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Citric acid production by *Aspergillus niger* through solid state fermentation using fruit wastes

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ABSTRACT

In this study, a fungal strain was isolated from soil contaminated with fruit wastes and screened for citric acid production and it was identified as *Aspergillus niger*. The solid wastesubstrates like fruit peels (grapes, mosambi peel) and bagasse with varying particle sizes of M_1 (0. 250mm), M_2 (0.150mm) and M_3 (0.63mm) and 4% methanol, 70% moisture level was maintained throughout the solid state fermentation process for the citric acid production from the fungal culture. The *Aspergillus niger* produces the higher yields of citric acid where grape peel with particle size 0.63mm as a substrate in the medium. Higher fungal biomass and protein contents estimated in grapes (M_3), mosambi (M_1) and bagasse (M_3) wastes substrates respectively in the solid state fermentation.

INTRODUCTION

Citric acid (2-hydroxy2, -propanetricarboxylic acid) is one of the most versatile and important carboxylic acid intermediate of metabolism in most plants and animals. Due to its innocuous nature, citric acid is extensively used in food preparations, pharmaceuticals and cosmetics. About 70% citric acid is used in food industry and remaining 30% in other industries[1]. Now a day, citric acid is also increasingly utilized as a monomer for the manufacture of biodegradable polymers which are widely used in various medical applications[2]. Citric acid (CA) is found in a variety of acidic fruit juices, particularly in the citric ones, although its extraction from natural sources, primarily lemon, was gradually replaced by biological procedures, mainly based on the use of microfungi, which are currently the most widely used[3]. One of the most important fungi used in the industrial microbiology Aspergillus niger has been employed for many years in the production of citric acid. Citric acid is produced from bulk hydrated materials and as a by-product of sugar production by Aspergillus niger[4]. In recent years, considerable interest has been shown in solid state production of citric acid by Aspergillus niger using agro residues like bagasse, corncob, carob pod and waste of food processing industries like apple and grape pomace and fruit peel due to its several advantages like solid waste management, biomass energy conservation, production of high value products and little risk of bacterial contamination [5]. Variety of substrates, such as sucrose, starch

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from various sources, cane and beet molasses, apple pomace, orange / pine apple wastes, banana waste have been utilized for the economical production of citric acid[6,7]. The present objective of the study is to screen and identify the potent fungal strain for citric acid production using cheaply available substrates through solid state fermentation (SSF).

MATERIAL AND METHODS

The soil sample contaminated with fruit waste was collected from local Fruit Market of Tirupati Town, Chittoor District, Andhra Pradesh, India.

Isolation, identification and screening of citric acid producing fungi

The fungal culture was enumerated by soil serial dilution technique and the isolates were maintained on Czapek-Dox agar slants. According to Kareem et al, **t**he fungal cultures were screened[8] for citric acid production. The potent citric acid producing fungal culture was identified based on its macroscopic and microscopic characteristics as followed by Narasimha et al[9] by referring to the standard book entitled "Compendium of soil fungi"[10].

Inoculum

Spore suspension of *A. niger* with concentration of 2 x 10^7 spores/ml was prepared by adding 25ml of sterile distilled water with Tween-80 (0.1%) on Czapek-Dox agar slant and was shaken vigorously for a minute on vortex mixer. It was suitably diluted to obtain a spore concentration of 2 x 10^7 spores/ml.

Collection of substrate samples

Two different fruits wastes mosambi peel and Grape residues was collected from local fruit market of Tirupati. Baggase was collected from Sri Venkateswara Sugar Factory, Gajulamandyam, Chittoor, Andhra Pradesh, India.

Solid State Fermentation

The solid state fermentation was carried out with Bagasse, Grape and mosambi peels were dried in an oven (70^0C) and cut in to small pieces. Grounded and screened to collect three fractions of different particle sizes (M1)0.250mm, (M2) 0.150mm, (M3)0.63mm. Bagasse of desired particle size was taken in 250ml Erlenmeyer flasks and 70% moisture was maintained in the following composition (gm/l). Sucrose 15, NH_4NO_3 0.25, $MgSO_4$ 0.025, H_2PO_4 0.1, $CuSO_4$ 0.004 and the medium pH was adjusted with pH 4.0. To set the desired moisture levels media were sterilized at 121^0C for 15 min.

For grape and mosambi substrates, the peels were dried in an oven at 70^0 C for 2 days grounded and screened to collect the particles of the size M1, M2, M3. Five gram grounded fruit waste was taken in 250ml conical flask and moistened with distilled water to desired moisture level 70%. Since mosambi peel waste contained less sugar it was moistened with sucrose solution to increase the sugar level to 31.8 g/100g of dry solid (Previously optimized). The flasks containing medium were sterilized at 121^0C for 15 min. After sterilization, the flasks containing medium were allowed to cool to room temperature and inoculated with 1ml of spore suspension (2x10^7 spores/ml) of *A.niger* followed by proper mixing. After inoculation flasks were incubated at 30^{o}C in a humidity controlled incubator. Methanol (4%v/w) was added to the medium before inoculation and after sterilization of the medium.

