Research progress on production methods of ε-poly-l-lysine

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Abstract. ε-poly-L-lysine (ε-PL) is a safe and environmentally friendly natural biological preservative, by 25~35 lysine residues through the intermolecular carboxyl group and amino amino condensation to form an amide bond to form L-lysine homopolymer, with high antibacterial activity, degradation in the human body must L-lysine, used in food and cosmetics and other industries. It is widely used in the United States, Japan and China. At present, the production cost of ε-PL is too high, and it is mainly produced by the fermentation of *Streptomyces*, but the *Streptomyces* is a non-GRAS(Generally Recognized As Safe) strain, not a general food-grade strain certified by the US Food and Drug Administration. In this review, the current biotechnological fermentation methods of *Streptomyces albicans* to produce ε-PL and the research progress of heterogenic expression of ε-PL through genetic engineering were reviewed. Increasing the output of ε-PL is of great significance for breaking the technological blockade and improving the core competitiveness of our country. The purpose of this review is to use genetic engineering technology to combine fermentation production, improve the yield of ε-PL from the genetic level, construct food-grade production strains, and further expand the method of ε-PL production.

Keywords: ε-poly-L-lysine fermentation production genetic engineering food grade.

1. Introduction

In recent years, food safety problems have become more and more frequent, and the abuse of preservatives has emerged endlessly[1]. With the improvement of people's quality of life, people have begun to look for safe and healthy preservatives. The research and application of natural preservatives has gradually become the focus of relevant researchers. Natural preservatives are mostly extracted from plants, animals and microorganisms, and biological preservatives that do not affect the sense and taste of food have become the object of research. Natural biological preservatives have been developed rapidly. Shima[2] is equal to ε-poly-L-lysine (ε-PL), which was first found in *Streptomyces*. 346 in 1977. It is light yellow or white in appearance, has good moisture absorption, has good water solubility, and is insoluble in organic solvents such as ethyl acetate and ether. ε-PL also has strong thermal stability, good safety, high biodegradability and good biological properties. In 1989, Japan has applied ε-PL to the industrial production stage, and has built an annual production line of 1,000 tons of industrial production. But at present in the domestic industrial production of ε-PL is still in the initial stage, how to improve the production of ε-PL, to reach the world's advanced level there is still a long way to go. The highest international ε-PL fermentation level has reached 48.3 g/L[3], but the domestic fermentation level is generally around 25 g/L. It is an effective way to reduce the cost of fermentation products to increase the concentration of metabolites in fermentation solution through bioprocess optimization. It is of great significance for our country to break the technological monopoly and improve the core competitiveness by improving the production of ε-PL, screening efficient production strains and reforming them, optimizing the late fermentation process and reducing the production cost.

In the past period of time, people used fermentation to optimize ε-PL production strains to increase the production of ε-PL, by increasing the intermediate metabolites, changing the shape and size of

the production strains, inducing the mutation of the production strains. With the rapid development of genetic engineering, more and more industrial production of genetic engineering technology, ε-PL production strain *Streptomyces* is a non-GRAS strain, there are still many problems in the production of food grade preservatives. The purpose of this review is to use genetic engineering technology to combine fermentation production and construct food-grade production strains while increasing ε-PL production at the genetic level.

2. Fermentation production of ε-PL

At present, ε-PL is mainly produced by the fermentation of Streptomyces albicolor. It is an effective way to reduce the cost of fermentation products to increase the concentration of metabolites in fermentation solution through bioprocess optimization. Optimization of fermentation conditions and breeding modification were used to improve the ε-PL content of ε-PL producing strains.

The optimal medium was optimized by means of experiment to screen out the most suitable nutrient ratio of production strains and seek the maximum yield. Good medium optimization is the most important step from experiment to industrial production. As early as 1977, Shima[4] and Sakai found that wild-type Streptococcus alba produced only 0.3g/L -PL after 96h culture in a medium containing glycerol, ammonium sulfate and yeast extract. The yield of S-2-aminoethyl-L-cysteine and glycine resistant mutant was increased to 1.2g/L in M3G medium after 96h culture

In 1998, Hiraki[5] found that a pH below 4.2 also significantly increased ε-PL production. ε-PL is a secondary metabolite of Streptomyces albicans. Wang[6] et al. discussed the research progress on the improvement of microbial secondary metabolite synthesis under pH stress, reviewed the influence of acid stress on microbial synthesis ability, and the bright prospect of microbial adaptive evolution. The pH strategy can effectively optimize product synthesis.

