

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

Xiaojing Wang (✉ wxj2055@126.com)

Shanghai University of Medicine and Health Sciences <https://orcid.org/0000-0002-9636-6511>

Kai Wu

Shanghai University of Medicine and Health Sciences

Hanzhi Zhang

Abiochem biotechnology Co., Ltd

Jing Liu

Shanghai University of Medicine and Health Sciences

Zhijun Yang

Shanghai University of Medicine and Health Sciences

Jing Bai

Suzhou University of Science and Technology

Hao Liu

Shanghai institute for food and drug control

Lei Shao

Shanghai University of Medicine and Health Sciences <https://orcid.org/0000-0003-4130-2817>

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Abstract

Objectives: Polymyxins are antibacterial polypeptides used as “last resort” therapy option for multidrug-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 catalysed hydrolysis of polymyxin B1, yielding cyclic heptapeptide. The efficiency of recombinant *S. albus* (SAL701) was higher than others, whereby 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 Thr190 in deacylase can facilitate hydrolysis with high regioselectivity.

Conclusions: These results show that SAL701 is effective in increasing cyclic heptapeptide moiety of polymyxin B1. These properties of the biocatalyst may enable its development in the industrial production of polymyxins antibiotics.

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. 1.¹ The core scaffold contains a cyclic heptapeptide linked to the side chain of a linear tripeptide with an N-terminal fatty acyl.² They have been reused as “last resort” therapy option for multidrug-resistant Gram-negative bacteria.¹ The situation is worsen by the emergence of polymyxin-resistant infections. The development of novel polymyxin derivatives increase its activity and reduce toxicity.² The antibacterial activity of polymyxin B1 is mainly affected by changing the amino acid of the side chain.³ Numerous novel compounds have been semi-synthesized by deacylating and replacing their side chains with a variety of different chains.^{4,5} 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.^{6,7} Therefore, polymyxin B1 analogues^{6,7} with increased antibacterial activity can generate by direct modifications of the cyclic peptide.⁶

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.⁸⁻¹⁰ The enzyme hydrolyze the acyl moieties of antibiotic echinocandins, and forms a cyclic hexapeptide moiety and long-chain fatty acid.^{11,12} The resulting cyclic hexapeptide can be further reacylated to produce a series of analogs useful as therapeutic antibiotics in clinical practice, such as anidulafungin.^{13,14} Ramoplanin is a lipoglycopeptide with antibacterial activity against major Gram-positive bacteria.^{15,16} Numerous novel ramoplanin derivatives have been semi-synthesized by deacylating and replacing their side chains with different kinds of carboxylic acids.¹⁷ Among them, the 2-methylphenylacetic acyl group, displays increased tolerability and high activity, indicating its potential for overcoming the limitations of ramoplanin *in vivo*.^{18,19} 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.^{20,21}

In this study, we attempted to expand the substrate spectrum of recombinant *Streptomyces* with deacylase gene, and we found that the enzyme could hydrolyze polymyxin B1. In addition, we tried to predict the specificity of the catalytic site through structure analysis, and payed attention to its potential biotechnological application.

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.²¹ All chemicals, biochemicals, restriction enzymes, media and molecular biological reagents were of analytical grade and obtained from standard commercial sources.

Gausserime 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*.²¹

Heterologous over-expression of deacylase gene

Plasmid pDS701 was introduced into different *Streptomyces* by intergeneric conjugation from *E. coli* ET12567 according to standard procedures.²² 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. Wild-type and recombinant cultures were grown on Gauseme 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 fresh fermentation medium and further incubated for 48 h. *S. albus* without the plasmid was fermented as a control.

Determination of bioconversion efficiency

We used the whole-cell reaction system for bioconversion, because it is convenient, high efficiency, reusability and safety. 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.⁷ 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 daptomycin cyclic heptapeptide polymyxin B1]/substrate addition) × 100%.²³ 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 by 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°C to 70°C in 0.1 M Tris buffer pH 8.0 by 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.

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 3000 ×g for 30 min, and the supernatant was measured using HPLC as described above. The recovered mycelia were washed with Tris buffer for three times and used for another bioconversion cycle.

