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OPTIMIZATION OF PHYSICAL CONDITIONS FOR THE PRODUCTION OF L-GLUTAMIC ACID BY A MUTANT *Micrococcus glutamicus* AB_{100}.

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ABSTRACT

Experimental studies were carried out to improve L-glutamic acid production using a biotin requiring auxotrophic mutant *Micrococcus glutamicus* AB100 by optimizing different physical parameters namely initial pH, period of incubation, volume of medium, age of inoculum, volume of inoculum and temperature. Maximum production of L-glutamic acid was obtained with initial pH, 6.5; period of incubation, 72h; volume of medium, 20ml; age of inoculum, 48h; volume of inoculum, 4% and temperature, 29^{o}C. Production was increased significantly ($p < 0.01$) from 6.8 to 10.4 mg/ml. Dry cell weight changed significantly ($p < 0.01$) from 2.3 to 4.6 mg/ml.

KEY WORDS

L-glutamic acid, physical parameters, incubation, medium, inoculum

INTRODUCTION

L-glutamic acid is widely used as flavor enhancer, food additive, feed supplements and therapeutic compound [1]. Monosodium L-glutamate (MSG), the largest amino acid product, used mainly as a flavor enhancer, whose production level is about 1.5 million tons and market demand is increasing rapidly at a rate of about 6% per year [2]. In the last fifty years, several microbial strains had been employed for the production, among them *Micrococcus glutamicus* was proved to be the most suitable [3]. Regarding the industrial production of L-glutamic acid, much efforts were still going on to improve its production, especially from the view point of its production cost [4,5]. Physical environment to which an organism is exposed is known to exert a significant influence on the growth and L-glutamic acid production [6,7]. Many reviews are available on the effects of different physical parameters for the production of L-amino acids by using different microorganisms [1,8,9].

In our previous investigation, we had already developed a high L-glutamic acid yielding biotin requiring auxotrophic mutant *Micrococcus glutamicus* AB_{100} from a regulatory mutant *Micrococcus glutamicus* AB_1 by induced mutation [12]. This present study is aimed to optimize different physical parameters namely, initial pH, period of incubation, volume of medium, age of inoculum, volume of inoculum and the temperature to maximize the production.

MATERIALS AND METHODS

Microorganism : *Micrococcus glutamicus* AB100, a biotin-auxotrophic mutant developed from a regulatory mutant *Micrococcus glutamicus* AB_1 by induced mutation in our laboratory and was employed throughout the investigation [10].

Composition of Basal Salt medium : Basal Salt medium contains, glucose, 10%; urea, 0.8%; K_2HPO_4, 0.1%; MgSO4, $7H_2O$, 0.025%; biotin, 0.2 µg/ml and pH was adjusted to 7.0 using 1(N) HCl and 1(N) NaOH.

Analysis of amino acid : Desending paper chromatography was employed for detecting L-glutamic acid in the culture broth and was run for 16 – 18h on a Whatman No. 1 Chromatographic paper. Solvent System used include : n-butanol : acetic acid : water (2 : 1 : 1). The spots were visualized by spraying with a solution of 0.02% ninhydrin in acetone and quantitative estimation of L-glutamic acid in the suspension was done using colonimetric estimation method [13,12].

Estimation of Dry cell weight (DCW) : After centrifugation, a few ml of 1(N) HCl was poured into the precipitate of the bacterial cells of calcium carbonate to dissolve calcium carbonate. The remaining bacterial cells were washed with water and dried at 100°C until the cell weight remain constant [13].

Statistical analysis : All data were expressed as mean ± SEM, where n = 6. The data were analysed by one way ANOVA followed by Dunett's post-hoc multiple comparison test using "4.0" software (Graph pad Inc., USA). A "p" value less than 0.05 was considered significant and less than 0.01 as highly significant.

RESULTS AND DISCUSSION

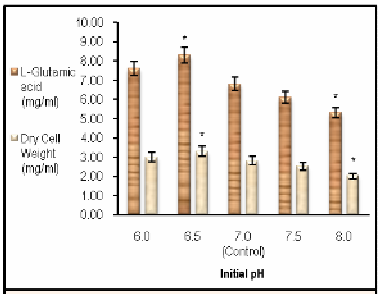

Fig.1 : Effect of initial pH on growth and L-glutamic acid production by *Micrococcus glutamicus* AB_{100}. Values were expressed as mean ± SEM, where n=6; * p<0.05 when compared to control)

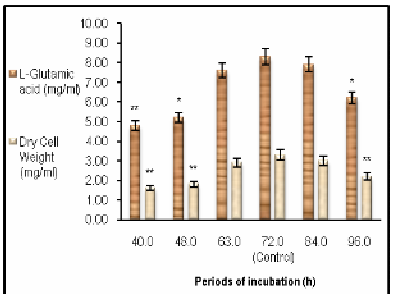

Fig.2 : Effect of periods of incubation (h) on growth and L-glutamic acid production by *Micrococcus glutamicus* AB_{100}. Values were expressed as mean ± SEM, where n=6; * p<0.05, ** p<0.01 when compared to control)

