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OPTIMIZATION OF MEDIUM COMPONENTS AND FEEDING STRATEGIES FOR EPSILON POLY-L-LYSINE PRODUCTION BY *STREPTOMYCES NOURSEI* NRRL 5126

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ABSTRACT: Epsilon poly-L-lysine (ε-PL) is a nontoxic biopolymer widely used in food and pharmaceutical industry. The present work reports the nutritional requirements for submerged fermentation of *Streptomyces noursei* NRRL 5126 using a combination of one factor at a time and statistical orthogonal array method. Two-fold increase in ε-PL productivity (74 mg/L) was observed using optimized medium containing glycerol as carbon source along with proteose peptone and ammonium sulphate as nitrogen source. Various metabolic precursors such as amino acids, tricarboxylic acid cycle intermediates were investigated for improved production of ε-PL. Four fold increase (74 mg/L to 310 mg/L) in ε-PL production was achieved by incorporation of L-aspartate (2mM) and citric acid (5mM). Fed batch fermentation for ε-PL production with intermittent feeding with glycerol and in combination with metabolic precursors was studied. Fed batch fermentation with intermittent feeding of glycerol gave a maximum yield of 233 mg/L. Productivity was further enhanced and reached a maximum of 408 mg/L by addition of glycerol along with 5 mM citric acid after 24 h and 2 mM L-aspartate after 36 hours respectively.

INTRODUCTION: Epsilon-Poly-L-lysine (ε-PL) is an unusual, naturally occurring homopolyamide of L-lysine with amide linkages between ε-amino and α-carboxyl groups [1-3]. It is biodegradable, edible and non-toxic and hence of great interest to the food and pharmaceutical industry. It was accidently discovered as an extracellular material produced by filamentous bacterium *Streptomyces albulus* 346 as a result of a screening for a Dragendorff positive substance. Currently ε–PL is mainly used as a natural preservative as it is harmless to human.

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Moreover it shows a wide spectrum of applications as an emulsifying agent, disinfectant, drug delivery carrier, endotoxin remover and biosensor and hence have attracted a great deal of attention [4].

Industrially ε-PL is produced by aerobic fermentation with *Streptomyces albulus* [1-3]. But the enzymatic and pH induced degradation of secreted ε-PL in the culture medium limits its use. Therefore there is a need to explore alternative organisms for ε-PL production [5, 6]. For a plentiful supply of ε-PL, it is necessary to develop an efficient production process. Several methods have been investigated in the hope to optimise its production for commercial usage. For example, a two phase pH control strategy [7], immobilization of cells on baggase, synthetic sponge, macroporous silica gel, and loofah sponge [8], etc. To achieve maximum production, knowledge regarding the

environmental and fermentation factors affecting this process needs to be well identified. The nutritional and environmental conditions which greatly influence ε-PL production include pH, temperature, aeration, agitation, carbon and nitrogen sources, inoculum size, etc [9].

However, because of the variation in genetic diversity in different microbial sources, there is no defined medium for ε-PL production. Therefore for economic production process, it is necessary to optimize all fermentation parameters including medium composition [10]. Medium optimization by changing one variable at a time is laborious and time consuming. Statistical methods are preferred as they reduce the total number of experiments, understand the interactions among nutrients at varying concentration and minimize the error in determining the most significant parameters [11].

Hence in the present study a sequential optimization method was used where a combination of one factor at a time and statistical orthogonal array method was used. Effect of addition of various amino acids and TCA cycle intermediates on ε-PL production was studied. We also describe enhancement in ε-PL production using fedbatch fermentation with intermittent feeding of glycerol and metabolic precursors.

MATERIALS AND METHODS:

Chemicals:

All media components were purchased from Hi-Media Limited, Mumbai. All the chemicals were of analytical grade and procured from SD Fine Chemicals Ltd, Mumbai and Merck India Limited. ε -PL was obtained from Zhengzhou Bainafo Bioenegineering Co. Ltd., China.

Microbial Culture and maintenance:

Streptomyces noursei NRRL 5126 was a gift from the ARS Culture Collection, USA. It was grown on agar slants on medium containing glucose 4 g/L; yeast extract 4 g/L; malt extract 10 g/L and agar 15 g/L (pH 7.2-7.4) at 32 ± 2 °C for 5 days and then stored at 4 ^{0}C.

Fermentations:

Experiments were carried out in 100 ml Erlenmeyer flasks with 25 ml of basal medium (g/L): yeast extract 5; glucose 50; $(NH_4)_2SO_4$ 10; $MgSO_4$ 0.5; K_2HPO_4 0.8; KH_2PO_4 1.4, $FeSO_4$ 0.03; $ZnSO_4$ 0.04; pH 6.8[12]. The initial pH of the medium was adjusted to 6.8 with 1 M NaOH before autoclaving at 121°C. The production medium was inoculated with 4 % ($^v/_v$) of a 48 h old culture. Shake flask cultures of organism were incubated at temperature 32 ± 2°C with continuous agitation at 150 rpm for 96 h. All experiments were carried out in triplicates.

