



Understanding high ϵ -poly-L-lysine production by *Streptomyces albulus* using pH shock strategy in the level of transcriptomics

Long Pan¹ · Xusheng Chen¹ · Kaifang Wang¹ · Zhonggui Mao¹

Received: 23 June 2019 / Accepted: 26 September 2019 / Published online: 8 October 2019
© Society for Industrial Microbiology and Biotechnology 2019

Abstract

ϵ -Poly-L-lysine (ϵ -PL) is a natural food preservative, which exhibits antimicrobial activity against a wide spectra of microorganisms. The production of ϵ -PL was significantly enhanced by pH shock in our previous study, but the underlying mechanism is poorly understood. According to transcriptional and physiological analyses in this study, the *mprA/B* and *pepD* signal transduction system was first proved to be presented and activated in *Streptomyces albulus* M-Z18 by pH shock, which positively regulated the transcription of ϵ -PL synthetase (Pls) gene and enhanced the Pls activity during fermentation. Furthermore, pH shock changed the ratio of unsaturation to saturation fatty acid in the membrane through up-regulating the transcription of fatty acid desaturase genes (SAZ_RS14940, SAZ_RS14945). In addition, pH shock also enhanced the transcription of cytochrome c oxidase (SAZ_RS15070, SAZ_RS15075), ferredoxin reductase (SAZ_RS34975) and iron sulfur protein (SAZ_RS31410) genes, and finally resulted in the improvement of cell respiratory activity. As a result, pH shock was considered to influence a wide range of proteins including regulators, fatty acid desaturase, respiratory chain component, and ATP-binding cassette transporter during fermentation. These combined influences might contribute to enhanced ϵ -PL productivity with pH shock.

Keywords ϵ -Poly-L-lysine · pH shock · Comparative transcriptomics · Signal transduction system · Electron respiratory chain

Introduction

ϵ -Poly-L-lysine (ϵ -PL) is a cationic polypeptide made of 25–35 L-lysine residues linked together via amide bonds between the ϵ -amino group and the α -carboxylic acid group [12]. It is mainly produced by bacteria belonging to the family of *Streptomycetaceae* as a secondary metabolite [30]. ϵ -PL and its hydrochloride are used as a natural food

preservative in Japan, South Korea, the United States, China and other countries for many years due to their antimicrobial properties and high food safety [37]. In addition, ϵ -PL has other numerous applications, such as drug carrier, nanoparticle, gene carrier, liposome, interferon inducer, lipase inhibitor, hydrogel, and coating material [1]. Currently, ϵ -PL has been industrially produced by aerobic fermentation using *Streptomyces albulus*. However, the high cost of production remains a major hurdle to widespread use of this natural antimicrobial agent and highly functional material.

It has been proved that improving the concentration of metabolite in fermentation broth through bioprocess optimization is an effective way to reduce the cost of fermentation products. As a result, medium optimization [8], nutrition feeding [7], dissolved oxygen regulation [35], in situ product removal fermentation [22], solid-state fermentation [34] and fermentation with immobilized cells [38] have been developed and achieved ϵ -PL production exceeding 30 g/L. pH is an important parameter for ϵ -PL accumulation. Kahar et al. [15] reported that no ϵ -PL was produced when pH was

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10295-019-02240-z>) contains supplementary material, which is available to authorized users.

✉ Xusheng Chen
chenxs@jiangnan.edu.cn

✉ Zhonggui Mao
maozg@jiangnan.edu.cn

¹ The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, China

maintained above 5.0 during fermentation, but this pH range benefited cell growth; however, pH in the range of 3.5–4.5 achieved ϵ -PL accumulation a lot. Accordingly, a two-stage pH control strategy was developed and enhanced ϵ -PL production to 48.3 g/L. Further investigations found that the control of pH 3.5–4.5 during fermentation could inhibit the activity of ϵ -PL-degrading enzyme and accumulate enough ATP for ϵ -PL synthetase activation [36]. Interestingly, we precisely adjusted pH in the range of 3.5–3.8 during fermentation and enhanced the ϵ -PL production of *Streptomyces albulus* M-Z18 by 16.6% [5]. Recently, we allowed pH to spontaneously decrease from 4.0 to 3.0 and then it was kept constant for 12 h at pH 3.0, and then pH was shifted back to pH 3.8 and maintained until the end of fermentation. Finally, this method, called pH shock, significantly improved the ϵ -PL production reaching 54.70 g/L [28]. Our previous study suggested that some key enzymes in the central metabolic and ϵ -PL biosynthetic pathways in *S. albulus* M-Z18 were generally strengthened by pH shock [27]. However, the underlying molecular mechanism is poorly understood.

To investigate the relationship between pH shock and high ϵ -PL production in *S. albulus* M-Z18, a steady fermentation process is deemed essential. Therefore, we have modified the fermentation process and divided the process into two stages: the first stage (cultivation stage) comprised mycelia growth, pH shock and mycelia viability recovery and was performed in one fermenter, while the second stage (fermentation stage) only included ϵ -PL production at a constant pH 3.8 and was performed in the other fermenter. As a result, the second stage improved ϵ -PL productivity by 36.6% and successfully reduced the fermentation time by 31.25% than the traditional fermentation process with pH shock [25]. In this study, RNA-seq analysis was employed to explore the profiles of genome-wide gene transcription in *S. albulus* M-Z18 in this steady fermentation process (the second stage), and the constant pH 3.8 (pH non-shock) fermentation process was used as the control. Meanwhile, biochemistry, quantitative real-time PCR and gene disruption experiments were used to validate the results of transcriptional analysis. Finally, the reason responsible for the pH shock enhancing ϵ -PL production in *S. albulus* M-Z18 was proposed.

Materials and methods

Bacterial strains, plasmids, primers

Bacterial strains and plasmids used in this study are listed in Table S1. The primers used in this study are listed in Table S2. *E. coli* DH5 α was used as a general host for routine cloning experiment. *E. coli* ET12567/pUZ8002 was used for transferring plasmids from *E. coli* to *Streptomyces*

by intergeneric. *Streptomyces albulus* M-Z18, a mutant from *S. albulus* Z-18 (CGMCC 10479) was used as a wild-type strain. pKC1132 (gifted from Professor Kecheng Zhang) was used as *E. coli*–*Streptomyces* shuttle vector for gene disruption inactivation.

Culture conditions

The seed medium and fermentation medium were used in this study as described previously [15, 21]. All media components were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) with analytical and biochemical grades. One liter multi-bioreactor system and 5-L fermenter were employed for different processes of fermentation, with the optimized conditions as reported previously [25]. pH shock process was applied in the first stage of the fermentation (1-L multi-bioreactor system) and developed as follows: pH was spontaneously dropped from initial pH 6.80 to pH 5.00, then kept constant at pH 5.00 by automatic addition of ammonia water (12.5%, v/v) for 8 h. After that, the pH was uncontrolled and maintained for 12 h (including pH decline from 4.0 to 3.0), and then the pH was recovered to 3.85 and kept until the DO recovery to below 50% air saturation. The same regulation was developed in control group except for pH shock. The second stage of the fermentations was developed in the 5-L fermenter and inoculated by different fermentation broth from the first stage.

