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ORIGINAL PAPER

Improving conjugation efficacy of *Sorangium cellulosum* by the addition of dual selection antibiotics

Zhi-Jie Xia · Jing Wang · Wei Hu · Hong Liu · Xiu-Zhen Gao · Zhi-Hong Wu · Peng-Yi Zhang · Yue-Zhong Li

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Abstract The conjugation protocols in myxobacterium *Sorangium cellulosum* are often inapplicable due to the strain-specific sensitivity to the presence of *Escherichia coli* cells or the resistances to many antibiotics. Here we report that the conjugative transfer of the mobilizable plasmid pCVD442 from *E. coli* DH5α (*λ pir*) to *Sorangium* strains could be greatly increased by the presence of low doses of dual selection antibiotics in the mating medium. The improvement was efficient in either *E. coli*-tolerant or sensitive *Sorangium* strains. For those phleomycin and hygromycin tolerant *Sorangium* strains, chloramphenicol-resistance gene was developed as a new selectable marker by driving the resistance gene with the *aph*II promoter. Using the improved protocol, the epothilone biosynthetic pathway was inactivated by an insertion mutation in the biosynthetic genes of the producing *Sorangium* strains.

Keywords Conjugation · *Sorangium cellulosum* · Antibiotics

Introduction

Myxobacteria are Gram-negative gliding bacteria that have complicated social lives [1] and an excellent ability to produce various bioactive compounds [2]. As the complete genome sequences of *Myxococcus xanthus* DK1622 and *S. cellulosum* So ce56 are available [3, 4], more functional loci, such as the biosynthetic genes for diversified secondary metabolites have been found. The ability to make mutations in myxobacteria is deeply required, especially to identify genes responsible for the biosynthesis of secondary metabolites. Transduction [5] and electroporation [6] have been well applied in the model species *M. xanthus*, while genetic transfer is usually difficult to achieve or low-efficient in other myxobacterial taxa and even different *Myxococcus* species or strains. For instance, in *Sorangium*, the producer of almost half of the discovered myxobacterial metabolites [7], there are many barriers limiting genetic transfers [8–11]. The sorangial cells grow slowly, tolerate many antibiotics, produce abundant extracellular polysaccharides, and tend to aggregate [12]. These characteristics make the DNA transfer manipulation more discommodious in *Sorangium*.

The major genetic tool in the genus *Sorangium* is conjugation, which was first reported by Jaoua et al. in 1992 [9]. It works in some *Sorangium* strains, such as *S. cellulosum* So ce26 and *S. cellulosum* So ce90 [13, 14]. For example, Pradella et al. disrupted the biosynthetic pathway of chivosazole in *S. cellulosum* So ce56 using the established protocol with hygromycin as the selection antibiotic [15]. Julien et al. [8] further developed a *mariner*-based transposon for DNA conjugative transfers in *S. cellulosum* So ce90, and obtained many mutants. However, the conjugative transfer of pMycoMarHyg did not produce any mutants in *S. cellulosum* So ce12, which was attributed to the sensitivity of So ce12 cells to the presence of living *E. coli* cells during the long mating time [10]. Kopp et al. suggested two alternative methods to circumvent the problem, using a markedly unequal ratio of sensitive *Sorangium* cells and *E. coli* cells (1×10^9 vs. 1×10^3 cells mL^{-1}), or liquid conjugation with a reduced incubation time (from 24 to

Z.-J. Xia and J. Wang contributes equally to this work.

Z.-J. Xia · J. Wang · W. Hu · H. Liu · X.-Z. Gao · Z.-H. Wu · P.-Y. Zhang · Y.-Z. Li (✉)
State Key Laboratory of Microbial Technology,
College of Life Science, Shandong University,
250100 Jinan, People's Republic of China
e-mail: lilab@sdu.edu.cn

6 h). However, this suggestion led to reduced efficiencies because of low number of donor cells or insufficient mating time. Thus, the protocols for genetic manipulation in *Sorangium* strains have not yet been adequately developed. The genetic methods established in one *Sorangium* strain probably cannot be applied to others, although they are close related in phylogeny [16].

In our genetic experiments with *Sorangium* strains, e.g., two epothilone producers So0157-2 and So02007-3 [17], the reported conjugation protocols were impractical, although the two strains are either tolerant or sensitive to the presence of *E. coli* cells. In this paper, the method of conjugation was improved by adding low concentrations of dual selection antibiotics in the mating media. Besides, chloramphenicol-resistance gene was developed as a new selectable marker for the phleomycin and hygromycin tolerant strains. Using the improved conjugation method, the biosynthesis of epothilones in the producing *Sorangium* strains was disrupted by inserting a DNA fragment in the biosynthetic gene cluster, leading to disappearance of the epothilone products.