Analysis of ε-PL:

The ε-PL concentration was measured based on selective binding of trypan blue with ε-PL [13]. Samples from duplicate flasks were taken periodically centrifuged at 10000 x *g* for 10 min at 15°C. The clear supernatant was used for measuring ε-PL concentration. The reaction mixture contained 1ml of appropriately diluted supernatant, 2.88 ml of 0.1 mM phosphate buffer (pH 7), and 120µl of trypan blue solution (1 mg/ml). After thorough mixing the mixture was incubated at 37°C for 60 min and then centrifuged (10000 x *g*, 10 min at 15°C). Supernatant was collected and the absorbance was measured at 580 nm on UV-VIS spectrophotometer. A standard curve was derived from measurements with known amounts (0-50 mg/L) of ε-PL.

Optimization of fermentation medium using one factor at a time:

To investigate the effect of carbon sources, glucose in the basal medium was substituted with different carbohydrates such as fructose, maltose, sucrose, mannitol, glycerol, starch and cellulose. To select the best nitrogen source for ε-PL production different organic and inorganic nitrogen sources such as peptone bacteriological, beef extract, yeast extract, proteose peptone, meat extract, ammonium sulphate and sodium nitrate were screened. To study the effect of inducers different mineral salts like $CoSO_4$, $FeSO_4$, $MnSO_4$, $CuSO_4$ at concentration 0.03 g/L were incorporated in the basal medium.

Medium optimization by statistical orthogonal array method:

To investigate the relationship between variables of medium components and optimize their concentrations for ε-PL production the $L_{16}(4^5)$ orthogonal array was used as shown in Table 1. The design for the $L_{16}(4^5)$ orthogonal array was

developed and analyzed using "MINITAB 13.30" software.

Effect of addition of amino acids:
Effect amino acids of the Asp family which includes L-aspartate, L-methionine, L-lysine and L-threonine were studied. These amino acids were added in the optimized fermentation medium individually with initial concentration of 2 mM. Further the concentration of selected amino acids were optimized by varying the concentration from 0.5 to 4 mM. The optimized medium with no amino acids was considered as control.

Effect of addition of TCA cycle intermediates:
To study the effect of different TCA cycle intermediates on production of ε-PL, citric acid, α-ketoglutaric acid, oxaloacetic acid, fumaric acid, malic acid and succinic acid were added at a concentration of 10 mM individually. Further screening was done with citric acid, α-ketoglutaric acid and oxaloacetic acid at concentration ranging between 2-15 mM. The optimized medium without incorporation of TCA cycle intermediates was considered as control.

Optimization of time of addition of L-Asp and citric acid on production of ε-PL:
Experiments were carried out using six, 250 ml Erlenmeyer flasks with 100 ml of optimized medium which were inoculated with 4 % ($^{v}/_{v}$) of a 48 h old culture. The effect of addition of L-Asp and citric acid was studied independently by supplementing the optimized fermentation medium with 2 mM L-Asp and 5 mM citric acid respectively at different stages of fermentation (**Table 3**). Shake flask cultures of organism were incubated at 32 ± 2°C with continuous agitation at 150 rpm for 96 h.

Fed Batch fermentation with intermittent feeding of glycerol for ε-PL production:
Experiments were carried out in 250 ml Erlenmeyer flasks with 100 ml of optimized medium which were inoculated with 4 % ($^{v}/_{v}$) of a 48 h old culture. Shake flask cultures of organism were incubated at temperature 32 ± 2°C with continuous agitation at 150 rpm for 96 h. Three set of experiments were carried out simultaneously. In the first set, the effect of glycerol feeding on ε-PL production was studied by incorporating glycerol after 24 h of fermentation. 1ml of glycerol (10 g/L) was added every 12 h in the optimized medium.

The second set was to study the combined effect of metabolic precursors along with intermittent feeding with glycerol. In this set, 1 ml (10 g/L) glycerol was incorporated every 12 hours in the optimized medium in which 5mM citric acid was added after 24 hours and 2mM L Asp was added after 36 hours. The third set was the control with 100 ml optimized medium without metabolic precursors and with no intermittent feeding with glycerol. Samples were withdrawn after every 12 h and analyzed for ε-PL production and growth of *Streptomyces noursei* NRRL 5126.

RESULTS AND DISCUSSION:
Optimization of fermentation medium and time using one factor at a time:
Preliminary studies were carried out to study the growth profile of *Streptomyces noursei* NRRL 5126 in basal medium. It was observed that in batch culture, cells exhibited a lag phase of 12 h, followed by exponential phase for 24 h. Beyond 24 h, decelerating log phase was observed. The stationary phase extended for 120 hours followed by the decline phase. ε-PL production started in the exponential phase after 24 h of incubation and showed highest titre of 35.5 mg/l after 96 h of incubation. Hence for all further experiments fermentation was carried till 96 hours.

