Efficient side-chain deacylation of polymyxin B1 in recombinant *Streptomyces* strains

Xiaojing Wang · Kai Wu · Hanzhi Zhang · Jing Liu · Zhijun Yang · Jing Bai · Hao Liu · Lei Shao

Received: 14 May 2022 / Accepted: 4 August 2022 / Published online: 8 September 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

Objectives Polymyxins are antibacterial polypeptides used as “last resort” therapy option for multi-drug-resistant Gram-negative bacteria. The expansion of polymyxin-resistant infections has inspired development of novel polymyxin derivatives, and deacylation is one of the critical steps in generating those antibiotics. Deacylase from *Actinoplanes utahensis* hydrolyze the acyl moieties of echinocandins, and also efficiently deacylates daptomycin, ramoplanin and other important antibiotics. Here, deacylase was studied considering its potential usefulness in deacylating polymyxin B1.

Results All the six recombinant strains containing the deacylase gene catalyzed hydrolysis of polymyxin B1, yielding cyclic heptapeptides. The efficiency of recombinant *S. albus* (SAL701) was higher than that of the others, and deacylation was the most efficient at 40 °C in 0.2 M Tris buffer (pH 8.0) with 0.2 M Mg²⁺. The optimal substrate concentration of SAL701 was increased from 2.0 to 6.0 g/L. SAL701 was highly thermostable, showing no loss of activity at 50 °C for 12 h, and the mycelia could be recycled at least three times without loss of catalytic activity. SAL701 could not deacylate β-lactam substrate such as penicillin G and cephalosporin C. Deacylase catalyzes the amide bond 1 closest to the nucleus of polymyxin B1 rather than the other bond, suggesting that it has high catalytic site specificity. Homology modeling and the docking results implied that Thr190 in deacylase could facilitate hydrolysis with high regioselectivity.

Conclusions These results show that SAL701 is effective in increasing the cyclic heptapeptide moiety of polymyxin B1. These properties of the biocatalyst may enable its development in the industrial production of polymyxins antibiotics.
Keywords  Deacylase · Polymyxin B1 ·  
*Streptomyces* host · Bioconversion · Antibiotic

Introduction

Polymyxins are antibacterial polypeptides first isolated from cultures of *Bacillus polymyxa* strains, and polymyxin B1 is one of the main components, which is shown in (Fig. 1, Brown and Dawson 2017a). The core scaffold contains a cyclic heptapeptide linked to the side chain of a linear tripeptide with an N-terminal fatty acyl (He et al. 2013). They have been reused as “last resort” therapy option for multidrug-resistant Gram-negative bacteria (Brown and Dawson 2017a). The situation is worsened by the emergence of polymyxin-resistant infections.

The development of novel polymyxin derivatives increases its activity and reduce toxicity (He et al. 2013). The antibacterial activity of polymyxin B1 is mainly affected by changing the amino acid of the side chain (Voitenko et al. 1990). Numerous novel compounds have been semi-synthesized by deacylating and replacing their side chains with a variety of different chains (Bairamashvili et al. 1989; Cui et al. 2020). Cui replaced the amino acid l-Dab in position 3 of polymyxin B1 with d-Ser to synthesize polymyxin S2, which showed enhanced activity against *Escherichia coli*, *Acinetobacter baumannii*, and *Klebsiella pneumonia*, and reduced toxicity (Cui et al. 2018; Zhang et al. 2021). Therefore, polymyxin B1 analogues with increased antibacterial activity can be generated by direct modifications of the cyclic peptide (Cui et al. 2018).

Fig. 1  Bioconversion of polymyxin B1 to its cyclic heptapeptide by deacylase
Enzyme modification is an efficient strategy for deacylation. Deacylase from *Actinoplanes utahensis* hydrolyzes the amide bonds of cyclic lipopeptides, which has broad substrate specificity for both the acyl side chain and cyclic peptide analogs of echinocandins, ramoplanin, daptomycin and other important antibiotics (Boeck et al. 1988; Hormigo et al. 2010; Debono et al. 1988a). The enzyme hydrolysis of the acyl moieties of antibiotic echinocandins, and forms a cyclic hexapeptide moiety and long-chain fatty acid (Kreuzman et al. 2000; Boeck et al. 1989). The resulting cyclic hexapeptide can be further reacylated to produce a series of analogs useful as therapeutic antibiotics in clinical practice, such as anidulafungin (Shao et al. 2013; Debono et al. 1988b). Ramoplanin is a lipoglycodepsipeptide with antibacterial activity against major Gram-positive bacteria (Gandolfi et al. 2012; Cudic et al. 2002). Numerous novel ramoplanin derivatives have been semi-synthesized by deacylating and replacing their side chains with different kinds of carboxylic acids (McCafferty et al. 2002). Among them, the 2-methylphenylacetic acyl group, displays increased tolerability and high activity, indicating its potential for overcoming the limitations of ramoplanin in vivo (Di et al. 2007; Ciabatti et al. 2007). Deacylation is the key step in generating the aforementioned antibiotics, and deacylase may be useful in the antibiotic industry for catalyzing the hydrolysis of not only echinocandins and ramoplanin, but also teicoplanin, daptomycin and its derivatives, as well as other related antibiotics with corresponding acyl side chains (Petraitiene et al. 1999; Wang et al. 2021).