Materials and methods

Strains and culture conditions

The strains and plasmids used in this study are listed in Table 1. The *Sorangium* strains were cultured at 30 °C in liquid M26 medium as previously described [18] and on VY/2 agar [12]. If required, the media were supplemented with 15 μg mL^{-1} gentamicin or 10 μg mL^{-1} chloramphenicol. *E. coli* cells were grown at 37 °C in LB medium supplemented, when required, with 30 μg mL^{-1} kanamycin, 50 μg mL^{-1} ampicillin, or 30 μg mL^{-1} chloramphenicol.

Construction of plasmids

Standard techniques were used for genetic manipulation. A 371-bp *aph*II promoter element and a 682-bp chloramphenicol resistant (*cat*) gene were PCR amplified from pSUP2021 separately with primers 5′-GAGATCTAGAG CTTCACGCTGC-3′ and 5′-CAATCATATGAAACGAT CCTCATCC-3′, and primers 5′-GCTCATATGGAGAAA AAAATC-3′ and 5′-AACTGTCTAGAAAAAATTACG C-3′. The two products were digested with *Nde*I and cloned into the pGEM-T Easy vector (Promega, USA) together. The ligation mixture was transferred into the competent cells of *E. coli* GM48, resulting in the plasmid pT19. The *aph*II promoter-*cat* gene cassette from pT19 was subcloned into pCVD442 digested with *Xba*I, which was used to transform *E. coli* DH5α (λ *pir*). The resulting plasmid was designated pCC11 (Fig. 1). Two fragments of the epothilone synthase gene (0.7 and 3.2 kb, Table 1) were PCR separately amplified from the genome of *S. cellulosum* strain So0157-2 with the primers 5′-TGACACCTGGC TGTGGAC-3′ and 5′-GGCAGCCAATGCCTACGATG-3′, and 5′-CGATCGTACGGATTGTTCGGGCTGC-3′ and

Table 1 Strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
Sorangium		
So0157-2	Ap^r, Km^r, Gm^r, Sm^r, Hm^r, Pm^r, Cm^s	[17]
So02007-3	Ap^r, Km^r, Gm^r, Sm^r, Hm^r, Pm^r, Cm^s	[17]
E. coli		
DH5α (λ *pir*)		Gift from H. Kaplan
GM48	*dam-*, *dcm-*	Gift from M. Marinus
Plasmid		
pRK2013	Km^r	[26]
pCVD442	Ap^r, *R6K ori, mobRP4, sacB*	[27]
pSUP2021	pBR325::Tn5; mobilizible	[28]
pT19	pGEM-T Easy vector with *aph*II and *cat*	Present work
pCC11	pCVD442 inserted with *aph*II and *cat*	Present work
pCCK700	pCC11 inserted with a 0.7 kb fragment of *epoF* from So0157-2	Present work
pCDM2	pCC11 inserted with a 1.5 kb fragment of the *epo* gene cluster from So0157-2	Present work
pCCMT61	pCC11 inserted with a 3.2 kb fragment of the *epo* gene cluster from So0157-2	Present work

The lethal concentration of living *E. coli* cells in a 40 h mating with 1×10^{10} *Sorangium* cells mL^{-1} is about 1×10^9 cells mL^{-1} for So0157-2 and less than 1×10^6 cells mL^{-1} for So02007-3

The similarity of 16s rRNA sequence between So0157-2 and So02007-3 was 99%

Ap ampicillin, *Km* kanamycin, *Gm* gentamicin, *Sm* streptomycin, *Hm* hygromycin, *Pm* phleomycin, *Cm* chloramphenicol, *Tet* tetracycline

