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Supplemental information

Re-wiring the regulation of the formicamycin biosynthetic gene cluster to enable the development of promising antibacterial compounds

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Supporting information

GUS assay results from *for* BGC promoters

Table S1: The activities of *for* gene cluster promoters were measured by fusing the promoters upstream of the β-glucoronidase reporter gene in pMF96. Activity of β-glucoronidase was measured by hydrolysis of PNPG using absorbance at 420 nm as described in **Method Details: GUS assay**.

Strain Name	Mean Miller Units/ mg protein ± Standard Error
S. formicae WT pMF96	0.37 ± 1.10
S. formicae WT pMF23	91.75 ± 21.75
S. formicae ΔforJ pMF96	0.00 ± 1.67
S. formicae ΔforJ pMF23	86.98 ± 18.81
S. formicae ΔforGF pMF96	2.01 ± 2.49
S. formicae ΔforGF pMF23	100.83 ± 12.22
S. formicae ΔforZ pMF96	0.37 ± 0.56
S. formicae ΔforZ pMF23	94.44 ± 16.37
S. formicae WT p*forJ*	32.82 ± 12.83
S. formicae ΔforJ p*forJ*	84.85 ± 10.05
S. formicae WT p*forG*	50.56 ± 14.34
S. formicae ΔforGF p*forG*	26.68 ± 8.11
S. formicae WT p*forH*	33.62 ± 12.52
S. formicae ΔforGF p*forH*	11.86 ± 5.07
S. formicae WT p*forZ*	0.38 ± 1.65
S. formicae ΔforZ p*forZ*	11.41 ± 7.68

Table S2: Media used in this study, related to **Method Details**.

Media	**Recipe (per litre)**	**Water**	**pH**
SFM	20 g soy flour 20 g mannitol 20 g agar	Tap	
MYM	4 g maltose 4 g yeast extract 10 g malt extract +/- 18 g agar	50:50 Tap:deionised	7.3
LB	10 g tryptone 5 g yeast extract 10 g NaCl (omitted when selecting with Hygromycin) +/- 20 g agar	Deionised	7.5
2YT	16 g tryptone 10 g yeast extract 5 g NaCl	Deionised	7.0

Table S3: Bacterial strains used and generated in this study, related to **Experimental Model and subject details.**

Strain	Description/Genotype	Plasmid	Resistance	Reference/Source
E. coli Top10	F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G			Invitrogen™
E. coli BW25113	λ⁻, Δ(*araD-araB*)*567,* Δ*lacZ4787*(*::rrnB-4*), *lacIp-4000*(*lacIQ*), *rpoS369*(*Am*), *rph-1,* Δ(*rhaD-rhaB*)*568, hsdR514*	pIJ790	Cml^R	(Datsenko and Wanner, 2000)
E. coli ET12567	*dam⁻ dcm⁻ hsdS⁻*	pUZ8002	Cml^R/Tet^R	(MacNeil *et al.*, 1992)
S. formicae wild-type				Lab stock
MSSA	ATCC 6538P			American Type Culture Collection
MRSA	ATCC BAA-1717			American Type Culture Collection
S. formicae ΔforJ	*forJ* deletion strain			This work
S. formicae ΔforGF	*forGF* deletion strain			This work
S. formicae ΔforZ	*forZ* deletion strain			This work
S. formicae ΔforJ: ΦBT1 forJ pforM	*forJ* complementation under forM promoter	pRD030	Hyg^R	This work
S. formicae ΔforJ: ΦBT1 forJ	*forJ* complementation under forJ promoter	pRD063	Hyg^R	This work
S. formicae ΔforJ: ΦBT1 pErmE forJ*	*forJ* complementation under ErmE* promoter	pRD06	Hyg^R	This work
S. formicae ΔforGF: ΦBT1 forGF	*forGF* complementation under native promoter	pRD031	Hyg^R	This work
S. formicae Δfor: ΦC31 *for 1-4 aac(3)IV*	Whole *for* cluster deletion complemented with pESAC-13 215-G with genes 1-4 (as annotated by AntiSMASH) replaced with apramycin gene	pRD037	Apr^R	This work
S. formicae Δfor: ΦC31 *for 1-7 aac(3)IV*	Whole *for* cluster deletion complemented with pESAC-13 215-G with genes 1-7 replaced with apramycin gene	pRD038	Apr^R	This work
S. formicae Δfor: ΦC31 *for 32-43 aac(3)IV*	Whole *for* cluster deletion complemented with pESAC-13 215-G with genes 32-43 replaced with apramycin gene	pRD039	Apr^R	This work