The chemical approach often require multistep schemes, which lead to the overall low yield. Okimura obtained the polymyxin nonapeptide PMB (PMBN) by employing trichloroethoxycarbonyl (Troc) group for side chain protection; but the yield was low (Brown and Dawson 2017b; Okimura et al. 2007). Enzymes efficiently catalyze thousands of metabolic processes in vivo. But one of the major disadvantages is the relatively low production of active enzymes; also the extraction and purification from organisms can easily inactivate the enzyme. Kimura prepared Polymyxin B Heptapeptide (PBHP) by Nagarse, the alkaline serine protease of *B. subtilis*. However, the reaction produced several peptide fragments as by-products, and the yield of PBHP was rather low (Kimura et al. 1992). So we used the whole-cell reaction system for bioconversion, because of its convenience, high efficiency, reusability, and safety.

In this study, we expanded the substrate spectrum of recombinant *Streptomyces* containing the deacetylase gene and found that these strains could hydrolyze polymyxin B1. In addition, we predicted the specificity of the catalytic site through structural analysis, and we found that Thr190 in deacetylase can facilitate hydrolysis with high regioselectivity. Also, we focused on the industrial production of polymyxin antibiotics.

**Materials and methods**

Strains and culture medium

*S. lividans* TK24, *S. coelicolor, S. albus, S. avermitilis* K139, *S. ambofaciens* 2283, *S. griseus*, and *A. utahensis* NRRL 12052 were stored in our laboratory. *Escherichia coli* ET12567/PUZ8002, a donor strain for conjugation between *E. coli* and *Streptomyces*, was purchased from Huayueyang Biological Technology Co., Ltd. (Beijing, China). Plasmids pDS701, pSET152 containing a deacylase gene under the control of PermE, were constructed in our laboratory as described earlier (Wang et al. 2021). All chemicals, biochemicals, restriction enzymes, media, and molecular biological reagents were of analytical grade and obtained from standard commercial sources.

Gauserime synthetic agar plates (2% soluble starch, 0.05% NaCl, 0.05% K₂HPO₄·3H₂O, 0.1% KNO₃, 0.05% MgSO₄·7H₂O, 0.001% FeSO₄·7H₂O, and 1.5% agar powder [pH 7.4]) were used to culture strains for sporulation. Seed medium (2.5% sucrose, 2.0% oatmeal, 0.25% yeast powder, 0.1% K₂HPO₄, 0.05% KCl, 0.05%MgSO₄·7H₂O, and 0.0002% FeSO₄·7H₂O) and fermentation medium (2% sucrose, 1% peanut meal, 0.1% KH₂PO₄, and 0.025% MgSO₄·7H₂O) were used to ferment *A. utahensis*. And seed medium (1.0% glucose, 0.5% yeast powder, and 1% peptone) and fermentation medium (2.5% glucose, 1% beanflour, 0.3% NaCl, and 0.3% CaCO₃) were used to ferment *Streptomyces* (Wang et al. 2021).
Heterologous over-expression of deacylase gene

Plasmid pDS701 was introduced into different *Streptomyces* by intergeneric conjugation from *E. coli* ET12567 according to standard procedures (Luzhetskii et al. 2001). Recombinant strains were cultured in medium supplemented with 50 μg/mL apramycin, and further confirmed using PCR amplification with universal primers M13F-47 and M13R-48. The wild-type and recombinant cultures were grown on Gause-rime synthetic agar plates at 30 °C for sporulation. An agar piece was inoculated into 50 mL seed medium for 30 h at 30 °C on a shaker at 220 rpm. The resulting mycelial suspension was added (2%) to 50 mL fresh fermentation medium and further incubated for 48 h. *S. albus* without the plasmid was fermented as a control.

Determination of bioconversion efficiency

The wet mycelia were sedimented by centrifugation and washed twice with 0.1 M Tris buffer (pH 8.0). The 10 g cell pellet (20% m/v) was resuspended in a 50 mL Tris buffer. The enzymatic reaction was initiated by adding polymyxin B1 (2 g/L) and allowed to continue for 5 h at 30 °C with shaking at 50 rpm. The reaction mixture was immersed in an ice bath and stopped by adding an equivalent volume of methanol. The mixtures were centrifuged, and the supernatants were filtered through a 0.22 μm pore diameter membrane.

