microorganisms

MDPI

Supplementary files.

Supplementary files. Table S1. Bacterial strains used in this study.

	Strains	N	Genotype /description	Source / reference
Escherichia coli	XL1-Blue	/	endA1 gyrA96 (Nalr) thi-1 recA1 relA1 lac glnV44 hsdR17(rK – mK +) F= [::Tn10 (Tetr) proAB+ lacIqZΔM15]	Stragene
Streptococcus agalactiae	12/111 (WT)	1	Isolated from maternal bacteraemia	[16]
	ΔphiA strain	1	Isogenic 12/111phiA deletion mutant of 12/111	This study
	Δ*relB-metK* strain	2	Isogenic Δ*relB-metk* (BIH62_0555 to BIH62_05470) region deletion mutant of 12/111	This study
	Δ*relB-yafQ* strain	2	Isogenic Δ*relB-yafQ* (BIH62_0555 toB1H62_05460) region deletion mutant of 12/111	This study
	Δ*endonuclease-metK* strain	2	Isogenic Δ*endonuclease-metK* (B1H62_05465 to B1H62_05470) region deletion mutant of 12/111	This study
	Δ*endonuclease* strain	2	Isogenic Δ*endonuclease* (B1H62_05465) deletion mutant of 12/111	This study
	Δ*metK* strain	2	Isogenic Δ*metK* (B1H62_05470) deletion mutant of 12/111	This study
	Δ*relB-metK*::*relB-metK* strain	2	*relB-metK* region in situ complementation of 12/111Δ*relB-metK*	This study
	Δ*relB-yafQ*::*relB-yafQ* strain	2	*relB-yafQ* region in situ complementation of 12/111Δ*relB-yafQ*	This study
	Δ*endonuclease-metK* ::*endonuclease-metK* strain	2	*Endonuclease-metK* region in situ complementation of 12/111Δ*endonuclease-metK*	This study
	Δ*endonuclease*:: *endonuclease* strain	2	endonuclease in situ complementation of 12/111Δ*endonuclease*	This study
	Δ*metK* ::*metK* strain	2	metK in situ complementation of 12/111Δ*metK*	This study
	12/111/ pTCV-P_{Tet}	2	12/111 caring empty complementation plasmid for control	This study
	ΔphiA /pTCV-P_{Tet}	1	12/111ΔphiA caring empty complementation plasmid for control	This study
	Δ*relB-metK*/pTCV-P_{Tet}	1	12/111Δ*relB-metK* caring empty complementation plasmid for control	This study
	ΔphiA / pTCV-P_{Tet} :: *relB-yafQ* strain	1	*relB-yafQ* plasmid complementation of 12/111ΔphiA	This study
	ΔphiA /pTCV-P_{Tet} :: *endonuclease-metK* strain	1	*endonuclease-metK* plasmid complementation of 12/111ΔphiA	This study
	ΔphiA /pTCV-P_{Tet} :: *endonuclease* strain	1	*endonuclease* plasmid complementation of 12/111ΔphiA	This study
	ΔphiA / pTCV-PTet :: *metK* strain	1	*metK* plasmid complementation of 12/111ΔphiA	This study
	Δ*relB-metK*/pTCV-P_{Tet} :: *relB-yafQ* strain	2	*relB-yafQ* plasmid complementation of 12/111ΔphiA	This study
	Δ*relB-metK*/pTCV-P_{Tet} :: *endonuclease-metK* strain	1	*endonuclease-metK* plasmid complementation of 12/111 ΔrelB-metK	This study
	Δ*relB-metK*/pTCV-P_{Tet} :: *endonuclease* strain	2	*endonuclease* plasmid complementation of 12/111 ΔrelB-metK	This study
	Δ*relB-metK*/pTCV-P_{Tet} :: *metK* strain	1	*metK* plasmid complementation of 12/111Δ*relB-metK*	This study

Supplementary files. Table S2. Plasmid used in this study.

Plasmids	Genotype /description	Source / reference
pG+host1^{TS}	Replication-thermosensitive shuttle (TS) plasmid; Ery^{r}	[28]
pTCV-P_{Tet}	Mob^{+} (IncP); oriR pACYC184; oriR pAM_1; complementation vector, promoter P_{Tet}	[29]
pTCV-P_{Tet} :: *relB-yafQ*	*relB-yafQ* complementation vector, promoter P_{Tet}	This study
pTCV-P_{Tet} :: *endonuclease-metK*	*Endonuclease-metK* complementation vector, promoter P_{Tet}	This study
pTCV-P_{Tet} :: *endonuclease*	*endonuclease* complementation vector, promoter P_{Tet}	This study
pTCV-P_{Tet}:: *metK*	*metK* complementation vector, promoter P_{Tet}	This study

Supplementary files. Table S3: Primers used in this study.

