J. Microbiol. Biotechnol. (2015), 25(3), 358–365
http://dx.doi.org/10.4014/jmb.1405.05069

jmb

Efficient Production of ε-Poly-L-Lysine by *Streptomyces ahygroscopicus* Using One-Stage pH Control Fed-Batch Fermentation Coupled with Nutrient Feeding

Sheng-Rong Liu, Qing-Ping Wu*, Ju-Mei Zhang, and Shu-Ping Mo

Guangdong Institute of Microbiology, Ministry-Guangdong Province Jointly State Key Laboratory of Applied Microbiology, Guangzhou 510070, P.R. China

Received: May 27, 2014
Revised: September 2, 2014
Accepted: September 30, 2014

First published online
October 1, 2014

**Corresponding author*
Phone: +86-20-87688132;
Fax: +86-20-87688132;
E-mail: wuqp203@163.com

pISSN 1017-7825, eISSN 1738-8872

ε-Poly-L-lysine (ε-PL) is a homopolymer of L-lysine molecules connected between the epsilon amino and alpha carboxyl groups. This polymer is currently used as a natural preservative in food. Insufficient biomass is a major problem in ε-PL fermentation. Here, to improve cell growth and ε-PL productivity, various nitrogen-rich nutrients were supplemented into flask cultures after 16 h cultivation, marking the onset of ε-PL biosynthesis. Yeast extract, soybean powder, corn powder, and beef extract significantly improved cell growth. In terms of ε-PL productivity, yeast extract at 0.5% (w/v) gave the maximum yield (2.24 g/l), 115.4% higher than the control (1.04 g/l), followed by soybean powder (1.86 g/l) at 1% (w/v) and corn powder (1.72 g/l) at 1% (w/v). However, supplementation with beef extract inhibited ε-PL production. The optimal time for supplementation for all nutrients examined was at 16 h cultivation. The kinetics of yeast-extract-supplemented cultures showed enhanced cell growth and production duration. Thus, the most commonly used two-stage pH control fed-batch fermentation method was modified by omitting the pH 5.0-controlled period, and coupling the procedure with nutrient feeding in the pH 3.9-controlled phase. Using this process, by continuously feeding 0.5 g/h of yeast extract, soybean powder, or corn powder into cultures in a 30 L fermenter, the final ε-PL titer reached 28.2 g/l, 23.7 g/l, and 21.4 g/l, respectively, 91.8%, 61.2%, and 45.6% higher than that of the control (14.7 g/l). This describes a promising option for the mass production of ε-PL.

Keywords: *Streptomyces*, ε-poly-L-lysine, one-stage pH control, fed-batch fermentation, nutrient feeding

Introduction

ε-Poly-L-lysine (ε-PL), initially isolated from *Streptomyces* liquid cultures, consists of 25–35 residues of L-lysine connected by unique linkages between the epsilon amino and alpha carboxyl groups [25]. In recent studies, *Kitasatospora* and *Bacillus* strains were reported to be capable of producing ε-PL [5, 20]. This extends our knowledge about the diversity of ε-PL-producing strains in the natural environment. ε-PL is a membrane-active agent owing to its polycationic property, and is effective on diverse microorganisms, including bacteria, filamentous fungi and yeast, and viruses [26, 27]. Moreover, ε-PL is biodegradable, and is non-toxic to humans and the environment [11]. Given these properties, ε-PL is an excellent material for utilization as a food preservative, and its use has increased in the food industry in Japan, South Korea, and the United States. ε-PL can also act as an anti-obesity agent, a hydrogel, an endotoxin neutralizer, a biosensor, and a carrier for drug delivery [21, 24, 31]. Recently, various forms of ε-PL and its analog have been synthesized in labs to improve the delivery efficiency of nucleic acids for gene therapy [6, 18].

The high demand for ε-PL in the food industry, in medicine, and in agriculture has inspired global interest in

its production, and numerous studies have been performed. These have mainly focused on the isolation of ε-PL-producing bacterial strains [5, 12, 19, 20], strain improvement [10, 16], determination of the biosynthetic mechanisms of ε-PL [8, 14, 22, 29, 30], characterization of ε-PL-degrading enzymes and its gene [7, 9, 15], optimization of culture conditions [1, 3, 13, 23], and on the inhibitory effect of ε-PL on food-borne microorganisms [2, 28].

Kahar *et al*. [13] investigated the influence of pH on ε-PL biosynthesis in batch fermentation. It was found that almost no ε-PL was formed when the pH of the broth was higher than 5.0, despite cell growth being desirable. Interestingly, large amounts of ε-PL were produced when the pH was lowered to 4.0, even though cell growth was poor. To address the inconsistent effect of pH on cell growth and ε-PL production, a two-stage pH control fed-batch fermentation method was developed. The operating procedures for this method are as follows: during fermentation, the pH of the broth naturally decreases from 7.0 to 5.0; when this level is reached, it is maintained to facilitate cell growth and increase biomass density. The cultivation period from the time of inoculation to the end of the pH 5.0 control period was designated as Phase one. After Phase one, the pH was allowed to decrease to around 4.0, and was thus maintained until the end of the fermentation process. The pH 4.0-controlled period was defined as Phase two.

