Role of Complex Nutrients in Alteration of Aconitase Activity during Citric Acid Fermentation by *Aspergillus niger*

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ABSTRACT

A mutant strain, *Aspergillus niger* AB_{1801} showed decrease and then disappearance of aconitase activity during citric acid fermentation when supplemented with peddy soak liquor, corn steep liquor and peptone. Extracts of malt, wheat and rice bran, defatted soybean and beef showed increase in enzyme activity but decreased acid production. Cell mass did not change appreciably.

INTRODUCTION

The use of byproducts of food production processes for citric acid production enhance the economy of the main production. Defatted rape seed or rice liquor slopes or potato pulp or corn steep liquor or maize starch or cerelose have been used [1-5]. Other complex nutrients, viz. wheat bran, wheat flour, malt extract, date seeds, cheese whey, barnidate coat sugar, sugar cane, banana extract, apple pomace, grape pomace, mandarin orange waste or even cotton waste have been exploited for citric acid production since three decades [6-12].

Besides serving as carbon and nitrogen source, complex nutrients contain a wide range of amino acids, vitamins and growth factors apart from the characteristics stimulator or inhibitor in them. In the present work the effect a host of complex nutrients have been probed into.

MATERIAL AND METHODS

Microorganism : The present strain *Aspergillus niger* AB_{910} isolated from North Bengal Soil was exposed to UV rays and then ethyleneimine, to obtain the maximum citric acid producing mutant, *Aspergillus niger* AB_{1801} [13].

Preparation of inoculum : The spores were harvested in czapex Dox medium, suspensions were prepared in to a concentration of 2.6×10^7 spores / ml. From this, 5 ml was used as inoculum [13].

Fermentation medium and cultural conditions : 150 ml chemically defined medium (pH 3.0) was composed of sucrose, 10%; urea, 0.2%; KH_2PO_4, 0.15%; $MgSO_4.7H_2O$, 0.01%; Ni^{2+}(10µg/ml) was added before inoculatin along with extracts of complex nutrients. Surface culture fermentations were then carried out in 1L bottle for 8 days at 28°-30°C [14].

Preparation of the complex nutrients : The different items were separately washed, incubated at 55°C for different periods, filtered, sterilized and stored at 4°C. Solid contents were calculated [15]. Corn was incubated with 0.52% potassium-bisulfite[16].

Preparation of enzyme extracts : The mycelia mat was neutralized with 1(M) KOH ground in borate buffer, centrifuged at 500g and then at 21, 600g at 4°C. The supernatant was treated with 1% protamine sulfate, stirred on magnetic stirrer before centrifuging again at 4,600g at 4°C. This supernatant was used for aconitase assay. All steps of experiment were carried out on ice[17].

Aconitase assay : The assay was carried out in HATACHI U2000 double beam spectrometer with the temperature control unit set at 30°C. Out unit of enzyme activity was defined as an initial rate of increase in extraction of 0.001/min/ml enzyme extract. The activity of the enzyme was determined by measuring the rate of appearance of cis-aconitate at 240mm from Na-citrate [18].

Citric acid assay : The assay was done from the broth and mycelia colorimetrically at 420mm using acetic anhydride and pyridine [19].

Estimation of dry cell weight : Dry cell weight was done by the method as described by shah et al (2002) [20].

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 a prism 4.0" software (Graph pad Inc., USA). A "p" value less

than 0.05 was considered significant and less than 0.01 as a highly significant.

RESULTS AND DISCUSSION

From fig. 1, it is evident that corn steep liquor at 0.01%-0.02% (solid content, 5.1%) concentration, increases citric acid production by 25%. Cell mass was fairly steady. No aconitase activity was observed then. Fig. 2 shows 12.5% increase in fermentation with 0.2% paddy soak liquor (solid content, 1.4%). Aconitase activity was absent then. Fig. 3 depicts that peptone at 0.03% - 0.5% concentration enhanced citric acid production. Enzyme activity and cell weight varied as before. Besides, the effect of extracts of malt, beef, defatted soybean, rice and effect of extracts of malt, beef, defatted soybean, rice and wheat bran were also performed, but no stimulatory effect was obtained. Usually, in such fermentations, the complex nutrients serve as carbon source to enhance the economic efficiency, but presently sucrose has been added and the effect of other factors in the nutrient has been assessed. All the nutrients contain mixtures of different amino acids, vitamins and growth factors. Yet not all complex nutrients are conductive to fermentation [21,22].