Construction of the recombinant strains

To construct the gene disruption mutants of *S. albulus* M-Z18, the partial DNA fragments of the target genes (*mprA*, *mprB*, *pepD*, *desk*, *desR* and *sigE*) were amplified by PCR, and then they were cloned into *Hind*III and *Eco*RV sites of pKC1132 plasmid using ClonExpress MultiS One Step Cloning Kit (Vazyme Biotech) to construct disruption plasmids. Besides, *attP* (a homologous sequence of ϕ 31 *attB* site in *S. albulus* M-Z18) was amplified by PCR from pIB139 plasmid and cloned into pKC1132 plasmid by the above method to construct pKC1132-*attp* as control. Subsequently, these plasmids (pKC1132-*attp*, pKC1132-*mprA*, pKC1132-*mprB*, pKC1132-*pepD*, pKC1132-*desk*, pKC1132-*desR* and pKC1132-*sigE*) were introduced into *S. albulus* M-Z18 via ET12567/pUZ8002 by conjugal transfer. Apramycin resistance gene was verified by PCR using primers Apr-F and Apr-R.

Analytical methods

Assay of dry cell weight (DCW), residual glucose, NH₄⁺-N and ϵ -PL production was performed as described previously [28].

Fatty acid component analysis

Mycelia were harvested by centrifugation at $3000\times g$ for 15 min, and the pellet was washed three times with distilled water. The fatty acids in the cells (40–50 mg in wet weight) were saponified and methylated. The methyl ester mixtures were determined by gas chromatography (TSQ 8000, Triple Quadrupole GC–MS/MS, Thermo Fisher Scientific, USA). Fatty acids were identified by the MIDI microbial identification system. Minor fatty acids (<0.6% of the total) are not considered. The data were expressed as a relative percentage of each fatty acid compared to the total area. For each condition, three repetitions of three independent experiments were considered.

Heme measurements

Heme was determined as described by Michener with appropriate modifications [23]. Mycelia were harvested by centrifugation at $12,000\times g$ for 5 min. The mycelia was washed with water, transferred to an amber centrifuge tube, and centrifuged again at $4\text{ }^{\circ}\text{C}$ and $8000\times g$ for 5 min. The pellet was then resuspended in 500 μL of 20 mM oxalic acid and stored at $4\text{ }^{\circ}\text{C}$ in the dark for 16 h. After the acid extraction, 500 μL of 2 M oxalic acid was added to each tube. Half of the resulting suspension was transferred to a new centrifuge tube and heated to $95\text{ }^{\circ}\text{C}$ for 30 min. The other half was unheated and acted as control. After centrifugation, 200- μL supernatant of each sample (heated and unheated) was measured by the Synergy H4 Microplate Reader (Biotek, Winooski, VT, USA) at the excitation wavelength of 400 nm and an emission wavelength of 620 nm. The assays were performed in triplicate, and the results were expressed as μmol per mg of total protein ($\mu\text{mol}/\text{mg}$ protein). All assays were performed in triplicate.

Assay of ϵ -PL synthetase activity

ϵ -PL synthetase (PLs) activity was determined as described by Chen [6]. The mycelia were suspended in 100 mM Tris–HCl buffer (pH 7.5) that contained 20% glycerol and 1 mM dithiothreitol and were then immediately processed by ultrasonic decomposition in an ice bath for 30 min with 2 s running and 2 s intervals with a 650 W sonicator (SM-650D; Shunma Tech., Nanjing, China). Unbroken cells and debris were removed by centrifugation at $12,000\times g$ for 20 min. The supernatant was used as a crude extract for the determination of PLs activity. One unit of PLs activity was defined as the amount of enzyme which catalyzed the consumption of 1 pmol of L-Lysine per second in the assay at $30\text{ }^{\circ}\text{C}$. The

assays were performed in triplicate, and the results were expressed as units per mg of total protein (U/mg protein).

Assay of respiratory activity

The redox dye had been widely used to determine the respiratory activity of bacteria. 5-cyano-2, 3-ditolyl-tetrazolium chloride (CTC), a soluble and non-fluorescing stain, could be adsorbed and reduced into an insoluble red-fluorescent substance (CTC formazan) by respiring cells via the electron transport chain and accumulating in the cell. Higher fluorescence intensity represents higher respiratory activity. The mycelia were collected from the shake flask by centrifugation at $12,000g$ for 5 min, washed twice and resuspended in saline (0.9% NaCl). CTC staining was conducted in a 1.5 mL Eppendorf tube using a Bacstain-CTC rapid staining kit (Dojindo, Kumamoto, Japan) for 30 min at $37\text{ }^{\circ}\text{C}$ according to the instructions [28]. Two hundred microliters of stained samples was deposited on a 96-well plate and measured under the Synergy H4 Microplate Reader (Biotek, Winooski, VT, USA) at the excitation wavelength of 488 nm and an emission wavelength of 630 nm. All assays were performed in triplicate.

RNA extraction and RNA-seq analysis

The samples were taken from 5-L fermenter at 24 h in the second stage of the fermentation. About 50 mL of fermentation broth was extracted and centrifuged to remove the supernatant. After washing with sterile water, the bacteria were treated with liquid nitrogen for 20 min, and then used for RNA-seq analysis. Total RNA was extracted using RiboPure™ -Yeast Kit (Life technologies, USA), following the manufacturer's protocols. Total RNA was digested using DNase I (New England Biolabs, USA), mRNA was enriched by removing the rRNAs using RiboZero™ Magnetic Kit and then purified with RNA Clean XP Beads (Agencourt, USA). cDNA library was constructed and sequenced by Illumina HiSeq 2000. Raw sequencing fragments were filtered to remove adaptors and low-quality reads. Transcriptome assembly was carried out with short reads assembling program. Only more than twofold differentially transcriptions ($p < 0.01$, FDR < 0.01) were considered to be significant and these genes were selected.

Identification of differentially expressed genes (DEGs) and gene ontology (GO) enrichment of DEGs

Transcript abundance of all unigenes was calculated using the fragments per kilobase per million reads (FPKM) method [31]. In this work, the significance of gene transcription differences was assessed using the $|\text{fold change}| \geq 2.0$ ($p < 0.01$, FDR < 0.01). GO enrichment of DEGs was conducted using

the Blast2GO program. We mapped all DEGs to each term of the GO database (<http://www.geneontology.org/>) and then calculated the unigenes number corresponding to each GO term. GO terms with $p < 0.05$ were defined as significantly enriched.

Quantitative real-time PCR

Reverse transcription was achieved with PrimeScript™ RT reagent Kit (Takara, Dalian, China). The transcriptional level of genes was determined by quantitative real-time PCR (qRT-PCR) on StepOne Real-Time PCR (Applied Biosystems) with SYBR® Premix Ex Taq™ (Ti RNaseH Plus) (Takara, Dalian, China). The PCR conditions were pre-denaturation at 95 °C for 1 min followed by 40 cycles of 15 s denaturation at 95 °C, 35 s annealing at 60 °C and 15 s extension at 95 °C [19]. All of these reactions were repeated thrice and qRT-PCR results were subjected to $2^{-\Delta\Delta C_t}$ method for relative quantification with endogenous gene [20]. Primers were designed according to the sequences from the *S. albulus* M-Z18 genome. For each gene, the fermentation samples in pH non-shock group were defined as the expression level of 1.0, and results were expressed as the fold increase of mRNA level over the control samples.

