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# Enhancement of ε-poly-L-lysine production by overexpressing the ammonium transporter gene in *Streptomyces albulus* PD-1

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**Abstract**

The antibacterial polymer ε-poly-L-lysine (ε-PL) has been widely used as a safe food preservative. As the synthesis of ε-PL requires a rich supply of nitrogen, the efficiency of nitrogen translocation and utilization is extremely important. The objective of this study was to improve the production of ε-PL by overexpressing the ammonium transporter gene *amtB* in *Streptomyces albulus* PD-1. Using the recombinant bacteria, the optimum carbon-to-nitrogen ratio in the synthesis stage of fermentation increased from 3 to 4.71, compared with that obtained using the wild-type strain, and the utilization efficiency of ammonium was improved too. Ultimately, the production of ε-PL increased from 22.7 to 35.7 g/L upon fed-batch cultivation in a 5 L bioreactor. Determination of the expression of the genes and enzymes associated with ammonium metabolism and ε-PL synthesis revealed that the overexpression of *amtB* in *S. albulus* PD-1 enhanced ε-PL biosynthesis by increasing the activity of the corresponding metabolic pathways. To the best of our knowledge, this is the first report on enhancing ε-PL production by overexpression of the *amtB* gene in an ε-PL-producing strain.

**Keywords** ε-poly-L-lysine · Nitrogen source · Ammonium transporter · *Streptomyces albulus* PD-1

## Introduction

The biopolymer ε-poly-L-lysine (ε-PL) is composed of 25–35 L-lysine residues with linkages between the α-carboxyl and ε-amino groups [1]. It is mainly produced by the members of the family Streptomycetaceae [2, 3].

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ε-PL is regarded as safe for human consumption owing to its broad-spectrum antimicrobial properties, water solubility, thermal stability, and non-toxicity [4]. As such, ε-PL has been approved as a food preservative in Japan, Korea, the United States and China [5, 6]. Furthermore, because it is biocompatible and biodegradable, ε-PL and its derivatives have been widely used as emulsifiers, biodegradable fibers, high-strength hydrogels, drug carriers, and biochips [7–9]. For example, recent studies demonstrate that the ε-PL-based hydrogels can be easily integrated into biological tissues, thus serving as wound dressings [10].

Nitrogen is a constituent element of cellular components such as proteins, nucleic acids and several cofactors [11]. It also regulates primary and secondary metabolism in different bacteria, including *Streptomyces* [12–14]. ε-PL is synthesized from L-lysine monomers by a non-ribosomal peptide synthetase, and the polymerization process requires a large amount of L-lysine, which acts as the direct precursor. The supply of a sufficient amount of nitrogen is essential for ε-PL fermentation, because each L-lysine molecule contains two nitrogen atoms, although most of the other amino acids have only one. Among the many nitrogen sources, inorganic $(NH_4)_2SO_4$ was found to have a strong positive effect on ε-PL production

[4, 15]. Furthermore, some organic nitrogen sources, such as yeast extract and beef extract, were found to contribute to the production of ε-PL by promoting cell growth [16, 17]. However, the excessively high nitrogen concentrations affect both the synthesis of enzymes involved in primary and secondary metabolism, as well as the utilization of different nitrogen sources from the fermentation medium [18]. To alleviate its negative effects, the residual nitrogen concentration is often maintained at a suitable level using fed-batch fermentation strategies [19]. With the development of molecular biotechnology, it has become possible to increase the utilization efficiency of nitrogen sources through genetic manipulation [20].

Ammonium transport proceeds in two ways, via free diffusion in the form of $NH_3$ gas and active transport in the form of the $NH_4^+$ cation. When $NH_3$ diffusion across the membrane is limited during metabolism, the ammonium transporter *amtB* is expressed to facilitate the transport of $NH_4^+$ from the extracellular environment into the cells [12, 21]. Because ε-PL is synthesized under acidic conditions of pH 4.0 in the fermentation process [22], ammonia primarily exists in the form of $NH_4^+$ ions in the fermentation broth. Therefore, $NH_4^+$ ions enter the cell primarily through the ammonium transporter, providing a nitrogen source for cell growth and ε-PL synthesis. Several studies have demonstrated that increasing the expression of genes related to nitrogen transport can significantly enhance the production of many valuable compounds. For example, Sindelar et al. improved L-lysine production by overexpressing the *amtA-ocd-soxA* operon in *Corynebacterium glutamicum* [23]. Similarly, Meng et al. enhanced the synthesis of antibiotics via heterologous expression of nitrate/nitrite transporter genes in *actinomycetes* [24]. Judging by the findings of these studies, the question whether the overexpression of *amtB* in *S. albulus* PD-1 can be used to produce more ε-PL remains of great significance.