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 (-79981) was selected for docking.²⁴ 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

Enzymatic modification is more efficient than chemical modification, 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.^{25,26} To investigate the effect of deacylase gene, pDS701 plasmid consist of 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 SGR701.¹³ There were no apparent phenotypic differences between *Streptomyces* and its recombinant strains. Genotypes of six recombinant strains were verified by 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, 5 h and then analysed by high-performance liquid chromatography (HPLC). All seven samples showed the new peak, which almost having the same retention time with the standard cyclic heptapeptide moiety of polymyxin B1 (4.56 min, Fig. 3b), and a peak of remaining polymyxin B1 substrate (31.10 min, Fig. 3a). The overlapped peak also obtained by injecting the mixture of 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 activity 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, the *S. albus* were considered as the preferred host for enzymatic deacylation. SAL701 strain has many advantages such as easy-cultivating, rapid-growing and high bioconversion efficiency, so it could be applied to bioconversion of polymyxin B1 in a short time.

Table 1 Bioconversion rate by wild-type and different recombinant *Streptomyces* strains

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

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 the polymyxin B1 cyclic heptapeptide moiety was verified by 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 $C_{35}H_{59}N_{11}O_8$ (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 $C_{56}H_{98}N_{16}O_{13}$ (Fig. 4b).⁷ Impure preparations, 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 reaction mixture strongly affects the bioconversion rate. Hydrolysis of 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 found that Tris buffer (60.8% and 65.2%) was more suitable for reaction than phosphate buffer (60.5% and 62.9%) and disodium hydrogen phosphate-citrate buffer (53.2% and 52.3%).

Table 2 Effect of reaction buffer

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	31.9 ± 2.2	-
6.0	42.8 ± 3.5	41.3 ± 4.0	-
6.5	41.4 ± 2.2	50.9 ± 3.1	-
7.0	42.9 ± 4.1	55.6 ± 3.7	-
7.5	53.2 ± 0.8	60.5 ± 0.8	60.8 ± 3.0
8.0	52.3 ± 1.2	62.9 ± 0.9	65.2 ± 4.0
8.5			64.7 ± 1.2
9.0	-	-	61.5 ± 1.9
9.5	-	-	62.2 ± 2.1
10.0			60.3 ± 2.0

Also, the activities were increased when the ionic strength of 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).

Table 3 Effect of ionic and ionic strength

Buffer	Ionic	Bioconversion rate (%)			
		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	Mg ²⁺	-	-	74.7 ± 2.1	-
Tris buffer	Cu ²⁺	-	-	69.2 ± 2.0	-
Tris buffer	Fe ²⁺	-	-	72.7 ± 1.3	-

Deacylase activity did not require metal ions as previously studied. But 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 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 during 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.

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 2.0 to 6.0 g/L, under conditions in which the bioconversion efficiency was more than 60%, and SCO701, SAM701 were increased from 2.0 to 4.0 g/L (Table 4).

Table 4 Effect of substrate concentration

polymyxin B1 concentration (g/L)	Bioconversion rate (%)			
	SAL701	SCO701	SAM701	<i>A. utahensis</i> 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

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 in activity 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, 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 mycelia

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

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 a β -lactam ring and a side chain. Different β -lactam substrates were used to determine the bioconversion rate. But 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.²⁷⁻²⁹ SAL701 catalyzes hydrolysis of amide bond 1 (Fig. 1), which is the closest amide bond to the cyclic heptapeptide of polymyxin B1, rather than at other amide bonds, suggesting its high specificity for the catalytic site. The specificity of deacylase could avoid the existence 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 was 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 are 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 Ser1 β , which act as a catalytic residue in MacQ, the docking results indicated that Thr190 in deacylase

from *A.utahensis* NRRL 12052, which located near amide bond 1, can facilitate hydrolysis with high regioselectivity.^{24,30,31} 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.

