Supplemented information

Cloning and functional analysis of the naphthomycin biosynthetic gene cluster in *Streptomyces* sp. CS

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Plasmid construction for *nat* gene inactivation

To construct the large DNA fragments deletion, a 10-kb KpnI fragment of the fosmid 14F11 was ligated to the KpnI-digested pJTU1289. The resultant plasmid pJTU3230 was used for targeted replacement of a 7.2-kb DNA fragment internal to the 10-kb KpnI fragment of 14F11 with the 1.40-kb *aac(3)IV* to generate pJTU3231. To inactivate *nat1*, an 8.2-kb BamHI/EcoRI fragment carrying *nat1* was ligated to the KpnI/BamHI-digested pJTU1289 to give pJTU3241. This plasmid was then used for targeted replacement of a 1.34-kb DNA fragment internal to *nat1* with the 1.40-kb *aac(3)IV* to generate pJTU3245. For *nat2* inactivation, a 9.7-kb AgeI/EcoRI DNA fragment of fosmid 14F11 was ligated to the XmaI/EcoRI-digested pIJ2925 to construct pJTU3248. The 9.7-kb fragment with *nat2* was then cleaved by EcoRI and XbaI from the pJTU3248 and ligated to the EcoRI/XbaI-digested pJTU1289 to construct pJTU3249. This plasmid was then used for targeted replacement of a 0.98-kb DNA fragment internal to *nat2* with the 1.40-kb *aac(3)IV* to generate pJTU3250.

Figure legends

Fig. S1 Alignments of AHBA synthases. RifK, AHBA synthase of rifamycin biosynthesis; NapF, AHBA synthase of naphthomycin C biosynthesis from *Streptomyces collinus* Tü 1892; RubK, AHBA synthase of rubradirin biosynthesis; Asm43, AHBA synthase of ansamitocin biosynthesis; GelK, AHBA synthase of geldanamycin biosynthesis; AsnF, AHBA synthase of ansatrienin biosynthesis.

Fig. S2 Deletion of a large fragment in *Streptomyces* CS. A, schematic representation for the deletion of large fragment. **B**, validation of the large fragment deletion mutant WYY1 by HPLC.

Fig. S3 AHBA biosynthetic gene sets from ansamycin biosynthetic gene clusters (A) and phylogenetic tree of AHBA synthases (B)

Fig. S4 Alignment of Nat1 and Asm12. Nat1, halogenase for naphthomycin biosynthesis; Asm12, halogenase for ansamitocin biosynthesis.

Fig. S5 Alignment (A) and phylogenetic tree (B) of oxidoreductases. RubP1, oxidoreductase for rubradirin biosynthesis; Orf19, oxidoreductase for rifamycin biosynthesis; Nat2, oxidoreductase for naphthomycin biosynthesis from CS; GdmM, oxidoreductase for geldanamycin; McbM, oxidoreductase for macbecin biosynthesis.

Fig. S6 ESI-MS analysis of ansamitocins and naththomycins

Table S1. Strains and plasmids used in this work

Strains or Plasmids	**Properties or products**	**Reference or source**
Strains		
Streptomyces sp. CS	wild-type, naphthomycins	1
WYY1	large fragment deletion mutant,	This study
DH10B	F$^-$*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80d *lac*ZΔM15Δ*lac*X74*deo*R *rec*A1*end*A1*ara* Δ139D(*ara*,*leu*)7697*gal*U*gal*Kλ$^-$*rps*L *nup*G	Invitrogen
ET12567 (pUZ8002)	*dam dcm hsd*S pUZ8002	2
Actinosynnema pretiosum	Wild-type, ansamitocins	3
HGF054	*asm12* with MscI site inserted, 19-DCl-ansamitocins	4
BL21(DE3)plysE	F$^-$ *ompT hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm* (DE3) plysE (Cm^R)	Invitrogen
WYY2	*nat1* mutant, Apr^R	This study
WYY3	*nat2* mutant, Apr^R	This study
WYY4	WYY2 complemented with pJTU824, Thio^R	This study
WYY5	WYY2 complemented with *asm12*, Thio^R	This study
WYY6	WYY2 complemented with *nat1*, Thio^R	This study
KW3	HGF054 complemented with pJTU139, Apr^R	This study
KW4	HGF054 complemented with *asm12*, Apr^R	This study