Statistical analysis

Each value is a mean of three replications. The results are expressed as mean \pm standard deviation (mean \pm S.D.). The

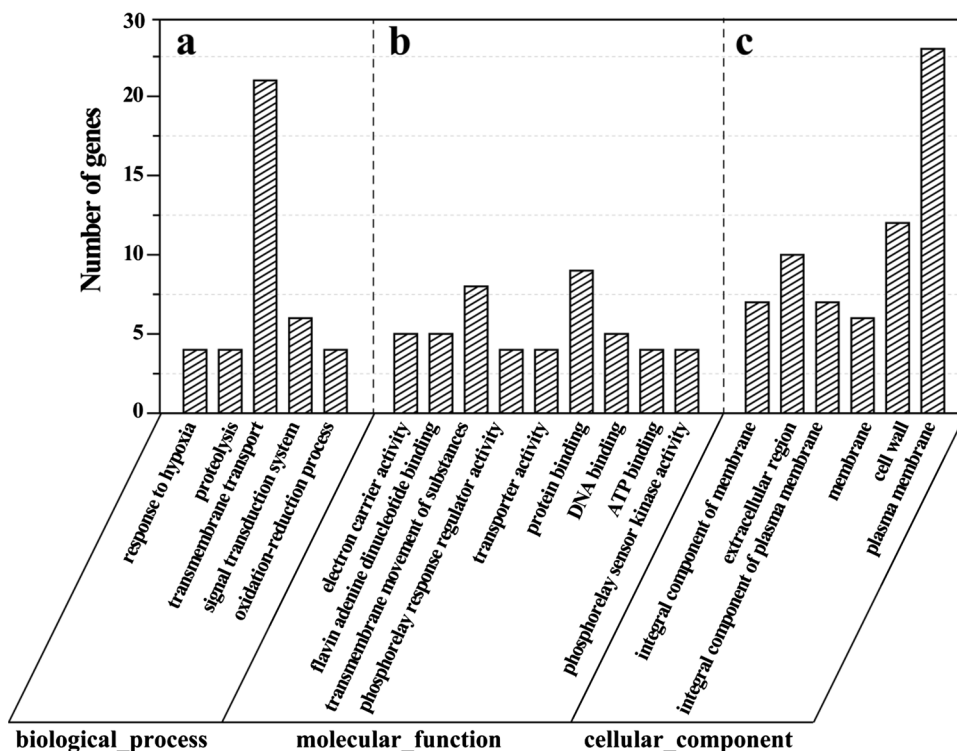
difference between experimental group and control group in this study was distinguished by the statistical analysis with the GraphPad Prism 6.0 and $p < 0.05$ was considered significant.

Results and discussion

Comparative transcriptomic profiles analysis

To understand the effects of pH shock on ϵ -PL biosynthesis and cell growth in the level of gene transcription, comparative transcriptomic analysis was conducted. A total of 7792 differentially expressed genes (DEGs) were detected by RNA-seq technology. Using statistical criteria of |Fold change| ≥ 2 and $p < 0.01$, a total of 199 significant DEGs were identified. Among which, 130 unigenes were up-regulated while 69 unigenes were down-regulated (Table S3). To further investigate these significant DEGs, GO classification was conducted and the results were summarized in three main categories: biological process, molecular function and cellular component (Fig. 1). Within the biological process category (Fig. 1a), DEGs assigned to ‘transmembrane transport’ (GO:0055085) and ‘signal transduction system’ (GO:0000160) were the most, indicating pH shock affected the transport of substrate and signal transduction process in *S. albulus* M-Z18. Unigenes were also enriched in ‘oxidation–reduction’ (GO:0055114), in which four unigenes were up-regulated while two unigenes were down-regulated.

Fig. 1 Gene ontology classification of DEGs. The results are summarized in three main categories: biological process (a), molecular function (b) and cellular component (c)



Within the molecular function category (Fig. 1b), most of the DEGs were assigned to ‘ATPase activity, coupled to transmembrane movement of substances’ (GO:0042626). Unigenes were also enriched in ‘phosphorelay sensor kinase activity’ (GO:0000155), in which all of unigenes were up-regulated. Within the cellular component category (Fig. 1c), DEGs were mainly assigned into terms related to membrane, including ‘plasma membrane’ (GO:0005886), ‘membrane’ (GO:0016020), ‘integral component of membrane’ (GO:0016021) and ‘integral component of plasma membrane’ (GO:0005887), and related to ‘cell wall’ (GO:0005618). The results indicated that membrane and signal transduction system could be significantly affected by pH shock. Thus, the genes involved in cell membrane and signal transduction system were further investigated.

The transcriptional changes related to cell membrane

Three DEGs related to lipid metabolism were identified from the transcriptome data, which were involved in steroid hormone biosynthesis (SAZ_RS40140), fatty acid degradation (SAZ_RS04465) and synthesis/degradation of ketone bodies (SAZ_RS02815) (Table S3). Two DEGs (SAZ_RS14940, SAZ_RS14945) annotated to regulate fatty acid desaturase were up-regulated by 2.27 and 2.01-fold, respectively (Table S3). Further qRT-PCR showed that the expression levels of both genes were enhanced by 2.33 and 2.18-fold, respectively (Fig. 2). The result was consistent with the transcriptome data. The fatty acids components of *S. albulus* M-Z18’s membrane were analyzed and shown in Fig. 3a, b. With the treatment of pH shock, tetradecanoic acid (C14:0),

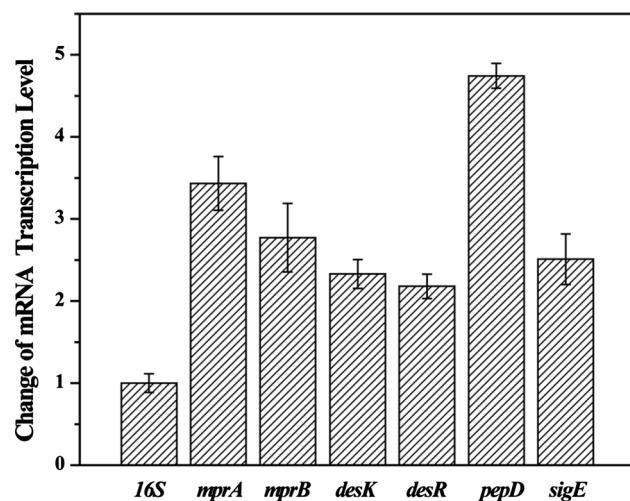


Fig. 2 Transcription level of genes related to signal transduction system in *S. albulus* M-Z18 during fermentation with pH shock. *mprA*: SAZ_RS24700, *mprB*: SAZ_RS24705, *desK*: SAZ_RS14940, *desR*: SAZ_RS14945, *pepD*: SAZ_RS24695, *sigE*: SAZ_RS22690

hexadecanoic acid (C16:0) and heptadecanoic acid (C17:0) in *S. albulus* M-Z18 were significantly decreased, whereas tetradecanoic acid (C14:1), hexadecanoic acid (C16:1) and heptadecanoic acid (C17:1) were increased, especially hexadecanoic acid (C16:1), which only existed after pH shock. The change of fatty acid composition in cell membrane not only lead to the decrease of average chain length, but also resulted in increase in the ratio of unsaturated to saturated fatty acids in the membrane from 5.18 to 7.49 (Table 1). The ratio of saturated to unsaturated fatty acids is an important factor modulating the permeability of cell membrane. In addition, phospholipids with unsaturated fatty acids were more elastic than phospholipids with saturated fatty acids [17]. Therefore, cell membrane fluidity was enhanced after pH shock, which was beneficial for substance transportation and energy metabolism. The increase of membrane fluidity could also enhance the acid tolerance of the cells [2, 4, 13]. Foster [9] reported that *Salmonella typhimurium* could obtain stronger viability after an acid shock in log phase. Interestingly, we also found *S. albulus* M-Z18 obtained stronger acid tolerance after pH shock [25].