Next, ε-PL-degrading enzymes present in the cell membrane of ε-PL-producing strains, including *S. albulus* and *Kitasatospora* sp., were isolated and characterized as membrane proteins [7, 15]. It was reported that the activity of ε-PL-degrading enzymes was maintained between 9.0 and 5.0, and no activity remained at pH 4.0 or lower. The degradation of ε-PL was performed by an exo-type mode. Therefore, it is likely that the accumulation of ε-PL in broth at pH 4.0 or lower was due to the inactivation of ε-PL-degrading enzymes under conditions of low pH. However, cells of ε-PL-producing strains are very sensitive to low pH, resulting in poor cell growth during the production phase. For this reason, high biomass cannot be achieved under normal culture conditions, and thus, insufficient biomass is a major problem during the fermentative production of ε-PL.

To increase cell biomass, immobilized cell fermentation of ε-PL was developed using a loofah sponge as the carrier material [32], significantly improving the productivity of ε-PL. Another advantage of this method is that the immobilized cells can be re-used. In our group, fermentation using immobilized cells combined with *in situ* adsorption was employed for the production of ε-PL at a flask level and the results were promising [17]. However, the use of these methods for ε-PL production in an industrial process is difficult. Currently, a two-stage pH control fed-batch fermentation is the most commonly used method for ε-PL fermentation.

In the present study, to improve cell growth and ε-PL production, a variety of nitrogen-rich nutrients were individually supplemented into flask cultures growing in the ε-PL phase. Furthermore, the kinetics of ε-PL fermentation was determined using a yeast extract supplement as a reference in the flasks. Thereafter, the most commonly used two-stage pH control fed-batch fermentation was modified by omitting the pH 5.0-controlled period and, instead, coupling nutrient feeding with the pH 3.9-controlled phase. Cultures were examined in a 30 L fermenter.

Materials and Methods

Chemicals

Yeast extract and beef extract were purchased from Guangdong Huankai Microbiological Science and Technology Limited Company. Soybean powder and corn powder were prepared by passing through a 60-mesh gauze screen after grinding. All other chemicals were of analytical purity and obtained from commercial sources.

Microorganisms

The organism used was *Streptomyces ahygroscopicus* GIM8, an ε-PL-producing strain isolated from soil samples [12]. The strain is stored in the China Center for Type Culture Collection under the collection number CCTCC M2011191.

Medium

Cultures in a fermentor were grown in M3G medium [13]; the composition (per liter) was as follows: 50.0 g glucose, 5.0 g yeast extract, 10.0 g $(NH_4)_2SO_4$, 1.36 g KH_2PO_4, 0.8 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.04 g $ZnSO_4{\cdot}7H_2O$, and 0.03 g $FeSO_4{\cdot}7H_2O$, at pH 7.2. The medium was used in fed-batch fermentation. For flask cultures, 50 g/l glucose was modified as 30 g/l, while other components were just the same. The medium was autoclaved at 121°C for 15 min.

The feeding medium comprised 800 g glucose and 80 g ammonium sulfate in 1 L of distilled water. The components of this solution were individually prepared, separately autoclaved at 121°C for 15 min, and then mixed after cooling.

Separate 200 g/l solutions of yeast extract, beef extract, soybean powder, and corn powder solutions were prepared and autoclaved at 115°C for 30 min. These were supplemented as nitrogen-rich nutrients.

Seed Culture

A 5 ml spore suspension prepared previously from a slant was

transferred to a 1,000 ml shake flask containing 250 ml of sterilized M3G medium. The inoculum was allowed to grow for 20 h in a 150 rpm rotary shaker at 30°C and used as seed culture in subsequent experiments.

Flask Fermentation

Aliquots of seed culture (2 ml) were inoculated in 50 ml of medium contained in 250 ml flasks and cultivated in a 190 rpm rotary shaker at 30°C for 96 h for ε-PL production. For achieving more cells involved in ε-PL biosynthesis, nutrient solutions of yeast extract, beef extract, soybean powder, and corn powder were separately added at 16 h cultivation, just at the onset of ε-PL biosynthesis. Supplementation of the nutrients at 0, 16, and 32 h cultivation was also performed to optimize the time of addition for ε-PL production.

Fermenter Fermentation Procedures

The fermentation was carried out in a 30 L fermenter (BioSTAT DL30; B.Braun Biotech International, Germany) under the following conditions. The precultured seed of 1 L was inoculated into 19 L of sterilized M3G medium in the fermenter with an initial pH of 6.8. The fermentation culture was agitated by a standard six-blade impeller operating at a stirring speed of 300 rpm; the aeration rate was 1 vvm and the culture temperature was 30°C. Changes in pH were detected online with a pH electrode. For inhibition of foam formation, an antifoaming agent was added to the culture broth *via* an automatically operated antifoam probe.