KW5	HGF054 complemented with *nat1*, Apr^{R}	This study
KW6	WYY3 complemented with *nat2*, Thio^{R}	This study
KW7	WYY3 complemented with *rif-orf19*, Thio^{R}	This study
KW8	HGF065 complemented with *nat2*, Apr^{R}	This study
Plasmids/ fosmids		
pBluescript KS(+)	*bla*, *lac*Z, orif1	Novagen
14F11, 4C11, 23G9	Fosmids containing naphthomycin biosynthetic gene cluster	This study
pJTU1289	*ori*(pIJ101), *tsr*, *bla*, *lacZ*	5
pJTU3231	*bla*, *aac(3)IV*, cloning of a KpnI fragment from 14F11 to pJTU1289, contains a linked 1.25-kb left arm, 1.4-kb *aac(3)IV*,1.25-kb right arm for large fragment inactivation	This study
pRSETb	*Bla*. T7	Invitrogen
pIJ2925	*bla*，*lacZa*，*ori*	6
pIB139	pSET152 derived vector with *PermE** promoter	7
pJTU824	*bla*，*tsr*，*rep*^{puc}，*att*$^{\phi C31}$，*oriT*，*PermE**	L Bai, unpublished
pJTU3245	*bla*, *aac(3)IV,* cloning of a BamHI/EcoRI fragment from 14F11 to pJTU1289, contains a linked 3.24-kb left arm, 1.4-kb *aac(3)IV*, and 3.72-kb right arm for *nat1* inactivation	This study
pJTU3243	pIB139 cloned with *asm12* for complementation	This study
pJTU3244	pIB139 cloned with *nat1* for complementation	This study
pJTU3246	pJTU824 cloned with *asm12* for complementation	This study
pJTU3247	pJTU824 cloned with *nat1* for complementation	This study
pJTU3250	*bla*, *aac(3)IV*, cloning of a EcoRI/AgeI fragment	This study

	from 14F11 to pJTU1289, contains a linked 3.94-kb left arm, 1.4-kb *aac(3)IV*, and 3.98-kb right arm for *nat2* inactivation	
pJTU5156	pJTU824 cloned with *nat2* for complementation	This study
pJTU5157	pJTU824 cloned with *rif-orf19* for complementation	This study
pJTU5158	pIB139 cloned with *nat2* for complementation	This study

