Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Development and optimization of an intergeneric conjugation system and analysis of promoter activity in *Streptomyces rimosus* M527*

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Received May 24, 2019; Revision accepted June 20, 2019; Crosschecked Sept. 12, 2019

Abstract: An efficient genetic transformation system and suitable promoters are essential prerequisites for gene expression studies and genetic engineering in streptomycetes. In this study, firstly, a genetic transformation system based on intergeneric conjugation was developed in *Streptomyces rimosus* M527, a bacterial strain which exhibits strong antagonistic activity against a broad range of plant-pathogenic fungi. Some experimental parameters involved in this procedure were optimized, including the conjugative media, ratio of donor to recipient, heat shock temperature, and incubation time of mixed culture. Under the optimal conditions, a maximal conjugation frequency of 3.05×10^{-5} per recipient was obtained. Subsequently, based on the above developed and optimized transformation system, the synthetic promoters SPL-21 and SPL-57, a native promoter *potrB*, and a constitutive promoter *permE** commonly used for gene expression in streptomycetes were selected and their activity was analyzed using *gusA* as a reporter gene in *S. rimosus* M527. Among the four tested promoters, SPL-21 exhibited the strongest expression activity and gave rise to a 2.2-fold increase in β-glucuronidase (GUS) activity compared with the control promoter *permE**. Promoter SPL-57 showed activity comparable to that of *permE**. Promoter *potrB*, which showed the lowest activity, showed a 50% decrease in GUS activity compared with the control *permE**. The transformation system developed in this study and the tested promotors provide a basis for the further modification of *S. rimosus* M527.

Key words: *Streptomyces rimosus* M527; Intergeneric conjugation; Promoter; β-Glucuronidase (GUS)
https://doi.org/10.1631/jzus.B1900270 **CLC number:** Q812

1 Introduction

Streptomycetes are soil-dwelling Gram-positive filamentous bacteria well known for their ability to produce several bioactive secondary metabolites, including therapeutic molecules such as antibiotic (Schlatter and Kinkel, 2015), antiparasitic (Cao et al., 2018), anticancer (Noomnual et al., 2016), and immunosuppressive agents (Yoo et al., 2017). Therefore, *Streptomyces* species have important commercial value in agriculture and medicine (Rey and Dumas, 2017; Kemung et al., 2018). Although *Streptomyces* attract a great deal of attention in genetic engineering and synthetic biology due to their outstanding ability to produce various secondary metabolites, the limited production and long fermentation period remain a bottleneck for their further application.

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* Project supported by the National Natural Science Foundation of China (Nos. 31772213 and 31972320) and the Excellent Youth Fund of Zhejiang Province, China (No. LR17C140002)
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A strain of antagonistic microorganism named *Streptomyces rimosus* M527 (China Center for Type Culture Collection (CCTCC) M2013270) has been isolated from the plant rhizosphere of wetlands. This strain has been shown to produce rimocidin, a 28-membered tetraene macrolide, comprising a large lactone ring with a sugar moiety. Rimocidin exhibits strong antifungal activity and is recognized as a promising fungicide that can be used to control plant-pathogenic fungi (Jeon et al., 2016). However, systems for genetic manipulation of *S. rimosus* M527, such as transformation of plasmids or gene over-expression, have not been developed. This situation hampers the genetic improvement of *S. rimosus* M527 using molecular biotechnological approaches. The development of an efficient genetic transformation method and expression system for this special strain is essential for enhancing the production of rimocidin.

Intergeneric conjugation between *Escherichia coli* and *Streptomyces*, has been proven to be a reliable and efficient transformation method (Du et al., 2012) and has been widely used as a means of transferring plasmids into *Streptomyces* strains (Zhou et al., 2012). However, there is still no universal protocol applicable to all *Streptomyces* strains.

Promoter elements are of indisputable importance in gene expression systems because they are responsible for efficient transcription, which is the first stage of gene expression (Myronovskyi and Luzhetskyy, 2016). In *Streptomyces*, only a small number of native promoters have been described (Luo et al., 2015; Li et al., 2018). Promoter engineering, which is a strategy for the design and construction of synthetic promoters (Seghezzi et al., 2011; Siegl et al., 2013; Sohoni et al., 2014; Ji et al., 2018) and engineered native promoters (Wang et al., 2013; Kakule et al., 2015; Yi et al., 2017), has been successfully employed to modulate and optimize gene expression in *Streptomyces*. However, the transcriptional activity of a promoter can change in different *Streptomyces* hosts.

