

Discovery of Alternative Producers of the Eneidyne Antitumor Antibiotic C-1027 with High Titers

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Dedicated to Professor Susan Band Horwitz, of Albert Einstein Medical College, Bronx, NY, for her pioneering work on bioactive natural products.

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Table S1. Bacterial strains used in this study.

Strain	Description	reference
<i>Streptomyces globisporus</i> C-1027	The original C-1027 producer, isolated from a soil sample collected in Qianjiang County, Hubei Province, China.	S1
<i>Streptomyces globisporus</i> AF40	A C-1027-non-producing strain, generated by treating the <i>S. globisporus</i> wild-type strain with acriflavine.	S2
<i>Streptomyces</i> sp. CB00657	C-1027 alternative producer, isolated from Panlong District of Kunming City, in the southwest of China.	This study
<i>Streptomyces</i> sp. CB02329	C-1027 alternative producer, isolated from Benxi City, Liaoning Province, in the northeast of China.	This study
<i>Streptomyces</i> sp. CB02366	C-1027 alternative producer, isolated from a soil sample collected on the beach in Dubai, the United Arab Emirates.	S3
<i>Streptomyces</i> sp. CB03608	C-1027 alternative producer, isolated from a primary evergreen broadleaf forest located between Yunxian to Gengma, Yunnan Province, in the southwest of China.	This study
<i>Kocuria rhizophila</i> ATCC 9341	Indicator strain for antimicrobial assay	S4

Table S2. Primers used in this study.

Primer	Nucleotide sequence (from 5' to 3')	Function
16SrRNA_for	AGAGTTTGATCCTGGCTCAG	Phylogenetic analysis
16SrRNA_rev	ACGGCTACCTTGTACGACTT	Phylogenetic analysis
rpoB-2	CATCGACCACTTCGGCAAC	Phylogenetic analysis
ActRpoB3303R	GAANCGCTGDCCRCCGAAGCTG	Phylogenetic analysis
trpBfor	TAATACGACTCACTATAGGGGCGCGAGGACCTGAACC ACAC	Phylogenetic analysis
trpBrev	GCTAGTTATTGCTCAGCGGCATGGCCGGGATGATGC CC	Phylogenetic analysis
cagA_Fnew	TCCGCTCCCGAAGGTGGAG	Sequencing the <i>cagA</i> genes
cagA_Rnew	AGATCGAGATCGCGGCCTG	Sequencing the <i>cagA</i> genes
sgcR_F	CAGGTTTGCCAAGCCGCGCG	Sequencing the <i>sgcR</i> genes
sgcR_F2	TGTGGGCCGAGGCGTTGTG	Sequencing the <i>sgcR</i> genes
sgcR_R	CGGTCCGAGGCGAGCACGTA	Sequencing the <i>sgcR</i> genes
sgcR1_F	GTGCTCGAACTCCTTGCTCTCC	Sequencing the <i>sgcR1</i> genes
sgcR1_R	GCGGGAATGAGTTCATCGATCCG	Sequencing the <i>sgcR1</i> genes
EKSAT-S	TGTA AACGACGGCCAGTATGGGSTTCGGCGGSATCA AC	Sequencing the 1-kb internal fragment of <i>pkcE</i>
EKSAT-AS	CAGGAAACAGCTATGACCAGMGGNGAGTGGAANGCG TG	Sequencing the 1-kb internal fragment of <i>pkcE</i>
C1027southernF	GCATTGCGGGCGTAGAAGGTG	Amplifying the probe for Southern hybridization
C1027southernR	CTGGTCACGGGCACATCCATC	Amplifying the probe for Southern hybridization

N=A, C, G, or T; D=A, G, or T, R=A or G; M=A or C; S=C or G

Figure S1. Structures of the 13 enediyne natural products known to date. (A) The five 9-membered enediynes. (B) The eight 10-membered enediynes. The years when each of the enediyne structures were elucidated are given in parentheses. The enediyne cores are highlighted in red.

