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# ROLE OF COMPLEX NUTRIENTS ON PRODUCTION OF L-GLUTAMIC ACID BY A MUTANT *Micrococcus glutamicus* $AB_{100}$.

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## ABSTRACT

The effect of some complex nutrients like wheat bran extract, rice bran extract, paddy soak liquor, tryptone, soybean extract, corn steep liquor, peptone, yeast extract, malt extract and beef extract on l-glutamic acid fermentation by a mutant *Micrococcus glutamicus* $AB_{100}$ were investigated. Different concentrations (02-0.5%) of each of the complex nutrients were added to the fermentation broth. Production of l-glutamic acid was decreased with wheat bran extract, rice bran extract, paddy soak liquor and tryptone but increased significantly with soybean extract, 0.3%; corn steep liquor, 0.25%; peptone, 0.3%; yeast extract, 0.4% malt extract, 0.3% and beef extract, 0.4%.

## KEY WORDS

Complex, L-glutamic acid, fermentation, *Micrococcus glutamicus*

## INTRODUCTION

Interest in the microbial production of different amino acids has increased greatly since the development of large scale production of l-glutamic acid could stimulate market development, much of recent efforts were still going on to improve the l-glutamic acid fermentation process especially from the stand point of saving its production cost[2,3]. Considerng the fact, various complex nutrients had been used for the fermentative accumulation of different amino acids[4-25].

The aim of the present work, therefore was to study the effect of some complex nutrients on l-glutamic acid fermentation by a biotin requiring auxotrophic mutant *Micrococcus glutamicus* $AB_{100}$.

## MATERIALS AND METHODS

***Microorganism :*** A biotin requiring auxotrophic mutant *Micrococcus glutamicus* $AB_{100}$ developed from a regulatory mutant *Micrococcus glutamicus* $AB_1$ by induced mutation in our laboratory was used throughout this study[26].

***Growth medium and growth conditions :*** The culture was maintained on agar slants having composition : glucose, 1.0%; peptone, 0.5%; beef extract, 0.3%; yeast extract, 0.1% and agar, 4.0%. The pH was adjusted to 6.5 using 1.0 (N) HCl and 1.0 (N) NaOH. The fermentation was carried out for 48h at 29°C on a rotary shaker with a shaking speed of 150 rpm[27].

***Preparation of inoculum :*** A full grown slant of 48h old of *Micrococcus glutamicus* $AB_{100}$ was scrapped off and suspended in 100 ml sterile water. The cell suspension of 4.0% (v/v) of the seed culture (6.0% X $10^7$ cells/ml) of the organism was used as inoculum[27].

***Composition of the selected synthetic medium for l-glutamic acid production :*** The following synthetic medium was used for the production of l-glutamic acid by this mutant : glucose, 9.0%; $(NH_4)_2HPO_4$, 1.4%; $K_2HPO_4$, 0.15%; $MgSO_4.7H_2O$, 0.03%; $CaCO_3$, 0.4%; $FeSO_4.7H_2O$, 5.0 µg/ml; $ZnSO_4.7H_2O$, 1.0 µg/ml; $MnSO_4.4H_2O$, 1.0 µg/ml and biotin, 0.2 µg/ml. pH was adjusted to 6.5[28].

***Preparation and addition of complex nutrients :***

(a) Preparation of rice bran and wheat bran extract : 40 gm of each material was taken into 250 ml of warm distilled water separately. The suspension was kept at 28°C for 48h. The extracts were filtered separately through cotton and evaporated to dryness under vaccum to recover solid content.

(b) Preparation of corn steep liquor : About 150 gm of corn was taken into 300 ml distilled water and kept it for 24h at 28°C. The extract was filtered through cotton and evaporated to dryness under vaccum to recover solid content.

(c) Preparation of paddy soak liquor : 100 gm paddy was added to 250 ml of distilled water and kept it at 28°C for 24h. The extract was filtered through cotton and evaporated to dryness under vaccum to recover solid content.

(d) Preparation of soybean extract : 50 gm of soybean was poured into 250 ml of distilled water and allowed to swallow for 24h at 28°C. The soaked water was extracted thoroughly and filtered through cotton. It was then dried for determination of solid content.