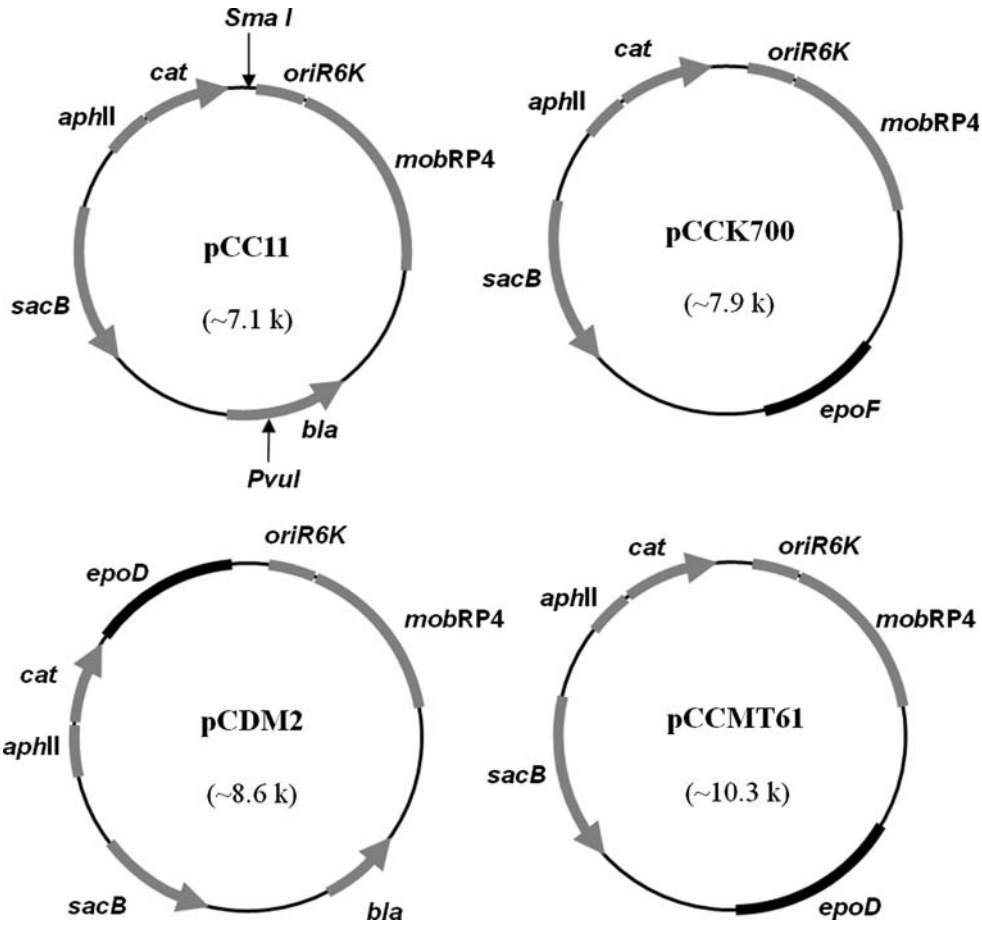

Fig. 1 Map of the plasmids pCC11, pCCK700, pCDM2, and pCCMT61. The *arrowheads* in pCC11 indicate the *Sma*I and *Pvu*I sites to insertion of epothilone biosynthetic gene fragments, and the resulting plasmids were termed pCCK700 (0.7-kb insert at *Pvu*I), pCDM2 (1.5-kb insert at *Sma*I), and pCCMT61 (3.2-kb insert at *Pvu*I). *mob*RP4 RP4-specific mobilization site, *bla* ampicillin-resistance gene, *sacB* sucrose-sensitive gene of Gram-negative bacteria, *epoD* and *epoF* homologous fragments of *Sorangium* strains

5′-CGATCGCAGACATGGGGGCCTCTAC-3′. After verification by DNA sequencing, the fragments were cloned into the *Pvu*I site of plasmid pCC11, resulted in plasmids pCCK700 and pCCMT61. The above 3.2 kb fragment was further digested using *Sma*I and the 1.5 kb product was cloned into the *Sma*I site of pCC11, resulting in the plasmid pCDM2 (Fig. 1; Table 1).

Matings

The *epo*-fragment-containing plasmids were introduced into the pRK2013-containing *E. coli* strain DH5α (*λ pir*), respectively. Normal conjugation was performed according to the methods reported [9, 10, 15]. The improved conjugation protocol proceeded as follows. The plasmid-containing *E. coli* cells were cultured to late log-phase in LB containing kanamycin and chloramphenicol. The cells were harvested and re-suspended in M26 to attain a cell density of 2×10^8 cells mL^{-1}. The *Sorangium* strains were cultured in M26 for 5 days. The harvested cells were re-suspended in M26 to attain a density of 1×10^{10} cells mL^{-1}. 150 µl of each suspension was mixed, spotted onto a 0.45-µm pore-size membrane spread on a VY/2 plate containing 7 µg mL^{-1} gentamicin and 5 µg mL^{-1} chloramphenicol and incubated at 30 °C for 40 h. The cells were then scraped from the membrane, suspended in 1 mL liquid M26 medium, and dispersed using a small tissue grinder. Before spreading onto the VY/2 selection plate containing gentamicin and chloramphenicol, the suspension was diluted by 10 and 100 times. An aliquot of 100 µl suspension was inoculated per plate. After 8–12 days of incubation at 30 °C, the colonies were counted and transferred to fresh selection plates. *Sorangium* cells without *E. coli* were spotted onto the mating and selection plates as the blank control.

