Original Article

Enhancing A82846B production by artificial attB-assisted overexpression of orf10–orf11 genes in Kibdelosporangium aridum SIPI-3927

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Abstract

A82846B, producing by Kibdelosporangium aridum, is an important precursor of the semi-synthetic glycopeptide antibiotic Oritavancin. K. aridum produces three components A82846A, B and C, so it is essential to increase A82846B titer and reduce A82846A and C titers by overexpressing halogenase and glycosyltransferase genes. Firstly, we constructed the genetically engineered strain SIPI-3927-attB harboring artificial attB site via homologous recombination. Secondly, two strains SIPI-3927-C1 and C2 were also constructed by integrating halogenase genes vcm8 and orf10 into artificial attB sites of SIPI-3927-attB, respectively. Meantime, three strains SIPI-3927-C3, C4 and C5 containing overexpressing glycosyltransferase A, B and C genes were obtained, respectively. Through fermentation analyses, the results showed that SIPI-3927-C1 and C2 could increase A82846B ratios, in which SIPI-3927-C1 showed a better performance. Moreover, the titer of SIPI-3927-C3 was highest in those of three strains. Finally, the strain SIPI-3927-C6 was constructed by integrating both orf10-encoded halogenase and orf11-encoded glycosyltransferase A, of which the yield and ratio of A82846B in shake-flask fermentation reached 1200 mg/L and 73.6%, respectively. Besides, the yield and ratio of A82846B in SIPI-3927-C6 grew up to 2520 mg/L and 86.5% in the 5-L fermenter culture, respectively. In conclusion, overexpressing orf10 gene can increase A82846B ratio, while overexpressing orf11 gene can increase A82846B titer as well. The artificial attB site is effective for inserting new genes.

Keywords: A82846B, Kibdelosporangium aridum, Halogenase, Glycosyltransferase, attB

Key points

It is first time to construct engineering strains of K. aridum for A82846B production. The attB-site has inserted into K. aridum SIPI-3927 by homologous recombination. The double-gene overexpressing engineered strain was constructed. The highest titer of A82846B was obtained in K. aridum SIPI-3927-C6.

Introduction

Oritavancin is a semi-synthetic antibiotic, which was recently approved by the Food and Drug Administration (FDA) (Brade et al. 2016; Corey et al. 2014) for treatment of gram-positive pathogens induced Acute Bacterial Skin and Skin Structure Infections (ABSSSIs) and Methicillin-resistant Staphylococcus Aureus (MRSA) (Edelsberg et al. 2014; Kaatz et al. 1998; Rupp et al. 2001). Compared with previous glycopeptide antibiotics, Oritavancin has a prolonged half-life about 245 h, which allows a 7-day course of treatment for one single dose (Brade et al. 2016).

Oritavancin is chemically synthesized by adding a 4-(4-chlorophenyl) benzyl group to A82846B via reductive alkylation (Leadbetter et al. 2010). A82846B, along with two structurally similar components A82846A and A82846C, is produced by Nocardia, Amycolatopsis, Kibdelosporangium (Rafai et al. 2016). Since A82846A,
A82846B, and A82846C only differ in their chlorine atom number, the purification of these compounds requires very cumbersome steps (Hamill et al. 1998), which definitely decreases the recovery ratio. Furthermore, the probable side effects of those impurities make it more necessary to reduce ratios of A82846A and C in fermentation broth.

The biosynthetic pathway of A82846B, including a total of 39 putative genes, was firstly reported in *A. orientalis* NRRL 18098 (Van Wageningen et al. 1998). The current dilemma was that halogenase encoded *orf10* gene activity was not high enough (NCBI Accession No: AJ223998.1). It was proposed that the catalytic activity of halogenase was not sufficient for complete halogenation of A82846. Therefore, improving the activity of halogenase may be a good strategy to solve this predicament. Wang et al. (2018) tried to overexpress the three-copy halogenase coding gene in *A. orientalis* SIPI-18099, and successfully increased the A82846B yield as well as the purity in shake-flask. This result partially demonstrated the hypothesis of insufficient halogenase activity accounting for incomplete halogenation of A82846A and A82846C and enhancement of halogenase activity can help to solve this problem.

