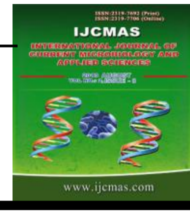

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Original Research Article

Production of Citric acid by Mutants of *Aspergillus niger* in Solid State Fermentation

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Keywords

Aspergillus niger,
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A B S T R A C T

Development of methodology for the recovery of citric acid from the fermented broth involves standardization of the various parameters to achieve maximum performance in terms of recovery and optimum conditions. In this study, experimental strains were designed using the UV irradiation to understand the efficiency of citric acid production using sesamum oil cake and rice chaff. The irradiation was done at various distances from 6 to 20 cm. The best irradiated mutants were obtained at the distance of 9 cm and these were selected for the mutational studies. The mutant strains isolated were designated as *A. niger* ETGP12-m1 and ETGP18-m2.

Introduction

Development of methodology for the recovery of citric acid from the fermented broth involves standardization of the various parameters to achieve maximum performance in terms of recovery and optimum conditions. The present investigation was carried out to study the purification of citric acid from fermented broth by calcium carbonate precipitation and subsequent acid hydrolysis. The parameters affecting the recovery were identified and empirical equation proposed to predict the recovery by precipitation method. Citric acid is the principal organic acid found in citrus fruits. To meet increasing demands it is produced from carbohydrate feedstock by fermentation with the fungus *Aspergillus niger* and the yeasts of *Candida* sp.

Effect of various fermentation conditions and the biochemistry of citric acid formation by *A. niger* have been discussed. Commercially citric acid is produced by surface, submerged and solid state fermentation techniques. Recovery of pure acid from fermentation broth is done primarily by precipitation with lime and also by solvent extraction

Materials and Methods

In this study the experimental strains were mutated using the UV irradiation to understand the efficiency of citric acid production using sesamum oil cake and rice chaff. The irradiation was done at various distances from 6 to 20 cm. The best irradiated mutants were obtained at the distance of 9 cm and these were selected for the mutational studies. The experimental strains were mutated using the UV irradiation to understand the efficiency of citric acid production using sesamum oil cake and rice chaff. The irradiation was done at various distances from 6 to 20 cm. The best irradiated mutants were obtained at the distance of 9 cm and these were selected for the mutational studies. The mutant strains isolated were designated as *A. niger* ETGP12-m1 and ETGP18-m2

The strains of *A. niger* ETGP12 (Plate 7a) and ETGP18 (Plate 8a) were subjected to UV irradiation to induce mutation and to understand the better yield of citric acid from different mutants. Spore suspensions of the parent strain *A. niger* ATCC9142, ETGP12 and ETGP18 were irradiated using a 15W UV lamp at varying distances (2, 4, 6 to 20 cm). The irradiations were performed in a dark room and the irradiated suspensions were shielded to avoid the light until plating was done on Czapekdox agar in order to minimize any photo-reactivation effects (Gardener *et al*, 1956; Banik 1975).

After the process of irradiation, the different strains were allowed to grow on Czapekdox agar and then the spores obtained from the first generation were evaluated for citric acid producing capability by following the paper culture technique and acid unitages achieved by each strain was noted.The mutant strains isolated were designated as *A. niger* ETGP12-m1 and ETGP18-m2 . The mutant strain that exhibited acid unitages higher than that exhibited by the parents were selected for further studies involving citric acid production from both substrates under solid state fermentation.

Results and Discussion

It is interesting to note that, ETGP12-m1, has showed the highest yield in the citric acid in both the substrates while the ETGP18-m2 has not showed considerable variations in the production of the citric acid. The process of mutagenesis is achieved by different methods like, irradiation, chemicals and fast neutrons (Bradley 1966). The use of fast neutrons is a costly affair. The chemical mutagens are inefficient, less potent and cause side effects to the researchers (Lawely 1966). The irradiation mutagens used generally are X-ray, Gamma rays and UV rays. Out of these, X-rays and Gamma rays have high penetrating power and cause grater nuclear damage as well as lethal effects in the organisms. Hence, these powerful mutagens are avoided.

The mutant strains isolated were designated as *A. niger* ETGP12-m1 and ETGP18-m2. The acid unitage of the *A. niger* ETGP12-m1 was 6.6, while the acid unitage of *A. niger* ETGP18-m2 was 6.2. The mutant strains of *A. niger* ETGP12-m1 and *A. niger* ETGP18-m2 were found to produce citric acid using the experimental substrates such as sesamum oil cake and rice chaff. These mutants were subjected to act on the substrates at the optimal conditions such as 50% moisture, 4.5 pH, 30°C and 4 mm particle size as recorded earlier. The results revealed that the mutant strain *A. niger* ETGP12-m1 has produced 113.3 and 119.6 g/kg of citric acid using sesamum oil cake and rice chaff respectively when compared to the parent strain. The mutant strain *A. niger* ETGP18-m2 produced the 92.6 and 96.4 g/kg of the citric acid un sesamum oil cake and rice chaff respectively at 72 hrs of fermentation (Figure 1).