This may be attributed to the fact that *A. niger* AB_{1801} is auxoheterotroplric for the specific amino acids and vitamins present in peptone or paddy soak liquor or corn steep liquor [23]. Mixtures of definite amino acids is generally reported to be superior to any individual amino acid probably favourable combinations are present in these nutrients [24]. The growth factors in them may accelerate the process. Grover (1964) recorded substantial increase in hyphal output of *A. flavum* on a mixture of amino acids [25]. Experiments with different amino acids and vitamins revealed substantial output with dl-valine, l-serine, l-histidine, l-lysine (yet unpublished). But amino acids and vitamins other than these are also present in corn steep liquor, paddy or peptone [25]. Again, these are also present in other complex nutrients which failed to show positive results. Over and above all, these components are permeable and easily utilizable by *A. niger* AB1801 [27]. It may also by that sudden substrate dilution induces a higher rate of production [28].

Malt extract was absolutely inhibitory to fermentation, where as wheat bran extract was unsuitable at higher concentrations. Rice bran extract was defatted soybean meal gradually antagonized fermentation at higher concentrations. Probably there is some ingredient in these complex mixtures. Which directly inhibits fermentation or indirectly inhibits utilization of some major components in the media [29].

Aconitase activity varies inversely with citric acid production. Aconitase is responsible for furthering the steps of citric acid cycle, using it as the substrate. The lower its substrate availability is decreased in the mitochondria, when its accumulation in the organellae exceeds a critical level [30]. Aconitase here probably shows end product allosteric inhibition [31].

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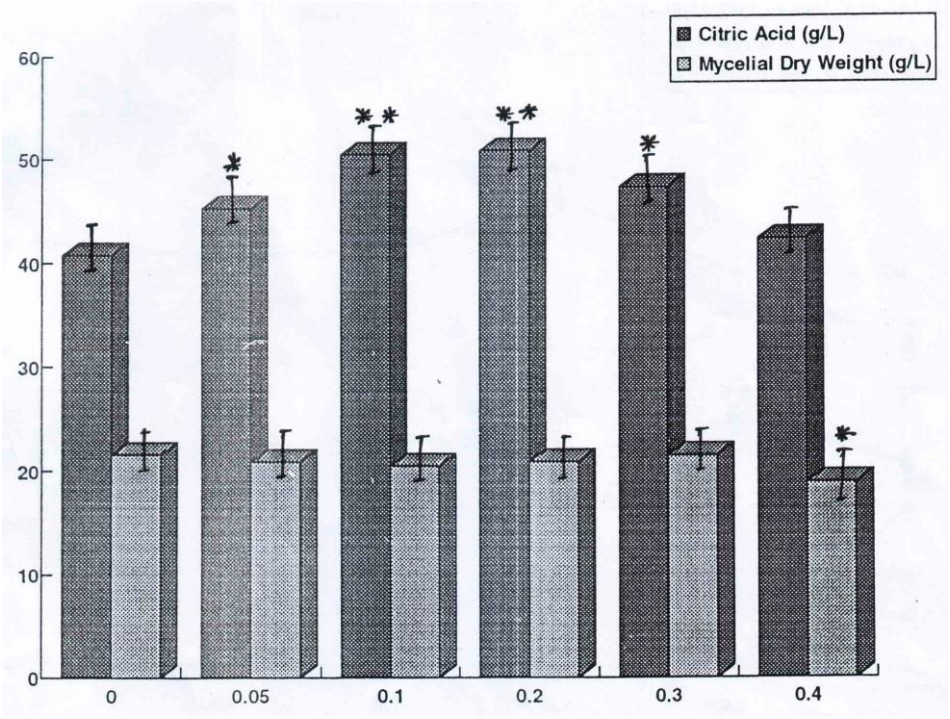

Fig. 1. Effect of varying cornsteep liquor concentration on citric acid fermentation by *Aspergillus niger* AB_{1801}.

(Values were expressed as mean ± SEM; where n-6; "O" stands for control. *p <0.05, **p < 0.01 when compared to control).

