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# Studies on The Isolation and Screening of Indigenous Citric Acid Producing Strains

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Sixty three strains of *Aspergillus niger* were isolated and investigated for citric acid production. Three strains S-43, S-44 and S-63 were found to produce citric acid with approximately 40% yield on the basis of glucose or sucrose supplied. Media and carbohydrate concentrations were varied to optimize the citric acid production.

***Key words:*** Citric acid, *Aspergillus niger,* Mutation.

## Introduction

Citric acid is a versatile organic acid of commercial importance. Recently developing countries are interested in establishing facilities for citric acid production within their boundaries. Commercially it is manufactured by fermentation of carbohydrates (David *et al* 1952). Media composition plays a vital role in its production in high yield (Jim and Zhang 1987). The most suitable substrates have been proved to be various types of molasses (Wang 1998) which are carbohydrate rich by-products of the sugar industry. A highly active organism is a pre-requisite for obtaining good yields. In this regards some specialized strain of *Aspergillus niger* is reported to be of industrial importance. Commercial strains for fermentation technology are guarded by secret (Jin and Peng 1997). It is, therefore, imperative to adapt indigenous laboratory strains either through growing in a given environment or by mutation (Gardner *et al* 1956) through chemical or physical treatment. Fermentation can be carried out by both surface and submerged processes. For developing countries surface process has better prospects (Wolfgang and Reiner 1976). Earlier studies (unpublished results) indicated that the citric acid is extremely sensitive to trace metals which was also reported in the literature (Vasilev and Kristova 1987). Biochemistry of citric acid production (Jim 1987) plays a significant role. Hence, it was considered worthwhile to isolate and screen the indigenous strains of *Aspergillus niger*. In the present studies those strains were selected which showed good yields of citric acid in the laboratory conditions. In future, mutation and modified cultural conditions will be searched out for high yield producers.

## Materials and Methods

*Microbial strains.* Several fungi were isolated from air and soil of different localities of Karachi. These isolates were purified and identified. From these isolates, 63 strains of *Aspergillus niger* were tested for citric acid production. Ten strains showed noticeable quantities of citric acid. These observations were made on medium No.1 (Table 1). The selected 10 strains were also tried on media No 2 and 3 (Table 1). Of these ten strains, three were more viable (S-43, S-44, S-63) for citric acid production (Table 2).

*Media and growth conditions.* All the isolates were grown on Czapek-Dox medium and also maintained on the same medium slants.

The constituents of media (1,2,3) were dissolved in distilled water, made upto 1 litre, pH was adjusted to 2.0 with the help of 1:1 HCl. 200 ml of each medium was distributed in 1 litre conical flasks and autoclaved at 15lb pressure for 20 min. The *Aspergillus niger* strains were inoculated on the above mentioned media and incubated at 28°C for 10 days. The flasks were then worked up and the broths were tested for citric acid formation by gravimetric analysis (Snell and Ettre 1970) pH of broth at the time of work up was between 3 and 4.

Media 1,2 and 3 were varied for their carbohydrate sources taking four percentages. The results are illustrated in Fig 1-3.

*Analytical methods.* For nalysis, method of Snell and Ettre (1970) was followed. An aliquot, 10ml of broth was taken in a 250 ml glass stoppered conical flasks and diluted to 100ml with distilled water. To 19ml $H_2SO_4$ (1:1) 10ml KBr (40%) was added. It was warmed to 55°C and 5% $KMnO_4$ solution was added portionwise with occasional shaking till $MnO_2$ ppt appeared, the flask left for 10 min. A solution of 250g $FeSO_4.7H_2O$ and 10ml $H_2SO_4$ was prepared and made upto 1 litre. 50ml of this $FeSO_4$ solution was added to the reaction flask washing down the walls of the flask and was left overnight in the refrigerator. The next day the solution was filtered through sintered glass funnel. The volume of filtrate was noted (s),