Functions	Primers	Sequence (5′–3′)
Deletion of *relB-metK* region	AR1	CTTACAGGATCCATGTTCTCCTTCATCAAGCTG
	AR2	TTTTTGCGTGGACTCCTACAGTTTCTTTAATTGTATC
	AR3	AAACTGTAGGAGTCCACGCAAAAAGCCCAACC
	AR4	GCCGTGGAATTCTTCATAATTGGCGTAGAAAGTTATTGGAAC
Deletion of *relB-yafQ* region	AR1	CTTACAGGATCCATGTTCTCCTTCATCAAGCTG
	AR64	GACTTTGATGAGAGTATCCTCCTACAGTTTCTTTAATTGTATC
	AR65	TAGGAGGATACGCTCATCAAAGTCACTTTATG
	AR66	ATTATCGAATTCAACAACTAGTAGGAGTGGTT
Deletion of *endonuclease-metK* region	AR58	TCTTTGGGATCCTTGGTATTTCTGTTACTGACGC
	AR71	GTGGAGGAAAACACCATAAGAAAAAAGCCACTCG
	AR72	TTTTCTTATGGTGTTTTCCTCCACGCAAAAAG
	AR4	GCCGTGGAATTCTTCATAATTGGCGTAGAAAGTTATTGGAAC
Deletion of *endonuclease* region	AR67	TCTTTGGGATCCTTGGTATTTCTGTTACTGACGC
	AR68	GGGAGCAGTAAGACCATAAGAAAAAAGCCACTCG
	AR69	TTTTCTTATGGTCTTACTGCTCCCAAGGAAGA
	AR70	AAAGTCGAATTCCCTTATGGCATGAGAGATGTCA
Deletion of *metK* region	AR61	GGAAATGGATCCGGATTTAGAGAGTGCCACATTGAACC
	AR62	GTTGGGCTTTTTGGATGCCACGTCGACCAAGCA
	AR63	CGACGTGGCATCCAAAAAGCCCAACCCTTGTG
	AR4	GCCGTGGAATTCTTCATAATTGGCGTAGAAAGTTATTGGAAC
In situ complementation of *relB-metK* region	AR1	CTTACAGGATCCATGTTCTCCTTCATCAAGCTG
	AR4	GCCGTGGAATTCTTCATAATTGGCGTAGAAAGTTATTGGAAC
In situ complementation of *relB-yafQ* region	AR1	CTTACAGGATCCATGTTCTCCTTCATCAAGCTG
	AR66	ATTATCGAATTCAACAACTAGTAGGAGTGGTT
In situ complementation of *endonuclease-metK* region	AR58	TCTTTGGGATCCTTGGTATTTCTGTTACTGACGC
	AR4	GCCGTGGAATTCTTCATAATTGGCGTAGAAAGTTATTGGAAC
In situ complementation of *endonuclease*	AR67	TCTTTGGGATCCTTGGTATTTCTGTTACTGACGC
	AR70	AAAGTCGAATTCCCTTATGGCATGAGAGATGTCA
In situ complementation of *metK*	AR61	GGAAATGGATCCGGATTTAGAGAGTGCCACATTGAACC
	AR4	GCCGTGGAATTCTTCATAATTGGCGTAGAAAGTTATTGGAAC
Plasmid complementation of *relB-yafQ*	AR33	ATTAATGGATCCGTAGGAGGATACTAAAATGG
	AR34	TAAGAACTGCAGAAAAACTTTGAGCAATTTGCTCAAAGTTTTTCACTCGCATAAAGTGACTT
Plasmid complementation of *endonuclease-metK*	AR85	GGTTCTCTGCAGAAAAACTTTGAGCAATTTGCTCAAAGTTTTTCGAGTGGCTTTTTTCTTATGGT
	AR36	TTGAAAGGATCCGCGTGGAGGAAAACATGATTT
Plasmid complementation of *endonuclease*	AR85	GGTTCTCTGCAGAAAAACTTTGAGCAATTTGCTCAAAGTTTTTCGAGTGGCTTTTTTCTTATGGT
	AR86	TTTAAGGGATCCCTTCCTTGGGAGCAGTAAGATG
Plasmid complementation of *metK*	AR35	CTTACACTGCAGAAAAACTTTGAGCAATTTGCTCAAAGTTTTTTGCTTGGTCGACGTGGC
	AR36	TTGAAAGGATCCGCGTGGAGGAAAACATGATTT

Supplementary files. Table S4: Comparison of prophagic RelB, YafQ and Metk proteins with their bacterial homologs. NS corresponding to no significant similarity found.

Query	Subject (reference)	query cover (in %)	identity (in %)
12/111phiA_RelB	E.coli_RelB [45]	48	26.7
	E.coli_DinJ [45]	58	29.6
	P307_RelB [45]	NS	NS
	H.i._RelB [45]	66	25.8
	V.c._RelB [45]	NS	NS
12/111phiA_YafQ	E.coli_RelE [45]	NS	NS
	E.coli_YoeB [45]	44	32.5
	aRelE [45]	NS	NS
	E.coli_YafQ [45]	93	39.5
12/111phiA_MetK	12/111_MetK	97	44.3
	E.coli_MetK1 [47]	97	55.2
	E.coli_MetK [47]	95	56.6

Supplementary files. Table S5: Search for *S. agalactiae* strains carrying prophages similar to 12/111phiA using blastn analysis from the NCBI database (Per. Query cover >60% and %Identity >90%).