UV rays with shorter wavelength than the visible lights are mild mutagens. Therefore they are considered to be ideal for effective induction of mutations (Elander 1969; Hopwood 1970). Mutagenesis and screening has been a method of choice for improvement of industrial citric acid production strains. Since *A. niger* lacks a sexual cycle and is refractory to classical genetic approaches, strains derived by this methodology exhibit improved yields as well as rates of citric acid production, utilize cruder and less expensive raw materials and are adopted for special conditions of fermentation process. Moreover, such mutant strains produced fewer byproducts and also exhibit a tolerance to impurities in the substrates.

Figure.1 Production of Citric Acid Using Mutant Strains (Average Values Plotted)

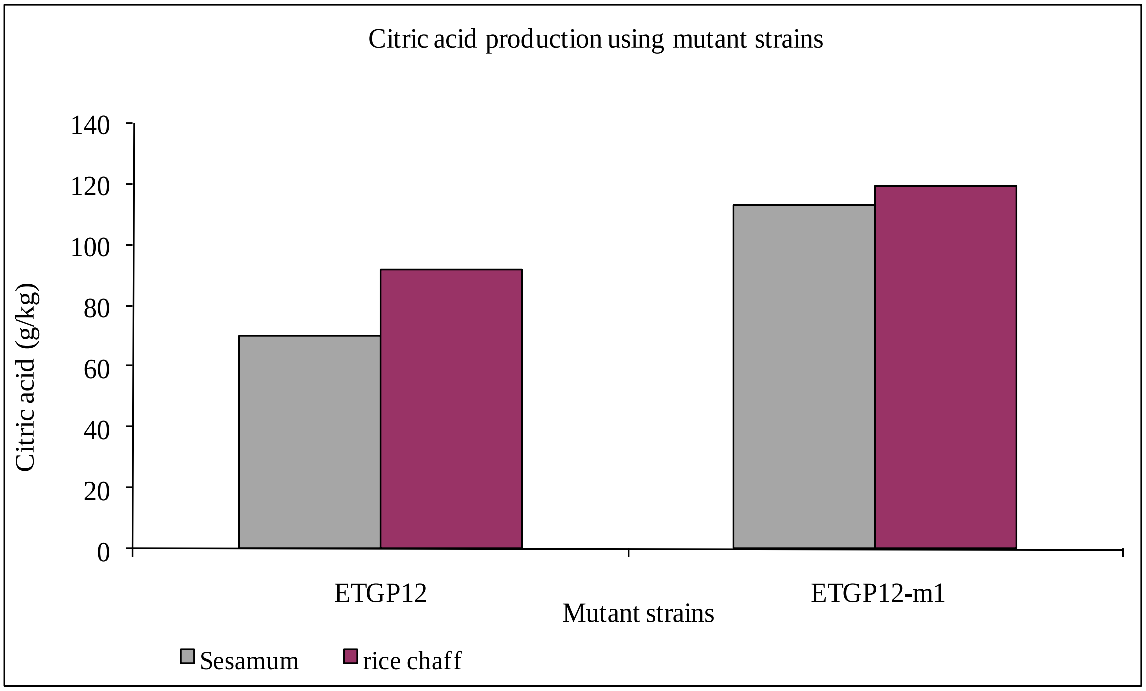

Figure 1. Production of citric acid using mutant strains (average values plotted)

In the present study also different strains were mutated using the UV irradiation to understand the citric acid production using sesamum oil cake and rice chaff. The irradiation was done at various distances from 6 to 21 cm. The best irradiated mutants were obtained at the distance of 9 cm and these were selected for the mutational studies. It is interesting to note that, the ETGP12-m1, has showed the highest yield in the citric acid with both the substrates, while the ETGP18-m2 has showed no considerable yield than that of the parent strain. Ikram-ul Haq *et al* (2004) have studied the production of citric acid by some selected mutant strains of *Aspergillus niger* from cane molasses in 250 ml Erlenmeyer flasks. For this purpose a conidial suspension of *A. niger* GCB-75, which produced 31.1 g/l citric acid from 15% (w/v) molasses sugar was subjected to UV-induced mutagenesis.

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