In this study, firstly, a reliable intergeneric conjugation system for *S. rimosus* M527 was developed by optimization of experimental parameters including the conjugative medium, heat-shock temperature, ratio of donor to recipient cell number, and incubation time of the conjugation plates. Subsequently, based on the developed transformation system, plasmids harboring different native promoters (*permE** and *potrB*) and synthetic promoters (SPL-21 and SPL-57) were separately transferred into *S. rimosus* M527, and their activity was determined using *gusA* encoding β-glucuronidase (GUS) as a reporter gene.

2 Materials and methods

2.1 Strains, plasmids, and primers

The strains, plasmids, and primers used in this study are described and listed in Table 1. The rimocidin producer *S. rimosus* M527 has been deposited at the CCTCC (M2013270), Wuhan, China. *E. coli* JM109 was used as a general host for gene cloning and expression. *E. coli* ET12567 harboring plasmid pUZ8002 was used as the donor for intergeneric conjugation.

2.2 Media and culture conditions

E. coli strains were cultured in liquid or on solid Luria-Bertani (LB) medium (Wang et al., 2018) containing appropriate antibiotics at 37 °C. Apramycin (50 μg/mL), chloramphenicol (25 μg/mL), ampicillin (100 μg/mL), kanamycin (50 μg/mL), and nalidixic acid (50 μg/mL) were added as needed. *S. rimosus* M527 and its derivates were incubated according to the method described by Zhao et al. (2019). *S. rimosus* M527 was incubated at 28 °C and grown in solid mannitol soya flour (MS) medium (soya flour 20 g/L, mannitol 20 g/L, agar 20 g/L, tap water) for sporulation. MS, tryptic soy broth (TSB), and 2CMC solid media were used for conjugation. TSB medium contains tryptone 17 g/L, soytone 3 g/L, D-glucose 2.5 g/L, NaCl 5 g/L, and K_2HPO_4 2.5 g/L (adjusted to pH 7.5 by NaOH). 2CMC solid medium, which is optimized based on 2CM medium (Wang, 2007), contains starch 10 g/L, tryptone 2 g/L, NaCl 1 g/L, $(NH_4)_2SO_4$ 2 g/L, $MgSO_4·7H_2O$ 2 g/L, $CaCO_3$ 2 g/L, casamino acids 2 g/L, $K_2HPO_4·3H_2O$ 1 g/L, $FeSO_4·7H_2O$ 1 g/L, $MgCl_2·6H_2O$ 1 g/L, $ZnSO_4·7H_2O$ 1 g/L, and agar 20 g/L (adjusted to pH 7.2 by NaOH).

S. rimosus M527 spores ($1×10^6$ mL^{-1}) were inoculated into a 250-mL Erlenmeyer flask containing 50 mL seed medium, and shaken at 28 °C and 180 r/min. The CP liquid medium used as seed medium had the same composition as that described in an earlier study (Zhao et al., 2018). Five percent of the

Table 1 Strains, plasmids, and primers used in this study

Strain, plasmid, primer	Description	Source or reference
Strain		
E. coli JM109	General cloning host	Our lab
E. coli ET12567/pUZ8002	Cm^r, Km^r, donor strain for conjugation	Our lab
S. rimosus M527	Parental strain, rimocidin producer	CCTCC M2013270
M527-21S	M527 with integrative vector pGUS-SPL-21	This work
M527-57S	M527 with integrative vector pGUS-SPL-57	This work
M527-ES	M527 with integrative vector pGUS-ermE*	This work
M527-BS	M527 with integrative vector potrB-GUS	This work
Plasmid		
pGUS-SPL-21	*gusA* under the control of synthetic promoter SPL-21	Siegl et al., 2013
pGUS-SPL-57	*gusA* under the control of synthetic promoter SPL-57	Siegl et al., 2013
pGUS-ermE*	*gusA* under the control of promoter *permE**	Siegl et al., 2013
potrB-GUS	*gusA* under the control of promoter *potrB*	This work
Primer		
P1	5'-ACGTCTAGAAGGCGGCCGTTGACCGCGAA-3' (*Xba*I)	This work
P2	5'-ACGGGTACCGGCCGTCAGGATCTGCCGAT-3' (*Kpn*I)	This work

Primers P1 and P2 were used for amplification of *otrB* promoter. *Xba*I and *Kpn*I restriction enzyme sites are underlined

seed culture was inoculated into 50 mL fermentation medium containing 1 g soya flour, 1 g D-mannitol, and 0.03 g $K_2HPO_4{\cdot}3H_2O$.