Southern hybridization

Genomic DNAs from *Sorangium cellulosum* So0157-2 and transconjugants were hydrolyzed with *PstI* and subjected to agarose gel electrophoresis. Separated DNA was transferred onto Hybond™ N+ membranes (Amersham Biosciences) by capillary blotting. Hybridization probe was generated by isolation of a 1.2 kb methyltransferase gene fragment from the 3.2 kb *epo* gene (Table 1). The DIG DNA Labeling and Detection Kit (Roche) was used to label DNA fragments and for hybridization experiments according to the manufacturer's instructions. The hybridization

temperature was 46 °C in a formamide buffer. Stringent washes took place at 68 °C.

Analysis of epothilone production

The *Sorangium* strains were inoculated on M26 agar [19] and incubated at 30 °C for 4–5 days. Then several beads of Amberlite XAD-16 resin (Rohm and Haas) were added on the colony (about 1 ml resin per plate containing 20 ml medium), and the cultures were incubated for additional 9–10 days. The resin from ten plates (about 200 ml culture) of each strain was harvested, washed with distilled water, air-dried, and extracted with 50 ml of methanol. The extracts were then dried in vacuo at 40 °C and stored at −20 °C. For HPLC analysis, the samples were redissolved in 300 µl methanol.

The detection of epothilones was performed on a Surveyor HPLC (Thermo Finnigan, USA) using our previously described method [18]. Eluted with 60% methanol and 40% buffer, the peaks of epothilone A appeared at 13.5 min and B at 16.3 min with baseline resolution. The production was qualitatively determined using authentic epothilone A and B.

Results and discussion

Construction of plasmids for conjugation in *Sorangium*

Sorangium cells can tolerate many antibiotics and the resistance is often strain-specific. Before this work, the mostly used selection antibiotics in *Sorangium* conjugation protocols were phleomycin and hygromycin [9, 15]. However, the two strains, So0157-2 and So02007-3 used in this work tolerated phleomycin and hygromycin, and the minimum inhibitory concentrations (MICs) were higher than 20 and 100 µg mL^{-1}, respectively. To select a competent selectable marker, a screening was performed on different *Sorangium* strains, and chloramphenicol was one of the few antibiotics to which all of the screened *Sorangium* strains were sensitive (MIC is less than 5 µg mL^{-1}). However, the chloramphenicol-resistance gene in pSUP2021 did not express in *Sorangium* cells, which is probably due to the identification of its promoter sequence in host cells. The Tn*5* *aph*II promoter element was reported to be recognized by a wide variety of *M. xanthus* and *S. cellulosum* strains [15, 20]. And the element was constructed into the plasmids to drive the chloramphenicol-resistance gene (Fig. 1), based on the method described by Pradella et al. [15]. The constructed mobilizable plasmids (Fig. 1) were used for DNA conjugation transfer from DH5 α (λ *pir*) to *S. cellulosum* strains with the help of pRK2013. The inserted homologous fragments were 0.7, 1.5, and 3.2-kb in size, and the conjugation efficiency of different lengths was compared. Plasmids are unable to replicate in *Sorangium* cells, and expression of the chloramphenicol-resistance gene was achieved only after the plasmids were site-specifically integrated into the chromosome by homologous recombination.

Conjugation between *S. cellulosum* So0157-2 and *E. coli*

S. cellulosum So0157-2 was able to produce epothilones [17, 18, 21, 22]. We had tried to make genetic engineering on the strain using the previously published protocols [9, 10, 15], but it was difficult to obtain a transconjugant. Each step in the conjugation process was adjusted such as changing the mating medium, the ratio of donor and recipient cells, the mating time, and the selection medium, but the efforts were in vain. So0157-2 strain was rather tolerant to the presence of living *E. coli* cells (Table 1) and was able to survive after a long mixing time. It was inferred there were probably two major reasons for the unsuccessful conjugation. One is the mutual inhibition of the donor and recipient cells during mating, while the other is the highly unequal doubling time of *Sorangium* and *E. coli* cells (about half an hour vs. 16–20 h).

To verify the speculation, one or both of the selection antibiotics gentamicin and chloramphenicol were added at different concentrations into the mating medium to decrease the interferences from the opposite cells. The MIC of gentamicin for *E. coli* cells was about 5 µg mL^{-1}, while the MIC of chloramphenicol for *S. cellulosum* So0157-2 cells was less than 5 µg mL^{-1}. Interestingly, when low concentrations of dual selection antibiotics, i.e., 7 µg mL^{-1} gentamicin and 5 µg mL^{-1} chloramphenicol were added into the mating medium, conjugation transfer of pCCMT61 into *S. cellulosum* So0157-2 was achieved, whereas single selection antibiotics had no effect. Addition of lower concentrations of the two selection antibiotics (4 µg mL^{-1} gentamicin and 3 µg mL^{-1} chloramphenicol or lower) decreased the effects or had no effects, while higher concentrations (10 µg mL^{-1} gentamicin and 10 µg mL^{-1} chloramphenicol) led to massive death of both cells. The appropriate concentrations of both antibiotics were similar to or small higher than the MICs of the separate mating cells. The reason was probably produced by the enzymatic metabolism of the tolerant counterpart cells. Besides, some other antibiotics such as hygromycin, phleomycin, and kanamycin or their combinations were also tested, but no effects for the improvement of the conjugational DNA transfer. To exclude false positives from contamination of *E. coli* cells, randomly selected pCCMT61 resistant colonies were transferred onto selection plates several times followed by inoculation of the cells in LB medium [12]. The chromosomes from the pure resistant colonies were determined for the existence of the chloramphenicol-resistance