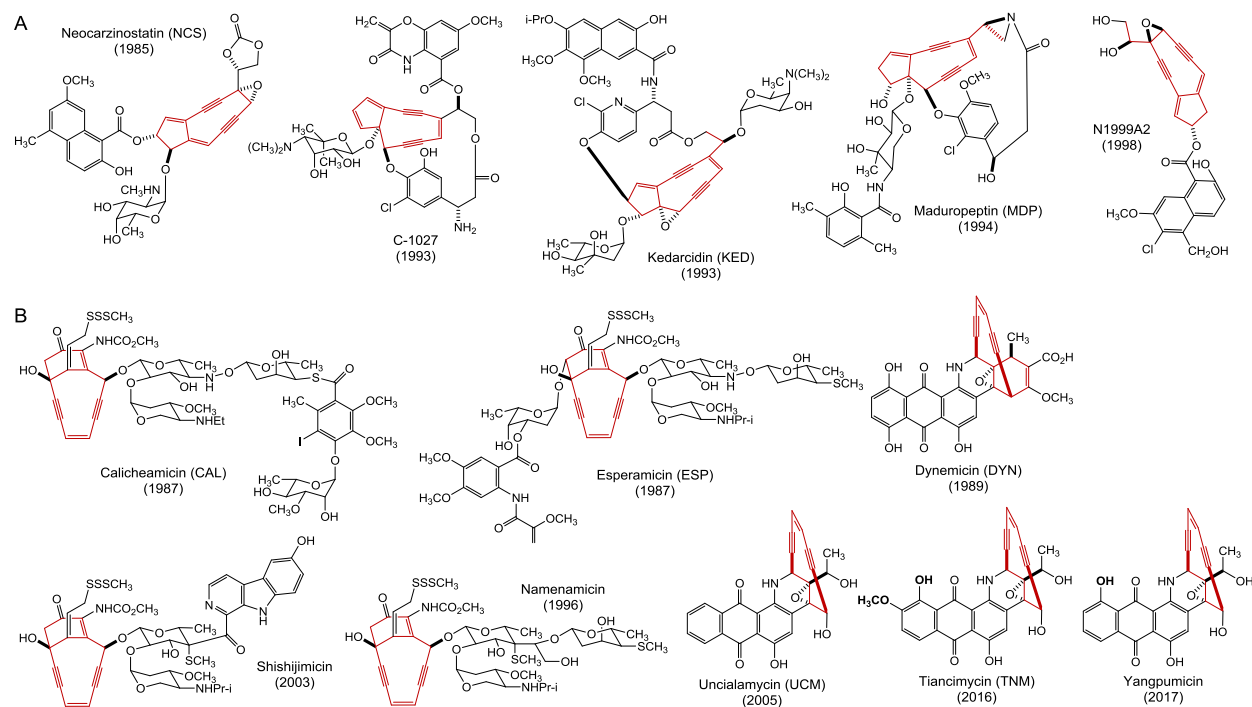


Figure S2. Phylogenetic analysis revealing four potential C-1027 alternative producers. The phylogenetic tree was constructed based on the translated 1-kb internal fragments of *pksEs* from the 81 enediyne gene clusters from the 3,400 actinomycetes strains, in comparison with the 11 known enediyne producers.^{S3} The four strains cladding together with the C-1027 original producer *S. globisporus* was highlighted in blue box. The strains subjected to genome sequencing were marked red.

