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# Biochemical changes in the fermentation broth during L-glutamic acid production by a mutant *Corynebacterium glutamicum* X680

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**Abstract**

The present study was undertaken to investigate the biochemical changes in the fermentation broth during submerged fermentation of L-glutamic acid using a biotin auxotroph *Corynebacterium glutamicum X680*. Dry cell weight increased with the concomitant rise of L-glutamic acid production along with the accumulation of α-ketoglutarate, pyruvate and succinate in the fermentation broth. residual sugar content decreased gradually. But the production decreased after 72h of incubation period. The pH of the medium decreased gradually with time due to accumulation of metabolic acids. The amino nitrogen increased up to 72h of incubation as an indicator of the product (L-glutamic acid) formation. Cell nitrogen and ammonical nitrogen also increased gradually throughout the fermentation period. This study focused on the pattern of byproduct formation and overall metabolic status of the microorganism during L-glutamic acid production.

L-glutamic acid fermentation was successfully industrialized through the discovery of *Corynebacterium glutamicum*[14]. Since then scientists urged to find out the metabolic aspects of L-glutamic acid production by the microorganism especially from the stand point of glucose and nitrogen utilization in various forms, pH changes in the production medium as well as metabolic byproducts formation[2,4,8,15]. Most of the literature revealed that glucose and urea or ammonium salts serve as carbon and nitrogen sources for L-glutamic acid production. Several acidic metabolic byproducts also formed during the course of L-glutamic acid production in microorganism[3]. Furthermore, transaminases enzyme plays pivotal role in microbial fermentation of L-glutamic acid[8].

Considering the literature survey, the present study was undertaken to assess the biochemical changes in the fermentation broth during L-glutamic acid production by the mutant *Corynebacterium glutamicum* X680

emphasizing on residual sugar changes, nitrogen changes, alterations of pH and transaminase activity.

*Microorganism:*

*Corynebacterium glutamicum* X680 developed from *Corynebacterium glutamicum* 60 by induced mutation was used throughout the study[6].

*Culture conditions and the composition of synthetic medium:* The following synthetic medium was selected for the present study: 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.

*Estimation of total nitrogen:* Total nitrogen was measured by the micro-Kjeldahl method as described by Allen[1].

*Estimation of ammonical nitrogen:* Ammonical nitrogen was estimated by micro-Kjeldahl method as described by Allen[1].

*Estimation of α-ketoglutarate and pyruvate:* To estimate α-ketoglutarate and pyruvate, the colorimetric method of Fridekinand Haugen[5].

*Estimation of succinate:* Manometric method was applied to determine succinate using succinate dehydrogenase[7].

*Estimation of dry cell weight (DCW):* The cell paste was obtained from the fermentation broth by centrifugation and dried at 100°C until constant cell weight was obtained[13].

*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[13].

*Estimation of residual sugar:* Residual sugar was determined by the DNS method as proposed by Miller[11].

*Estimation of pH*: pH of the broth was estimated using pH meter (model: MXCL20X1).