During the fermentation, the pH of the fermentation broth continued to decrease, and when it reached 3.9 (at approximately 18 h cultivation), this value was maintained by automatically feeding a 12% NH_4OH solution into the fermenter until the end of cultivation. Meanwhile, yeast extract, soybean powder, or corn powder of 0.5 g in solution (200 g/l) per hour was continuously fed to the fermentor from this time point until the end of fermentation. In the process of fermentation, once the glucose concentration had decreased to approximately 10 g/l, fed-batch cultivation (continuous supplementation of the feeding medium) was initiated to prevent further decline. For the control, operating conditions were identical, but excluded feeding of yeast extract solution.

Analytical Methods

Samples were centrifuged at 12,000 ×*g* for 10 min prior to analysis of residual glucose and ε-PL. Glucose was measured using the 3,5-dinitrosalicylic acid method. ε-PL was measured with high-performance liquid chromatography as previously reported [13].

To determine biomass accumulation, 5 ml of culture was centrifuged at 12,000 ×*g* for 10 min; the cells were resuspended in distilled water, deposited onto preweighed filter paper, and washed twice with distilled water. The filter paper covered with the mycelia was dried to constant weight in a 90°C oven for 12 h and allowed to cool in a desiccator. The biomass was calculated from the weight of the dried paper.

Results

Effects of Nitrogen-Rich Nutrient Supplementation into the ε-PL-Producing Cultures on Cell Growth and ε-PL Production

Table 1 indicates that cell growth was significantly increased following the addition of nutrients at all supplemented concentrations, suggesting that the addition of nutrients can effectively support cell growth at an unfavorably low pH. Maximum cell growth was achieved in cultures supplemented with beef extract at 1.0% (w/v) and reached 16.30 g/l, whereas only 11.23 g/l was produced in the control condition. Furthermore, with the exception of beef extract, all tested nutrients at all concentrations boosted ε-PL production. Of these, yeast extract at 0.5% (w/v) enhanced ε-PL production the greatest, resulting in a yield of 2.24 g/l in comparison with the control (1.04 g/l). Notably, the cultures supplemented with beef extract, which showed significantly enhanced growth, did not exhibit an increase in ε-PL production (0.74 g/l) as compared with other treatments and the control. This suggests that some components in beef extract may inhibit ε-PL biosynthesis, without an effect on cell growth.

Influence of Time of Nutrient Supplementation on ε-PL Production

Supplementation of nutrients after 0, 16, and 32 h of cultivation, representing the time points before, just, and

Table 1. Results of flask cultures supplemented with different nutrients ranging from 0.25%–1.0% (w/v) after 16 h cultivation and without supplementation.

Nutrients	Concentration (%, w/v)	[a]Maximal biomass (g/l)	[a]Final ε-PL (g/l)
Yeast extract	0.25	13.23 ± 0.35	1.64 ± 0.13
	0.5	15.27 ± 0.28	2.24 ± 0.16
	1	14.13 ± 0.41	2.22 ± 0.12
Beef extract	0.25	13.14 ± 0.26	0.64 ± 0.08
	0.5	14.64 ± 0.56	0.57 ± 0.10
	1	16.30 ± 0.32	0.74 ± 0.07
Soybean powder	0.25	12.30 ± 0.47	1.32 ± 0.11
	0.5	14.12 ± 0.26	1.67 ± 0.14
	1	15.47 ± 0.53	1.86 ± 0.07
Corn powder	0.25	12.80 ± 0.29	1.23 ± 0.12
	0.5	13.67 ± 0.46	1.57 ± 0.13
	1	15.23 ± 0.34	1.72 ± 0.14
Control	No addition	11.23 ± 0.42	1.04 ± 0.11

[a]Each data point represents the mean ± SD from three independent samples.

Table 2. Final ε-PL concentration in flask cultures supplemented with nutrients at (0.5% (w/v)) after 0, 16, and 32 h cultivation and without supplementation.

Nutrients	Time of addition (h)	[a]ε-PL concentration (g/l)
Yeast extract	0	0.91 ± 0.14
	16	2.21 ± 0.14
	32	1.96 ± 0.11
Soybean powder	0	1.44 ± 0.12
	16	1.73 ± 0.08
	32	1.64 ± 0.10
Corn powder	0	1.54 ± 0.08
	16	1.63 ± 0.09
	32	1.58 ± 0.13
Control	No addition	1.08 ± 0.10

[a]Each data point represents the mean ± SD from three independent samples.

after the onset of ε-PL production, respectively, was examined on ε-PL production. The results are shown in Table 2. It can be seen that for all nutrients examined, the supplementation at 16 h cultivation (just at the onset of ε-PL production) yielded the highest ε-PL yields, and a slightly lower production was observed at supplementation at 32 h cultivation. There were significant differences among the nutrients upon supplementation at 0 h cultivation. For soybean and corn powders, the initial addition increased ε-PL production but was decreased by yeast extract. This may be due to an inhibitory effect by a high level of yeast extract in the broth since it was one of the components in the formulation.