The optimal pH value of Streptomyces albus is neutral, while the optimal pH of ε-PL synthase is 4.0. With the progress of fermentation, Streptomyces albus spontaneously carries out acid stress, and the ambient pH decreases, and eventually spontaneously drops to about pH3.0. At this time, the activity of some enzymes in the production strain was greatly reduced, even inactivated, and the ε-PL yield was reduced. Controlling pH3.5-4.5 during fermentation can inhibit the activity of ε-Pl-degrading enzyme and accumulate sufficient ATP. Yamanaka[7] K et al. found that the catalytic function of ε-PL synthase is regulated by intracellular ATP, and high concentration of ATP is an important condition for the activation of ε-PL synthase.

In order to solve the problem of decreasing ε-PL production due to uncontrollable pH during fermentation, Kahar et al.[8] designed a two-phase pH control and substrate feeding strategy in a 5-liter fermenting tank. This method increased the output of ε-PL from 5.7g to 48.3g/L. Chen et al. proposed a two-stage pH control strategy based on kinetic analysis for efficient ε-PL fermentation. This method proved to be a better strategy for increasing e-PL concentration, yield, and productivity. With this pH shift control strategy, the e-PL concentration, yield and productivity were increased by 16.6, 7.04 and 52.1%, respectively, compared with a single pH control process (pH3.5). Ren et al. applied a flask pH control strategy to ε-PL producing strain Streptomyces sp.M-Z18. The effects of four buffer systems on the fermentation process of ε-PL in Streptomyces sp.M-Z18 were studied. It was found that the addition of 0.1mol/L citric acid at 18h of fermentation had a controlling effect on the pH value of the whole process. The pH value can be stabilized at about 3.8, and the ε-PL yield can reach 5.00±0.05g/L, which increases 167% and 75% compared with natural fermentation (uncontrollable pH). PAN et al.[9] applied the pH shock strategy to shock S.albulus M-Z18, and after the shock, the transcription level and enzyme activity level of ε-PL synthase were significantly improved. pH shock may enhance pls activity of S.albulus M-Z18 by increasing pls transcription, thus promoting ε-PL production. However, after acidic pH shock, transcription levels of methylenomycin biosynthetic gene(mmy) are enhanced, eventually leading to increased production of methylenomycin.

Ren[10] et al. found that during the fermentation process, the cell morphology of the production strain Streptomyces sp.M-Z18 was changed by physical methods, the microspheres were reduced by talc with a median diameter of 5 microns, and 10g/L was added. At the end of fermentation, the yield of ε-PL was 2.01±0.06g/L, which was 59.5% and 119.6% higher than that of the control. The buffer system and talc powder added in the fermentation process, although the yield of ε-PL was greatly increased, the composition of fermentation liquid was complex, which was not conducive to the separation and purification in the later stage.

Traditional breeding methods are time-consuming, labor-intensive, complex and inefficient, which seriously restrict the further improvement of ε-PL strain yield. In 2007, Ochi[11] proposed a new concept of "ribosome engineering" that focuses on activating dormant genes to fully induce cell function. Ribosome engineering can be applied to strain improvement and screening of new metabolites. Bacillus subtilis and Streptomyces, these prokaryotes exhibit a wide range of adaptations under conditions of extreme nutritional restriction.

3. Modification of ε-PL producing bacteria

In essence, the unilateral biological process optimization synthesis of ε-PL is only limited to a class of organisms. Genetic engineering can combine the dominant genes of each species at the gene level, and has the advantage of naturally crossing the species barrier, overcoming the limitations between biological species, and having unlimited possibilities, which is also the biggest feature of genetic engineering[12]. It is not possible to unilaterally increase the yield through genetic engineering, if the traditional biological process optimization can be combined, the production of ε-PL will not only change the amount, but also a qualitative leap. Aixia Wang et al.[13] combined the two methods, as shown in Fig.1, connecting the strong promoter pro2 and the ribosome binding site (Rbs2) of phage ©C31 capsid protein with pls, obtaining pro-rbs2-pls fragments, and then introducing Streptomyces albiculus CICC 11022 into the recombinant cell named Q-PL2. Streptomyces albicularis CICC 11022 and Q-PL2 were analyzed by fluorescent quantitative PCR, as shown in Figure 1. The pls expression efficiency of Streptomyces albicans CICC 11022 increased gradually with time, while Q-PL2 had a higher pls expression efficiency at the initial stage, but the expression efficiency decreased with time, but it was still much higher than that of Streptomyces albicans CICC 11022.