gene by slot blot hybridization (not shown), and all were positive.

The plasmid pCCMT61 contained a DNA fragment of the epothilone synthase encoding gene, truncated at the 5′-end and 3′-end. Therefore, single cross-over integration into the epothilone biosynthesis gene cluster should result in destruction of the production. Several transconjugants were assayed of the production of epothilone A. In contrast to the wild-type strain, none of these mutants was able to produce epothilone A (Fig. 2), which indicated that the epothilone biosynthetic pathway had been disrupted.

Applicability of the improved protocol

Using the optimized conditions, the mating for transfer of pCCMT61 into *S. cellulosum* So0157-2 produced 15–50 transconjugants on one plate (inoculated of about 1.5×10^7 *Sorangium* cells). The transconjugation efficiency of pCCMT61 was improved more than 100 times by adding low concentrations of dual selection antibiotics into the mating plates (Table 2). To demonstrate the effect of dual selection antibiotics in mating plates, the transconjugation of different plasmids pCDM2 and pCCK700 was also performed. For the plasmid pCDM2 with smaller sizes of cloned inserts, the resulting transconjugant efficiency for *S. cellulosum* So0157-2 was also enhanced, almost the same as that of pCCMT61 (Table 2). Southern blot was done to verify site-specific integration of pCDM2 (Fig. 3). There are many repeated domains in epothilone biosynthesis gene cluster [14], so that the only methyltransferase gene fragment was used as a probe to improve hybridization specificity. For plasmid pCCK700, many transconjugants were obtained by using the improved protocol, even the efficiency was some lower than that of pCCMT61. The homologous fragment in plasmid pCCK700 was less than 1-kb, and the efficiency reached 1×10^{-7} (Table 2). The transconjugant efficiency was calculated as the proportion of transconjugants from the total number of *Sorangium* cells used at the start of mating, and the efficiency could be much higher when calculated according to the viable sorangial cells [9]. The improved protocol met the needs for genetic manipulation of different sizes of homologous fragments in the epothilone-producer *S. cellulosum* So0157-2, and the metabolic engineering is being undertaken.

For the strains employed in this study, *S. cellulosum* So02007-3 exhibits sensitivity to the existence of living *E. coli* cells (Table 1), as the case in *S. cellulosum* So ce12 [10]. During a 24-h mating without antibiotics, the lethal concentration of living *E. coli* cells is less than 1×10^6 cells mL^{-1} for 1×10^{10} So02007-3 cells mL^{-1}. With the presence of dual selection antibiotics in the mating plate, survivals of *S. cellulosum* So02007-3 cells with the presence of *E. coli* cells was greatly improved (the performing protocol was the same as So0157-2). Thus, in the present conjugation process with dual selection antibiotics, the ratio of donor and recipient cells was rather high, not only in the