Conclusion

We previously reported the hydrolytic activity for the side chains of echinocandin B and daptomycin by the recombinant *Streptomyces*, this drove us to explore different antibiotics of great significance.^{13,21} In this study, we efficiently bioconversion polymyxin B1 to its cyclic heptapeptide, a starting material synthesizing antibiotics, which has not been reported before, and deacylase may be useful in the antibiotic industry for catalyzing the hydrolysis of polymyxin B1. We also tried to hydrolyze other important antibiotics with a similar structure, 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⁺. Also the mycelia of SAL701 was high thermostability and reusability. Additionally, catalytic site specificity of the deacylase was described and predicted by homology modeling and docking, which showed Thr190 facilitates hydrolysis with high regioselectivity. All above results show that recombinant *Streptomyces* strain SAL701 is effective in catalysing polymyxin B1 to its cyclic heptapeptide moiety. The recombinant strain may be used for the industrial production of polymyxin antibiotics.

Declarations

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Author Contributions Xiaojing Wang, Kai Wu and Hanzhi Zhang performed the experiments and data analyses, and wrote the manuscript. Lei Shao and Hao Liu designed the study plan. Jing Liu, Zhijun Yang and Jing Bai revised the manuscript. All authors read and approved the final manuscript.

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Figures

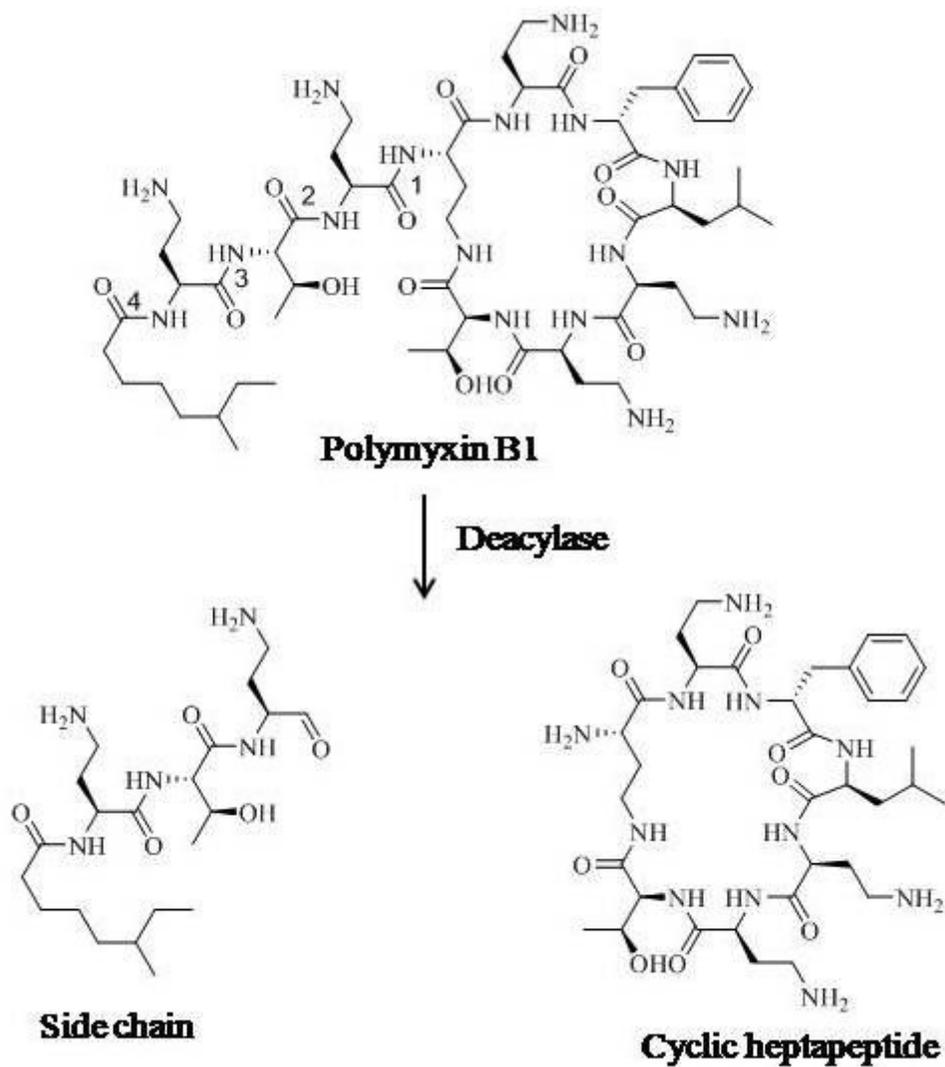


Figure 1

Bioconversion of polymyxin B1 to its cyclic heptapeptide by deacylase.

