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CITRIC ACID FERMENTATION BY A UV-TREATED MUTANT OF *ASPERGILLUS NIGER*

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Abstract

The production of citric acid of by *Aspergillus niger* was investigated with wild-type (GCB-1) and UV-treated mutant (UV-6) using 7.5, 9.0 and 10.5 L medium in stirred fermentor. Yield of citric acid was found to be seven-fold higher as compared to the parent in 9.0 L medium and the corresponding increase was two-fold in the 7.5 L medium. With 10.5 L / fermentation, the yield was low with both the parent and the mutant strain, though the mutant gave higher yield compared to the parent.

Introduction

Aspergillus niger is an organism of choice for citric acid fermentation (Roukas & Kotzekidou, 1987). The demand for this acid is increasing day by day due to its multifarious uses in industries, food and pharmaceuticals. *Aspergillus niger,* a filamentous fungus, shows a wide-range of pellet and mycelial morphology under submerged liquid culture. Any structural change in DNA due to mutation can alter the enzyme behaviour of the mould (Pazouki *et al.*, 2000). Some superior strains of *Aspergillus niger* producing citric acid have been isolated following ultraviolet irradiation and their preliminary characterizations were made (Hannan *et al.*, 1973, 1976). However, their productivities were mostly studied at the laboratory scale, mostly in 250 ml conical flasks containing 25 ml medium in which the mutants showed 2-5 times higher yield of citric acid compared to the parent strain. For commercial exploitation it is necessary to test their performance at semi-pilot or pilot scale level (Islam *et al.*, 1984; Rajoka *et al.*, 1998). The present report describes the results of the experiments on citric acid fermentation using a UV-treated mutant, UV-6 vis-à-vis GCB-1, the parent in semi-pilot scale fermentor.

Materials and Methods

Ultraviolet treated first step mutant, UV-6 and the wild-type GCB-1 of *Aspergillus niger* were used. Both of these cultures of *Aspergillus niger* were obtained from the culture collection of *Biotechnology Research Laboratories, Department of Botany, Government College, Lahore, Pakistan.* The strains were maintained on agar slants containing 1% Malt extract, 1% Yeast extract, 1.5% Dextrose and 1.5% Bacto-agar and stored at 5°C in a referigerator. Conidia were harvested in sterile distilled water, after 3-5 days of growth at 30°C and inoculated to the fermentation media at a density of 5×10^6 – 1×10^7 conidia/ml of the medium. The fermentation medium (Doelger & Prescott, 1934) contained in 1 litre of tap water: 140 g sucrose, 2.23g NH_4NO_3, 0.23 g $MgSO_4.7H_2O$ and 1 g K_2HPO_4 adjusted to pH 3.5 with 1N HCl. The medium was sterilized @ 15 p.s.i. (121°C) for 20 minutes.

Table 1. Citric acid fermentation by GCB-1 (wild type) and UV-6 (radiation induced mutant) of *Aspergillus niger* in semi pilot scale fermentor.

Vol. of medium (L)	Strains of *A. niger*	Peak day of acid production	TA expressed as ml of 0.1N NaOH to titrate 1.0 ml of medium	DCM (gm/ml)	SS (gm/ml)	CA produced in the medium (gm/ml)	Residual sugar in the medium	CA in relation to SS (%)	Mycelial Morphology
7.5	GCB-1	6	3.30	0.1200	0.15	0.017	0.052	11.33	Gummy mass
	UV-6	6	7.25	0.0950	"	0.039	0.028	26.00	Shiny pellets
9.0	GCB-1	6	1.90	0.0910	"	0.008	0.103	5.33	Round pellets
	UV-6	6	9.05	0.0132	"	0.059	0.035	39.33	Mixed pellets
10.5	GCB-1	7	0.65	0.1150	"	0.003	n. d.	2.00	Viscous
	UV-6	7	1.85	0.0790	"	0.012	n. d.	8.00	Small pellets

Temperature = 30±0.25 °C, pH = 3.5
'n. d.' Estimates of sugar were not done, as the yield of citric acid was very poor.
Symbols: TA = total acid, DCM = dry cell mass, SS = sugar supplied, CA = citric acid

A stainless steel stirred fermentor (Model: GLSC-AF-199-10, Pak. made) of 15L capacity was used for citric acid fermentation. Three different volumes of media i. e., 7.5 L, 9.0 L, 10.5 L were employed to find out the best one suitable for the semi-pilot scale production of citric acid. Total titratable acid values were determined with 0.1N NaOH from the 6th day of incubation at 30°C and citric acid & residual sugar were estimated between 5-8 days after culture growth. Citric acid was determined by pyridine – acetic anhydride method (Marrier & Boulet, 1958) and residual sugar by DNS method (Tasun *et al.*, 1970). The conversion efficiency of sugar to citric acid was later estimated from the spectrophotometric data.

Results and Discussion

Aspergillus niger mutant UV-6 showed better results than the wild-type, GCB-1 in citric acid production capacity at the semi-pilot scale (Table 1). This has been observed both in the 7.5L as well as 9.0L media. In 7.5L media the increase was two - fold UV-6 (26.0%), GCB-1 (11.33%) whereas in 9.0L media it was seven – fold UV-6 (39.33%), GCB-1 (5.33%). With 10.5L of media the yield was very poor in both the cases UV-6 (8.0%), GCB-1 (2.0%). Production of citric acid in submerged culture is dependent on the depth of the media (Haq *et al.*, 1998). It has been reported that in submerged culture the production of the acid declines if the depth of the media exceeds (Hamissa & Radwan, 1977). In the present studies 7.5 L of the medium was found to equal 25cm and the production of the acid was 0.039g/ml. With 9.0 L gave an yield of culture condition UV-6 (39.33%) as compared to 5.33% yield when GCB-1 was used.

The result seems to indicate that the mutant UV-6 has a good potentiality for large-scale production of citric acid in submerged culture. Both genetic and environmental manipulations have been applied in the improvement of citric acid production by *Aspergillus niger* and improvement in yield has been reported by Jernejc *et al.*, (1982) by using deionised commercial sucrose solutions, proper phosphate concentrations, low initial pH and suitable amount of copper as growth inhibiting agent. In the present study, commercial sucrose was used without any pre-treatment while media composition was similar to that used by Doelger & Prescott (1934). It is expected that yield of citric acid by the mutant UV-6 could be further increased if optimal culture conditions can be formulated.

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