*Kibdelosporangium aridum*, a natural A82846B producer, has a higher yield (900 mg/L) and ratio (34%) of A82846B in shake-flask compared with *A. orientalis*. Thus, overexpression of halogenase gene was more promising to generate a better performed high-yield A82846B producer of *K. aridum*. The genes coding glycosyltransferases involved in the biosynthesis of various glycopeptide antibiotics were predicted to be used to link heptapeptide and three glycosyl groups in A82846B biosynthesis (Van Wageningen et al. 1998). It was found that glycosyltransferases (GtfA, GtfB, GtfC, reaction site as Fig. 1) were encoded by *orf11, orf12* and *orf13* (NCBI Accession No: AJ223998.1), and could have a positive regulatory effect on the biosynthesis of A82846B (Solenberg et al. 1997).

Fig. 1 Structure and the key biosynthesis gene of A82846. Green box is a glycosyltransferase reaction site; red box is a halogenase reaction site; encoded gene of enzyme in brackets.
The **attB/P** integration system was widely used in *Streptomyces*, which depends on homologous recombination between the **attB** site and the **attP** site mediated by the ΦC31 integrase (Patricia et al. 2002). This system allows exogenous genes to be integrated into chromosome and expressed stably and efficiently (Yuan et al. 2016; Lee et al. 2016). In this study, we provide a shortcut for recombinant strains construction by inserting an artificial **attB** site into *K. aridum* chromosome via homologous recombination. Based on the engineered chassis, the co-expression of **orf10** coding halogenase and **orf11** coding glycotransferase led to significant boost of A82846B titer and ratio. The constructed engineering strains can be reducing the industrialization cost of A82846B. This strategy of constructing engineered strains can provide a new method for other strains that lack similar **attB** sites.

**Materials and methods**

**Strains, plasmids, and primers**

Bacterial strains and plasmids used in this study are listed in Table 1; primers are listed in Table 2.

### Table 1 Bacterial strains and plasmids

| Strains or plasmids       | Description                                      | Source                      |
|---------------------------|--------------------------------------------------|-----------------------------|
| **Plasmid vectors**       |                                                  |                             |
| pSET152                   | acc(3)IV, oriTRK2, pC31, int                     | Bierman et al. (1992)       |
| pKC1139                   | acc(3)IV, pSG5, oriTRK2                          | Bierman et al. (1992)       |
| pSB153                    | acc(3)IV, onTRK2, kasO*P promoter                | Wang et al. (2013)          |
| pKC1139-attB              | acc(3)IV, pSG5, onTRK2, attB                     | This study                  |
| pSET152-vcm8              | acc(3)IV, onTRK2, Aprα, kasO*P, vcm8             | This study                  |
| pSET152-orf10             | acc(3)IV, onTRK2, Aprα, kasO*P, orf10            | This study                  |
| pSET152-orf11             | acc(3)IV, onTRK2, Aprα, kasO*P, orf11            | This study                  |
| pSET152-orf12             | acc(3)IV, onTRK2, Aprα, kasO*P, orf12            | This study                  |
| pSET152-orf13             | acc(3)IV, onTRK2, Aprα, kasO*P, orf13            | This study                  |
| pSET152-orf10-orf11       | acc(3)IV, onTRK2, Aprα, kasO*P, orf10-orf11      | This study                  |
| **Strains**               |                                                  |                             |
| E. coli DH5α              | Host for general cloning                         | FENGHBIO, China             |
| E. coli ET12567/pUZ8002    | Donor strain for intergenetic conjugation between E. coli and *Streptomyces*, Kan’, Chl’ | FENGHBIO, China             |
| A. orientalis ATCC 43491  | Vancomycin-producing the parental strain         | ATCC                        |
| *K. aridum* SIPI-3927 (CGMCC 4.7675) | A82846B-producing strain                        | CGMCC                       |
| SIPI-3927-attB            | SIPI-3927, attB                                  | This study                  |
| SIPI-pSET152              | SIPI-3927-attB, pSET152                          | This study                  |
| SIPI-3927-C1              | SIPI-3927-attB, pSET152-vcm8                     | This study                  |
| SIPI-3927-C2              | SIPI-3927-attB, pSET152-orf10                    | This study                  |
| SIPI-3927-C3              | SIPI-3927-attB, pSET152-orf11                    | This study                  |
| SIPI-3927-C4              | SIPI-3927-attB, pSET152-orf12                    | This study                  |
| SIPI-3927-C5              | SIPI-3927-attB, pSET152-orf13                    | This study                  |
| SIPI-3927-C6              | SIPI-3927-attB, pSET152-orf10-orf11              | This study                  |