S. formicae Δfor: ΦC31 *for 36-43 aac(3)IV*	Whole *for* cluster deletion complemented with pESAC-13 215-G with genes 36-43 replaced with apramycin gene	pRD040	Apr^R	This work
S. formicae ΔforJ: ΦBT1 forJ 3x Flag	*forJ* deletion mutant complemented in-trans with 3x flag-tagged *forJ* for ChIP	pRD034	Hyg^R	This work
S. formicae ΔforGF: ΦBT1 forGF 3x Flag	*forGF* deletion mutant complemented in-trans with 3x flag-tagged *forGF* for ChIP	pRD035	Hyg^R	This work
S. formicae ΔforZ: ΦBT1 forZ 3x Flag	*forZ* deletion mutant complemented in-trans with 3x flag-tagged *forZ* for ChIP	pRD036	Hyg^R	This work
S. formicae ΔforV	*forV* deletion strain			(Qin *et al.*, 2017)
S. formicae ΔforX	*forX* deletion strain			(Qin *et al.*, 2020)
S. formicae ΔforY	*forY* deletion strain			(Qin *et al.*, 2020)
S. formicae ΔforS	*forS* deletion strain			(Qin *et al.*, 2019)
S. formicae ΔforJ, ΔforV	*forV* deletion strain with *forJ* deletion			This work
S. formicae ΔforJ, ΔforX	*forX* deletion strain with *forJ* deletion			This work
S. formicae ΔforJ, ΔforY	*forY* deletion strain with *forJ* deletion			This work
S. formicae ΔforJ, ΔforS	*forS* deletion strain with *forJ* deletion			This work
S. formicae: pMF96	Wildtype strain with GUS but no promoter controlling expression (negative control)	pMF96	Hyg^R	This work
S. formicae: pMF23	Wildtype strain with GUS but no promoter controlling expression (negative control)	pMF23	Apr^R	This work
S. formicae ΔforJ: pMF96	*forJ* deletion strain with GUS but no promoter controlling expression (negative control)	pMF96	Hyg^R	This work
S. formicae ΔforJ: pMF23	*forJ* deletion e strain with GUS but no promoter controlling expression (negative control)	pMF23	Apr^R	This work
S. formicae ΔforGF: pMF96	*forGF* deletion strain with GUS but no promoter controlling expression (negative control)	pMF96	Hyg^R	This work
S. formicae ΔforGF: pMF23	*forGF* deletion e strain with GUS but no promoter controlling expression (negative control)	pMF23	Apr^R	This work
S. formicae ΔforZ: pMF96	*forZ* deletion strain with GUS but no promoter	pMF96	Hyg^R	This work