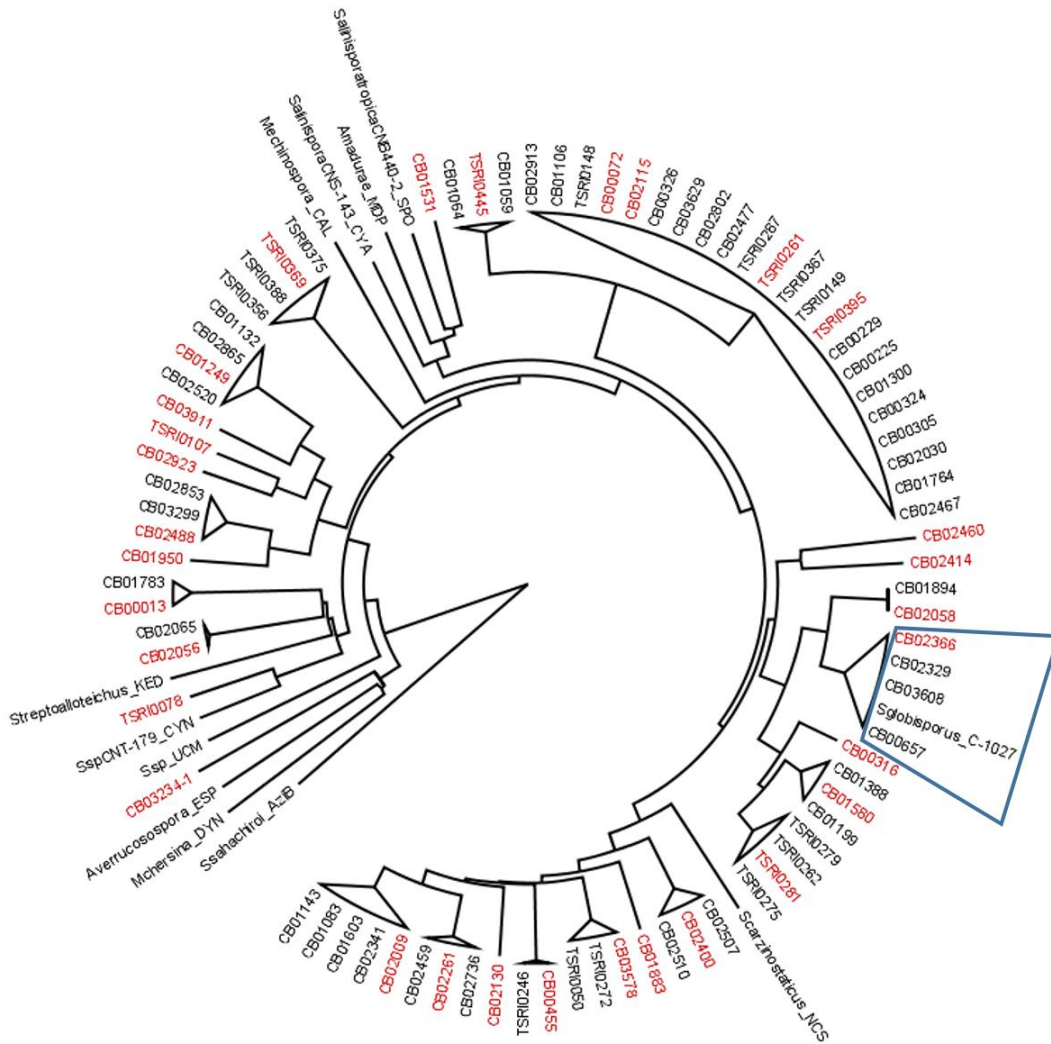


Figure S3. The C-1027 biosynthetic gene clusters from *S. globisporus* and CB02366 have an identical genetic organization, with amino acid sequence identities for individual gene products ranging from 83% to 99%.^{S3}

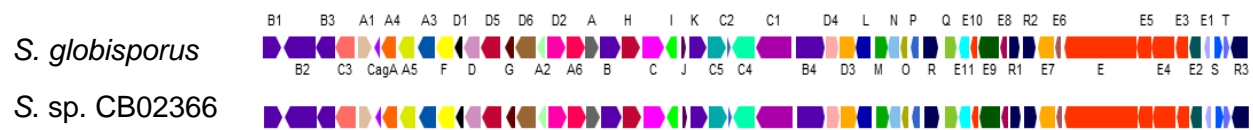


Figure S4. HR-ESI-MS analysis of the fermentation broths confirming production of the C-1027 chromoprotein complex by *S. sp.* CB00657, CB02329, CB02366, and CB03608, with the original producer *S. globisporus* as a positive control, yielding the $[M + H]^+$ ions at m/z 844.2486, 844.2477, 844.2485, and 844.2485, respectively, for the C-1027 enediyne chromophore (calculated $[M + H]^+$ ion for $C_{43}H_{43}N_3O_{13}Cl$ at m/z 844.2485).