In this study, the *amtB* gene was overexpressed using different promoters in *S. albulus* PD-1 to investigate its effect on ε-PL production. The fermentation results showed a significant increase in the production of ε-PL. Furthermore, to elucidate the effects of *amtB* in *S. albulus* PD-1, we analyzed the transcriptional level and enzyme activity of ε-PL synthetase and proteins related to nitrogen absorption and assimilation. The findings of this study, thus, broaden our understanding of nitrogen metabolism in *S. albulus* PD-1, and provide an efficient fermentation strategy to improve the industrial fermentation of ε-PL.

## Materials and methods

### Strains and plasmids

The ε-PL producer *S. albulus* PD-1 has been deposited in the China Center for Type Culture Collection (Accession

No. M2011043). *Escherichia coli* DH5α (Tiangen Biotech CO., Beijing, China) was used as the host strain for maintenance and propagation of plasmids. *Escherichia coli* ET12567 (pUZ8002) [25] was used as the non-methylating plasmid donor strain for intergeneric conjugation with *S. albulus* PD-1. The chromosomally integrated shuttle vector (*E. coli*/*Streptomyces*), pSET152, which can integrate specifically into the *attB* sites on the *Streptomyces* chromosome via integrase-*attp*-directed site-specific recombination, was used for overexpression of *amtB*.

### Culture media

The Luria–Bertani (LB) medium for *E. coli* strains comprised 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl; solid medium was produced by adding 20 g/L agar. Mannitol-soy flour (MS) agar medium comprising 20 g/L mannitol, 20 g/L soy flour, 40 mM $MgCl_2$ and 20 g/L agar was used for intergeneric conjugation. Medium 3G (M3G) that was used for fermentation consisted of 50 g/L glucose, 10 g/L $(NH_4)_2SO_4$, 5 g/L yeast extract, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.8 g/L $K_2HPO_4$, 1.36 g/L $KH_2PO_4$ 0.03 g/L $FeSO_4 \cdot 7H_2O$, 0.04 g/L and $ZnSO_4 \cdot 7H_2O$. When required, antibiotics were used at the following concentrations: 30 μg/mL apramycin, 25 μg/mL kanamycin, 25 μg/mL chloramphenicol and 25 μg/mL nalidixic acid.

### Molecular manipulations

For the construction of *amtB* overexpression plasmids, the strong promoter *PermE** and the original promoter ($P_{amtB}$) of *amtB* were compared (Fig. 1). The nucleotide sequences of *amtB* gene and *amtB* gene with $P_{amtB}$ were amplified from genomic DNA of *S. albulus* PD-1 using the primers *amtB*-F/*amtB*-R and *amtB*-F'/*amtB*-R, respectively. The promoter *ermE** was amplified from plasmid pIB139 using the primers *PermE**-F/*PermE**-R. The resulting PCR products were inserted into the vector backbone pSET152 which had been digested with the corresponding enzymes to create plasmids pSET152-*PermE**-*amtB* and pSET152-P$_{amtB}$-*amtB* by seamless cloning [26]. The resulting recombinant vectors were introduced into *E. coli* ET12567 and then transferred into *S. albulus* PD-1 by intergeneric conjugation. All the constructed plasmids were verified by restriction enzyme digestion and DNA sequencing. The manipulations were conducted as described before with some modifications [27]. Furthermore, to investigate the effect of pSET152 on ε-PL biosynthesis, the empty plasmid pSET152 was also integrated into the *S. albulus* PD-1 genome, generating *S. albulus* PD-1-pSET152. The corresponding primers are listed in Table 1.
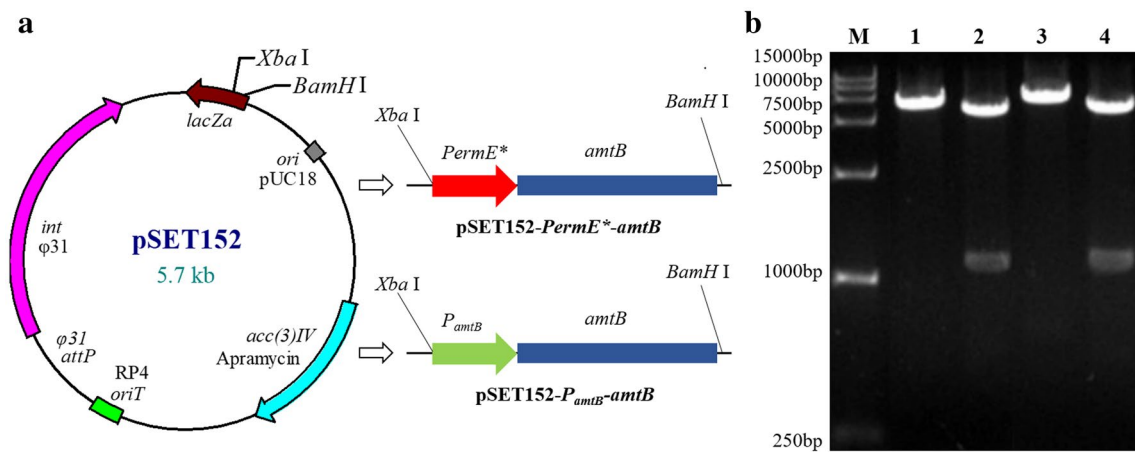
**Fig. 1 a** Construction of recombinant expression vectors. **b** Confirmation of recombinant plasmids by restriction enzyme digestion. Marker: DNA molecular weight standard; 1: pSET152-$P_{amtB}$-amtB/XbaI; 2: pSET152-$P_{amtB}$-amtB/XbaI + BamHI; 3: pSET152-PermE*-amtB/XbaI; 4: pSET152-$P_{amtB}$-amtB/XbaI + BamHI