**DNA manipulation**

The manipulations of genomic DNA, plasmid DNA isolation, restriction endonuclease digestion, and DNA ligation were performed according to standard procedures (Sambrook and Russell 2001). The enzymes were purchased from Takara, Japan and Thermo Fisher Scientific (Thermo, USA). ClonExpress MultiS One Step Cloning Kit (CMOSTK) was purchased from Vazyme Biotech Co., Ltd. The A82846B standard was purchased from MedKoo Biosciences, Inc. The defoamer of SAG471 was purchased from Beijing BaiYuan Chemical Co., Ltd. All chemicals used were molecular biology grade and commercially available.

**Construction of plasmids**

In order to construct pKC1139-**attB** plasmid containing **attB** site, orf35-**attB**-F/R and orf36-**attB**-F/R were used as primers, respectively, and SIPI-3927 genome was used as a template to obtain two 1000 bp homology arms by PCR amplification. This PCR products and pKC1139 plasmid with BamH1/Xba1 restriction enzyme digestion were ligated by CMOSTK to obtain a pKC1139-**attB** plasmid.
Table 2 Primers used in this study

| Primers         | Purpose                          | Sequence (5′–3′)                                                                 |
|-----------------|----------------------------------|-------------------------------------------------------------------------------|
| orf35-att8-F/R  | Amplification of orf35-att8 for construction of pSET152-att8 | CTGCAGTGTGGACTCTAGAAGCTCTTGATCAGGACACGAGACACGACCCGGGGGAGGCGGAC|CCGCTGATCGGCTGAGCGAGGAGGCTGCAATA |
| orf36-att8-F/R  | Amplification of orf36-att8 for construction of pSET152-att8 | CGGGGGGAGGCGGGACGCGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| kasO*P-F/R      | Amplification of kasO*P promoter for construction of pSET152-vcm8  | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| vcm8-F/R        | Amplification of vcm8 for construction of pSET152-vcm8            | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| orf10-F/R       | Amplification of orf10 for construction of pSET152-orf10          | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| orf11-F/R       | Amplification of orf11 for construction of pSET152-orf11          | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| orf12-F/R       | Amplification of orf12 for construction of pSET152-orf12          | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| orf13-F/R       | Amplification of orf13 for construction of pSET152-orf13          | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| orf10–orf11-F/R | Amplification of orf10–orf11 for construction of pSET152-orf10–orf11 | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |
| attB-F/R        | Amplification of artificial attB site in K. aridum                 | CGGGGAGGCGGGTACGCGGCTGAGCGAGGACACGCGGTCGATGCTTACGCGGCTG |

The italicized part represents attB sequence; underline represents restriction enzyme cutting site.

The pSET152 linear vector was obtained by digesting the pSET152 plasmid with Xba1/BamH1 restriction enzyme. The kasO*P promoter was amplified by PCR using kasO*P-F/R as primers and plasmid pSB153 as a template. The vcm8 (NCBI Accession No: HQ679900.1) was amplified by PCR using vcm8-F/R as primers and strain ATCC43491 as template. These three products were then ligated by CMOSTK to obtain a pSET152-vcm8 plasmid.

The orf10, orf11, orf12, orf13 and orf10–orf11 productions were amplified, which SIPI-3927 genome as a template, and orf10-F/R, orf11-F/R, orf12-F/R, orf13-F/R and orf10–orf11-F/R as primers, respectively. The pSET152-vcm8 plasmid with Nde1/BamH1 restriction enzyme digestion was used as a linear vector. The linear vector and the PCR products which we amplified above were ligated by CMOSTK to obtain plasmids pSET152-orf10, pSET152-orf11, pSET152-orf12, pSET152-orf13 and pSET152-orf10–orf11, respectively.

