Supporting Materials

Genomic Insights and Synthetic Biology Applications of Marine Actinomycete *Streptomyces griseoincarnatus* HNS054

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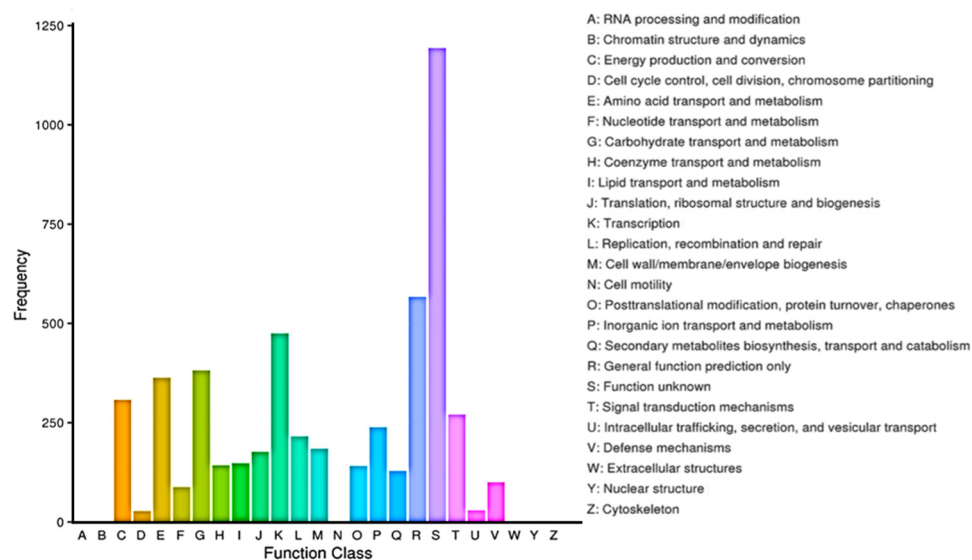
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A: RNA processing and modification
B: Chromatin structure and dynamics
C: Energy production and conversion
D: Cell cycle control, cell division, chromosome partitioning
E: Amino acid transport and metabolism
F: Nucleotide transport and metabolism
G: Carbohydrate transport and metabolism
H: Coenzyme transport and metabolism
I: Lipid transport and metabolism
J: Translation, ribosomal structure and biogenesis
K: Transcription
L: Replication, recombination and repair
M: Cell wall/membrane/envelope biogenesis
N: Cell motility
O: Posttranslational modification, protein turnover, chaperones
P: Inorganic ion transport and metabolism
Q: Secondary metabolites biosynthesis, transport and catabolism
R: General function prediction only
S: Function unknown
T: Signal transduction mechanisms
U: Intracellular trafficking, secretion, and vesicular transport
V: Defense mechanisms
W: Extracellular structures
Y: Nuclear structure
Z: Cytoskeleton