Effects of Nutrient Supplementation on the Kinetics of Flask Fermentation

To clarify how supplemented nutrients affect ε-PL biosynthesis, yeast extract at 0.5% (w/v) was used as a reference, and the kinetics of pH, biomass, ε-PL formation, and glucose consumption were determined. As shown in Fig. 1A, the pH of the culture broth decreased from its initial value of 6.8 to around 4.0 after 16 h cultivation. This pH was favorable for ε-PL biosynthesis. During prolonged cultivation, the pH continued to decrease and was 2.83 at the end of fermentation. Conversely, when 0.5% (w/v) yeast extract was fed to cultures after 16 h cultivation, the pH rose slightly soon after addition, suggesting that cellular metabolism was altered. Then, the decline in pH continued and reached 2.76 at the end of the cultivation

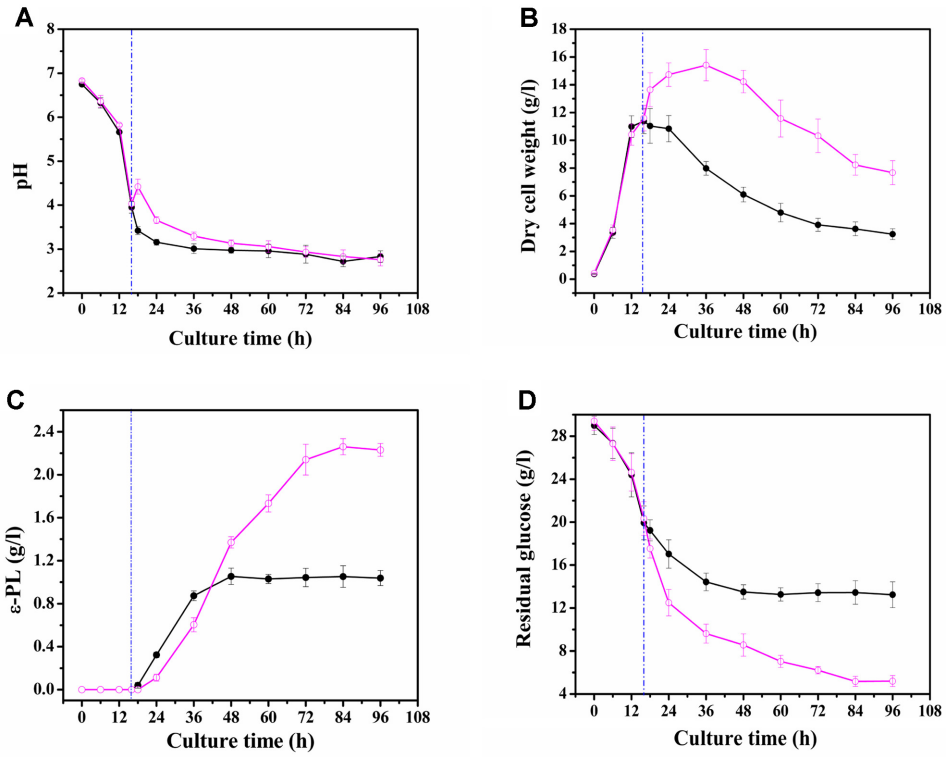

Fig. 1. Kinetics of pH, biomass, ε-PL formation, and glucose consumption between *Streptomyces ahygroscopicus* GIM8 cultures supplemented with yeast extract (0.5% (w/v)) (*open circles*) and control cultures (*solid circles*).
Each data point represents the mean ± SD from three independent samples.

period. The difference in pH was not statistically significant between the two cultures. The continuous decrease of pH during cultivation is likely due to the consumption of glucose [13, 23].

Fig. 1B shows that after inoculation, the biomass increased rapidly and almost reached the maximum level (11.38 g/l) after approximately 16 h cultivation, when the cells began to synthesize ε-PL in the control culture. However, a continuous decline in biomass was associated with the ε-PL production phase, probably due to the extremely low pH following cell lysis. At the end of fermentation, only 3.24 g/l biomass remained. In the yeast-extract-supplemented cultures, cell growth continued up to 36 h cultivation and reached 15.41 g/l, which was 1.36-fold higher than that in the control (11.38 g/l). After this maximum value had been reached, the biomass began to decrease and fell to 7.67 g/l. Based on these comparative data, it was concluded that nutrient supplementation is an effective means of improving cell growth even at the low-pH ε-PL production phase.