Construction of recombinant strains K. aridum

The plasmids were introduced into K. aridum by the E. coli–Streptomyces conjugation method described previously (Kieser et al. 2000). The E. coli ET12567/pUZ8002 containing the plasmid was grown in the presence of antibiotics (50 mg/L apramycin, 25 mg/L chloramphenicol, and 50 mg/L kanamycin) to an OD600 of 0.4–0.6. The cells were washed twice with an equal volume of LB medium, and resuspended in 2 mL LB medium. The K. aridum was incubated in 30 mL YEME medium (yeast extract 0.3%, tryptone 0.5%, malt extract 0.3%, sucrose 34%) at 30 °C for 48 h, then 10% of the culture was transferred into 30 mL YEME medium for 36 h growth. Mycelium was collected by centrifugation and resuspended in 2 mL LB. 2 mL ET12567/pUZ8002 cells mixed with the resuspended K. aridum, and spread the mixture on MISP-4 plates (mannitol 0.5%, glucose 1.0%, soluble starch 0.5%, soybean cake powder 0.5%, tryptone 0.2%, yeast extract 0.1%, ammonium sulfate 0.2%, sodium chloride 0.1%, dipotassium hydrogen phosphate 0.1%, carbonic acid calcium 0.2%, trace element 1 mL, agar powder 2% and pH 6.8–7.0). The plates were incubated for 16–20 h at 30 °C and then overlaid with 1 mL water containing 400 μg nalidixic acid and 800 μg apramycin. The plates were incubated again for 7–12 days at 30 °C until the recombinant strains were obtained. The recombinants were then transferred onto slant medium containing 50 mg/L apramycin.
Firstly, the pKC1139-attB plasmid was transferred into K. aridum SIPI-3927 as described above. Secondly, the K. aridum SIPI-3927-attB recombinant strain with loss of apramycin resistance was obtained by double exchange screening. Finally, the other pSET152 plasmids were transferred to K. aridum SIPI-3927-attB as described above to obtain different recombinant strains.

Culture of K. aridum
The parental type or recombinant K. aridum strains were grown on solid medium (glucose 1%, starch 2%, yeast extract 0.5%, hydrolyzed casein 0.5%, calcium carbonate 0.1%, agar 2%, pH 6.8–7.0) at 30 °C for 6–7 days. For fermentation culture (Tian et al. 2020), a 1 cm² 2 cm agar piece was transferred into a 250-mL flask with 30 mL production medium (glucose for 48 h. Then 10% seed culture was inoculated into a 250-mL flask with 30 mL production medium (glucose 2%, starch 0.5%, corn syrup 0.5%, yeast powder 0.5%, ammonium sulfate 0.5%, and calcium carbonate 0.5%, pH 6.8) and incubated at 30 °C, 220 rpm for 48 h. Then 10% seed culture was inoculated into a 250-mL flask with 30 mL production medium (glucose 6%, corn starch 1%, hydrolyzed casein 0.5%, soy flour 1%, yeast powder 0.5%, beef extract 1%, potassium dihydrogen phosphate 0.05%, magnesium sulfate heptahydrate 0.18%, sodium chloride 0.3%, calcium carbonate 0.5%, SAG471 defoamer 0.03%, pH 6.6–6.8) and incubated at 34 °C, 250 rpm for 48 h. Then 10% seed culture was inoculated into a 250-mL flask with 30 mL production medium (glucose 6%, starch 2%, yeast extract 1%, potassium dihydrogen phosphate 0.05%, magnesium sulfate heptahydrate 0.18%, sodium chloride 0.3%, calcium carbonate 0.5%, SAG471 defoamer 0.03%, pH 6.6–6.8) and incubated at 34 °C, 250 rpm for 44 h. Shake-flask fermentations were carried out in three independent experiments.

Production of A82846B in 5-L fermenter
To research A82846 production, 2 cm × 3 cm agar pieces of the K. aridum SIPI-3927 or SIPI-3927-C6 from slant medium were cultured in 750-mL flasks with 100 mL seed medium at 30 °C, 220 rpm, for 48 h. Then 10% seed culture was inoculated into 3 L of production medium in a 5-L fermenter. The fermentation temperature was kept at 34 °C, and the pH was adjusted to 6.8 with NaOH at the beginning. 30% glucose solution was added when the content of glucose dropped below 1.0%, and its concentration was kept at about 1–2% during the fermentation period.