The transcriptional changes of genes involved in transmembrane transportation

As shown in Fig. 1a, 21 DEGs were significantly enriched in transmembrane transport (GO:0055085) process. Eighteen unigenes were annotated as ATP-binding cassette (ABC) transporters (Table 2), and other three unigenes (SAZ_RS34335, SAZ_RS29210, SAZ_RS05735) were annotated as sugar porter family major facilitator superfamily (MFS) transporter, mycobacterial membrane protein large (MMPL) family transporter, respectively. Among these transmembrane transport DEGs, 16 unigenes were up-regulated and 5 unigenes were down-regulated. Interestingly, five unigenes (SAZ_RS25245, SAZ_RS25250, SAZ_RS06610, SAZ_RS06615, SAZ_RS11800) related to iron transportation were all up-regulated. It is reported that iron is a component of heme, the up-regulated unigenes may promote the synthesis of heme, thereby further enhancing the metabolic activity of cells [18]. This inference has been confirmed by the determination of heme. As shown in Fig. 3c, the content of intracellular heme increased significantly after pH shock by 50.69%, 41.88% and 140.88% during different periods of the fermentation. In addition, the related cation ion transport protein is closely connected with pH changes and respiratory chain in the fermentation process of *Streptomyces* [32]. The expression level of one ABC lipid transporter unigene (SAZ_RS40565) was highly up-regulated by 8.81-fold after pH shock, suggesting that cells’ response to pH shock was by enhancing lipid metabolism and transport [3]. Transmembrane transporters play important roles in a variety of cellular functions, including cellular metabolism,

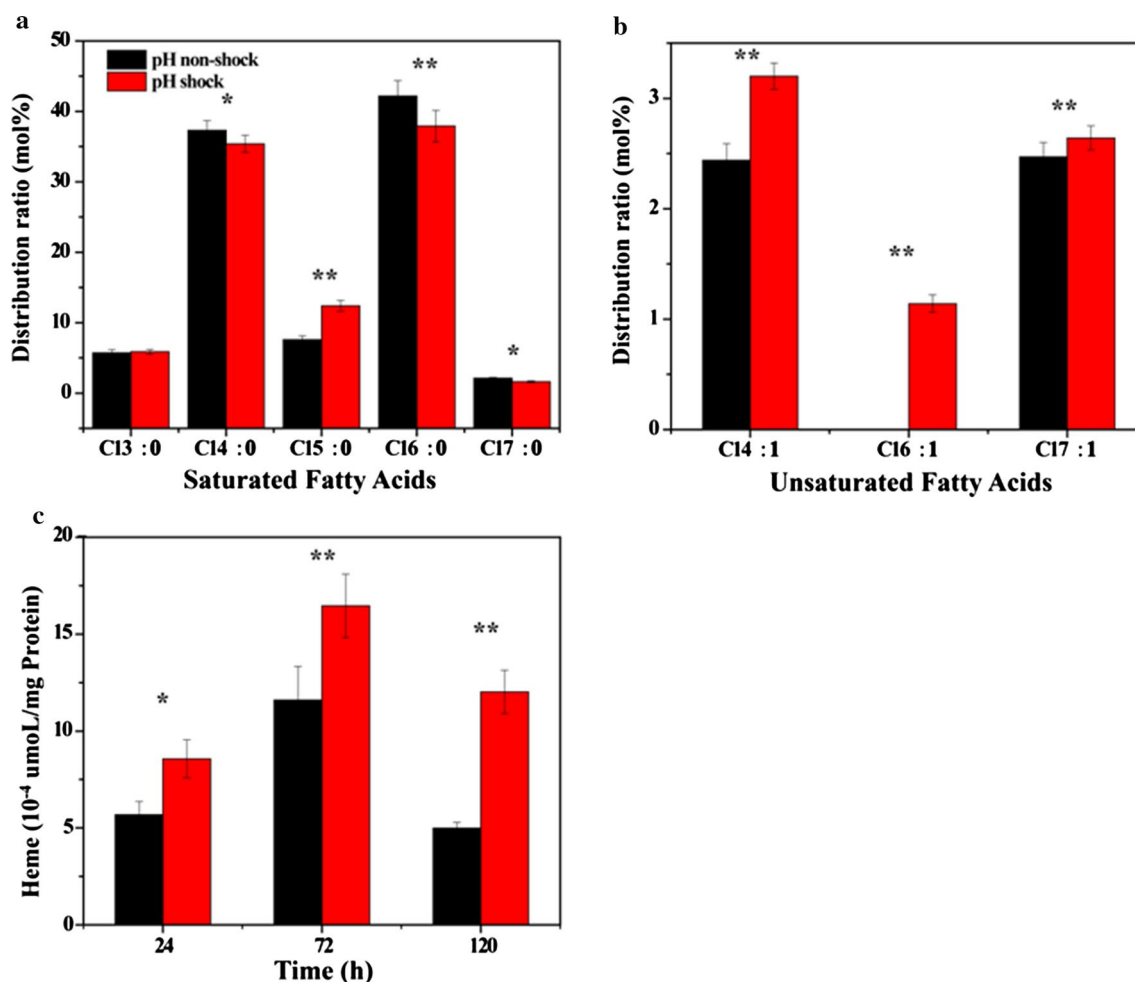


Fig. 3 Fatty acid composition analysis of cellular membrane and intracellular heme measurements (a saturated fatty acids, b unsaturated fatty acids, c intracellular heme content)

Table 1 The effects of pH shock on average chain length and unsaturation of membrane fatty acids in *S. albulus* M-Z18

	pH non-shock	pH shock
Average chain length	15.01 ± 0.02	14.87 ± 0.03**
Unsaturated/saturated fatty acid ratio ^a	5.18 ± 0.05	7.49 ± 0.07**

Values are mean ± SD from three independent experiments

Asterisks represents significant differences between the pH non-shock and

pH shock groups, ** $p < 0.01$

^aRatio of unsaturated/saturated fatty acid = $(C14:1 + C16:1 + C17:1) / (C13:0 + C14:0 + C15:0 + C16:0 + C17:0)$

ion homeostasis, intracellular pH homeostasis and signal transduction [24]. The up-regulated expression of these genes showed that transporters in *S. albulus* M-Z18 cells can respond to pH shock timely, which might be conducive to nutrient uptake and secondary metabolites excretion.

