Research Article

EFFECTS OF MACRO-MINERAL ELEMENTS ON GROWTH AND L-GLUTAMIC ACID FERMENTATION BY A MUTANT *MICROCOCCUS GLUTAMICUS* AB_{100}

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ABSTRACT

An experimental study was carried out to examine the effects of different macro-mineral elements on growth and l-glutamic acid fermentation by a mutant *Micrococcus glutamicus* AB_{100}. It was required for improved production of l-glutamic acid by this mutant. K_2HPO_4, 0.15%; $MgSO_4.7H_2O$, 0.03% and $CaCO_3$, 0.04% were required for improved production of l-glutamic acid by this mutant. KH_2PO_4, $NaH_2PO_4.H_2O$, HCl, NaCl and $CaCl_2$ showed no effect on growth and l-glutamic acid accumulation, where as $K_2B_4O_7.X\ H_2O$ showed negative impact on growth and the production. L-glutamic acid production was increased significantly ($p<0.01$) from 14.8 mg/ml to 18.8 mg/ml and dry cell weight was increased significantly ($p<0.01$) from 7.3 mg/ml to 9.8 mg/ml after addition of the necessary macro-mineral elements in the fermentation broth.

Keywords: Macro-mineral, growth, L-glutamic acid, mutant, *Micrococcus glutamicus*.

INTRODUCTION

L-glutamic acid, a non-essential amino acid has a wide spectrum of commercial use as flavor enhancer, food additive, infusion compounds etc[1]. The industrial production of l-glutamic acid is mainly carried out by fermentation using bacterial species namely *Micrococcus glutamicus, Brevibacterium roseum, Brevibacterium flavum* etc.[2] For l-glutamic acid over production, *Micrococcus glutamicus* strains have been developed in our laboratory by induced mutation in our laboratory[3]. However, each bacterium has a defined range of growth conditions including requirements of macro-minerals elements[4]. Roy and Chatterjee (1989) reported the influence of culture conditions on l-glutamic acid production by *Arthrobacter globiformis*[5]. Lee et al (2006) claimed the requirements of different macro-mineral elements on growth and l-threonine production by *E.Coli* mutant[6]. Kase and Nakayama, Banik and Majumdar (1975), tani et al (1988) reported that microbial production of l-methionine required different macro elements[7-11].

Considering all these facts, the present study was intended to examine the effects of different macro-mineral elements on growth and l-glutamic acid fermentation by a biotin-auxotrophic mutant *Micrococcus glutamicus* AB_{100}.

MATERIALS AND METHODS

Microorganism: *Micrococcus glutamicus* AB_{100}, a biotin requiring auxotrophic mutant derived from a regulatory mutant derived from a regulatory mutant *Micrococcus glutamicus* AB_1 by induced mutation was used throughout this study[3].

Minimal salt medium: Minimal salt medium contained glucose, 9.0%; $(NH_4)_2HPO_4$, 1.4%; $MgSO_4.7H_2O$, 0.025%; K_2HPO_4, 0.1%; biotin, 0.2 μg/ml. pH was adjusted to 6.5.

Fermentation conditions: Fermentation was carried out using shake-flask method on a rotary shaker rotating at 150 rpm, in 100 ml Erlenmayer conical flask containing 20 ml minimal salt medium for 72h at 29°C. The medium was inoculated with 4.0% (v/v) of a 48h old seed culture (6.0 x 10^7 cells) of *Micrococcus glutamicus* AB_{100}[12].

Addition of different macro mineral elements : Different macro-mineral elements (namely, K_2HPO_4, KH_2PO_4, $NaH_2PO_4.H_2O$, $MgSO_4.7H_2O$, KCl, NaCl, $CaCl_2.2H_2O$, $CaCO_3$ and $K_2B_4O_7.XH_2O$) at varying concentrations (0.02-0.2%) were added to the minimal salt medium one by one to assess their effects on growth and l-glutamic acid production by this mutant[7].

Analysis of amino acid: Descending paper chromatography was used to detect l-glutamic acid in culture broth using a solvent system composed of n-butanol : acetic acid : water (2:1:1) which was run for 18h on a whatman no. 1 chromatography paper. The spots were visualized by spraying with a solution of 0.2% ninhydrin in acetone and quantitative estimation of l-glutamic acid was done using clorimetric estimation method[13,14].

