Supplemented information

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

Yingying Wu, Qianjin Kang, Yuema Shen, Wenjin Su, Linquan Bai

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

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<tr>
<th>Strains or Plasmids</th>
<th>Properties or products</th>
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from 14F11 to pJTU1289, contains a linked 3.94-kb left arm, 1.4-kb \textit{aac(3)IV}, and 3.98-kb right arm for \textit{nat2} inactivation

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Fig. S1
**Fig. S2**

(A) Diagram showing the genetic modification in the wild-type strain to create WYY1. The genetic elements and their orientations are depicted, with the introduction of a "bla" cassette via double-crossover recombination.

(B) Chromatograms comparing WT and WYY1 strains. The chromatograms show the presence of naphthomycin E and naphthomycin A in WYY1, indicating a successful genetic modification.
A  Naphthomycins from S. CS

Rifamycin

Naphthomycin C from S. collinus

Rubradirin

Ansamitocin

Geldanamycin

Ansatrienin

B

0.05

RubK.seq

NapF.seq

NatK.seq

RffK.seq

AnsF.seq

GdnA.seq

Asm43.seq

naphthalenic ansamycins

bezenic ansamycins

Fig. S3
Fig. S4
Fig. S5
Fig. S6

References