2.3 Construction of plasmids

Vectors pGUS-SPL-21, pGUS-SPL-57 and pGUS-ermE* were gifts from Prof. LUZHETSKYY (Siegl et al., 2013). The 300-bp *otrB* promoter fragment (*potrB*) (nucleotides −300 to −1 upstream from gene *otrB* translational start codon) was amplified from genomic DNA of *S. rimosus* M527 using primers P1 and P2 (Table 1). The PCR amplification procedure was performed as described by Xu et al. (2017). The synthetic promoter SPL-57 in vector pGUS-SPL-57 was removed by digesting pGUS-SPL-57 with *Xba*I/*Kpn*I, and was replaced by *Xba*I/*Kpn*I *potrB* to create the plasmid potrB-GUS. Sequencing of the inserted fragment containing *potrB* confirmed that the gene contained no mutations.

2.4 Intergeneric conjugation procedure and analysis of exconjugants

Intergeneric conjugation between *E. coli* and *Streptomyces* was conducted as described by Phornphisutthimas et al. (2010) and Ma et al. (2014) with minor modifications. *S. rimosus* M527 spores (1×10^8 mL^{-1}) were collected from solid medium, filtered by sterile absorbent cotton to remove mycelia and then incubated at different test temperatures for 10 min to induce germination. A culture of the spores was cultured in 2× yeast extract tryptone (YT) broth (Ma et al., 2014) at 28 °C for 2 h. Then the spores, serving as recipients, were washed twice with an equal volume of LB broth and resuspended in 500 μL fresh LB medium. The donor strain, *E. coli* ET12567/pUZ8002 harboring the constructed plasmids, was grown in LB with appropriate antibiotics until an optical density at 600 nm (OD_{600}) of 0.4–0.6 was reached. The cells were washed twice with LB and resuspended in a final volume of 500 μL of LB. *E. coli* donor cells and the recipients were mixed and spread on 2CMC, MS, and TSB agar plates containing 10 mmol/L $MgCl_2$. The plates were incubated at 28 °C for 10–20 h and overlaid with 500 μL fresh LB medium containing 100 μg/mL nalidixic acid and 300 μg/mL apramycin. The plates were further incubated at 28 °C for 3–4 d, and the exconjugants were then counted. For conjugation with mycelia, *S. rimosus* M527 was incubated in CP liquid medium for 48 h at 28 °C. Mycelia were collected and mixed with donor cells, and spread on the agar plates.

All the putative exconjugants were purified on solid 2CMC plates supplemented with 300 μg/mL apramycin to confirm the integration of the target plasmids. After transferring into *S. rimosus* M527, a constructed plasmid can be maintained only in a chromosomally integrated state. Total exconjugant DNA was isolated according to the methods

described by Kieser et al. (2000). The existence of the apramycin resistance (*apr*) gene from the constructed plasmids in the chromosome of exconjugants was verified by PCR amplification. The average value of the conjugation frequency of three independent experiments was calculated. Each conjugation experiment was carried out three times.

