BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2011. Vol. **8**(2), p. 717-722

Effect of Metabolic Inhibitors on Growth and Production of L-glutamic Acid by the Mutant *Micrococcus glutamicus* AB_{100}

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(Received: 05 July 2011; accepted: 29 August 2011)

An experimental study was carried out to examine the effects of different metabolic inhibitors namely 6-marcaptopurine, 2, 4 dinitrophenol, sodium arsenate, mercuric chlorice, sodium arsenite, 2-thiouracil, sodium azide, malonic acid and sodium fluoride on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100}. All these inhibitors showed detrimental effect on growth of l-glutamic acid production.

Key words: Metabolic inhibitors, L-glutamic acid, Growth, Mutant, *Micrococcus glutamicus*.

Microbial production of l-glutamic acid is predominantly operated through Embden-Meyerhof-parnas (EMP) Pathway and the early steps of Kerb's cycle in presence of oxygen which serves as a terminal electron accepted[1]. Thus, different metabolic inhibitors may alter the production of l-glutamic acid by inhibiting either the path ways at different steps or the growth of the microorganism as a whole.

The present investigation was under taken to examine the effect of different metabolic inhibitors with different concentrations on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100}.

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MATERIAL AND METHODS

Microorganism

A biotin-auxotrophic *Micrococcus glutamicus* AB_{100}, derived from *Micrococcus glutamicus* AB_1, by induced mutation by ethyleneimine as chemical and UV irradiation as physical mutagens respectively[2].

Synthetic medium for l-glutamic acid production

The following composition of the synthetic medium was used for l-glutamic acid fermentation by the *Micrococcus glutamicus* AB_{100} : glucose, 9.0%; diammonium hydrogen phosphate, 1.4%; magnesium sulfate, hepatahydrate, 0.03%; calcium carbonate, 0.04%; ferrous sulfate heptahydrate, 5.0 µg/ml; Zinc sulfate, hypatahydrate, 1.0 µg/ml; manganese sulfate, tetrahydrate, 1.0 µg/ml; and biotin, 0.2 µg/ml; pH 6.5[3].

Fermentation conditions

Fermentation was conducted with shake-glask method on a rotary shaker totating at 150 rpm in 10 ml Erlenmyer Conical flask containing 20 ml synthetic medium for 72h at 29°C, inoculated with 4.0% (v/v) of 48 h old seed culture (6.0×10^7

cells) of *Micrococcus glutamicus* AB_{100}[4].

Addition of metabolic inhibitors

Different concentrations (0.100-0.001 mole / ml) of different metabolic inhibitors were added to the fermentation broth one by one at different time intervals (0-48h) to examine their effects on growth and l-glutamic acid production.

Analysis of amino acid

Chlorimetric estimation method using descending chromatography was employed for the estimation of l-glutamic acid[5,6].

Estimation of Dry cell weight (DCW)

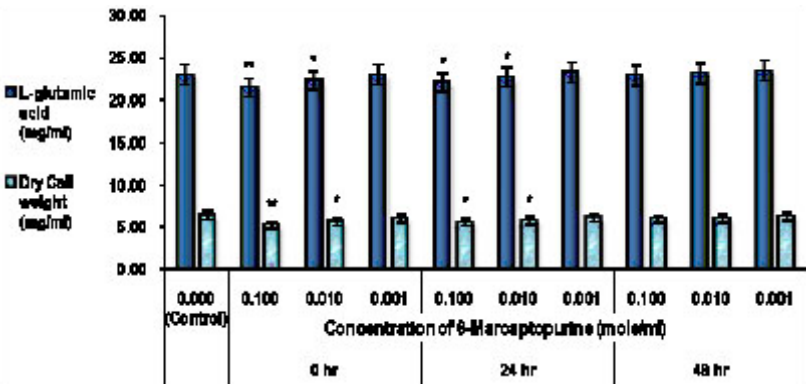
DCW was estimated using the method of Shah et al[7].

Statistical analysis

Values were expressed as mean ± SEM, where n=6. The data were statistically estimated by one way ANOVA followed by Dunett's post hoc. Multiple comparison test using "prism 4.0" soft ware (Graph pad Inc., USA). A "p" value less than 0.05 was considered significant an less than 0.01 as highly significant.