## Shake flask two-stage culture

Firstly, cells were cultured 24 h in M3G medium at 30 °C and 200 rpm. The resulting seed cultures were used to inoculate the second stage medium at 10% (v/v), after which fermentation was conducted at 30 °C and 200 rpm for 7 days. In the second stage, the glucose concentration was kept constant at 10 g/L, and the nitrogen content in $(NH_4)_2SO_4$ were controlled at 0.2, 0.3, 0.4, 0.5, 1 g/L, 1.5 and 2, respectively. The carbon nitrogen ratio (C/N) (mol/mol) was varied from 23, 16.5, 11.5, 9.17, 4.71, 3 and 2.36 by calculation of the carbon nitrogen ratio formula, respectively. The concentration of $NH_4^+$–N was determined according to Zhou et al. [28]. The standard solution of

ammonium was diluted with water to different concentrations and their absorbance determined separately after indophenol blue reaction for 35 min to generate a standard curve of $NH_4^+$–N concentrations. The content of carbon and nitrogen in the fermentation broth was based on the initial concentration of glucose and ammonium nitrogen ($C_{Glu}$ and $C_{NH4^+-N}$). The carbon-to-nitrogen ratio (mol/mol) was calculated according to the following formula

$$\frac{C}{N} = \frac{C_{carbon}}{C_{nitrogon}} = \frac{(C_{Glu} \times 6)/180}{(C_{NH4^+-N})/14}.$$

**Table 1** Primers used in plasmid construction and qRT-PCR analysis

| Primer | DNA sequence (5′–3′) |
|---|---|
| amtB-F | GGATCCAGTGAACCTCTCAGGTTCCGATG |
| amtB-R | GCGCGGCCGC**GGATCC**CTACTTCTGCCGCTTGTAGAAGG (**Bam**HI) |
| amtB-F' | GCAGGTCGAC**TCTAGA**TTGCATACCGGCCGCT (**Xba**I) |
| PermE*-F | GCAGGTCGAC**TCTAGA**ATGCATGCGAGTGTCCGT (**Xba**I) |
| PermE*-R | AGGTTCACTGGATCCTACCAACCGGCAC |
| amtB | F: ATCCTCAAGAAGCTCACCGA |
| | R: GACGAACAGCTCGAAGCC |
| gdh | F: AGACCTTCATCTCGGGTCTG |
| | R: GAGGTAGGTGTCGTCCTCGT |
| glnA | F: TACTTCCTCGACACCGTCCT |
| | R: AGGAGGACATCGCGTAGC |
| ask | F: GAGTTCGACATGCTGCTGAC |
| | R: ACGGAGTCAGTGATCACACC |
| pls | F: CGGATTCGTCCAACTCCT |
| | R: GACGATGATCAGCCACCA |
| hrdB | F: CGACTACACCAAGGGCTACA |
| | R: TTGTTGATGACCTCGACCAT |

## Fed-batch fermentation and analysis of key parameters

Fed-batch fermentation was performed in a 5-L jar-fermenter (Baoxing Corp., Shanghai, China) using a published two-stage pH control method [29]. The pH was initially maintained at 6.0 by adding ammonia for 24 h to promote cell growth, after which it was reduced to 4.0 to stimulate ε-PL production. The residual sugar in the fermentation broth was measure using an SBA-40C biological analyzer (Shandong Academy of Sciences, China). The broth was centrifuged, and the resulting precipitate was collected, washed twice with distilled water, and dried at 105 °C to a constant weight to determine the dry cell weight (DCW). The concentration of ε-PL was determined via high-performance liquid chromatography (HPLC) using TSKgel ODS-120T column (4.6 × 250 mm, Tosoh, Tokyo), according to a previously reported method [30].The detection wavelength was recorded at 215 nm at 30 °C.