2.5 GUS assay

GUS activity was measured according to a method described previously (Myronovskyi et al., 2011; Siegl et al., 2013; Xu et al., 2017). Spores of recombinant strains were inoculated into 100 mL of TSB liquid medium and grown in shaking flasks for 48 h at 28 °C. A 40-mL sample was collected into an Eppendorf (EP) tube and dried at 60 °C. Another 40-mL sample was used for the GUS assay. The culture was centrifuged, and the deposited cells were collected and resuspended in 20 mL of GUS buffer 2 (50 mmol/L phosphate-buffered saline (PBS; pH 7.0), 5 mmol/L dithiothreitol (DTT), 0.1% Triton X-100, 1 mg/mL lysozyme). After incubating at 37 °C for 30 min, the lysates were subsequently diluted with GUS buffer 1 (50 mmol/L PBS (pH 7.0), 5 mmol/L DTT, 0.1% Triton X-100) to 45 mL and centrifuged at 11000 r/min at 4 °C for 10 min. A 500-μL sample of the supernatant was mixed with 500 μL of GUS buffer 3 (50 mmol/L PBS (pH 7.0), 5 mmol/L DTT, 0.1% Triton X-100, supplemented with 2 mmol/L *p*-nitrophenyl-β-D-glucuronide), and the absorption was spectrophotometrically measured at 415 nm for 30 min (UV-1800, Shimadzu, Japan). As a control, 500 μL of GUS buffer 1 was mixed with 500 μL of the sample.

Specific enzyme activity is indicated as unit per gram of protein. The following equation was used to calculate the enzymatic activity (EA): $EA=2V\times\Delta A_{415}/14\times DCW$, where V equals the total end volume of the sample (45 mL), ΔA_{415} equals the absorbance which was spectrophotometrically measured at 415 nm per minute, and DCW equals the dry weight in grams of the original sample size (40 mL). All experiments were performed in triplicate, and the reported values are the average from three assays with their calculated standard deviations (SDs).

2.6 Statistical analysis

All experiments were carried out at least three times, and the results were expressed as mean± SD. Statistical analysis was performed using Student's *t*-test (Zhou et al., 2017).

3 Results

3.1 Development and optimization of intergeneric conjugation transformation for *S. rimosus* M527

It has been reported that both mycelia and spores of streptomycetes can be used as recipients in intergeneric conjugation (Phornphisutthimas et al., 2010). To determine the appropriate status of the recipient for transformation efficiency, mycelia and spores of *S. rimosus* M527 were assessed. When mycelia were used as the recipient in conjugation, almost no exconjugants appeared when using MS or TSB as the conjugation medium. Exconjugants were obtained only when 2CMC medium was used, and the conjugation efficiency was about 1×10^{-8}–1×10^{-7}. After using spores as the recipient, exconjugants were generated at a frequency of 1×10^{-7}–1×10^{-5}, an almost 100-fold improvement compared with using mycelia as the recipient. Our results confirmed that both spores and mycelia of *S. rimosus* M527 could be used as recipient, but the conjugation efficiency achieved with spores was much higher than that obtained with mycelia.

The selection of an appropriate conjugation medium has an obvious influence on the conjugation efficiency in streptomycetes (Du et al., 2012; Sun et al., 2014), so three applicable solid media, MS, TSB, and 2CMC, were tested to determine the appropriate medium. The 2CMC medium proved to be optimal for the intergeneric conjugation of *E. coli*-*S. rimosus* M527, whether mycelia or spores were chosen as recipient (Table 2). Therefore, 2CMC was used as the conjugation medium in follow-up experiments.

It has been reported that an appropriate ratio between the number of donor cells and recipient spores is an important parameter in the case of *Streptomyces* species (Enríquez et al., 2006; Ma et al., 2014). In this study, 1×10^{8} mL^{-1} *E. coli* ET12567 as donor cells and a range of *S. rimosus* M527 spores (1×10^{6}–1×10^{9} mL^{-1}) as recipients were tested. When the number of the spores reached 1×10^{7} mL^{-1}, the conjugation efficiency reached the highest value of 1.67×10^{-5} (Table 2), indicating that maximum efficiency was achieved with a donor-to-recipient ratio of 10:1.

Table 2 Effects of conjugation media and the number of recipient spores on the efficiency of intergeneric conjugation

Conjugation medium	Number of recipient spores (mL^{-1})[b]	Number of exconjugants	Conjugation frequency[c]
MS	1×10^9	330	3.30×10^{-7}
	1×10^8	168	1.68×10^{-6}
	1×10^7	64	6.40×10^{-6}
	1×10^6	5	5.00×10^{-6}
TSB	1×10^9	264	2.64×10^{-7}
	1×10^8	106	1.06×10^{-6}
	1×10^7	28	2.80×10^{-6}
	1×10^6		
2CMC[a]	1×10^9	583	5.83×10^{-7}
	1×10^8	397	3.97×10^{-6}
	1×10^7	167	1.67×10^{-5}
	1×10^6	13	1.30×10^{-5}

