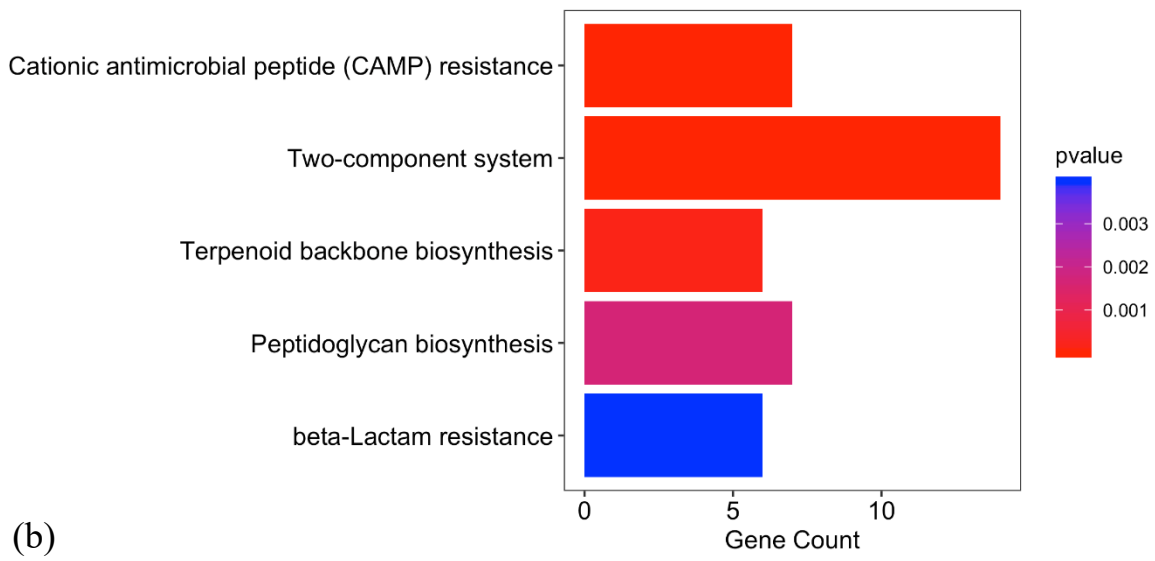
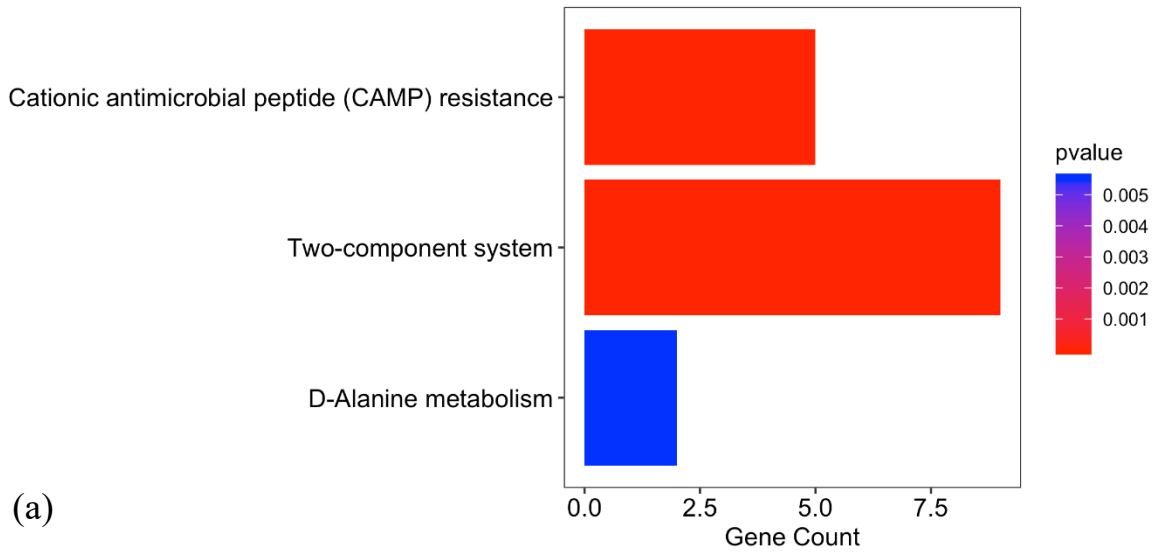
Locus	Description	Fold change in EFC3Py
OG1RF_11766	multidrug ABC transporter ATP-binding protein	28.3
OG1RF_11767	multidrug ABC transporter ATP-binding protein	27.4

Supplementary Table S9. List of primers used to construct the plasmids and verify knockout strains.

