Genome engineering and direct cloning of antibiotic gene clusters via phage \(\phi BT1\) integrase-mediated site-specific recombination in \(Streptomyces\)

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Several strategies have been used to clone large DNA fragments directly from bacterial genome. Most of these approaches are based on different site-specific recombination systems consisting of a specialized recombinase and its target sites. In this study, a novel strategy based on phage \(\phi BT1\) integrase-mediated site-specific recombination was developed, and used for simultaneous \(Streptomyces\) genome engineering and cloning of antibiotic gene clusters. This method has been proved successful for the cloning of actinorhodin gene cluster from \(Streptomyces coelicolor\) M145, napsamycin gene cluster and daptomycin gene cluster from \(Streptomyces roseosporus\) NRRL 15998 at a frequency higher than 80%. Furthermore, the system could be used to increase the titer of antibiotics as we demonstrated with actinorhodin and daptomycin, and it will be broadly applicable in many \(Streptomyces\).

\(Streptomyces\) are high-GC Gram-positive bacteria well known for their ability to produce a wide variety of medically and agriculturally useful antibiotics and related compounds\(^1\). Genes responsible for the biosynthesis of a specific secondary metabolite are usually arranged in clusters that vary in size from a few to over 100 kb\(^2\). To gain insight into the biosynthesis and regulation of antibiotics in \(Streptomyces\), it is of great importance to clone their gene clusters. Recently, various approaches have been developed to clone gene clusters directly from bacterial genomic DNA. These methods include RecET-mediated linear-plus-linear homologous recombination (LLHR)\(^3\), \(oriT\)-directed capture system\(^4\) and transformation-associated recombination (TAR)\(^5\). The RecET-mediated LLHR was successful in cloning gene clusters (10 to 52 kb in length) from the genome of \(Photorhabdus luminescens\) into expression vectors in \(Escherichia coli\)\(^3\). The \(oriT\)-directed capture system has been used to clone regions up to 140 kb from the genome of \(Burkholderia pseudomallei\)\(^6\) and 200 kb from megaplasmid of \(Sinorhizobium meliloti\)\(^4\). However, the use of this system was limited to Gram-negative bacteria that can be established as conjugation donors\(^6\). Taking advantage of the natural in vivo homologous recombination of \(Saccharomyces cerevisiae\), TAR cloning strategy was used to capture a 21.3 kb enterocin gene cluster from \(Salinispora pacifica\) CNT-150\(^7\) and a 67 kb taromycin A biosynthetic gene cluster from \(Saccharomonospora\) sp. CNQ-490\(^8\).

The ability to delete large genomic fragments within \(Streptomyces\) genome is of great interest for genetic manipulations of \(Streptomyces\). Several strategies have been developed for a number of bacteria. Some methods are based on the meganuclease I-SceI system which involves the meganuclease I-SceI of \(Saccharomyces cerevisiae\) and its 18 bp recognition sequence\(^9,10\). Many of them are based on site-specific recombination systems consisting of a specialized recombinase and its target sites. Nearly all site-specific recombinases fall into two families, the tyrosine recombinases and the serine recombinases\(^11\). The recombination systems of the tyrosine recombinase family include Cre/loxP from the P1 phage\(^12\), Dre/rox from the P1-like transducing phage D6\(^13\) and the Flp/FRT from yeast\(^14\). The Cre, Dre and Flp proteins are the tyrosine recombinases which catalyze reciprocal site-specific recombination of DNA at loxP, rox and FRT sites, respectively. Integrases (Int) from \(Streptomyces\) temperate phage \(\phi C31\) and \(\phi BT1\) belong to serine recombinase family. They catalyze site-specific recombination of the phage attachment site (attP) with the bacterial attachment site (attB), resulting in the formation of two hybrid sites (attL and attR)\(^15,16\). Both \(\phi C31\) and \(\phi BT1\) attP-int loci have been used to construct versatile vectors which can
integrate into different attB sites in *Streptomyces*\textsuperscript{15,16}. To increase the diversity of attP-attB pair of \(\Phi BT1\), 15 mutated attP-attB pairs (\(\text{attP}_{\text{acr}}\text{-attB}_{\text{ori}1} \rightarrow \text{attP}_{\text{acr}}\text{-attB}_{\text{ori}2}\)) were generated by PCR mutagenesis of the central dinucleotide sequence of attP and attB\textsuperscript{7}. The Cre/loxP system was successfully used for the deletion of large fragments in *Magnetospirillum gryphiswaldense* and several *Streptomyces* species\textsuperscript{18–20}. However, the use of \(\Phi C31\) and \(\Phi BT1\) integrase in this aspect has not been exploited.