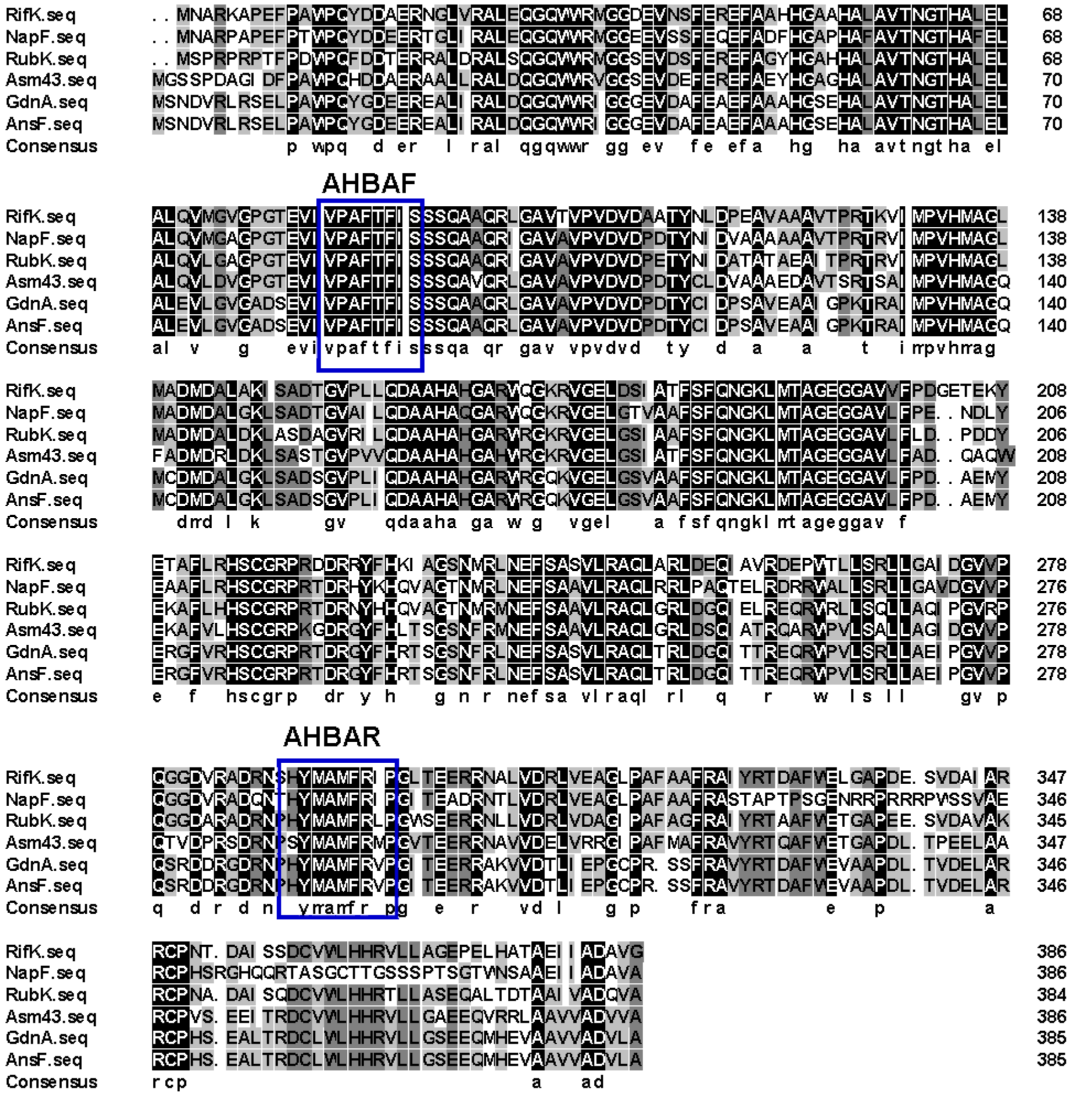

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Fig. S1