Figure S1. COG annotation of the HNS054 genome. Functional categories were assigned to 5121 genes in the HNS054 genome using the COG annotation. The majority of these genes fell into categories such as S (function unknown), R (general function prediction only), K (transcription), G (carbohydrate transport and metabolism), E (amino acid transport and metabolism), C (energy production and conversion), and T (signal transduction mechanisms). Notably, 129 genes were associated with secondary metabolite biosynthesis, transport, and metabolism (Q).
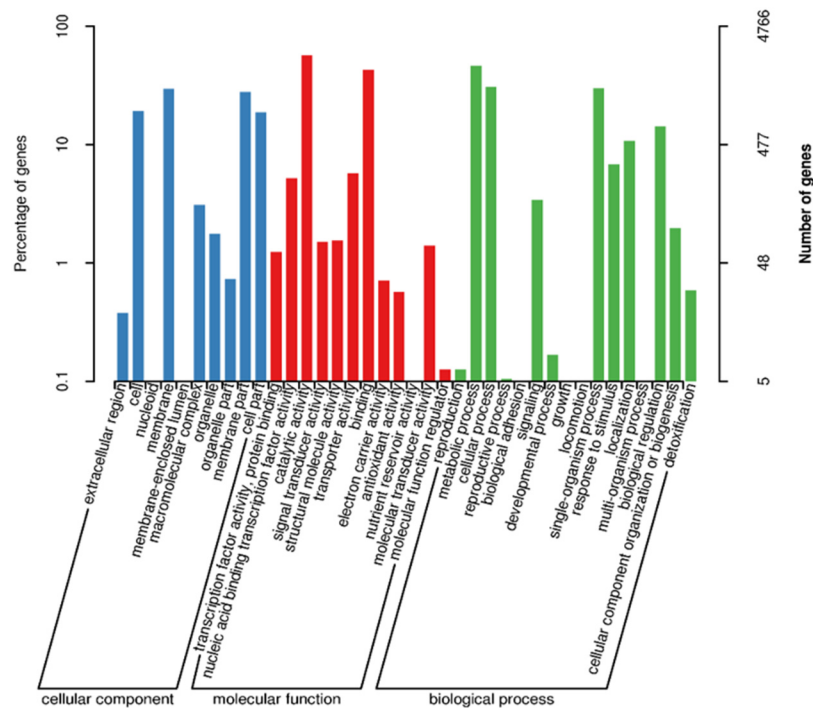
Figure S2. GO annotation of the HNS054 genome. Using the GO database annotation, 4766 genes were categorized into three major groups: cellular component, molecular function, and biological process. Enriched gene categories included membrane part, cell, membrane, and macromolecular complex (cellular component), as well as catalytic activity and binding (molecular function). Additionally, genes were associated with metabolic process, single-organism process, and biological process (biological process).
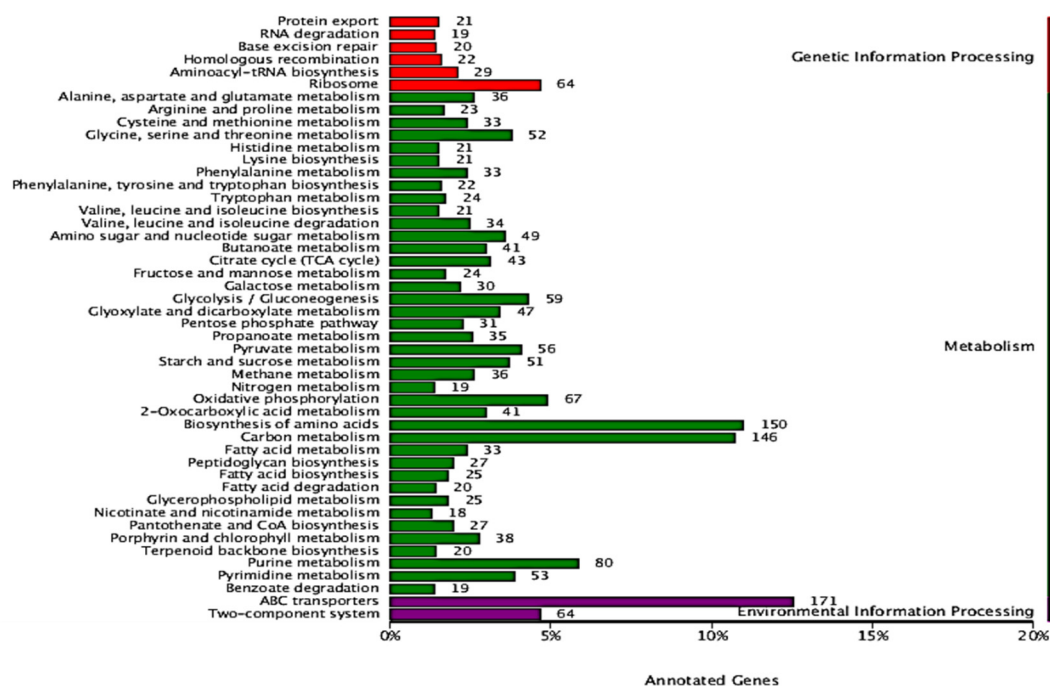
Figure S3. KEGG annotation of the HNS054 genome. The KEGG database annotated 2380 genes, classifying them into three main categories: genetic information processing, metabolism, and environmental information processing. The predominant genes were identified in ABC transporters, amino acid biosynthesis, and carbon metabolism, with notable contributions from purine metabolism and two-component systems.
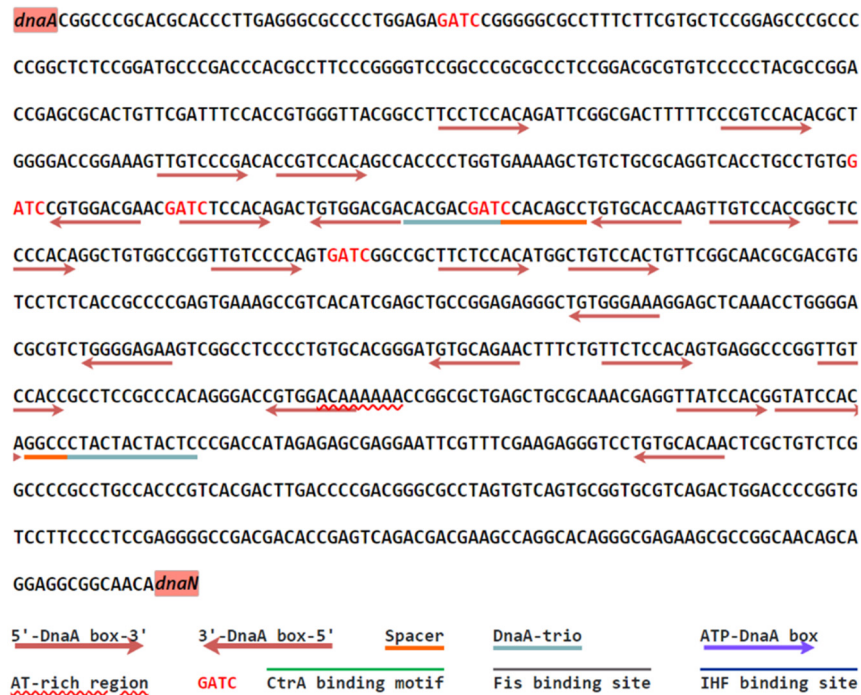
Figure S4. Characterization of the replication origin site (OriC) on the HNS054 chromosome. The replication origin comprises an AT-rich region (ACAAAAAA), 22 DnaA boxes, five GATC sites, and two DnaA-trios.
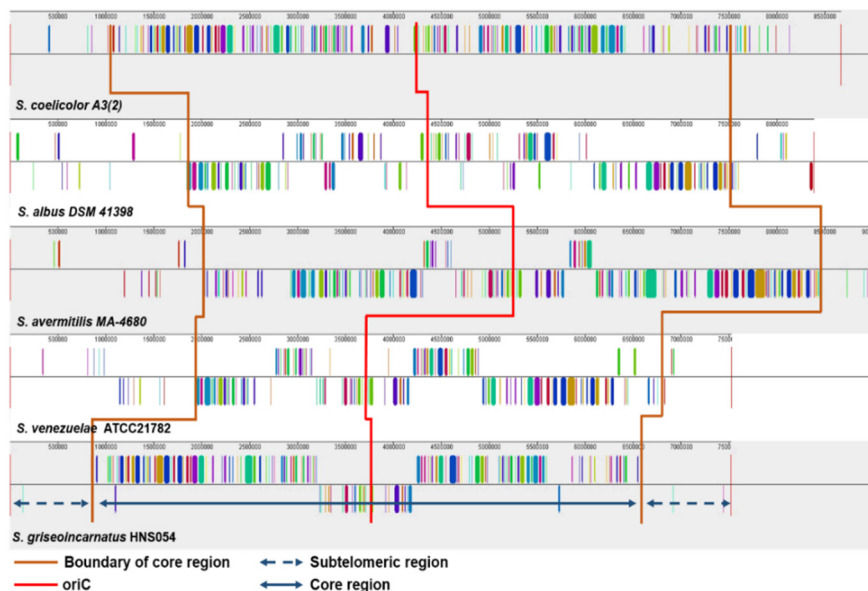
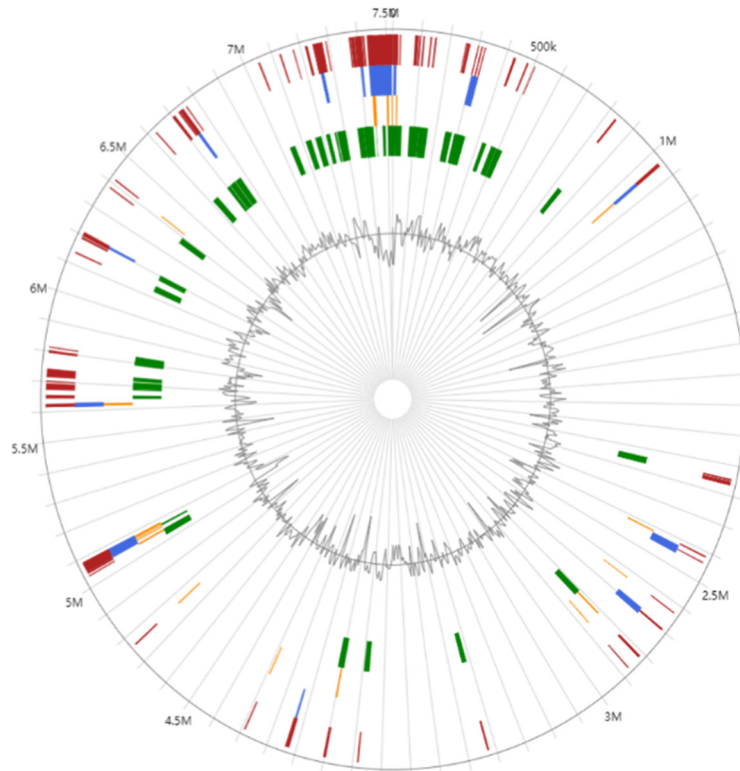**Figure S5**. Multigenome comparison of HNS054 and other *Streptomyces* chromosomes.

