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DETERMINATION OF AUXOTROPHIC NATURE OF THE MUTANT *Micrococcus glutamicus* AB_{100}

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ABSTRACT

An experimental study was carried out to investigate the auxotrophic nature of the newly developed mutant *Micrococcus glutamicus* AB_{100}. To investigate the role of biotin on growth and l-glutamic acid production by this strain, yeast extract from the minimal salt medium was replaced by 0.2 µg/ml of biotin. Biotin (0.2 µg/ml) was proved to be an essential element for growth production was decreased significantly ($p<0.01$). But excess biotin created over growth, leading to anaerobic condition which could adversely affect l-glutamic acid production. Thus, in this present study, it was proved that this strain a biotin auxotroph.

KEY WORDS

Experimental, *Micrococcus glutamicus,* biotin, auxotroph.

INTRODUCTION

Interest in microbial fermentation of amino acids has increased greatly since development of large-scale microbial processes to make glutamic acid[1]. Though the large-scale manufacture of inexpensive amino acids could stimulate market development, much of the recent interest is due to the potential production of natural isomers at a cost permitting their use as dietary supplements[2].

A great variety of microorganisms has been isolated or induced for amino acid production. They are classified into wild strain, auxotrphic mutants and regulatory mutants[3]. Among auxotrophic mutants derived from regulatory mutants of *Micrococcus glutamicus*, strains required biotin for their growth and l-glutamic acid accumulation[5-9].

Our present study was intended to examine the auxotrophic nature of the newly developed high l-glutamic acid yielding strain *Micrococcus glutamicus* AB_{100}.

MATERIALS AND METHODS

Microorganism : *Micrococcus glutamicus* AB_{100} developed by induced mutation in our laboratory was used throughout this study[10].

Basalt salt medium for l-glutamic acid production : Basalt sale medium contained glucose, 10%; urea, 0.2%; K_2HPO_4, 0.1%; $MgSO_4.7H_2O$, 0.025%; yeast extract, 0.2%; pH 7.0.

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.

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[11].

Analysis of Amino acid : Descending paper chromatography was employed for detecting L-glutamic acid in culture medium and was run for 18h on a watman 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 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[12,13].

RESULTS AND DISCUSSION

Fig. 1 showed that production of L-glutamic acid was maximum when yeast extract in the basal salt medium was replaced by 0.2 µg/ml biotin. When biotin concentration was increased above 0.2 µg/ml, dry cell weight was increased significantly ($p<0.05$) but production of l-glutamic acid was decreased. But when both 0.1% yeast extract and biotin (0.2 µg/ml) were simultaneously added to the medium, the production of l-glutamic acid was decreased significantly ($p<0.05$) along with significant increase (0.01) or dry cell weight.

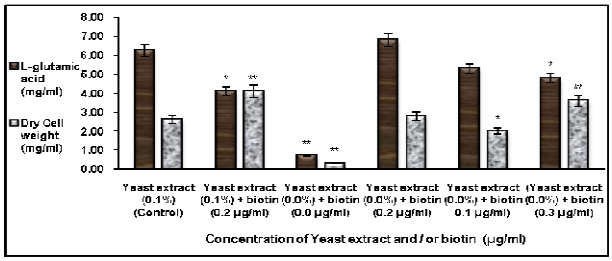

Fig. 1

Determination of auxotrophic nature of Micrococcus glutamicus AB_{100}. (Values were expressed as mean ± SEM; where n=6; *p<0.05, **p<0.01 when compared to control)

Kinoshita et al (1957) studied on l-glutamic acid production and reported similar pattern of result with *Micrococcus glutamicus*[4]. Shiio et al (1962) studied the effect of biotin on the bacterial formation of glutamic acid and reported that in presence of higher concentration of biotin, growth of the bacterial cells increased tremendously leading to creation of anaerobic state. Thus, the production of l-glutamic acid decreased gradually as it was an oxygen dependent process and accumulation of lactic acid occurred in the fermentation broth[7].

The trend of our present study also supported the above mentioned reviewers views. Yeast extract is a complex nutrient and may also contained biotin. Production or l-glutamic acid might be decreased due to presence of excess biotin when both yeast extract (0.1%) and biotin (0.2 µg/ml) were added in the broth. Thus, from the present study it was tentatively concluded that the mutant *Micrococcus glutamicus* AB_{100} was a biotin-auxotroph and its maximum production of l-glutamic acid can be obtained in presence of 0.2 µg/ml biotin.

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