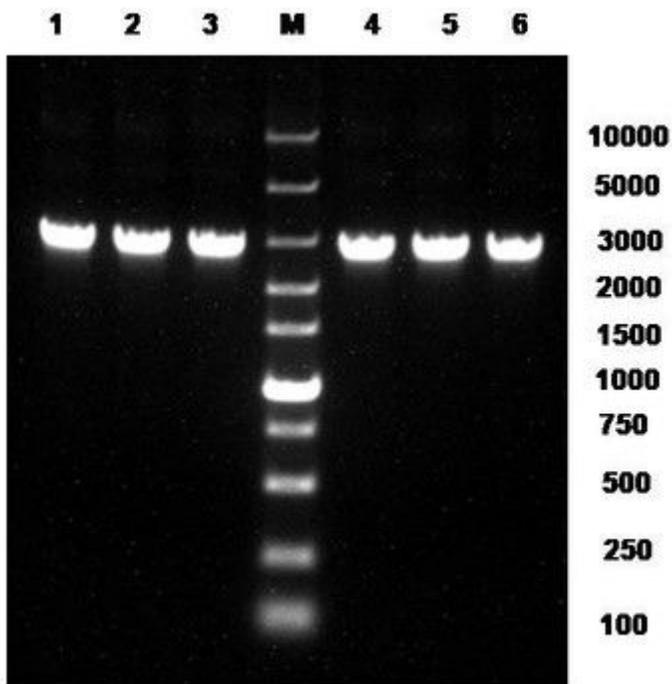


Figure 2

Genotype identification of six *Streptomyces* recombinant strains by PCR reaction. Lane 1-6, 3.3 kb products for SLI701, SCO701, SAL701, SAV701, SAM701 and SGR701. Marker: 100, 250, 500, 750, 1000, 1500, 2000, 3000, 5000 and 10000 bp.

Figure 3

HPLC profiles of deacylation reaction mixtures by *A. utahensis* NRRL 12052 or different recombinants. Standard polymyxin B1 and its cyclic heptapeptide were shown as control. (A) Standard polymyxin B1; (B) standard polymyxin B1 cyclic heptapeptide; (C) *A. utahensis* NRRL 12052; (D-I) SLI701, SCO701, SAL701, SAV701, SAM701 and SGR701.

