Metabolomic analysis of biosynthesis mechanism of ϵ -polylysine produced by *Streptomyces diastatochromogenes*

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Research

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Abstract

ϵ -polylysine (ϵ -PL) is a polypeptide that shows broad-spectrum inhibition against both Gram-positive and Gram-negative bacteria, and it's mainly produced by *Streptomyces sp.* However, the biosynthesis mechanism of ϵ -PL by *Streptomyces sp.* is still unclear. Herein, the metabolomic analysis of the biosynthesis mechanism of ϵ -PL in the original strain TUST and the high-yield mutant strain 6#-7 were investigated. Results show that the difference on metabolisms between TUST and 6#-7 was significant during fermentation periods. And based on further analyses of the results of both metabolomics and enzymatic activity, a possible metabolic regulation mechanism of the high-yield mutagenized strain 6#-7 was proposed. The transport and absorption capacity for glucose of strain 6#-7 is improved. And the activity of enzymes relating to ϵ -PL synthesis, including Hexokinase (HK) et al., is strengthened. On the contrary, the activity of enzymes in the branched-chain pathways, such as Succinate dehydrogenase (SDH) et al. is decreased. Meanwhile, the increase of trehalose, glutamic acid and proline makes the strain 6#-7 more resistant to ϵ -PL. Moreover, the strain 6#-7 has stronger ability to transfer ϵ -PL out the cell. Thus the ability of the mutagenized strain to synthesize ϵ -PL is enhanced and the strain 6#-7 can produce more ϵ -PL compared with the original strain. These findings provide a theoretical basis for further improving the production of ϵ -PL.

Introduction

ϵ -polylysine (ϵ -PL) is one of the two amino acid homopolymers that have been found in nature. It consists of 25–35 L-lysine residues linked via the α -carboxyl and ϵ -amino groups (Fig. S1) (El-Sersy et al. 2012), and it's produced by microbial metabolism (mainly by actinomycetes) (Shukla et al. 2012). It has various functions such as antibacterial (Shima et al. 1984) and antiphage activities (Shima et al. 1982). In addition, it is biodegradable and has good water solubility. And mouse feeding experiments verified that the polymer has almost no acute or chronic toxicity (Hiraki et al. 2003). ϵ -PL was firstly found in the fermentation broth of *Streptomyces sp.* NBRC 14147 (Shima and Sakai 1977). Currently, ϵ -PL produced by mutant strains of NBRC 14147 had been approved as a food additive and commercialized (Oppermann-Sanio and Steinbuchel 2002). In order to improve the yield of ϵ -PL, Zong et al. obtained a mutagenized strain of *Streptomyces albulus* and the yield of ϵ -PL increased from 0.40 to 1.59 g/L (Zong et al. 2012). By using genome shuffling and gentamicin resistance, Wang et al. improved the yield of ϵ -PL in *S. albulus* AG3-28 to 3.43 g/L, which was 49.1% greater than the starting strain (Wang et al. 2016). Shima et al. reported that L-lysine can be directly utilized to produce ϵ -PL in the biosynthesis (Shima et al. 1983). By investigating the feedback of aspartate kinase, Hamano et al. obtained a high-yield ϵ -PL producing strain (M68V) (Hamano et al. 2007). By knocking out the homoserine dehydrogenase gene in *Streptomyces clavuligerus*, Yilmaz et al. obtained a mutagenized strain with a 4.3-fold ϵ -PL yield compared with the original strain (Yilmaz et al. 2008). Vanooyen et al. found that reducing the activity of citrate synthase can buffer the increase of the substrate and thus obtained a high-yield lysine producing strain of *Corynebacterium glutamicum* (Vanooyen et al. 2012).

In previous work by our group, a high-yield ϵ -PL mutant *Streptomyces diastatochromogenes* strain (6#-7) was obtained from the original strain TUST which was isolated from the soil samples of Hainan Island. Specifically, the strain 6#-7 was got by atmospheric room temperature plasma (ARTP) and nitrosoguanidine mutagenesis combined with methylene blue and streptomycin resistance screening systems (Li et al. 2013). However, the biosynthesis mechanism of ϵ -PL by the strains is still unclear. Herein, the starting strain TUST and the high-yield mutant strain 6#-7 were investigated to reveal the biosynthesis mechanism of ϵ -PL. Through multi-level analysis, the biosynthesis mechanism of the high-yield mutants was elucidated at the aspects of metabolic regulation and enzymology.