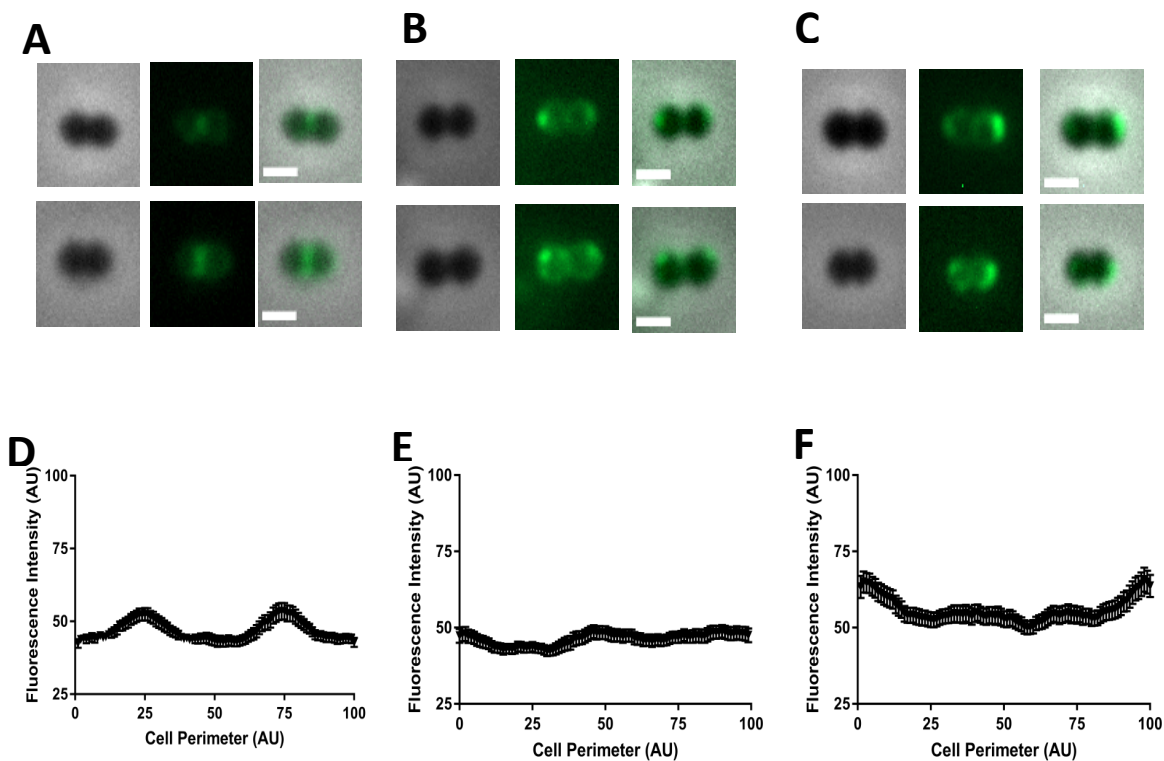
Primers	Sequence	Description
<i>liaF_INF_F</i>	gatatctgcagaattcccgaagccgac cattt	Primer used during PCR amplification of <i>liaF</i> from EFC3C.
<i>liaF_INF_R</i>	taccgagctcggatccatattggttcagg agtttctgt	Primer used during PCR amplification of <i>liaF</i> from EFC3C.
<i>liaR_INF_F</i>	gatatctgcagaattcgagaacgtgttgt cggc	Primer used during PCR amplification of <i>liaR</i> from EFC3Py.
<i>liaR_INF_R</i>	tgattacgccaagctctgccgcaaac gtcct	Primer used during PCR amplification of <i>liaR</i> from EFC3Py.
<i>liaFcomp_INF_F</i>	gcttgatatcgaatttaggcgaccaagt gattat	Primer used during PCR amplification of <i>liaF</i> from OG1RF.
<i>liaFcomp_INF_R</i>	tagaactagtgatctcaagcataatctg gaacatcatatggataagcgtaatctgg aacatcgtatgggtatacacggatcacc tcaac	Primer used during PCR amplification of <i>liaF</i> from OG1RF.
<i>liaRcomp_INF_F</i>	gtgaggaaactgaattcgtgatcaaagta atgtagtgatgacc	Primer used during PCR amplification of <i>liaR</i> from OG1RF.& to check gene insertion
<i>liaRcomp_INF_R</i>	acctgttgaatgatctgcagttaagcata atctggaacatcatatggataagcgtaat ctggaacatcgtatgggtatttgctaaa ccatg	Primer used during PCR amplification of <i>liaR</i> from OG1RF and to check gene insertion.
M13F(-20)	gtaaacgacggccagt	Forward primer to check insertion in pGCP123
M13F(-40)	gtttcccagtcacgac	Forward primer to check insertion in pGCP213
M13R-pUC (-26)	caggaaacagctatgac	Reverse primer to check insertion in pGCP213 & pGCP123