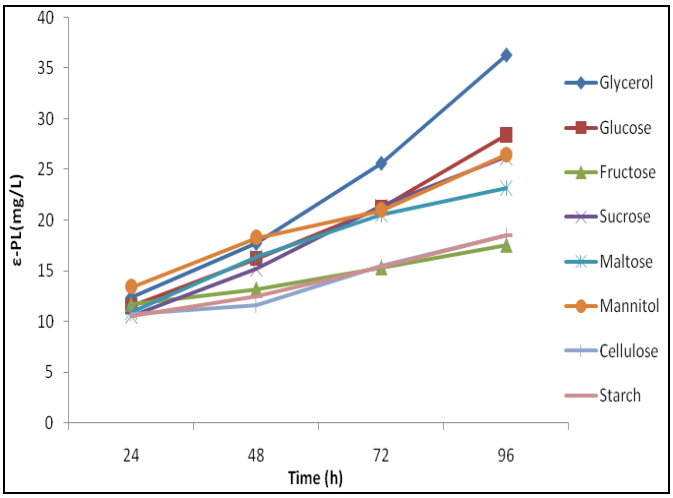

FIGURE 1A: EFFECT OF CARBON SOURCES ON ε - PL PRODUCTION BY *S. NOURSEI* NRRL 5126

Effect of carbon source on ε-PL production:
For all microbial fermentations, the carbon source is used as energy source and act as a major cell

building component. Hence the first step was to select the best carbon source for ε-PL production.

Figure 1a depicts the effect of different carbon sources on ε-PL production. Fructose, starch and maltose gave least ε-PL yield. This may be due poor utilization of these sugars by the organism. Of the remaining carbon sources tested mannitol resulted in 26.5 mg/L of ε-PL while maximum ε-PL production of 36.19 mg/L was achieved using glycerol. Similar results were obtained using *S. albulus* [2, 3, 7, 12, 14, 15, 16, 17].

This may be because many organisms possess glycerol transporters for the uptake of glycerol, which is easily metabolized by glycerol kinase and glycerol-P-dehydrogenase to give dihydroxyacetone phosphate which enters the glycolytic pathway [18]. Moreover studies suggest that use of short chain aliphatic hydroxy compounds like glycerol yield short chain ε-PL which is more preferred as a food preservative due its pleasant taste [19]. Hence glycerol was selected as best carbon source.

Effect of nitrogen source:

Nitrogen sources are known to affect ε-PL production; hence different organic and inorganic nitrogen sources were screened. Figure 1b represents the effect of varying nitrogen sources on ε-PL production.

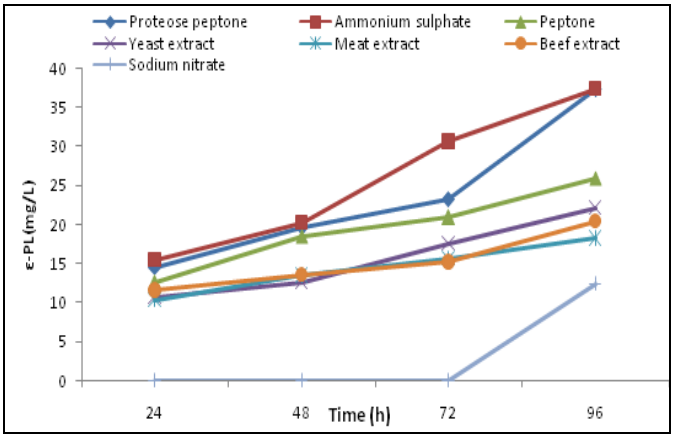

FIGURE 1B: EFFECT OF NITROGEN SOURCE ON ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126

Among the various organic nitrogen sources tested proteose peptone (37.25 mg/L) showed higher ε-PL yield. Many researchers have suggested that yeast extract is the best nitrogen source for ε-PL production [7, 6, 12]; however it was not as effective as proteose peptone for the production of ε-PL with *S. noursei*.

Among the inorganic nitrogen sources ammonium sulphate (37.36 mg/L) yielded maximum ε-PL than other inorganic nitrogen sources. This is because free amino groups are necessary for the biosynthetic pathway of ε-PL which is readily available from ammonium sulfate [20]. These results are in-line to that reported by [2, 3, 6, 12]. Since equivalent ε-PL yield was achieved with proteose peptone and ammonium sulfate hence both of them were selected as source of organic and inorganic nitrogen source respectively for further optimization.

Effect of mineral salts:

Studies suggest that mineral salts such as iron, cobalt, manganese are involved in the expression of ε-PL synthesis genes hence effect of these salts on ε-PL production was studied [21]**. Figure 2** represents the inducing effect of various mineral salts on ε-PL production. It was observed that among the various salts tested, ferrous salt showed a pronounced effect in enhancing ε-PL yield to 45 mg/L. This may be because ferrous salt is essential for carbon metabolism, nitrogen assimilation, and ε-PL production.