We devised a novel strategy for *Streptomyces* genome engineering and cloning of antibiotic gene clusters. This method is based on phage \(\Phi BT1\) attP-attB-int system and requires two single crossovers for targeted integration of mutated attB and attP into the recipient chromosome. Using the system, we easily cloned 25 kb fragment containing actinorhodin (act) gene cluster from *S. coelicolor* M145, 45 kb fragment containing napsamycin (nap) gene cluster and 157 kb fragment containing daptomycin (dap) gene cluster from *S. roseosporus* NRRL 15998. In addition, this method could be used to improve the titer of antibiotics by increasing copy numbers of anti-biotic gene clusters.

**Results**

**Construction of pUC119- and pKC1139-based plasmids.** Our strategy used in this study requires both homologous and site-specific recombinations. The homologous recombinations were used for targeted integration of the mutated attB and attP into *Streptomyces* chromosome, while the \(\Phi BT1\) integrase-mediated site-specific recombination was employed to excise targeted region of interest from the chromosome (Fig. 1). The mutated attB and attP sites were chosen to avoid site specific recombination with the endogenous attB site in *Streptomyces* genome and consequently undesirable DNA rearrangements. Sites of attB\textsubscript{6} and attP\textsubscript{6} were randomly chosen from the 15 mutated attP-attB pairs. For the integration of attB\textsubscript{6} into *Streptomyces* chromosome, pUC119-based suicide plasmids (pSV::attB\textsubscript{6}-act, pSV::attB\textsubscript{6}-nap and pSV::attB\textsubscript{6}-dap) were constructed. These plasmids are derivatives of pUC119 containing the kanamycin-resistance gene (neo), the origin of transfer (oriT) from plasmid RK2 (for the intergeneric conjugation between *E. coli* and *Streptomyces*), attB\textsubscript{6} and a 2.0 kb homologous region flanking 5’ end of the targeted regions (Fig. S1a). We also constructed pKC1139-based plasmids (pKC1139::attP\textsubscript{6}-act, pKC1139::attP\textsubscript{6}-nap and pKC1139::attP\textsubscript{6}-dap) for the integration of attP\textsubscript{6} into *Streptomyces* chromosome. These plasmids are derivatives of pKC1139 containing attP\textsubscript{6} and a 2.0 kb homologous region flanking 3’ end of the targeted regions (Fig. S1b).

**Cloning of act gene cluster from *S. coelicolor* M145.** To test this strategy, we first chose to clone the well-studied act gene cluster (SC05070-SC05092) from *S. coelicolor* M145. For this purpose, pSV::attB\textsubscript{6}-act and pKC1139::attP\textsubscript{6}-act were introduced into the recipient chromosomes via single-crossover homologous recombination to obtain double-cointegrate strain Sco-actB\textsubscript{6}P\textsubscript{6} (Fig. 1). Further introduction of pIJ10500 (an integrative plasmid containing the \(\Phi BT1\) integrase gene) into Sco-actB\textsubscript{6}P\textsubscript{6} allowed subsequent excision of 23 kb act gene cluster from *S. coelicolor* M145, leaving behind the suicide vector pUC119::neo, a scar of 42 bp attL site and pIJ10500 integrated within SCO4848. Excision of the gene cluster was confirmed by PCR analysis using both genomic and plasmid DNA as templates (Fig. 2). For 9 out of 10

