SUPPLEMENTARY INFORMATION for

In vivo characterization of phosphotransferase-encoding genes *istP* and *forP* as interchangeable launchers of the C3′,4′-dideoxygenation biosynthetic pathway of 1,4-diaminocyclitol antibiotics

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Table S1 Bacterial strains and plasmids used in this study.

Strains/plasmids	Relevant characteristics	Source
Strains		
E. coli XL1 Blue MRF'	General cloning host	Stratagene
E. coli ET12567/ pUZ8002	Demethylation host (*recE dam*$^{-}$ *dcm*$^{-}$ *hsdS* Cm^{R} Str^{R} Tet^{R} Km^{R}), *E. coli-Streptomyces* intergeneric conjugation	Stratagene
M. olivasterospora	Wild-type strain, fortimicin producer	DSM43868
ΔforP	*forP* disrupted mutant strain of *M. olivasterospora*	This study
ΔforP::*forP*	*forP* disrupted mutant strain of *M. olivasterospora* containing an complementation plasmid pFORP	This study
ΔforP::*istP*	*forP* disrupted mutant strain of *M. olivasterospora* containing an complementation plasmid pISTP2	This study
S. tenjimariensis	Wild-type strain, istamycin producer	ATCC31603
ΔistP	*istP* disrupted mutant strain of *S. tenjimariensis*	This study
ΔistP::*istP*	*istP* disrupted mutant strain of *S. tenjimariensis* containing an complementation plasmid pISTP	This study
ΔistP::*forP*	*istP* disrupted mutant strain of *S. tenjimariensis* containing an complementation plasmid pFORP1	This study
Plasmids		
pGEM^{R}-T -easy vector	*E. coli* general sub-cloning vector, *amp*^{R}	Promega (USA)
pKC1139	*Streptomyces- E. coli* bifunctional vector, *apr*^{R}	Stratagene
pKCE	pKC1139 containing *apr*^{R}, *erm*^{R}	[12]
pIP501	pKCE carrying *istP* right-hand and left-hand PCR fragments, used for *istP* disruption, *apr*^{R}	This study
pFP501	pKC1139 carrying *forP* right-hand and left-hand PCR	This study

	fragments, used for *forP* disruption, *apr*^{R}	
pSET152	Integration vector, *apr*^{R}	[12]
pNPBE1	pSET152 carrying the *Streptomyces* constitutive promoter P*erm*E*, and chloramphenicol resistance gene (*cm*^{R}), *apr*^{R}	[14]
pISTP	pNPBE1 containing the complete *istP* under control of P*erm*E*, *cm*^{R}, *apr*^{R}	This study
pISTP2	pNPBE2 containing the complete *istP* under control of P*erm*E*, *tsr*^{R}, *apr*^{R}	This study
pNPBE2	pSET152 carrying the *Streptomyces* constitutive promoter P*erm*E*, and thiostreptone resistance gene (*tsr*^{R}), *apr*^{R}	This study
pFORP	pNPBE2 containing the complete *forP* under control of P*erm*E*, *tsr*^{R}, *apr*^{R}	This study
pFORP1	pNPBE1 containing the complete *forP* under control of P*erm*E*, *cm*^{R}, *apr*^{R}	This study

Table S2 Deoxy-oligonucleotide primers used for the disruption and complementation of the specific genes involved in the biosynthesis of fortimicins and istamycins.

Primers	Oligonucleotide sequence (5' to 3')	Restriction site
forP-L1	GTCAAGCTTGCCTGCGGCCACGATGTCGTC	*Hin*dIII
forP-L2	GGAGGATCCTGCCTGGACAACTTCCTCGTCGCG	*Bam*HI
forP-R1	GCCGGATCCGCGGCTTGCCACATGCGTCCACG	*Bam*HI
forP-R2	GGCGAGAATTCCGCGGTGATCGTGACGCCCGAG	*Eco*RI
forP-F	GGCGAAGATCTCTCGAGCGCGGTGATCGTGACGCCCGA	*Bgl*II / *Xho*I
forP-R	GGGAGAATTCTCTAGACGCGGTGATCGTGACGCCC	*Eco*RI / *Xba*I
istP-L1	GCAAGCTTTTCACCCGTCGCTTCGGGGAGA	*Hin*dIII
istP-L2	TCCTCTAGATGGCAGAGGTTGTACCGCCCCTC	*Xba*I
istP-R1	ACCGTCTAGACTCGACGACTGGGCGGACGA	*Xba*I
istP-R2	GGCGAATTCGACGATGCTGGAGAGGTTCGCC	*Eco*RI
IstP-F	GGAAGATCTCTCGAGCGCGGTCGATCTTGACGGCTG	*Bgl*II / *Xho*I
IstP-R	GGCTGAATTCTCTAGACTACCGCTGCCCGGGTC	*Eco*RI / *Xba*I

Primer pairs for amplification of left- or right-flanking fragments of a target gene, for PCR/sequencing confirmation, and for cloning target genes for over-expression are marked with suffixes –L1/-L2, -R1/-R2 or –F/-R, respectively. Restriction enzyme sites are indicated by underline.