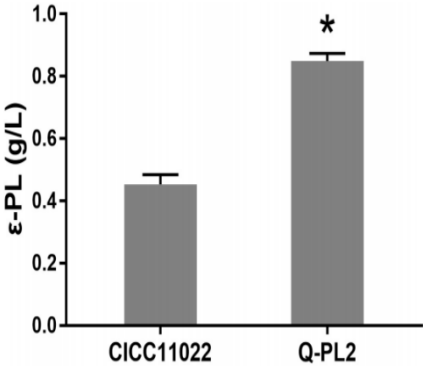

Fig.1 CICC 11022 and Q-PL2 ε-PL production.

Then *Streptomyces* albicolor CICC 11022 and Q-PL2 were fermented in M3G medium for 72h to measure the ε-PL yield in Fig. 1. The production of ε-PL was successfully increased under the action of unnatural strong promoter. On the basis of increasing the yield of ε-PL, the fermentation of recombinant strain Q-PL2 was optimized. Aixia Wang[7] et al. also studied the effects of sodium citrate and other metabolic intermediates on the e-PL production capacity of strain Q-PL2, which overexpressed pls gene, and compared the effects of sodium citrate on the ε-PL production capacity of the two strains. By adding 2g/L sodium citrate, the concentration of ε-PL produced by Streptomyces parvus CICC11022 increased from 0.03 in 0.45 soil to 0.07g/L in 0.58 soil, while the concentration of strain Q-PL2 increased from 0.02 in 0.85 soil to 0.17g/L in 1.81 soil. The e-PL titer

ratio of sodium citrate to strains CICC11022 and Q-PL2 was 7.0 for 28.5 soil and 13.3% for 113.2 soil. Therefore, the addition of sodium citrate can have a synergistic effect with the overexpression of pls genes. Under the synergistic effect of 2g/L sodium citrate, the ε-PL produced by Q-PL2 was 211.2% higher than that by wild strain by 17.4%.

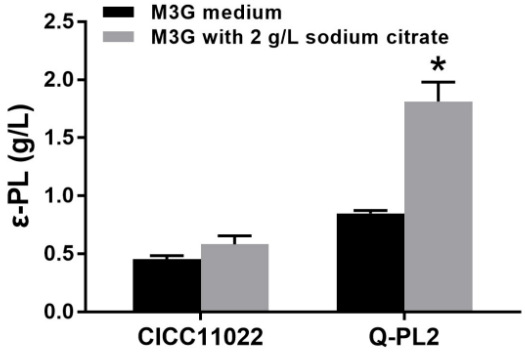

Fig.2 CICC 11022 and Q-PL2 ε-PL production.

As shown in Fig.2, although the yield of ε-PL has been greatly increased by genetic engineering, Streptomyces albicolor is a non-GRAS strain, and the components in the product are complex, which is not conducive to separation and purification. Bacillus subtilis is a United States Food and Drug Administration (FDA) certified GRAS (Generally recognized as safe) strain, no endotoxin, commonly used as a cell factory for the production of recombinant proteins, amino acids and chemicals, is a good carrier for the production of food grade ε-PL. However, the safety of ε-PL produced by non-GRAS strains is of concern, and its application in the food and pharmaceutical industries may have certain limitations. At present, there are few studies on food-grade heterologous expression of ε-PL synthase by genetic engineering. According to the existing literature, in 2012, Nermeen A. El-Sersy[14] et al. realized the heterogenic expression of ε-PL synthase in Bacillus subtilis for the first time. Subsequently, Marie Claudine[15] et al. established a food-grade ε-PL expression system, and used genetic engineering methods to optimize the pls gene on Streptomyce parvus, connect it with pMA5 plasmid, and introduce it into Bacillus subtilis 168. The heterologous expression of ε-PL synthase was successfully achieved, and the recombinant strain B. subtilis 168/pMA5-pls was obtained. E-PL synthase was successfully expressed in B. subtilis 168/pMA5-pls by MALDI-TOF-MS analysis. The whole cell catalytic system was constructed, and the conditions of the recombinant strain *B. subtilis* 168/pMA5-pls were optimized in terms of temperature, initial pH and substrate concentration. Although the ε-PL produced did not reach a high level, it provided a new strategy for the production of food grade ε-PL.

4. Conclusion

Increasing the yield of ε-PL by a single species will inevitably have limitations, and the dominant genes of other species cannot be transferred to the production strain, which has a natural barrier. With the continuous development of biotechnology, genetic engineering is a kind of science and technology that is currently at the forefront of The Times, breaking the barrier between species. The emergence of genetic engineering is like adding a new horizontal coordinate on the basis of the vertical coordinate of ε-PL production, which not only improves the yield of ε-PL, but also broadens the method of producing food-grade ε-PL. The combination of new genetic engineering and traditional biological process optimization, based on the production of food-grade recombinant strains, and then the biological process optimization, to play a "1+1 > 2" effect, better meet the needs of people's lives, which is the significance of this review.

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