An analytical HPLC system was used to measure the cyclic heptapeptide polymyxin B1 released during the hydrolysis of polymyxin B1. Chromatographic separation was carried out on a Waters 2695 HPLC System (Milford, MA, USA) consisting of analytical C18 column (250×4.6 mm; 5 μm; Agilent Technologies, Santa Clara, CA, USA) with a mixture of sodium sulfate solution (30 mM, pH was adjusted to 2.3 with phosphoric acid) and acetonitrile in a ratio of 77.5:22.5 V/V as the mobile phase (Zhang et al. 2021). HPLC was performed at a flow rate of 0.8 mL/min for 35 min with UV detection at 215 nm. The compounds were identified using ESI–MS analysis performed on an Agilent HPLC 1260 coupled with a 6550 quadrupole time-of-flight mass spectrometry system. The method was linear at concentrations of 0.5–1500 μM with a correlation coefficient of 0.999. A standard curve was drawn using different amounts of cyclic heptapeptide polymyxin B1 and peak areas. The molar bioconversion rates were compared according to the following formula: bioconversion rate=((product of cyclic heptapeptide polymyxin B1/substrate addition)×100% (Tabakov et al. 1994). All enzyme measurements were performed in triplicate, and the maximum error was less than 5%.

Optimization of bioconversion conditions

Enzymatic activity was determined using the standard assay conditions for each case but using different buffers. The effect of pH was examined at different pH values ranging from 3.0 to 8.0 in disodium hydrogen phosphate-citrate buffer, from pH 5.5 to 8.0 in phosphate buffer, and from pH 7.5 to 10.0 in Tris buffer at 0.1 M constant ionic strength and 30 °C using 2 g/L polymyxin B1. The effect of metal ions was evaluated by adding 0.2 M KCl, NaCl, MgCl2, CuSO4 or FeSO4 to the Tris buffer. The effect of the ion concentration in the phosphate buffer and Tris buffer was also tested from 0.05 to 0.3 M.

The effect of temperature of SAL701 was measured at temperatures ranging from 25 to 70 °C in 0.1 M Tris buffer pH 8.0 using 2 g/L polymyxin B1. The reaction time was prolonged to 96 h to determine the biotransformation efficiency.

The optimum concentration of substrate was determined by adding different amounts of polymyxin B1 (1–12 g/L) under the optimized conditions in a total reaction volume of 200 mL using wild-type *A. utahensis* NRRL 12052, recombinant strains SAL701, SCO701 and SAM701. All enzyme measurements were performed in triplicate as above.

Thermostability and recycling of SAL701

The mycelia of *Streptomyces* strains carrying the deacylase gene were incubated at 4, 30, and 50 °C under the optimized conditions. At different time points during incubation, mycelia samples were withdrawn and assayed to determine their deacylase activity using polymyxin B1 as a substrate.

The mycelia were added to a 50 mL reaction buffer containing 2 g/L polymyxin B1 under the optimized conditions. After the enzymatic reaction for 5 h, the solution was centrifuged at 2000 rpm for 30 min, and the supernatant was measured using HPLC as
described above. The recovered mycelia were washed thrice with Tris buffer and used for another bioconversion cycle. All enzyme measurements were performed in triplicate as above.

Molecular docking

The deacylase homology protein structure model was built based on the crystal structure (PDB ID 5C9I; 39.1% identity) using Discovery Studio 2016, and the model with the lowest DOPE score (−79,981) was selected for docking (Kimura et al. 1992). Substrates polymyxin B1 for deacylase were modeled in Chem3D. Substrate docking was performed using AutoDock4. The substrate was docked into the binding pocket using flexible docking. AutoDock tools were used for enzyme and substrate preparations. To encompass the entire substrate-binding pocket, the docking box was set to 60×60×60 grid points with a grid spacing of 0.375 Å. The box center was set as X = 19.336, Y = −0.931, and Z = 52.155.

Results and discussion

Over-expression of deacylase gene in Streptomyces hosts

Enzyme engineering is a powerful tool for laboratory-studies and industrial production in vitro, but deacylase was rate-limiting in the antibiotics production process, as the bioconversion efficiency of deacylases in the original strain is low. Streptomyces strains are suitable heterologous hosts for producing enzymes, particularly GC-rich deacylases, for industrial applications (Ueda et al. 2011; Junji et al. 1993). To investigate the effect of deacylase gene, pDS701 plasmid consisting of the deacylase gene was introduced into six Streptomyces species (S. lividans TK24, S. coelicolor, S. albus, S. avermitilis K139, S. ambofaciens 2283 and S. griseus), yielding recombinant strains SLI701, SCO701, SAL701, SAV701, SAM701 and SGR701 (Shao et al. 2013). There were no apparent phenotypic differences between Streptomyces and its recombinant strains. Genotypes of six recombinant strains were verified using polymerase chain reaction (PCR). The 3.3-Kb DNA fragments were amplified. Sequencing and alignment confirmed that all six segments had 100% identity with the deacylase gene of recombinant plasmid pDS701 (Fig. 2). As negative controls, PCR products could not be amplified from S. albus host and wild-type A. utahensis.