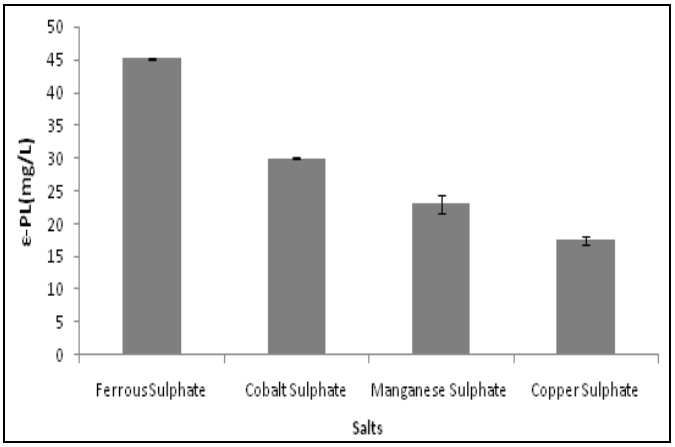

FIGURE 2: EFFECT OF MINERAL SALTS ON ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126

In addition ferrous ions stimulates the activities of proteinase and promotes the activities of ammonia assimilating enzymes, which convert ammonium ions into amino acid lysine, the precursor for ε-PL biosynthesis [22]. Further varying concentration of $FeSO_4$ from 0.01-0.1 g/L was studied but maximum ε-PL yield was obtained with 0.03 g/L. Further increase in $FeSO_4$ concentration showed a

detrimental effect on cell growth and ε -PL yield. Hence for further studies 0.03 g/L of $FeSO_4$ was used.

Medium optimization by statistical orthogonal array method:

ε-PL expression is influenced by culture conditions like nature of carbon source, nitrogen source and amount of salts. Hence concentration of these components were optimized using orthogonal array method. Table 1 represents the experimental design and concentrations of medium components for ε-PL production by $L_{16}(4^5)$ orthogonal array method.

TABLE 1: L_{16} (4^5) ORTHOGONAL ARRAY FOR ε-PL PRODUCTION BY *STREPTOMYCES NOURSEI* NRRL 5126

Run	Media Components					Concentration of media components					
	Glycerol	Proteose Peptone	$(NH_4)_2SO_4$	$MgSO_4$	$ZnSO_4$	Glycerol	Proteose Peptone	$(NH_4)_2SO_4$	$MgSO_4$	$ZnSO_4$	ε-PL (mg/L)
1	1	1	1	1	1	20	5	2	0.125	0.02	28.23
2	1	2	2	2	2	20	10	4	0.25	0.04	36.13
3	1	3	3	3	3	20	15	6	0.5	0.06	40.13
4	1	4	4	4	4	20	20	8	0.75	0.08	29.33
5	2	1	2	3	4	30	5	4	0.5	0.08	65.45
6	2	2	1	4	3	30	10	2	0.75	0.06	67.23
7	2	3	4	1	2	30	15	8	0.125	0.04	65.87
8	2	4	3	2	1	30	20	6	0.25	0.02	69.09
9	3	1	3	4	2	40	5	6	0.75	0.04	63.12
10	3	2	4	3	1	40	10	8	0.5	0.02	60.13
11	3	3	1	2	4	40	15	2	0.25	0.08	56.11
12	3	4	2	1	3	40	20	4	0.125	0.06	53.19
13	4	1	4	2	3	50	5	8	0.25	0.06	34.11
14	4	2	3	1	4	50	10	6	0.125	0.08	46.13
15	4	3	2	4	1	50	15	4	0.75	0.02	43.55
16	4	4	1	3	2	50	20	2	0.5	0.04	43.23

The variables optimised for ε-PL production include concentration of glycerol, proteose peptone, $(NH_4)_2SO_4$, $MgSO_4$ and $ZnSO_4$. The last column indicates the ε-PL yield. The data obtained from orthogonal array method was analyzed using MINITAB13.3 SOFTWARE. **Table 2** represents the response table for means (larger is better) obtained for L_{16} (4^5) orthogonal array. The last two rows in the table document delta values and the ranks for the system. Rank and delta values shows which factors have the greatest effect on ε -PL production. The size of the effect, Delta is measured by taking the difference between the highest and the lowest characteristic average for a factor. Higher the delta value more is the effect of that component on ε-PL production. Rank, based on delta values, orders the factors from the greatest effect to the least effect on ε-PL production. The magnitude of order of effect of factors on ε-PL production was found to be Glycerol >$(NH_4)_2SO_4$ >Proteose Peptone > $MgSO_4$ > $ZnSO_4$.

TABLE 2: RESPONSE TABLE FOR MEANS FOR ε-PL PRODUCTION BY *STREPTOMYCES NOURSEI* NRRL 5126

Levels	Glycerol	Proteose Peptone	Ammonium Sulphate	Magnesium Sulphate	Zinc Sulphate
	Mean	Mean	Mean	Mean	Mean
1	33.45	47.73	48.70	48.35	50.25
2	66.91	52.40	49.58	48.86	52.08
3	58.13	51.41	54.62	52.23	48.66
4	41.75	48.71	47.36	50.80	49.25
Delta	33.45	4.68	7.26	3.88	3.42
Rank	1	3	2	4	5