[a] Different kinds of medium were tested to determine the appropriate medium. 2CMC medium was proven to be optimal for the conjugation of *E. coli*-*S. rimosus* M527. [b] 1×10^8 mL^{-1} *E. coli* ET12567 donor cells were used, and a range of *S. rimosus* M527 spores (1×10^6–1×10^9) were tested as recipients. [c] Values are presented as the number of conjugations per colony forming units of the recipient, and each value represents the average efficiency from three independent experiments

Table 3 Effects of heat-shock temperature on transformation frequency

Heat-shock temperature (°C)	Average transformation frequency
35	4.89×10^{-6}
40	7.66×10^{-6}
45	1.08×10^{-5}
50	2.23×10^{-5}
55	1.67×10^{-5}
60	8.39×10^{-6}

S. rimosus M527 spores (1×10^7 mL^{-1}) were collected and heat-treated at various temperatures for 10 min to induce germination

Table 4 Effect of different incubation time before the plates were flooded with antibiotics on transformation frequency

Incubation time (h)	Average transformation frequency
0–6	2.74×10^{-6}
6–12	7.07×10^{-6}
12–18	3.05×10^{-5}
18–24	1.16×10^{-5}
24–30	5.13×10^{-6}

S. rimosus M527 spores (1×10^7 mL^{-1}) in mixed culture with the donor cells *E. coli* ET12567 (1×10^8 mL^{-1}) were incubated for various lengths of time at 28 °C before the plates were flooded with fresh LB medium containing antibiotics

Heat-shock treatment of *Streptomyces* spores has generally been carried out prior to mixing with donor *E. coli* (Sun et al., 2014; Zhang et al., 2018). To determine the optimal temperature for heat treatment and incubation time of heat-shocked spores, the effects of temperature and different incubation time on conjugation efficiency were investigated. In this study, high conjugation efficiencies were achieved when spores were treated at 45–55 °C for 10 min (Table 3), and the efficiencies rapidly decreased when spores were treated above 55 °C or below 45 °C.

In addition, before the plates are flooded with fresh LB medium containing antibiotics, the incubation time of the mixed culture of *Streptomyces* and *E. coli* plays an important role in conjugation efficiency (Zhang et al., 2018). If the time of mixed culture is too short, *Streptomyces* mycelia grow weakly and are easily scraped off, leading to a lower conjugation efficiency. Conversely, the longer the incubation time of mixed culture, the greater the possibility of false positives, which hinders the selection of the positive exconjugants. Our results demonstrated that the optimal incubation time of mixed culture plates was 12–18 h (Table 4).

The optimal conjugation conditions were determined: pre-incubated *S. rimosus* M527 spores were selected as recipients, the appropriate donor-to-recipient ratio was 10:1, and spores were heat-treated at 50 °C for 10 min prior to mixing with donor *E. coli* ET12567/pUZ8002. After incubating at 28 °C for 12–18 h, the plates were overlaid with 500 μL fresh LB medium containing nalidixic acid and apramycin (100 and 300 μg/mL, respectively). Incubation was continued at 28 °C until exconjugant cells appeared, and the highest efficiency of conjugation was 3.05×10^{-5} when 2CMC medium was used as the conjugation medium.

3.2 Comparison of promoter activity in *S. rimosus* M527 using GUS as reporter

To test the transcriptional activity of four promoters (synthetic promoters SPL-21 and SPL-57, native promoter *potrB*, and constitutive promoter *permE**), gene *gusA* was used as a reporter gene and was placed under the control of the tested promoters. The construction of plasmid potrB-GUS was performed as follows: the 300-bp *otrB* promoter fragment was amplified from genomic DNA of *S. rimosus* M527 and replaced the synthetic promoter SPL-57 in vector pGUS-SPL-57 to create plasmid potrB-GUS

(Fig. 1). Plasmids potrB-GUS, pGUS-SPL-21, pGUS-SPL-57, and pGUS-ermE* were separately introduced into *E. coli* ET12567/pUZ8002 and then transferred into *S. rimosus* M527 using the optimized intergeneric conjugation conditions described above to generate exconjugants M527-BS, M527-21S, M527-57S, and M527-ES, respectively. GUS activity was visible in all resulting recombinant strains and compared with that of the wild-type strain *S. rimosus* M527 (Fig. 2). Strains M527-21S and M527-BS exhibited darker and lighter blue color than the other strains, respectively. No significant difference in the intensity of blue color was detectable in the recombinant strain M527-57S or M527-ES.