Figure S6. Genomic island (GI) analysis of the HNS054 genome. The core region exhibits highly conserved and homologous genes, while the accessory genome includes dispensable elements, including 18 GIs predicted by IslandViewer4. The red module corresponds to comprehensive analysis, the green module to IslandPick analysis, the yellow module to SIGI-HMM analysis, and the blue module to IslandPath-DIMOB analysis.
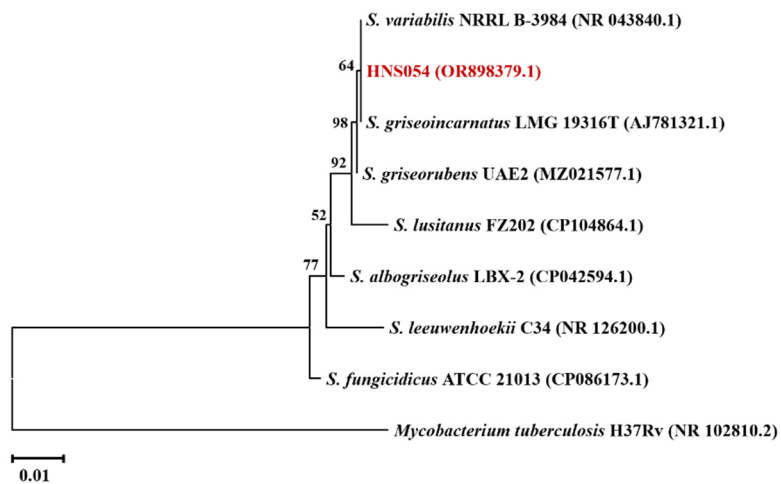

Figure S7. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of HNS054. In 2021, following the reclassification of the *Streptomyces* genus (https://lpsn.dsmz.de/genus/streptomyces), *S. variabilis* was officially recognized as a heterotypic synonym of *S. griseoincarnatus*.
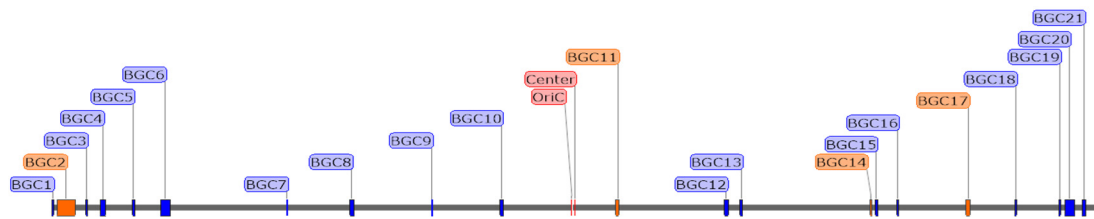
Figure S8. Distribution of BGCs in the HNS054 genome. The antiSMASH web server was employed to upload the HNS054 genome sequences and identify individual BGCs, followed by a comprehensive examination of their genomic locations. A total of 21 BGCs were found to be evenly distributed across the genome.

Figure S9. Nucleotide sequence alignment at *attB* sites of HNS054. *Sco*: *S. coelicolor*; *Sgr*: *S. griseoincarnatus*.
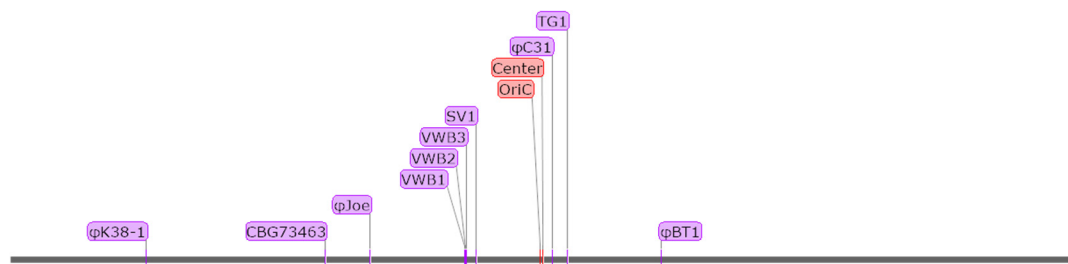
Figure S10. Distribution of 10 natural *attB* sites in the HNS054 chromosome.
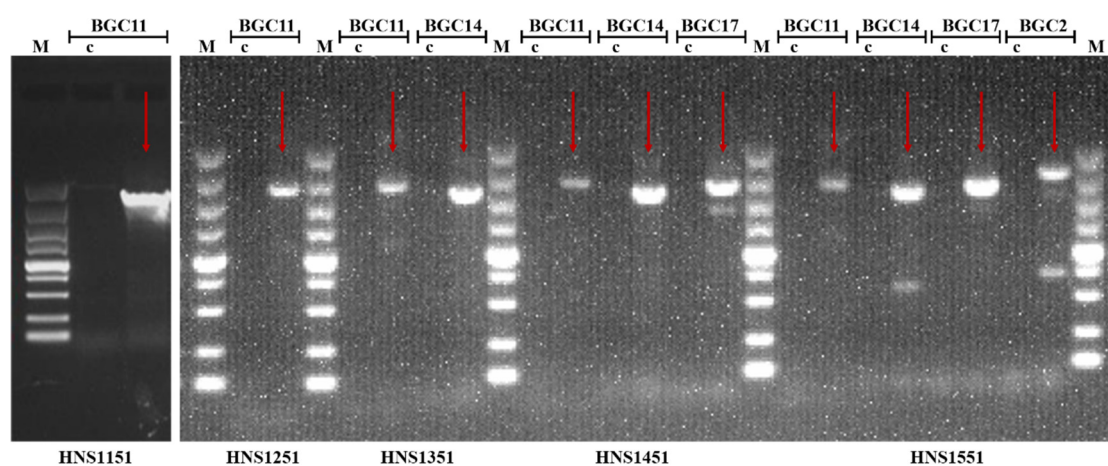
Figure S11. PCR verification of strains featuring BGC knockout and φC31 *attB* site introduction. M: 5 Kb DNA marker; c: negative control.
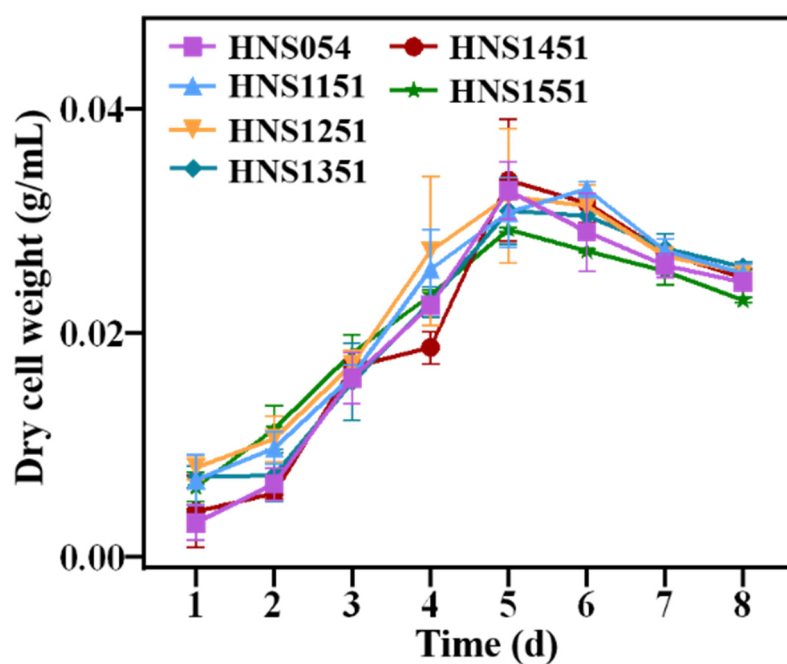
Figure S12. Growth curve of strains. *Streptomyces* spores were scraped from MS plates and inoculated into the seed medium for 24 h. The seed culture was then transferred to the fermentation medium to achieve an initial OD_{600} of 0.2. 1 mL samples were collected every 24 h and centrifuged. The supernatant was discarded and the pellets were dried at 65 °C for 3-5 d until the weight was constant. The biomass weight at different time points was calculated and the growth curve was plotted.