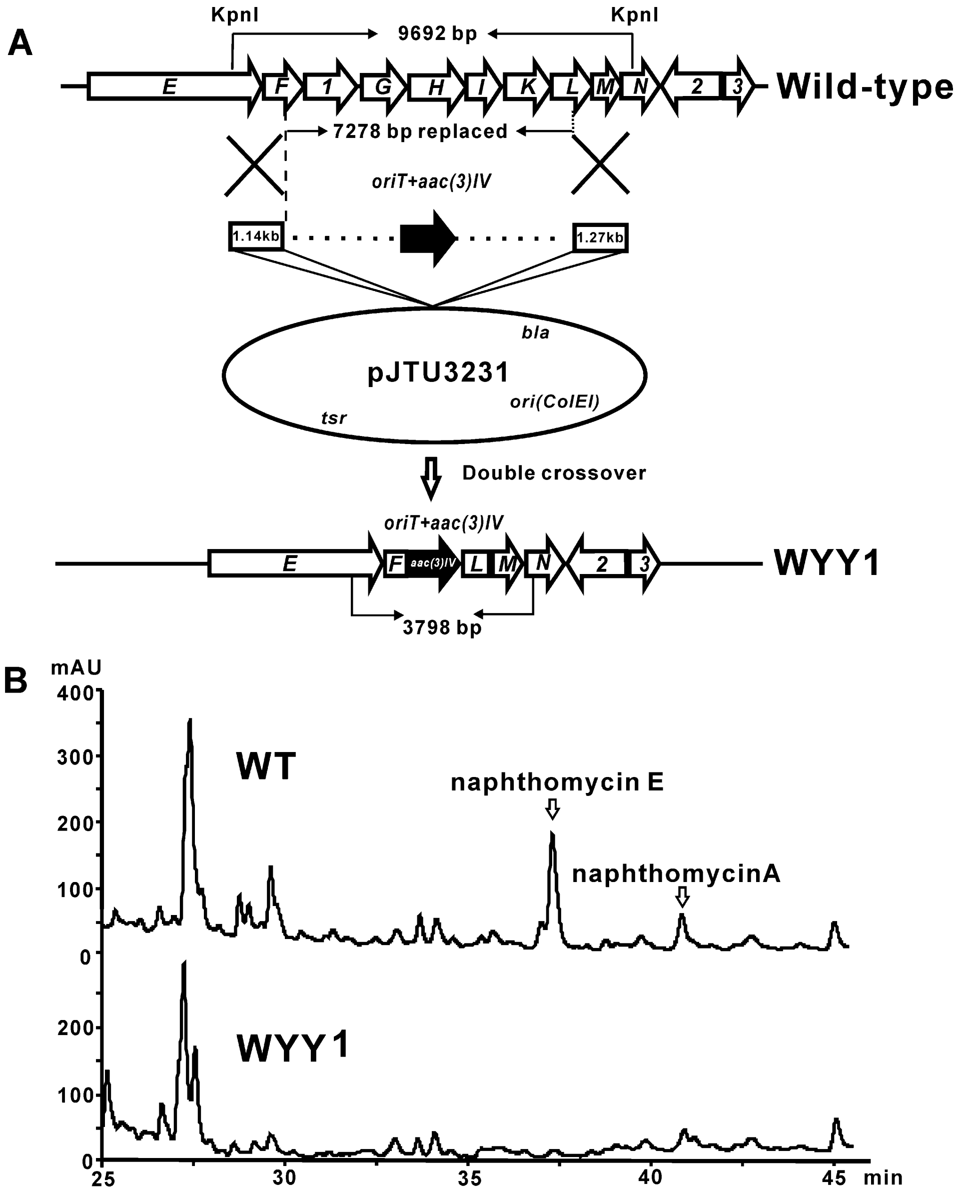
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Fig. S2