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**Figure 1 | Schematic diagram of antibiotic gene cluster cloning from *Streptomyces* chromosome.** Initially, a pUC119-based suicide plasmid (pSV::attB\textsubscript{6}Up) carrying attB\textsubscript{6} and a region homologous to 5’ end of the cluster is introduced into the chromosome by a single crossover. A second plasmid pKC1139::attP\textsubscript{6}Dn is based on pKC1139 carrying attP\textsubscript{6} and a region homologous to 3’ end of the cluster. When the incubation temperature is higher than 34°C, pKC1139::attP\textsubscript{6}Dn turns into a non-replicating plasmid and then is integrated into the chromosome by a single crossover. Expression of \(\Phi BT1\) integrase (encoded in the plasmid pIJ10500) leads to excision of the pKC1139 backbone with gene cluster of interest, leaving behind the suicide vector pUC119::neo and 42 bp attL site. **ampC**: apramycin resistance gene; **neo**: kanamycin resistance gene; **ori**: temperature-sensitive origin of replication from pSG5; **rep**: encoding a replication initiator protein from pSG5.
Deletion of act gene cluster from S. coelicolor M145. To delete the act gene cluster from S. coelicolor M145, a single colony of M145-MCact was randomly selected for the removal of pKC1139::act. After three rounds of nonselective growth at 28°C and subsequent cultivation at 40°C, approximately 5% of M145-MCact colonies lost pKC1139::act. Strains lacking the act gene cluster (M145-Dact) were first confirmed by PCR (data not shown), and then patched on R5MS solid agar plate for visual comparison of actinorhodin production. Unlike S. coelicolor M145 that could produce both blue pigment actinorhodin and red pigment undecylprodigiosins, M145-Dact could only produce the red pigment undecylprodigiosins (Fig. 4a). This was further validated by no actinorhodin production of M145-Dact in R5MS liquid culture (Fig. 4b).

Figure 2 | Confirmation of the excision events by PCR amplifications. (A) The schematic diagram showing the position of primers in the chromosome of double-cointegrate strains. (B) Agarose gel electrophoresis showing PCR amplified fragments. PCR templates in the upper panels are genomic DNAs from S. coelicolor M145 or S. roseosporus NRRL 15998 (G) and ten randomly selected double-cointegrate strains with pIJ10500 (M145-MCact, Sro-MCnap or Sro-MCdap), while PCR templates in the lower panels are plasmid DNAs including pKC1139 (P) and ten different clones of pKC1139::act, pKC1139::nap and pKC1139::dap. The primers used and the expected size of amplification fragments were indicated.

Cloning and deletion of nap and dap gene cluster from S. roseosporus NRRL 15998. To clone gene cluster of medium and large sizes, we used the same strategy to clone nap and dap gene cluster from S. roseosporus NRRL 15998. Excision of nap gene cluster from S. roseosporus NRRL 15998 occurred in 9 out of 10 exconjugates, and excision of dap gene cluster from S. roseosporus NRRL 15998 occurred in 8 out of 10 exconjugates (Fig. 2). Like pKC1139::act, plasmid containing nap gene cluster (pKC1139::nap) was passed through E.coli Top10 and isolated plasmid DNA was confirmed by BamHI and EcoRI digestion, respectively (Fig. S2b). The cloned fragment covers a contiguous DNA region of 45 kb from SSGG02973 to SSGG03009. For pKC1139::dap, the plasmid was isolated directly from Streptomyces and confirmed with restriction digestion (Fig. S2c). The 157 kb fragment covering SSGG00215-SSGG00287 contains the complete dap gene cluster. Similar to that of act gene cluster, the removal of pKC1139::nap and pKC1139::dap from Sro-MCnap and Sro-MCdap generated strains lacking nap and dap gene clusters (Sro-Dnap and Sro-Ddap).