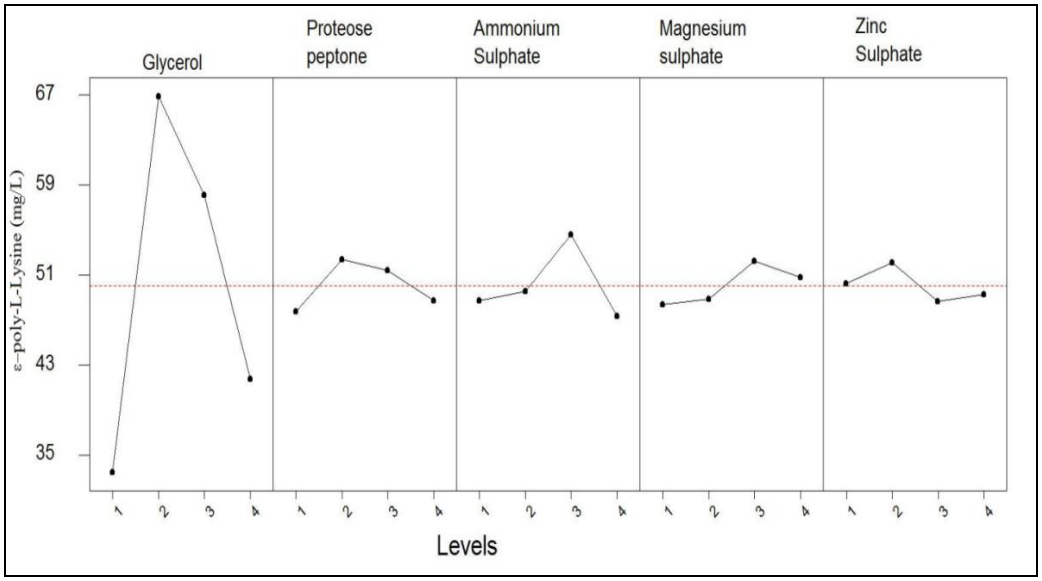

FIGURE 3: MAIN EFFECT PLOT FOR MEANS BY ORTHOGONAL ARRAY METHOD FOR ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126

To verify the predicted results, growth profile of the organism using optimized medium was studied and compared with the growth profile with the basal medium (**Figure 4**).

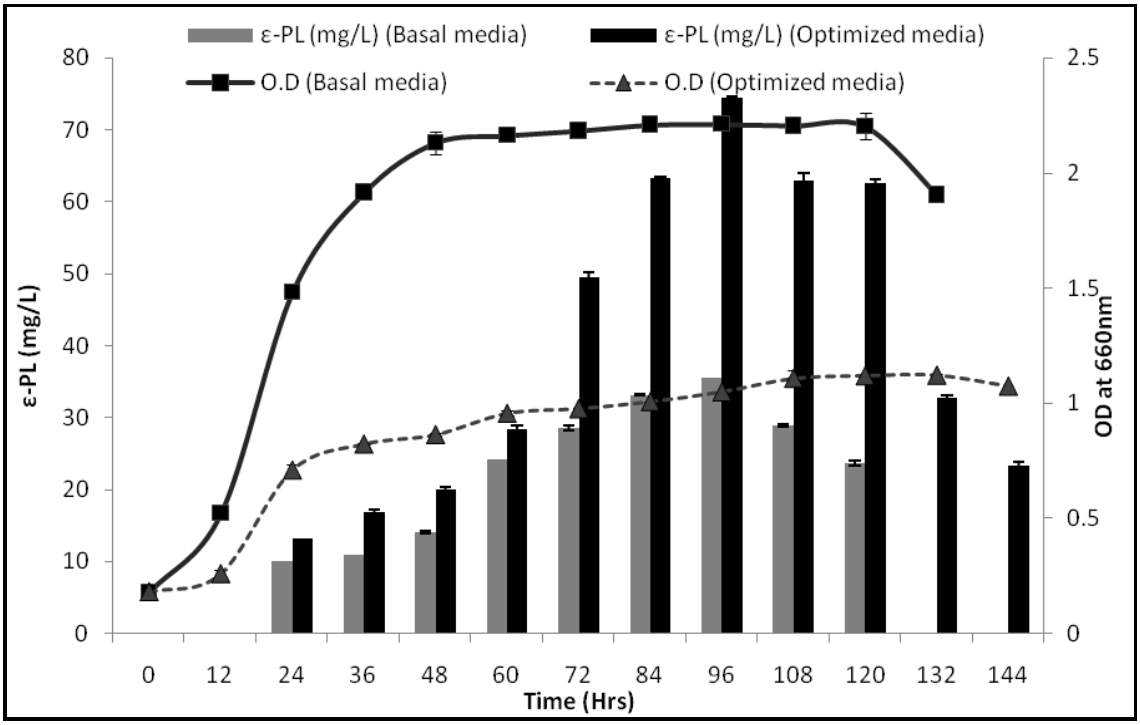

FIGURE 4: COMPARATIVE GROWTH PROFILE OF *S. NOURSEI* NRRL 5126 IN OPTIMIZED MEDIUM AND BASAL MEDIUM.

It can be seen that after optimization lag phase was reduced, the exponential phase extended for 36 h followed by decelerating log phase and the stationary phase till 120 h. ε-PL production started after 24 h of growth and showed highest titre (74.42 mg/L) after 96 h of incubation. Thus the experimental results are comparable to that predicted by the software and more than two times increase in ε-PL yield was achieved after medium optimization. Therefore it can be said that the medium optimized by this combinatorial approach improved the yield of ε-PL.

Effect of addition of amino acids on ε-PL production:

Lysine is the direct monomer precursor in the biosynthesis of ε-PL. In most bacteria, L-Lysine is biosynthesized through diaminopimelate pathway (DAP) in which TCA cycle intermediates aspartate and oxaloacetate combine with ammonium ions from nitrogen source to give DAP. Hence the effect of addition of different amino acids on ε-PL production was studied (**Figure 5**).