Description	Query Cover	Per. Ident	E-value	Hit Lengh (bp)	Hit ac. numbers
Streptococcus agalactiae CUGBS591	97%	99,05%	0	2227680	CP021862
Streptococcus agalactiae FDAARGOS_670	97%	99,04%	0	2210718	CP044090
Streptococcus agalactiae Sag153	97%	99,04%	0	2174504	CP036376
Streptococcus agalactiae Sag37	97%	99,04%	0	2198785	CP019978
Streptococcus urinalis NCTC13766	97%	95,52%	0	2144000	LR134323
Streptococcus agalactiae 32790-3A	95%	99,05%	0	2148904	CP029561
Streptococcus agalactiae B105	95%	99,05%	0	2273717	CP021773
Streptococcus agalactiae FDAARGOS_512	95%	99,05%	0	2134138	CP033822
Streptococcus agalactiae NGBS128 (phiStag1)	95%	99,05%	0	2074179	CP012480
Streptococcus sp. 'group B' FDAARGOS_229	95%	99,05%	0	2178261	CP020432
Streptococcus agalactiae NJ1606	84%	94,19%	0	2136438	CP026084
Streptococcus agalactiae YZ1605	84%	94,06%	0	2281602	CP026082
Streptococcus agalactiae C001	83%	91,83%	0	2121372	CP008813
Streptococcus MR1-Z1-201	78%	93,41%	0	41765	LR134293
Streptococcus constellatus FDAARGOS_1015	75%	93,03%	0	2038583	CP066055
Streptococcus constellatus FDAARGOS_1208	75%	93,04%	0	1978680	CP069558
Streptococcus constellatus subsp. pharyngis C1050	75%	93,04%	0	1991156	CP003859
Streptococcus constellatus subsp. pharyngis C232	75%	93,04%	0	1935414	CP003800
Streptococcus constellatus subsp. pharyngis C818	75%	93,04%	0	1935662	CP003840
Streptococcus phage Javan639	73%	94,51%	0	38797	MK448825
Streptococcus phage Javan95	73%	94,50%	0	38797	MK448836
Streptococcus canis HL_77_2	72%	90,13%	0	2152128	CP053790
Streptococcus phage Javan101	67%	93,04%	0	37952	MK448666
Streptococcus phage Javan107	67%	93,04%	0	37952	MK448668
Streptococcus phage Javan113	67%	93,04%	0	37901	MK448670
Streptococcus phage Javan93	67%	94,42%	0	37554	MK448835
Streptococcus phage Javan25	65%	98,93%	0	35085	MK448717
Streptococcus phage Javan32	65%	98,93%	0	35085	MK448906
Streptococcus phage Javan38	65%	98,93%	0	35085	MK448922
Streptococcus phage Javan65	65%	98,93%	0	35085	MK448827
Streptococcus equi subsp. zooepidemicus ATCC 35246	63%	92,06%	0	2167264	CP002904
Streptococcus equi subsp. zooepidemicus OH-71905	63%	92,06%	0	2189155	CP046040
Streptococcus equi subsp. zooepidemicus TN-714097	63%	92,06%	0	2188654	CP046042

Supplementary files. Table S6: Microbial count after 24h culture in TH/MRS.

Strains	CFU/mL	Standard Deviation
12/111	$3.8x10^5$	$8.6x10^4$
12/111ΔphiA	$8.0x10^4$	$5.9x10^4$

Supplementary files. Table S7: Identification of non-synonymous mutations in in situ deleted and complemented mutants. This analysis was performed using the Variation analysis service from the PATRIC server. Nucleotides modification was identified by "type" column. Syn: refers to no change on the encoded protein. Nonsyn: refers to non-synonymous mutation leading to amino acid change. "Ref-nt" and "Var-nt" corresponding respectively to sequence using like reference and sequence identified as modified. "Ref_nt_pos_change" indicated nucleotide position of the change in reference genome. "Ref_aa_pos_change" corresponding to position of amino acid (aa) involved by mutation and indicated aa replacing reference aa. "Upstream feature" refers to gene located upstream to the mutated gene. "downstream feature" refers to gene located downstream to the mutated gene. "snpEff_type" indicated location and nature of mutation. "snpEff _impact" informed of impact of detected mutations.

Samples	Contig	Type	Ref_nt	Var_nt	Ref_nt_pos_change	Ref_aa_pos_change	Gene_ID	Function	Upstream feature	Downstream feature	snpEff_type	snpEff_impact
12/111Δ*relB-metK*:: *relB-metK* n°1 VS	1311.3476.con.0004	Nonsyn	taa	Caa	253T>C	Ter85Gln ext*	fig|1311.3476.peg.661	ABC transporter membrane-spanning permease, Pep export, Vex1	hypothetical protein	ABC transporter membrane-spanning permease, Pep export, Vex1	stop_lost	HIGH
12/111Δ*relB-metK* n°1	1311.3476.con.0007	Nonsyn	taa	Caa	328T>C	Ter110Gln ext*	fig|1311.3476.peg.1037	Xylulose kinase (EC 2.7.1.17)	Xylulose kinase (EC 2.7.1.17)	hypothetical protein	stop_lost	HIGH