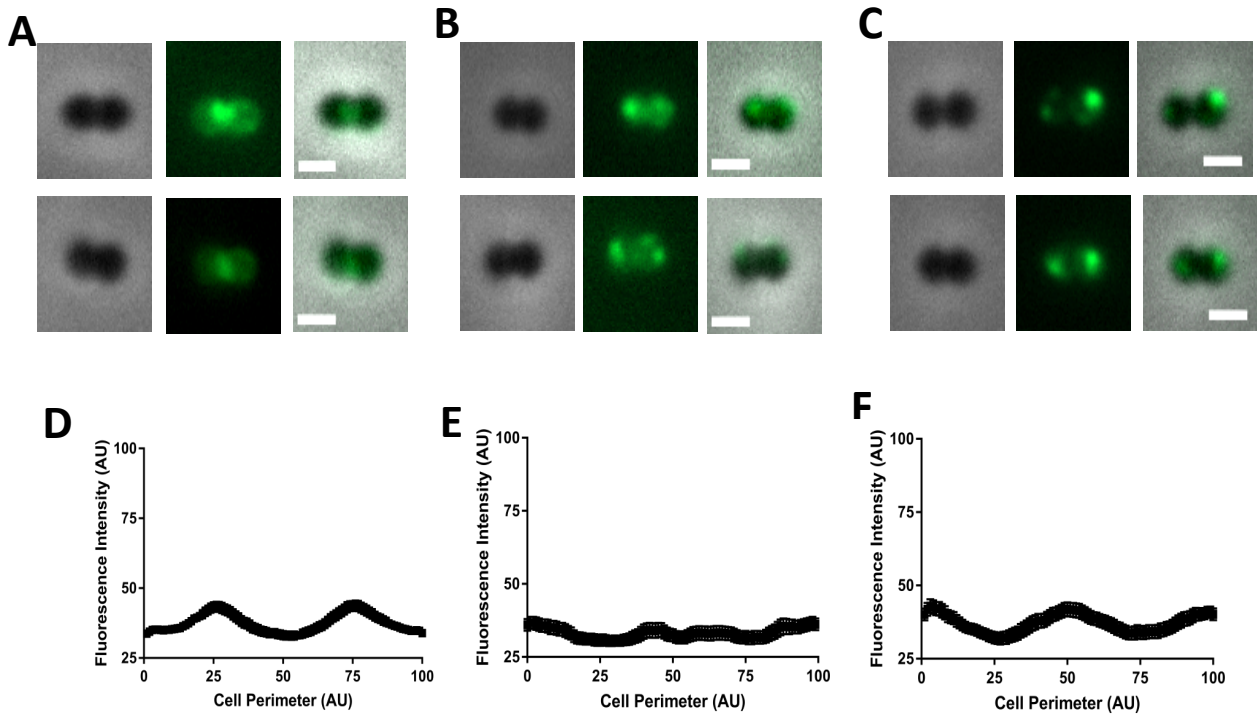
Supplementary Figure S1. Bar plot depicting the most enriched KEGG pathways on treatment of wild type OG1RF with (a) COE1-3C and (b) COE1-3Py. The colours of the bar chart represent the p value of the enriched KEGG pathway. Height represent the gene count.



Supplementary Figure S2. Bar plot depicting the most enriched KEGG pathways on treatment of wild type OG1RF with (a) EFC3C and (b) EFC3Py. The colours of the bar chart represent the p value of the enriched KEGG pathway. Height represent the gene count.