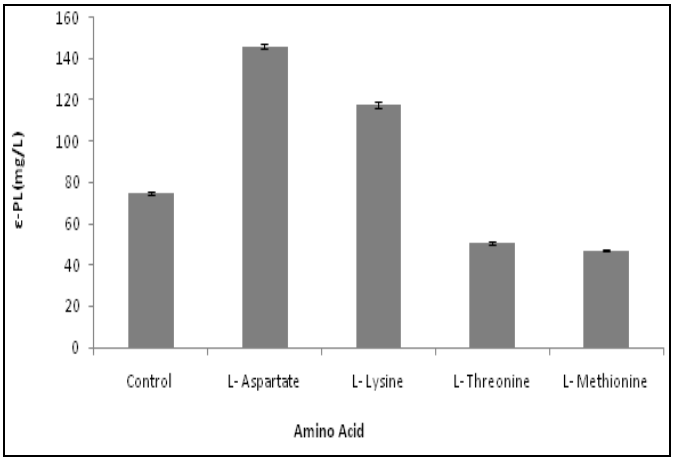

FIGURE 5: EFFECT OF AMINO ACIDS ON ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126

Among the different amino acids added, L-Lys and L-Asp supported maximum ε-PL production of 118 mg/l and 146 mg/l respectively. This may be because L-Asp molecule is directly utilized in synthesis of L-Lys which is a precursor in biosynthesis of ε-PL and hence enhances its yield [2, 6].

However L-methionine (47 mg/l) and L-threonine (51 mg/l) showed a negative effect and gaveε-PL yield less than control which might be probably due to the shift in metabolic pathway. Addition of L-methionine and L-threonine would result in L-isoleucine synthesis, instead of L-lysine biosynthesis, thereby decreasing ε-PL production. No significant change was observed in control flask and yielded 75 mg/l of ε-PL.

The next step was optimization of L-Lys and L-Asp concentration for ε-PL production. Varying concentrations ranging from 0.5mM to 4mM of L-Lys and L-Asp were tested. With increase in concentration of L-Lys and L-Asp increase in ε-PL titres was observed. Maximum yield was achieved with 2mM L-Asp acid (133 mg/l) and 2.5 mM L-Lys (125 mg/l) respectively. Further increase in concentration, resulted in decrease in accumulation of ε-PL. Since among the two amino acids, L-Asp supported the maximum ε-PL yield, it was used for further studies.

Effect of addition of TCA cycle intermediates on ε-PL production

Tricarboxylic acid (TCA) cycle act as a major metabolic hub of the cell and provides intermediates for biosynthesis of amino acids. Hence effect of various TCA cycle intermediates such as citric acid, α-ketoglutaric acid, oxaloacetate, fumaric acid, mallic acid, succinic acid on ε-PL production were studied (**Figure 6**).

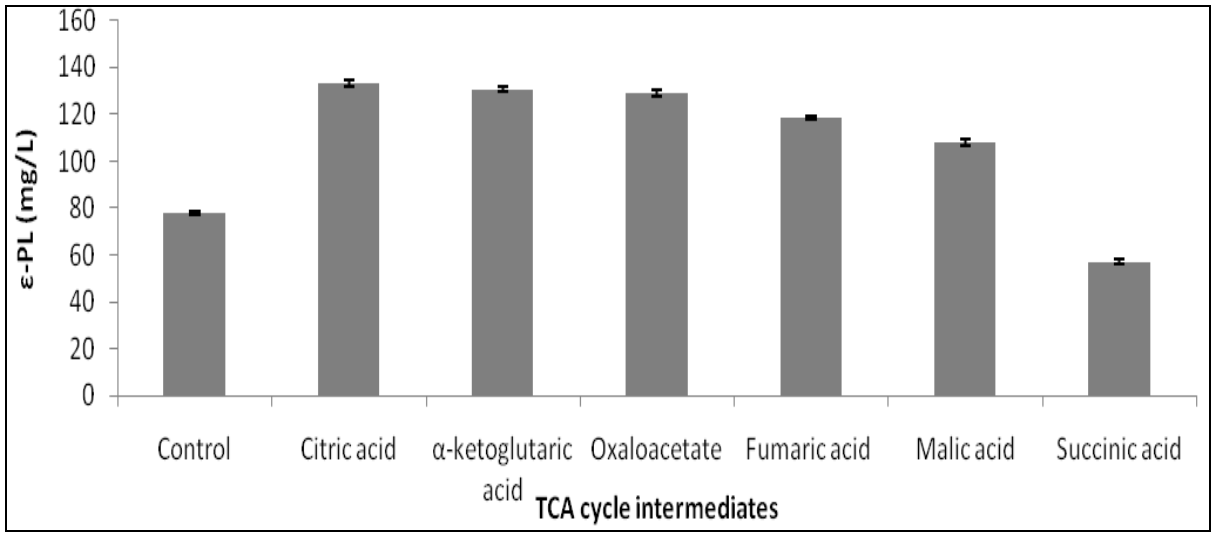

FIGURE 6: EFFECT OF TCA CYCLE INTERMEDIATES ON ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126

Among the various TCA cycle intermediates, succinic acid showed negative effect on the production of ε-PL possibly due to inhibition of reactions from fumarate to malate as well as from oxaloacetate to aspartate thereby affecting TCA cycle and cellular metabolism.