The transcriptional changes of genes involved in signal transduction system

With pH shock, unigenes involved in ‘signal transduction system’ (GO:0000160) were all up-regulated (Table 2). The results showed that the signal transduction pathway existed in response to stress caused by pH shock in *S. albulus* M-Z18. Unigene SAZ_RS22690 encoding RNA polymerase sigma factor (*sigE*) had a 2.80-fold up-regulation, which was significant for the transcription of genes in prokaryotes. Two pairs of two-component system (TCS), *mprA/B* (SAZ_RS24700, SAZ_RS24705) and *desK/R* (SAZ_RS14940, SAZ_RS14945) were up-regulated by 2.27, 2.01, 3.86 and 2.78-fold, respectively, together with a serine protease *pepD* (SAZ_RS24695) had a 5.08-fold up-regulation. The expressions of these unigenes were verified through qRT-PCR, the results were consistent with transcriptome (Fig. 2). TCSs play major roles in signal transduction and thus it is essential for its host to be able to adapt to a stress environment. It has

Table 2 The DEGs involved in transmembrane transport, electron carrier activity and signal transduction system

Unigene ID	Fold change	Description
Transmembrane transport		
SAZ_RS25245	2.19	Iron ABC transporter permease
SAZ_RS25250	2.14	Iron ABC transporter permease
SAZ_RS06610	2.22	Iron ABC transporter permease
SAZ_RS06615	2.30	Iron-siderophore ABC transporter protein
SAZ_RS11800	2.55	Iron ABC transporter permease
SAZ_RS40565	8.81	ABC lipid transporter
SAZ_RS18410	3.10	ABC transporter ATP-binding protein
SAZ_RS07390	2.07	ABC transporter ATP-binding protein
SAZ_RS05070	3.82	ABC transporter ATP-binding protein
SAZ_RS22405	2.17	ABC transporter ATP-binding protein
SAZ_RS22415	2.03	ABC transporter ATP-binding protein
SAZ_RS27445	2.65	ABC transporter ATP-binding protein
SAZ_RS05065	3.02	ABC transporter permease
SAZ_RS18420	3.14	ABC transporter permease
SAZ_RS05720	−2.36	ABC transporter permease
SAZ_RS39675	−2.86	ABC transporter permease
SAZ_RS40085	−2.55	ABC transporter ATP-binding protein
SAZ_RS24490	−2.18	Phosphate ABC transporter substrate-binding protein PstS
SAZ_RS29210	2.16	MMPL family transporter
SAZ_RS34335	2.19	Sugar porter family MFS transporter
SAZ_RS05735	−2.22	MFS transporter
Electron carrier activity		
SAZ_RS15070	4.48	Cytochrome c oxidase
SAZ_RS15075	4.77	Cytochrome c oxidase
SAZ_RS34975	2.12	Ferredoxin reductase
SAZ_RS31410	2.60	Iron sulfur protein
Signal transduction system		
SAZ_RS24695	5.08	Serine protease pepD
SAZ_RS14940	2.27	Two-component system, NarL family, sensor histidine kinase DesK
SAZ_RS14945	2.01	Two-component system, NarL family, response regulator DesR
SAZ_RS24700	3.86	Two-component system, OmpR family, response regulator MprA
SAZ_RS24705	2.78	Two-component system, OmpR family, response regulator MprB
SAZ_RS22690	2.80	SigE family RNA polymerase sigma factor

been reported that pH shock can cause significant changes in the transcription of TCS-related genes of *Streptomyces coelicolor* in a wide range [16].

To confirm the relationship between these unigenes involved in the signal transduction system and ϵ -PL biosynthesis, the corresponding gene disruption mutants were constructed and shake flask fermentation results shown in Fig. 4. The disruption of *desK*, *desR* and *sigE* showed no significant effect on the cell growth of mutants, ϵ -PL production, and the consumption of glucose and time profile of pH change compared with the control strain (Fig. 4a–d). However, the ability of spores' production of mutant strains with *desK*, *desR* and *sigE* disruption decreased when they were cultivated on the plate (Fig. 4f), which means that these three

genes may be related to the formation of spores. However, the disruption of *mprA*, *mprB* and *pepD* genes reduced the production of ϵ -PL by 52.11%, 60.56% and 55.63%, respectively. Interestingly, the final DCW of three mutant strains reached 7.48, 7.87 and 7.72 g/L, respectively, but there were no significant differences with the control strain (7.84 g/L). It was indicated that the inactivation of these three genes had little effect on the cell growth of mutants. Besides, the final pHs (3.46, 3.53 and 3.42) in the mutant strains' fermentation broth were higher than that of the control strain (pH 2.98), and the glucose consumption rate was relatively low during the whole fermentation (Fig. 4a–d). To investigate whether the decrease of ϵ -PL production in three mutants is related to the transcription of ϵ -PL synthetase gene (*pls*,

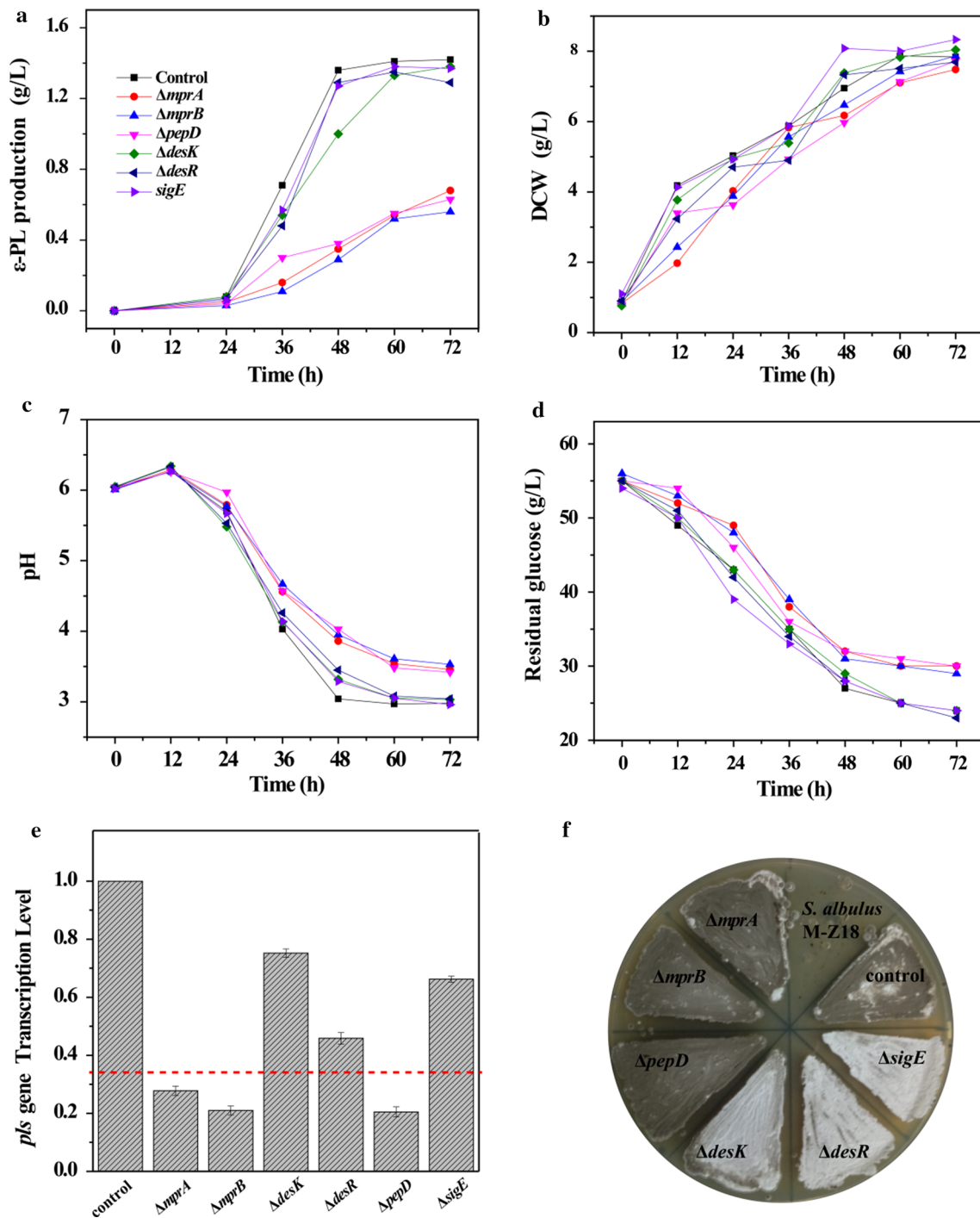


Fig. 4 Time profiles of batch fermentation parameters of mutant strains (a–d), *pls* transcriptional levels in mutants at 24 h (e), and growth of wild and mutant strains on plate with 50 μ g/ml apramycin (f)