## RNA sample preparation and qRT-PCR analysis

As the fed-batch fermentation reached 110 h, the maximum specific ε-PL production rate was reached. At this timepoint, the transcriptional levels of *amtB*, glutamate dehydrogenase (GDH) gene (*gdhA*), GS (*glnA*), GOGAT (*gltB*), aspartate kinase (*ask*) and ε-PL synthetase (*pls*) were determined by quantitative real-time PCR (qRT-PCR) using a previously described method [31]. RNA extraction was performed using an RNeasy MiniKit (TaKaRa Biotechnology Company, Dalian, China), and reverse transcription reactions were performed using a PrimeScript RT Reagent Kit (TaKaRa Biotechnology Company, Dalian, China). qRT-PCR was carried out in 25μL reaction mixtures comprising SYBR Premix ExTaq (12.5 μL), forward primer (1 μL; 5 pmol), reverse primer (1 μL; 5 pmol), and cDNA (2 μL; 25 ng/μL) in ddH$_2$O. The PCR reaction conditions were as follows: 30 s at 95 °C, followed by 40 cycles comprising 5 s at 95 °C and 30 s at 60 °C each, and a final cycle comprising 15 s at 95 °C, 1 min at 60 °C, and 15 s at 95 °C. All reactions were repeated three times and the qRT-PCR results were subjected to the $2^{-\Delta\Delta Ct}$ method [32] for relative quantification with the *hrdB* gene as endogenous control [33, 34]. Primers were designed according to the *S. albulus* PD-1 genome sequence and are listed in Table 1.

## Enzyme activity assays

When the fed-batch fermentation reached 110 h, the mycelia from 10 mL of culture broth were harvested by centrifugation, washed twice with 0.85% (w/v) saline, and suspended in 1 mL buffer containing 40% glycerol, 10 mM dithiothreitol and 100 mM Tris–HCl buffer (pH8.0). The suspended

mycelia were disrupted by sonication. Cell debris was removed by centrifugation at 16,000×*g* for 20 min, and the supernatant was collected as crude extract for enzymatic activity assays. The GDH and GOGAT activities were measured spectrophotometrically by monitoring the rate of NADPH oxidation at 340 nm [35]. The GDH activity was measured by adding a crude enzyme solution to a solution containing 50 mM Tris-HC1 buffer (pH 7.6), 150 mM NH$_4$Cl, 7 mM α-ketoglutarate and 0.12 mM NADPH. The GOGAT activity was measured by adding a crude enzyme solution to a solution containing 50 mM Tris-HC1 buffer (pH 7.6), 15 mM glutamine, 3 mM α-ketoglutarate, 0.05 Mm NADPH. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the oxidation of 1 μmol of NADPH per min. The GS activity [35] was measured by adding a crude enzyme solution to a solution containing 50 mM imidazole–HCl buffer (pH 7.0), 15 mM MgCl$_2$, 55 mM glutamate (sodium salt-pH was adjusted to 7.0 with 1 N NaOH), 2.5 mM ATP, 25 mM NH$_4$Cl. One unit of GS activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol γ-glutamyl hydroxamate per min in the transferase; Aspartate kinase (Ask) was measured according to Xu et al. [31], the reaction mixture with a total volume of 100 mM Tris–HCl (pH 7.0), 600 mM (NH$_4$)$_2$SO$_4$, 600 mM hydroxylamine–NaOH (pH7.0), 10 mM MgSO$_4$, 10 mM ATP and 10 mM aspartate–NaOH (pH7.0) and crude extract. One unit of Ask activity catalyzed the formation of 1 μmol of aspartyl-β-hydroxamate per minute in the assay at 30 °C. ε-PL synthetase (Pls) were measured according to Chen et al. [36]. Briefly, the Pls activity was determined by monitoring the reduction of L-lysine using HPLC in a 1 mL reaction mixture with the following composition: 100 mM Tris–HCl (pH 8.0), 100 mM L-lysine, 50 mM ATP, 50 mM MgCl$_2$, 50 mM dithiothreitol, and 20μL membrane fraction solutions. One unit of enzyme activity is defined as the amount of enzyme catalyzing the consumption of 1 pmol L-lysine per second at 30 °C.

## Results and discussion

### Construction of recombinant plasmids and strains

As depicted in Fig. 1, two plasmids, pSET152-*PermE*\*-*amtB* and pSET152-$P_{amtB}$-*amtB*, were constructed and verified via single- and double restriction enzyme digestions. The results suggested that the two recombinant plasmids were successfully constructed. Subsequently, intergeneric conjugation was used to individually transfer these two recombinant plasmids and the empty vector control pSET152 into *S. albulus* PD-1, resulting in the three strains *S. albulus* PD-1-pSET152, *S. albulus* PD-1-pSET152-*PermE*\*-*amtB* and *S. albulus* PD-1-pSET152-$P_{amtB}$-*amtB*.