Results And Discussion

Comparison of fermentation performance between the original strain TUST and the high-yield strain 6#-7

The difference between the original strain TUST and high-yield strain 6#-7 in fermentation performance of ϵ -PL production was compared by shake flask fermentation, which provided a data foundation for the exploration of ϵ -PL biosynthesis mechanisms.

Fermentation characteristics of the wild-type strain TUST and the mutant strain 6#-7 are shown in Fig. 1. With the increase of fermentation time, the pH value of the fermentation broth showed a downward trend because of the production of primary metabolic organic acid. During 24–48 h, the pH value decreased obviously due to the rapid growth of the strain in the early stages of fermentation, while the downward trend became slower during 48–72 h. In addition, the pH value of fermentation broth of the strain 6#-7 was slightly larger than that of the starting strain TUST during the whole fermentation process. As for residual sugar (RG), it also showed a downward trend indicating that both strains were continuously consuming the carbon source. And there was no significant difference in the sugar consumption rate between the two strains. In addition, the biomass of both strains reached a maximum of 5–7 g/L at 24 h and then decreased during 24–48 h and afterwards increased. And the biomass of strain 6#-7 was 1.47 times larger than that of strain TUST at 72 h. During 24–48 h there was little difference in the amount of ϵ -PL between the two strains, while in the middle and late stages of fermentation 6#-7 produced more ϵ -PL than that of TUST. For both strains the amount of ϵ -PL reached a maximum at 72 h and the value of strain 6#-7 was 0.24 g/L greater than that of the original strain.

Metabolomics Analysis By GC-MS

101 metabolites including 21 amino acids, 18 sugars, 6 alcohols and their derivatives were detected and quantified by GC-MS. The meaningful data are shown in Table 1 and the contents of other metabolites are shown in Table S1.

Table 1
Metabolite contents of cells identified by GC-MS

Metabolites	TUST		6#-7					
	12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
L-Aspartic acid 1	ND	ND	ND	ND	0.11 ± 0.02	ND	0.29 ± 0.04	0.01 ± 0.01
L-Aspartic acid 2	ND	ND	ND	ND	0.40 ± 0.09	0.21 ± 0.01	1.15 ± 0.09	0.03 ± 0.01
L-Asparagine	ND	0.63 ± 0.20	0.21 ± 0.03	0.22 ± 0.03	0.05 ± 0.01	1.15 ± 0.09	1.35 ± 0.05	0.26 ± 0.01
L-Lysine	0.06 ± 0.01	0.03 ± 0.01	0.16 ± 0.05	0.11 ± 0.05	0.04 ± 0.01	0.05 ± 0.01	0.33 ± 0.02	0.21 ± 0.03
D-Glucose	55.07 ± 1.74	109.51 ± 17.11	13.12 ± 1.97	4.70 ± 0.80	145.32 ± 4.04	80.47 ± 3.69	63.76 ± 2.42	0.38 ± 0.03
Maltose 1	0.06 ± 0.03	0.06 ± 0.01	0.07 ± 0.02	0.14 ± 0.01	ND	ND	ND	ND
Maltose 2	0.10 ± 0.04	0.16 ± 0.03	0.04 ± 0.02	0.05 ± 0.01	0.09 ± 0.03	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
D-(+)-Trehalose	1.07 ± 0.15	11.12 ± 0.91	3.58 ± 0.55	2.07 ± 0.26	0.99 ± 0.10	35.58 ± 1.92	70.92 ± 2.73	1.40 ± 0.13
Hexadecenoic acid 1	ND	ND	0.13 ± 0.01	0.25 ± 0.04	ND	ND	ND	0.07 ± 0.01
Hexadecenoic acid 2	15.67 ± 0.59	7.32 ± 0.52	2.91 ± 0.18	2.76 ± 0.23	4.35 ± 0.45	2.88 ± 0.39	2.83 ± 0.03	2.70 ± 0.28
Hexadecenoic acid 3	6.81 ± 0.56	3.63 ± 1.20	2.91 ± 0.18	2.76 ± 0.23	4.35 ± 0.45	2.88 ± 0.39	2.83 ± 0.03	2.70 ± 0.28
Octadecanoic acid 1	16.18 ± 0.42	8.34 ± 2.40	8.85 ± 0.70	7.80 ± 0.11	12.11 ± 0.50	8.86 ± 0.50	8.26 ± 0.40	7.88 ± 0.27
Octadecanoic acid 2	9.89 ± 0.76	5.16 ± 1.76	4.43 ± 1.20	3.69 ± 0.40	6.69 ± 0.29	3.98 ± 0.60	3.89 ± 0.14	3.74 ± 0.39
l-Norvaline	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	ND	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Sedoheptulose	ND	ND	0.01 ± 0.01	ND	ND	ND	0.01 ± 0.01	0.01 ± 0.01
1-Dodecanol	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.03 ± 0.01