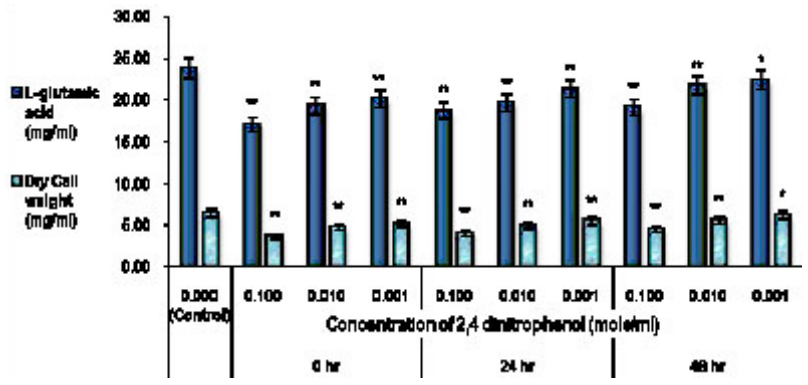
RESULTS AND DISCUSSION

The effects of different metabolic inhibitors namely 6-marcaptopurine, 2, 4 dinitrophenol, sodium arsenate, mercuric chloride, sodium arsenite, 2-thiouracil, sodium azide, malonic acid and sodium fluoride were examined on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} as depicted in Fig. 1 – 9 as follows :



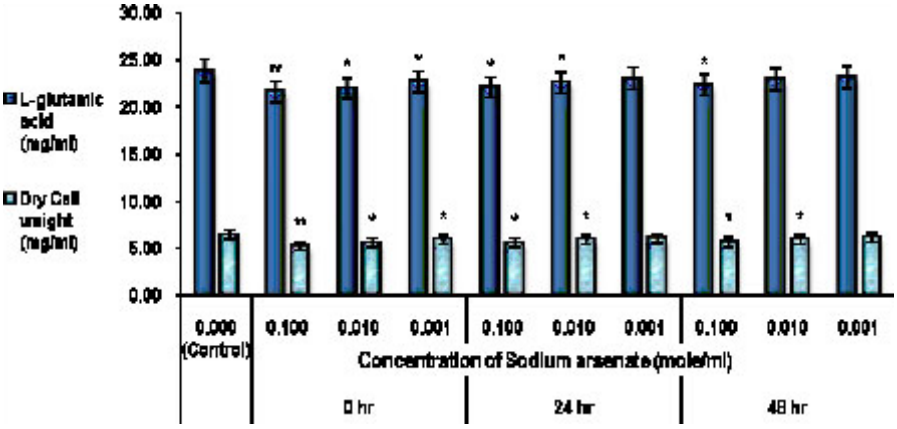
(Values were expressed as mean ± SEM, where n = 6; *p < 0.05, **p < 0.01 when compared to control)

Fig. 1. Effect of different concentrations of 6-marcaptopurine on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



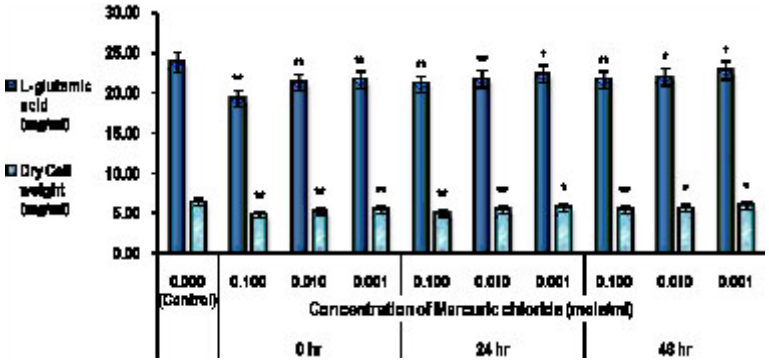
(Values were expressed as mean ± SEM, where n = 6; *p < 0.05, **p < 0.01 when compared to control)

Fig. 2. Effect of different concentrations of 2,4 dinitrophenol on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