Improvement of antibiotic titers. The pKC1139 contains a temperature-sensitive origin of replication from pSG5, which is a medium copy plasmid with an approximate 20–50 copy numbers per chromosome. When cultured at 28°C, pKC1139 exists as autonomous plasmid in Streptomyces. In S. coelicolor M145, there is only one copy of act gene cluster in the chromosome. After the BT1 integrase-mediated excision, the act gene cluster was transferred into pKC1139. An increase in copy number of act gene cluster will improve actinorhodin production. This was confirmed both on R5MS agar plate (Fig. 4a) and in R5MS liquid culture (Fig. 4b). It should be noted that the titer of actinorhodin in M1146-MCact was even higher than that of M145-MCact. Similarly, daptomycin titer
could also increase after the excision of dap gene cluster from its chromosome location in S. roseosporus NRRL 15998. Cultures of Sro-MCdap and S. roseosporus NRRL 15998 were subjected to bioassay against S. aureus, the results showed that Sro-MCdap exhibited bigger inhibition zones against S. aureus than S. roseosporus NRRL 15998 at time intervals from 2–5 days (Fig. 5a). This was further verified by comparison of daptomycin from fermentation broth of S. roseosporus NRRL 15998 and Sro-MCdap by high-performance liquid chromatography (HPLC) analysis (Fig. 5b). In addition, we noticed that existence of extra copy numbers of antibiotic gene clusters caused a slowdown in growth of Streptomyces. When cultured on AS-1 agar medium, growth of Sro-MCnap and Sro-MCdap are severely impaired, especially at earlier stages of cultivation (Fig. S3). This phenotype was most likely attributed to the metabolic burden of extra copy numbers of antibiotic gene clusters. This assumption is based on the observation that growth of Sro-Dnap (devoid of nap gene cluster) and Sro-Ddap (devoid of dap gene cluster) are converted back to that of S. roseosporus NRRL 15998 (Fig. S3).

To examine the stability of multiple copy plasmids in Streptomyces, two randomly chosen strains of Sro-MCdap were passaged consecutively for five or ten times on AS-1 plates supplemented with or without apramycin. Biological activities of these strains (G5 and G10) were compared with that of the original Sro-MCdap (G0). All Sro-MCdap strains exhibited similar inhibitory activity against S. aureus (Fig. S3). This is the largest size ever reported in Gram positive bacteria and should be good enough for most antibiotic gene clusters. Last, our strategy can be used to improve the titer of industrial important antibiotics by creating strains with extra copy numbers of antibiotic biosynthetic gene clusters.

The \( \phi BT1 \ attP-attB-int \) system is helpful for genetic modifications of Streptomyces genome at multiple sites. In addition to the intact \( attP-attB \) pair, there are 15 mutated \( attP-attB \) pairs which can be recognized by \( \phi BT1 \) integrase\(^{17} \). Multiple rounds of large fragment deletion can be achieved with the following modifications.

1. Relocation of the \( attB_6 \) sequence (or any other mutated \( attB \)) to the downstream of the 2.0 kb homologous fragment in pSV::attBUp. This change will allow the excision of the pUC119::neo backbone from Streptomyces genome together with pKC1139.
2. Construction of an autonomous helper plasmid containing a temperature-sensitive origin of replication from pSG5, the origin of transfer (oriT) from plasmid RK2 and \( \phi BT1 \) integrase gene.

This plasmid can ensure the high efficient excision of large fragment from Streptomyces genome and subsequent removal of \( \phi BT1 \) inte-
grase. With these modifications, there is only 42 bp attL site left in the chromosome of Streptomyces.

Genome analysis suggested that S. roseosporus NRRL 15998 has potential capacity to produce napsamycins. However, the production of napsamycins in S. roseosporus NRRL 15998 has not been reported. With this strategy, we cloned nap gene cluster in pKC1139 to generate pKC1139::nap. It can be manipulated extensively in E. coli. These manipulations include replacement of vector backbone with integrative plasmid and deletion or constitutive expression of regulatory gene by PCR targeting. The modified gene cluster can be transferred into heterologous hosts for expression. It can also be transferred back into the mutant devoid of nap gene cluster after removal of pKC1139::nap. Detection of napsamycin in these strains will shed light on the activation of cryptic gene clusters in Streptomyces.

In some industrial overproducing strains generated by traditional mutagenesis, amplification of biosynthetic gene cluster has been observed. Based on these observations, controlled amplification of gene cluster was used to increase the productivity of commercially important antibiotics. Integration of an additional copy of gene cluster for nikkomycin and gougerotin biosynthesis led to an increased production of nikkomycin and gougerotin by Streptomyces ansichromogene and Streptomyces graminearus, respectively. The zouA-mediated gene amplification of act gene cluster in S. coelicolor M145 led to a 20-fold increase in actinorhodin production. The zouA encodes a site-specific relaxase similar to TraA protein which catalyzes RecA-independent site-specific recombination. The recombination sites of ZouA are oriT-like RsA and RsB. In this study, we reported the amplification of gene clusters mediated by phage φBT1 integrase and improved antibiotic titers in the engineered Streptomyces strains. We believe that the system described here could be used readily to increase antibiotic titers in many Streptomyces and possible other actinomycetes.