2, 3 refers to the different configurations of the same substance

Metabolites	TUST				6#-7			
	12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
Acetamide 1	0.07 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	0.04 ± 0.01
Acetamide 2	0.19 ± 0.06	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
2, 3 refers to the different configurations of the same substance								

As seen in Table 1, after 48-h fermentation, the contents of amino acids, especially for the ϵ -PL precursors such as asparagine, aspartic acid, and lysine, in the high-yield strain 6#-7 were larger than those in the original strain TUST, indicating that the high-yield strain can produce more products in key steps of the ϵ -PL synthesis pathway. Among the 18 sugars, the glucose content in strain 6#-7 was significantly lower than that in strain TUST indicating that 6#-7 has a stronger glucose-consumption ability and thus can product more ϵ -PL. And maltose content in the branched-chain ϵ -PL metabolic pathway was lower in 6#-7, which benefits to the accumulation of ϵ -PL. On the contrary, the trehalose content increased in 6#-7 which enhances the resistance to ϵ -PL of the strain. The contents of 28 organic acids, hexadecenoic acid, octadecanoic acid and other fatty acids in strains 6#-7 were significantly lower than those in TUST, indicating that the fatty acid branch metabolism pathway in 6#-7 is weakened and thus the consumption of acetyl-CoA is reduced. And all of these benefit the accumulation of ϵ -PL in 6#-7. The contents of other compounds, such as norvaline, sedoheptulose, dodecanol, and acetamide, did not show any obvious difference between the two strains.

Principal component analysis (PCA) and Partial least squares analysis (PLSA)

Unsupervised (PCA) and supervised (PLSA) approaches were used to classify the dataset of different strains. The values of R^2 and Q^2 represent the accuracy of fit and the predictability of the model respectively. It is considered that if R^2 and Q^2 are greater than 0.9 the model is valid, and generally, it's reasonable if the values are greater than 0.5. The main statistics are shown in Table S2. It shows that the values of R^2 and Q^2 are close to 1.0 except the Q^2 of PCA, indicating that the model is credible.

The scores and loadings represent the discrete (Jackson 1980) and central tendency of the data (Kettaneh et al. 2005). Specifically, discrete tendency reflects the differences among the variables, while central tendency reflects the similarity among the variables (Wold et al. 1987).

Each experiment was repeated five times to ensure reproducibility of the results and the data were initially classified by PCA analysis. As shown in Fig. 2(A), data of the same group were centralized indicating the model has a good testing repeatability. The distribution of each group, except for TUST at 12 h and 24 h, was discrete from other groups meaning there were significant differences in the metabolisms between TUST and 6#-7 during fermentation periods. The data of TUST at 12 h and 24 h were clustered indicating the metabolism was stable for TUST at initial stage. The loading plot was used to find biomarkers. The

farther a metabolite is away from the origin of coordinates, the greater contribution it makes (Teague et al. 2007). As seen in Fig. 2(B), the metabolites with high contribution rate during the whole fermentation process were D-(+)-trehalose, D-glucose, D-galactose, butanoic acid, L-alanine, oxalic acid, glutamic acid, glycerol and L-valine.

To further confirm the results of PCA, PLSA was used for statistical analysis (H 1999). As shown in Fig. 3(A), the results were consistent with those of the PCA indicating the metabolisms of TUST and 6#-7 were significant different during fermentation periods. And in Fig. 3(B), there were other major metabolites, namely palmitic acid and stearic acid, besides those found in the PCA.