While citric acid, α-ketoglutaric acid and oxaloacetate increased ε-PL yield, probably due to the conversion of oxaloacetate to aspartate thereby promoting ε-PL biosynthesis [23, 24, 25].Further the effect of varying concentration of TCA cycle intermediates ranging from 2mM to 15mM of citric acid, α-ketoglutaric acid and oxaloacetate respectively on the production of ε-PL was studied (**Figure 7**).

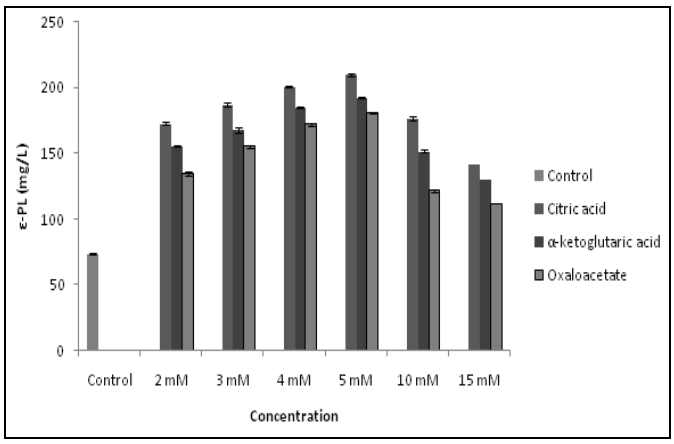

FIGURE 7: EFFECT OF VARYING CONCENTRATION OF CITRIC ACID, α- KETOGLUTARIC ACID AND OXALOACETATE ON ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126.

With increase in concentration increase in ε-PL was achieved, but among the three, citric acid at a concentration of 5 mM (210 mg/L) gave maximum ε-PL yield and hence was considered as the best and used for further studies. Further increase in concentration showed a negative effect on ε-PL production.

Optimization of time of addition of L-Asp and citric acid on production of ε-PL

In fermentative production the time of addition of precursor plays an important role in the productivity of any metabolite. Hence the next step was optimization of time of addition of aspartic acid and citric acid. Table 3 documents the effect of time of addition of L-Asp and citric acid on production of ε-PL.

TABLE 3: OPTIMIZATION OF TIME OF ADDITION OF L-ASP AND CITRIC ACID ON PRODUCTION OF ε-PL

Time of addition	ε-PL Yield (mg/L)	
	L- Aspartic acid (2mM)	Citric acid (5mM)
0 hours	78.99 ± 0.9	18.53 ± 0.44
12 hours	99.62 ± 0.61	31.94 ± 0.175
24 hours	124.66 ± 0.43	**228.58 ± 0.605**
36 hours	**175.59 ± 1.38**	175.73 ± 0.5
48 hours	153.55 ± 0.54	151.16 ± 0.93
60 hours	143.70 ± 0.61	131.67 ± 0.56

Fed Batch Fermentation with intermittent feeding of glycerol for ε-PL production The effect of intermittent feeding of glycerol on ε-PL production by *Streptomycesnoursei* NRRL 5126 in shake flask culture was studied (**Figure 9**).

It was observed that incorporation of precursors at the initial stage of fermentation did not increase the production of ε-PL probably due to utilization of these precursors by the organism for its growth. Addition of citric acid at 24 h and L- Asp at 36 h in the fermentation medium individually gave maximum biopolymer production (229 mg/L, 176 mg/L respectively). To study the synergistic effect citric acid and L-Asp were added in combination at 24 h and 36 h respectively and growth profile of *S. noursei* NRRL 5126 was studied. Initially no substantial increase in ε-PL production was observed. Further, the cells reached its exponential phase at 36 hrs of incubation. Maximum ε-PL yield of 310 mg/L was obtained in 96 hours. After 96 hrs there was a considerable decrease in the yield of ε-PL (**Figure 8**).

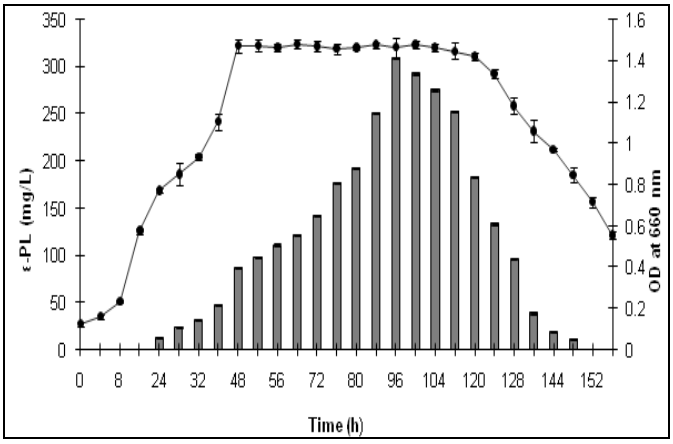

FIGURE 8: GROWTH PROFILE OF *S. NOURSEI* NRRL 5126 AFTER ADDITION OF L-ASPARTIC ACID AND CITRIC ACID

The decrease in yield of ε-PL can be due to peptide hydrolase produced by ε-PL producers. Thus medium supplementation with citric acid and L-Asp improved the yield of ε-PL from 74 mg/L to 310 mg/L. Thus a fourfold increase in ε-PL yield was achieved after incorporation of precursors in the optimized medium.