All the above mentioned complex nutrients were added to the synthetic medium according to them solid content in a sterile condition, but peptone, yeast extract, beef extract, malt extract and tryptone were added directly to the synthetic medium[29].

The effect of varying concentrations (0.2-0.5%) of each of the complex nutrients were examined on growth and l-glutamic acid production by this mutant.

***Estimation of l-glutamic acid :*** Descending paper chromatography was employed for the detection of l-glutamic acid in a culture broth and was run for 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 0.2% ninhydrin in acetone and quantitative estimation of l-glutamic acid in the suspension was done by using clorimetric estimation method[30,31].

***Estimation of Dry cell weight (DCW) :*** After centrifugation, a few ml of 1.0(M) HCl was poured into the precipitate of the bacterial cells and calcium carbonate to dissolve calcium carbonate. The remaining bacterial cells were washed with water and dried at 100°C until cell weight remain constant[32].

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

## RESULTS AND DISCUSSION

The effects of different complex nutrients on cellular growth and l-glutamic acid production by the mutant were depicted in Fig. 1 to 10.

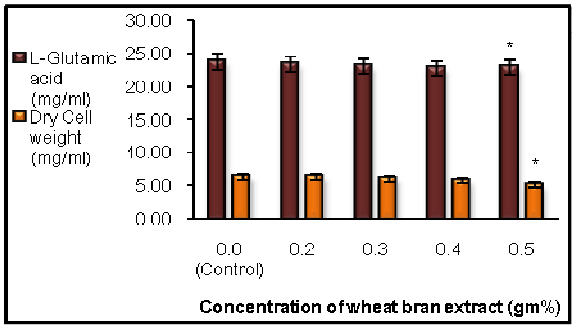


**Fig.1**

***Effect of wheat bran extract on 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)***

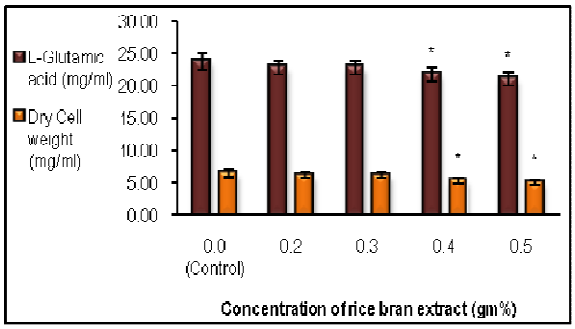


**Fig.2**

***Effect of rice bran extract on 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)***

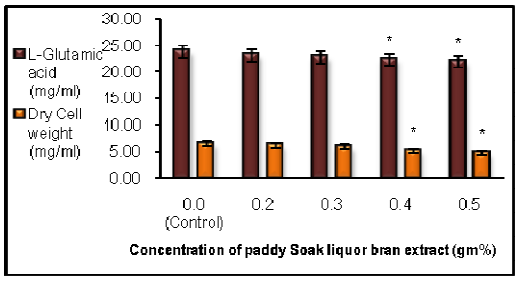


**Fig.3**

***Effect of paddy soak liquor bran extract on 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)***

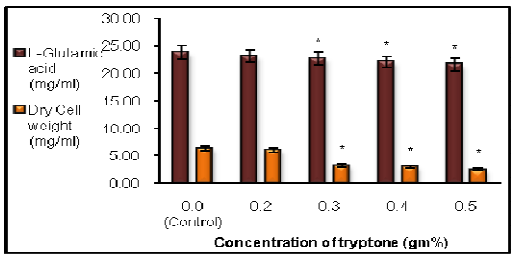


**Fig.4**

***Effect of tryptone on 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)***

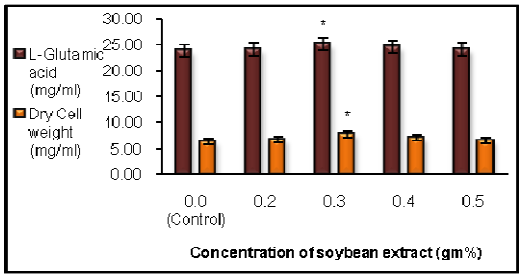