A- RelB

```
P307_RelB            MPNIILSDTSASVSELKKNPMATVSAGDGFPVAILNRNQPAFYCVPAELYEKMLDA----
         56
V.c._RelB            MTTRILADVAASITEFKANPMKVATSAFGAPVAVLNRNEPAFYCVPASTYEIMMDK----
         56
12/111phiA_RelB      --MAKTANINLRIEPSTKAQAESLFGSFGISV--------------TDAINIFLNTSIME
         44
E.coli_DinJ          --MAANAFVRARIDEDLKNQAADVLAGMGLTI--------------SDLVRITLTKVARE
         44
E.coli_RelB          -----MGSINLRIDDELKARSYAALEKMGVTP--------------SEALRLMLEYIADN
         41
H.i._RelB            -MALTNSSISFRTVEKTKLEAYQVIEQYGLTP--------------SQVFNMFLAQIAKT
         45


P307_RelB            -----------LDDQELVKLVAERSNQPL-----HDVDLDKYL-----------------
         83
V.c._RelB            -----------LEDLELLAIAKERLSEDS-----VSVNIDDL------------------
         82
12/111phiA_RelB      GGFPFQIKQPRYNRETELAMEEARQIMEGKVTTKSYASVSDLMADLNED-----------
         93
E.coli_DinJ          KALPFDLRE--PNQLTIQSIKNSEAGIDV----HKAKDADDLFDKLGI------------
         86
E.coli_RelB          ERLPFKQTLLSDEDAELVEIVKER-----------LRNPKPVRVTLDE------L-----
         79
H.i._RelB            RSIPVDLNYLRPNKETLAAIDELD-----------SGNAESFFIEASENYSAEEFTKRIL
         94

P307_RelB            ---- 83
V.c._RelB            ---- 82
12/111phiA_RelB      ---- 93
E.coli_DinJ          ---- 86
E.coli_RelB          ---- 79
H.i._RelB            NGGQ 98
```

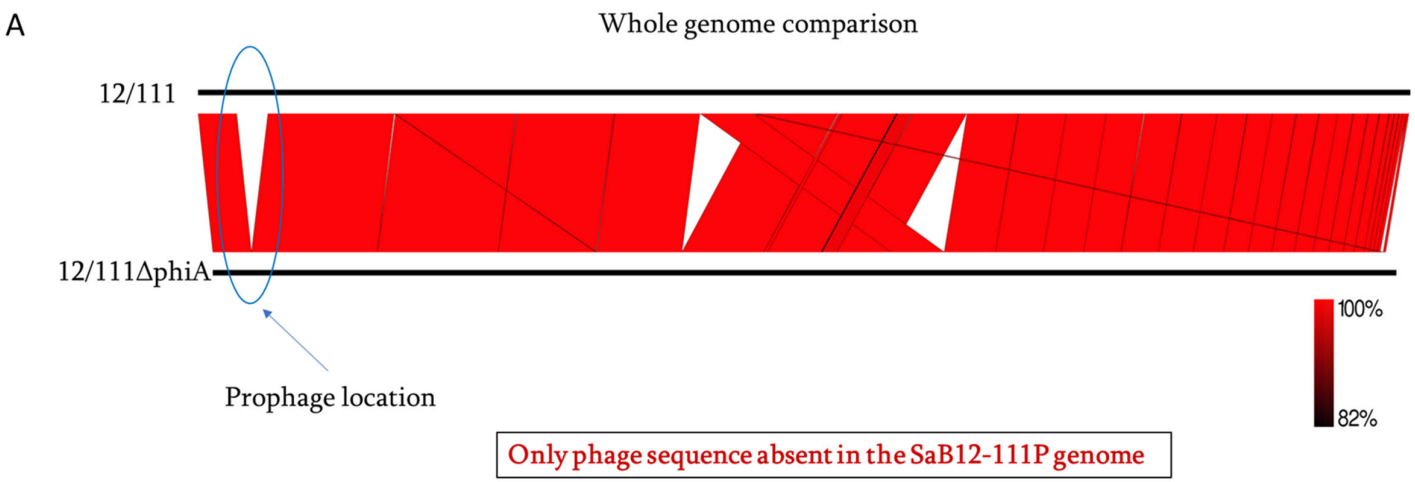
B- YafQ

```
E.coli_YoeB          ---MKLIWSEESWD-DYLYWQETDKRIVKKINELIK--DTRRTPFEG-KGKPEPLKHNLS
         53
E.coli_YafQ          MIQRDIEYSGQYSK-DVKLAQKR-HKDMNKLKYLMTLLINNTLPLPA-VYKDHPLQGSWK
         57
12/111phiA_YafQ      --MLQLVTTNQFRK-DVKRAKKR-GLNLKKLEAVLDPL-QKEETLDE-KHRDHALVGNYM
         54
E.coli_RelE          -----MAYFLDFDERALKEWRKLGSTVREQ-LK-KKLVE----VLESPRIEANKLRGMPD
         49
aRelE                -----MTYRVKIHKQVVKALQSLPKAHYRRFLEFRDILE--YEPVPREKFDVIKLEGTGD
         53

E.coli_YoeB          GFWSRRITEEHRLVYAVTDDSLLIAACRYHY---------------
         84
E.coli_YafQ          GYRDAHVEPDWILIYKLTDKL--LRFERTGTHAA--LFG-------
         92
12/111phiA_YafQ      GFRECHIEPDWLLVYAIDKGQLILTASRTGSHSD--LF--------
         90
E.coli_RelE          CYKIKLRSSGYRLVYQVIDEKVVVFVISVGKRERSEVYSEAVKRIL
         95
aRelE                LDLYRARLGDYRVIYSVNWKDKVIKILKLKPRGR--AYK-------
         90
```