The GUS enzymatic assay, which precisely reflected the promoter strength, was performed to determine the expression of these four promoters in detail. In consideration of variation among different exconjugants, three independent exconjugants were selected for screening in the GUS assay. Promoter SPL-21 exhibited the strongest expression activity among the four tested promoters (a 2.2-fold increase in GUS activity compared with *permE**) and promoter *potrB* the weakest (a 50% decrease in GUS activity compared with *permE**). Promoter SPL-57 showed the activity comparable to that of *permE** (Fig. 3). The data were in accordance with aforementioned intuitive observations.

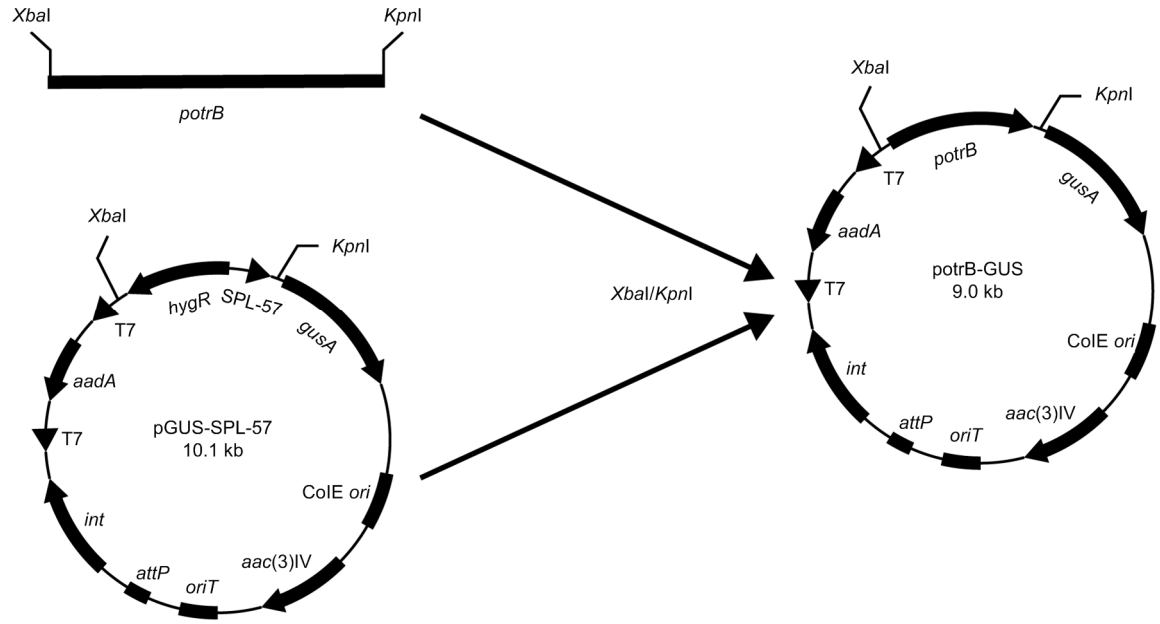

Fig. 1 A map of plasmid potrB-GUS constructed in this study

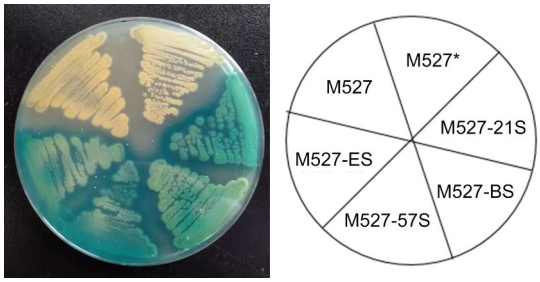

Fig. 2 Visual observation of GUS activity in solid medium of wild-type strain *S. rimosus* M527 and different recombinant strains

