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Research Article

PRODUCTION AND PARTIAL CHARACTERIZATION OF CITRIC ACID BY LOCAL ISOLATE OF *ASPERGILLUS NIGER* USING SORGHUM

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ABSTRACT
In the present study, the citric acid producing organisms were isolated from soil sample using serial dilution technique and the isolate were identified on the basis of their microscopic and morphological characteristics. The isolated fungal species were screened on the citric acid production using Czapek-Dox agar medium. From the isolates, fungal species *Aspergillus niger* were used for citric acid production, and the fungal species subculture using potato dextrose medium. The fresh culture of isolated *Aspergillus niger* were mixed to the fermentative medium along with the substrates, sorghum flour, corn starch and wheat flour separately and the fermentative medium was incubated 6-7 days at 30^0C, after incubation the citric acid content was estimated by titrimetric method. Among the three substrates highest citric acid production was observed in corn starch supplemented medium than sorghum and wheat flour medium. Further characterization of citric acid was by paper chromatography, thin layer chromatography and IR. So it is highly recommended that the usage of corn starch supplemented medium with *Aspergillus niger* will be effective in the production of citric acid than the other substrates.
Keywords: citric acid, *Aspergillus niger*, carbon source

INTRODUCTION

Citric acid (2-hydroxy2, -propane tri carboxylic acid) is one of the most versatile and important carboxylic acid. Citric acid worldwide demand is about 6.0 X 105 tons per year[1]. Approximately 75 % commercial use of citric acid is for food and 12 % for pharmaceutical industries[2]. Until about 1920, all commercial CA was produced from lemon and lime juices[3]. Rohr *et al* 1983 reported that CA can be produced by fermentation process using species of microorganisms namely *Aspergillus niger*; a fungus which was used commercially for the first time in 1923. They also indicated that factors affecting the production of CA by fermentation include the nutritional composition of the media, environmental conditions, deficiency of manganese and other metals, pH and dissolved oxygen tension. The influence of types and concentrations of sugars[5] chelating effect on metal ions[6], ammonium nitrate and aeration[7] on CA production by *A. niger* have also been studied. Many microorganisms have been evaluated for the production of citric acid including bacteria such as *Bacillus licheniformis, B. subtilis* and *Corynebacterium* spp.[8] fungi such as *A. nige*, *A. awamori*, *A. foetidus* and *Penicillium restrictum*[9]; Yeast such as *Candida lipolytica*, *C. intermedia* and *Saccharomyces cerevisiae*[10]. However, *A. niger* a filamentous fungus remained the organism of choice for citric acid production due to ease of handling, its ability to ferment a variety of cheap raw materials and high yields[11]. A cost reduction in citric acid production can be achieved by using cheap agricultural wastes such as apple and grape pomace, orange peel, kiwi fruit peel, cotton waste, okra soy-residue and cane molasses[12]. Other carbohydrates and wastes that have been considered, experimentally, to produce CA by *A. niger* includes inulin[13], date fruit syrup[6], sugar cane molasses[14], soya whey[15], kumara[16] and Carob pod[17] and cheese whey[18]. At present time CA is produced commercially using mutant strains of *A. niger* and with a significant amount by *Saccharomycopsis lipolytica*[19], *Pencillium simplicissimum*[20] and *A. foeitidus*[21]. The aim of this study production and optimization of citric acid by using *Aspergillus niger* using cheapest raw material such as corn starch, sorghum flour and wheat flour.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from various locations in orange fruit juice shops at chopda, Maharashtra, India for isolation of fungal strain. The soil samples were placed in sterile polythene bags, closed tightly and stored in refrigerator.

Isolation and Identification Fungal Strain

Serial dilutions were performed by using the collected soil samples to isolate the fungi. The isolated colonies were examined by lacto phenol cotton blue technique, and identified according to their morphological characteristics.

Preparation of the Medium for Growth of Fungi

The identified colonies were streaked on czapekdox medium and incubated at 25^0C for 48-72 h. After incubation separate out the required *Aspergillus niger* fungal culture and also check acid produce or not by using $CaCo_3$.