Analytic method
One milliliter of the culture was centrifuged at 12,000 rpm for 20 min to remove the precipitate, and then the supernatant was diluted 5- to 20-fold. Processed sample was assayed by HPLC with a gradient elution program (0 → 20 min, A:B = 95:5 → 80:20; 20 → 22 min, A:B = 80:20 → 95:5; 22 → 27 min, A:B = 95:5; A: 0.1% trifluoroacetic acid, B: acetonitrile) in a phenyl chromatographic column (4.6 × 250, 5 μm, Welch, China) with at 1.0 mL/min and detection at 225 nm.

To measure biomass, 10 mL fermentation culture was centrifuged in a graduated centrifuge tube for 10 min, 5000 rpm. Measure the volume of supernatant (v), the value of (10 – v)/10 was the biomass which could reflect the growth conditions of the strains.

Data analysis
The data were analyzed using Excel 2010, SPSS 20.0, OriginPro8.5 data analysis and statistical software.

Results
Construction of K. aridum SIPI-3927-attB strain containing attB site
Conjugal transfer is the main method of molecular manipulation in Streptomyces. Because there is no attB site in K. aridum, it is difficult to insert an exogenous gene into the genome through the ΦC31 integrase (Kim et al. 2008). To solve this predicament, we constructed an attB site into the chromosome of K. aridum by homologous recombination (Fig. 2a, b). The single-exchange transformants were generated at several times, and a total of 580 transformants were selected for obtaining the second homologous recombination. Three strains were obtained without apramycin resistance by double-exchange. The double-exchange efficiency was about 0.5%. The product was verified thought PCR amplification procedure which three strains were used as a template and the attB-R/F as primers (Fig. 2c, d). Finally, we obtained two strains containing the attB site, and 1# was named as K. aridum SIPI-3927-attB. In addition, the attB site insertion has no significant effect on the biosynthesis of A82846B (Fig. 3a).

Overexpression of orf10 and vcm8 in K. aridum SIPI-3927-attB
In the biosynthesis of A82846B, due to insufficient expression of the halogenase, the impurities A82846A and A82846C have more ratio than A82846B in whole A82846. In order to increase the ratio of A82846B, we overexpressed the halogenase gene. Two plasmids pSET-vcm8 and pSET-orf10 were constructed which controlled by a strong promoter kasO*P. They contained the extra copy gene of vcm8 and orf10, respectively. Subsequently, these plasmids were inserted into the K. aridum SIPI-3927-attB by conjugate transformation. Then, the recombinant strains of SIPI-3927-C1 and SIPI-3927-C2 were obtained by resistance selection with 50 μg/mL apramycin. The result showed that there was no significant difference about A82846B yields among strains of SIPI-3927-attB, SIPI-3927-C1, SIPI-3927-C2 and the parental strain (Fig. 3a), of which the ratio of SIPI-3927-C2 for A82846A, B, and C were 24.8%, 72.8%, and 2.4%, respectively, which were significantly different from the parental strain SIPI-3927 (60.4%, 34%, and 5.6%). Meanwhile, the ratio of
A82846B in the SIPI-3927-C1 was increased to 44.7%. These results suggest that the insertion of *K. aridum* orf10 gene is more efficient than *A. orientalis* vcm8 gene during enhancing the ratio of A82846B.

**Effect of orf11, orf12 and orf13 genes encoded glycosyltransferases on A82846B production**

Glycosyltransferases were catalytic enzymes that linked the heptapeptide and three glycosyl groups in the A82846B biosynthesis. The GtfA, GtfB and GtfC of A82846B biosynthesis were encoded by the orf11, orf12 and orf13, respectively. Three plasmids, pSET152-orf11, pSET152-orf12 and pSET152-orf13, containing the extra gene of orf11, orf12 and orf13, respectively, controlled by a strong promoter kasO*P* were constructed. These plasmids were transferred into the SIPI-3927-attB strain by conjugative transfer. Finally, we obtained recombinant strains of SIPI-3927-C3, SIPI-3927-C4 and SIPI-3927-C5. As shown in Fig. 3b, these recombinant strains of A82846B titer were 1100 mg/L, 920 mg/L and 910 mg/L, respectively. In SIPI-3927-C3, the yield of A82846B was increased by 1.22-fold compared with parental type strain SIPI-3927. In addition, it was found that the Gtfs did not increase the ratio of A82846B. The results showed that the orf11 gene can significantly increase the yield of A82846B.