Figure S13. Identification of the plasmid pSET152::gul introduced into the strains HNS1151-1551. M: 5 Kb DNA marker; c: negative control. N: native; 11: BGC11; 14: BGC14; 17: BGC17; 2: BGC2.
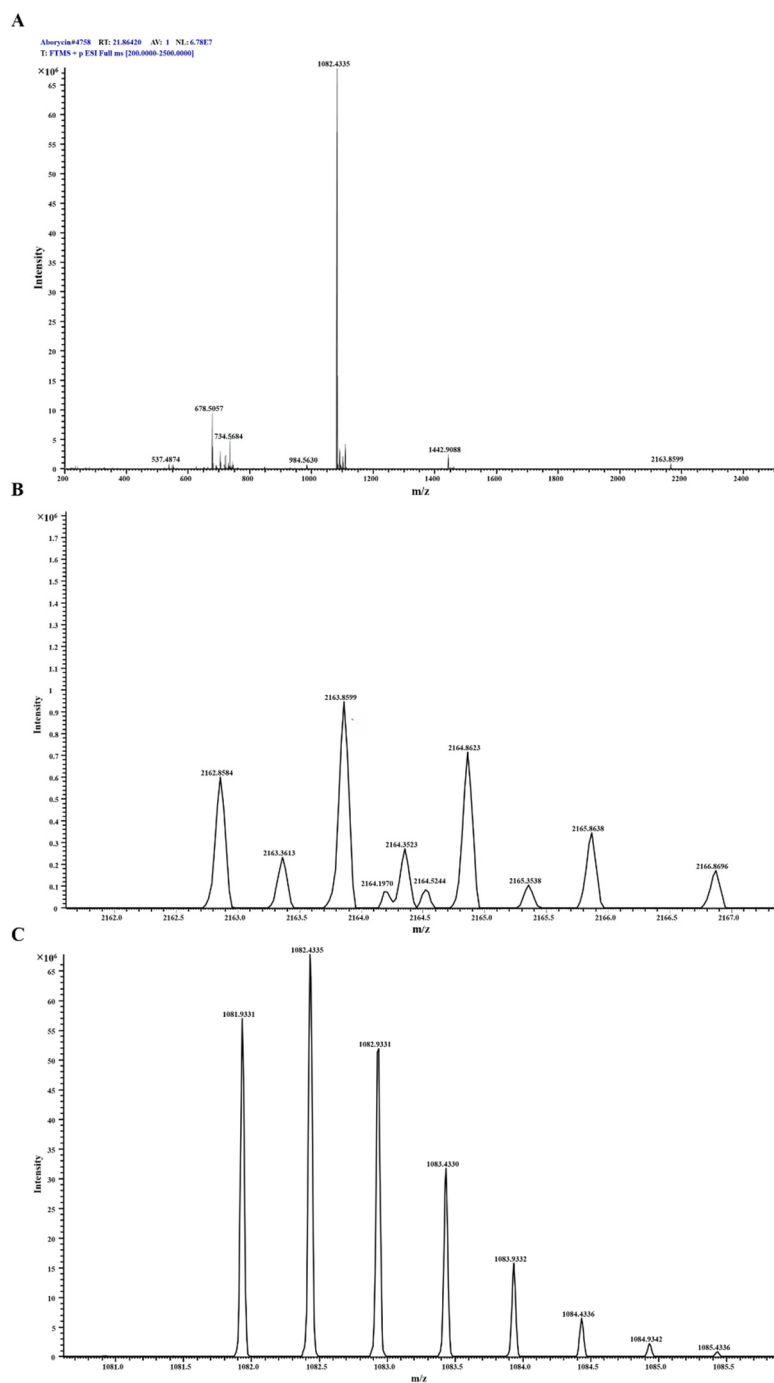
Figure S14. Positive ion peak in the mass spectra of aborycin. The ESI-MSMS data revealed [M + H]+ at m/z = 2162.8584, calculated for C97H132N23O26S4: 2162.8591, and [M + H]2+ at m/z = 1082.4335. Figure S14A displays the full mass spectrum at 21.9 min, while Figure S14B-C present magnified views of Figure S14A.
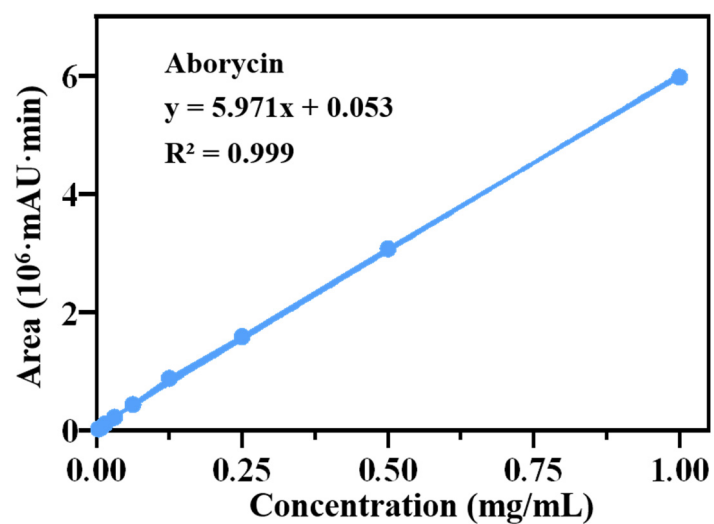
Figure S15. The standard curve depicting the relationship between aborycin concentrations and the HPLC peak areas.
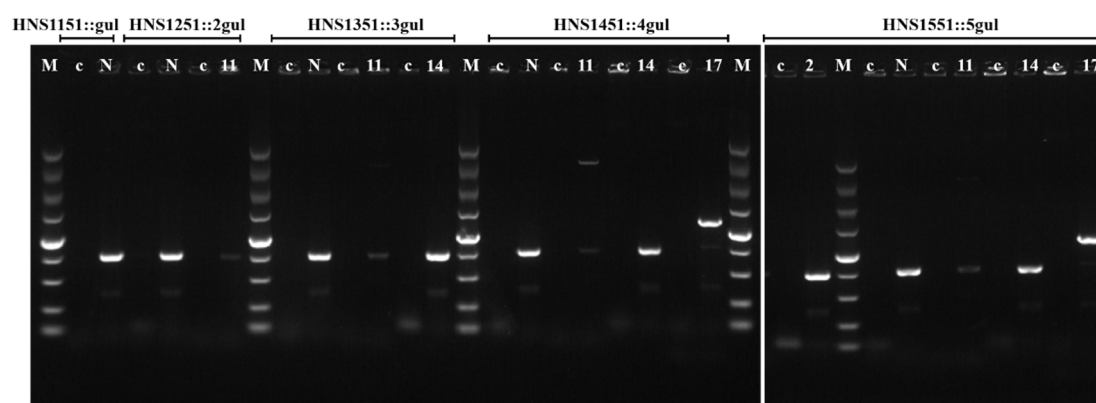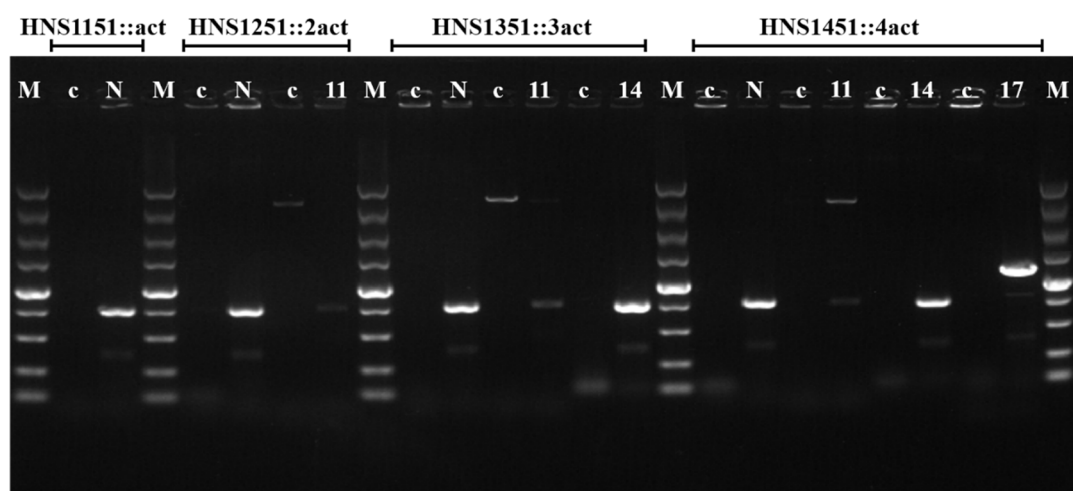