Under the PLSA model, multidimensional analysis of differential metabolite screening can be performed by Variable importance plot analysis (VIP), which can screen potential biomarkers. VIP value more than 0.5 was used as the cutoff value for statistical significance. As in Fig. 4, biomarkers extracted from the loading plot can be sorted according to the contribution rate: D-glucose > D-galactose > D-(+)-trehalose > stearic acid > palmitic acid > glycerol > L-alanine and oxalic acid. Among them, D-glucose, D-galactose and D-(+)-trehalose are the main intermediates related to sugar metabolism. Stearic acid and palmitic acid are important products of fatty acid metabolism pathway. L-alanine and oxalic acid are closely related to cell center carbon metabolism.

Hierarchical cluster analysis (HCA) was performed to further validate the prediction accuracy of the PCA model. Heatmap generated from HCA based on the first principal component is shown in Fig. S2. The samples have obvious distinctions and can be sorted according to strains indicating the HCA results are in consistent with those of PCA.

Comparative Analysis Of Intracellular Metabolites Related To ϵ -PL Synthesis

The changes of intracellular metabolite concentration in the strains are shown in Fig. 5. It shows that strain 6#-7 has strong ability to absorb glucose. And the weakening of fatty acid metabolism pathway liberates more acetyl-CoA to tricarboxylic acid cycle (TCA), which can promote the TCA pathway and increase the accumulation of oxaloacetate (Oaa). The enhancement of diaminopimelate pathway increases the content of threonine, homoserine and isoleucine, and that promotes the accumulation of lysine, which is the precursor of ϵ -PL. In addition, it is presumed there may be a pathway that can compensate for the loss of intermediate metabolites in the pentose phosphate pathway (PPP) and allow the PPP to be restored, which in turn provides large amount of NADPH for ϵ -PL synthesis. Moreover, the increase of α -ketoglutaric acid and succinic acid promotes the production of proline and glutamic acid, which enhances the ability of the strain to resist ϵ -PL itself. Thus, the ability of the mutagenized strain to synthesize ϵ -PL is enhanced.

In general, based on the metabolomics analysis, it was found that the intracellular metabolism of the strain significantly changed after mutagenesis, which is consistent with the results of PCA and PLSA.

Combing the findings of main metabolites by the above loading plot analysis and the analysis of related metabolic intermediates in the metabolite network, a possible metabolic regulation mechanism of the high-yield mutagenized strain was proposed. Firstly, the transport and absorption capacity for glucose of strain 6#-7 is improved. Secondly, the TCA pathway is promoted and that can increase the accumulation of the precursor Oaa. Thirdly, the diaminopimelate pathway is enhanced to produce more lysine which is essential precursor for ϵ -PL synthesis. Fourthly, the increase of trehalose, glutamic acid and proline makes the strain 6#-7 more resistant to ϵ -PL.

Comparative analysis of key enzyme activities in metabolic pathways during ϵ -PL synthesis

Enzyme plays an important role in the growth and metabolism of microorganisms (Teague et al. 2007), thus the enzyme activities in metabolic pathways associated with ϵ -PL synthesis were measured to gain further insight into the biosynthesis mechanism of ϵ -PL in the high-yield strain 6#-7 at the enzymatic level.

Results are shown in Fig. 6, compared with the original strain TUST, the activities of Hexokinase (HK) and Pyruvate kinase (PK) in the high-yield strain 6#-7 are strengthened, which provides sufficient glucose-6-phosphate (G6p) and pyruvate (Pyr). The increase of the activities of Phosphoenolpyruvate carboxylase (PEPC) and Succinate dehydrogenase (SDH) benefits the accumulation of Oaa. Besides, the synthesis of ϵ -PL requires a large amount of NADPH, and the increasing activity of PEPC can also provide more NADPH for ϵ -PL synthesis. And the enhancement of Aspartokinase (ASK) activity can produce more β -aspartic acid (β -Asp) for the synthesis of lysine, which is the precursor of ϵ -PL. Meanwhile, the decrease of the activities of Glucose-6-phosphate dehydrogenase (G6PDH) and Homoserine dehydrogenase (HSD) leads to less of G6p and homoserine to the branched-chain pathways. All of these promote the produce of ϵ -PL in the high-yield strain 6#-7. In addition, the activity of Polylysine synthetase (PLS) has almost no change between the two strains.