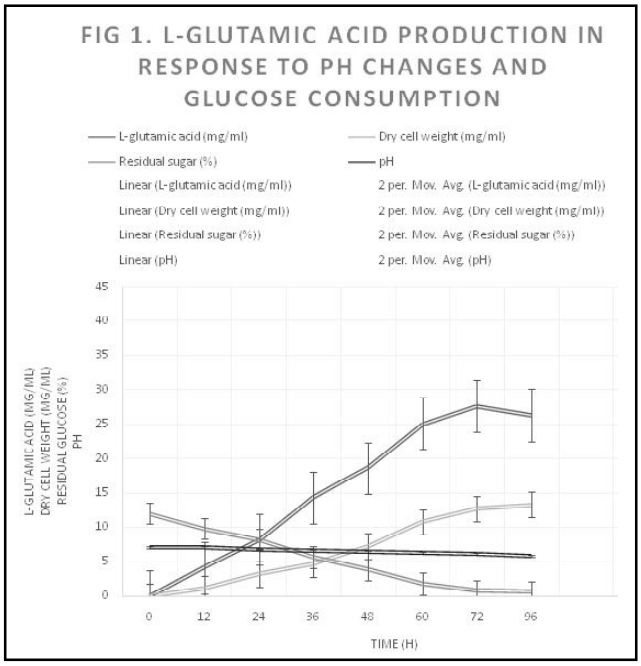
*Extraction and assay of transaminase activity*: Freshly cultured (24h) broth was centrifuged at 10,000rpm at 4°C for 10min. The pellet was washed thrice with deionized double distilled water. The pellet was then suspended in 100ml of 0.2(M) phosphate buffer (pH 7.5) followed by disruption using sonication from an ultrasonic generator DXM-68 at 4°C for 20min. Crude enzyme extract was collected by centrifugation at 10,000rpm for 20min at 4°C. The extract was further centrifuged at 40,000rpm for 30min at 4°C. The clear supernatant was used for enzyme assay. Transaminase activity was measured in terms of product (L-glutamic acid) formation. The reaction was carried out at 30°C for 4h. The effect of pH and temperature on transaminase activity was also assayed to optimize it[10].

*Statistical analysis:* All the data were presented as mean ± SEM. Data were analyzed using one way ANOVA followed by Dunett's post hoc multiple comparison test using a software Prism 4.0, considering *p<0.05 as significant and **p<0.01 as highly significant.

*Corynebacterium glutamicum* coluld able to produce organic acids such as succinic acid, acetic acid etc as by products during L-glutamic acid production in the fermentation with gradual decline in pH[3]. Under aerobic state, succinic acid is produced via carboxylation of phosphoenolpyruvate or oxaloacetate followed by reduction[9]. However, Okino *et al.*[12] observed succinic acid production by this strain without accumulation of lactate[12].

*Carbon and nitrogen utilization and pH changes:* Submerged fermentation of L-glutamic acid was initiated with glucose, 12% and pH, 7.0. However, from Fig 1, it is evident that, with the progress of the fermentation process, residual glucose concentration decreased gradually in the fermentation broth along with the rise of L-glutamic acid production and cell mass. Decrease in pH was likely to be due to accumulation of L-glutamic acid and other metabolic acids.

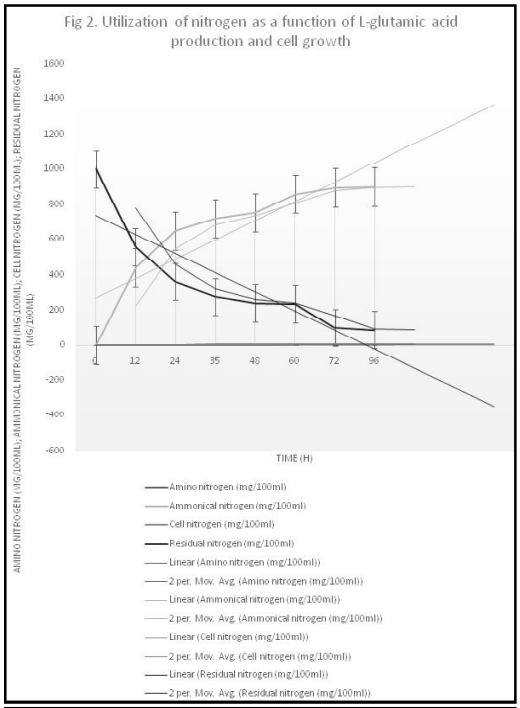
FIG 1. L-GLUTAMIC ACID PRODUCTION IN RESPONSE TO PH CHANGES AND GLUCOSE CONSUMPTION