Figure S16. Identification of the plasmid pSET152::act introduced into the strains HNS1151-1451. M: 5 Kb DNA marker; c: negative control. N: native; 11: BGC11; 14: BGC14; 17: BGC17.
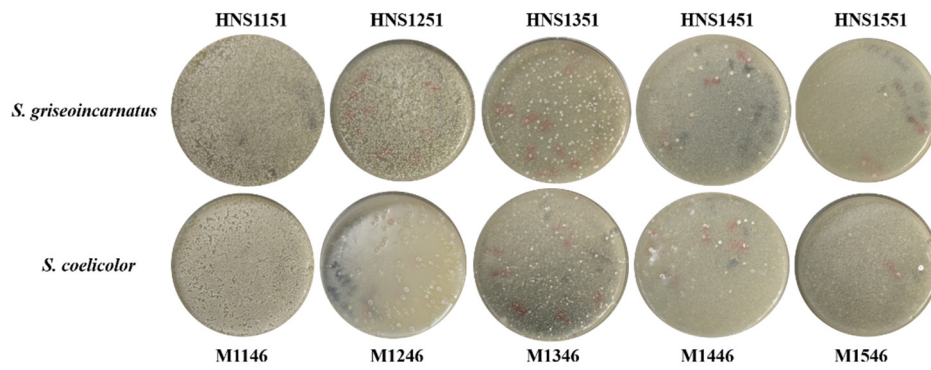
Figure S17. The conjugation effect of *attB* numbers on strains. Plasmid pSET152 was transferred from *E. coli* ET12567/pUZ8002 to various *Streptomyces* strains by intergeneric conjugation, and the conjugants were grown on apramycin (50 μg/mL) plates. The plates were incubated for 7 d and the number of colonies was counted. The results reveal a decrease in conjugation efficiency with an increase in the number of identical *attB* sites within the same strain.

Table S1. Basic genome sequencing features of HNS054

Features	Chromosome
Sequencing technology	PacBio Sequel II, Illumina NovaSeq 6000
Contig	1
Scaffold	1
Genome topology	Linear
Assembly size (bp)	7523030
GC content (%)	72.3
No. of ORFs	6678
No. of tRNA	72 (45 species)
No. of rRNA (5S, 16S, 23S)	18
No. of BGCs	21

Table S2. Endogenous CRISPR sequences in HNS054 genome

Element	Id	Start	End	Spacer	Repeat consensus
CRIPSR	1	794940	795069	1	CAGCACGTGCAGAGGGCGCAGGAGTCCGTGCACAGC
CRIPSR	2	811402	811513	2	CCACCGGGGCCGCCCGGACCGCCGG
CRIPSR	3	935947	936046	1	GGCTTCCACTGAGTGCGAGGGCTCCCGCCGAGTGGGCG
CRIPSR	4	1081083	1081182	1	GGGGCGCGGGGAACCGCGCGAACGCCCACCACG
CRIPSR	5	1209035	1209141	1	GGGCGTTCACCCGCCGCCCACTGC
CRIPSR	6	1512962	1513061	1	GGTTGTTCGCGCAGTTCCCCGCGCCCCTGGGGGC
CRIPSR	7	2004747	2004824	1	GTCCAGCTCGGCGCGCAGCCGCTC
CRIPSR	8	2282208	2282299	1	TCGCGCAGTTCCCCGCGCCCCTG
CRIPSR	9	2449899	2449991	1	CGCGGGGAACTGCGCGACCAGCCAC
CRIPSR	10	2505449	2505549	1	GGGTCGTGGGCGGGCTGCTGGTGGCCGCC
CRIPSR	11	2534316	2534420	1	GTCAGCACCCACCGGTACAGCCAC
CRIPSR	12	3292081	3292170	1	TCGCGCAGTTCCCCGCGCCCCTTC
CRIPSR	13	3772603	3772701	1	GGCCGCGGCCGGCGGGTAGCCGTA
CRIPSR	14	3853567	3853692	1	GCGGGGCTCGTGGTCGGTCCCCGGGGGTTATGCCG
CRIPSR	15	4517185	4517292	1	CTCGGCGGCCTAGCTGCGCAGGCCAGGTC
CRIPSR	16	4867640	4867699	1	AGGGGCGCGGGGAACTGCGCGAC
CRIPSR	17	5142849	5142939	1	CGTCGCGCAGCCGCTCGGCCTCCT
CRIPSR	18	5143275	5143365	1	CGTCGCGCAGCCGCTCGGCCTCCT
CRIPSR	19	5143494	5143583	1	CGTCGCGCAGCCGCTCGGCCTCCT
CRIPSR	20	5292916	5293019	1	GGGGCGCGGGGAACTGCGCGAGCA
CRIPSR	21	6004602	6004714	1	GGCCGACGGCGTGCTCCGTGCCACACGGCCGC
CRIPSR	22	6787654	6787745	1	GTTCGCGCAGTTCCCCGCGCCCCTT
CRIPSR	23	7066800	7066893	1	AGGGGCGCGGGGAACTGCGCGCTC
CRIPSR	24	7138726	7138844	1	ACGGCCTGCCCGGTCCCGGCACCCTGGTCCGG

Table S3. ANI and dDDH analysis of HNS054 to related species

Strain	ANI (%)	dDDH (%)
S. griseoincarnatus RB7AG	98.45	85.70
S. variabilis JCM 4422	98.23	83.50
S. lusitanus FZ202	95.28	59.60
S. griseorubens JCM 4383	95.28	59.70
S. albogriseolus LBX-2	94.17	53.90
S. fungicidicus ATCC 21013	88.42	31.80
S. leeuwenhoekii C34	86.71	26.90