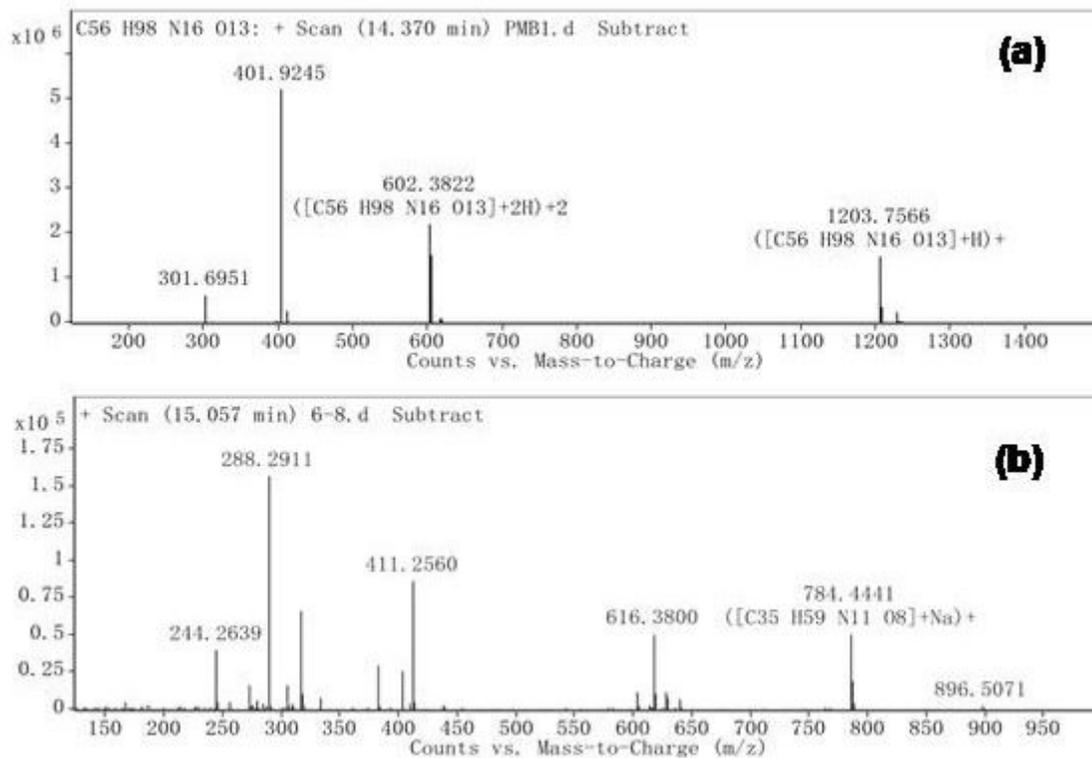


Figure 4

MS data of polymyxin B1 (4a) and its cyclic heptapeptide (4b)