**Methods**

**Bacterial strains, plasmids, primers and growth conditions.** Bacterial strains and plasmids used in this study are listed in Table 1, and primers are listed in Table S1. S. coelicolor M145 and S. roseosporus NRRL 15998 were used for cloning of act, nap and dap gene clusters. S. coelicolor M1146 is an engineered derivative of S. coelicolor M145 that lacks gene clusters for actinorhodin (ACT), undecylprodigiosins (RED), cryptic polyketide (CPK) and calcium-dependent antibiotic (CDA) biosynthesis. Staphylococcus aureus was used as an indicator strain for daptomycin bioassay. E. coli Top10 was used as a general host for propagating plasmids. E. coli ET100 were used for transferring DNA from E. coli to Streptomyces by intergeneric conjugation.

For general purpose, S. coelicolor M145 and its derivatives were grown on mannitol soya flour medium (MS) agar or in yeast extract-malt extract (YEME) liquid medium.
| Strains/plasmids | Genotype/description | Reference/source |
|------------------|----------------------|-----------------|
| **E. coli** | | |
| Top10 | F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) φ80 lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara leu)7697 galU galK rpsL (StrR) endA1 nupG | Invitrogen |
| ET1567 | F⁻ dam-13: Tn9 dcm-6 hsdM hsdR zjy-220:: Tn10 recF143 galK2 gal122 ara-14 lacY1 xyl-5 leuB6 thi-1 tonA31 rpsL136 hisG4 tex-78 mtl-1 glnV44 | 40 |
| **Staphylococcus** | | |
| S. aureus | A indicator strain | 41 |
| **Streptomyces** | | |
| S. coelicolor M145 | Prototrophic; SCP1− SCP2− Pgl+ | 32 |
| S. coelicolor M146 | Δact Δred Δapk Δcda | 31 |
| S. coelicolor M145-Dact | A derivative of S. coelicolor M145 with attB6 and attP6 flanking act gene cluster | This study |
| S. coelicolor M146-Δact | Δact | This study |
| S. coelicolor M145-pKC1139 | A derivative of S. coelicolor M145 containing pKC1139 | This study |
| S. coelicolor M1146-Δact | Δact | This study |
| S. coelicolor M1146-pKC1139 | A derivative of S. coelicolor M1146 containing pKC1139 | This study |
| S. roseosporus NRRL 15998 | A daptomycin-producing strain | Broad Institute |
| S. roseosporus NRRL 15998-Dact | A derivative of S. roseosporus NRRL 15998 containing pKC1139 | This study |
| S. roseosporus NRRL 15998-Dred | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking dred gene cluster | This study |
| S. roseosporus NRRL 15998-Dcpk | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking cpk gene cluster | This study |
| S. roseosporus NRRL 15998-Dcda | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking cda gene cluster | This study |
| S. roseosporus NRRL 15998-DactB6P6 | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking act gene cluster | This study |
| S. roseosporus NRRL 15998-DredB6P6 | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking dred gene cluster | This study |
| S. roseosporus NRRL 15998-DcpkB6P6 | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking cpk gene cluster | This study |
| S. roseosporus NRRL 15998-DcdaB6P6 | A derivative of S. roseosporus NRRL 15998 with attB6 and attP6 flanking cda gene cluster | This study |
| S. roseosporus NRRL 15998-ΔactB6P6 | A derivative of S. roseosporus NRRL 15998 containing multicopy of act gene cluster | This study |
| S. roseosporus NRRL 15998-ΔdredB6P6 | A derivative of S. roseosporus NRRL 15998 containing multicopy of dred gene cluster | This study |
| S. roseosporus NRRL 15998-ΔcdaB6P6 | A derivative of S. roseosporus NRRL 15998 containing multicopy of cda gene cluster | This study |
| **Plasmids** | | |
| pUZ8002 | tra neo RP4 | 42 |
| pUC119::neo | pUC119 containing kanamycin resistance gene (neo) | 43 |
| pKC1139 | E. coli-Streptomyces shuttle plasmid contains a Streptomyces temperature-sensitive origin of replication | 15 |
| pJJ10500 | A derivative of pMS82 containing qBT1 integrase gene | 36 |
| pUC19-attB6-act | A derivative of pUC19:: neo containing attB6 and 2.0 kb homologous region flanking the 5’ end of act gene cluster | This study |
| pSV::attB6-act | A derivative of pUC19:: neo containing the origin of transfer (attI) from plasmid RK2, attB6 and 2.0 kb homologous region flanking the 5’ end of act gene cluster | This study |
| pSV::attB6-nap | A derivative of pUC19::neo containing the origin of transfer (attI) from plasmid RK2, attB6 and 2.0 kb homologous region flanking the 5’ end of nap gene cluster | This study |
| pSV::attB6-dap | A derivative of pUC19::neo containing the origin of transfer (attI) from plasmid RK2, attB6 and 2.0 kb homologous region flanking the 5’ end of dap gene cluster | This study |
| pKC1139::attP6-act | A derivative of pKC1139 containing attP6 and 2.0 kb homologous region flanking the 3’ end of act gene cluster | This study |
| pKC1139::attP6-nap | A derivative of pKC1139 containing attP6 and 2.0 kb homologous region flanking the 3’ end of nap gene cluster | This study |
| pKC1139::attP6-dap | A derivative of pKC1139 containing attP6 and 2.0 kb homologous region flanking the 3’ end of dap gene cluster | This study |
| pKC1139-act | A derivative of pKC1139 containing 25 kb fragment including actinorhodin gene cluster and its flanking sequences | This study |
| pKC1139-nap | A derivative of pKC1139 containing 45 kb fragment including napsamycin gene cluster and its flanking sequences | This study |
| pKC1139:dap | A derivative of pKC1139 containing 157 kb fragment including daptomycin gene cluster and its flanking sequences | This study |
Actinorhodin quantification. To quantitate actinorhodin production, S. coelicolor M145 and its derivatives were grown in 50 ml of R5 medium at 28 °C, 1 ml culture was harvested in a time-course and treated with KOH (1 N final concentration), and titer was calculated by measuring the absorbance at 640 nm.