Metabolomics And Enzymatic Analyses In ϵ -PL Synthesis

Based on the results of metabolomics and enzymatic analyses, a possible metabolic regulation mechanism of the high-yield mutagenized strain was proposed (Fig. 7). Firstly, the transport and absorption capacity for glucose of strain 6#-7 is improved. Secondly, the activity of enzymes relating to ϵ -PL synthesis, including HK, PK, PEPC, SDH, ASK, is strengthened. And on the contrary, the activity of enzymes in the branched-chain pathways, such as G6PDH and SDH is decreased. Thirdly, the increase of trehalose, glutamic acid and proline makes the strain 6#-7 more resistant to ϵ -PL. Fourthly, the strain 6#-7 has stronger ability to transfer ϵ -PL out the cell. Thus, the ability of the mutagenized strain to synthesize ϵ -PL is enhanced and the strain 6#-7 can produce more ϵ -PL compared with the original strain.

Conclusion

In this study, the biosynthesis mechanism of ϵ -PL produced by the high-yield strain 6#-7 were studied. It was found that the metabolism map of the high-yield strain changed significantly compared with the origin strain. Analysis of PCA and PLSA scores showed that there were significant differences in intracellular metabolism during different periods of fermentation. The main metabolites that cause cell variability by loading plot analysis may be D-glucose, D-galactose, D-(+)-trehalose, stearic acid, palmitic acid, glycerol, L-alanine and oxalic acid. Unlike the origin strain, the ability of strain 6#-7 cells to transport and absorb glucose is improved. The EMP and TCA pathways are activated earlier, and the diaminopimelate pathway is enhanced by providing a large amount of ϵ -PL precursor-lysine. Moreover, increased protective substances such as trehalose, glutamic acid and proline enhance the ability of the strain 6#-7 itself to resist ϵ -PL.

The activity of key enzymes involved in the metabolism pathway related to ϵ -PL synthesis was also examined, and the reasons for the differences between the two strains in the ability to synthesize ϵ -PL were explained at the enzymatic level. The increase of HK, PK, PEPC and ASK activities together with the reduction of HSD and G6PDH activities promote the production of ϵ -PL. To the best of our knowledge, it's the first time the metabolomic analysis of biosynthesis mechanism of ϵ -PL produced by *Streptomyces diastatochromogenes* was systematically studied. These findings provide a theoretical basis for further improving the production of ϵ -PL.

Methods

Strains, medium, and culture conditions

The strain was inoculated into Bennett's slant medium and cultured at a temperature of 30 °C with a humidity of 50% for 5–7 days to harvest gray spores. A ring of spores was picked from the Bennett's slope and inserted into 100 mL of M3G seed medium. The flasks were incubated at 30 °C and 180 rpm on a shaker for 30 h. Subsequently, the seed culture was transferred to another 500 mL flask containing 100 mL of fermentation medium at an inoculation amount of 6.4% (v/v), and cultured at 30 °C 180 rpm for 72 h.

Bennett's solid medium (pH 7.7) is comprised of 10 g/L glucose, 1 g/L beef extract, 2 g/L peptone, 1 g/L yeast extract, and 20 g/L agar. The M3G seed and fermentation medium contained 10 g/L ammonium sulfate, 1.36 g/L potassium dihydrogen phosphate, 0.8 g/L dipotassium phosphate, 5 g/L yeast extract and the initial pH was adjusted to 7.2 with ammonium hydroxide solution.

In order to evaluate the effects on the synthesis of ϵ -PL by high-yield strains, the fermentation parameters including pH, biomass, residual sugar (RG), and ϵ -PL yield were measured every 24 h. The pH value was measured by using a FE20 pH meter (METTLER TOLEDO, Shanghai). Biomass concentrations were calculated from the dry cell weight. Briefly, 8 mL culture aliquots were taken every 24 h, centrifuged at 6000 $\times$ g for 10 min, the obtained residues were washed three times with sterile water, and finally dried at 95 °C to constant weight. The corresponding biomass was calculated from the dried samples. The supernatant after centrifugation of 1 mL of fermentation broth at 6000 $\times$ g for 10 min was diluted 50

times with distilled water, and the residual glucose concentration in the fermentation broth was measured by SBA-40E biosensor analyzer. The ϵ -PL yield was determined by spectrophotometry (Thermo Fisher, China). The fermentation broth cultured for a certain period was centrifuged at $8000 \times g$ for 5 min and the supernatant was appropriately diluted. Afterward, the diluted solution was mixed with 1 mM methyl orange solution 1:1 and shaken at 30 °C, 140 rpm for 30 min, and centrifuged at $4000 \times g$ for 15 min. One mL of the supernatant was diluted to 50 mL with 0.1 M Na_2HPO_4 - NaH_2PO_4 buffer (pH 6.6) and then the absorbance at 465 nm was measured (pure buffer was used as a blank control).