	controlling expression (negative control)			
S. formicae ΔforZ: pMF23	*forZ* deletion e strain with GUS but no promoter controlling expression (negative control)	pMF23	Apr^R	This work
S. formicae ΔforJ: ΦBT1 GUS pforJ	*forJ* deletion strain with GUS expressed under *pforJ*	pRD062	Hyg^R	This work
S. formicae: ΦBT1 GUS pforJ	Wildtype strain with GUS expressed under *pforJ*	pRD062	Hyg^R	This work
S. formicae: ΦBT1 GUS pforG	WIldtype strain with GUS expressed under *pforG*	pRD054	Hyg^R	This work
S. formicae ΔforGF: ΦBT1 GUS pforG	*forGF* deletion strain with GUS expressed under *pforG*	pRD054	Hyg^R	This work
S. formicae: ΦBT1 GUS pforH	Wildtype strain with GUS expressed under *pforH*	pRD055	Hyg^R	This work
S. formicae ΔforGF: ΦBT1 GUS pforH	*forGF* deletion strain with GUS expressed under *pforH*	pRD055	Hyg^R	This work
S. formicaa: ΦBT1 GUS pforZ	Wildtype strain with GUS expressed under *pforZ*	pRD058	Hyg^R	This work
S. formicae ΔforZ: ΦBT1 GUS pforZ	*forZ* deletion strain with GUS expressed under *pforZ*	pRD058	Hyg^R	This work

Table S4: ePACs and plasmids used or generated in this study, related to **Experimental Model and subject details.**

Plasmid	Description	Resistance	Reference
pUZ8002	RK2 derivative with a mutation in *oriT*	Kan^R	(Keiser *et al.*, 2000)
pMS82	*ori,* pUC18, *hyg, oriT,* RK2, int ΦBT1	Hyg^R	(Gregory, Till and Smith, 2003)
pIJ773	*aac(3)IV oriT bla*	Apr^R	(Gust *et al.*, 2004)
pR9604	pUB307 derivative	$Carb^R$	(Piffaretti, Arini and Frey, 1988)
pESAC-13 215-G	*aph*II, *tsr*	Kan^R/Tsr^R	BioS&T and (Qin *et al.*, 2017)
pCRISPomyces-2	Apr^R, *oriT,* $rep^{pSG5(ts)}$, ori^{ColE1}, *sSpcas9,* synthetic guide RNA cassette	Apr^R	(Cobb, Wang and Zhao, 2015)
pIJ10257	*oriT*, ΦBT1 *attB-int*, *ermEp**, pMS81 backbone	Hyg^R	(Hong *et al.*, 2005)
pMF96	ΦBT1 *attB-int*, uidA CDS, GUS plasmid	Hyg^R	(Feeney *et al.*, 2017)
pIJ10740 (pMF23)	ΦC31 *attB-int, ermEp**	Apr^R	(Feeney *et al.*, 2017)
pRD026	pCRISPomyces-2 f*orJ* flanking DNA and gRNA	Apr^R	This work
pRD027	pCRISPomyces-2 f*orGF* flanking DNA and gRNA	Apr^R	This work
pRD028	pCRISPomyces-2 *ForZ* flanking DNA and gRNA	Apr^R	This work

pRD030	pMS82 *pforM* forJ	Hyg^R	This work
pRD031	pMS82 *pforG* forGF	Hyg^R	This work
pRD032	pMS82 *pforZ* forZ	Hyg^R	This work
pRD034	pMS82 *pforM* f*orJ 3x Flag*	Hyg^R	This work
pRD035	pMS82 *pforG* f*orGF 3x Flag*	Hyg^R	This work
pRD036	pMS82 *pforZ* *forZ 3x Flag*	Hyg^R	This work
pRD037	*pESAC-13* 215-G *1-4 aac(3)IV oriT*	Kan^R/Tsr^R	This work
pRD038	*pESAC-13* 215-G *1-7 aac(3)IV oriT*	Kan^R/Tsr^R	This work
pRD039	*pESAC-13* 215-G *32-43 aac(3)IV oriT*	Kan^R/Tsr^R	This work
pRD040	*pESAC-13* 215-G *36-43 aac(3)IV oriT*	Kan^R/Tsr^R	This work
pRD054	pMF96 pforGF GUS	Hyg^R	This work
pRD055	pMF96 pforH GUS	Hyg^R	This work
pRD058	pMF96 pforZ GUS	Hyg^R	This work
pRD062	pMF96 pforJ GUS	Hyg^R	This work

pRD063	pMS82 *pforJ* forJ	Hyg^R	This work
pRD064	pIJ10257 forJ	Hyg^R	This work

Table S5: Primers designed and used for this study (5'-3'), related to **Experimental Model and subject details.** Capital bases indicate overhangs, restriction sites etc.