Colonies of wild-type strain M527, vector control strain M527* (containing the empty plasmid pSET152), and recombinant strains (M527-ES, M527-BS, M527-21S, M527-57S) containing different promoters were covered with X-Gluc (1 mmol/L) on 2CMC medium and incubated at 28 °C for 3–4 d. Blue halos are 5,5'-dibromo-4,4'-dichloro-indigo, formed by β-glucuronidase (GUS) activity

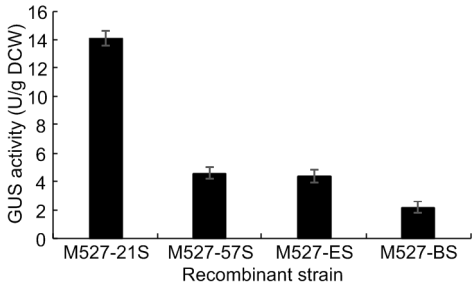

Fig. 3 Detection of GUS activity in different recombinant strains

β-Glucuronidase (GUS) was used as a reporter gene for examining protein synthesis. GUS activity was measured in cell lysates of strains M527-21S, M527-57S, M527-ES, and M527-BS from fermentation broth. Data are expressed as mean±SD

4 Discussion

There are three main genetic transformation methods for *Streptomyces*: polyethylene glycol (PEG)-mediated protoplast transformation, electroporation, and intergeneric conjugation. To genetically engineer *S. rimosus* M527, the most suitable transformation system needs to be identified. In this study, the three transformation methods were performed to introduce plasmids into *S. rimosus* M527. The results showed that the transformation efficiency obtained from intergeneric conjugation was significantly higher than those from the other two methods (data not shown).

Intergeneric conjugation between *E. coli* cells and *Streptomyces* spores was initially reported by Mazodier et al. (1989). The conjugation takes advantage of the donor *E. coli* ET12567 host with a transfer function (*tra* gene in pUZ8002), which allows for mobilization of a plasmid harboring an origin of transfer (*oriT*). Conjugation is advantageous over other *Streptomyces* transformation protocols for two reasons: (1) it can readily transform large plasmids with an *oriT* into a *Streptomyces* recipient; and (2) many *oriT*-harboring plasmids allow direct integration into a chromosomal specific attachment site (*attB*) (e.g., via *int-attP* on pSET152).

Intergeneric conjugation allows the transfer of plasmids manipulated in *E. coli* into *Streptomyces* spores. Thus, higher conjugation efficiencies have been reported in several *Streptomyces* when spores rather than mycelia were used. Mycelia are the vegetative and propagative forms of actinobacteria. Recently, mycelia have shown potency in intergeneric conjugation. Some research results concerning intergeneric conjugation using mycelia as recipient have been reported for several *Streptomyces* (Du et al., 2012; Rocha et al., 2018; Zhang et al., 2018). The conjugation conditions were highly strain-specific and usually ineffective for different actinobacteria. Due to interspecific variation, appropriate conditions for intergeneric conjugation have to be developed and optimized for each particular strain (Sun et al., 2014; Wang and Jin, 2014; Zhang et al., 2018).

In this study, both the mycelia and spores of *S. rimosus* M527 were chosen as recipients for establishment of intergeneric conjugation. The results showed that conjugation efficiencies achieved with spores were higher than those achieved with mycelia. From the point of morphology, spores are more suitable for the introduction or acceptance of plasmid DNA than mycelia. In particular, plasmid DNA can be effectively transferred from the donor to the recipient through the germ tube when the spores are in a germination state after heat-shock treatment, leading to high conjugation efficiency.

MS medium is usually used for sporulation and conjugation. Unfortunately, using the mycelia as the recipient for conjugation failed despite considerable effort. On TSB medium *S. rimosus* M527 grows well but does not produce spores. This hampers subsequent conjugation. Whether spores or mycelia were used as the recipient, the conjugation efficiency was lower on TSB medium than on the other two media. In this study, 2CMC medium was shown to be optimal for the intergeneric conjugation of *E. coli*-*S. rimosus* M527, whether mycelia or spores were chosen as recipient. The components of 2CMC medium may be more effective for conjugation. Some other experimental parameters involved in conjugation were further optimized. Under optimal conditions, a maximal conjugation frequency of 3.05×10^{-5} per recipient was obtained.