As shown in Fig. 1C, the cells were stimulated to produce ε-PL after approximately 16 h cultivation owing to a favorable pH being reached for ε-PL biosynthesis (pH 4.0). ε-PL concentration continued to increase until 48 h of cultivation with a ε-PL titer of 1.05 g/l. Further cultivation did not lead to an increase in ε-PL production, probably due to nutrient depletion and/or product ε-PL feedback inhibition. Under this nutrient condition, the production period lasted 32 h. As for the supplemented cultures, ε-PL concentrations were below those of the control in the early production phase. This may be due to a rise in pH soon after the addition of yeast extract, which resulted in a pH that was unfavorable for ε-PL biosynthesis. With the pH decline, ε-PL levels increased rapidly and reached a maximum of 2.26 g/l after 84 h cultivation. The production duration was around 68 h, which was significantly higher than that in the control (32 h) cultures, indicating that the ε-PL production period was extended by the nutrient supplement.

As revealed in Fig. 1D, a significant amount of glucose was consumed in the growth phase, which continued into the production phase in both cultures. In the control, 13.24 g/l glucose remained at the end of the cultivation period, from an initial glucose concentration of 30 g/l, whereas only 5.20 g/l glucose remained in the cultures supplemented with yeast extract at the end of the experiment. This suggests that an additional 8.04 g/l was consumed by the cells, due to the addition of yeast extract. The increased glucose consumption may be related to increases in cell growth and ε-PL production.

Profiles of One-Stage pH Control Fed-Batch Fermentation Coupled with Continuous Feeding of Yeast Extract, Corn Powder, or Soybean Powder Solutions and No Nutrient Feeding

Based on the results obtained during flask fermentation, the most commonly used two-stage pH control fed-batch fermentation was modified by omitting the pH 5.0-controlled phase while coupling with nutrient feeding in the pH 3.9-controlled ε-PL production phase. This improved process was referred to as one-stage pH control fed-batch fermentation coupled with nutrient feeding.

The profiles of one-stage pH control fed-batch fermentations in the presence of yeast extract, soybean powder, and corn powder feeding, and without nutrient feeding, are shown in Fig. 2. This figure shows that the pH of broth decreased rapidly from an initial pH value of 6.8 to approximately 3.9 in the first 18 h of fermentation. The reason for the decrease in pH was the same as that described during flask fermentation. Next, cultures were maintained at pH 3.9 by supplementing with ammonium solution until the end of fermentation. We refer to this pH control scheme as one-stage pH control. This pH 3.9-controlled phase favored ε-PL production.

As illustrated in Fig. 2, after inoculation, the cells adapted to the cultivation conditions quickly and a continuous increase in biomass was observed. At 18 h cultivation, when the cells began to produce ε-PL, the biomass reached around 2.4 g/l in all fed-batch cultures. Notably, cell growth was significantly improved from time of yeast extract, soybean powder, or corn powder feeding compared with the control. In yeast extract supplemented cultures (Fig. 2A), the biomass continued to increase up to 264 h, reaching 24.8 g/l. A slight increase in biomass was observed during the prolonged cultivation period. Following supplementation with soybean and corn powder (Figs. 2B and 2C), the maximum biomass achieved was 25.6 g/l at 288 h fermentation, for each. In the control culture (Fig. 2D), cell growth had almost ceased by 168 h, yielding a maximum biomass of only 15.6 g/l, and further cultivation did not lead to increased cell growth, possibly implying a state of nutrient depletion. Therefore, yeast extract supplementation yielded 59% more biomass than the control, and this value was 64.1% for both soybean powder and corn powder supplementation. Therefore, continuous feeding of nutrients is an efficient means of improving cell growth during ε-PL fermentation.