Deacylation of different recombinant Streptomyces strains

Six recombinant Streptomyces strains and A. utahensis NRRL 12052 were inoculated; the mycelia were collected to determine the bioconversion efficiency. The reaction mixture containing 2 g/L polymyxin B1 in 0.1 M Tris buffer pH8.0, was incubated at 30 °C for 5 h and then analysed using HPLC. All seven samples showed a new peak, which had almost the same retention time as that of the standard cyclic heptapeptide moiety of polymyxin B1 (4.56 min, Fig. 3b), and a peak of the remaining polymyxin B1 substrate (31.10 min, Fig. 3a). The overlapped peak was also obtained by injecting the mixture of the SAL701 sample and standard cyclic heptapeptide polymyxin B1. So we preliminary verified that all six recombinant species and wild-type strain could deacylate polymyxin B1, with different activities dependent on the host strains. As shown in Table 1, five of the six recombinant strains showed similar bioconversion efficiencies, and although SAV701 showed a lower conversion efficiency (Fig. 3g), it was still higher
than that of the wild-type strain (34.0% compared with 23.3%). SAL701 (Fig. 3f) showed the highest efficiency with the highest percentage of bioconversion rate (65.2%). No polymyxin B1 or its cyclic heptapeptide moiety was detected in the whole-cells of all seven species examined. Therefore, S. albus containing the deacylase gene (SAL701) was considered the preferred strain for enzymatic deacylation. The SAL701 strain has many advantages, such as easy-cultivation, rapid-growth and high bioconversion efficiency; therefore, it could be applied to bioconversion of polymyxin B1 in a short time.

Qualitative analysis of polymyxin B1 and its cyclic heptapeptide

Polymyxin B1 and its cyclic heptapeptide moiety were extracted from the reaction mixture, and their identities were confirmed. The structure of polymyxin B1 and its cyclic heptapeptide was 2 g/L. (a) Standard polymyxin B1; (b) standard polymyxin B1 cyclic heptapeptide; (c) A. utahensis NRRL 12052; (d-i) SLI701, SCO701, SAL701, SAV701, SAM701 and SGR701.
the polymyxin B1 cyclic heptapeptide moiety was verified using electrospray ionization-mass spectrometry (ESI–MS). The parent ion was observed at m/z 784.4441 [M + Na]⁺ (calculated 784.4440), and its mass spectrum was consistent with the molecular formula C₃₅H₅₉N₁₁O₈ (Fig. 4a). Polymyxin B1 showed parent ions at m/z 602.3822 [M + 2H]²⁺ (calculated 602.3822) and m/z 1203.7566 [M + H]⁺ (calculated 1203.7572), and its mass spectrum was consistent with the molecular formula C₅₆H₉₈N₁₆O₁₃ (Fig. 4b) (Zhang et al. 2021). Impure preparations,

Table 1  Bioconversion rate by wild-type and different recombinant *Streptomyces* strains on bioconversion of 2 g/L polymyxin B1

| Strains               | Description                        | Bioconversion rate (%) |
|-----------------------|------------------------------------|------------------------|
| *A. utahensis* NRRL 12052         | Wild-type                          | 23.3 ± 3.2             |
| SLI701                 | pDS701 integrated into *S. lividans* TK24 | 58.1 ± 2.1             |
| SCO701                 | pDS701 integrated into *S. coelicolor* | 60.7 ± 0.4             |
| SAL701                 | pDS701 integrated into *S. albus*   | 65.2 ± 4.5             |
| SAV701                 | pDS701 integrated into *S. avermitilis* K139 | 34.0 ± 3.9            |
| SAM701                 | pDS701 integrated into *S. ambofaciens* 2283 | 59.8 ± 0.9             |
| SGR701                 | pDS701 integrated into *S. griseus*  | 52.3 ± 1.0             |
| *S. albus*             | *Streptomyces* without plasmid as negative control | 0                      |

Fig. 4  MS data of polymyxin B1 (a) and its cyclic heptapeptide (b)
polymyxin B containing polymyxin B1, were also successfully deacylated but at a slower rate.

Effect of reaction buffer on bioconversion of polymyxin B1 by SAL701

The buffer of the reaction mixture strongly affects the bioconversion rate. Hydrolysis of 2 g/L polymyxin B1 catalyzed by SAL701 was evaluated in different 0.1 M buffer pH range of 3.0–10.0 at 30 °C for 5 h. As shown in Table 2, the strain displayed the highest activity at pH 8.0 in Tris buffer (65.2%), which was selected as the standard buffer for deacylation. The same pH value (pH 7.5 or 8.0) in three different buffers were compared, and it was found that Tris buffer (60.8% and 65.2%) was more suitable for the reaction than phosphate buffer (60.5% and 62.9%) and disodium hydrogen phosphate-citrate buffer (53.2% and 52.3%).

Furthermore, the activities were increased when the ionic strength of the buffer was increased (0.05–0.3 M phosphate buffer and Tris buffer). The bioconversion efficiency increased from 56.3 to 70.1% in Tris buffer and from 52.8 to 61.2% in phosphate buffer (Table 3).

Deacylase activity does not require metal ions as previously reported (Shao et al. 2013). However, the inclusion of an ion (0.2 M K⁺, Na⁺, Mg²⁺ or Fe²⁺), enhanced the deacylase activity (73.9%, 72.1%, 74.7% and 72.7% respectively, compared to 70.1% for the enzyme alone, Table 3). Addition of Cu²⁺ did not increase the activity. Therefore, 0.2 M Tris buffer pH 8.0 with 0.2 M Mg²⁺ was selected as the standard condition for the deacylation reaction.