Figure S1 Schematic data on the in-frame disruption of *istP* (ΔistP) in the genome of *Streptomyces tenjimariensis* ATCC31603.

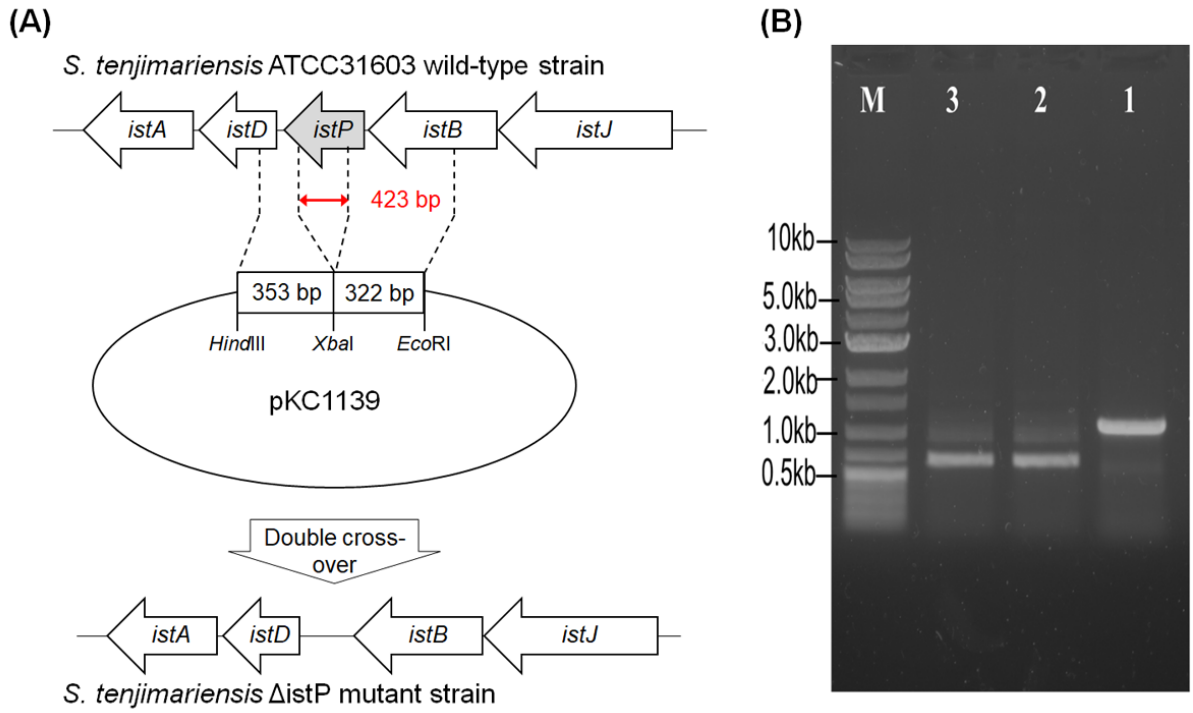


Figure S2 Schematic data on the in-frame disruption of *forP* (ΔforP) in the genome of *Micromonospora olivasterospora* DSM43868.

