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# IMPROVEMENT OF CITRIC ACID YIELD OF *ASPERGILLUS NIGER* BY RADIATION

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Ionizing and non-ionizing radiations 'like X-rays and UV rays were found to be effective in the improvement of citric acid yield of the strain *Aspergillus niger* 6 N3. Repeated irradiation of the strain produced strains with about three fold increase in the yield of citric acid. Contrary to the earlier observations it was found that the increase in the rate of mutation was not increasing proportional to the increase in the energy rate.

## INTRODUCTION

Industrially important biochemical products of a microorganism can be improved qualitatively and quanititatively by the improvement of parent strain by mutagenic techniques, as the genetic make up of the organism plays an important role in the cellular metabolism and accumulation of organic by-products. In the naturally occuring strains of *Aspergillus niger* the capacity for the production of acid is rather limited, whatever the physiological conditions of nutrition are given. Possibilities of maximum exploitation of microbial fermentation was, therefore, considered by the employment of mutation techniques. The work was started under a research project sponsored by the International Atomic Energy Agency and a preliminary report on the results of investigation was communicated earlier by one of the authors (Chaudhuri, 1974.)

Single exposures and repeated exposures of the strain *A. niger* 6N3 to ionizing and non-ionizing radiations (X-rays and UV rays) were made to improve the citric acid yielding capacity of the parent strain.

## MATERIALS AND METHODS

The parent strain used in the experiment was *Aspergillus niger* 6N3 producing 31. 4 g of citric acid per litre of the culture filtrate and was isolated from the soil of Naihati, West Bengal. For the second step mutation an UV mutant of the above parent, 50UV24, isolated from the UV irradiated population was used.

The source of UV radiation was a 4 watt General Electric Germicidal Lamp of bent tube construction emitting about 95% of radiant energy at 2537A° unit and the energy dose 100 ergs/mm²/sec. The source of X-ray was an X-ray tube manufactured by Messers Machlett Laboratory Inc. Pvt. Ltd. U.S.A. A run at 100KV

with a current of 9mA and the energy emitted per 35 seconds was calculated at 1000 r units.

Conidial suspensions were prepared from 5 day old cultures grown on Shu and Johnson's sporulating medium ( Shu and Johnson, 1948.). For UV irradiation 7 ml quantity of conidial suspension was poured into each petridish. Treatment of 3, 6, 9, 12, 15, 21, 24, and 30 minutes for the first step mutation and 6. 12, 18, 24 and 30 minutes for the second step mutation were given to the samples at 25°C. During irradiation the conidial suspensions were subjected to uniform shaking with a vibratory shaker so as to ensure unifrom exposure to UV rays.

Fox X-radiation small aluminium planchets containing one ml of conidial suspensions were arranged in a sterile petridish which was placed against the target emitting X-rays at a distance of 10 cm. Treatment of 5, 10, 15, 20, 25, 30, 35 and 40 Kr. were given to the suspensions in case of the first step mutation treatment and 10, 20, 30, 35 and 40 Kr. were applied for the second step mutation.

The irradiated spores after necessary dilutions were plated out in complete medium (Pontecorvo, 1953). The detection and isolation of high acid yielding strains were done by transferring the colonies to Czapek-Dox agar plates containing 0.02% bromocresol green as indicator. The high acid yielding strains were isolated on the basis of 'acid unitage' ( Diameter of the acid zone/Diameter of the mycelial colony). From each treated population 500 transfers were made for the selection of high acid yielding strains. Out of the strains thus isolated 50 strains from each treatment were selected and tested for citric acid production in 100 ml capacity Erlenmeyer flasks containing 25 ml of Shu and Johnson's medium ( Shu and Johnson, 1947) at 30°C for 9 days. Those strains which gave higher yield of citric acid compared to the parent strain wereselected and statisticaly evaluated. The citric acid content was estimated following the method of Marrier and Boulet (1958).

## RESULTS

UV and X-rays treatment on the conidia of *A. niger* 6N3 was found to be effective in the improvement of citric acid production of the parent strain. A good number of strains with appreciably high yield of citric acid were isolated from the UV and X-ray treated population.

Statistical analysis of the results and the level of significance of the mutation rate in connection with various treatments confirm the improved production of citric acid by mutation due to radiation. It has been noted that the increase in the citric acid yield is not gradual along with the increase in dose (energy ) except in case of those treated with UV rays for the second step mutation. (Table 1).

The maximum increase in the production of citric acid was noticed with 24

minutes treatment (47.90±0.98) with UV rays and 35 Kr. (46.48±1.98) with X-rays. In case of the second step mutation, maximum yield was found to be an exposure of 30 minutes ( 85.37 ± 3.46) to UV rays and at a dosage of 35 Kr. ( 81.60±3.95 ) with X-rays.

Table 1. *Citric acid ( mg/ml ) yield ( Mean ± S E. ) of the selected strains from UV and X-ray irradiated populations*

I step mutation

| UV Treatment | Control | 3 minutes | 6 minutes | 9 minutes | 12 minutes | 15 minutes | 21 minutes | 24 minutes | 30 minutes |
|---|---|---|---|---|---|---|---|---|---|
| Mean ± S.E yield of citric acid | 30.04 ±0.08 | 30.52 ±0.56 | 32.22** ±0.52 | 44 15*** ±0.43 | 45.45*** ±1.30 | 33.84 ±2.78 | 33.44** ±1.04 | 47.>0*** ±0.98 | 39.06*** ±1.78 |
| X-ray Treatment | Control | 5 Kr. | 10 Kr. | 15 Kr. | 20 Kr. | 25 Kr. | 30 Kr. | 35 Kr. | 40 Kr. |
| Mean ± S E yield of citric acid | 30.04 ±0.08 | 31.0 ±0.65 | 34.22* ±1.29 | 33.44** ±0.87 | 34.72** ±1.10 | 42.28** ±2.66 | 37.60*** ±1.22 | 46.48*** ±1.98 | 40.65*** ±0 88 |

II step mutation

| UV Treatment | Control | 6 minutes | 12 minutes | 18 minutes | 24 minutes | 30 minute |
|---|---|---|---|---|---|---|
| Mean ± S.E yield of citric acid | 49.62±0.41 | 62.38***±0.81 | 62.38***±0 92 | 65 66***±0.86 | 82.62***±3.02 | 85.37.***±3.46 |
| X-ray Treatment | Control | 10 Kr. | 20 Kr. | 30 Kr. | 35 Kr. | 40 Kr. |
| Mean ± S.E yield of citric acid | 49.62±0.41 | 73.24**±5.04 | 73.92**±5.79 | 66.90**±4.97 | 81.60***±3.95 | 70.98***±47.7 |

***=P<0.001 ; **=P<0.01 *=P<0.02

## DISCUSSION

Results of the experiments indicate that the mutation rate and also the yield of citric acid can be increased by subjecting the conidia to repeated irradiation.

It has been reported that species of *Aspergillus* the number of mutants obtained following the exposure to the various radiations, increase with increasing doses in linear fashion ( Stapleton and Martin, 1949 ; Hollaender, 1955. ). But in the present study increase in the yield of citric acid and percentage of mutation could not however, be correlated with the increase in the energy rate.

Even though increase in the yield of citric acid by *Aspergillus niger* by repeated irradiation had been reported earlier (Das and Nandi, 1969 ; Scichertova and Leopold, 1969.), the yield reported was not too high. In the present study a few

strains isolated from the repeatedly irradiated population had shown an increased yield of citric acid which is more than three times the yield of the parent strain.

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