Effect of reaction temperature and time on bioconversion of polymyxin B1 by SAL701

The enzymatic activity varies over a temperature range of 25–70 °C. Maximum deacylase activity was achieved at 40 °C under the optimized reaction buffer (0.2 M Tris buffer, pH 8.0 with 0.2 M Mg²⁺); the conversion rate of polymyxin B1 was 76.9% (Fig. 5).

The ability of SAL701 to deacylate polymyxin B1 was examined within 96 h. The time course was determined under the optimized buffer conditions. As shown in Fig. 6, the deacylated product occurred at the earliest time tested (5 h), and the bioconversion efficiency was 74.8%, and then gradually increased to 77.7% at 96 h. Therefore, we concluded that

### Table 2 Effect of reaction buffer on bioconversion of 2 g/L polymyxin B1

| pH     | Bioconversion rate (%) |
|--------|------------------------|
|        | disodium hydrogen phosphate-citrate buffer | phosphate buffer | Tris buffer |
| 3.0    | 21.2 ± 1.2             |
| 3.5    | 19.3 ± 3.4             |
| 4.0    | 23.6 ± 0.9             |
| 4.5    | 25.9 ± 4.6             |
| 5.0    | 26.9 ± 3.0             |
| 5.5    | 33.3 ± 2.7             |
| 6.0    | 42.8 ± 3.5             |
| 6.5    | 41.4 ± 2.2             |
| 7.0    | 42.9 ± 4.1             |
| 7.5    | 53.2 ± 0.8             |
| 8.0    | 52.3 ± 1.2             |
| 8.5    | 52.3 ± 1.2             |
| 9.0    | 52.3 ± 1.2             |
| 9.5    | 52.3 ± 1.2             |
| 10.0   | 60.3 ± 2.0             |

### Table 3 Effect of ions and ionic strength on bioconversion of 2 g/L polymyxin B1

| Buffer         | Ionic | 0.05 M | 0.1 M | 0.2 M | 0.3 M |
|----------------|-------|--------|-------|-------|-------|
| phosphate buffer | –     | 52.8 ± 1.7 | 55.6 ± 3.9 | 59.0 ± 2.2 | 61.2 ± 1.6 |
| Tris buffer     | –     | 56.3 ± 4.2 | 65.2 ± 1.1 | 70.1 ± 2.9 | 69.2 ± 1.0 |
| Tris buffer K⁺  | –     | –       | –     | 73.9 ± 3.0 | –     |
| Tris buffer Na⁺ | –     | –       | –     | 72.1 ± 1.5 | –     |
| Tris buffer Mg²⁺| –     | –       | –     | 74.7 ± 2.1 | –     |
| Tris buffer Cu²⁺| –     | –       | –     | 69.2 ± 2.0 | –     |
| Tris buffer Fe²⁺| –     | –       | –     | 72.7 ± 1.3 | –     |
extending the time can improve the conversion rate, but the efficiency increases slowly after 5 h. So 5 h can be selected in future experiments.

Effect of substrate concentration on bioconversion of polymyxin B1 by SAL701

The optimum concentration of polymyxin B1 in the bioconversion mixture was confirmed using SAL701, SCO701, SAM701 recombinant strains and wild-type A. utahensis NRRL 12052, the relatively highly active recombinant Streptomyces strains. The optimal substrate concentration of SAL701 was increased from 2 to 6 g/L, under conditions in which the bioconversion efficiency was more than 60%, those of SCO701 and SAM701 were increased from 2 to 4 g/L (Table 4).

It is necessary to deploy engineered deacylase for practical, industrial scale applications. In this section, we studied the potential of using recombinant strains containing the deacylase gene for industrial-scale polymyxin B1 cyclic heptapeptide production by preliminary increasing the substrate amount. The catalytic activity of enzyme can be further improved through chassis metabolic engineering and fermentation process optimization.

**Table 4** Effect of substrate concentration on bioconversion of polymyxin B1

| Polymyxin B1 concentration (g/L) | Bioconversion rate (%) |
|----------------------------------|------------------------|
|                                  | SAL701 | SCO701 | SAM701 | A. utahensis NRRL 12052 |
| 1.0                              | 78.4 ± 3.0 | 74.8 ± 2.5 | 75.1 ± 2.1 | 39.4 ± 2.2 |
| 2.0                              | 73.9 ± 1.7 | 72.4 ± 1.7 | 72.3 ± 2.8 | 31.8 ± 1.8 |
| 3.0                              | 70.2 ± 1.3 | 69.4 ± 1.5 | 68.9 ± 0.6 | 26.4 ± 3.0 |
| 4.0                              | 70.7 ± 1.3 | 66.4 ± 2.5 | 64.3 ± 2.8 | 22.5 ± 0.3 |
| 6.0                              | 61.2 ± 0.8 | 58.7 ± 3.1 | 56.1 ± 0.5 | 21.0 ± 0.7 |
| 8.0                              | 43.7 ± 0.3 | 43.6 ± 3.2 | 50.4 ± 1.5 | 23.0 ± 1.7 |
| 10.0                             | 47.3 ± 1.3 | 36.8 ± 2.7 | 41.4 ± 1.7 | 19.5 ± 1.2 |
| 12.0                             | 33.9 ± 1.6 | 37.9 ± 2.8 | 30.1 ± 3.9 | 21.2 ± 3.9 |