## Effects of *amtB* overexpression on ε-PL synthesis

The modified *S. albulus* strains PD-1-pSET152, PD-1-pSET152-*PermE\**-*amtB* and PD-1-pSET152-$P_{amtB}$–*amtB*, as well as the wild-type *S. albulus* PD-1 were cultured in shake flasks to investigate the effects of *amtB* overexpression on DCW, ε-PL synthesis and ammonium utilization. As shown in Table 2, the empty vector control pSET152 that was integrated into the chromosome of *S. albulus* PD-1 had no effect on ε-PL production, as expected. By contrast, the two recombinant strains overexpressing the target gene showed a great production improvement over the wild type. The results revealed that the plasmids pSET152-$P_{amtB}$-*amtB* and pSET152-*PermE\**-*amtB* led to increases of ε-PL production by 11.5 and 22.1% over the parental strain, respectively (Table 2). Similarly, Sindelar et al. reported that overexpression of *amtA-ocd-soxA* enhanced L-lysine production, and they speculated that this was caused by increased ammonium uptake due to higher AmtA levels [23]. In this study, the residual $NH_4^+$–N of the *amtB*-overexpressing strains was lower than that of the wild type (Table 2). This result indicated that the overexpression of the ammonium transporter improved the efficiency of nitrogen source utilization. Moreover, the effect was stronger with *PermE\** than with the native promoter of *amtB*. As a strong promoter, *PermE\** showed a good performance in the genetic manipulation of many ε-PL-overproducing strains [37, 38]. Other studies have shown that the strong constitutive *ermE\** promoter could overwhelm the complex regulatory cascade in the metabolic process and increase the production of target chemicals [39]. Furthermore, the biomass of these two recombinant strains showed a slight improvement over the wild type. These results showed that the overexpression of *amtB* enhanced the transport of ammonium ions and promoted the synthesis of ε-PL. The *S. albulus* strain PD-1-pSET152-*PermE\**-*amtB* was selected for further experiments and renamed as *S. albulus* PD-1-*amtB* for sake of brevity.

## Optimum carbon-to-nitrogen ratio during the synthesis stage

The ratio of carbon and nitrogen is an important parameter in microbial fermentations, and a suitable ratio can greatly increase the synthesis of target products [40]. Previous studies focused on optimizing the initial C/N ratio of fermentation materials, and ignored the direct influence of the C/N ratio during the phase of active ε-PL synthesis. In view of this problem, *S. albulus* PD-1-*amtB* and *S. albulus* PD-1 were subjected to two stages of flask fermentation [4] at different carbon-to-nitrogen ratios at a constant carbon concentration.

As shown in Fig. 2, with the decrease of C/N concentration in the medium, the synthetic ability of *S. albulus* PD-1 and *S. albulus* PD-1-*amtB* presented a trend that changed from increase to decrease. Thus, it was found that too low or too high concentrations of nitrogen sources had inhibitory effects on cell growth and product synthesis, and similar results were also observed in other studies [41–43]. Moreover, under the same C/N ratio, the ε-PL yield of *S. albulus* PD-1-*amtB* was higher than that of *S. albulus* PD-1. Furthermore, the optimal C/N ratio of *S. albulus* PD-1-*amtB* was 4.71, higher than that of *S. albulus* PD-1, and their ε-PL titers were 1.475 and 1.236 g/L, respectively. This implied that the ammonium utilization efficiency of strain *S. albulus*
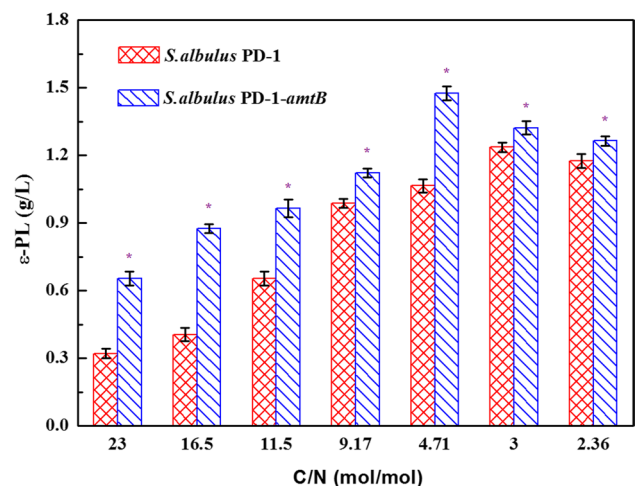
**Fig. 2** Influence of different C/N ratios at a constant C concentration on the ε-PL yield in two-stage shake flask fermentations for 7 days. The initial carbon source in the production culture medium was 10 g/L glucose, and the nitrogen content in $(NH_4)_2SO_4$ were controlled at 0.2, 0.3, 0.4, 0.5, 1 g/L, 1.5 and 2, respectively. *$p < 0.05$; Duncan's multiple range test