Table S4. AntiSMASH-predicted BGCs in the HNS054 genome

No.	Cluster No.	Type	Length (nt)	Most similar known cluster	Similarity (%)
1	BGC1	Ectoine	13753	Ectoine	100
2	BGC2	NRPS	153463	Naphthyridinomycin	100
3	BGC3	RiPP-like	10484	Streptamidine	75
4	BGC4	T3PKS	41073	Alkylresorcinol	100
5	BGC5	Terpene	22502	Carotenoid	54
6	BGC6	T2PKS	72509	Spore pigment	83
7	BGC7	Ectoine	8869	Ectoine	100
8	BGC8	NRPS	32799	Scleric acid	17
9	BGC9	Siderophore	10632	Desferrioxamine B/E	83
10	BGC10	Lanthipeptide-class-iv	22672	Venezuelin	100
11	BGC11	Lasso peptide	22493	Aborycin	100
12	BGC12	Phenazine	20446	–	–
13	BGC13	Terpene	20783	Albaflavenone	100
14	BGC14	Ripp-like	11016	–	–
15	BGC15	Terpene	19936	Geosmin	100
16	BGC16	Siderophore	13239	Grincamycin	8
17	BGC17	Terpene	26696	Hopene	92
18	BGC18	Ripp-like	10215	Informatipeptin	57
19	BGC19	Ectoine	15409	Ectoine	100
20	BGC20	NRPS-PKS	100080	Polyoxypeptin	48
21	BGC21	Lanthipeptide-class-III	22657	SapB	100

Table S5. Identification of natural *attB* sites in strains

Type of *attB* in SSR system	HNS054	*S. griseus* NBRC 13350 NC_010572	*S. coelicolor* A3(2) NC_003888.1	*S. albus* J1074 NC_020990	*S. atratus* SCSIO ZH16 NZ_CP027306.1
φBT1 (*Sco*)	59/62 (95%)	46/48 (95%)	62/62 (100%)	38/41 (92%)	57/62 (91%)
φC31 (*Sco*)	47/51 (92%)	46/51 (90%)	51/51 (100%)	48/51 (94%)	47/51 (92%)
SV1 (*Sco*)	54/55 (98%)	47/52 (90%)	55/55 (100%)	18/18 (100%)	18/18 (100%)
TG1 (*Sco*)	33/33 (100%)	28/30 (93%)	46/46 (100%)	31/33 (93%)	43/46 (93%)
VWB1(*Sco*)	51/51 (100%)	53/53 (100%)	53/53 (100%)	53/53 (100%)	50/50 (100%)
VWB2 (*Sco*)	43/44 (97%)	27/27 (100%)	43/43 (100%)	43/43 (100%)	43/44 (97%)
VWB3 (*Sco*)	38/39 (97%)	Not found	27/27 (100%)	27/27 (100%)	27/27 (100%)
φJoe (*Sven*)	34/38 (89%)	27/27 (100%)	26/26 (100%)	33/36 (91%)	38/38 (100%)
φK38-1 (*Sco*)	47/48 (97%)	50/55 (90%)	61/61 (100%)	30/32 (93%)	35/38 (92%)
CBG73463 (*Sco*)	54/57 (94%)	16/16 (100%)	57/57 (100%)	15/15 (100%)	15/15 (100%)
R4 (*Sco*)	Not found	37/41 (90%)	41/41 (100%)	14/14 (100%)	14/14 (100%)

Sco: *S. coelicolor*; *Sven*: *S. venezuelae*

Table S6. The antibiotic sensitivity of HNS054

Antibiotic	Concentration (μg/mL)					
	0	12.5	25	50	75	100
Ampicillin	++	+	+	+	+	+
Chloramphenicol	++	++	++	++	++	++
Kanamycin	++	+	+	−	−	−
Apramycin	++	+	+	−	−	−
Thiostrepton	++	++	++	++	++	++
Nalidixic acid	++	++	++	++	++	++
Tetracycline	++	+	−	−	−	−
	50	100	150	200	250	300
Spectinomycin	++	++	+	−	−	−
Hygromycin	+	−	−	−	−	−

Note: −: not grown; +: weak growth; ++: normal growth

Primer	Sequence (5′-3′)	Description
Del-BGC11-up-fwd	tttttgagatctgaattccacATCCGTTGGCAAGGTTTGAT	Primers for the plasmid pKY01dB11 construction
Del-BGC11-up-rev	CCAAGGAGATCCTCACCGACTT	Primers for the plasmid pKY01dB11 construction
Del-BGC11-down-fwd	gtcggtgaggatctccttggACCCGAGACCTACGCATTACG	Primers for the plasmid pKY01dB11 construction
Del-BGC11-down-rev	acgacggccagtgccaagcttCGCAGCAGCCCTGTCCAC	Primers for the plasmid pKY01dB11 construction
ID-BGC11-fwd	gggaatccattggccttatgt	Primers validation of the BGC11 knockout in strain
ID-BGC11-rev	gaggagcttgtcactcatccg	Primers validation of the BGC11 knockout in strain
Del-BGC11-up-B-fwd	tttttgagatctgaattccacgtcccagacgagcgagatgcc	Primers for the plasmid pKY01dB11::attB construction
Del-BGC11-up-B-rev	aagggcacgccctggcacccgcaccgGTCCGTCGTCTCGGTCAGCAC	Primers for the plasmid pKY01dB11::attB construction
Del-BGC11-down-B-fwd	CGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACCCGCCAGCCACGACAGATG	Primers for the plasmid pKY01dB11::attB construction
Del-BGC11-down-B-rev	acgacggccagtgccaagcttGGTCGCCGCCCTCCTCACGC	Primers for the plasmid pKY01dB11::attB construction
BGC11-sgRNA-B-fwd	agtcctaggtataatactagtCGGGCAAGACTTAGTTTCATgttttagagctagaaatagca	Primers for the plasmid pKY01dB11::attB, pKY01dB11 construction
sgRNA-rev	gtggaattcagatctcaaaaa	Primers for the plasmid pKY01dB11::attB, pKY01dB11, pKY26dB17::attB, pKY44dB2::attB, pKY10dB14::attB construction
ID-BGC11-B-fwd	gtgccaggaccgccccacg	Primers validation of the BGC11 knockout and $attB_{\varphi C31}$ site introduction in strain
ID-BGC11-B-rev	gctgcgcggtgaggaccgg	Primers validation of the BGC11 knockout and $attB_{\varphi C31}$ site introduction in strain
Del-BGC17-up-fwd	tttttgagatctgaattccacCTGATGCCGTTCGTGCTG	Primers for the plasmid pKY26dB17::attB construction
Del-BGC17-up-rev	aagggcacgccctggcacccgcaccgCGTTCTACACTGCTGACCCAA	Primers for the plasmid pKY26dB17::attB construction
Del-BGC17-down-fwd	CGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACCAGGGAGTGACCACAGATGACC	Primers for the plasmid pKY26dB17::attB construction
Del-BGC17-down-rev	acgacggccagtgccaagcttTCGCCTCTTCGATCAGGGT	Primers for the plasmid pKY26dB17::attB construction
BGC17-sgRNA-fwd	agtcctaggtataatactagtCAGGCACGCAACTTCGCCTAgttttagagctagaaatagca	Primers for the plasmid pKY26dB17::attB construction
ID-BGC17-fwd	CCCGTGAGTGAGACTACGCA	Primers validation of the BGC17 knockout and $attB_{\varphi C31}$ site introduction in strain
ID-BGC17-rev	TCGGCTGGAAGTGGATGTC	Primers validation of the BGC17 knockout and $attB_{\varphi C31}$ site introduction in strain
Del-BGC2-up-fwd	ttttgagatctgaattccacCATGGAGAACATCACGTCGAAC	Primers for the plasmid pKY44dB2::attB construction
Del-BGC2-up-rev	aagggcacgccctggcacccgcaccgGCGGGAACCCAGTGAGCA	Primers for the plasmid pKY44dB2::attB construction
Del-BGC2-down-fwd	CGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACCCCAACATCAGAGCGATTACCG	Primers for the plasmid pKY44dB2::attB construction
Del-BGC2-down-rev	acgacggccagtgccaagcttCCGCTTGGTTGCCGTGTG	Primers for the plasmid pKY44dB2::attB construction

