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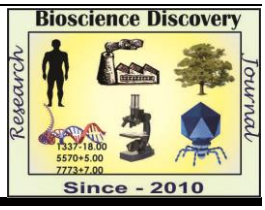

Citric acid production by *Aspergillus niger* and *Aspergillus flavus* isolated from soybean rhizospheric soil

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Abstract

Citric acid is a good, natural preservative. *Aspergillus niger* and *Aspergillus flavus* were isolated from soybean rhizospheric soil. The 1 ml conidial suspension containing 5×10^5 spores/ml was used as an inoculum. Citric acid was produced by surface and submerged fermentation. Dry cell mass was determined.Citric acid was determined titrimetrically.The maximum production of citric acid was obtained by using *A. niger*. *A. niger* strain produced maximum amount of citric acid in submerged fermentation as compared to the surface fermentation. *Aspergillus* species produce the citric acid and inhibit the pathogens present in the rhizospheric region of soybean and increase the crop yield.

INTRODUCTION

Use of Soybean oil can reduce the heart problem, as it has low cholesterol content. Citric acid finds applications as a function of additive detergents, pharmaceuticals, cosmetics and toiletries (Carlos *et al.*, 2006).Citric acid is a weak organic acid. It is a good natural preservative (Alam *et al.*, 2010). Citric acid is used in preservation of food (Baig *et al.*, 2010). Harsha *et al.*, 2013 mentioned that citric acid is a good, natural preservative , adds an acidic taste to foods and soft drinks.*Aspergillus niger* is most commonly used for citric acid production. Citric acid is one of the organic acid synthesized and released by the fungus, *A. niger* when cultivated on synthetic growth medium. It is widely used for preparation and preservation of various food products, apart from its industrial uses (Bharadb *et al*., 2011). Lade *et al.* in 2006 studied the minimal inhibitory concentration of citric acid against the pathogens *E.coli, P. acruginasa, Proteus species, S. aureus* and *Klebsiella* species by microbroth dilution method. The effective range of citric acid was found to be 2000-4000 µg/ml with mean MIC value of 2205 µg/ml. There are various soybean diseases caused by soil borne microorganisms. Charcoal rot, ashy or stem blight or dry root rot is caused by *Macrophomina phaseolina (Tassi) Goid.*Collar rot / Sclerotial blight is caused by *Sclerotium rolfsii Sacc*. Rhizoctonia aerial blight / Web blight is caused by *Rhizoctonia solani Kuhn.*, Sudden death syndrome by Fusarium crassistipithatum. *Aspergillus* species produce the citric acid and inhibit the pathogens present in the rhizospheric region of soybean and increase the crop yield. Javed *et al.* in 2002, produced this acid from Aspergillus niger using cane molasses in a stirred fermentor.In 2010, the submerged fermentation for the citric acid production was done by Baig *et al*. Harsha *et al.* in 2013 done the comparative study of citric acid production between U.V.induced mutants of *A. niger* and *A. flavus*. In the present investigation,an attempt was made to produce citric acid by the fungus *A. niger* and *A. flavus* isolated from soybean rhizospheric soil by the surface and submerged fermentation.

MATERIALS AND METHODS

Isolation of the fungus

The strains used were *Aspergillus niger* and *Aspergillus flavus* for the citric acid production. These were isolated in petriplates containing Czapek Dox agar medium. Fungal cultures were identified on the basis of cultural characteristics by mounting with cotton blue (Awasthi and Sandikar, 2010).

Preparation of conidial suspension

Inoculum was prepared by adding sterile distilled water into the five day old slant. With the help of inoculating loop, the mycelium was mixed and was used as an inoculum (Mohmmad., 2011). The inoculum size i.e. the number of spores was measured by using haemocytometer as described by Aneja, 1996 and Sharma in 1989.

Fermentation methodology

The medium used for the citric acid production was as follows:

Sucrose-14gram,K_2HPO_4-0.5gram,$MgSo_4.7H_2O$-.25gram,AmmoniumNitrate-0.5gram,$CuSO_4$-.06,$ZnSO_4$-0.25gram,$MnSO_4$-1.0gram,$FeSO_4$-1.5gram,Distilled water-1000ml and PH-2.0 to 2.5. After steaming for 20 minutes for the three successive days, media was inoculated with 2% spore suspension and incubated at 30^{o}C with agitation speed of 120 rpm for five days in case of submerged fermentation, In the surface fermentation, flasks were incubated at 30^{o}C on flat platform for five days. After termination of fermentation period the culture filtrate was centrifuged at 8000 rpm for 10 min at 4°C to remove unwanted particles and spores. The supernatant obtained after centrifugation was used as the crude citric acid source (Mohmmad, 2011).