C- MetK

```
12/111phiA_MetK      ----MIFTSEQVSSGHPDKLCDQISDAIVTECLKHDKNSRVAVETLIKDNQVVVAGEVST
         56
12/111_MetK          MSERKLFTSESVSEGHPDKIADQISDAILDAILEQDPDAHVAAETAVYTGSVHVFGEIST
         60
E.coli_MetK1         -MNDYLFTSESVAEGHPDKMADQISDAILDAILLQDPWGKVACECLVKTGATIVAGEIST
         59
E.coli_MetK          -MAKHLFTSESVSEGHPDKIADQISDAVLDAILEQDPKARVACETYVKTGMVLVGGEITT
         59

12/111phiA_MetK      KYYFNLEGIVKKVLEPYGM---------RDVMVTNLLGVQSPDIAQGVDKG---------
         98
12/111_MetK          TAYVDINRVVRNTIAEIGYDKAEYGFSAESVGVHPSLVEQSPDIAQGVNEALEVRGSLEQ
         120
E.coli_MetK1         HAAVDIEKIVRNTIKEIGYDHSRLGFDGNTCCVLNILGKQSANIADGIRGH---------
         110
E.coli_MetK          SAWVDIEEITRNTVREIGYVHSDMGFDANSCAVLSAIGKQSPDINQGVDRA---------
         110

12/111phiA_MetK      ------SAGDQGIMFGYATDETPEFLPLPYVLATRVLEKLTNL-----------------
         135
12/111_MetK          DPLDLIGAGDQGLMFGFAVDETPELMPLPISLAHQLVKKLTDLRKSGELTYLRPDAKSQV
         180
E.coli_MetK1         -SMEELGAGDQGITFGYACDETSELMPATLVYAHRLMERQAQLRKSQRLPFLLPDAKSQV
         169
E.coli_MetK          -DPLEQGAGDQGLMFGYATNETDVLMPAPITYAHRLVQRQAEVRKNGTLPWLRPDAKSQV
         169

12/111phiA_MetK      ------------------------------------------------------------
         135
12/111_MetK          TVEYDDNDQPIRVDAVVISTQHDPNVTNDQLHKDVIEKVINEVIPSHYLDDQTKFFINPT
         240
E.coli_MetK1         TLRYQDNR-VHSVDTVVVSTQHSPDVSLDALREAVIEEIVKPVMPSHWLTPQTRFLVNPA
         228
E.coli_MetK          TFQYDDGK-IVGIDAVVLSTQHSEEIDQKSLQEAVMEEIIKPILPAEWLTSATKFFINPT
         228


12/111phiA_MetK      -------------------------------GHPALGKDPSKVDRSAAYMARKIAKDFVR
         164
12/111_MetK          GRFVIGGPQGDSGLTGRKIIVDTYGGYSRHGGGAFSGKDATKVDRSASYAARYIAKNIVA
         300
E.coli_MetK1         GSFVIGGPVGDCGLTGRKIIVDTYGGAACHGGGAFSGKDPSKVDRSAAYAARYVAKNIVA
         288
E.coli_MetK          GRFVIGGPMGDCGLTGRKIIVDTYGGMARHGGGAFSGKDPSKVDRSAAYAARYVAKNIVA
         288


12/111phiA_MetK      EGYAKRCEVQLAYAIGVAEPVGVYVNTFGTSDYPLEQLVGVVRERYDLTPQGIIKELNLL
         224
12/111_MetK          ADLAKKVEVQLAYAIGVAQPVSVRVDTFGTGVIAEADLEAAVRQIFDLRPAGIINMLDLK
         360
E.coli_MetK1         AGLASRCEVQLGWAIGLPRPVSVRINTFGTQTVSSEALLRGVNSHFDLSVFGIITTLDLL
         348
E.coli_MetK          AGLADRCEIQVSYAIGVAEPTSIMVETFGTEKVPSEQLTLLVREFFDLRPYGLIQMLDLL
         348


12/111phiA_MetK      DVDYTKTTCLGHFTKPY--LPWEQ--------------246
12/111_MetK          RPIYRQTAAYGHMGRTDIDLPWERVDKVQALKDFIASK 398
E.coli_MetK1         VPRYRKTACYGHFGRDS--FPWEVTDKAALLYEDVGR- 383
E.coli_MetK          HPIYKETAAYGHFGREH--FPWEKTDKAQLLRDAAGLK 384
```

Supplementary files. Figure S1. Alignment of prophagic RelB, YafQ and MetK proteins with their bacterial functional homologs. A- Alignment of prophagic RelB protein with bacterial member of RelB antitoxin family described by [45].B- Alignment of prophagic YafQ protein with bacterial member of RelE toxin family described by [45] with highlighting of amino acid essential for RelE toxin activity in bold and for YafQ toxin activity in red and bold [48,49].C- Alignment of prophagic MetK protein with functional bacterial MetK [47] and 12/111 bacterial protein.