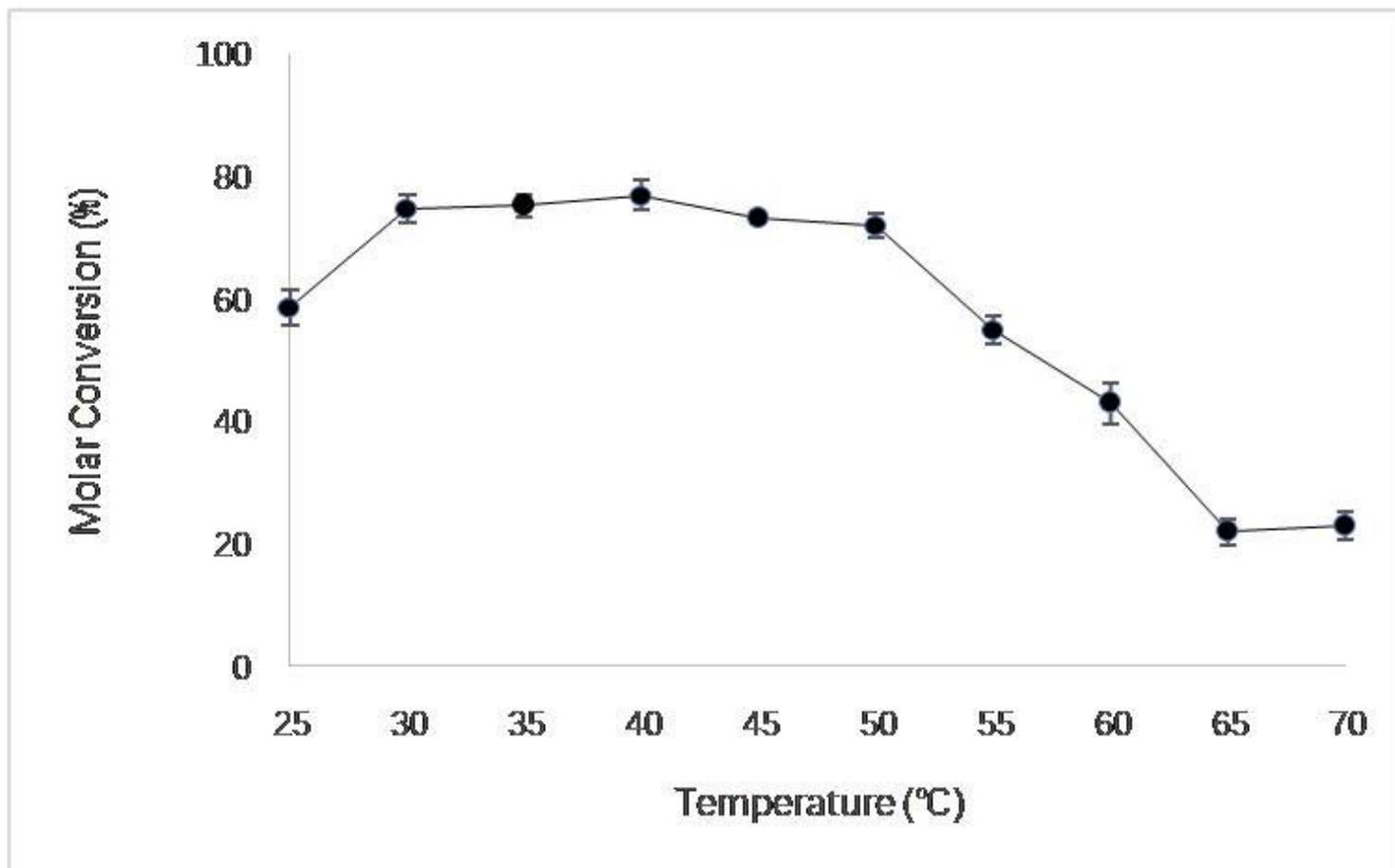


Figure 5

Effect of temperature on bioconversion of polymyxin B1 by SAL701

Figure 6

Effect of reaction time on bioconversion of polymyxin B1 by SAL701

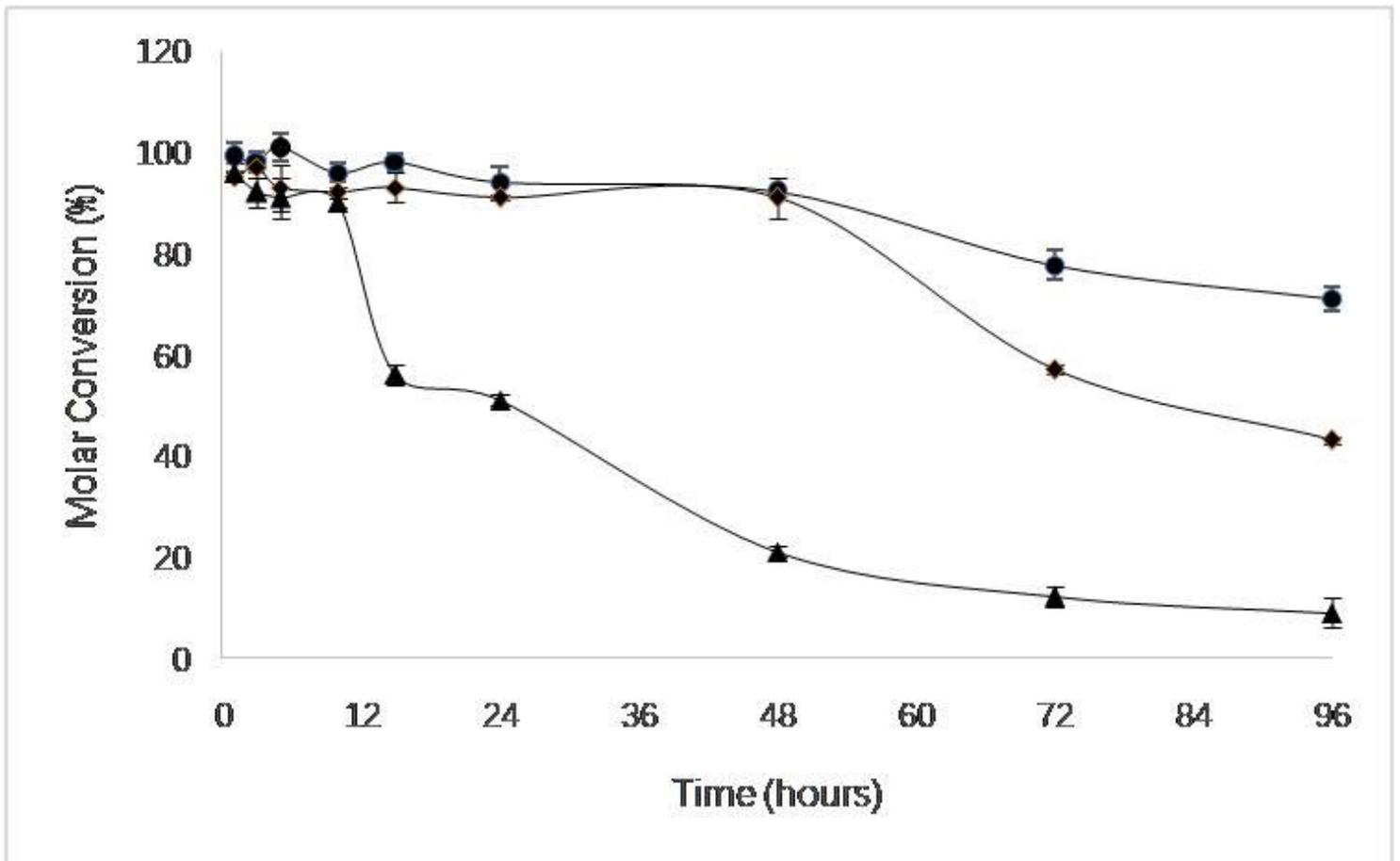


Figure 7