Production of Citric Acid

Take sterile Czapekdox agar medium with 0.5 g $CaCo_3$ Petri plates. Streak plates with isolated fungal culture and incubate plates at 25^0C for 48-72 h. After incubation we see that a clear zone obtained below *Aspergillus niger* species and white colour of $CaCo_3$ is destroyed in fungus region. This indicates the organic acid production.

Fermentation Media Preparation

Czapekdox medium; substrate - 20 g, distilled water one liter. The experiments were conducted in 250 ml five flask containing 50 ml czapekdox medium; add the sorghum flour, corn starch and wheat flour 1.0, 1.5, 2.0, 2.5, 3.0 g in each flask respectively, sterilize the flask in autoclave. After

sterilization transfer 5 ml of liquid broth containing *Aspergillus niger* in each flask. Incubate the flask in shaking incubator at 30^0C, 120 rpm for 6-7 days, then media was notified for citric acid production using pH strip.

Assay of Citric Acid

Citric acid was determined by using 0.1N NaoH and phenolphthalein as indicator[22]. In this procedure 1 ml of culture filtrate was taken using whatman filter paper and then 2-3 drops of phenolphthalein indicator was added and filtrated with 0.1N NaoH and the end point was noted. When the filtrate changes into colorless to pink colour then it was calculated according to the following formula.

$$\text{Amount of citric acid produced} = \frac{\text{B. R} \times 7 \times 10}{5}$$

Optimization of Citric Acid Production

In order to increase amount of citric acid production in various carbon and nitrogen source; it was done in corn starch, sorghum flour and wheat flour as substrates using *Aspergillus niger*.

Partial Characterization of Citric Acid

Citric acid was subjected to paper chromatography 1:1 butanol water system is used. Citric acid does not show any spotting on TLC plate using silica gel.

Spectroscopic Analysis of Citric Acid

Citric acid was analyzed by scanning the absorbance in the wavelength region of 200-1100 nm using UV visible spectrophotometer.

IR Analysis

The fraction of chromatography after determining R_f value was scrapped and dissolved in methanol and peaks were obtained by IR assay.

RESULT AND DISCUSSION

From the soil samples collected, the fungal species were isolated by using PDA medium, after serial dilution. More number of fungal colonies was observed. Among the colonies, observed *Aspergillus niger* was selected specifically for citric acid production. Those organisms were identified by lacto phenol cotton blue method based on their morphological characterstics. *Aspergillus niger* which shows black with white margin, colorless brownish shade conidiophores, globose, thick walled brown colour conidia. It was confirmed by Gillman fungi manual. Similar results have been reported by[23], that *Aspergillus niger* RCNM 17 was isolated from soil sample collected from rotten fruit dump outside a whole sale fruit stall, Kumar *et al*., (2003) also reported that *Aspergillus niger* isolated from the soil, where the pieces of lemon were placed at different places and then cultured in PDA plates. The production of citric acid was screened using czapekdox agar medium the *Aspergillus niger* culture was poured into individual sterile petri plates. The plates present yellow zone around the *Aspergillus niger* colonies due to citric acid formation. Citric acid productivity of *Aspergillus niger* was analyzed using the corn starch, sorghum flour and wheat flour as substrate with fermentative medium. The estimated results were presented in Table and Figure. The result showed that corn starch (385 mg) produce high amount of citric acid using *Aspergillus niger* compared than sorghum flour and wheat flour (298 mg, 280 mg). In this study highest citric acid production were noted in corn starch compared with sorghum flour and wheat flour. So the corn starch used for optimization study. The production of citric acid from carobpod by *Aspergillus niger* in solid state fermentation was investigated. The maximum citric acid concentration was observed[24].

Effect of Carbon Sources on Citric Acid Production

Citric acid production was analyzed by using carbon sources such as corn starch, sorghum flour, and wheat flour (Table 1). Maximum citric acid production was recorded in the corn starch supplemented medium (385 mg) and low amount of citric acid production was recorded in the wheat flour supplemented medium (280 mg).