BGC2-sgRNA-fwd	agtcctaggtataatactagtCGACGCTCCTCTCGTGGTCCgttttagagctagaaatagca	Primers for the plasmid pKY44dB2::attB construction
ID-BGC2-fwd	ccccgcgcgtccagcacac	Primers validation of the BGC2 knockout and $attB_{\varphi C31}$ site introduction in strain
ID-BGC2-rev	tgggccgtacggcggggttg	Primers validation of the BGC2 knockout and $attB_{\varphi C31}$ site introduction in strain
Del-BGC14-up-fwd	tttttgagatctgaattccacCTCGGAGGCCCGTATGCAC	Primers for the plasmid pKY10dB14::attB construction
Del-BGC14-up-rev	aagggcacgccctggcacccgcaccgGGCACTACACCTCGCTGAACAA	Primers for the plasmid pKY10dB14::attB construction
Del-BGC14-down-fwd	CGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACCGCAGCAGGGTGGTGAGGGT	Primers for the plasmid pKY10dB14::attB construction
Del-BGC14-down-rev	acgacggccagtgccaagcttAGGGCGTCGGGGTGGAACT	Primers for the plasmid pKY10dB14::attB construction
BGC14-sgRNA-fwd	agtcctaggtataatactagtCCGCCAGACGCTTCCAGCCCgttttagagctagaaatagca	Primers for the plasmid pKY10dB14::attB construction
ID-BGC14-fwd	gtcgcctccttctccctcg	Primers validation of the BGC14 knockout and $attB_{\varphi C31}$ site introduction in strain
ID-BGC14-rev	ggaacaagtgggcgtacacg	Primers validation of BGC14 knockout and $attB_{\varphi C31}$ site introduction in strain
pKCcas9sc-fwd	CCGGTCCAGTAATGACCTCAGA	Primers verification of pKY01dB11::attB, pKY26dB17::attB, pKY44dB2::attB, pKY10dB14::attB plasmid construction
pKCcas9sc-rev	TGCAAGGCGATTAAGTTGGGT	Primers verification of pKY01dB11::attB, pKY26dB17::attB, pKY44dB2::attB, pKY10dB14::attB plasmid construction
pSET152-fwd	GTCATAGCTGTTTCCTGTGTGAAATT	Linearized amplification of pSET152
pSET152-rev	ACTGGCCGTCGTTTTACAACG	Linearized amplification of pSET152
054 gul-fwd	gttgtaaaacgacggccagtTCCGTTGGCAAGGTTTGATG	Amplification of aborycin BGC
054 gul-rev	acacaggaaacagctatgacACGACGAGAAGGAGACCGAGG	Amplification of aborycin BGC
BGC11-fwd	TCCCGGGTGGTGCCGACGAC	Primers verification of pSET152::gul plasmid construction
BGC11-rev	GTCGGAACAGGAGAGCGCAC	Primers verification of pSET152::gul plasmid construction
BGC2 B-fwd	CCCTTCCTGGTTGGCTTGGTTT	Primers for $attB_{\varphi C31}$ integration detection
BGC2 B-rev	GCAGCCAGGCGAGTGGATTCTT	Primers for $attB_{\varphi C31}$ integration detection
BGC11 B-rev	ggcagctcctgcatcgactcg	Primers for $attB_{\varphi C31}$ integration detection
BGC17 B-rev	CCGACTTGACGGTGCCCTGGAAC	Primers for $attB_{\varphi C31}$ integration detection
BGC14 B-rev	CGCACCATCTCCTCTACAACACCACG	Primers for $attB_{\varphi C31}$ integration detection
ID-oriT-fwd	gcagagcaggattcccgttgagca	Primers for $attB_{\varphi C31}$ integration detection
Native B-rev	GGGGTGGCAGGAAGTTCAACGCTC	Primers for $attB_{\varphi C31}$ integration detection

Table S8. Plasmids and strains used in this study

Plasmid	Description	Source
pKCcas9dO	*ori^{pUC}, oriT, reppSG5, acc(3)IV, PJ23119(SpeI)-spacer-sgRNA-HR cassette, PtipA-SpCas9*	[36]
pKY01dB11	pKY01dB11 with two homologous arms and the sgRNA transcription cassette for deleting the cluster BGC11	This study
pKY01dB11::attB	pKY01dB11::attB with two homologous arms and the sgRNA transcription cassette for deleting the cluster BGC11 and introducing an artificial *attB$_{φC31}$*	This study
pKY26dB17::attB	pKY26dB17::attB with two homologous arms and the sgRNA transcription cassette for deleting the cluster BGC17 and introducing an artificial *attB$_{φC31}$*	This study
pKY10dB14::attB	pKY10dB14::attB with two homologous arms and the sgRNA transcription cassette for deleting the cluster BGC14 and introducing an artificial *attB$_{φC31}$*	This study
pKY44dB2::attB	pKY44dB2::attB with two homologous arms and the sgRNA transcription cassette for deleting the cluster BGC2 and introducing an artificial *attB$_{φC31}$*	This study
pSET152	*oriT, attP$_{φC31}$, int$_{φC31}$, lacZa, ori^{pUC}, acc(3)IV*	[37]
pKC-gul	*acc(3)IV, ori^{pUC}, rep^{pSG5}, oriT, tipA-Scocas9,* j23119-gulspacer-gRNA, homologous region flanking *gul* BGC	In the laboratory
pSET152::gul	*oriT, attP$_{φC31}$, int$_{φC31}$, acc(3)IV, ori^{pUC}, gul* BGC	In the laboratory
pSET152::act	*oriT, attP$_{φC31}$, int$_{φC31}$, acc(3)IV, ori^{pUC}, act* BGC	[34]