GC-MS Based Metabolomics Testing

Prior to the GC-MS analysis, the sample was derivatized by the method of Bo et al. (Bo et al. 2014). Briefly, 50 μL of methoxy ammonium hydrochloride/pyridine solution (20 mg/mL) was added to the freeze-dried sample, vortexed to fully dissolve it, placed in a 40 °C-water bath, and shaken for 80 min. Then 80 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added, vortexed, and placed back in the water bath for another 80 min. Finally, after centrifuged at $10000 \times g$ for 5 min, 100 μL supernatants of the derivatized sample were taken and placed at room temperature for 2 h.

The GC-MS system consisted of an Agilent 7890A gas chromatography system and an Agilent 5795C quadrupole mass selective detector (Agilent Technologies, Palo Alto, CA). GC was performed on a HP-5 column (60 m $\times$ 0.250 mm $\times$ 0.25 μm , Agilent Technologies). A total of 1 μL of the derivatized sample was injected with a split ratio of 1:10. The temperatures of injector, GC interface, and ion source were set at 280, 250, and 280 °C, respectively. The oven temperature was initially controlled at 70 °C for 2 min, then increased to 290 °C with a gradient of 5 °C/min, and then it was kept at 290 °C for another 3 min, and finally dropped to 70 °C. The carrier gas was helium at a constant flow of 1 mL/min. Mass spectra were recorded at two spectra per second with an m/z 50–800 scanning range.

Mass spectral peaks identification and quantification were performed by MESCHEM software (Agilent Technologies). Peak deconvolution was performed by AMDIS-32 previously. The NIST MS standard reference databases 2.0 were used for the identification of metabolites.

Data analysis

The mathematical model and the reliability verification of the model are important guarantees for the accuracy of later data analysis. And cross validation is a practical and accurate method to test whether the model is effective (Han and Yuan 2009). Therefore, the Principal component analysis (PCA) and Partial least squares analysis (PLSA) by SIMCA-P 11.5 were used. The spectra were reconstructed by peak area integration (Lv et al. 2016) and after being centralized and Orthogonal signal correction (OSC), PCA was performed. The raw data were converted from multiple metrics into comprehensive metrics and the clustering effect was previewed by Score plot. Besides, the loading plot was used to find biomarkers. Then PLSA was used for verification analysis. And the sample grouping, biomarker and contribution rate of the metabolites were verified by the score plot, the loading plot and Variable importance plot (VIP)

respectively. The data were intercepted for Hierarchical cluster analysis (HCA) for metabolites with a threshold greater than 0.5. Meanwhile, the data were statistically analyzed by SPSS 20.0 software.

Abbreviations

ϵ -PL: ϵ -polylysine; *S. albulus*: *Streptomyces albulus*; ARTP: atmospheric room temperature plasma; GC-MS: gas chromatography–mass spectrometry; RG: residual sugar; PCA: Principal component analysis; PLSA: Partial least squares analysis; VIP: Variable importance plot; HCA: Hierarchical cluster analysis; TCA: tricarboxylic acid cycle; PPP: pentose phosphate pathway; Glc: Glucose; GlcA: Gluconic acid; G6p: Glucose-6-Phosphate; 6pg: 6-phosphate gluconic acid; RI5p: Ribulose-5-phosphate; R5p: Ribose-5-phosphate; X5p: Xylulose-5-phosphate; S7p: Sedoheptulose-7-phosphate; G3p: Glyceraldehyde-3-phosphate; E4p: Erythrose-4-phosphate; F6p: Fructose-6-phosphate; Gly: Glycine; Ser: Serine; Cys: Cystathionine; Pep: Phosphoenolpyruvic acid; Pyr: Pyruvate; Ala: Alanine; AcCoA: Acetyl-CoA; Oaa: Oxaloacetate; Cit: Citrate; Ict: Isocitrate; Akg: α -ketoglutarate; Succ: Succinate; Fum: Fumarate; Mal: Malic acid; Asn: Asparagine; Asp: Aspartic acid; β -Asp: β -Aspartic acid; Hse: Homoserine; Thr: Threonine; Ile: Isoleucine; Lys: Lysine; Glu: Glutamic acid; Pro: Proline; Orn: Ornithine; Cite: Citrulline; Arg: Arginine; HK: Hexokinase; PK: Pyruvate kinase; PEPC: Phosphoenolpyruvate carboxylase; ASK: Aspartokinase; HSD: Homoserine dehydrogenase; G6PDH: Glucose-6-phosphate dehydrogenase; SDH: Succinate dehydrogenase; PLS: Polylysine synthetase.