Thermostability of SAL701 at 4 (●), 30 (☐) and 50°C (▲)

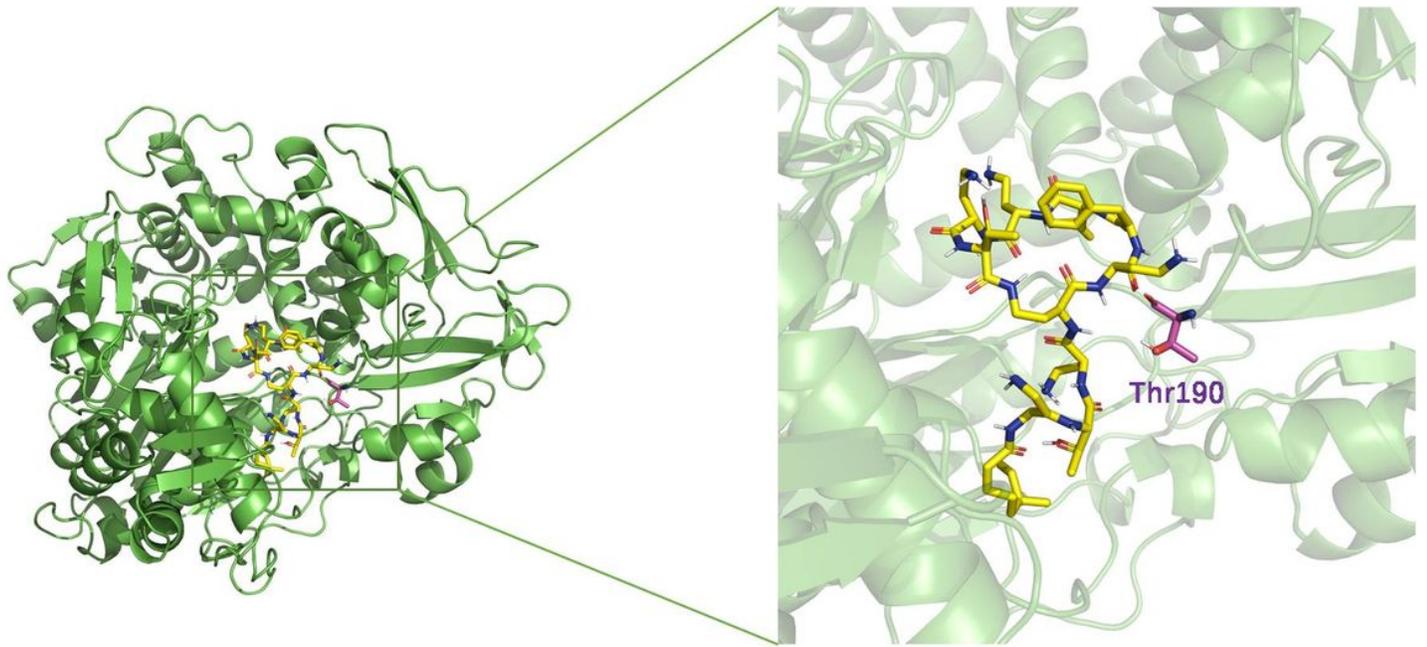


Figure 8

Substrate docking based on structure model of deacylase from *A. utahensis* NRRL 12052. The substrate is colored in yellow and potential catalytic residue Thr190 is colored in purple.