Strain	Description	Source
E. coli DH5α	*F-φ80 lac ZΔM15 Δ(lacZYA-arg F) U169 endA1 recA1 hsdR17(rk-,mk+) supE44λ-thi-1 gyrA96 relA1 phoA*	[38]
E. coli ET12567(pUZ8002)	*dam, dcm, hsdS, cat, tet, tra, neo*, RP4	[39]
S. coelicolor M1146	*S. coelicolor* M145 (contains one native *attB$_{φC31}$*) *Δact, Δred, Δcpk, Δcda*	[38]
S. coelicolor M1246	*S. coelicolor* M1146 *Δcpk::attB$_{φC31}$*	[27]
S. coelicolor M1346	*S. coelicolor* M1246 *Δred::attB$_{φC31}$*	[27]
S. coelicolor M1446	*S. coelicolor* M1346 *Δcda::attB$_{φC31}$*	[27]
S. coelicolor M1546	*S. coelicolor* M1446 *Δact::attB$_{φC31}$*	[27]
S. coelicolor M1346::3gul	*S. coelicolor* M1346 integrated three copy of pSET152::gul at the native *attB$_{φC31}$*	[15]
S. griseoincarnatus HNS054	Wide type strain, *S. griseoincarnatus* HNS054 with one copy of gul BGC at the native *attB$_{φC31}$*	[17]
S. griseoincarnatus HNS1151	*S. griseoincarnatus* HNS054 deleted BGC11	This study
S. griseoincarnatus HNS1251	*S. griseoincarnatus* HNS054 introduced X *attB$_{φC31}$* at the adjacent loci of the deleted BGC11	This study
S. griseoincarnatus HNS1351	*S. griseoincarnatus* HNS054 introduced 2X *attB$_{φC31}$* at the adjacent loci of the deleted BGC11 and BGC14	This study
S. griseoincarnatus HNS1451	*S. griseoincarnatus* HNS054 introduced 3X *attB$_{φC31}$* at the adjacent loci of the deleted BGC11, BGC14 and BGC17	This study
S. griseoincarnatus HNS1551	*S. griseoincarnatus* HNS054 introduced 4X *attB$_{φC31}$* at the adjacent loci of the deleted BGC11, BGC14, BGC17 and BGC2	This study
S. griseoincarnatus HNS1151::gul	*S. griseoincarnatus* HNS1151 integrated one copy of pSET152::gul at the native *attB$_{φC31}$*	This study
S. griseoincarnatus HNS054::pSET152	*S. griseoincarnatus* HNS054 integrated one copy of pSET152 at the native *attB$_{φC31}$*	This study
S. griseoincarnatus HNS1251::2gul	*S. griseoincarnatus* HNS1251 integrated two copy of pSET152::gul at the *attB$_{φC31}$*	This study
S. griseoincarnatus HNS1351::3gul	*S. griseoincarnatus* HNS1351 integrated three copy of pSET152::gul at the *attB$_{φC31}$*	This study
S. griseoincarnatus HNS1451::4gul	*S. griseoincarnatus* HNS1451 integrated four copy of pSET152::gul at the *attB$_{φC31}$*	This study

S. griseoincarnatus HNS1551::5gul	*S. griseoincarnatus* HNS1551 integrated five copy of pSET152::gul at the $attB_{\varphi C31}$	This study
S. griseoincarnatus HNS1151::pSET152	*S. griseoincarnatus* HNS1151 integrated one copy of pSET152 at the native $attB_{\varphi C31}$	This study
S. griseoincarnatus HNS1151::act	*S. griseoincarnatus* HNS1151 integrated one copy of pSET152::act at the native $attB_{\varphi C31}$	This study
S. griseoincarnatus HNS1251::2act	*S. griseoincarnatus* HNS1251 integrated two copy of pSET152::act at the $attB_{\varphi C31}$	This study
S. griseoincarnatus HNS1351::3act	*S. griseoincarnatus* HNS1351 integrated three copy of pSET152::act at the $attB_{\varphi C31}$	This study
S. griseoincarnatus HNS1451::4act	*S. griseoincarnatus* HNS1451 integrated four copy of pSET152::act at the $attB_{\varphi C31}$	This study
S. coelicolor M1346::3act	*S. coelicolor* M1346 integrated three copy of pSET152::act at the $attB_{\varphi C31}$	This study

Table S9. Basic information of *Streptomyces* genomes

No.	Strain	NCBI number	Size	Source
1	*S. griseoincarnatus* HNS054	CP139576	7.52	marine sponge
2	*S. violaceusniger* Tu 4113	NC_015957.1	10.66	soil
3	*S. bingchenggensis* BCW-1	NC_016582.1	11.94	soil
4	*S. griseus* NBRC 13350	NC_010572.1	8.55	soil
5	*S. avermitilis* MA-4680	NC_003155.5	9.03	soil
6	*S. scabiei* 87.22	NC_013929.1	10.15	plant-associated
7	*S. coelicolor* A3(2)	NZ_CP042324.1	8.67	soil
8	*S. collinus* Tu 365	NC_021985.1	8.27	soil
9	*S. davaonensis* JCM4913	NC_020504.1	9.47	soil
10	*S. hygroscopicus* 5008	NC_017765.1	10.15	soil
11	*S. reticuli* TUE45	LN997842.1	8.35	soil
12	*S. globisporus* C-1027	NZ_CP013738.1	7.61	soil
13	*S. venezuelae* ATCC21782	NZ_CP029190.1	7.53	soil
14	*S. ambofaciens* ATCC 23877	NZ_CP012382.1	8.30	soil
15	*S. glaucescens* GLA.O	NZ_CP009438.1	7.45	soil
16	*S. lividans* TK24	NZ_CP009124.1	8.35	soil
17	*S. vietnamensis* GIM4.0001	NZ_CP010407.1	8.87	soil
18	*S. leeuwenhoekii* C34	NZ_LN831790.1	7.90	soil
19	*S. xiamenensis* MCCC 1A01550	NZ_CP009922.3	5.96	mangrove
20	*S. noursei* DS30.6	NZ_JAJUFB000000000.1	8.86	soil
21	*S. lydicus* A02	NZ_CP007699.2	9.30	soil
22	*S. diacarni* LHW51701	NZ_QOIN00000000.1	7.66	marine sponge
23	*S. oceani* SCSIO 02100	NZ_LJGU00000000.1	6.31	marine
24	*S. reniochalinae* LHW50302	NZ_QOIM00000000.1	7.69	marine sponge
25	*S. microflavus* NA06532	NZ_CP054926.1	7.79	soil
26	*S. albus* DSM 41398	NZ_CP010519.1	8.38	soil
27	*S. violaceoruber* S21	NZ_CP020570.1	7.92	marine
28	*S. luteoverticillatus* CGMCC 15060	NZ_CP034587.1	7.37	marine
29	*S. formicae* KY5	NZ_CP022685.1	9.61	insect-associated
30	*S. niveus* SCSIO 3406	NZ_CP018047.1	7.99	marine
31	*S. spongiicola* HNM0071	NZ_CP029254.1	7.18	marine sponge
32	*S. atratus* SCSIO_ZH16	NZ_CP027306.1	9.64	marine
33	*S. pluripotens* MUSC 135	NZ_CP021080.1	7.35	mangrove
34	*S. aquilus* GGCR-6	NZ_CP034463.1	10.39	plant-associated
35	*S. indicus* CGMCC 4.5727	NZ_FNFF00000000.1	8.23	marine
36	*S. qinzhouensis* SSL-25	NZ_CP042266.1	8.15	mangrove

37	*S. chattanoogensis* NRRL ISP-5002	NZ_LGKG00000000.1	9.13	soil
38	*S. kronopolitis* 6G-OA-10	NZ_JAMFLE000000000.1	7.60	plant-associated
39	*S. griseoincarnatus* RB7AG	NZ_JAMQBH000000000.1	7.71	marine