ε-PL production was initiated at 18 h fermentation owing

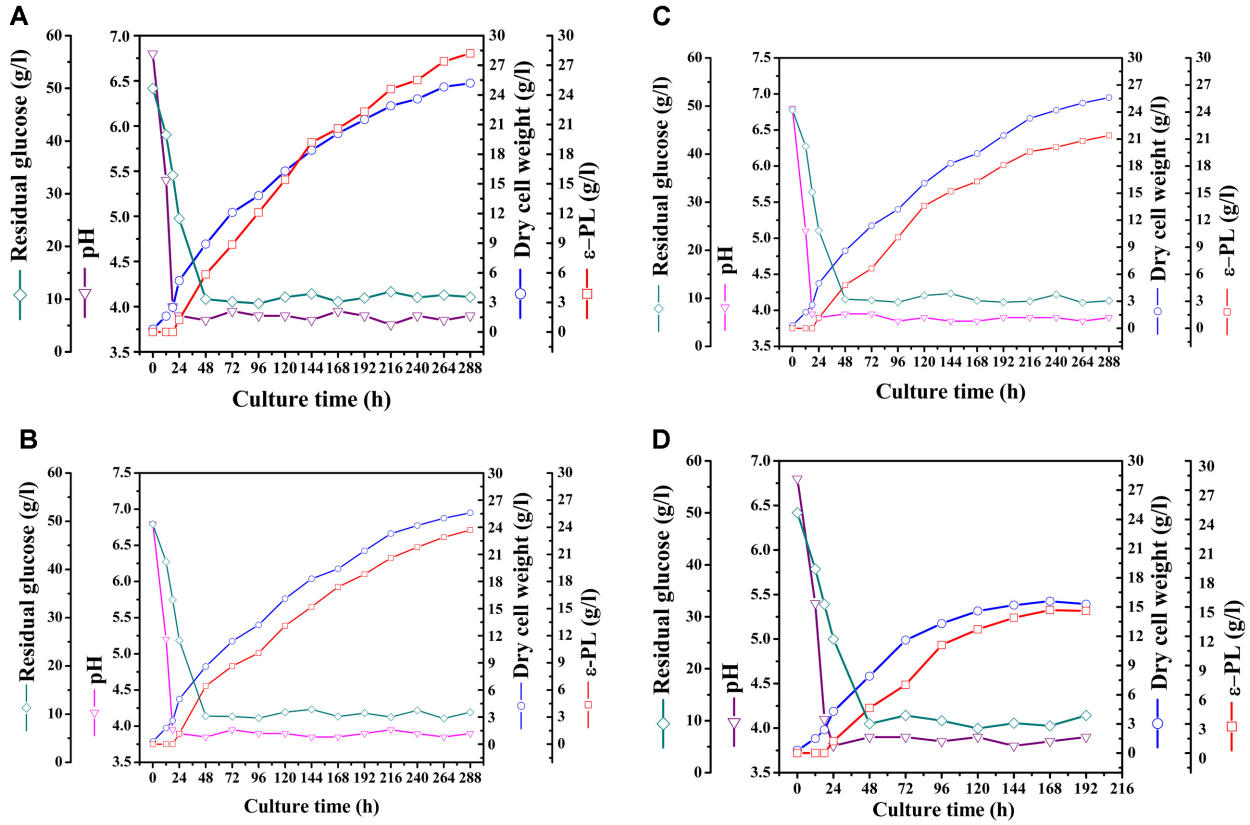

Fig. 2. Profiles of one-stage pH control fed-batch fermentation of ε-PL by *Streptomyces ahygroscopicus* GIM8 with continuously feeding of 0.5 g per hour of yeast extract (**A**), soybean powder (**B**), or corn powder (**C**), or without nutrient feeding (**D**).

to an optimal pH of around 4.0 being attained, and the maximum production of 14.7 g/l was reached by 168 h in the control culture. Prolonged cultivation yielded no further increase. The duration of ε-PL production was approximately 150 h. With continuous feeding of nutrients into cultures from 18 h to the end of fermentation, ε-PL production was sustained from 18 to 264 h (yeast extract), 288 h (soybean powder), and 288 h (corn powder) fermentation, with slight increases persisting throughout the remaining time (data not shown). This implies that the production of ε-PL continued for at least 246, 270, and 270 h for yeast extract, soybean powder, and corn powder supplements, respectively, 1.64-, 1.8-, and 1.8-fold longer than in the control culture. At the end of fermentation, the yeast extract supplement resulted in the highest amount of ε-PL production, reaching 28.2 g/l at 288 h fermentation, which was 91.8% higher than that produced in the control (14.7 g/l). In soybean powder and corn powder supplemented cultures, ε-PL production reached 23.7 g/l and 21.4 g/l, respectively, which was 61.2%, and 45.6% higher than that produced by the control.

Fig. 2 shows that glucose consumption was rapid during both the growth and production phases, and was mainly used for cell metabolism, cell growth, and ε-PL production. Furthermore, glucose consumption almost ceased with termination of ε-PL biosynthesis. The termination of glucose utilization may be related to a loss of cellular activity.

Discussion

Previous studies have demonstrated that as a secondary metabolite, the biosynthetic pathway of ε-PL is very complex [14, 22, 29, 30], but the stages of its biosynthesis process remain unclear. Usually, a variety of environmental and nutritional factors such as cell morphology, culture parameters, and nutritional status can greatly affect ε-PL biosynthesis. In the present study, a significant increase in the cell density and production duration was achieved by feeding nutrients to ε-PL-producing cultures in a flask or in a fermenter. These two variables may be responsible for the increase in ε-PL production. Furthermore, as these nutrients contain minerals, vitamins, and other bioactive compounds,

some important enzymes associated with ε-PL biosynthesis may be beneficially regulated by some components of these nutrients. Thus, these may act as coenzymes, resulting in increased ε-PL synthesis rate and production. To understand the possible mechanism for this, detailed molecular studies are needed.

During the last decade, a variety of fermentation processes such as immobilized cell fermentation [32], precursor L-lysine feeding fermentation [4], and two-stage pH control fermentation [13] have been developed. Among these, two-stage pH control fed-batch fermentation is the most commonly used method for the industrial production of ε-PL. However, one drawback with this approach is that the onset of ε-PL biosynthesis is artificially delayed owing to the setup of a pH 5.0-controlled period in this process. It is reasonable to suspect that the cells that grow before and in the pH 5.0-controlled period may be compromised and, therefore, the capacity of these cells to produce ε-PL may be affected when they enter the production phase. Moreover, the culture goal for high biomass remains difficult to achieve using an additional pH 5.0-controlled phase. Another disadvantage is that nutrient depletion may have occurred because the process lasts for over a week, during which only a mixture of glucose and ammonium sulfate was fed to the cultures.