**Table 2** Comparison of flask fermentation performance of the two *amtB*-overexpressing strains and the corresponding empty vector control with their wild-type parent

| Strains | DCW (g/L) | ε-PL (g/L) | Residual $NH_4^+$–N (g/L) |
|---|---|---|---|
| S. albulus PD-1 | 6.50 ± 0.23b | 1.13 ± 0.08c | 1.71 ± 0.04a |
| *S. albulus* PD-1-pSET152 | 6.61 ± 0.21b | 1.10 ± 0.06c | 1.72 ± 0.03a |
| S. albulus PD-1-pSET152-PermE*-amtB | 7.03 ± 0.15a[a] | 1.38 ± 0.05a | 1.40 ± 0.05c |
| S. albulus PD-1-pSET152-$P_{amtB}$-amtB | 6.71 ± 0.25ab | 1.26 ± 0.04b | 1.55 ± 0.03b |

The same lowercase letters in each column indicate no significant difference, whereas different lower case letters indicate a significant difference

PD-1-*amtB* was increased after overexpressing *amtB*. Thus, the optimal C/N ratio for *S. albulus* PD-1-*amtB* during the ε-PL synthesis period was found to be 4.71, which provided guidance for the following fed-batch fermentations.

## Fed-batch fermentation performance of the recombinant strains

Based on the optimization in two-stage shake-flask fermentations, 5 L fed-batch fermentations were performed with C/N ratios for *S. albulus* PD-1 and *S. albulus* PD-1-*amtB* of 3 and 4.71, respectively. The residual sugar concentration was maintained at approximately 10 g/L during the fed-batch fermentation, as described in a previous study [44].

As shown in Fig. 3a, b, *S. albulus*-PD-1-*amtB* showed a 15.8% increase of the final DCW (32.3 g/L) and 55.2% increase of ε-PL production (35.7 g/L) compared to the wild-type strain. To analyze the dynamic changes of pH, cell growth and ε-PL production, the specific cell growth rate and specific ε-PL production rate of the two strains were fitted based on the data shown in Fig. 3. The specific cell growth rate is shown in Fig. 4a. Notably, *S. albulus* PD-1-*amtB* maintained a certain growth advantage over the wild-type strain throughout the fermentation process. This was attributed not only to the increase of ammonium transport efficiency, but also to the low nitrogen supplement concentration in the culture supernatant of *S. albulus* PD-1-*amtB*, which may alleviate the repression that occurs at higher $NH_4^+$ concentrations to some extent [18].

The specific ε-PL production rate is shown in Fig. 4b. During the first 48 h, there was almost no difference in the synthetic ability of the two strains, because the early stage mainly encompasses the growth of the bacteria. After the pH was reduced to 4.0, the ε-PL began to enter the high-speed synthesis period. During this time, the specific ε-PL production rate of *S. albulus* PD-1-*amtB* was significantly higher than that of the wild-type strain, indicating that the ε-PL synthesis ability of the cells was strengthened after overexpressing *amtB*. Thus, this work offers great promise for the industrial production of ε-PL.

## Enhancement of relevant metabolic pathways

Ammonium is utilized as the main nitrogen source via two stages of uptake and assimilation [45]. After ammonium is transported into the cells, assimilation proceeds via glutamate dehydrogenase (GDH) or glutamine synthetase/glutamate synthase (GS/GOGAT), depending on the ammonium availability in the medium. At high concentrations (> 1 mM), it is primarily assimilated by GDH, while at low concentrations it is solely assimilated by GS/GOGAT to yield L-glutamate [46, 47]. However, to meet the demand for the synthesis of glutamine, the GS pathway is still active
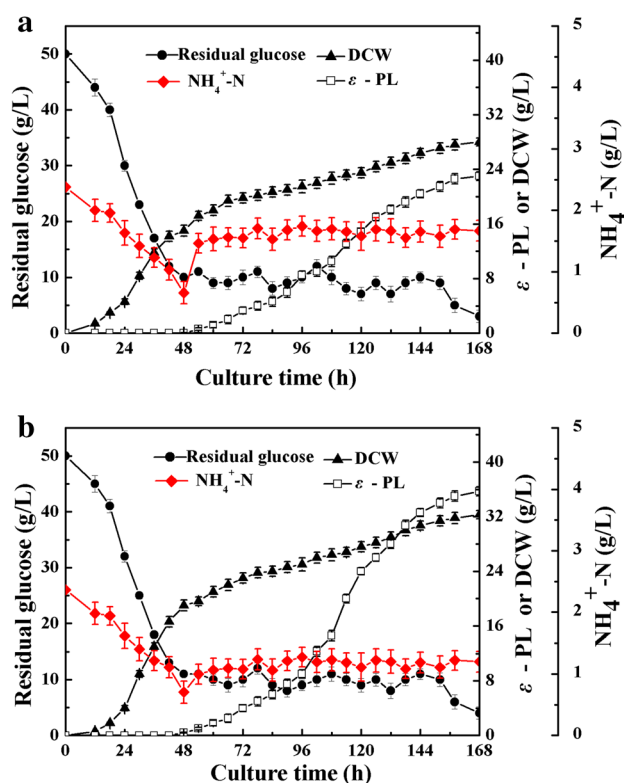