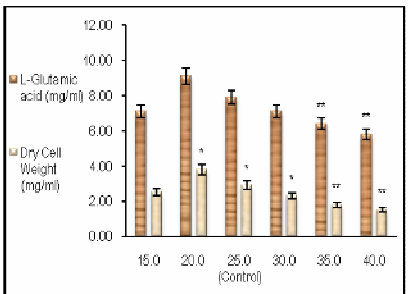

Fig.3 : Effect of volume of media (ml) on growth and L-glutamic acid production by *Micrococcus glutamicus* AB_{100}. Values were expressed as mean ± SEM, where n=6; * p<0.05, ** p<0.01 when compared to control)

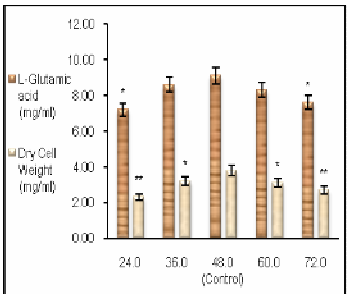

Fig.4 : Effect of volume of media (ml) on growth and L-glutamic acid production by *Micrococcus glutamicus* AB_{100}. Values were expressed as mean ± SEM, where n=6; * p<0.05, ** p<0.01 when compared to control)

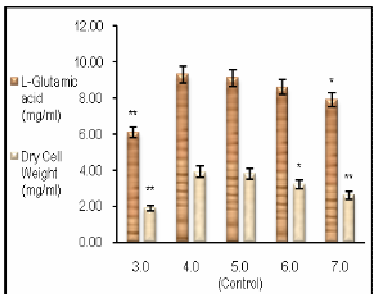

Fig.5 : Effect of different volume (ml) of inocula on growth and L-glutamic acid production by *Micrococcus glutamicus* AB_{100}. Values were expressed as mean ± SEM, where n=6; * p< 0.05, ** p< 0.01 when compared to control)

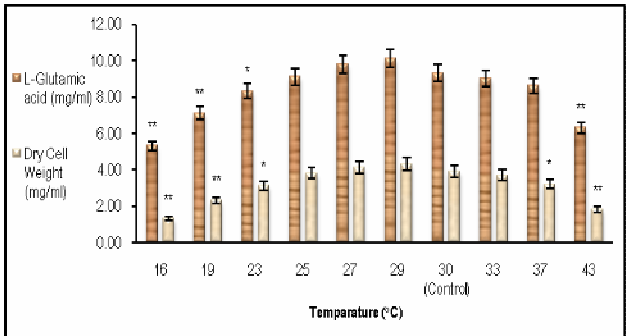

Fig.6 : Effect of Temperature (°C) on growth and L-glutamic acid production by *Micrococcus glutamicus* AB_{100}. Values were expressed as mean ± SEM, where n=6; * p<0.05, ** p<0.01 when compared to control)

Fig1 - 6

showed the effects of different physical conditions on the growth and L-glutamic acid production by the mutant Micrococcus glutamicus AB_{100}. Maximum production with highest dry cell weight were obtained with initial pH, 6.5; incubation period, 72h; volume of medium, 20 ml; age of inoculum, 48h; volume of inoculum, 4 ml ($6.0 \times 10^7 \pm 0.32 \times 10^2$ cells) and temperature, 29°C. After optimization of different physical conditions, production of L-glutamic acid was increased significantly ($p < 0.01$) from 6.8 to 10.4 mg/ml. Cellular growth changes from 2.3 to 4.6 mg/ml also showed similar pattern ($p < 0.01$).

Kinoshita et al (1961) [8] claimed that maximum L-lysine acid was obtained by *Micrococcus glutamicus* ATCC 13058 with initial pH 7.0, 48h period of incubation, 24h age of inoculum, 10% volume of inoculum at 28°C temperature. Tsunoda et al (1961) [9] studied on Brevibacterium flavum no 2247 and noted that it produced maximum L-glutamic acid between pH range of 7.5 to 8.0. Carito and Pisano (1966) [14] studied on the production of L-alanine by *Fusarium moniliforme* and reported maximum fermentative accumulation of L-alanine was resulted with 2% volume of medium in 250 ml Erlenmeyer flask, 72h period of incubation at 28°C. Shah et al (2002) [13] used *corynebacterium glutamicum* for the production of L-lysine and noted that 24h age of inoculum 20h period of incubation, initial pH 7.5, 10% inoculum volume, 50 ml fermentation medium in 500 ml Erlenmeyer flask at 30°C were proved to be the optimum. Ekwealor and obtata (2005) [15] obtained maximum L-lysine by *Bacillus megaterium* SP14 using initial pH 7.0, 10% inoculum volume, 20 ml fermentation medium in 100 ml Erlenmeyer flask, 72h fermentation period at 30°C Lee obtained L – threonine by a mutant *Escherichia coli* at 30oC with pH 6.016. Yugandhar et al (2006) [1] optimized the cultural conditions for *Brevibacterium roseum* which accumulated maximum L-glutamic acid in the fermentation broth with initial pH 6.0, 24h age of inoculum, 96h period of incubation, 5% inoculum volume at 30°C. Ekwealor and obeta (2007) [17] obtained maximum L-lysine by *Bacillus megaterium* SP86, *Bacillus megaterium* SP14 with initial pH 7.2, 10% inoculum volume, 24h age of inoculum, 72h period of incubation at 30°C.

Thus, from this present study, it was concluded that using minimum salt medium, by optimizing different physical conditions, production of L-glutamic acid by this mutant might be increased significantly.

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