Effect of Vitamin B-complex on Growth and L-glutamic acid Accumulation by a Mutant Micrococcus glutamicus AB$_{100}$

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An experimental study was carried out to investigate the effect of vitamin B-Complex on growth and l-glutamic acid accumulation by an auxotrophic mutant Micrococcus glutamicus AB$_{100}$. Different concentrations (0.1 – 2.0 µg/ml) vitamin B$_1$, Folic acid thiamine-HCl, riboflavin, nicotinic acid, pyridoxine-HCl, inositol, calcium pantothenate, paraamino-benzoic acid and biotin were examined. Production was maximum with vitamin B$_1$, 1.0 µg/ml; Folic acid, 0.5 µg/ml; thiamine-HCl, 1.0 µg/ml; riboflavin, 0.3 µg/ml; nicotinic acid, 0.3 µg/ml; pyridoxine-HCl, 0.7 µg/ml; inositol, 0.6 µg/ml; calcium pantothenate, 0.4 µg/ml; paraamino-benzoic acid 0.3 µg/ml; and biotin 0.2 µg/ml. Production was decreased with further increment of the vitamin concentrations, but dry cell weight increased continuously with increased levels of the vitamins.

Key words: Mutant, Micrococcus glutamicus, Vitamin, Dry cell weight.

Fermentative production of l-glutamic acid has been used during last fifty years and the production yield has been increased significantly over the years$^{1-3}$. Different nutrients parameters also known to significantly alter the product yield.

Many microorganisms used for the production of l-amino acids including l-glutamic acid required different vitamins for their growth and metabolites accumulation. Several reviews are available on the requirements of vitamin B-Complex for the microbial production of l-amino acids$^{4-15}$. Considering all these reviews, the present study was intended to study the effect of vitamin B-complex on growth and l-glutamic acid accumulation.

MATERIAL AND METHODS

Microorganism

Micrococcus glutamicus AB$_{100}$, a biotin requiring auxotrophic mutant derived from a regulatory mutant Micrococcus glutamicus AB$_1$ by induced mutation in our laboratory was used throughout the study$^{16}$.

Synthetic medium used for l-glutamic acid production

The composition of the synthetic medium used for L-glutamic acid production was as follows: glucose, 9.0%; diammonium hydrogen phosphate, 1.4%; dipotassium hydrogen phosphate, 0.15%; magnesium sulfate, hepta hydrate; 0.03%; calcium carbonate, 0.04; ferrous sulfate, hepta hydrate, 5.0 µg/ml; zinc sulfate, hepta hydrate, 1.0 µg/ml; manganese sulfate, tetra hydrate, 1.0 µg/ml; and biotin, 0.2 µg/ml; pH 6.5.

Fermentation was carried out using shake, flask method on a rotary shaker (150 rpm) in 100 ml
Erlenmayer conical flask containing 20 ml mineral salt medium for 72h at 29°C. The medium was inoculated with 4.0% (v/v) of 48h old seed culture (6.0 X 10^4 cells) of Micrococcus glutamicus AB, followed by addition of vitamin B-complex to the synthetic medium.

Initially, the basal medium contained only biotin (0.2 µg/ml) as a member of vitamin B-complex. Different members of vitamin B-complex namely vitamin B₁₂, folic acid, thiamine-HCl, riboflavin, nicotinic acid, pyridoxine-HCl, inositol, biotin, Calcium pantothanate, paraaminobenzoic acid and biotin were added separately to the medium at varying concentrations (0.1-2.0 µg/ml). Analysis of Amino acid

Descending paper chromatography was used for detecting l-glutamic acid in culture medium and was run for 18h on a Whatman no. 1 chromatography 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 using colorimetric estimation method.

Estimation of Dry Cell Weight

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 derived at 100°C until cells weight remain constant.

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 and less than 0.01 as a highly significant.

RESULTS AND DISCUSSION

Fig. 1 – 10 showed the effect of different members of vitamin B-complex on growth and l-glutamic acid production by the mutant Micrococcus glutamicus AB. All the vitamins studied showed positive effect on growth and the production. Maximum l-glutamic acid production was obtained with vitamin B₁₂, 1.0 µg/ml; Folic acid, 0.5 µg/ml; thiamine-HCl, 1.0 µg/ml; riboflavin, 0.5 µg/ml; nicotinic acid, 0.5 µg/ml; pyridoxine-HCl, 0.7 µg/ml; inositol, 0.6 µg/ml; calcium pantothanate, 0.4 µg/ml; paraamino-benzoic acid 0.3 µg/ml; and biotin 0.2 µg/ml. Production of l-glutamic acid was decreased significantly (p<0.01) after omission of biotin from the fermentation medium. Dry cell weight was increased continuously with the increment of the vitamin concentrations. Production of l-glutamic acid was decreased significantly (p<0.05 and p<0.01 respectively) from 0.4-2.0 µg/ml concentration of biotin.

Biotin was suggested as a co-factor for glucose oxidation, protein synthesis, cellular permeability and co-valent bond formation with cobalt. Lactic acid accumulation was increased with rining level of biotin due to excess cellular population which created anaerobic condition, and at higher biotin concentration, lactic acid should be treated as the main fermentation product, accompanied by low levels of succinic acid and...
Fig. 2. Effect of Folic acid on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.

Fig. 3. Effect of thiamine-HCl on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB_{100}.

Fig. 4. Effect of riboflavin 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)
Fig. 5. Effect of nicotinic acid on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB<sub>100</sub>

Values were expressed as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control

Fig. 6. Effect of inositol on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB<sub>100</sub>

Values were expressed as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control

Fig. 7. Effect of inositol on growth and production of L-glutamic acid by *Micrococcus glutamicus* AB<sub>100</sub>

Values were expressed as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control
Fig. 8. Effect of calcium pantothenate on growth and production of L-glutamic acid by *Micrococcus glutamicus AB*<sub>100</sub>

Values were expressed as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control.

Fig. 9. Effect of paraamino benzoic acid on growth and production of L-glutamic acid by *Micrococcus glutamicus AB*<sub>100</sub>

Values were expressed as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control.

Fig. 10. Effect of biotin on growth and production of L-glutamic acid by *Micrococcus glutamicus AB*<sub>100</sub>

Values were expressed as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control.
malic acid. Takahashi et al (1965) reported that among different vitamins tested, thiamine-HCl significantly stimulated l-glutamic acid production by a newly isolated strain (S10B1) of *corynebacterium*. Ekwealor and obeta (2007) studies the effect of thiamine-HCl, nicotinic acid, biotin, pyridoxine-HCl, folic acid and riboflavin on l-lysin production by *Bacillus sp* and reported that biotin was essential for l-lysin production by this strain.

However, in our present investigation, all the vitamins examined simulated the growth and l-glutamic acid production up to certain concentrations which were depicted in Fig. 1 – 10, but higher concentrations of vitamins simulated the growth, but production was decreased gradually, probably due to anaerobic conditions created by excess cellular population.

REFERENCES