SAZ_RS35855), we detected the transcription of *pls* gene in three mutant strains by qRT-PCR. As shown in Fig. 4e, only the transcription of *pls* gene in mutant strains with *mprA*, *mprB* and *pepD* disruption showed significant reduction by 83.51%, 92.62% and 93.21%, respectively, compared with the control strain. It confirmed the signal transduction

related to *mprA*, *mprB* and *pepD* genes played a positive regulatory role in the transcription of *pls* gene.

Unfortunately, the functions of *mprA*, *mprB* and *pepD* were not reported in *Streptomyces* until now. However, the MprAB has been reported as an intact signal transduction system in *Mycobacterium tuberculosis* [26]. *M. tuberculosis*

is often exposed to various environmental and physiological stresses during growth in the host, including hypoxia, nutrient limitation, reactive oxygen and low pH. To adapt to these conditions, MprAB is found participating in these stress responses [29]. Therefore, the relationship between MprAB and PepD was investigated [11]. It was reported that PepD was participated in the mycobacterial stress response mediation through MprAB and SigE. Authors proposed that MprB was kept inactive under non-stress condition by a protein repressor. When the membrane was exposed to a stress condition, this repressor disengaged from MprB. This leads to the activation of the MprAB system and subsequent up-regulation of genes comprising the MprAB regulon, including *mprAB*, *pepD*, and *sigE* [11, 33]. Herein, we have proved the disruption of *mprA/B* and *pepD* could down-regulate the transcription of *pls*, suggesting that *mprA/B* and *pepD* might sense the pH shock signal and subsequently regulate the transcription of *pls*. To the best of our knowledge, this is the first TCS (MprAB) proved for regulation of ϵ -PL biosynthesis in *S. albulus*. However, the detailed regulation process needs to be elucidated further in a future study.

The transcriptional changes of genes involved in the respiratory activity

pH shock significantly affected the ‘electron carrier activity’ (GO:0009055) within the biological process category shown in Fig. 1b. The unigenes cytochrome c oxidase (SAZ_RS15070 and SAZ_RS15075), ferredoxin reductase (SAZ_RS34975) and iron sulfur protein (SAZ_RS31410) were up-regulated by 4.48-, 4.77-, 2.12- and 2.60-fold, respectively, which are the major components of the respiratory chain (Table 2). Jiang et al. [14] reported that the respiratory vitality of the cells is significantly enhanced after alkaline pH shock. The up-regulated expression of these genes means that respiratory activity was strengthened in *S. albulus* M-Z18 by pH shock. To confirm this speculation, the respiratory activity of *S. albulus* M-Z18 was analyzed during fermentation. As shown in Fig. 5, the respiratory activity of mycelia in pH shock and pH non-shock fermentations showed the same trend, but the respiratory activity of *S. albulus* M-Z18 was always higher in pH shock fermentation. At the end of fermentation, the respiratory activity of *S. albulus* M-Z18 in pH non-shock fermentation was reduced to 66.77%, but it was only reduced by 51.67% in pH shock. The final respiratory activity in pH shock group was significantly higher than that in pH non-shock group ($p < 0.01$).

The transcriptional changes of ϵ -PL synthetase gene

ϵ -PL synthetase (Pls) is a non-ribosomal peptide synthase that catalyzes intracellular L-lysine to form ϵ -PL, which is the key enzyme in the ϵ -PL biosynthesis and might be

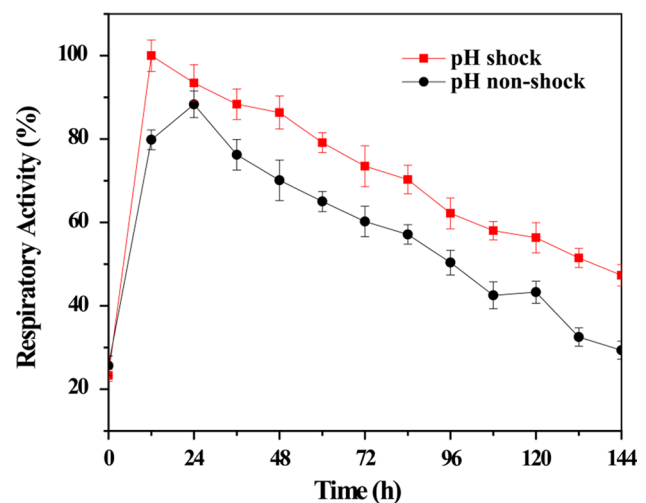


Fig. 5 Time profile of respiratory activity of *S. albulus* M-Z18 during fermentations with pH shock and pH non-shock

influenced by pH shock. However, *pls* was only up-regulated 1.79-fold in the transcriptome. As the key enzyme, the transcription of *pls* is important for ϵ -PL synthesis. The transcription of *pls* in pH shock and pH non-shock fermentation is monitored and shown in Fig. 6a. The changes in *pls* had the same trend, and the highest transcriptional level of *pls* was increased by 2.73-fold and 1.45-fold at 72 h, respectively. Interestingly, the transcription of *pls* in pH shock is significantly higher than that in pH non-shock during fermentation. In addition, we also measured the *pls* activity during the whole fermentation. As described in Fig. 6b, it reached the highest level (469.32 and 298.28 U/mg protein) at 48 h and then decreased along with fermentation. *pls* activity was always higher during the fermentation with pH shock strategy. Therefore, pH shock might increase the *pls* activity in *S. albulus* M-Z18 through improving the transcription of *pls*, and finally promoted the ϵ -PL production. Similarly, the transcript levels of the methylenomycin biosynthetic gene (*mmy*) were enhanced after acidic pH shock, eventually leading to an increase in the production of methylenomycin [10]. Thus, pH shock can effectively increase the transcription of the synthetic gene related to metabolite, thereby promoting the synthesis of the metabolites.