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washed with ice cold distilled water (50ml). The residue on sintered funnel was dried in dessicator over sulfuric acid and weighed for pentabromo acetone (p). The citric acid x, in g 100 $ml^{-1}$ of media was calculated through the following formula:

$$x = 0.445P + 0.018S$$

where x = of citric acid in aliquot (mg)
P = wt. of pentabromo acetone (mg)
S = volume of filtrate (ml)

## Results and Discussion

Locally isolated strains are more liable to achieve optimization in the indigenous environment. Out of the 63 strains tested for citric acid production only ten showed higher ability of producing citric acid. These 10 strains were also examined on media 2 and 3 when three strains out of ten,were found to be more potent. These were subjected to optimization by varying % of carbohydrate source (Fig 1-3). It is established that variation in constituents highly affects the yields of metabolites (Singh *et al* 1998.) and addition of certain chemicals also enhances the yields (Qadeer *et al* 1968). The highest yield (7.4%) was observed in medium I by strain S-44 with 15% glucose. The yields were calculated on the basis of the filtered broth at the time of work up which was 60% of the original volume of the broth taken.

The yields of citric acid when calculated on the basis of the available carbohydrate came to be 40% which is half of the

**Table 1**
Concentration of nutrients in three media

| Sr. No. | Nutrients ($gl^{-1}$) | Medium-I | Medium-II | Medium-III |
|---|---|---|---|---|
| 1. | Carbohydrate | 150 (Glucose) | 125 (Sucrose) | 140 (Glucose) |
| 2. | $NH_4NO_3$ | 2.23 | 2.00 | 1.00 |
| 3. | $KH_2PO_4$ | 1.00 | 0.75 | -- |
| 4. | $MgSO_4.7H_2O$ | 0.50 | 0.20 | 0.23 |
| 5. | $ZnSO_4.7H_2O$ | 0.005 | -- | -- |
| 6. | $FeSO_4.7H_2O$ | 0.0023 | 0.001 | 0.001 |

**Table 2**
Citric acid production by *A. niger* strains

| Sr. No. | *Aspergillus niger* strain isolates | Citric acid produced (g $100ml^{-1}$) | | |
|---|---|---|---|---|
| | | Medium-I | Medium-II | Medium-III |
| 1. | 43 | 2.4 | 3.0 | 4.4 |
| 2. | 44 | 2.5 | 4.1 | 0.8 |
| 3. | 55 | 2.0 | 1.3 | 1.4 |
| 4. | 56 | 2.6 | 1.0 | 3.1 |
| 5. | 58 | 2.0 | 3.4 | 2.0 |
| 6. | 59 | 3.4 | 1.7 | 2.3 |
| 7. | 60 | 3.5 | 2.7 | 2.5 |
| 8. | 61 | 3.0 | 3.1 | 2.6 |
| 9. | 62 | 2.9 | 1.1 | 0.7 |
| 10. | 63 | 3.4 | 2.2 | 4.7 |