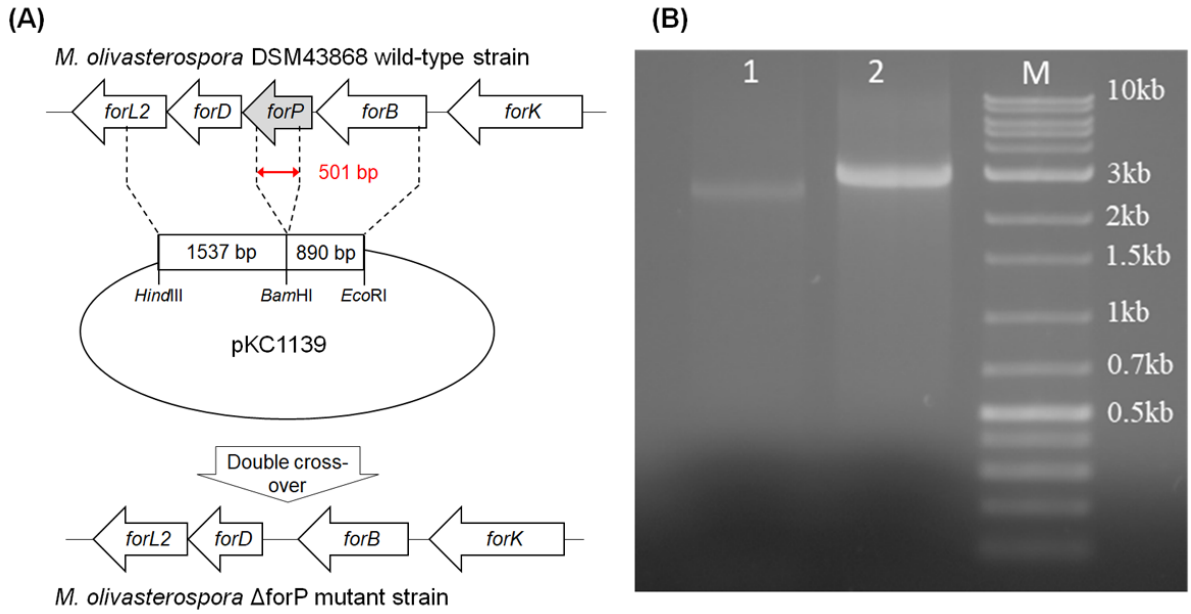


Figure S3 Fragmentation pattern and ESI-MS/MS spectra of both a natural fortimicin (FOR) biosynthetic intermediate FOR-KK1 (**A** and **C**) and a new FOR shunt product 3-*O*-methyl-FOR-KK1 (**B** and **D**).

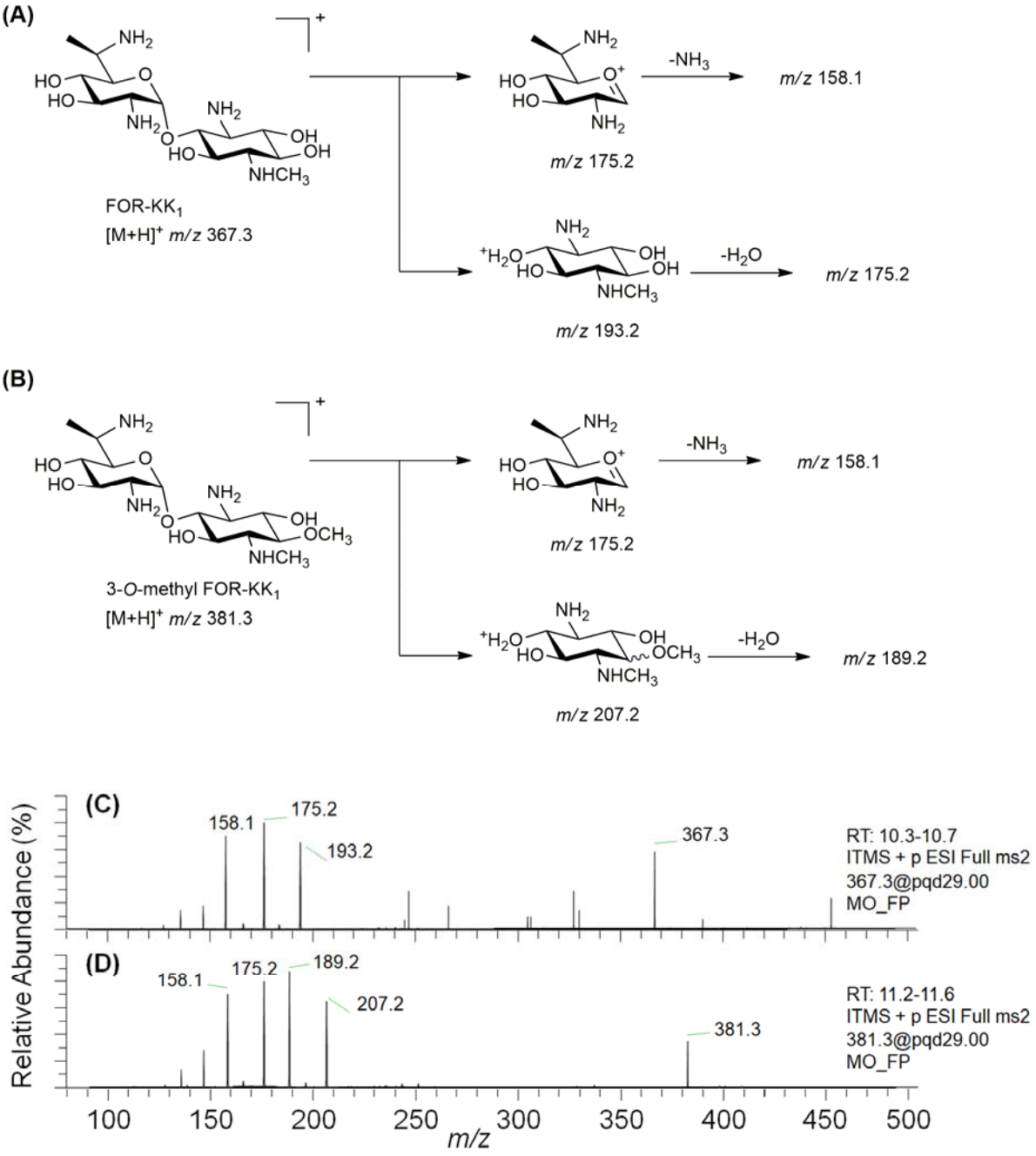
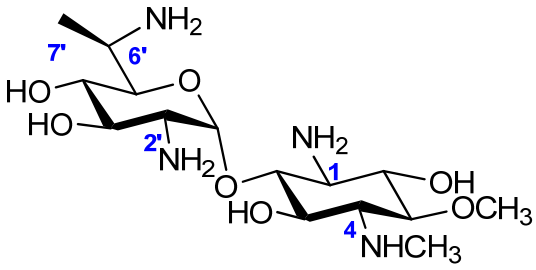