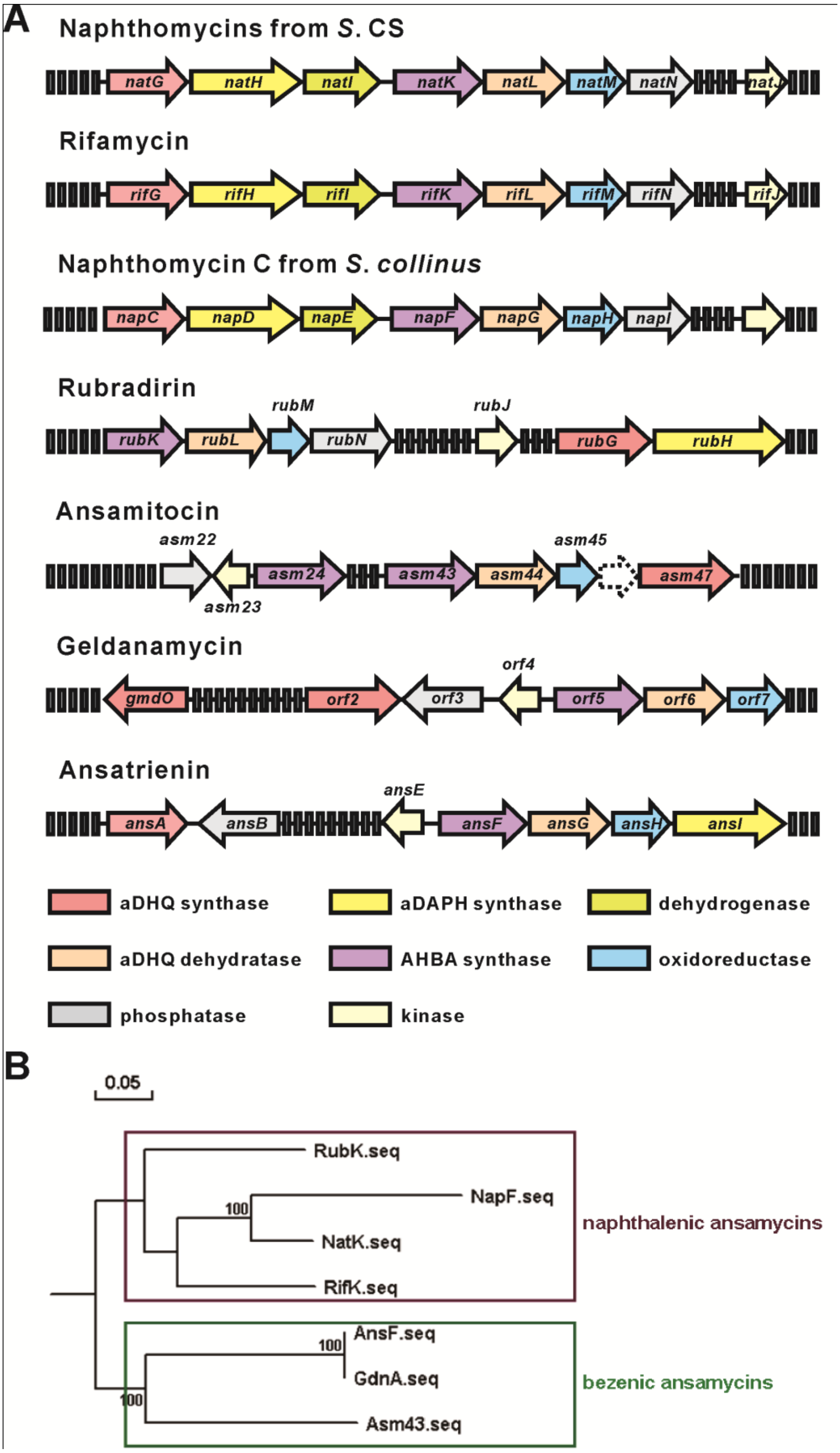
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Fig. S3