Estimation of Dry Cell Weight: After proper centrifugation, 2 ml of 1.0 (M) HCl was poured into the precipitate of the bacterial cells and $CaCO_3$ to dissolve $CaCO_3$. The remaining bacterial cells were washed with water and derived at 100°C until cells weight remain constant[15].

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 Inc., USA). A "p" value less than 0.05 was considered significant and less than 0.01 as a highly significant.

RESULTS AND DISCUSSION

The effect of different macro-mineral elements on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} were depicted in Fig 1-9. Production was maximum with KH_2PO_4, 0.15%; $MgSO_4.7H_2O$, 0.03% and $CaCO_3$, 0.04% along with maximum dry cell weight KH_2PO_4, $NaH_2PO_4.H_2O$, KCl, NaCl, and $CaCl_2.2H_2O$ did not have any significant impact on both cellular growth and l-glutamic acid production. But $K_2B_4O_7.XH_2O$ was proved to be detrimental for both cellular growth and l-glutamic acid production.

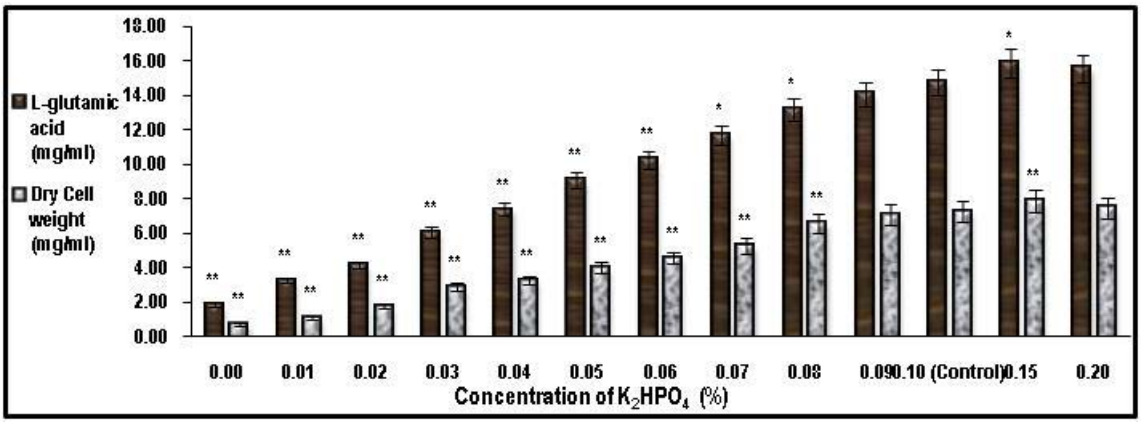

Figure 1: Effect of K_2HPO_4 on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

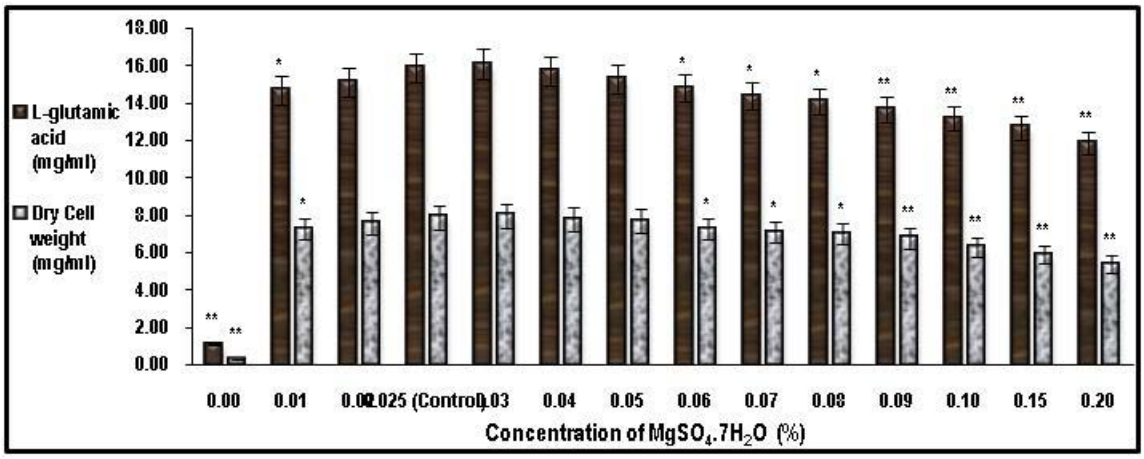

Figure 2: Effect of $MgSO_4.7H_2O$ on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