To overcome these limitations, one-stage pH control fed-batch fermentation coupled with nutrient feeding was proposed and examined in the present study. The results are promising. In comparison with the two-stage pH control process, the process developed in this study has the following benefits: (i) nutrient feeding would continuously improve cell growth; (ii) large amounts of the biomass would be produced during the nutrient feeding phase, and these cells could immediately begin ε-PL biosynthesis without loss of any cellular production capacity; and (iii) supplementation can avoid nutrient depletion during the ε-PL production phase. These benefits may explain the significant enhancement in ε-PL production from this method.

From an economic point of view, the cost of supplements must be factored into the development of a commercially viable fermentation process for industrial application, because it would greatly influence the economic viability of the process. In the present study, using yeast extract supplementation as an example of fed-batch fermentation, the total amount of yeast extract supplemented was 135 g, calculated from the feeding amount per hour multiplied by feeding hour (0.5 g/h × 270 h). In this case, the additional production of ε-PL attained was 270 g [(28.2 g/l-14.7 g/l) × 20 L]. Specifically, 1 g of yeast extract supplement resulted in an additional increase of 2 g of ε-PL, based on glucose consumption. Thus, this strategy of nutrient feeding is economically viable, as the market price of ε-PL yeast extract was about 290.3 USD per kilogram, while this value was 19.2 USD for yeast extract, at the time of this study. Furthermore, the increased cost due to greater glucose consumption was low. Interestingly, soybean and corn powders also produced promising results; therefore, the ε-PL production costs could be further reduced by using these cheap nutrients. Overall, these factors suggest that this process is viable. Usually, ε-PL production would be further increased by optimizing the amount of supplement, feeding mode, formulation of nutrients, and other variables.

In conclusion, supplementation of the nitrogen-rich nutrients yeast extract, soybean powder, or corn powder into flask cultures during the ε-PL production period can greatly improve cell growth and ε-PL production. Yeast extract, followed by soybean powder and corn powder, had the most significant effect on enhancing ε-PL production. Conversely, beef extract decreased ε-PL production, despite being the most effective at increasing the biomass. The supplementation was optimum when added at a cultivation time just (16 h cultivation) at the onset of ε-PL biosynthesis in flask cultures. The one-stage pH control fed-batch fermentation coupled with nutrient feeding can significantly increase ε-PL production, and is economically viable. This process is a promising candidate for mass production of ε-PL on an industrial scale.

Acknowledgments

This work was supported by the Science and Technology Planning Project of Guangdong Province, China (2008A010900003 and 2009B011300004).