Primer name	Description	Sequence
pCRISP Test F	Test XbaI site pCRISP2	aggctagtccgttatcaacttgaaa
pCRISP Test R	Test XbaI site pCRISP2	tcgccacctctgacttgagcgtcga
Spacer test	Test BbsI site of pCRISP2	atacggctgccagataaggc
ForJ For1	Repair template *forJ* KO, left flank	gctcggttgccgccgggcgtttttaTCTAGAggtgtgcgcgaagaacggcc
ForJ Rev 1	Repair template *forJ* KO, left flank	GCTGCTGCGACCAGGCGAGCTCGCcactgacgcggtcgttcccg
ForJ For 2	Repair template *forJ* KO, right flank	GCGAGCTCGCCTGGTCGCAGCAGCtgacgtgcttcgagaccgcc
ForJ Rev 2	Repair template *forJ* KO, right flank	gcaacgcggccttttacggttcctggccTCTAGAcctcttcatgttcctggtgggcc
ForJ gRNA For	sgRNA *forJ* deletion	ACGCtgccgacaccttctccatga
ForJ gRNA Rev	sgRNA *forJ* deletion	AAACtcatggagaaggtgtcggca
ForJ KO Test 1F	Test *forJ* deletion in genome	cctcttcggtgagcgcttcgagg
ForJ KO Test 2R	Test *forJ* deletion in genome	cctgttggacttcgcgcaggc
ForJ KO Test 2F	Test *forJ* deletion in genome	gtacgccaggaggacgtgcg
ForJ KO Test 1R	Test *forJ* deletion in genome	gccgacgcggcacttctatcc
ForZ For1	Repair template *forZ* KO, left flank	gctcggttgccgccgggcgtttttaTCTAGAcgaacaggccgacgctgaacag
ForZ Rev 1	Repair template *forZ* KO, left flank	GCTGCTGCGACCAGGCGAGCTCGCcatggcttgaagtccagcacgtcc
ForZ For 2	Repair template *forZ* KO, right flank	GCGAGCTCGCCTGGTCGCAGCAGCtcatccgtacctggcagctcgtcg
ForZ Rev 2	Repair template *forZ* KO, right flank	gcaacgcggccttttacggttcctggccTCTAGAccgaggcggacggatcgcgtcc
ForZ gRNA For	sgRNA *forZ* deletion	ACGCgtcggcggtcaactcgactg
ForZ gRNA Rev	sgRNA *forZ* deletion	AAACcagtcgagttgaccgccgac
ForZ KO Test 1F	Test *forZ* deletion in genome	gccggtgccgaaccggacgc
ForZ KO Test 2R	Test *forZ* deletion in genome	cgcacgccgccacgacgagc
ForZ KO Test 2F	Test *forZ* deletion in genome	cgcacgccgccacgacgagc
ForZ KO Test 1R	Test *forZ* deletion in genome	cgcacgccgccacgacgagc
ForGF For1	Repair template *forGF* KO, left flank	gctcggttgccgccgggcgtttttaTCTAGAggagccggtcttggccatctgc
FoGF Rev 1	Repair template *forGF* KO, left flank	GCTGCTGCGACCAGGCGAGCTCGCggcagcctcgttcacagcagc
ForGF For 2	Repair template *forGF* KO, right flank	GCGAGCTCGCCTGGTCGCAGCAGCtgaggctcaggcgggttcgatgg
ForGF Rev 2	Repair template *forGF* KO, right flank	gcaacgcggccttttacggttcctggccTCTAGAcgagatcgtcatccacgcgcc
ForGF gRNA For	sgRNA *forGF* deletion	ACGCtggcgaagatgttgcgcaga