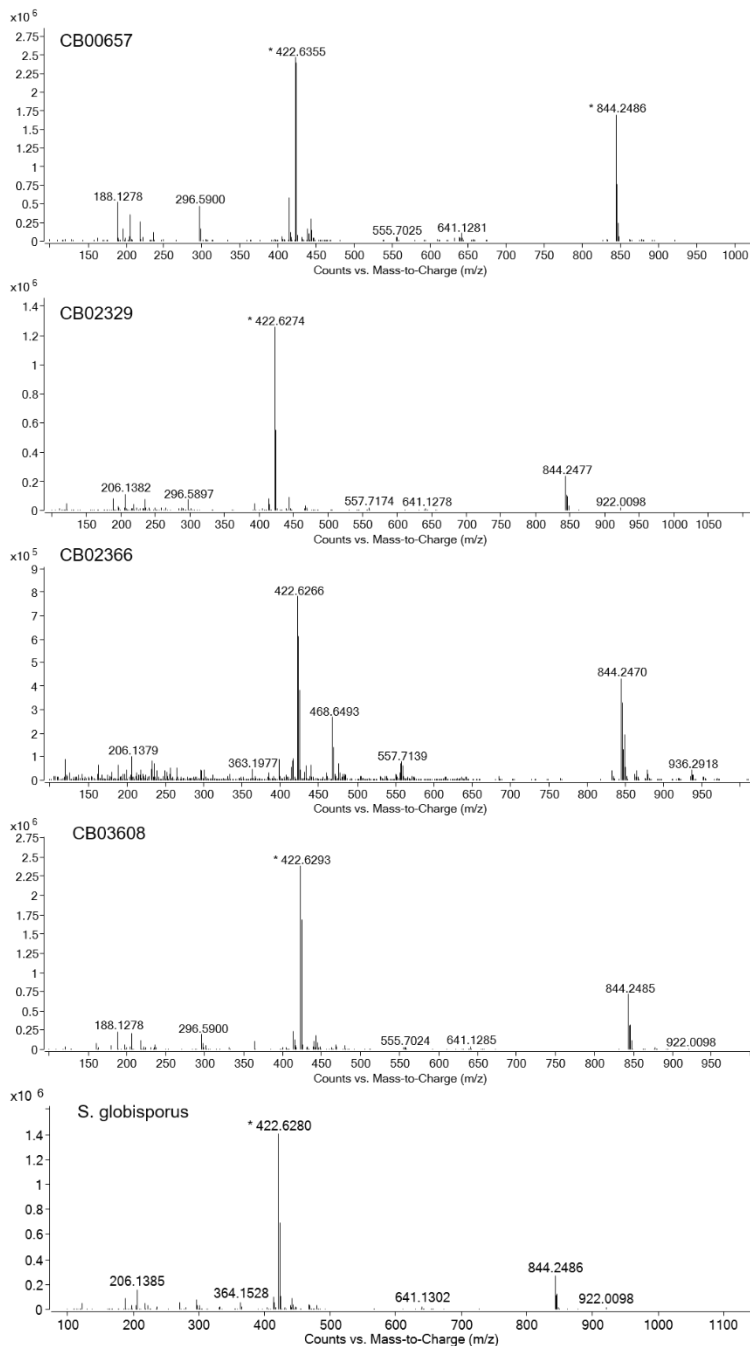


Figure S5. Alignment of nucleotide sequences of (A) the *cagA* genes and (B) the 1-kb internal fragment of *pksEs* showing the high sequence similarity among the C-1027 BGCs in the five C-1027 producers.



Figure S6. Alignment of nucleotide sequences of (A) the *sgcR* genes and (B) the *sgcR1* genes showing the high sequence similarity among the C-1027 BGCs in the five C-1027 producers.