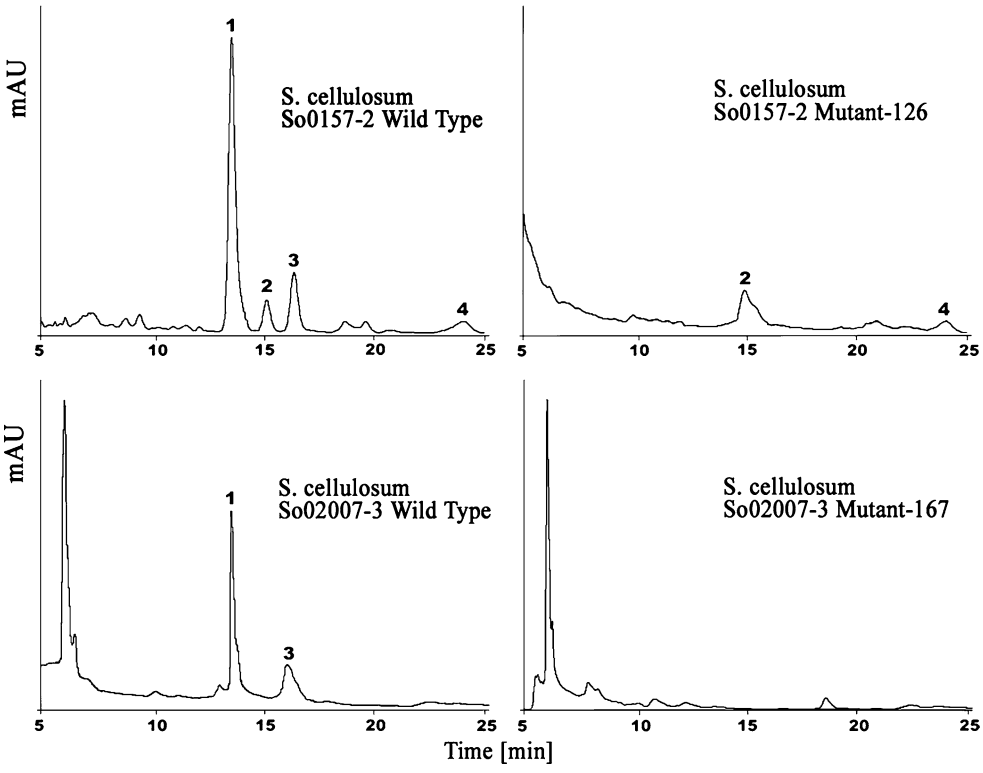

Fig. 2 HPLC profiles of epothilones production in *Sorangium* strains. Numbers correspond to substances as follows: *1* and *3* are epothilone A and B, *2* and *4* are unknown peaks; *mAU* mili absorption units

Table 2 Transfer of mobilizable plasmids into *S. cellulosum* So0157-2

Mobilizable plasmid	Size of the DNA insert in pCVD442 (kb)	Antibiotics in mating medium	Transconjugant efficiencies[a]
pCCMT61	3.2	None[b]	$<1 \times 10^{-8}$
pCCMT61	3.2	Gm, Cm	$1–3 \times 10^{-6}$
pCDM2	1.5	Gm, Cm	$1–2 \times 10^{-6}$
pCCK700	0.7	Gm, Cm	1×10^{-7}

[a] The transconjugant efficiencies were calculated as the proportion of transconjugants from the total number of *Sorangium* cells used at the start of mating

[b] The normal conjugation method was used

tolerant, but also in sensitive *Sorangium* strains. More donor cells certainly allow more sufficient mating. The transconjugant frequency for transferring the plasmid pCCMT61 into So02007-3 also reached $1–2 \times 10^{-6}$, almost the same frequency as that of *S. cellulosum* So0157-2. The epothilone biosynthesis in the So02007-3 mutants was also disrupted (Fig. 2).

In classical conjugation protocols, no antibiotic is added during the mating time. It is not clear why and how the addition of two selection antibiotics worked. The reason of achieving the conjugative plasmid transfers in *Sorangium* strains is inferred to be more than the presence of the high number of *E. coli* cells. Low concentrations of antibiotics not only inhibit the specific targets, but also probably behave as global regulators of cellular functions [23, 24]. The presence of antibiotics may repress some important metabolic pathways and consequently the growth of sensitive cells. The dual selection antibiotics may make the mating cells in a sick stage. DNA transfer is an invasion course for host cells, and the sick stage is probably competent for the conjugation transfer. On the other hand, stress of the presence of antibiotics may also induce expression of the genes involving in DNA transfer of the tolerant cells [25], which may strengthen cellular transferring ability. In our experiments, the concentrations of the dual selection antibiotics were either equal to or small higher than the MICs. The antibiotics concentrations were stepped down by the degradation of the tolerant counterpart cells, allowing the sensitive counterparts to survive. Thus, it is suggested that the presence of low and stepping-down concentrations of the two selection antibiotics have dual effects on the counterpart cells. The involved mechanisms are being studied.

In the present experiments, the presence of dual selection antibiotics in the mating plate is a crucial factor for the conjugational transfer of mobilizable plasmids from *E. coli* to *Sorangium* cells. For those phleomycin and hygromycin resistant *Sorangium* strains, chloramphenicol was developed as a new selectable marker by introducing the *aph*II promoter before the resistance gene. The improved protocol obviously enhanced the genetic manipulation efficiency of *S. cellulosum* strains. It improved the transconjugation