The conjugation transfer system developed in this study provides a basis for the introduction of genes into *S. rimosus* M527. However, the promoter is a crucial element for gene expression. Selection of a suitable promoter for gene expression is also important for further improving rimocidin production in *S. rimosus* M527 using genetic engineering technology. Promoter *permE** is widely used for heterologous expression of genes in streptomycetes. Ji et al. (2018) reported that the native promoter *potrB* was stronger than *permE** in *S. rimosus*. Also, two artificial synthetic promoters, SPL-21 and SPL-57, were shown to be much stronger than *permE** in different *Streptomyces*. In *Streptomyces diastatochromogenes* 1628, SPL-57 showed activity comparable to that of *permE**. In contrast, promoter SPL-21 showed a 5.2-fold increase in GUS activity compared with *permE** (Xu et al., 2017). One promoter may show different transcriptional activity due to differences in the genetic background of the host strain. In this study, two synthetic promoters, SPL-21 and SPL-57, a native promoter *potrB*, and a constitutive promoter *permE** were compared and analyzed for GUS activity. Similar results were obtained from *S. diastatochromogenes*

1628, in which the promoter SPL-21 showed the strongest activity among the tested promoters, and SPL-57 showed activity comparable to that of *permE**. Surprisingly, the promoter *potrB* showed the lowest activity compared with that of promoter *permE**, which is not consistent with the results reported by Ji et al. (2018). This inconsistency may have been caused by the different backgrounds or regulatory mechanisms of the host strains.

Note that the constructed plasmids used for detection of GUS activity of promoters were derived from the pSET152 backbone. The effects of the different plasmids on GUS activity driven by the tested promoters would have been excluded. In addition, the whole genome was sequenced. The results of scanning the genome sequence revealed that *S. rimosus* M527 contained a single copy of pSET152 integrated at a unique chromosomal attachment site (*attB*). The copy number of plasmid integration had no effect on the *gusA* expression driven by the tested promoters.

5 Conclusions

In conclusion, in this study a genetic transfer system based on intergeneric conjugation was developed and optimized for *S. rimosus* M527. The activity of four promoters using *gusA* as reporter gene was determined and analyzed. These four promoters exhibited different expression activity and may be classified as strong, medium, and weak, which will facilitate fine-tuning of the expression of different genes in *S. rimosus* M527. This work will provide a basis for further modification of *S. rimosus* M527.

Contributors

Zhang-qing SONG, Zhi-jun LIAO, and Ye-feng HU participated in the design. Zheng MA and Zhang-qing SONG wrote this article. Andreas BECHTHOLD revised the manuscript. Zheng MA and Xiao-ping YU checked the final version. All authors approved the final manuscript and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Zhang-qing SONG, Zhi-jun LIAO, Ye-feng HU, Zheng MA, Andreas BECHTHOLD, and Xiao-ping YU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：龟裂链霉菌 M527 接合转移体系的建立和优化以及启动子的活性分析

目　的：建立并优化适用于龟裂链霉菌（*Streptomyces rimosus*）M527 的属间接合转移体系，并在此基础上分析四个启动子的表达活性。

创新点：有效的遗传转化系统和合适的启动子是链霉菌基因表达和基因工程的必要前提。由于链霉菌的遗传背景复杂，接合转移实验参数对菌种具有高度特异性。因此，本研究建立并优化了一套适用于 *S. rimosus* M527 的属间接合转移体系，并在此基础上分析比较了四个启动子的表达活性。这将为进一步遗传修饰 *S. rimosus* M527 奠定基础。

方　法：以 *S. rimosus* M527 为研究对象，通过对接合转移体系中的一些重要的实验参数（如接合转移培养基、供受体比例、热激温度和混合培养时长等）进行了优化。在此基础上，构建以 *gusA* 为报告基因的质粒，通过直观显色反应以及 GUS 酶活的定量检测，分析比较了四个启动子的表达活性。

结　论：建立了一种有效的适用于 *S. rimosus* M527 接合转移体系，优化后的接合效率最高达 3.05×10^{-5}。分析测试的四个启动子中，合成启动子 SPL-21 表现出最高表达活性，比常用强组成型启动子 *permE**活性高出 2.2 倍，合成启动子 SPL-57 与 *permE**的活性无显著差异，内源启动子 *potrB* 的活性比 *permE**降低了 50%。

关键词：龟裂链霉菌 M527；接合转移；启动子；GUS