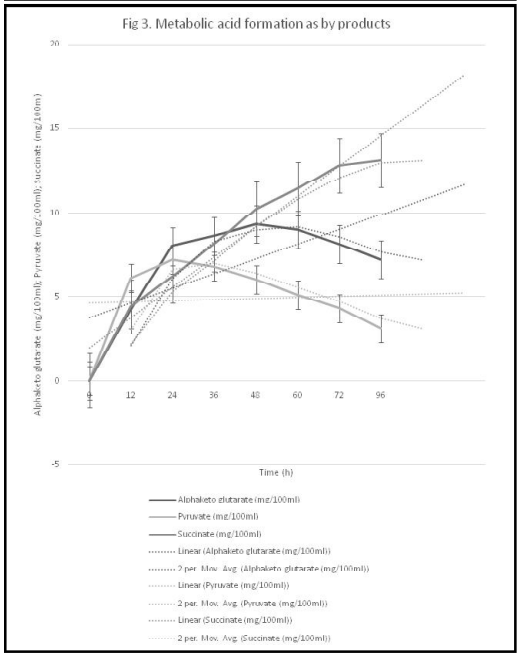



Though theproduction of L-glutamic acid increased up to 72h of incubation, and then decreased gradually, but the total cell mass increased continuously. Decrease in L-glutamic acid in the broth was not only due to entry of the cells into death phase, but might be due to reuptake by the cells.

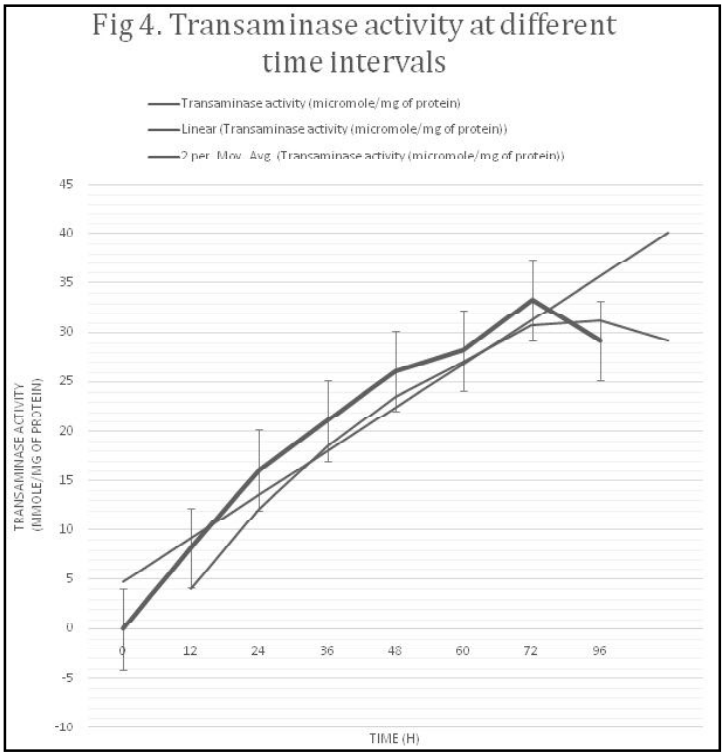
*Nitrogen utilization:* Fig 2 and 3 depicted the pattern of changes in different forms of nitrogen contents as well as α-ketoglutarate, puruvate and succinate in the

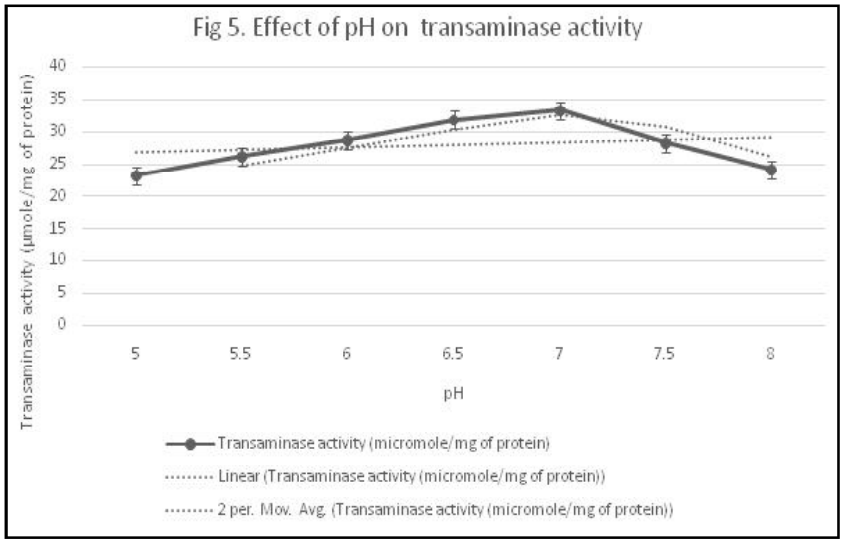
broth with the progress of the fermentation. Though the amino nitrogen content raised upto 72h of incubation, it indicated the optimum production of L-glutamic acid.

