E2-01: Improvement of citric acid production from *Aspergillus sp* strain UV_1 using methanol

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Bioconversion of sugars to citric acid could be enhanced by using *Aspergillus sp* mutants and incorporating specific simulators into the culture medium. Such simulators reported for citric acid production are methanol, quaternary ammonium compounds, oximes and fluor acetate. It is suggested that citric acid production is stimulated by removing heavy metal ions present as contaminants in the medium. Further addition of organic solvents such as lower alcohols has been reported for improving the membrane permeability and thus transport of the metabolite is made easy. This study was carried out to find the role of methanol on citric acid production.

Locally isolated and UV mutated *Aspergillus sp* strain UV_1 was grown in basic medium. Basic medium contained (gl^{-1}) glucose, 50; NH_4NO_3, 0.5; $KH_2PO_4.7H_20$, 0.5; $MgSO_4.7H_2O$, 0.1; peptone 7.0; $ZnSO_4.7H_2O$, 0.1 x 10^{-3}; ferrous ammonium sulphate, 0.1 x 10^{-3} and $CuSO_4.5H_2O$, 0.06 x 10^{-3}.

Basic medium was supplemented with varying concentrations of methanol (0, 20, 30 and 40 mll^{-1}). To each test, respective controls were prepared by taking additional glucose (0, 18.18, 27.28 and 36.38 gl^{-1}) instead of methanol to equalize the total carbon content. Spores (6 day old) in suspension (7 x 10^6 spore ml^{-1}) were inoculated and incubated at 30°C under diffused light. Citric acid and reducing sugar in the media were monitored.

Basic medium (medium A), basic medium containing optimized amount of methanol (medium B), basic medium without glucose containing methanol equivalent to the elemental carbon content contributed by glucose and methanol of medium B (medium C) and basic medium without glucose containing methanol equivalent to total carbon content of medium A (medium D) were prepared. The experiment was proceeded as above. In addition intracellular citric acid was monitored.

Basic medium containing 3 different concentrations of glucose (50, 100 and 150 gl^{-1}) were prepared with (Test) and without (Control) the addition of optimized amount of methanol. The experiment was continued as above.

In basic medium the fungus produced 4.6gl^{-1} extracelluar citric acid at 96th hour. Citric acid production increased to 7.8gl^{-1} with an increase in methanol concentration to 30ml^{-1} and further increase in methanol concentration did not improve citric acid production. Citric acid produced in the media containing glucose equivalent to different concentrations of methanol in basic medium was very much less than that produced in basic medium and in the respective test media.

To find whether methanol was used as a carbon source, medium C was prepared (85ml^{-1} methanol). In medium C no citric acid production and surface film formation were observed with no spore germination. In medium D the same observations were made. Extracellular and intracellular citric acid produced in medium B were more than in medium A while the percentage of citric acid secreted into the medium in both media A and B was almost same (96.0 and 95.3% respectively).

More citric acid production was observed in tests than in controls. In tests containing 50, 100 and 150gl^{-1} glucose with 30ml^{-1}, 7.6, 12.1 and 12.1 gl^{-1} extracelluar citric acid was respectively produced. Intracellular citric acid in tests increased with an increase in the concentration of glucose upto 100 gl^{-1} and further increase in glucose decreased the intracellular citric acid. In controls the intracellular citric acid concentration decreased with concomitant increase in glucose concentration from 50 to 150 gl^{-1}. No significant difference in the percentage of citric acid excreted in the presence and absence of methanol was observed.

Citric acid produced by *Aspergillus* sp strain UV_1 was increased from 4.6 to 7.8 gl^{-1} by methanol supplementation. Methanol alone in the medium has inhibited the germination of the spores and it was not used as a carbon source for citric acid production. From the experiment it was evident that methanol has not improved the membrane permeability for the acid and it has stimulatory effect on citric acid production.