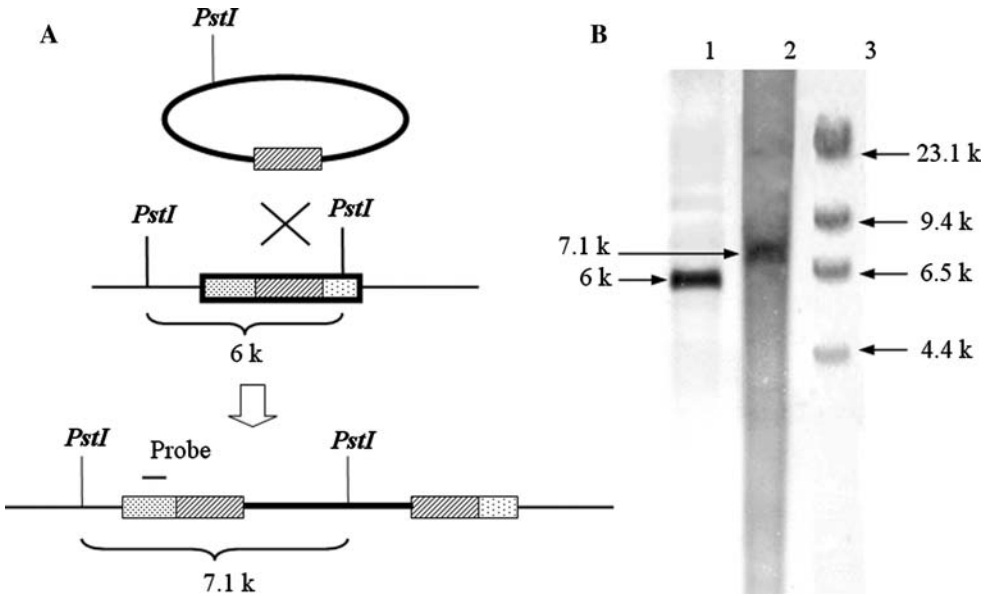

Fig. 3 Insertional inactivation of the *S. cellulosum* So 0157-2 *epoD* gene. **a** Schematic representation of the *S. cellulosum* So 0157-2 *epoD* integration event. *Heavy box S. cellulosum* So 0157-2 *epoD* gene, *hatched boxes* the fragment of the *S. cellulosum* So 0157-2 *epoD* gene used for the disruption, *black line S. cellulosum* So 0157-2 chromosome, *heavy black line* pCDM2 vector sequences. The result of the homologous integration event is two truncated copies of the *epoD* gene interspersed with vector sequences. **b** Southern hybridization of *PstI*-digested *S. cellulosum* chromosomal DNA with the DIG-labeled *epoD* gene fragment as a probe. *Lane 1* chromosomal DNA from the wild type So 0157-2 strain, *lane 2* chromosomal DNA from mutant-167, *lane 3* size marker

efficiency of not only non-sensitive *Sorangium* strains to living *E. coli* cells but also the sensitive ones. The method described here provides a valuable genetic manipulation choice for newly isolated *Sorangium* strains or non-model organisms for genetic research.