ForGF gRNA Rev	sgRNA *forGF* deletion	AAACtctgcgcaacatcttcgcca
ForGF KO Test 1F	Test *forGF* deletion in genome	gcagttcctggacgatgcgc
ForGF KO Test 1R	Test *forGF* deletion in genome	cgagggtctggagaacgcgc
ForGF KO Test 2F	Test *forGF* deletion in genome	cgtcggcaccttctaccaccg
ForGF KO Test 2R	Test *forGF* deletion in genome	gcctgcgtgattcatcggctg
pMS82 f*orJ pforM* F1	*forJ* complementationunder *pforM*	gccgagaaccTAGGATCCAAGCTTgatgccggtgagcagggcgag
pMS82 *forJ pforM* R1	*forJ* complementationunder *pforM*	ggcgccgtggtcgtggtcataccggctcccatcggttgctg
pMS82 *forJ pforM F2*	*forJ* complementationunder *pforM*	cagcaaccgatgggagccggtatgaccacgaccacggcgcc
pMS82 *forJ pforM R2*	*forJ* complementation under *pforM*	CTGGTACCATGCATAGATCTAAGCTTgcggaggcggaccgtgcctag
pMS82 *forZ* F	*forZ* complementation	gccgagaaccTAGGATCCAAGCTTccggtcaccacccattggag
pMS82 *forZ* R	*forZ* complementation	CTGGTACCATGCATAGATCTAAGCTTaggagttgtgcgccctcgc
pMS82 *forGF F*	*forGF* complementatio	gccgagaaccTAGGATCCAAGCTTcgtgtaccccctgtgcacg
pMS82 *forGF* R	*forGF* complementatio	CTGGTACCATGCATAGATCTAAGCTTccgctgctcgccatcgaac
pMS82 TEST F	Test HindIII site pMS82	gcaacagtgccgttgatcgtgctatg
pMS82 TEST R	Test HindIII site pMS82	gccagtggtatttatgtcaacaccgcc
ForJ-3xFLAG R	*forJ 3x*Flag for ChIP	gcctgaaccgcctccaccgtgccccgcgggcacctg
ForJ-3xFLAG F	*forJ 3x*Flag for ChIP	caggtgcccgcggggcacggtggaggcggttcaggc
FLAG-pMS82 R	3*x*Flag in pMS82	CTGGTACCATGCATAGATCTAAGCTTtcaCTTGTCGTCATCGTCCTTG
pMS82 ForF prom F	*forGF 3x*Flag for ChIP	gccgagaaccTAGGATCCAAGCTTcgtgtaccccctgtgcacg
ForF-prom R	*forGF 3x*Flag for ChIP	ggtcaccacggtctgcatagcagcctccccggttcg
ForGF F	*forGF 3x*Flag for ChIP	cgaaccggggaggctgctatgcagaccgtggtgacc
ForF-3xFLAG R	*forGF 3x*Flag for ChIP	gcctgaaccgcctccaccgccccggtcgccctgcg
FLAG-pMS82 F	3*x*Flag in pMS82	cgcagggcgaccggggcggtggaggcggttcaggc
ForZ-3xFLAG R	*forZ 3x*Flag for ChIP	gcctgaaccgcctccaccccgctcgcacgccgccacg
ForZ-3xFLAG F	*forZ 3x*Flag for ChIP	cgtggcggcgtgcgagcggggtggaggcggttcaggc
ForJ Test F	To check expression by RT-PCR	gcaaggcggcgcagagcg
ForJ Test R	To check expression by RT-PCR	gccgacaccttctccatgagg
ForZ Test F	To check expression by RT-PCR	gaaccggacgcagccgcag
ForZ Test R	To check expression by RT-PCR	cctcgacgcgtgccacgag
ForF Test F	To check expression by RT-PCR	cctcgacgcgtgccacgag
ForF Test R	To check expression by RT-PCR	gcgaccagggtcatgacctcg
pMF96 HindIII Test For	Test HindIII site of pMF96	gctcaatcaatcaccggatcc
pMF96 HindIII Test Rev	Test HindIII site of pMF96	catgtccgtacctccgttg
16S r RNA For	qPCR reference gene	cgggtctgcagtcgatacgg