Conclusions

Based on transcriptional analysis combined with these physiological validation experiments, the mechanism of *Streptomyces* response to pH shock was revealed as Fig. 7. pH shock activated the MprA/B and PepD signal transduction system in *S. albulus* M-Z18, then resulted in up-regulating the transcription of *pls*, and finally enhanced

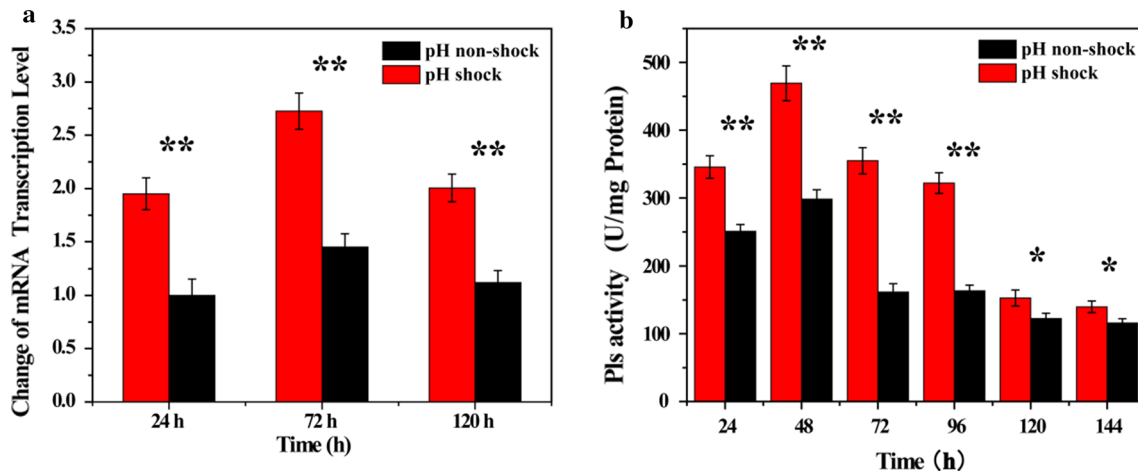


Fig. 6 Time profile of transcriptional changes of *pls* (a) and *pls* activity (b)

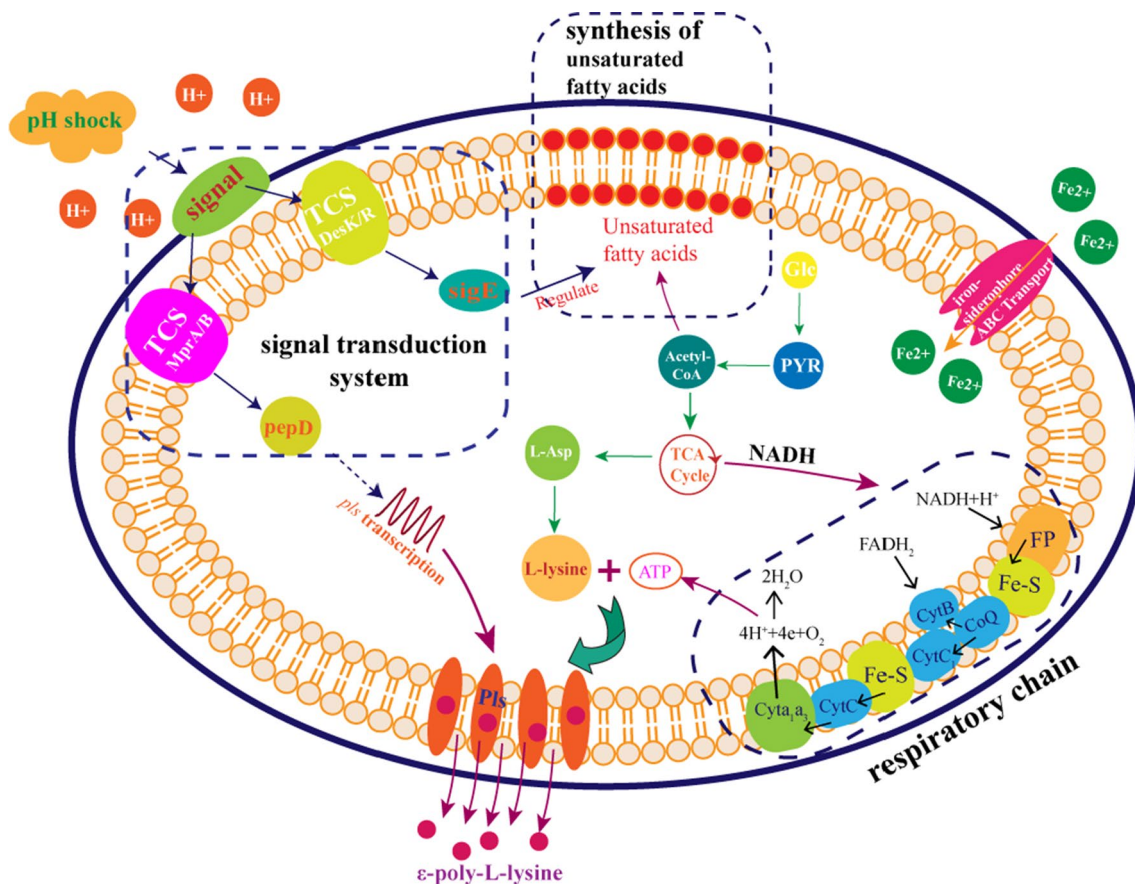


Fig. 7 The response mechanism of *Streptomyces* response to pH shock

the Pls activity during fermentation. Meanwhile, the pH shock increased the membrane permeability through the regulation of unsaturation/saturation fatty acid ratio by DesK/R, which might be helpful for intracellular and extracellular substances transportation. In addition, the pH

shock also enhanced the cell respiratory activity through up-regulating the transcription of genes involved in the major components of the respiratory chain, which might provide sufficient energy for cell growth and ϵ -PL biosynthesis. These combined observations were considered to

have contributed to the enhanced ϵ -PL productivity with the pH shock.

Acknowledgements This work was supported by the Program of the National Natural Science Foundation of China (31671846, 31301556), and the National First-Class Discipline Program of Light Industry Technology and Engineering (LITE2018-27), the Cooperation Project of Jiangsu Province among Industries, Universities and Institutes (BY2016022-25).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interests.