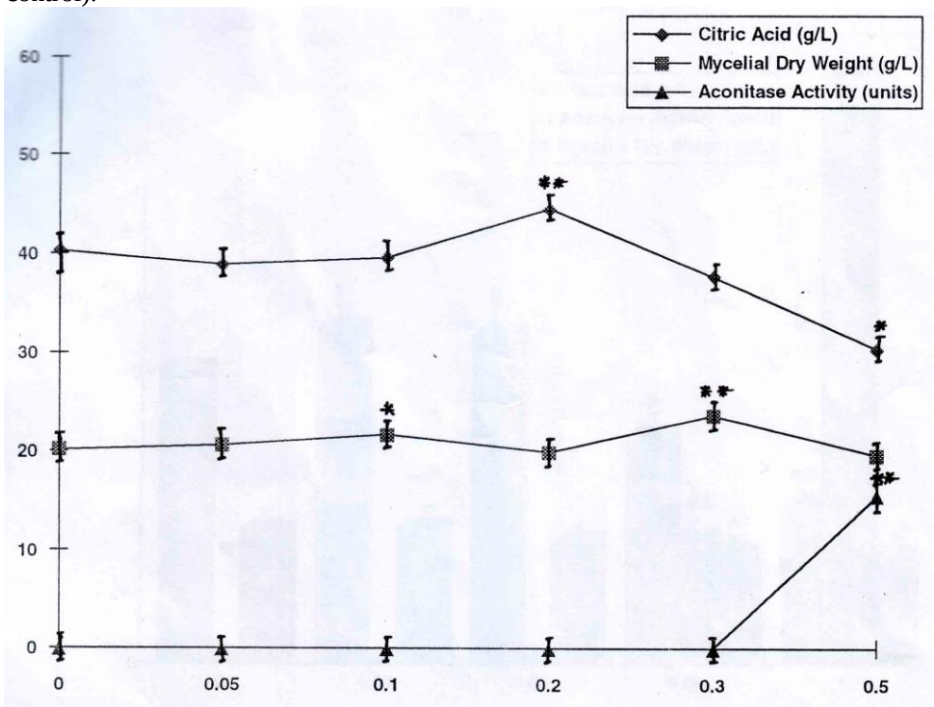

Fig. 2. Effect of varying concentration of paddy soak liquor on citric acid fermentation by *Aspergillus niger* AB_{1801}.

(Values were expressed as mean ± SEM; where n-6; "O" stands for control. *p <0.05, **p < 0.01 when compared to control).

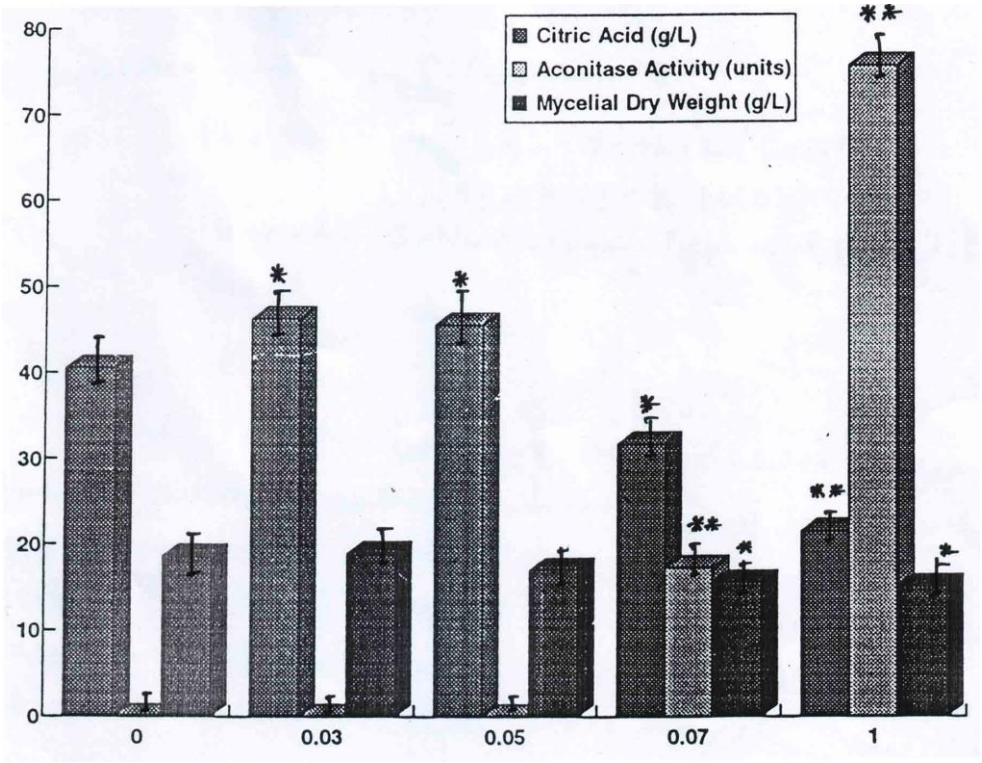

Fig. 3. Effect of varying peptone concentration on citric acid fermentation by *Aspergillus niger* AB_{1801}.
(Values were expressed as mean ± SEM; where n-6; "O" stands for control. *p <0.05, **p < 0.01 when compared to control).