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Effect of antibiotics on L-glutamic acid production by *Corynebacterium glutamicum* X680

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Abstract

L-glutamic acid is a proteinigenic, non-essential amino acid with immense industrial value[1]. Its fermentation in industrial scale was started in 1957[7]. Addition of antibiotics was found to be effective for its production improvement. In my previous investigation, I showed stimulatory effect of penicillin G on extracellular accumulation of L-glutamic acid by the mutant[5]. In connection of this study, the investigation was undertaken to examine the effect of some other antibiotics on extracellular accumulation of L-glutamic acid by the biotin auxotroph *Corynebacterium glutamicum* X680.

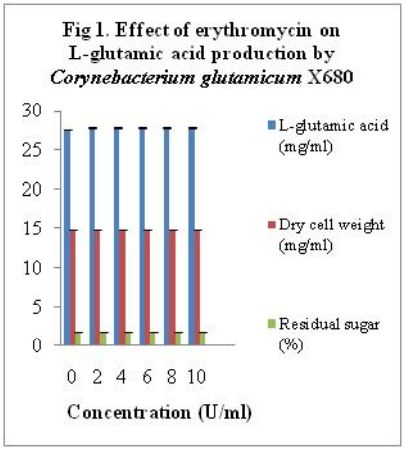
Microorganism: A biotin auxutrophic mutant*Corynebacterium glutamicum* X680 was used throughout the study[4].

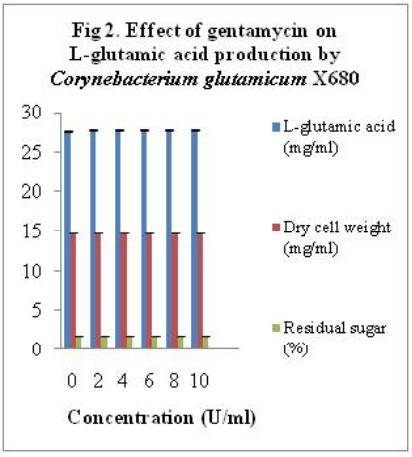
Composition of the growth medium: The bacterial growth medium was composed of (g%): glucose, 2%; peptone, 0.5%; yeast extract, 0.1%; beef extract, 0.3%; K_2HPO_4, 0.1%; KH_2PO_4, 0.1%; $MgSO_4.7H_2O$, 0.0025%; agar, 4% in double distilled deionized 1L water. The pH was neutrally adjusted[5].

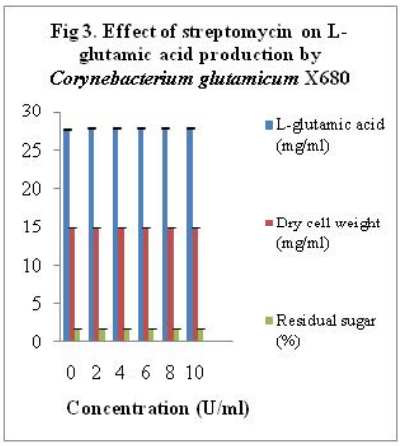
Culture conditions: The following-synthetic medium was recommended for improved and steady rate of L-glutamic acid production (27.6 mg/ml)with the free cells of *Corynebacterium glutamicum* X680 in submerged fermentation: pH, 7.0; period of incubation, 72h; volume of medium, 30ml; size of inoculum, 4%($8X10^6$ cells); age of inoculum, 48h; temperature, 30°C; shaker's speed (agitation), 150rpm; glucose, 12g%; urea, 1g%; calcium carbonate, 4g%; biotin, 3µg/ml; potassium dihydrogen phosphate, 0.3g%; dipotassium hydrogen phosphate, 0.3g%; magnesium sulphate, heptahydrate, 2mg%; zinc sulphate, heptahydrate, 10µg/ml ; ferrous sulphate, heptahydrate, 10µg/ml andbiotin, 3µg/ml[5].

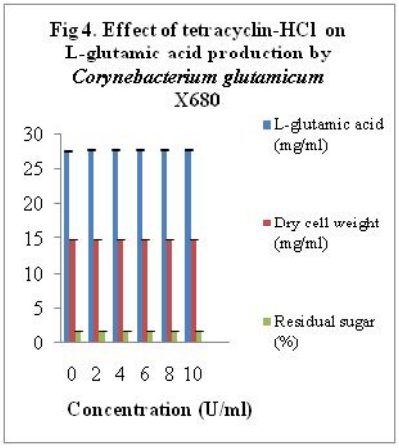
Estimation of dry cell weight: After centrifugation, 2 ml 1(N) HCl was poured into the precipitate of the bacterial cells to dissolve it. Calcium carbonate was added to neutralize it. The remaining cells were washed twice and dried at 100°C for 16h till the cell weight remained constant[9].