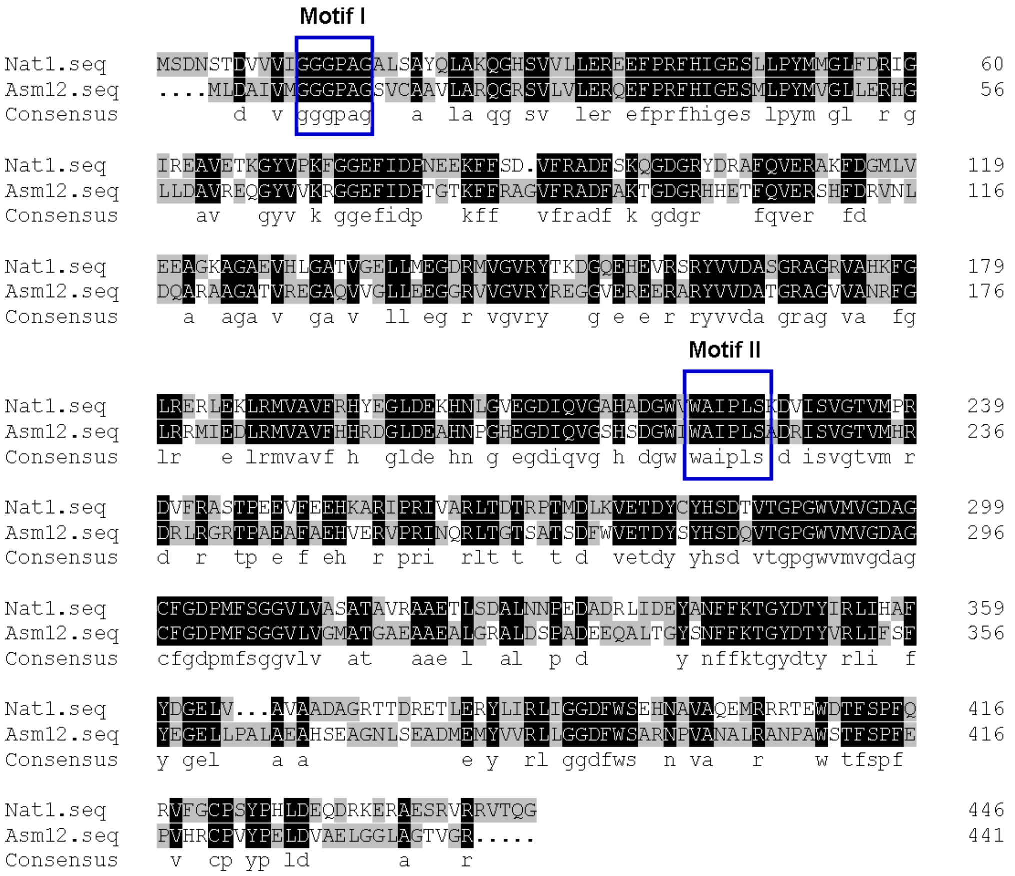

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Fig. S4

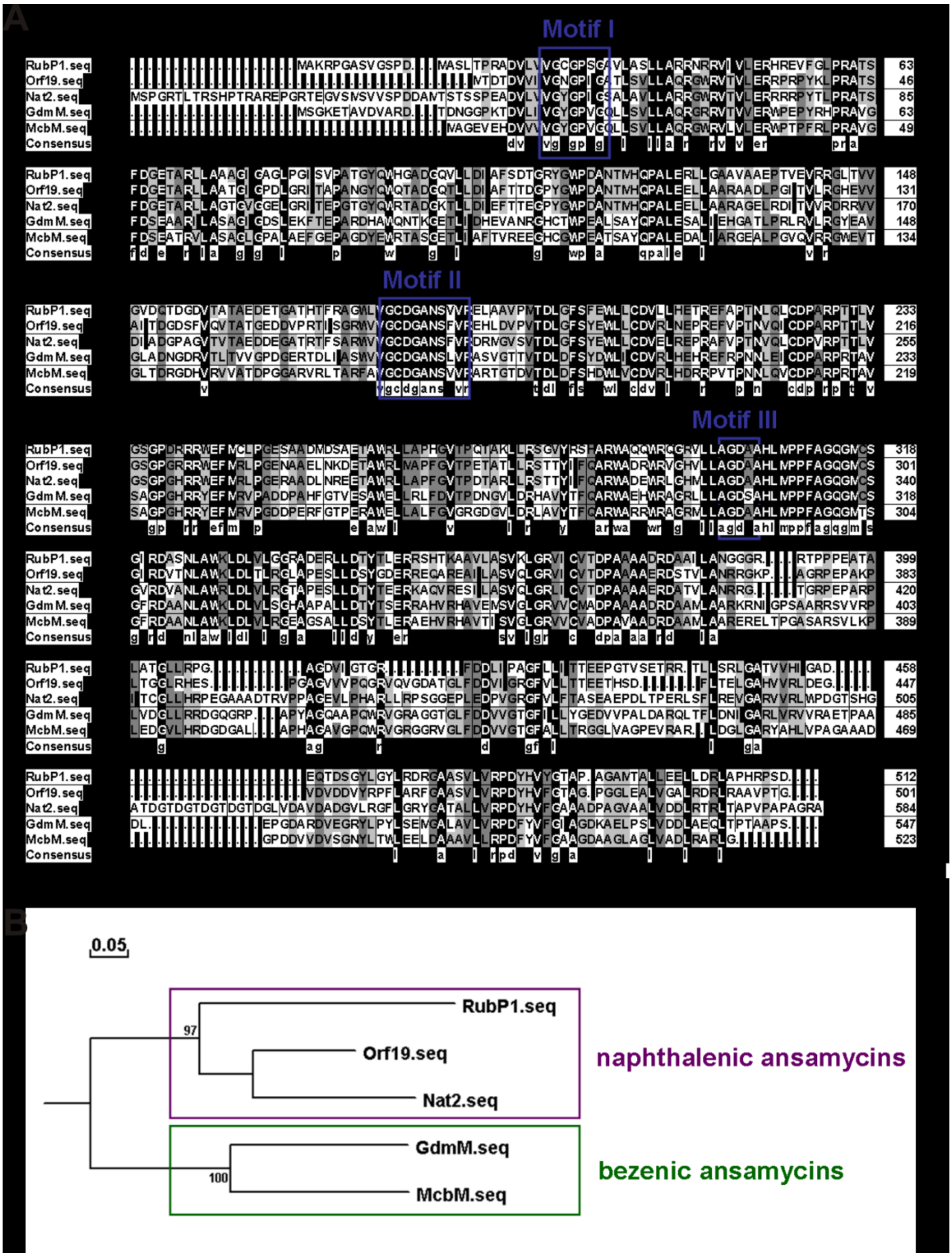
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Fig. S5