**Fig. 3** Comparison of the wild type and *amtB*-overexpressing strain in fermentations in a 5-L jar bioreactor using a two-stage pH control strategy. The residual sugar concentration was maintained at approximately 10 g/L during the fed-batch fermentation; the C/N ratios for *S. albulus* PD-1 and *S. albulus* PD-1-*amtB* were maintained at 3 and 4.71 during the fed-batch fermentation, respectively. **a** *S. albulus* PD-1, **b** *S. albulus* PD-1-*amtB*

at high nitrogen concentrations [48]. Since the concentration of ammonium in our study was maintained at a high level during the synthesis phase of ε-PL, the GDH pathway was utilized for the synthesis of glutamate. Thus, to investigate the mechanism by which the overexpression of the ammonium transport protein increased ε-PL production, the transcription profiles of genes related to nitrogen metabolism and ε-PL synthesis were measured.

The transcription level of *amtB* was increased 2.1-fold (Fig. 5a), which indicated that *amtB* was successfully overexpressed under the control of the strong *PermE\** promoter. Thus, it is conceivable that ammonium uptake was increased due to higher *amtB* levels [23]. As shown in Fig. 5a, b, the transcription level and enzyme activity of the GDH gene (*gdhA*) were increased 1.5 and 1.3 times compared to the control group, respectively. The higher *amtB* transcription level could in turn have stimulated ammonium assimilation. Consequently, the high expression of GDH, could enhance the accumulation of glutamate [49]. Furthermore, the glutamate could provide nitrogen for the synthesis of the precursor L-lysine through transamination, while the formation
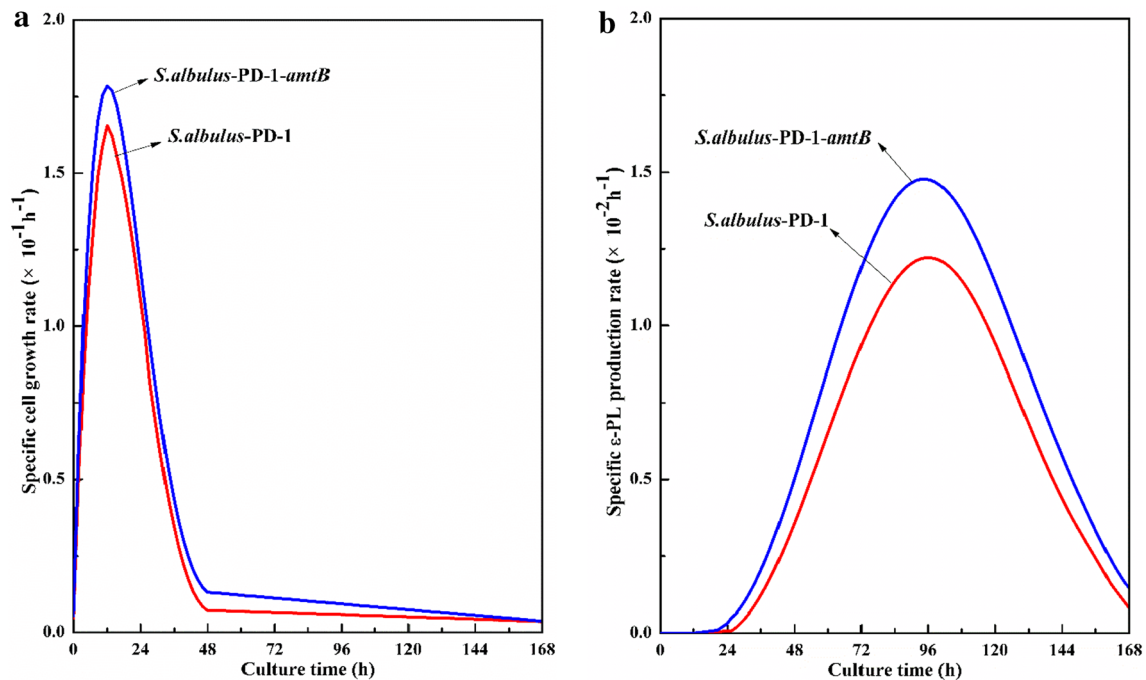
**Fig. 4** Comparison of fermentation process parameters in a 5 L bioreactor. **a** Specific cell growth rate. **b** Specific ε-PL production rate

of α-ketoglutaric acid could promote the TCA cycle at the same time, providing carbon for L-lysine synthesis [50]. Ultimately, these metabolic changes led to an increase of ε-PL production.