Extraction and analytical methods

Fermented material of the flask was dried in an oven at 50^o C and extracted by the addition of 100ml of distilled water. The mixture was agitated on a rotary shaker for 2 hour and then filtered through Whattman filter paper No.1. The supernatant was used for the estimation of total residual sugar and citric acid. Total reducing sugar was determined[11] and citric acid was estimated by the acetic anhydride and pyridine method[12], Protein estimation[13] and pH and Biomass was also estimated [14].

RESULT AND DISCUSSION

The fugal isolated from soil contaminated with fruit waste was screened according to the method of Kareem et al[8]. In this method the formation of yellow zone surrounding the fungal colony in the plate is the indication for the citric acid ability of the culture and the fungal culture was

identified as *A.niger* based on its macroscopic and microscopic characteristics listed in the TABLE 1. Similarly the fungal culture *A.niger* was isolated from soil contaminated with effluents of cotton ginning mill[9] and dairy industries[15] were identified.

The maximum biomass and protein content was observed in grape substrate with particle size of M_1 (3.2g, 4.56µg/ml), M_2 (3.4g, 4.24 µg/ml) respectively (TABLE 2). The pH value maintained at the beginning of fermentation was important for a specific biomass formation. Normally, citric acid production occurred after 24 h of fermentation, this study shows that as incubation time increased more citric acid is produced and pH values decreased. Thus, the drop in pH observed during the process was due to the formation and accumulation of citric acid[8].

The fungal culture *A.niger* was used for the citric acid production on different substrates (bagasse, mosambi, grape) with particle sizes M_1 (0.250mm), M_2 (0.150mm) M_3 (0.63mm) and the results were represented in TABLES 3-5. In the present study, maximum citric acid production was observed in grape as substrate in the medium (34.4g/Kg) with particle size of M_3 (0.63mm). The increase in citric acid production and biomass values was accompanied with steady decrease in sugar along the incubation time[8]. The parallel relationship between citric acid produc-

TABLE 1: Macroscopic and microscopic characteristic of *Aspergillus niger*

Macroscopic	Color	Texture	Colony Diameter (cm)
	Black	Filamentous	10.2
Microscopic	Conidiophores length (µm)	Conidiophores diameter (µm)	Conidia shape
	3.31	3.21	Globose

TABLE 2 : pH, Biomass and protein content of *Aspergillus niger* on different substrates with particle size of bagasse, grape and mosambi

Substrates	Particle size	pH	Biomass (gm)	Protein (µg/ml)
Bagasse	M_1(0.250mm)	3.2	2.82	0.78
	M_2(0.150mm)	3.1	2.53	0.60
	M_3(0.63mm)	3.0	2.20	0.64
Grape	M_1(0.250mm)	2.40	3.20	4.56
	M_2(0.150mm)	2.08	3.40	4.24
	M_3(0.63mm)	1.5	3.0	3.20
Mosambi	M_1(0.250mm)	2.14	3.12	4.16
	M_2(0.150mm)	2.70	3.40	3.12
	M_3(0.63mm)	2.80	3.10	2.80

TABLE 3: Citric acid production by *A.niger* on different particle sizes of bagasse substrate z

Bagasse substrate different particle size	sugar consumption (%)	Citric acid production (g/kg)
M_1(0.250mm)	89.2	23.6
M_2(0.150mm)	92	24.9
M_3(0.63mm)	88.3	22.1

***Values represented in the table are mean of duplicates**

TABLE 4: Citric acid production by *A.niger* on different particle sizes of mosambi substrate

Mosambi substrate different particle size	Sugar consumption (%)	Citric acid production (g/kg)
M_1(0.250mm)	94.1	28.6
M_2(0.150mm)	92	26.7
M_3(0.63mm)	89.5	23.3

***Values represented in the table are mean of duplicates**

TABLE 5 : Citric acid production by *A.niger* on different particle sizes of grape substrate

Grape substrate different particle size	Sugar consumption (%)	Citric acid production (g/kg)
M_1(0.250mm)	94.3	29.8
M_2(0.150mm)	96.6	31.9
M_3(0.63mm)	99.2	34.4

***Values represented in the table are mean of duplicates**

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tion and the consumption of sugar was also observed (TABLES 3-5). These results were agreed with the report of El-Holi and Al-Delamy[16]. However larger particles of the medium reduced substrate availability to the microbe, resulting in lower citric acid production. In contrast to Kumar et al[17] reported 1.2mm particle size for the maximum citric acid production. From this study, we observed that the lower particle size (0.63mm) was suitable substrate particle size for the citric acid production by *A.niger*. The increase in citric acid yield with methanol is a general phenomenon and is commonly used in citric acid production.

CONCLUSION

The potent citric acid producing fungal strain was isolated from fruit waste contaminated soil and it was identified as *A.niger*. Among the substrates with different particle size used in the study, grape peel with particle size 0.63mm showed the best chiefly available substrate for the citric acid production for fungal strain, *A.niger*.

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