Table 1: Effect of different carbon source on citric acid production

Starch	pH	Citric acid production in mg% on different Carbon Sources		
		Corn starch	Sorghum flour	Wheat flour
0.5 %	3.5	163 mg	132 mg	120 mg
1 %	2	385 mg	275 mg	203 mg
1.5 %	2	385 mg	290 mg	280 mg
2 %	2	380 mg	285 mg	271 mg
2.5 %	2	381 mg	282 mg	270 mg

Effect of Nitrogen Sources on Citric Acid Production

Citric acid production was analyzed by using nitrogen sources such as Ammonium sulphate, Yeast extract, Ammonium nitrate, casein, and Sodium nitrate (Table 2). Maximum citric acid production was recorded in the Ammonium sulphate incorporated medium (350 mg) and low amount of citric acid production was recorded in the casein supplemented medium (140 mg). Nitrogen has been reported to be an important factor in fermentation processes due to an increase in C/N ratio[25]. Thus the present study revealed that the *Aspergillus niger* strain and corn starch was most efficient for the production of citric acid, the commercial industrial production.

Table 2: Effect of nitrogen sources on production of citric acid on different substrates (After 6 days)

Sources	pH	Citric acid production in mg% on different nitrogen sources		
		Corn starch	Sorghum flour	Wheat flour
Ammonium sulfate	2	350 mg	330 mg	290 mg
Yeast extract	3	264 mg	250 mg	265 mg
$NaNO_3$	3.5	238 mg	220 mg	230 mg
Casein	3.9	140 mg	130 mg	125 mg
$NaNO_2$	3.6	210 mg	190 mg	150 mg

Purification of Citric Acid

Concentrated citric acid was subjected to paper chromatography 1:1 butanol water system was used and for development of chromatography paper spray of bromo cresol green was used. Measure the R_f value from paper.

$R_f = 0.36$ (Compound) $R_f = 0.35$ (Standard citric acid)

By performing thin layer chromatography compound does not show any spotting on chromatography plate.

Spectrophotometric Analysis

Absorption Spectra of Compound

Absorption spectra of product observed by using spectrophotometer from 200-1100 nm, by using Water as a solvent and compared with standard citric acid. By observing graph which shows the near about same absorption such as absorption wavelength at 576 nm of compound same as the original citric acid it shows the produced citric acid is may be citric acid.

IR Spectra

It is taken by dry methanol as a solvent for compound and methanol was used for the finding infra red spectra which gives a peak which shows the spectra at the value near about for the value of spectra for OH group that may conclude produced compound may be acid (Figure 1)

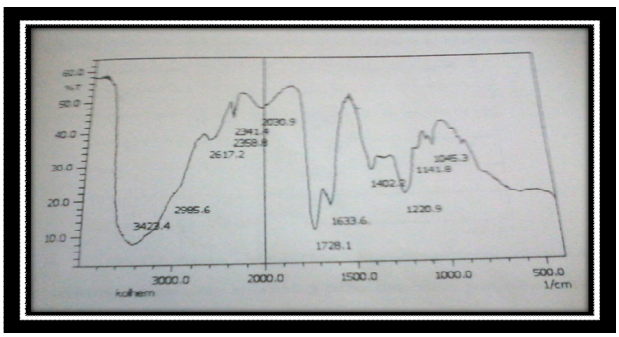

Figure 1: IR Spectroscopy of citric acid produced from *Aspergillus niger*

Table 3: Peaks positions and percent transmission

No.	Pos. (1/cm)	Inten. (% T)
1	1045.3	35.58
2	1141.8	34.52
3	1220.9	21.06
4	1402.2	28.63
5	1633.6	18.14
6	1728.1	11.34
7	2030.9	47.76
8	2341.4	46.51
9	2358.8	44.51
10	2617.2	37.92
11	2985.6	21.94
12	3423.4	8.09

CONCLUSION

At last concluded that the maximum amount of citric acid was produced by *Aspergillus niger* in corn starch supplemented medium. So it is highly recommended the convention of corn starch supplemented medium with *Aspergillus niger* will be valuable in the invention of citric acid than the other substrates.

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