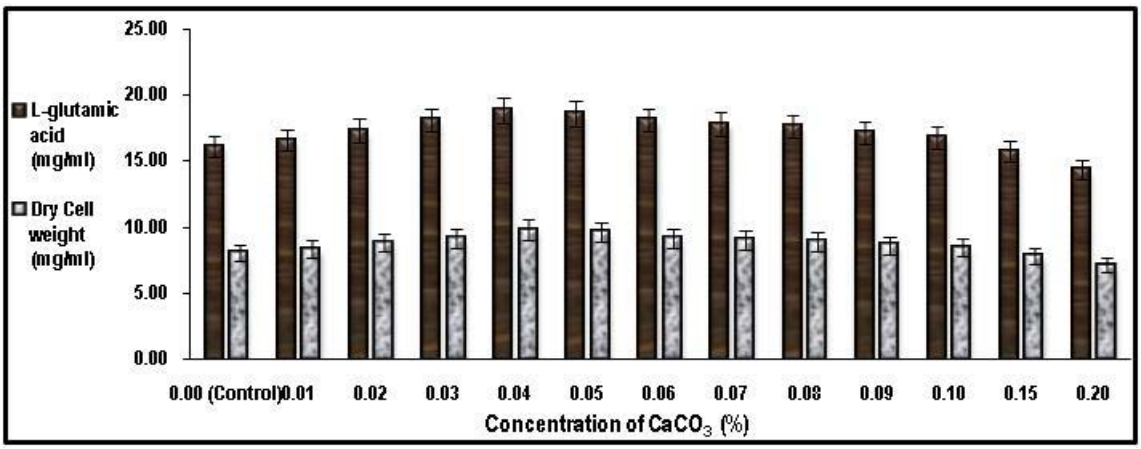

Figure 3: Effect of $CaCO_3$ on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

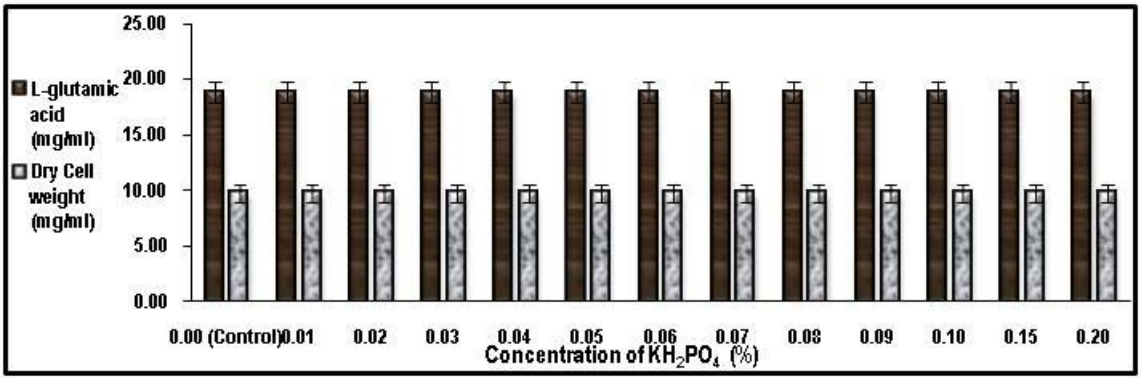

Figure 4: Effect of KH_2PO_4 on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

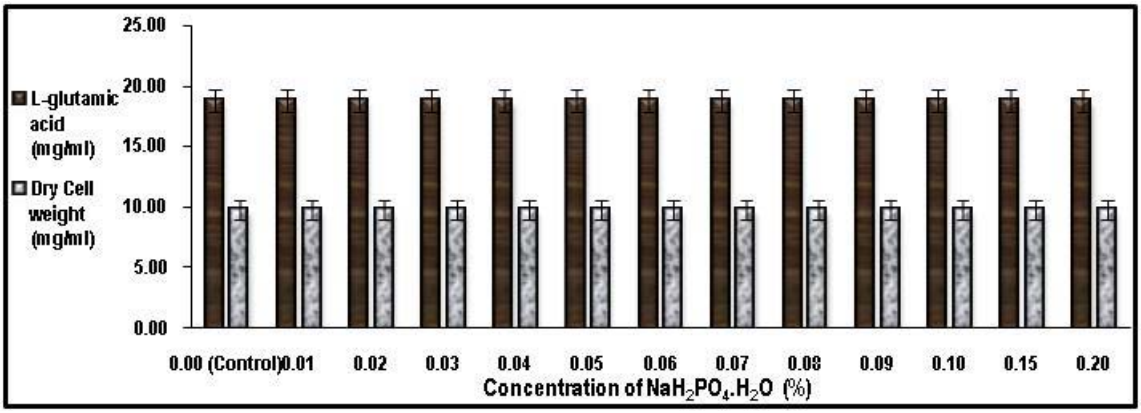

Figure 5: Effect of $NaH_2PO_4.H_2O$ on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