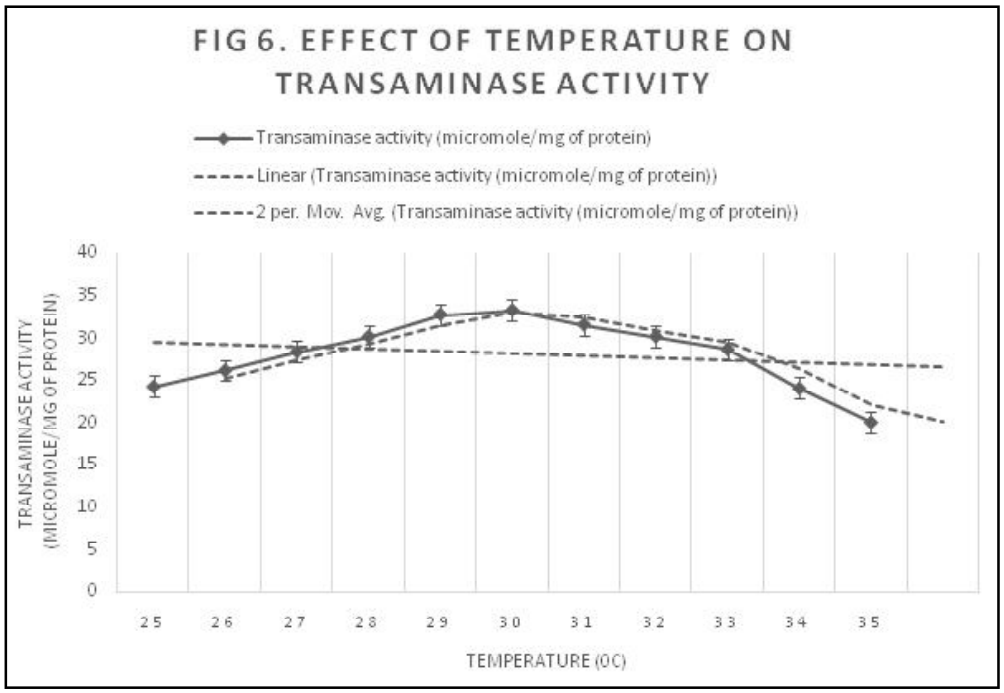


**Transaminase activity during L-glutamic acid fermentation:** Transaminase activity increased uo to 72h of incubation and then declined (Fig 4). Maximum activity was obtained with pH, 7.0 and temperature, 30ºC (Fig 5 and 6).






Maximum L-glutamic acid production was obtained with highest transaminase activity with pH 7.0 and temperature, 30°C. Ammino nitrogen level rose as the production of L-glutamic acid increased and then declined gradually. Ammonical nitrogen and cell nitrogen levels increased as the liberation of ammonia and cell mass rose gradually with sharp decline of residual sugar level and increase in pH due to accumulation of metabolic acids.

This study reveals that during the course of L-glutamic acid fermentation, *Corynebacteriumglutamicum* X680 several secondary metabolic acids (like pyruvate, alpha ketoglutarateetc) were accumulated during the course of fermentation led to sharp decline of pH in the medium and rapid utilization of sugar. Transaminase activity was showed maximum with pH 7.0 at $30^0$C.

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*Conflict of interest :*

The authors clearly declare there is no conflict of interest.

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