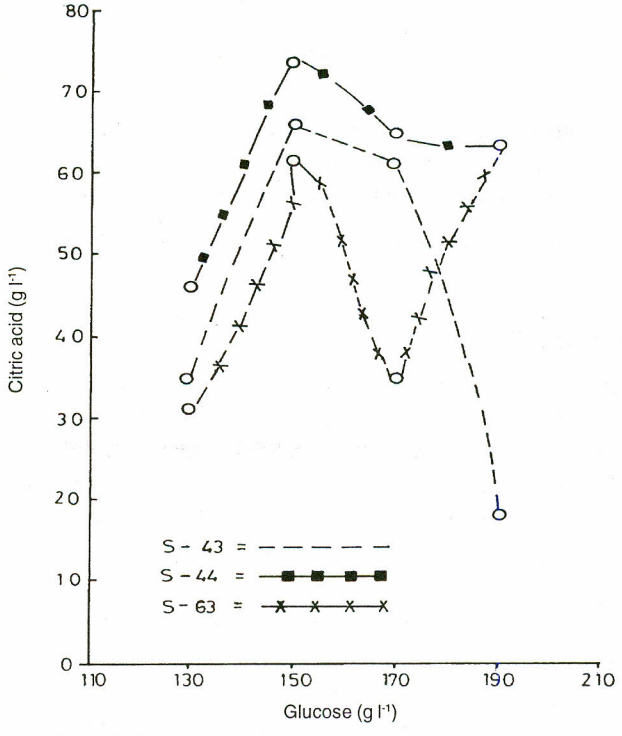


**Fig 1.** Effect of glucose concentration on citric acid production by *Aspergillus niger* in Medium-I.

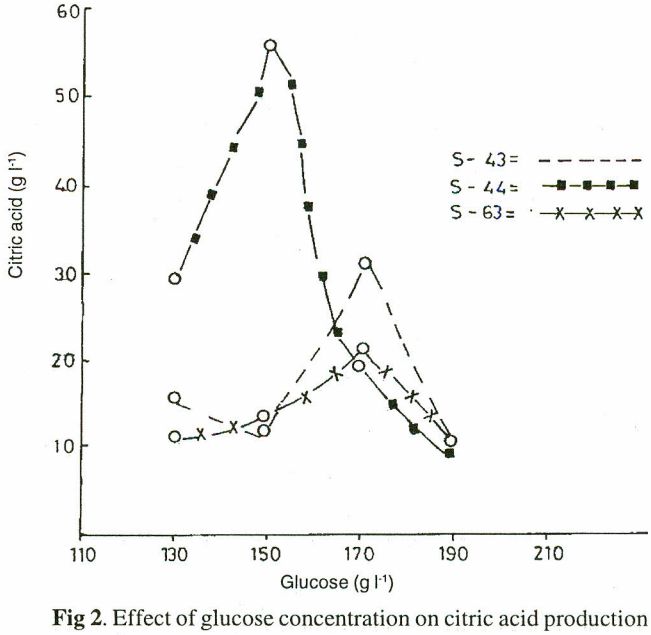


**Fig 2.** Effect of glucose concentration on citric acid production by *Aspergillus niger* in Medium-II.

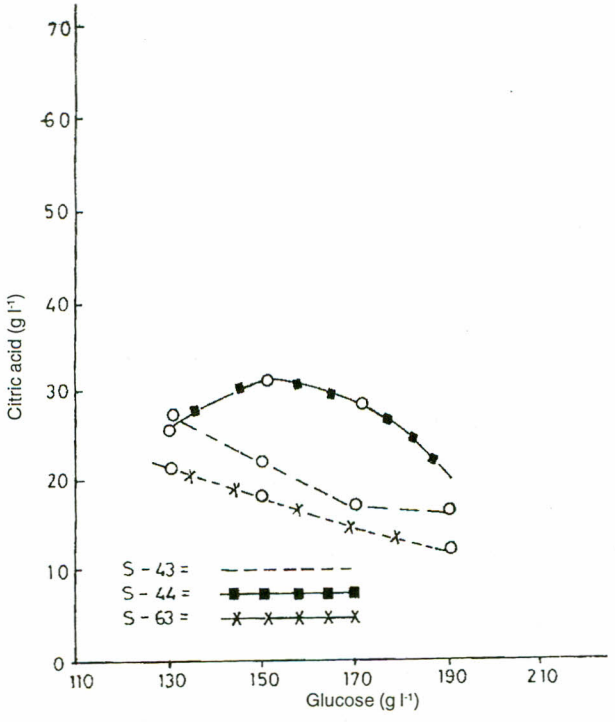


**Fig 3.** Effect of glucose concentration on citric acid production by *Aspergillus niger* in Medium-III.

desired yield for commercially viable strain. Gravimetric method for analysis was used for citric acid determination in culture media because other acids do not interfere. After 10 days incubation the sugar consumption did not increase the yield of citric acid.

In the present study, the results of screening of indigenous strains were quite encouraging. Now need was felt to optimize the yield by repeated subculturing and mutation (Gardner *et al* 1956) and using cheaper raw materials. Molasses is available locally as a by-product of sugar industry. The mycelium which is obtained during worked up after fermentation is non toxic and has a high content of protein (Wolfgang and Reiner 1976). It has been reported that after pressing and drying it makes an excellent animal feed supplement and is worth recovery as a by-product.

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