**Fig. 5** Effect of reaction temperature on bioconversion of 2 g/L polymyxin B1

**Fig. 6** Effect of reaction time on bioconversion of 2 g/L polymyxin B1

**Fig. 7** Thermostability of SAL701 at 4 (filled circle), 30 (filled diamond) and 50 °C (filled triangle) on bioconversion of 2 g/L polymyxin B1

**Fig. 7** Thermostability of SAL701 at 4 (filled circle), 30 (filled diamond) and 50 °C (filled triangle) on bioconversion of 2 g/L polymyxin B1

**Thermostability of SAL701**

The thermostability of the mycelia of recombinant S. albus was evaluated at different temperatures under the optimized conditions above (Fig. 7). The enzyme showed no decrease inactivity after storage at 4 °C and 30 °C for at least 48 h. However, the mycelia also maintained 90% of its activity following storage at 50 °C for at least 12 h.
Recycling of mycelia of SAL701

The mycelia was used to bioconvert polymyxin B1, and the number of times required for the mycelia to be reused was investigated. The mycelia of SAL701 could be recycled at least three times without loss of catalytic activity on the basis of maintaining the bioconversion efficiency at 70.2% under the optimized conditions above (Table 5).

Table 5 Number of times of reutilizing the SAL701 mycelia on bioconversion of 2 g/L polymyxin B1

| Number of times | Bioconversion rate (%) |
|----------------|------------------------|
| 1              | 72.4 ± 0.2             |
| 2              | 73.5 ± 3.1             |
| 3              | 70.2 ± 3.0             |
| 4              | 50.3 ± 3.7             |
| 5              | 30.9 ± 1.8             |
| 6              | 23.4 ± 1.6             |
| 7              | 6.3 ± 1.2              |
| 8              | 6.9 ± 2.4              |

for the catalytic site. The specificity of deacylase could prevent the formation of impurities, reduce the difficulty in the final purification process, and improve the economic benefits of the process.

An enzyme’s specificity depends on its structure. As a representative functional deacylase, the sequence similarity to many other deacylases. Although a few deacylase structures have been reported, that of a functional deacylase has not been determined. Homology protein structure models for deacylase were constructed to identify the structural basis for the programming of this distinct specificity (Fig. 8) based on the known crystal structure of the MacQ (PDB ID 5C9I; resolution: 1.80 Å, and identity of 39.1% with deacylase). MacQ derived from Acidovorax sp. strain MR-S7 is comprised of 806 amino acid residues, and exhibits acylase activity against β-lactam antibiotics and N-acylhomoserine lactones (AHLs). Structural comparison with MacQ revealed that deacylase has a similar structure and active site organization, indicating that the key enzyme-co-product interactions among MacQ are well-conserved, deacylase and similar enzymes such as the catalytic site specificity. Ser/Thr/Cys is the necessary residue of catalysis, and initiates amide bond cleavage of various substrate compounds as a nucleophile. Similar to Ser1β, which act as a catalytic residue in MacQ, the docking results indicated that Thr190 in deacylase from A. utahensis NRRL 12052, which is located near amide bond 1, can facilitate hydrolysis with high regioselectivity (Yasutake et al. 2017; Seemüller et al. 1995; Duggleby et al. 1995). The relatively close distance of the hydroxyl group to the amide carbon atom of the peptide bond suggests an important role for the residues in catalysis, as revealed by substrate docking. Based on the above research, we could predict similar enzyme-catalyzed reactions mediated by this class of enzymes.

Substrate spectrum of SAL701

The aforementioned results established the characteristic of SAL701 and necessitated further research about their substrate spectrum. The chemical structure of β-lactam antibiotics also consists of a β-lactam ring and a side chain. Different β-lactam substrates were used to determine the bioconversion rate. However, no enzymatic deacylation was observed for the β-lactam compounds penicillin G and cephalosporin C.

Hydrolysis of specific amide bonds of SAL701

Enzyme-catalyzed reactions are specific. Amidohydrolase from E. coli N.C.I.B. 8743 is stereospecific for the deacylation of α-amino acids, acylated L-α-amino acids, but not D-α-amino acids are hydrolyzed, whereas DL-amino acids are only 50% hydrolyzed (Parmar et al. 1992; Cole 1969; Liu et al. 2020). SAL701 catalyzes hydrolysis of amide bond 1 (Fig. 1), which is the closest amide bond to the cyclic heptapeptide of polymyxin B1, rather than hydrolysis of other amide bonds, suggesting its high specificity.

Conclusion

We previously reported the hydrolytic activity for the side chains of echinocandin B and daptomycin by the recombinant Streptomyces. This compelled us to explore different antibiotics of great significance (Shao et al. 2013; Wang et al. 2021). In this study, we describe an efficient bioconversion process of polymyxin B1 to its cyclic heptapeptide, a starting
material in the synthesis of antibiotics, which has not been reported before. And this recombinant Streptomyces strain SAL701 may be useful in the antibiotic industry for catalyzing the hydrolysis of polymyxin B1. We also attempted to hydrolyze other important antibiotics with similar structures, to expand the application of recombinant strain in the future.