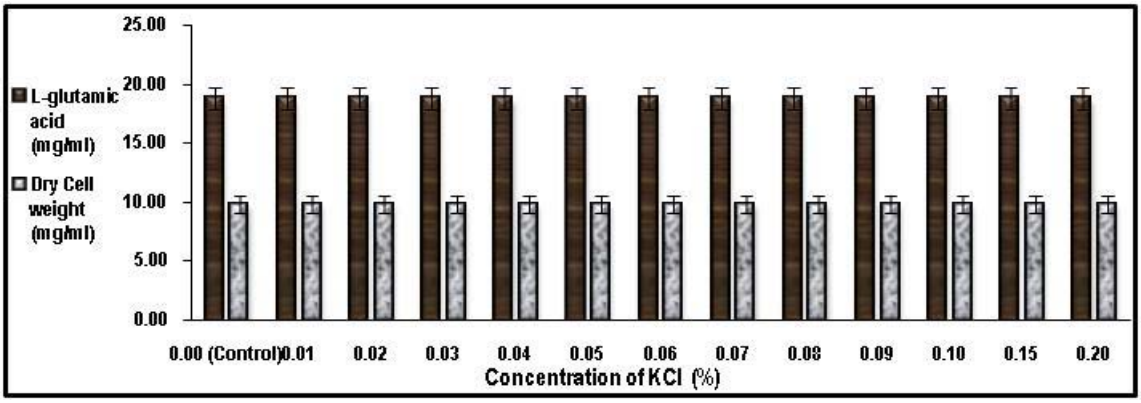

Figure 6: Effect of KCl on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

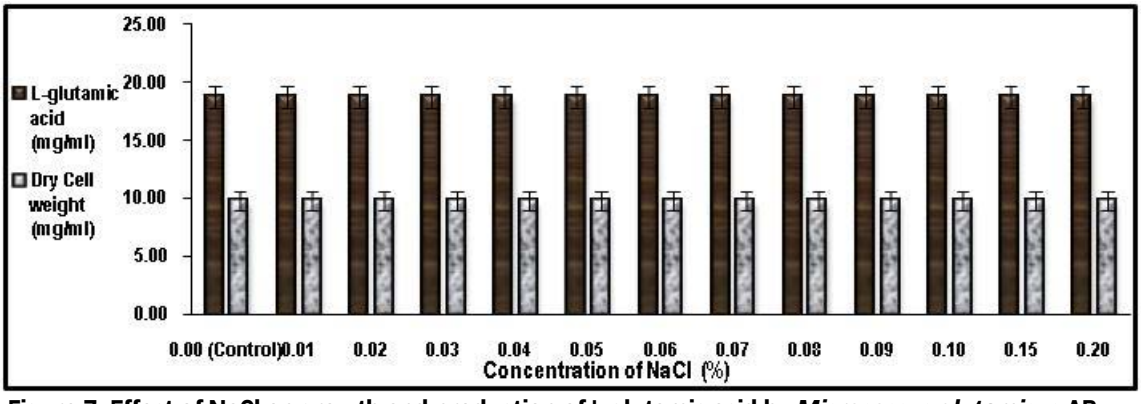

Figure 7: Effect of NaCl on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

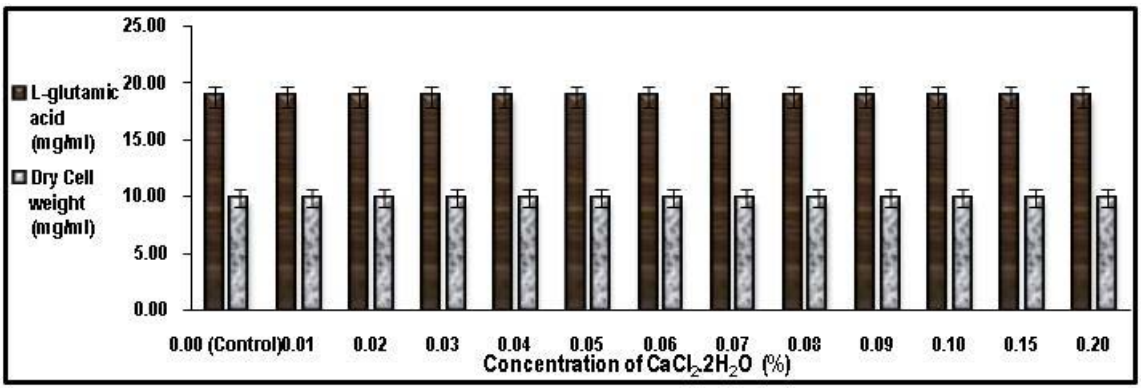

Figure 8: Effect of $CaCl_2.2H_2O$ on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

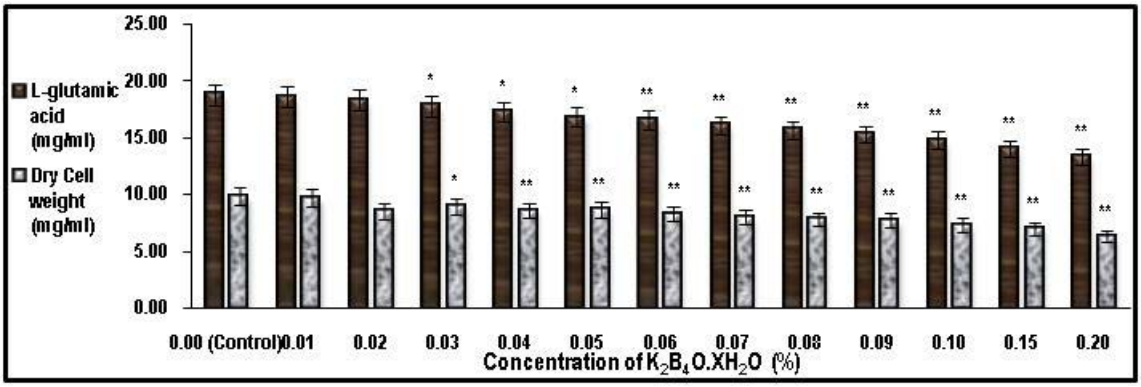

Figure 9: Effect of $K_2B_4O.XH_2O$ on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.
(values were expressed as mean ± SEM, where n=6; *p<0.05; **p<0.01 when compared to control)

Carito et al (1966) reported that *Fusarium moniliforme* required K_2HPO4, 0.6%; KH_2PO_4, 0.4%; $MgSO_4.7H_2O$, 0.4%; NaCl, 0.2% and $CaCl_2.2H_2O$, 0.2% for l-alanine production[16]. Birnbacum et al (1969) used $MgSO_4.7H_2O$, 0.05%; as macro-mineral element for l-glutamic acid production by corynebacterium glutamicum[16]. Kase and Nakayama (1974) have reported K_2HPO_4, 0.05%; KH_2PO_4, 0.1%; $MgSO_4.7H_2O$, 0.01% were needed for l-methionine production[18]. Banik and Majumdar (1975) reported that production of l-methionine required K_2HPO_4, 0.03%; $MgSO_4.7H_2O$, 0.1% as macro-mineral element[7]. Lee et al (2006) used KH_2PO_4, 0.85%; $MgSO_4.7H_2O$, 0.1%; $CaCl_2.2H_2O$, 1.32%; $K_2B_4O_7.XH_2O$, 0.006% as macro-mineral element for l-throeonine production *E. Coli* mutant[6]. Yugandhar et al (2007) reported that *Brevibacterium roseum* required K_2HPO_4, 0.12%; $CaCO_3$, 0.16%; $MgSO_4.7H_2O$, 0.01% and NaCl, 0.01% for l-glutamic acid production[19]. But there is no review available on the effect of KCl on growth and l-glutamic acid production by micro-organisms. In our present study we have observed that KCl showed no significant effect on growth and l-glutamic acid production by this mutant.

Thus, in this present study it was concluded that production of l-glutamic acid was increased significantly (p<0.01) from 18.8 mg/ml to 18.8 mg/ml after addition of K_2HPO_4, 0.15%; $MgSO_4.7H_2O$, 0.03% and $CaCO_3$, 0.04% in the fermentation broth.

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