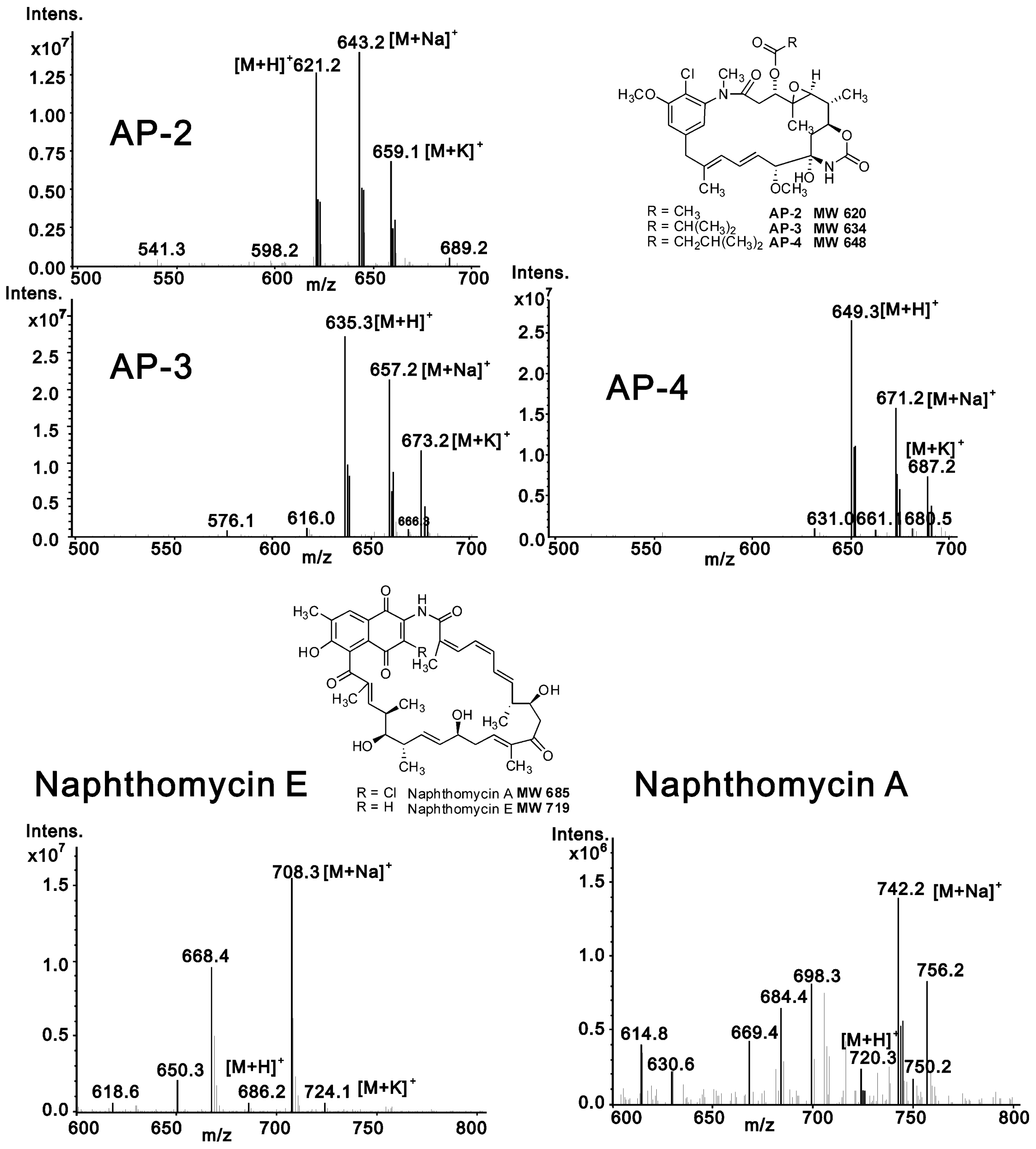

Fig. S6

References

1. C. Lu and Y. Shen, *J Antibiot (Tokyo)*, 2007, **60**, 649-653.

2. D. J. MacNeil, K. M. Gewain, C. L. Ruby, G. Dezeny, P. H. Gibbons and T. MacNeil, *Gene*, 1992, **111**, 61-68.

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4. P. Spiteller, L. Bai, G. Shang, B. J. Carroll, T. W. Yu and H. G. Floss, *J Am Chem Soc*, 2003, **125**, 14236-14237.

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