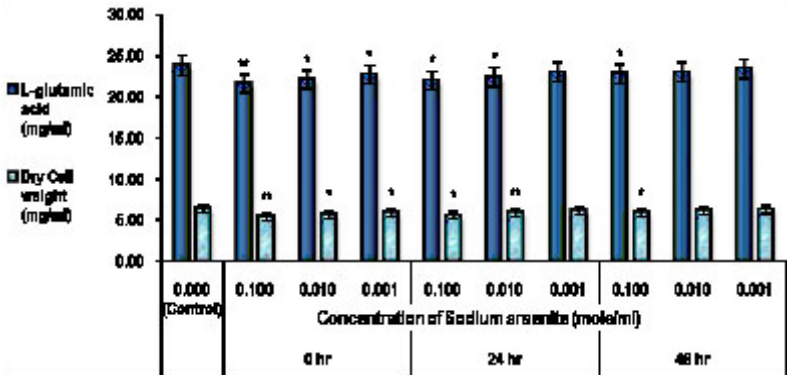
(Values were expressed as mean ± SEM, where n = 6; *p < 0.05, **p < 0.01 when compared to control)

Fig. 3. Effect of different concentrations of Sodium arsenate on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



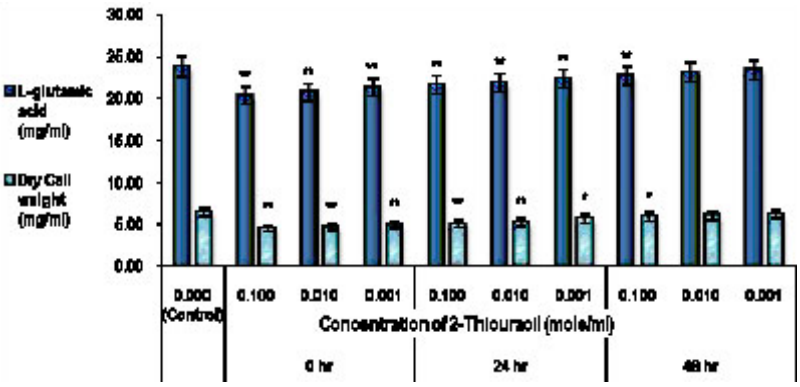
(Values were expressed as mean ± SEM, where n = 6; *p < 0.05, **p < 0.01 when compared to control)

Fig. 4. Effect of different concentrations of Marcuric chloride on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



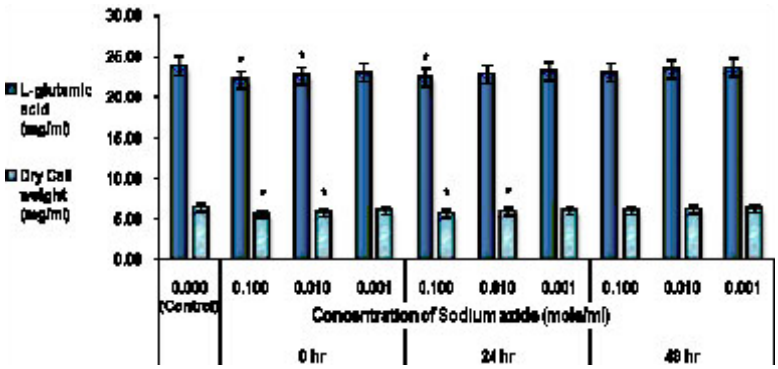
(Values were expressed as mean ± SEM, where n = 6; *p < 0.05, **p < 0.01 when compared to control)

Fig. 5. Effect of different concentrations of Sodium arsenite on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