In addition, another assimilation system was investigated by measuring the transcription level of the GS gene (*glnA*). It was found to be increased 1.2 times (Fig. 5a) and its enzyme activity accordingly increased from 1.45 to 1.92 U/mg protein (Fig. 5b). Glutamine functions as a nitrogen donor in the synthesis of carbamoyl phosphate, histidine, purines, and glucosamine-6-phosphate, a precursor of peptidoglycan [51]. Therefore, the stronger GS activity promoted the synthesis of more glutamine, which could have improved the growth of the bacteria. By contrast, the transcriptional level of the GOGAT gene (*gltB*) and its enzyme activity were almost unchanged (Fig. 5a, b), which also proved that the GOGAT system does not play an important role at high nitrogen concentrations [12].

Further investigations of the key enzymes in the ε-PL synthesis pathway were also conducted. The transcription level of the aspartate kinase gene (*ask*) was found to be increased 1.4 times (Fig. 5a) and its enzyme activity accordingly increased from 37.45 to 52 U/mg protein (Fig. 5b). As the key enzyme of the diaminopimelic acid pathway (DAP), the increase of *ask* activity indicated that more L-lysine was used for the synthesis of ε-PL [19]. Furthermore, the transcription level of the ε-PL synthetase gene (*pls*) was found to be increased 1.6 times (Fig. 5a) and the enzyme activity increased from 59.87 to 73.5 U/mg protein (Fig. 5b). These results suggested that *amtB* overexpression has a profound effect not only on ammonium assimilation but also on ε-PL biosynthesis. In conclusion, the overexpression of *amtB* strengthened the transport of ammonium, thereby promoting its assimilation, which in turn resulted in intracellular accumulation of L-lysine and increased the yield of ε-PL. Nevertheless, the specific relationship between nitrogen metabolism and the ε-PL synthesis pathway is still unclear and further studies are needed.

## Conclusions

In this study, the *amtB* gene was successfully overexpressed by increasing its transcriptional level in the ε-PL-producing strain *S. albulus* PD-1 for the first time. Consequently, the production of ε-PL increased from 22.7 to 35.7 g/L in fed-batch culture in a 5 L bioreactor. The analysis of transcriptional and enzyme activity levels showed that the strain's ammonium absorption and assimilation abilities were enhanced, which promoted the ε-PL synthesis pathway. Therefore, this study deepens our understanding of nitrogen utilization in the ε-PL-producing strain *S. albulus* PD-1, and will provide the basis for improving the production of biochemicals in other actinomycetes by rational engineering.
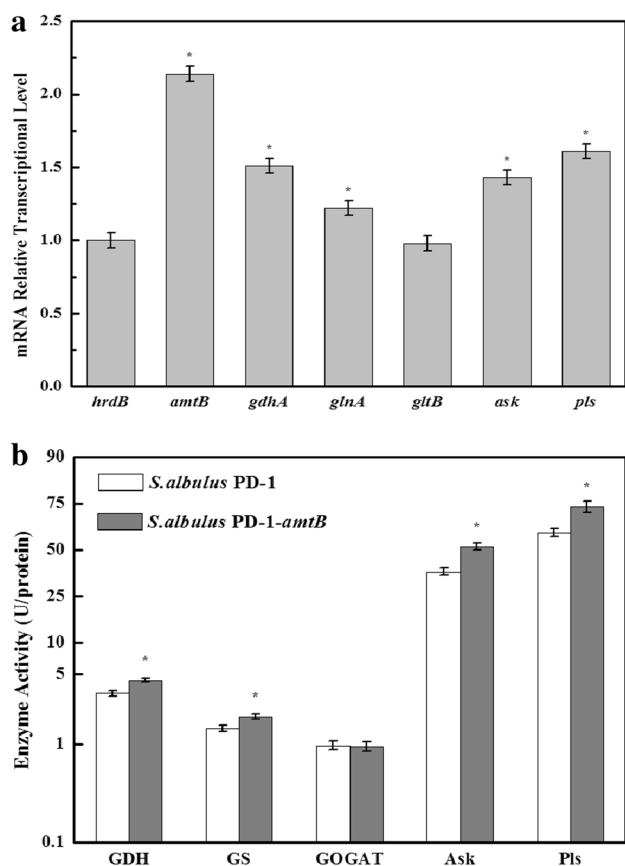
**Fig. 5** Relative mRNA expression levels of the key genes and activities of key enzyme of metabolic pathways related to ε-PL synthesis. **a** Relative mRNA expression levels, **b** key enzyme activities. *$p < 0.05$; Duncan's multiple range test

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