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JDC and SRJ designed the research, FZG performed the experiments, ZYW and TYD performed statistical analysis and presented data in figures and tables, ZYW, TYD and MA wrote the paper. All authors read and approved the manuscript.

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Figures

Figure 1

Fermentation performance of the strains

Figure 2

Principle component analysis (PCA) of the metabolites at different fermentation stages: Score plot (A) and Loading plot (B)

Figure 3

Partial least squares analysis (PLSA) of the metabolites at different fermentation stages: Score plot (A) and Loading plot (B)

Figure 4

VIP results of differential metabolites, 1: D-Glucose; 2: D-Galactose; 3: D-(+)-Trehalose; 4: Stearic acid; 5: Palmitic acid; 6: Glycerol; 7: L-Alanine; 8: Oxalic acid; 9: Butanoic acid; 10: Octadecanoic acid; 11: Hexadecanoic acid; 12: Glutamic acid; 13: L-Proline; 14: Ethanediodic acid; 15: Glucopyranose; 16: N-α-acetyl-L-lysine; 17: Cadaverine tri-TMS; 18: Androst-2-en-17-amine; 19: Glycyl-L-glutamic acid; 20: D-Erythro-pentose; 21: L-Valine; 22: D-Xylopyranose; 23: β-D-Galactopyranoside; 24: Propanoic acid; 25: L-Threonine; 26: Pregna-1,4,6-triene-3,20-dione; 27: L-Isoleucine; 28: L-Phenylalanine; 29: Tetradecanoic acid; 30: L-Serine; 31: 2-Monostearin; 32: Adenosine

Figure 5

Schematic showing changes of metabolite abundance mapped onto the metabolic network in strain 6#-7, Red ones: increase in content; Green ones: decrease in content; Yellow ones: first increased and then decreased. Glc: Glucose; GlcA: Gluconic acid; G6p: Glucose-6-Phosphate; 6pg: 6-phosphate gluconic acid; R15p: Ribulose-5-phosphate; R5p: Riobse-5-phosphate; X5p: Xylulose-5-phosphate; S7p: Sedoheptulose-7-phosphate; G3p: Glyceraldehyde-3-phosphate; E4p: Erythrose-4-phosphate; F6p: Fructose-6-phosphate; Gly: Glycine; Ser: Serine; Cys: Cystathionine; Pep: Phosphoenolpyruvic acid; Pyr: Pyruvate; Ala: Alanine; Accoa: Acetyl-CoA; Oaa: Oxaloacetate; Cit: Citrate; Ict: Isocitrate; Akg: α-ketoglutarate; Succ: Succinate;

Fum: Fumarate; Mal: Malic acid; Asn: Asparagine; Asp: Aspartic acid; β -Asp: β -Aspartic acid; Hse: Homoserine; Thr: Threonine; Ile: Isoleucine; Lys: Lysine; Glu: Glutamic acid; Pro: Proline; Orn: Ornithine; Cite: Citrulline; Arg: Arginine

Figure 6

Changes of enzyme activity of the strain before and after mutagenesis, Red ones: increase in activity; Green ones: decrease in activity; Yellow one: no obvious change. HK: Hexokinase; PK: Pyruvate kinase; PEPC: Phosphoenolpyruvate carboxylase; ASK: Aspartokinase; HSD: Homoserine dehydrogenase; G6PDH: Glucose-6-phosphate dehydrogenase; SDH: Succinate dehydrogenase; PLS: Polylysine synthetase

Figure 7

A possible metabolic regulation mechanism of the high-yield ϵ -PL mutagenized strains: the transport and absorption capacity for glucose of strain 6#-7 is improved; and the activity of enzymes relating to ϵ -PL synthesis is strengthened; on the contrary, the activity of enzymes in the branched-chain pathways is decreased; besides, the increase of trehalose, glutamic acid and proline makes the strain 6#-7 more resistant to ϵ -PL; moreover, the strain 6#-7 has stronger ability to transfer ϵ -PL out the cell

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