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References

1. Shimkets LJ (1990) Social and developmental biology of myxobacteria. Microbiol Rev 54:473–501
2. Reichenbach H, Höfle G (1993) Production of bioactive secondary metabolites. In: Dworkin M, Kaiser D (eds) Myxobacteria II. American Society for Microbiology, Washington DC, pp 347–397
3. Goldman BS, Nierman WC, Kaiser D, Slater SC, Durkin AS, Eisen JA et al (2006) Evolution of sensory complexity recorded in a myxobacterial genome. Proc Natl Acad Sci USA 103:15200–15205. doi:10.1073/pnas.0607335103
4. Schneiker S, Perlova O, Kaiser O, Gerth K, Alici A, Altmeyer MO et al (2007) Complete genome sequence of the myxobacterium *Sorangium cellulosum*. Nat Biotechnol 25:1281–1289. doi:10.1038/nbt1354
5. Kaiser D (1991) Genetic systems in myxobacteria. Methods Enzymol 204:357–372. doi:10.1016/0076-6879(91)04018-J
6. Kashefi K, Hartzell PL (1995) Genetic suppression and phenotypic masking of a *Myxococcus xanthus* frzF- defect. Mol Microbiol 15:483–494. doi:10.1111/j.1365-2958.1995.tb02262.x
7. Gerth K, Pradella S, Perlova O, Beyer S, Müller R (2003) Myxobacteria: proficient producers of novel natural products with various biological activities-past and future biotechnological aspects with the focus on the genus *Sorangium*. J Biotechnol 106:233–253. doi:10.1016/j.jbiotec.2003.07.015
8. Julien B, Fehd R (2003) Development of a *mariner*-based transposon for use in *Sorangium cellulosum*. Appl Environ Microbiol 69:6299–6301. doi:10.1128/AEM.69.10.6299-6301.2003
9. Jaoua S, Neff S, Schupp T (1992) Transfer of mobilizable plasmids to *Sorangium cellulosum* and evidence for their integration into the chromosome. Plasmid 28:157–165. doi:10.1016/0147-619X(92)90046-D
10. Kopp M, Irschik H, Gross F, Perlova O, Sandmann A, Gerth K et al (2004) Critical variations of conjugational DNA transfer into secondary metabolite multiproducing *Sorangium cellulosum* strains So ce12 and So ce56: development of a mariner-based transposon mutagenesis system. J Biotechnol 107:29–40. doi:10.1016/j.jbiotec.2003.09.013
11. Zirkle R, Ligon JM, Molnar I (2004) Heterologous production of the antifungal polyketide antibiotic soraphen A of *Sorangium cellulosum* So ce26 in *Streptomyces lividans*. Microbiology 150:2761–2774. doi:10.1099/mic.0.27138-0
12. Reichenbach H, Dworkin M (1992) The myxobacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd edn. Springer-Verlag, New York, pp 3416–3487
13. Schupp T, Toupet C, Cluzel B, Neff S, Hill S, Beck JJ et al (1995) A *Sorangium cellulosum* (myxobacterium) gene cluster for the biosynthesis of the macrolide antibiotic soraphen A: cloning, characterization, and homology to polyketide synthase genes from actinomycetes. J Bacteriol 177:3673–3679
14. Molnár I, Schupp T, Ono M, Zirkle R, Milnamow M, Nowak-Thompson B et al (2000) The biosynthetic gene cluster for the microtubule-stabilizing agents epothilones A and B from *Sorangium cellulosum* So ce90. Chem Biol 7:97–109. doi:10.1016/S1074-5521(00)00075-2
15. Pradella S, Hans A, Spröer C, Reichenbach H, Gerth K, Beyer S (2002) Characterisation, genome size and genetic manipulation of the myxobacterium *Sorangium cellulosum* So ce56. Arch Microbiol 178:484–492. doi:10.1007/s00203-002-0479-2
16. Bode HB, Müller R (2006) Analysis of myxobacterial secondary metabolism goes molecular. J Ind Microbiol Biotechnol 33:577–588. doi:10.1007/s10295-006-0082-7
17. Li ZF, Zhao JY, Xia ZJ, Shi J, Liu H, Wu ZH et al (2007) Evolutionary diversity of ketoacyl synthases in cellulolytic myxobacterium *Sorangium*. Syst Appl Microbiol 30:189–196. doi:10.1016/j.syapm.2006.06.002
18. Gong GL, Sun X, Liu XL, Hu W, Cao WR, Liu H et al (2007) Mutation and a high-throughput screening method for improving the production of Epothilones of Sorangium. J Ind Microbiol Biotechnol 34:615–623. doi:10.1007/s10295-007-0236-2
19. Nguimbi E, Li YZ, Gao BL, Li ZF, Wang B, Wu ZH et al (2003) 16S–23S ribosomal DNA intergenic spacer regions in cellulolytic myxobacteria and differentiation of closely related strains. Syst Appl Microbiol 26:262–268. doi:10.1078/072320203322346119
20. Gill RI, Shimkets LJ (1993) Genetic approaches for analysis of myxobacterial behavior. In: Dworkin M, Kaiser D (eds) Myxobacteria II. American Society of Microbiology, Washington DC, pp 129–155
21. Hu W, Dong H, Li YZ, Hu XT, Han GJ, Qu YB (2004) A high-throughput model for screening anti-tumor agents capable of promoting polymerization of tubulin in vitro. Acta Pharmacol Sin 25:775–782
22. Dong H, Li YZ, Hu W (2004) Analysis of purified tubulin in high concentration of glutamate for application in high throughput screening for microtubule-stabilizing agents. Assay Drug Dev Technol 2:621–628. doi:10.1089/adt.2004.2.62
23. Goh EB, Yim G, Tsui W, McClure J, Surette MG, Davies J (2002) Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. Proc Natl Acad Sci USA 99:17025–17030. doi:10.1073/pnas.252607699
24. Yim G, de la Cruz F, Spiegelman GB, Davies J (2006) Transcription modulation of Salmonella enterica serovar Typhimurium promoters by sub-MIC levels of rifampin. J Bacteriol 188:7988–7991. doi:10.1128/JB.00791-06
25. Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys JP (2006) Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. Science 313:89–92. doi:10.1126/science.1127912
26. Figurski DH, Helinski DR (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc Natl Acad Sci USA 76:1648–1652. doi:10.1073/pnas.76.4.1648
27. Donnenberg MS, Kaper JB (1991) Construction of an eae deletion mutant of enteropathogenic *Escherichia coli* by using a positive-selection suicide vector. Infect Immun 59:4310–4317
28. Simon R, Priefer U, Puhler A (1983) A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram negative bacteria. Bio/Technology 1:784–791. doi:10.1038/nbt1183-784