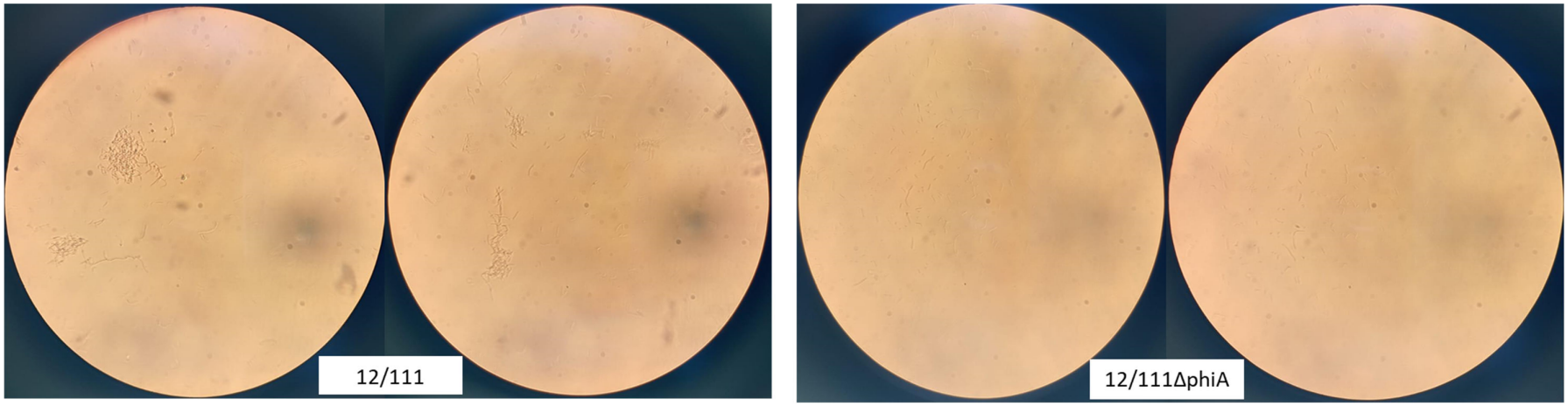


B

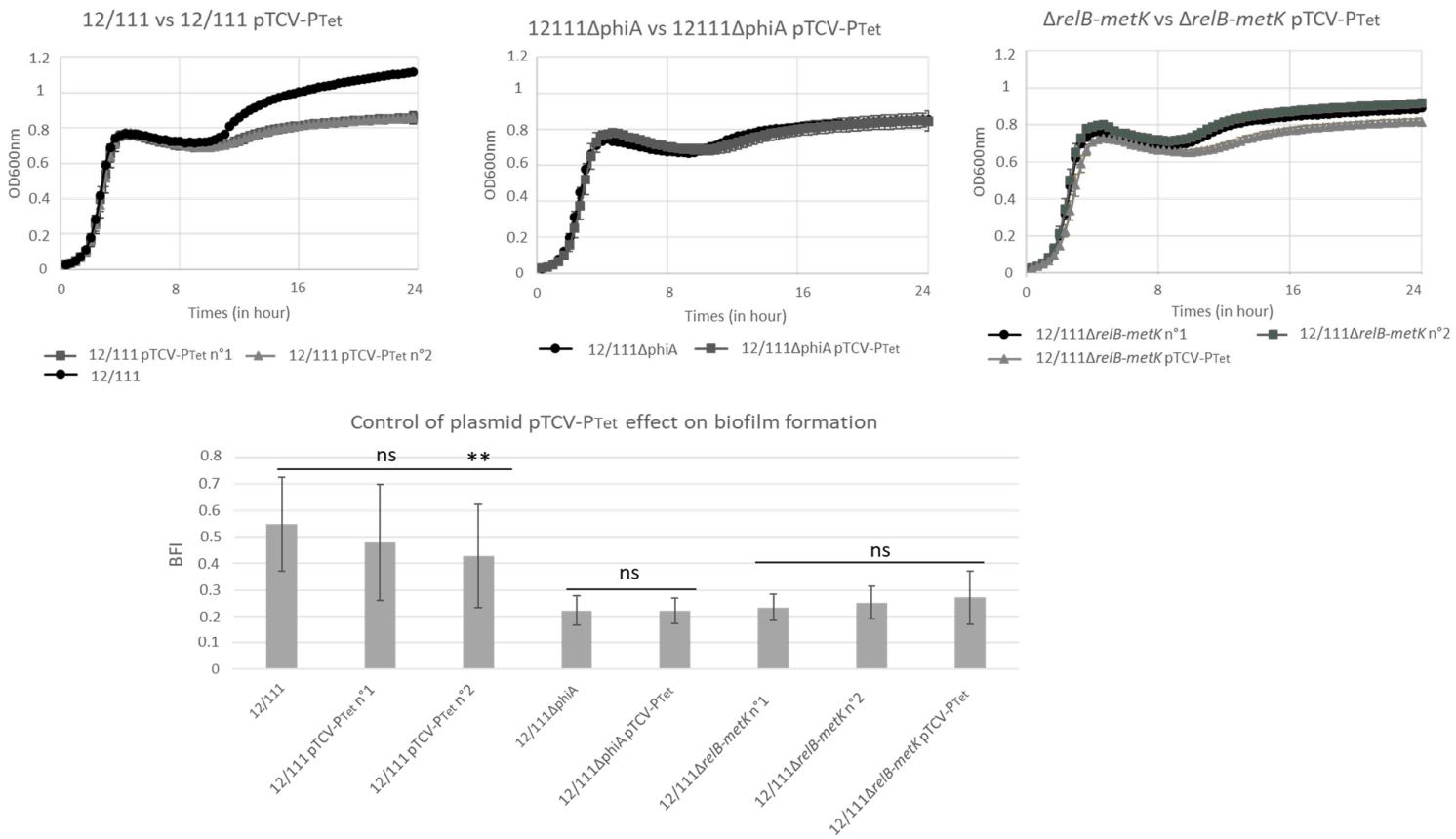
Pairwise Comparison of Genomic SNPs

	12/111ΔphiA	12/111
12/111ΔphiA	0	0
12/111	0	0

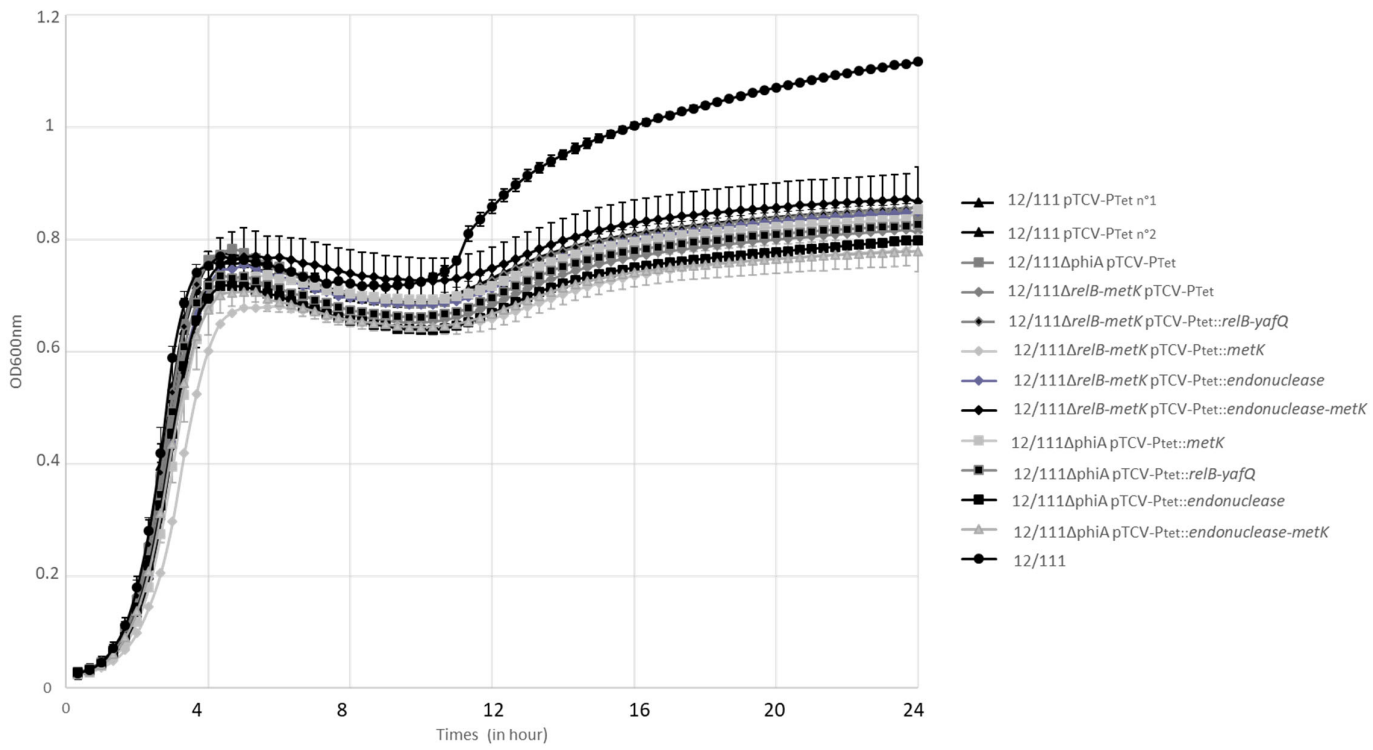
Supplementary files. Figure S2: 12/111 vs. 12/111ΔphiA genomic comparison. A- Whole genome alignment between 12/111 and 12/111ΔphiA; deletion of the phiA from the 12/111ΔphiA genome is indicated with arow. B-Single nucleotide polymorphism (SNP) pairwise comparison.