References

- Bankar SB, Singhal RS (2013) Panorama of poly- ϵ -lysine. *Rsc Adv* 3:8586–8603
- Beney L, Gervais P (2001) Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Appl Microbiol Biotechnol* 57:34–42
- Borst P, Zelcer N, Helvoort AV (2000) ABC transporters in lipid transport. *Biochim Biophys Acta* 1486:128–144
- Broadbent JR, Larsen RL, Deibel V, Steele JL (2010) Physiological and transcriptional response of *Lactobacillus casei* ATCC 334 to acid stress. *J Bacteriol* 192:2445–2458
- Chen X-S, Li S, Liao L-J, Ren X-D, Li F, Tang L, Zhang J-H, Mao Z-G (2011) Production of ϵ -poly-L-lysine using a novel two-stage pH control strategy by *Streptomyces* sp. M-Z18 from glycerol. *Bioprocess Biosyst Eng* 34:561–567
- Chen X-S, Mao Z-G (2013) Comparison of glucose and glycerol as carbon sources for ϵ -poly-L-lysine production by *Streptomyces* sp. M-Z18. *Appl Biochem Biotechnol* 170:185–197
- Chen X-S, Ren X-D, Zeng X, Zhao F-L, Tang L, Zhang H-J, Zhang J-H, Mao Z-G (2013) Enhancement of ϵ -poly-L-lysine production coupled with precursor L-lysine feeding in glucose–glycerol co-fermentation by *Streptomyces* sp. M-Z18. *Bioprocess Biosyst Eng* 36:1843–1849
- Chen X, Tang L, Li S, Liao L, Zhang J, Mao Z (2011) Optimization of medium for enhancement of ϵ -poly-L-lysine production by *Streptomyces* sp. M-Z18 with glycerol as carbon source. *Bioresour Technol* 102:1727–1732
- Foster JW (1991) Salmonella acid shock proteins are required for the adaptive acid tolerance response. *J Bacteriol* 173:6896–6902
- Hayes A, Hobbs G, Smith CP, Oliver SG, Butler PR (1997) Environmental signals triggering methylenomycin production by *Streptomyces coelicolor* A3(2). *J Bacteriol* 179:5511–5515
- He H, Hovey R, Kane J, Singh V, Zahrt TC (2006) MprAB is a stress-responsive two-component system that directly regulates expression of sigma factors SigB and SigE in *Mycobacterium tuberculosis*. *J Bacteriol* 188:2134
- Hiraki J, Ichikawa T, Ninomiya S, Seki H, Uohama K, Seki H, Kimura S, Yanagimoto Y, Barnett JW Jr (2003) Use of ADME studies to confirm the safety of epsilon-polylysine as a preservative in food. *Regul Toxicol Pharm* 37:328–340
- Huang R, Pan M, Wan C, Shah NP, Tao X, Wei H (2016) Physiological and transcriptional responses and cross protection of *Lactobacillus plantarum* ZDY2013 under acid stress. *J Dairy Sci* 99:1002–1010
- Jiang J, Sun YF, Tang X, He CN, Shao YL, Tang YJ, Zhou WW (2017) Alkaline pH shock enhanced production of validamycin A in fermentation of *Streptomyces hygroscopicus*. *Bioresour Technol* 249:234
- Kahar P, Iwata T, Hiraki J, Park EY, Okabe M (2001) Enhancement of ϵ -polylysine production by *Streptomyces albulus* strain 410 using pH control. *J Biosci Bioeng* 91:190–194
- Kim YJ, Moon AN, Song JY, Kim ES, Kim CJ, Chang YK (2009) Gene-expression analysis of acidic pH shock effects on two-component systems in *Streptomyces coelicolor*. *Biotechnol Bioprocess Eng* 14:584
- Kolbeck S, Behr J, Vogel RF, Ludwig C, Ehrmann MA (2019) Acid stress response of *Staphylococcus xylosum* elicits changes in the proteome and cellular membrane. *J Appl Microbiol* 126:1480–1495
- Köster W (2001) ABC transporter-mediated uptake of iron, siderophores, heme and vitamin B12. *Res Microbiol* 152:291–301
- Overbergh L, Giulietti A, Valckx D, Decallonne B, Bouillon R, Mathieu C (2003) The use of real-time reverse transcriptase PCR for the quantification of cytokine gene expression. *J Biomol Tech* 14:33–43
- Lara AR, Leal L, Flores N, Gosset G, Bolívar F, Ramírez OT (2010) Transcriptional and metabolic response of recombinant *Escherichia coli* to spatial dissolved oxygen tension gradients simulated in a scale-down system. *Biotechnol Bioeng* 93:372–385
- Li S, Li F, Chen X-S, Wang L, Xu J, Tang L, Mao Z-G (2012) Genome shuffling enhanced ϵ -poly-L-lysine production by improving glucose tolerance of *Streptomyces graminearus*. *Appl Biochem Biotechnol* 166:414–423
- Liu S, Wu Q, Zhang J, Mo S (2011) Production of ϵ -poly-L-lysine by *Streptomyces* sp. using resin-based, in situ product removal. *Biotechnol Lett* 33:1581–1585
- Michener JK, Jens N, Smolke CD (2012) Identification and treatment of heme depletion attributed to overexpression of a lineage of evolved P450 monooxygenases. *Proc Natl Acad Sci USA* 109:19504–19509
- Mishra NK, Chang J, Zhao PX (2014) Prediction of membrane transport proteins and their substrate specificities using primary sequence information. *PLoS One* 9:e100278
- Pan L, Chen X-S, Liu M-M, Liu Y-J, Mao Z-G (2017) Efficient production of ϵ -poly-L-lysine from glucose by two-stage fermentation using pH shock strategy. *Process Biochem* 63:8–15
- Pang X, Vu P, Byrd TF, Ghanny S, Soteropoulos P, Mukamolova GV, Wu S, Samten B, Howard ST (2007) Evidence for complex interactions of stress-associated regulons in an mprAB deletion mutant of *Mycobacterium tuberculosis*. *Microbiol* 153:1229–1242
- Ren X-D, Chen X-S, Tang L, Zeng X, Wang L, Mao Z-G (2015) Physiological mechanism of the overproduction of ϵ -poly-L-lysine by acidic pH shock in fed-batch fermentation. *Bioprocess Biosyst Eng* 38:2085–2094
- Ren X-D, Chen X-S, Zeng X, Wang L, Tang L, Mao Z-G (2015) Acidic pH shock induced overproduction of ϵ -poly-L-lysine in fed-batch fermentation by *Streptomyces* sp. M-Z18 from agro-industrial by-products. *Bioprocess Biosyst Eng* 38:1113–1125
- Riccardo M, Roberta P, Sebastien R, Jocelyn B, Luc G, Issar S, Roberta P (2004) Sigma factors and global gene regulation in *Mycobacterium tuberculosis*. *J Bacteriol* 186:895–902
- Shih IL, Shen MH, Van YT (2006) Microbial synthesis of poly(epsilon-lysine) and its various applications. *Bioresour Technol* 97:1148–1159
- Wang D, Yang C, Long D, Zhu J, Wang J, Zhang S (2015) Comparative transcriptome analyses of drought-resistant and—susceptible *Brassica napus* L. and development of EST-SSR markers by RNA-Seq. *J Plant Biol* 58:259–269
- Wei ZH, Bai L, Deng Z, Zhong JJ (2012) Impact of nitrogen concentration on validamycin A production and related gene transcription in fermentation of *Streptomyces hygroscopicus* 5008. *Bioprocess Biosyst Eng* 35:1201–1208

33. White MJ, He H, Penoske RM, Twining SS, Zahrt TC (2010) PepD participates in the *Mycobacterial* stress response mediated through MprAB and SigE. *J Bacteriol* 192:1498
34. Xu D, Yao H, Xu Z, Wang R, Xu Z, Li S, Feng X, Liu Y, Xu H (2017) Production of ϵ -poly-lysine by *Streptomyces albulus* PD-1 via solid-state fermentation. *Bioresour Technol* 223:149–156
35. Xu Z, Feng X, Sun Z, Cao C, Li S, Xu Z, Xu Z, Bo F, Xu H (2015) Economic process to co-produce poly (ϵ -L-lysine) and poly (l-diaminopropionic acid) by a pH and dissolved oxygen control strategy. *Bioresour Technol* 187:70–76
36. Yamanaka K, Kito N, Imokawa Y, Maruyama C, Utagawa T, Hamano Y (2010) Mechanism of ϵ -poly-L-lysine production and accumulation revealed by identification and analysis of an ϵ -poly-L-lysine-degrading enzyme. *Appl Environ Microbiol* 76:5669–5675
37. Yoshida T, Nagasawa T (2003) ϵ -Poly-L-lysine: microbial production, biodegradation and application potential. *Appl Microbiol Biotechnol* 62:21–26
38. Zhang Y, Feng X, Xu H, Yao Z, Ouyang P (2010) ϵ -Poly-L-lysine production by immobilized cells of *Kitasatospora* sp. MY 5-36 in repeated fed-batch cultures. *Bioresour Technol* 101:5523–5527

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