16S r RNA Rev	qPCR reference gene	gctttcgctcctcagcgtcag
MLK For	qPCR expression	ctgatcttcggtgccttcctgtcc
MLK Rev	qPCR expression	cggcgagcagtccgaggtc
J For	qPCR expression	ccgaccgtgcggaaactcg
J Rev	qPCR expression	gtccggatccacatgccgc
HI For	qPCR expression	ccttcgagttcgtcgtggacg
HI Rev	qPCR expression	gctgctcggcgaccagatc
GF For	qPCR expression	gctccaccactacgaacagcg
GF Rev	qPCR expression	ggagcgagtcctcgatcacg
TSRABCDE For	qPCR expression	cgacaccatcgacaccgcc
TSRABCDE Rev	qPCR expression	cgttccactccacgaccacc
UVWXY For	qPCR expression	gcagcttctccaggagttcc
UVWXY Rev	qPCR expression	gccaagaagatcctcgacagg
Z For	qPCR expression	ctcatccggctcgtcacgc
Z Rev	qPCR expression	cagatggcggttggcgagc
AA For	qPCR expression	gaccggaggaacgcctgg
AA Rev	qPCR expression	cggtgtcgaggtccttgctc
*pforH-G*F F For	*pforG* in pMF96	AAAAAcatatggcgctgctcacggtcatcg
*pforH-G*F Rev	*pforG* in pMF96	AAAAActcgaggcagcctcgttcacagcag
pforZ-AA F For	*pforZ* in pMF96	AAAAAcatatggaatccctgacgcgccgcg
pforZ-AA F Rev	*pforZ* in pMF96	AAAAActcgaggacgatggtggtgtcgagcac
forJ pMF96 360 bp prom for	*pforJ* in pMF96	caattaatctagaggatccatatgcctgttcgtcgcggtggc
forJ pMF96 360 bp prom rev	*pforJ* in pMF96	gtacctccgttgctcgactcgaggttcgctcacctctgctgtgacg
pforGF-H For	*pforH* in pMF96	AAAAAcatatggcagcctcgttcacagcagc
pforGF-H Rev	*pforH* in pMF96	AAAAActcgaggcgctgctcacggtcatcg
pMS82 *forJ pforJ* F1	*forJ* complementationunder *pforJ*	gccgagaaccTAGGATCCAAGCTTcctgttcgtcgcggtggc
pMS82 *forJ pforJ* R1	*forJ* complementationunder *pforJ*	ggcgccgtggtcgtggtcatgttcgctcacctctgctgtgacg
pMS82 *forJ pforJ* F2	*forJ* complementationunder *pforJ*	cgtcacagcagaggtgagcgaacatgaccacgaccacggcgcc
pMS82 *forJ pforJ* R2	*forJ* complementationunder *pforJ*	CTGGTACCATGCATAGATCTAAGCTTgcggaggcggaccgtgcctag
pIJ10257 *forJ* F	*forJ* complementationunder *pErmE**	gtctagaacaggaggccccatatgatgaccacgaccacggcgc
pIJ10257 *forJ R*	*forJ* complementationunder *pErmE**	catgagaacctaggatccaagcttggaacgaccgcgtcagtgcc

Figure S1. Calibration curve for Fasamycin E and Formicamycin I, related to **Figure 2, Table 1 and Method Details: Titre Determination.**