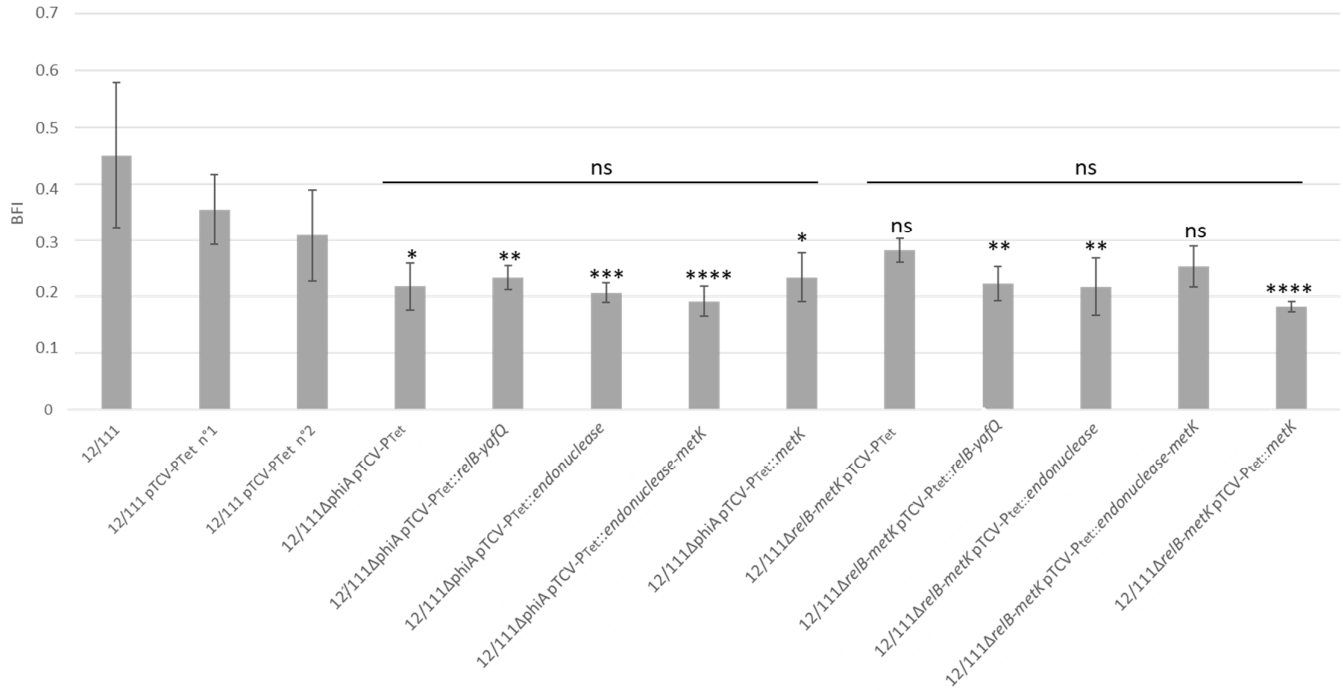
Supplementary files. Figure S3: Microscopic appearance of 12/111, 12/111ΔphiA. Bacterial examination by wet mount (x40).



Supplementary files. Figure S4: Comparative growth and biofilm formation of 12/111, 12/111ΔphiA and 12/111Δ*relB-metK* respectively with 12/111 pTCV-P_{Tet}, 12/111ΔphiA pTCV-P_{Tet} and 12/111Δ*relB-metK* pTCV-P_{Tet}. Growth on TH/MRS medium supplemented or not with erythromycin [10 µg/mL] at 37°C with agitation and measured every 20 min for 24h. Curve data represent mean OD600nm measurement from three independent experiments. Biofilm formation on TH 1% glucose medium and translated by biofilm formation index (BFI) value.** $p<0.01$; ns = no significance. Histogram data represent BFI mean from three independent experiments.



Supplementary files. Figure S5: Determination of *relB-yafQ,* endonuclease and *metK* ORFs involvement in *S. agalactiae* growth. Growth of 12/111 pTCV-P_{Tet}, 12/111ΔphiA pTCV-P_{Tet}, 12/111Δ*relB-metK* pTCV-P_{Tet} and their associated plasmid complemented mutants. Growth on TH/MRS medium supplemented with erythromycin [10 µg/mL] (except for 12/111 control strain) at 37°C with agitation and measured every 20 min for 24h. Data represent mean OD600nm measurement from three independent experiments.



Supplementary files. Figure S6: Determination of *relB-yafQ*, *endonuclease* and *metK* ORFs involvement in biofilm formation. 12/111 pTCV-P_{Tet}, 12/111ΔphiA pTCV-P_{Tet}, 12/111Δ*relB-metK* pTCV-P_{Tet} and their associated plasmid complemented mutants' ability to produce biofilm in TH 1% glucose translated by biofilm formation index (BFI). * corresponding $p<0.05$; ** to $p<0.01$;**** to $p<0.0001$; ns = no significance. Data represent mean of BFI from three independent experiment.