**Fig.5**

***Effect of soybean extract on 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)***

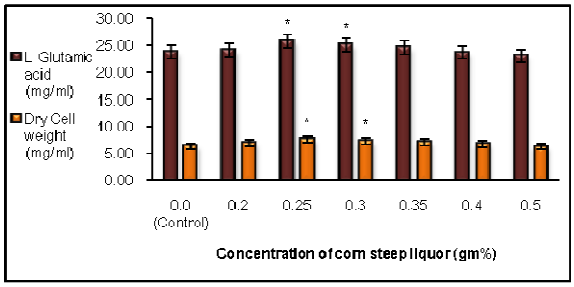


**Fig.6**

***Effect of corn steep liquor on 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)***

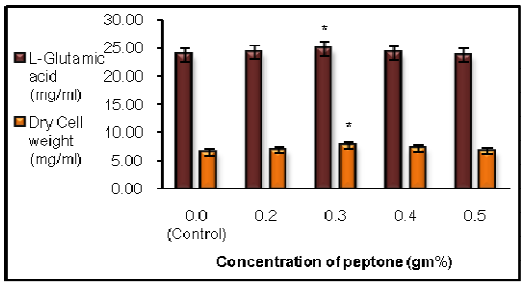


**Fig.7**

***Effect of peptone on 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)***

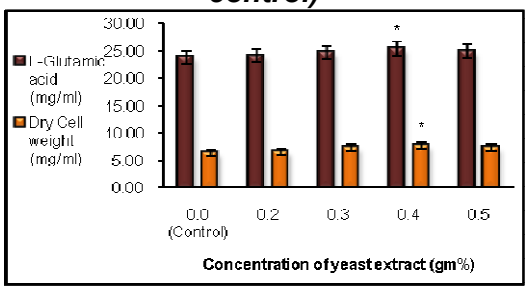


**Fig.8**

**Effect of yeast extract on 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)**

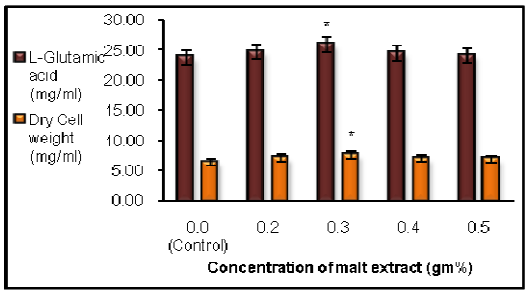


**Fig.9**

**Effect of malt extract on 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)**

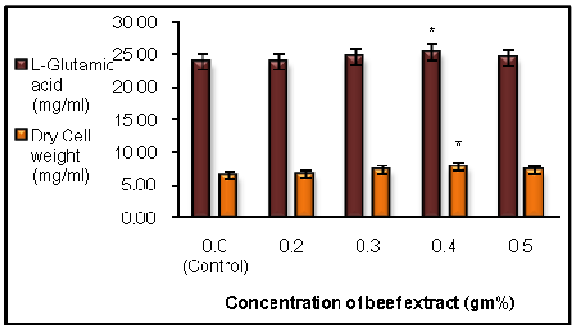


**Fig.10**

***Effect of beef extract on 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)***

Production of l-glutamic acid was decreased with wheat bran extract, rice bran extract, paddy soak liquor and tryptone but increased significantly ($p<0.05$) with soybean extract, 0.3%; corn steep liquor, 0.25%; peptone, 0.3%; yeast extract, 0.4% malt extract, 0.3% and beef extract, 0.4%. Dry cell weight changes also showed similar pattern of changes.

The detrimental effect of some complex nutrients like wheat bran extract, paddy soak liquor and tryptone were exerted on cellular growth and l-glutamic acid production probably due to osmotic inbalance[33]. Among the nutritional factors present in different complex nutrients, biotin seemed to be the most potential simulating factor to l-glutamic acid accumulation by fermentation[34,35]. But here peptone being a lower protein composed of multiple amino acids, also showed positive impact on l-glutamic acid production and cellular growth, indicating certain probable role of some amino acids in this process. However, it needs further investigation which is under way.

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