**Effect of orf10–orf11 on A82846B yield and ratio**

All above studies indicated that orf10 and orf11 can increase the ratio and yield of A82846B, respectively. These were positive regulatory genes for A82846B biosynthesis. The pSET152-orf10–orf11 plasmid was constructed to increasing yield and ratio of A82846B, which controlled by a strong promoter kasO*P*. The plasmid was transferred into the SIPI-3927-attB strain by conjugative transfer, then the recombinant strain SIPI-3927-C6 was obtained. It can be seen that orf10 significantly improves the A82846B ratio, while orf11 significantly increases the A82846B yield in the fermentation shake-flask (Fig. 4). Compared with the parental strain SIPI-3927, the A82846B yield in strain SIPI-3927-C6 was increased from the initial 930 mg/L to 1200 mg/L, and the A82846B ratio was increased from the initial 34.0% to 73.6% (Fig. 3c). The impurities A82846A and A82846C decreased from 60.4% and 5.6% to 25.2% and 1.2%, respectively (Fig. 3c). The results indicated that overexpression of the orf10–orf11 in the parental strain is an efficient method for reducing impurities and increasing yield.

**Shake-flask fermentation stability of recombinant strain SIPI-3927-C6**

Since the recombinant strains overexpress its own genes of orf10 and orf11, the extra gene loss could be caused by multiple generation culture. It is necessary to study the
stability of the recombinant strain SIPI-3927-C6 in shake-flask fermentation. SIPI-3927-C6 was generated to the F6 in solid medium, and the A82846B production of the F1–F6 strains were fermented by shake-flask. The A82846B yields of the F1–F6 strains were all above 1000 mg/L, and the highest titer was 1253 mg/L (Table 3). The ratios of A82846B were above 70.3%, and the highest ratio of A82846B was 74.1% (Table 3). The results indicated that SIPI-3927-C6 has good genetic stability.

Culture of *K. aridum* SIPI-3927-C6 and SIPI-3927 in 5-L fermenter

The A82846B production by *K. aridum* SIPI-3927-C6 and SIPI-3927 were studied in a 5-L fermenter. During the culture process, the glucose was rapidly consumed within 48 h in *K. aridum* strains, and then the glucose concentration was controlled between 1–2% by 30% glucose streaming (Fig. 5). At about 144 h, the A82846B yields of SIPI-3927 and SIPI-3927-C6 reached 1013 mg/L (A82846B ratio was 38.9%) and 2520 mg/L (A82846B ratio was 86.5%), respectively (Table 4). The A82846B yield of the recombinant strain SIPI-3927-C6 was 2.5-fold higher than the parental strain SIPI-3927. The ratio of A82846A and A82846C decreased to 13.4% and 0.1%, respectively, compared with that of SIPI-3927 (A82846A was 52.1% and A82846C was 9.0%). This change of A82846 ratio drastically reduced the purification pressure, providing a low-price substrate for the alkylation synthesis of Oritavancin. Therefore, the construction of the recombinant *K. aridum* SIPI-3927-C6 was an important method to reduce the cost of A82846B production.

Discussion

Conjugal transfer was an important method in *E. coli–Streptomyces* system, which can rapidly inserted genes into *Streptomyces* by gene overexpression (Bierman et al. 1992). Since exogenous genes had genetic instability in *Streptomyces*, it could be solved by the attB/P integration system. The attB/P integration system inserted exogenous genes into the genome of *Streptomyces*, which enables more stable heredity in multiple generations (Olano et al. 2008). However, the absence of natural attB site in *K. aridum* chromosome impeded the exogenous genes overexpression and genetic engineering of *K. aridum*, it was not possible to insert the integrin plasmid of pSET152 into *K. aridum*. In our study, we constructed an attB site on the genome of *K. aridum* by homologous recombination. The strain allows the pSET152 plasmid to quickly integrate the exogenous gene into the chromosome. Compared with Wang’s (2018) research, this genetic manipulation strategy could insert more attB sites into the genome for further study, which may greatly improve the overexpression ability in *K. aridum*, and the passage of the strain was more stable. It was the first time to propose a new strategy to recombinant DNA of the A82846B in the *K. aridum*.