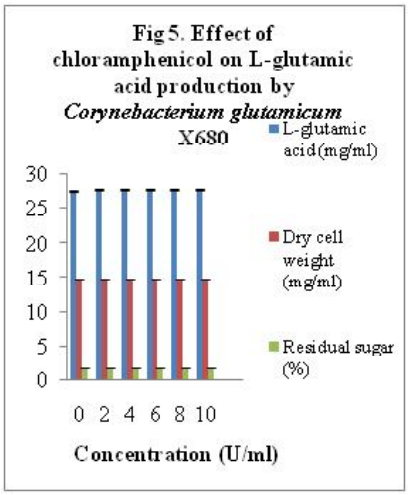
Estimation of L-glutamic acid : Descending paper chromatography was used for the detection of L-glutamic acid. Solvent system used was composed of n-butanol: aceticacid:water (2:1:1). The spots were











visualized by spraying 0.2% ninhydrin in acetone. The quantitative estimation was done by colorimetric estimation method[9].

Estimation of residual sugar: Residual sugar was estimated by DNS method[6].

Addition ofantibiotics: Erythromycin, gentamycin, streptomycin, tetracycline_HCl and chloramphenicol with varying concentrations (6U/ml) were added to the medium after 6h of incubation[5].

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 (Graph pad Inc., USA). A 'p' value less than 0.05 was considered significant and less than 0.01 as highly significant.

All the chemicals used are in AR grade. Borosil glass goods were used throughout the study.

The effects of different antibiotics (erythromycin, gentamycin, streptomycin, tetracycline-HCl and cholesterol) at a concentration of 6U/ml were added to the medium after 6h of incubation. However, none of the antibiotics showed significant stimulatory effect on L-glutamic acid production by the mutant *Corynebacterium glutamicum* X680 (Fig 1-5). Israilides *et al.*,[8] reported adverse effecst of chloramphenicol and novobiocin on L-lysine production by *Bacillussubtilis*[8]. Licomycin, neomycin and tetracycline_HCl stimulated L-lysine production by *Bacillus mehaterium*SP76 and SP14[3]. However, erythromycin stimulated both of these two strain in addition to *Bacillus mehaterium*SP 86. Dike *et al.*,[2] reported ampicillin and erythromycin do not affect L-methionine production in *Bacillus cereus* DS13. Tetracycline-HCl and chloramphenicol stimulated L-methionine production by both *Bacillus cereus* DS13 and *Bacillus cereus* AS9[2].

These experiments have clearly shown that unlike penicillin G, other antibiotics studied exhibited almost no effect on L-glutamic acid production by the biotin auxotroph *Corynebacterium glutamicum* X680.

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References :

1. Alharbi, N.S.,S. Kadaikunnan, J.M. Khaled, T.N. Almanaa, G.M. Innasimuthu, B. Rajoo, K.F. Alanzi and S.K. Rajaram (2020). *Journal of King SaudUniversity–Science*, *32*(2): 1403-1408.
2. Dike, K., I.A. Ekwealor and S. Eziuzor (2013). *Advances in Microbiology*, *3*(1): 26-31.
3. Ekwealor, I.A. and A.N. Obeta (2008). *African Journal of Biotechnology*, *7:* 1550-1553.
4. Ganguly, S. (2019). *Journal of the Indian Chemical Society*, *96*(6): 705-710.

5. Ganguly, S. (2020). *Indian Journal of Applied and Pure Biology, 35*(1): *In press*.
6. Garriga, M., M. Almaraz and A. Marchiaro (2017). *Actas de Ingeniería*, *3*(1): 173-179.
7. Ikeda, M. and R. Katsumata (1999). *Applied Environmental Microbiology*, *65*(6): 2497-2502.
8. Israilides, C.J., A.N.C. Weir and A.T. Bull (1989). *Agricultural and Biological Chemistry, 28*(2): 120-124.
9. Zamir, A. and N.A. Lichtenstein (1955). *Analytica Chemica Acta, 12:* 577-579.