Furthermore, we have determined the optimal reaction conditions for this process; 40 °C in 0.2 M Tris buffer (pH 8.0) with 0.2 M Mg2+, and the maximum bioconversion rate was 77.7%. Also the mycelia of SAL701 was highly thermostable and reusable. Additionally, catalytic site specificity of the deacylase was described and predicted by homology modeling and docking, which showed that Thr190 facilitates hydrolysis with high regioselectivity. All the above results illustrate that SAL701 strain is effective in catalyzing polymyxin B1 to its cyclic heptapeptide moiety. The recombinant strain may be used for the industrial production of polymyxin antibiotics.

**Author contributions** XW, KW and HZ performed the experiments and data analyses, and wrote the manuscript. LS and HL designed the study plan. JL, ZY and JB revised the manuscript. All authors read and approved the final manuscript.

**Funding** This research was financially supported by the Natural Science Foundation of Shanghai (20ZR1424600), the National Natural Science Foundation of China (81773616), the Shanghai Excellent Technology Leader Program (17XD1423200), and Nature Science Foundation of Jiangsu Higher Education Institutions of China (20KJB180002).

**Declarations**

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

**References**

Bairamashvili DI, Voitenko VG, Gushchin IS, Zinchenko AA, Miroshnikov AI, Zebrev AI (1989) Histamine releasing action of polymyxin B and its analogs. Bulletin Exp Biol Med 107(4):504–506. https://doi.org/10.1007/BF00842390

Boeck LD, Fukuda DS, Abbott BJ, Debono M (1988) Deacylation of A21978C, an acidic lipopeptide antibiotic complex, by *Actinoplanes utahensis*. J Antibiot (tokyo) 41(8):1085–1092. https://doi.org/10.7164/antibiotics.41.1085

Boeck LVD, Fukuda DS, Abbott BJ, Debono M (1989) Deacylation of echinocandin b by *Actinoplanes utahensis*. J Antibiot 42(3):382–388. https://doi.org/10.7164/antibiotics.42.382

Brown P, Dawson M (2017a) Development of new polymyxin derivatives for multi-drug resistant Gram-negative infections. J Antibiot 70:386–394. https://doi.org/10.1038/ja.2016.146

Brown P, Dawson MJ (2017b) Development of new polymyxin derivatives for multi-drug resistant Gram-negative infections. J Antibiot 70(4):386–394. https://doi.org/10.1038/ja.2016.146
Ciabatti R, Malfioli SI, Panzone G, Canavesi A, Michelucci E, Tiseni PS, Marzorati E, Checchia A, Giannionne M, Jabes D (2007) Synthesis and preliminary biological characterization of new semisynthetic derivatives of ramoplanin. J Med Chem 50(13):3077–3085. https://doi.org/10.1021/jm070042c

Cole M (1969) Deacylation of acylamino compounds other than penicillins by the cell-bound penicillin acylase of Escherichia coli. Biochem J 115(4):741–745. https://doi.org/10.1042/bj1150741

Cudic P, Behenna DC, Kranz JK, Kruger RG, Wang AJ, Veklich YI, McCafferty DG (2002) Functional analysis of the lipoglycodelpsipeptide antibiotic ramoplanin. Chem Biol 9(8):897–906. https://doi.org/10.1016/S1074-5521(02)00191-6

Cui AL, Hu XX, Chen Y, Jin J, Yi H, Wang XK, He QY, You XF, Li ZR (2018) Synthesis and preliminary biological characterization of new semisynthetic derivatives of ramoplanin. J Med Chem 50(13):3077–3085. https://doi.org/10.1021/jmc.8b00976

Duggleby HJ, Tolley SP, Hill CP, Dodson EJ, Dodson G, Baker PJ, Mcgilvray D, Yeh WK (2000) Membrane-associated echinocandin B deaclyase of Actinoplanes utahensis: purification, characterization, heterologous cloning and enzymatic deacylation reaction. J Ind Microbiol Biotechnol 24(3):173–180. https://doi.org/10.1016/sj.jimbiot.2900796

He J, Abdelraouf K, Ledesma KR, Chow DSL, Tam VH (2013) Pharmacokinetics and efficacy of liposomal polymyxin B in a murine pneumonia model. Int J Antimicrob Agents 42(6):559–564. https://doi.org/10.1016/j.ijantimicag.2013.07.009

Hormigo D, Mata IDL, Acebal C, Arroyo M (2010) Immobilized aculeacin A acylase from Actinoplanes utahensis: characterization of a novel biocatalyst. Biore sour Technol 101(12):4261–4268. https://doi.org/10.1016/j.biortech.41.1085

Junji I, Hideo T, Haruo IM (1993) Efficient production of aculeacin A acylase in recombinant Streptomyces strains. Appl Microbiol Biotechnol 39:532–536. https://doi.org/10.1007/BF00205046