References

1. Bankar SB, Singhal RS. 2011. Improved poly-ε-lysine biosynthesis using *Streptomyces noursei* NRRL 5126 by controlling dissolved oxygen during fermentation. *J. Microbiol. Biotechnol.* **21:** 652-658.
2. Cheng SS, Lu WY, Park SH, Kang DH. 2010. Control of foodborne pathogens on ready-to-eat beef slurry by ε-polylysine. *Int. J. Food Microbiol.* **141:** 236-241.
3. Chen XS, Li S, Liao LJ, Ren XD, Li F, Tang L, *et al.* 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.
4. Chen XS, Ren XD, Zeng X, Zhao FL, Tang L, Zhang HJ, *et al.* 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.
5. EI-Sersy NA, Abdelwahab AE, Abouelkhiir SS, Abou-Zeid DM, Sabry SA. 2012. Antibacterial and anticancer activity of ε-poly-L-lysine (ε-PL) produced by a marine *Bacillus subtilis* sp. *J. Basic Microbiol.* **52:** 1-10.
6. Eom KD, Park SM, Tran HD, Kim MS, Yu RN, Yoo H. 2007. Dendritic α,ε-poly(L-lysine)s as delivery agents for antisense oligonucleotides. *Pharm. Res.* **24:** 1581-1589.
7. Feng XH, Xu H, Xu XY, Yao J, Yao Z. 2008. Purification and some properties of ε-poly-L-lysine-degrading enzyme from *Kitasatospora* sp. CCTCC M205012. *Process Biochem.* **43:** 667-672.
8. Geng WT, Yang C, Gu YY, Liu RH, Guo WB, Wang XM, *et al*. 2014. Cloning of ε-poly-L-lysine (ε-PL) synthetase gene from a newly isolated ε-PL producing *Streptomyces albulus* NK660 and its heterologous expression in *Streptomyces lividans*. *Microbial Biotechnol*. **7:** 155-164.
9. Hamano Y, Yoshida T, Kito M, Nakamori S, Nagasawa T, Takagi H. 2006. Biological function of the *pld* gene product that degrades ε-poly-L-lysine in *Streptomyces albulus*. *Appl. Microbiol. Biotechnol*. **72:** 173-181.
10. Hiraki J, Hatakeyama M, Morita S, Izumi Y. 1998. Improved ε-poly-L-lysine production of an *S*-(2-aminoethyl)-L-cysteine resistance mutant of *Streptomyces albulus*. *Seibutsu-kogaku Kaishi* **76:** 487-493.
11. Hiraki J, Ichikawa T, Ninomiya S, Seki H, Uohama K, Seki H, *et al*. 2003. Use of ADME studies to confirm the safety of ε-polylysine as a preservative in food. *Regul. Toxicol. Pharmacol.* **37:** 328-340.
12. Huang JM, Wu QP, Liu SR, Zhang JM. 2011. Screening of new ε-polylysine producing strain and structure identification of its product. *Microbiol. China* **38:** 871-877.
13. Kahar P, Iwata T, Hiraki J, Park E, Okabe M. 2001. Enhancement of ε-polylysine production by *Streptomyces albulus* strain 410 using pH control. *J. Biosci. Bioeng*. **91:** 190-194.
14. Kawai T, Kubota T, Hiraki J, Izumi Y, Yoshikazu I. 2003. Biosynthesis of ε-poly-L-lysine in a cell-free system of *Streptomyces albulus*. *Biochem. Biophys. Res. Commun.* **311:** 635-640.
15. Kito M, Takimoto R, Yoshida T, Nagasawa T. 2002. Purification and characterization of an ε-poly-L-lysine-degrading enzyme from an ε-poly-L-lysine-producing strain of *Streptomyces albulus*. *Arch. Microbiol*. **178:** 325-330.
16. Li S, Li F, Chen XS, Wang L, Xu J, Tang L, Mao ZG. 2011. Genome shuffling enhanced ε-poly-L-lysine production by improving glucose tolerance of *Streptomyces graminearus*. *Appl. Biochem. Biotechnol*. **166:** 414-423.
17. Liu SR, Wu QP, Zhang JM, Mo SP, Yang XJ, Xiao C. 2012. Enhanced ε-poly-L-lysine production from *Streptomyces ahygroscopicus* by a combination of cell immobilization and *in situ* adsorption. *J. Microbiol. Biotechnol*. **22:** 1218-1223.
18. Moccia M, Roviello GN, Bucci EM, Pedone C, Saviano M. 2010. Synthesis of a L-lysine-based alternate alpha,epsilon-peptide: a novel linear polycation with nucleic acids-binding ability. *Int. J. Pharm.* **397:** 179-183.
19. Nishikawa M, Ogawa K. 2002. Distribution of microbes producing antimicrobial ε-poly-L-lysine polymers in soil microflora determined by a novel method. *Appl. Environ. Microbiol*. **68:** 3575-3581.
20. Ouyang J, Xu H, Li S, Zhu HY, Chen WW, Zhou J, *et al*. 2006. Production of ε-poly-L-lysine by newly isolated *Kitasatospora* sp. PL6-3. *Biotechnol. J.* **1:** 1459-1463.
21. Pandey AK, Kumar A. 2014. Improved microbial biosynthesis strategies and multifarious applications of the natural biopolymers epsilon-poly-L-lysine. *Process Biochem.* **49:** 496-505.
22. Saimura M, Takehara M, Mizukami S, Kataoka K, Hirohara H. 2008. Biosynthesis of nearly monodispersed poly(ε-L-lysine) in *Streptomyces* species. *Biotechnol. Lett*. **30:** 377-385.
23. Shih IL, Shen MH. 2006. Optimization of cell growth and poly(ε-lysine) production in batch and fed-batch cultures by *Streptomyces albulus* IFO 14147. *Process Biochem.* **41:** 1644-1649.
24. Shih IL, Shen MH, Van YT. 2006. Microbial synthesis of poly(ε-lysine) and its various applications. *Bioresour. Technol.* **97:** 1148-1159.
25. Shima S, Sakai H. 1977. Polylysine produced by *Streptomyces*. *Agric. Biol. Chem.* **41:** 1907-1909.
26. Shima S, Matsuoka H, Iwamoto T, Sakai H. 1984. Antimicrobial action of ε-poly-L-lysine. *J. Antibiot.* **37:** 1449-1455.
27. Shima S, Fukuhara Y, Sakai H. 1982. Inactivation of bacteriophages by ε-poly-L-lysine produced by *Streptomyces*. *Agric. Biol. Chem.* **46:** 1917-1919.
28. Sofos JN, Geornaras I. 2005. Activity of ε-polylysine against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*. *J. Food Sci*. **72:** M404-M408.
29. Yamanaka K, Maruyama C, Takagi H, Hamano Y. 2008. ε-Poly-L-lysine dispersity is controlled by a highly unusual nonribosomal peptide synthetase. *Nat. Chem. Biol*. **4:** 766-772.
30. 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.
31. Yoshida T, Nagasawa T. 2003. ε-Poly-L-lysine: microbial production, biodegradation and application potential. *Appl. Microbiol. Biotechnol*. **62:** 21-26.
32. Zhang Y, Feng XH, Xu H, Yao Z, Ouyang PK. 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.