Supplementary materials

Materials and methods

Polymerase chain reaction (PCR) reagents, restriction enzymes, Miniprep kits, Gel Extraction kits and SAM were purchased from TaKaRa Biotechnology Co., Ltd., Japan. Oligonucleotide primer synthesis and DNA sequencing of PCR products were performed by tsingke Biotechnology Co. Ltd. China (Ma et al., 2020).

The strains, plasmids and PCR primers (restriction sites are underlined) were summarized in Table S1. Luria–Bertani (LB) medium was used to culture the *E. coli* cells containing appropriate antibiotics (Eustáquio et al., 2005). *E. coli* DH5α was used as general cloning host. *E. coli* ET12567 (pUZ8002) was used as a donor for *E. coli–Streptomyces* intergeneric conjugations (Ma et al., 2014a). Intergeneric conjugation of *Streptomyces* and *E. coli* was performed as described by (Kieser et al., 2000). *S. diastatochromogenes* 1628 was isolated and deposited in the China General Microbiological Culture Collection Center with the number CGMCC 2060 (Ma et al., 2014a).

S. diastatochromogenes 1628 and its derivates were incubated and fermented as described by Wang et al. (2018). The seed medium and fermentation medium have the same compositions as the description of earlier study (Song et al., 2019; Tao et al., 2016; Zhao et al., 2018). The fermentations condition was performed as described by Ma et al. (2014b) and Lu et al. (2016).

After cultivation for 12 h, sterile-filtered aqueous solutions of exogenous SAM were added to the medium to achieve the final concentrations.

Cloning of the SAM synthetase gene *metK_{sd}* into the expression plasmid pIB139 and transformation into *S. diastatochromogenes* 1628

All recombinant DNA techniques were performed as described by Sambrook and Russell (2001). A 1209-bp gene fragment *metK$_{sd}$* (GenBank accession No. KX689260) was amplified from *S. diastatochromogenes* 1628 was using the primers P1/P2 which digested with *Nde*I and *Not*I. Then the PCR product harboring gene fragment *metK$_{sd}$* was inserted into corresponding site of vector pIB139 to pIB139-*metK$_{sd}$* (Fig. S1). The inserted gene fragment was sequenced as described by Zhao et al. (2018). Sequencing result confirmed that inserted gene fragment *metK$_{sd}$* did not contain any mutation.

The plasmid pIB139-*metK$_{sd}$* was introduced into *E. coli* ET12567/pUZ8002 and then transferred into strain 1628 by intergeneric conjugation to generate *S. diastatochromogenes* 1628-MetK (Ma et al., 2014b).

Construction of *metK$_{sd}$* disruption (ΔmetK$_{sd}$) mutant and its complementation

To further determine the role of *metK$_{sd}$* in secondary metabolites biosynthesis, knock-out plasmid pKC1132-Δ*metK$_{sd}$* was generated (Fig. 3a). Disruption of the *metK$_{sd}$* gene was performed by gene replacement *via* homologous recombination as described by Xu et al. (2019) and Ma et al. (2020). For construction of *metK$_{sd}$*-disruption(ΔmetK$_{sd}$) mutant, a 2.1-kb DNA fragment upstream homologous arm (UHA) of the *metK$_{sd}$* start codon was amplified by PCR using P3 and P4 (Table S1) and a 2.1-kb fragment downstream homologous arm (DHA) of the *metK$_{sd}$* stop codon was amplified by PCR using P5 and P6 (Table S1). The PCR products were digested with the *Hin*dIII/*Pst*I and *Pst*I/*Eco*RV endonucleases, respectively, and ligated together with the 3.5-kb *Hin*dIII/*Eco*RV fragment from pKC1132, yielding the *metK$_{sd}$* disrupted vector pKC1132-Δ*metK$_{sd}$*. Subsequently, introduction of the constructed pKC1132-Δ*metK$_{sd}$* into the wild-type strain *S. diastatochromogenes* 1628 was conducted by intergeneric conjugation. The ΔmetK$_{sd}$ mutants were

named *S. diastatochromogenes* 1628-ΔmetK$_{sd}$. It was obtained by a double-crossover recombination event between homologous regions of the plasmid and chromosome. Mutants were selected by apramycin sensitivity (Apr^{s}). The genotype of mutant *S. diastatochromogenes* 1628-ΔmetK$_{sd}$ was confirmed by PCR.

The construction of complemented strain was performed as described as Xu et al. (2019) and Ma et al. (2020). For the complementation of the $metK_{sd}$ gene in *S. diastatochromogenes* 1628-ΔmetK$_{sd}$, a 1409-bp DNA fragment containing the coding region of $metK_{sd}$ gene and its 200-bp upstream promoter region was amplified by PCR using P7 and P2 (Table S1) as primers. The DNA fragment was inserted into the *Xba*I and *Not*I sites of pSET152 to obtain pSET152::$metK_{sd}$. The plasmid pSET152::$metK_{sd}$ gene and the empty vector pSET152 as a control were introduced into mutant *S. diastatochromogenes* 1628-ΔmetK$_{sd}$ by conjugation, resulting in the complemented strain 1628-ΔmetK$_{sd}$/pSET152::$metK_{sd}$ and the control strain 1628-ΔmetK$_{sd}$/pSET152, respectively.