Figure S4 High-resolution MS data and ^{1}H (500 MHz, D_2O) and ^{13}C (125 MHz) NMR data chart on a new fortimicin (FOR) shunt product 3-*O*-methyl-FOR-KK1.



HR-qTOF-MS: *m/z* 381.2348 $[M+H]^+$ (calculated for $C_{15}H_{32}N_4O_7^+$, 381.2344)

Position	^{1}H (ppm) multiplets in Hz	^{13}C (ppm)
1	2.64 (ddd, 1H)	57.90
2	3.43 (dd, 1H)	71.13
3	3.51 (dd, 1H)	90.42
4	2.16 (m, 3H)	59.85
5	3.48 (dd, 1H)	68.82
6	3.43 (dd, 1H)	85.10
7 (OCH_3)	3.30 (s, 3H)	57.37
8 ($NHCH_3$)	3.25 (d, 3H)	34.44
1′	5.18 (d, 1H)	107.65
2′	3.13 (dd, 1H)	55.40
3′	3.64 (dd, 1H)	74.02
4′	3.41 (dd, 1H)	71.28
5′	3.90 (ddd, 1H)	91.24
6′	3.11 (dd, 1H)	43.89
7′	1.12 (s, 3H)	14.40

Figure S5 LC-ESI-MS/MS analysis of the istamycin congener profiles produced by (**A**) the wild-type strain and (**B**) the bilateral complemented (ΔistP::*forP*) strain of *Streptomyces tenjimariensis* ATCC31603.

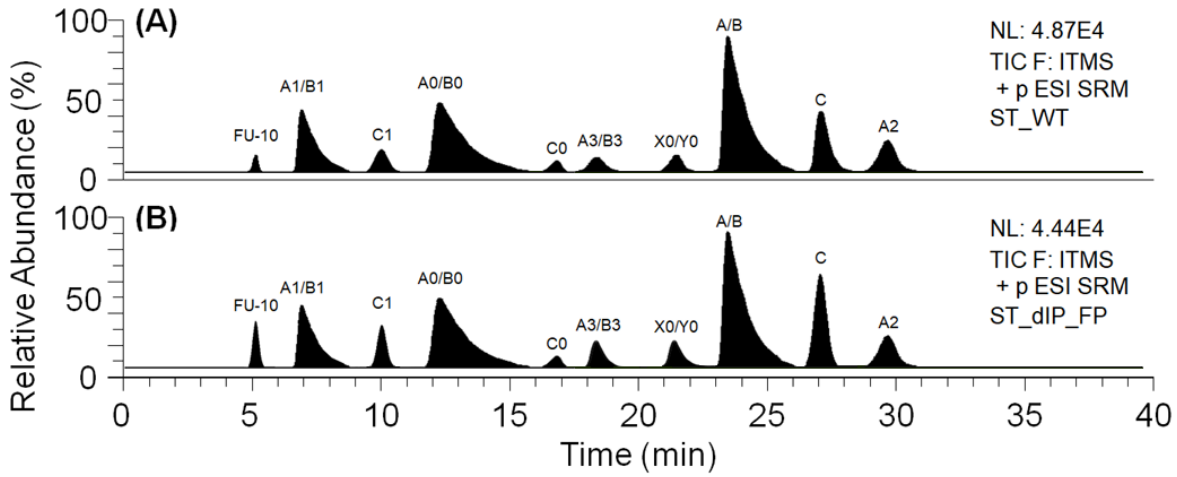


Figure S6 LC-ESI-MS/MS analysis of the fortimicin congener profiles produced by (**A**) the wild-type strain and (**B**) the bilateral complemented (ΔforP::*istP*) strain of *Micromonospora olivasterospora* DSM43868.