Van Wageningen et al. (1998) and Xu et al. (2014) reported that halogenases of A82846B and vancomycin
were encoded by orf10 and vcm8, respectively. Meantime, the vcm8 encoded halogenase may be theoretically eliminated the A82846A and A82846C impurities in current industrial vancomycin production, given the fact that vancomycin has two chlorine substitutes without any mono- or non-chlorine analogs. However, the experimental results showed that the halogenase encoded by the orf10 gene may specifically increase the halogenation ability in the biosynthesis of A82846B. Therefore halogenase of vancomycin was not an optimal choice on the biosynthesis of A82846B. Meanwhile, Solenberg et al. (1997) proposed that glycosyltransferases have a positive effect on increasing the production of A82846B. In our study, it is confirmed that orf11-encoded GtfA can effectively increase the yield of A82846B.

The yield and ratio of A82846B in 5-L fermenter reached 2520 mg/L and 86.5% by supplying the sufficient oxygen and carbon source compared with shake-flask fermentation in SIPI-3927-C6. It was evidenced that oxygen and glucose control may be a key factor in the production of A82846B. Similarly, Wang et al. (2018) constructed a recombinant strain, A. orientalis

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**Table 3 Comparison recombinant F1–F6 strains of SIPI-3927-C6 in shake-flask fermentation**

| Generation | Biomass (%) | Yield of A82846B (mg/L) | A82846B (%) |
|------------|-------------|-------------------------|-------------|
| F1         | 22          | 1025                    | 70.3        |
| F2         | 24          | 1201                    | 73.6        |
| F3         | 25          | 1253                    | 72.4        |
| F4         | 23          | 1198                    | 74.1        |
| F5         | 24          | 1122                    | 73.9        |
| F6         | 22          | 1234                    | 73.5        |

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**Fig. 4** HPLC analysis of the A82846 components production in fermentation broths
chal-3, containing three consecutive copies of halogenase encoding halogenase of A82846B biosynthesis, the yield and ratio of A82846B were reached 2200 mg/L and 88.2% in 5 L fermentor culture, respectively. Compared with Wang’s study, our research improved by 14.5% A82846B yield with almost the same A82846B ratio. Moreover, the fermentation yield and ratio were remained stable during culture for six consecutive generations. According to industrial production of recombinant strains, it was documented that continuous multiple-copy gene easily lost during generation due to secondary recombination (Myronovskyi et al. 2018; Olano et al. 2008). This method could insert more attB sites in the genome of K. aridum, which greatly avoids gene loss and improves applicant potential of our strain in commercial A82846B production. In addition, the fermentation process optimization of the engineering strain SIPI-3927-C6 was another key factor for the A82846B production in the future. The regulation of the fermentation process will become the focus of our next research.

In conclusion, it was the first time to constructing an artificial attB site in K. aridum and overexpression of orf10–orf11, and more attB sites can be inserted into the genome for further research. Furthermore, the recombinant strain SIPI-3927-C6 could be used for the industrial production of A82846B, and it was shown a great significance in reducing the production cost of Oritavancin.

Acknowledgements
Not applicable.

Authors’ contributions
TX and HHF conceived and designed research. TX and HH conducted experiments. TX and HHF analyzed data. HH contributed new reagents or analytical tools. TX wrote the manuscript. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
All authors agreed to the publication of data reported in this work.

Table 4 Comparison the yield and ratio of A82846 in 5-L fermentation

| Strains   | A82846B (%) | A82846A (%) | A82846C (%) | Yield of A82846B (mg/L) |
|-----------|-------------|-------------|-------------|-------------------------|
| SIPI-3927 | 38.9        | 52.1        | 9.0         | 1013                    |
| SIPI-3927-C6 | 86.5      | 13.4        | 0.1         | 2520                    |

Fig. 5 Comparison metabolic curves between recombinant strain SIPI-3927-C6 and parent strain SIPI-3927 in 5L fermentor. Solid line, SIPI-3927-C6; dotted line, SIPI-3927.
Competing interests
The authors declare that they have no competing of interests.

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Received: 26 February 2020 Accepted: 10 March 2020

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