Analysis of tetraene macrolides and TM

The sample was prepared and analyzed as described by Ma et al. (2014b). A 1 ml sample was taken from each culture every 24 h during the fermentation process. The fermentation broth was harvested by centrifugation, and the supernatants were extracted with ethyl acetate. The extract was filtered through a Millipore membrane (pore diameter 0.22 μm). Production of tetraene macrolide antibiotics was determined by high-performance liquid chromatography (HPLC) (Waters-e2695, USA) with an Agilent SB-C18 column (5 μm particle size; 4.6 mm×250 mm). The percentage volume of methanol was varied as follows: linearly increased from 65% to 80% (0-30min), linearly increased to 100% (30-31min), held at 100% (31-38min) and then linearly

decreased to 65% (38-39min) and held at 65% until 48min. The detection wavelength was 304 nm. The whole process was performed at 30 °C. The solvent flow was 1.0 ml/min.

TM was measured according to a method described by Wang et al. (2018) and Ma et al. (2014b).

Determine of dry cell weight, SAM synthetase and intracellular SAM concentration

The dry cell weight was determined as described by Zhao et al. (2013). For estimation of dry cell weight, the fermentation broth were centrifuged at 5,000 rpm for 10 min. Cell pellets were washed twice with distilled water and dried at 80°C to constant weight.

SAM synthetase was assayed using a method described by Okamoto et al. (2003). SAM concentration was determined by HPLC (Carlson and Riggin 2000; Zhao et al., 2010a; Zhao et al., 2013).

Analysis of gene transcriptional levels by qRT-PCR

The extraction of RNA, design of primers, and analysis of transcriptional level of *toy* genes were performed as described by Wang et al. (2018) and Ma et al. (2020).

Statistical analysis

Statistical analysis was performed as described by Song et al. (2020).

Table S1 Strains, plasmids, and primers used in this study

Strains or plasmids or primers	Description	Source
Strains		
E. coli DH5α	General cloning host	TaKaRa
E. coli ET12567 (pUZ8002)	Cm^r, Km^r, donor strain for conjugation	Our lab
S. diastatochromogenes 1628	Wild-type strain	CGMCC 2060
1628-MetK	Wild-type with overexpression plasmids pIB139-$metK_{sd}$	This work
1628-ΔmetK$_{sd}$	Wild-type with $metK_{sd}$ disrupted	This work
1628-ΔmetK$_{sd}$/pSET152	Mutant 1628-ΔmetK$_{sd}$ with integrative plasmid pSET152	This work
1628-ΔmetK$_{sd}$/pSET152::$metK_{sd}$	*metK* complemented strain, mutant 1628-ΔmetK$_{sd}$ with integrative plasmid pSET152::$metK_{sd}$	This work
Plasmids		
pIB139	Derivative of integrative plasmid pSET152, harboring an *ermE** promoter, Apr^r, *Ori*T$_{RK2}$, ΦC31 *int/att*P	Our lab
pIB139-$metK_{sd}$	Overexpression of $metK_{sd}$ gene in pIB139	This work
pKC1132	Used for disrupted $metK_{sd}$ gene	Our lab
pKC1132-Δ$metK_{sd}$	$metK_{sd}$ disruption plasmid, pIB139 containing $metK_{sd}$ upstream and downstream fragment	This work
pSET152	Integrative plasmid, Apr^r, *OriT RK*$_2$, *ΦC31 int/attP*	This work
pSET152::$metK_{sd}$	pSET152 harboring $metK_{sd}$ driven by its own promoter	
Primers		
P1	ACCGCATATGGTGTCCCGCCGCCTGTTTA(*Nde*I)	This work
P2	ACCGGCGGCCGCTTACAGGCCCGCGGCCTT(*Not*I)	This work
P3	CCCAAGCTTGTTCGACGTGACGCTCGTCA (*Hin*dIII)	This work
P4	AAACTGCAGGGACACATCGCTCCCTGGGG(*Pst*I)	This work
P5	AAACTGCAGCTGTAAGAAGGCGCCGCGCC(*Pst*I)	This work
P6	CGGATATCGCCCGGCGGGGGGTCCC (*Eco*RV)	This work
P7	CGCTCTAGACCCTTGACCCGGCTGATTC(*Xba*I)	This work

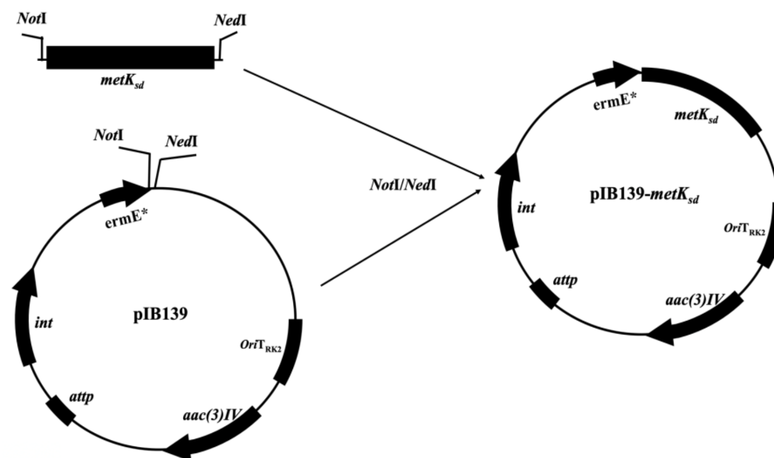

Fig. S1 Map of constructed plasmid pIB139-$metK_{sd}$.