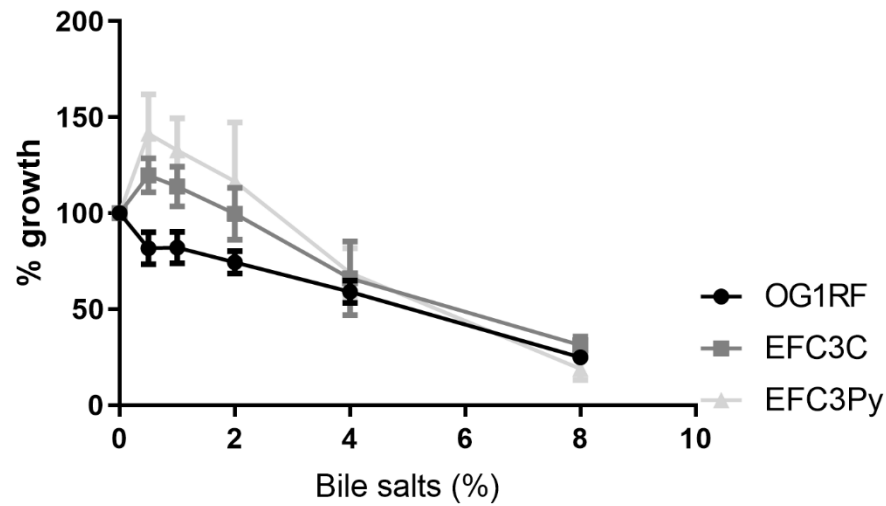


Supplementary Figure S3. Representative images of localization patterns of COE1-3Py in (A&D) *Enterococcus faecalis* OG1RF wild type, (B&E) EFC3C and (C&F) EFC3Py strains. Fluorescence intensity along the circumference of the cell was plotted against the cell perimeter of 1 to 100 units to generate a fluorescence distribution profile. Intensity analysis performed by PSICIC analysis software for two independent experiments with duplicates in each experiment. Excitation and emission wavelengths were 515/640 nm, respectively. The scale bar represents 0.5 μm .

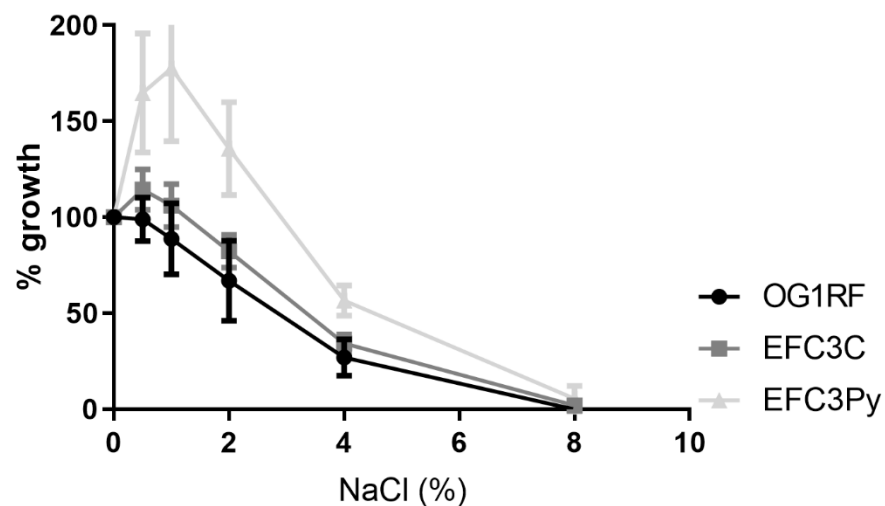


Supplementary Figure S4. Representative images of localization patterns of COE1-3C in (A&D) *Enterococcus faecalis* OG1RF wild type, (B&E) EFC3C and (C&F) EFC3Py strains. Fluorescence intensity along the circumference of the cell was plotted against the cell perimeter of 1 to 100 units to generate a fluorescence distribution profile. Intensity analysis performed by PSICIC analysis software for two independent experiments with duplicates in each experiment. Excitation and emission wavelengths were 515/640 nm, respectively. The scale bar represents 0.5 μm .

(A)



(B)



Supplementary Figure S5. Characterization of sodium chloride and bile salts tolerance in EFC3C, EFC3PY and OG1RF wild type. The effect of different concentrations of bile salts (0, 0.5%, 1%, 2%, 4% and 8%) or sodium chloride (0, 0.5%, 1%, 2%, 4% and 8%) was plotted as the % growth against the concentrations of bile salts or sodium chloride (NaCl). This error bars indicate the standard deviations of three biological replicates and three technical replicates (n =9). % growth = (OD₆₀₀ measured at 7 h/ OD₆₀₀ measured at 0 h) x 100. The error bars represent the standard deviation.