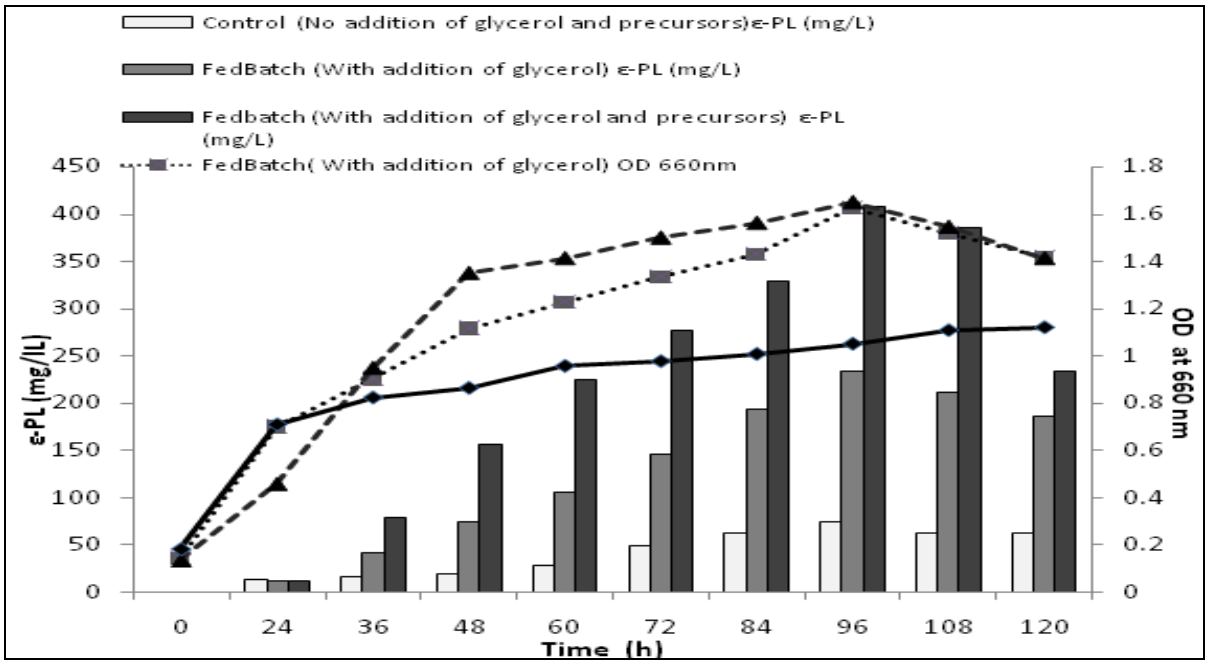

FIGURE 9: EFFECT OF FEEDING GLYCEROL ON ε-PL PRODUCTION BY *S. NOURSEI* NRRL 5126 IN PRESENCE AND ABSENCE OF PRECURSORS

In the control flask without metabolic precursors and no intermittent feeding, ε-PL production started from 24 h and reached maximum of 74 mg/L. In the feeding cultures, glycerol was incorporated after 24 h of cultivation, with the end of exponential phase, when the cells start to enter the stationary phase. 1 ml of glycerol (10g/L) was added every 12 hours.

The rate of ε-PL production increased with the increase in glycerol feeding time and was found to be maximum at 96 hours. Glycerol feeding resulted in enhanced ε-PL production from 74 mg/L to 233 mg/ L. Thus more than 3 fold increase in ε-PL production was achieved as compared to control.

The possible reason for the observed results can be due to enhanced utilization of glycerol by the organism for growth as well as ε-PL production. Earlier results showed that a considerable increase in ε-PL was obtained with the incorporation of citric acid and aspartic acid. So it was thought that incorporation of citric acid and aspartic acid along with intermittent addition of glycerol might have an added effect.

In line with this a prominent increase in ε-PL production with a yield of 408 mg/L was achieved. More than 5 fold increase in productivity was obtained as compared to control. Thus the combinatorial approach gave a marked increase in ε-PL production by *Streptomyces noursei* NRRL 5126.

CONCLUSION: Medium for ε-PL production by *S. noursei* NRRL 5126 was developed using a combination of one factor at a time method and statistical array method. After optimization ε-PL yield of 74 mg/L was obtained as compared to 35 mg/L with basal medium. Addition of 2mM L-Asp after 36 h and 5 mM citric acid after 24 h resulted in pronounced increase in ε-PL productivity to 310 mg/L. An appropriate feeding strategy of glycerol along with metabolic precursors was developed for ε-PL production by *Streptomyces noursei* NRRL 5126 resulting in further increase in ε-PL yield to 408 mg/L. Thus a substantial improvement in ε-PL yield was obtained. With further modifications this strain *S. noursei* NRRL 5126 could be an alternative ε-PL producer.

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