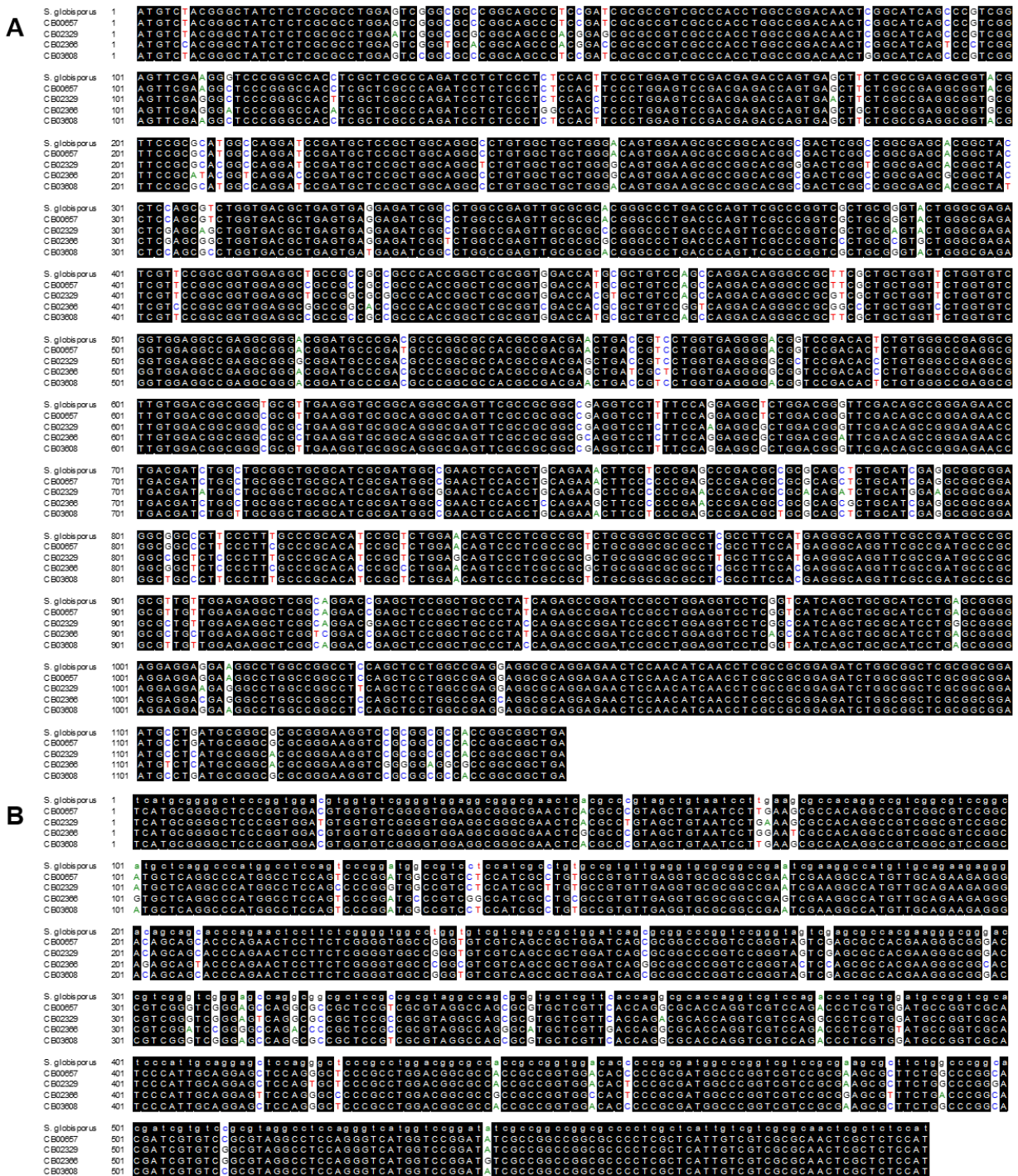
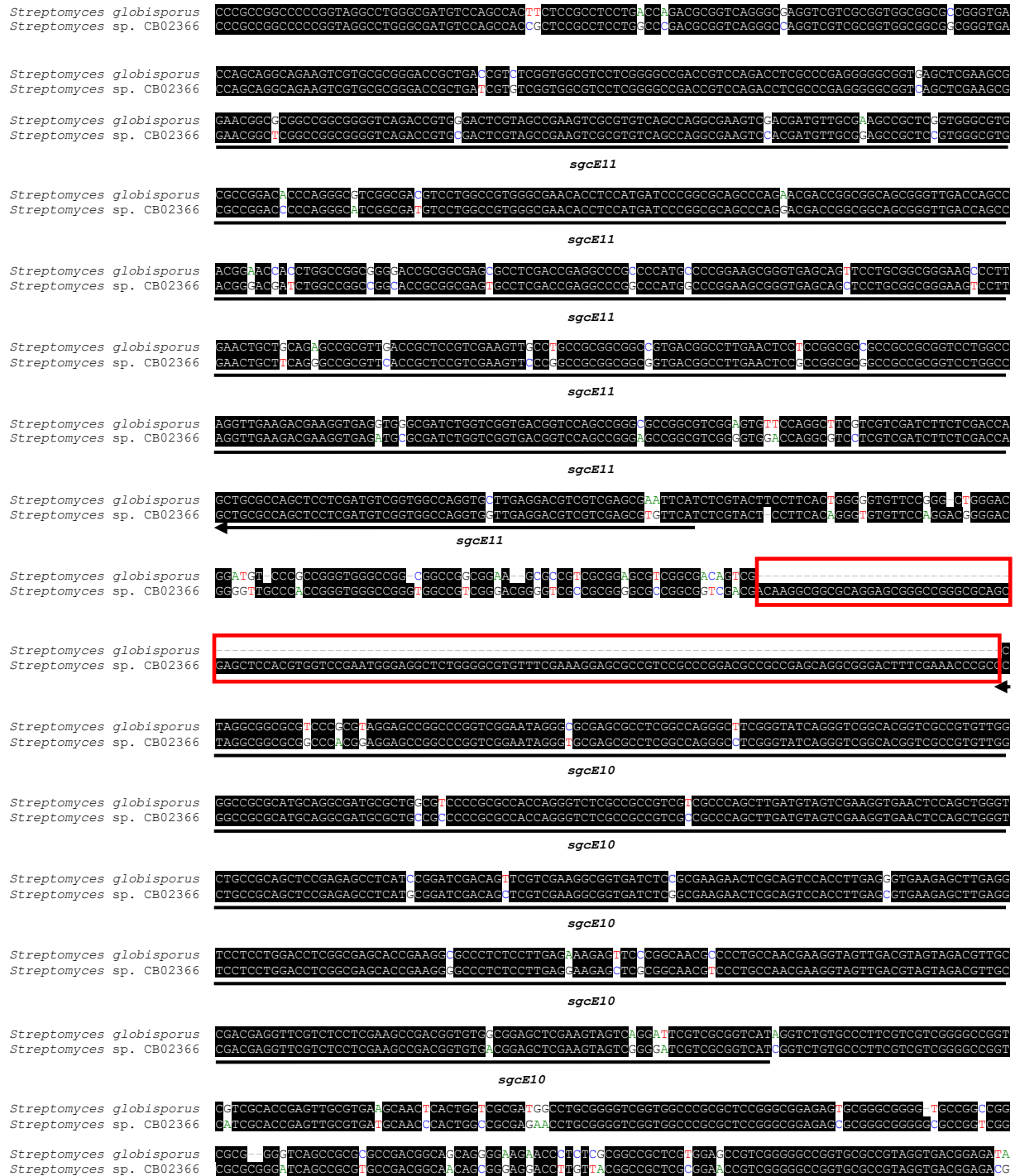


Figure S7. Alignment of the C-1027 BGCs in *S. globisporus* and *S. sp.* CB02366 revealing the frequent deletion or insertion of nucleotides beyond the open reading frames. The deletion of a 130-bp fragment between *sgcE10* and *sgcE11* genes in *S. globisporus* is highlighted in red.



REFERENCES

- (S1) Hu, J. L.; Xue, Y. C.; Xie, M. Y.; Zhang, R.; Otani, T.; Minami Y.; Yamada, Y.; Marunaka, T. *J. Antibiot.* **1988**, *41*, 1575–1579.
- (S2) Liu, W.; Shen, B. *Antimicrob. Agents Chemother.* **2000**, *44*, 382–392.
- (S3) Yan, X.; Ge, H.; Huang, T.; Yang, D.; Teng, Q.; Crnovcic, I.; Li, X.; Rudolf, J. D.; Lohman, J. R.; Gansemans, Y.; Zhu, X.; Huang, Y.; Zhao, L.-X.; Jiang, Y.; Van Nieuwerburgh, F.; Rader, C.; Duan, Y.; Shen, B. *mBio.* **2016**, *7*, e02104-16.
- (S4) Tang, J. S.; Gillevet, P. M. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 995–997.