Production and analysis of daptomycin. Small-scale fermentation of daptomycin was carried out by following the procedures described previously with minor modification. In brief, starter culture was grown in TSM (2% glucose, 1% D-glucose, 0.5% tryptone, 0.5% yeast extract, 0.1% [vol/vol] Tween 80 and 2% [vol/vol] MOPS, pH 7.0) and grown for 36 h as seed culture, and 1 ml of seed culture was transferred into a shake flask containing 50 ml of TSM at 150 rpm and 28 °C.

The cultures were incubated for different time points at 28 °C before fermentation broths were collected by centrifugation.

For daptomycin analysis, culture broths were centrifuged at 13,000 × g for 10 min to remove the mycelia. The supernatants were filtered through a Milipore membrane (pore diameter, 0.22 mm) and 50 μl of sample was used for HPLC analysis. Separation of daptomycin was achieved with an Agilent 1100 HPLC system and a ZORBAX SB-Aq column (5 μm pore size, 4.6 by 250 mm). HPLC conditions were described as follows: gradient elution with buffer A (0.01% [vol/vol] trifluoroacetic acid in acetone) and buffer B (0.01% [vol/vol] trifluoroacetic acid in diH2O), flow rate at 1.0 ml/min, temperature of 30 °C and detection at 214 nm. The elution profile was a linear gradient of 10%–100% buffer A over 22 min, a hold at 100% buffer A over 3 min, a linear gradient of 100%–10% buffer A over 2 min and a final hold at 10% buffer A over 3 min.

Bioassy against S. aureus was performed as previously described with modifications.

To insert apramycin to obtain double-cointegrate strain Sco-actB6P6. Similar procedures were used to reduce the antibiotic concentrations of apramycin and its derivatives were patched on AS-1 agar. After incubation for 2–5 days at 28 °C, agar plugs were prepared from the patches, placed on the surface of an empty Petri dish, and overlaid with culture of indicator strain in soft nutrient agar containing 5 mM CaCl2. The zone of inhibition was assessed after overnight incubation at 37 °C.
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Author contributions

D.D. and W.L. performed the experiments. T.Y. assisted with design of the project. N.G. conceived and designed the project, and wrote the manuscript. T.H. supervised the project and revised the manuscript.

Additional information

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