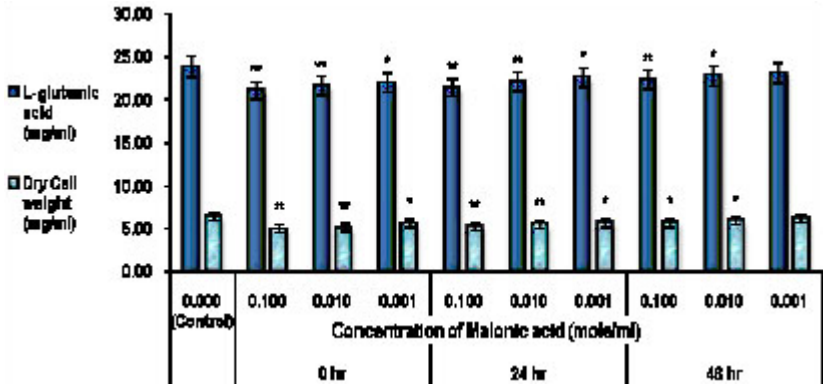
(Values were expressed as mean ± SEM, where n = 6; *p < 0.05, **p < 0.01 when compared to control)

Fig. 6. Effect of different concentrations of 2-Thiouracil on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



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

Fig. 7. Effect of different concentrations of Sodium azide on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals



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

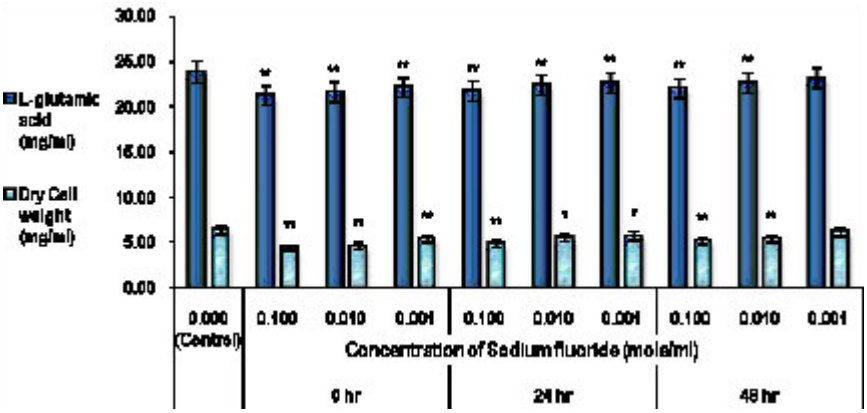
Fig. 8. Effect of different concentrations of Marcuric acid on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals

All these metabolic inhibitors showed either significantly negative impacts on growth and l-glutamic acid production ($p < 0.05$ and $p < 0.01$) or no significant effects.

6 – mercaptopurine being a thiopurine exerted its detrimental effects on cellular growth and l-glutamic acid production probably developing multiple cytotoxicities[8].

2, 4 dinitrophenol, is a cellular metabolic poison which uncouples oxidative phosphorylation, leading to rapid utilization of ATP without further generation of ATP, leading to impairement of cellular metabolism[9].

The toxic effect of sodium arsenate was recorded on cellular growth and l-glutamic acid production probably due to the fact that the



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

Fig. 9. Effect of different concentrations of Sodium fluoride on growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} added to the fermentation broth at different time intervals

arsenate can replace the inorganic phosphate in the step of glycolysis that generated 1, 3 bisphosphoglycerate, producing 1–arseno–3–phosphoglycerate. This compound is hydrolyzed immediately without generation of ATP, leading the cytotoxicity[10].

Mercuric chloride exerted its lethal effect on growth and l-glutamic acid production probably via blocking different degenerative metabolic pathways. However its exact mechanism of developing cytotoxicity is still not clear to us.

Due to multiple cytotoxic effects of sodium arsenite it might adversely affected the growth and l-glutamic acid production by the mutant *Micrococcus glutamicus* AB_{100} [10].

2-thiouracil may generate the toxic effects probably by enhancing the DNA stand breaking[12].

Sodium azide is a bacteriostatic compound which inhibits cytochrome oxidase in different Gram +ve and Gram –ve bacteria and thus may inhibit l-glutamic acid production[13].

L-glutamic acid production was inhibited by malonic acid which probably inhibited succinic dehydrogenase (Complex II) in the respiratory electron transport chain[14].

Similar to most soluble meterials, fluoride compounds like sodium fluoride are readily absorbed by different organisms and thereafter it binds calcium and interferes with the activities of various enzymes[15]. In the present study it might inhibit the bacterial growth and l-glutamic acid production by this method.

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