Kimura Y, Matsunaga H, Vaara M (1992) Polymyxin B octapeptide and polymyxin B heptapeptide are potent outer membrane permeability-increasing agents. J Antibiot 45(5):742–749. https://doi.org/10.1016/antibiotics.45.742

Kreuzman AJ, Hodges RL, Swartling JR, Pohle TL, Ghag SK, Baker PJ, Mcgilvray D, Yeh WK (2000) Membrane-associated echinocandin B deaclyase of Actinoplanes utahensis: purification, characterization, heterologous cloning and enzymatic deacylation reaction. J Ind Microbiol Biotechnol 24(3):173–180. https://doi.org/10.1016/sj.jimbiot.2900796

Liu X, Yang M, Liu Y, Ge F, Zhao J (2020) Structural and functional insights into a lysine deaclyase in the Cyanobacterium Synechococcus sp. PCC 7002. Plant Physiol 184(2):762–776. https://doi.org/10.1104/pp.20.00583

Luzhetzkii AN, Ostash BE, Fedorenko VA (2001) Intergeneric conjugation Er cherichia coli-Streptomyces globisporus 1912 using integrative plasmid pSET152 and its derivatives. Russ J Genet 37:1123–1129. https://doi.org/10.1023/a:1012344319564

McCafferty DG, Cudic P, Frankel BA, Barkallah S, Kruger RG, Li W (2002) Chemistry and biology of the ramoplanin family of peptide antibiotics. Biopolymers 66:261–284. https://doi.org/10.1002/bip.10296

Okimura K, Ohki K, Sato Y, Ohashi K, Sakura N (2007) Semi-synthesis of polymyxin B (2–10) and colistin (2–10) analogs employing the trichloroethoxy carbonyl (Troc) group for side chain protection of α, γ-diaminobutyric acid residues. Chem Pharm Bull 55(12):1724–1730. https://doi.org/10.1248/cpb.55.1724

Parrm VS, Prasad AK, Singh PK (1992) Lipase-catalysed transesterifications using 2, 2, 2-trifluoroethyl butyrate: effect of temperature on rate of reaction and enantioselectivity. Tetrahedron Asymmetry 3(11):1395–1398. https://doi.org/10.1016/0105-0020(92)80015-O

Petraitienė R, Petraitis S, Chandarlapalli M, Sein T, Bell A, Lyman CA, McMillian CL, Bacher J, Walsh TJ (1999) Antifungal activity of LY303366, a novel echinocandin B, in experimental disseminated candidiasis in rabbits. Anti microbe Agents Chemother 43:2148–2155. https://doi.org/10.1128/AAC.43.9.2148

Seemuller E, Lapes A, Stock D, Lüwe J, Huber R, Baumeister W (1995) Proteasome from Thermoplasma acidophilum: a threonine protease. Science 268(5210):579–582. https://doi.org/10.1126/science.7725107

Shao L, Li J, Liu AJ, Chang Q, Lin HM, Chen DJ (2013) Efficient biocconversion of echinocandin B to its nucleus by overexpression of deaclyase genes in different host strains. Appl Environ Microb 79(4):1126–1133. https://doi.org/10.1128/AEM.02792-12

Tabakov V, Voeikova TA, Tokmakova IL, Bolotin AP, Frollova E, Lomovskaia D (1994) Intergeneric Er cherichia coli-S treptomyces conjugation as a means for the transfer of conjugative plasmids into producers of antibiotics
chlortetracycline and bialaphos. Genetika 30:57–61. 
https://doi.org/10.1111/1468-5965.00207

Ueda S, Shibata T, Ito K, Oohata N, Yamashita M, Hino M, Yamada M, Isogai Y, Hashimoto S (2011) Cloning and expression of the FR901379 acylase gene from Streptomyces sp. no. 6907. J Antibiot (tokyo) 64:169–175. https://doi.org/10.1038/ja.2010.151

Voitenko VG, Bayramashvili DI, Zebrev AI, Zinchenko AA (1990) Relationship between structure and histamine releasing action of polymyxin B and its analogues. Agents Actions 30(1):153–156. https://doi.org/10.1007/BF01969025

Wang XJ, Liu J, Zhang HZ, Wu K, Yang ZJ, Ning RN, Huang JH, Shao L (2021) Efficient bioconversion of daptomycin to its nucleus by heterologous expression of deacylase genes in Streptomyces. J Chem Technol Biotechnol 96(11):3066–3073. https://doi.org/10.1002/jctb.6858

Yasutake Y, Kusada H, Ebuchi T, Hanada S, Kamagata Y, Tamura T, Kimura N (2017) Bifunctional quorum-quenching and antibiotic-acylase MacQ forms a 170-kDa capsule-shaped molecule containing spacer polypeptides. Sci Rep 7(1):1–11. https://doi.org/10.1038/s41598-017-09399-4

Zhang HZ, Li F, Dun JL, Sun N, Liu H, Chen GL (2021) Combination of derivatization-HPLC-MS and enzymatic hydrolysis-edman degradation for amino acid sequence and configuration of polymyxin B components. Chromatographia 84:1057–1064. https://doi.org/10.1007/s10337-021-04091-2

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.