Estimation of dry cell mass (DCM)

Dry cell mass was determined by filtering the culture broth through preweighed Whatman filter paper No. 1. Mycelium was thoroughly washed with tap water and dried in an oven at 105°C for 2 h. The dry cell mass was weighed and calculated as g/l by subtracting the initial weight from the final weight (Mohmmad., 2011).

Citric Acid Determination

Percentage of citric acid was determined titrimetrically by using 0.1 N NaOH and phenolphthalein as an indicator .The 10 ml filtrate was taken in a conical flask and 2-3 drops of phenolphthalein indicator was added to it. This was titrated against 0.1N NaOH till pale pink colour obtained. The titration was repeated till concordant values were obtained. The percentage of citric acid was determined from the following formula:

Normality of citric acid=[N(NaOH) x V (NaOH)] /V(Citric Acid)

% Citric acid = [Normality x Equivalent wt of citric acid x 100]/Volume of filtrate
(Delaimy *et al.,* 2003, Mader, 2012 and Porges, 1932).
N-Normality, V-Volume and Equivalent wt of citric acid-96.

RESULTS AND DISCUSSION

Aspergillus niger and *Aspergillus flavus* were isolated from soybean rhizospheric soil. They were identified on the basis of cultural characteristics by mounting with cotton blue. One ml of spore suspension (5×10^5) spores/ml was used as an inoculum. The inoculum size was measured by using haemocytometer. Dry weight of mycelium was measured after 5 days of incubation. The dry weight of the mycelium in submerged fermentation was more as compared to the surface fermentation. The weights are noted down as in the following table.

TableNo.1: Dry weight of *Aspergillus niger and Aspergillus flavus* isolated from soybean rhizospheric soil

Type of fermentation	Weight of whatmann filter paper No.1 in grams (X)		Weight of whatmann filter paper No.1+ weight of mycelium in grams (Y)		weight of mycelium in grams (Y-X)	
	A. niger	*A.flavus*	*A. niger*	*A.flavus*	*A.niger*	*A. flavus*
Surface fermentation	0.82	0.67	0..97	0..80	0.15	0.13
Submerged fermentation	0.64	1.04	1.04	1.31	0.40	0.27
S.E.					0.19	0.14
C.D.at P=0.01					0.93	0.69
C.D.at P=0.05					0.63	0.46

Citric acid was determined titrimetrically by using 0.1 N NaOH and phenolphthalein as indicator. The end point is colourless to pale pink. The citric acid produced in submerged fermentation was more as compared to the surface fermentation. The citric acid produced was noted down as in the following table.

TableNo.2: Percentage of citric acid produced from *Aspergillus niger and Aspergillus flavus* isolated from soybean rhizospheric soil

Type of fermentation	Mean amount of 0.1N NaOH required in ml		Percentage of citric acid produced	
	A. niger	*A.flavus*	*A.niger*	*A.flavus*
Surface fermentation	2.9	2.2	27.84	21.12
Submerged fermentation	3.4	2.5	32.64	24
S.E.			21.45	16
C.D.at P=0.01			105.85	78.96
C.D.at P=0.05			71.68	53.47

Above mentioned observations were supported by different investigators. Awasthi &Sandikarin 2010 identified and isolated the fungal cultures on the basis of cultural characteristics by mounting with cotton blue. spore suspension was prepared and used as inoculums according to the method described by Mohmmad. in 2011 one ml of spore suspension 5×10^5spores/ml was used as an inoculums.5×10^5spores/ml was used as an inoculum. He also described the citric acid medium composition. Aneja in 1996 and Sharma in 1989 have given the method to measure the inoculum size i.e. the number of spores by using haemocytometer and also described the citric acid medium composition. In the submerged fermentation, the agitation speed of 120 rpm for five days was adjusted as described by Mohmmad in 2011.Centrifugation at 8000 rpm for 10 min was described to remove the suspended particles and the spores. The supernatant obtained after centrifugation was used as the crude citric acid source. Akpan *et al*. in 2010, Delaimy *et al*. in 2003 and Mader in 2012 determined citric acid (CA) titrimetrically by using 0.1 N NaOH and phenolphthalein as an indicator.

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