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CITRIC ACID PRODUCTION BY ASPERGILLUS ORYZAE AD-2: PROCESS OPTIMIZATION AND YIELD ENHANCEMENT

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ABSTRACT

Results: In the present study, an attempt was made to produce citric acid using Aspergillus oryzae. The optimum condition for citric acid production was determined. pH at 3, temperature at 30 ˚C and 1% inoculums size was found to be optimum. The enhanced citric acid production was seen using UV- mutation; however immobilization method did not enhance the yield.

KEYWORDS: Citric acid, Aspergillus oryzae, UV- mutation, immobilization.

INTRODUCTION

Microorganisms are ubiquitous[1-4] and produce various important organic acids that are essential, as they act as building-block. Among various organic acids, citric production by microbes is one of oldest microbial process.[5] Citric acid is the most versatile organic acid and broadly used for multipurpose in different industries including food, cosmetics, pharmacy, beverages and many others.[6] At present, most of the citric acid is produced by the fungus rather than chemical synthesis because fungal fermentation is much cheaper than chemical synthesis. Nowadays, citric acid is produced by submerged fermentations because the process is very easy to operate.[7] The production of citric acid by submerged fermentation mainly depends on many factors such as pH, temperature, aeration, nutritional composition, concentrations of carbon, nitrogen, metal and others.[8] There are many reports on citric acid production by Aspergillus niger[6,8] but only scanty reports using other fungi. Hence, in the present study an effort was made to isolate citric acid producing fungi (other than Aspergillus niger), optimize the production parameters and enhancing the yield using UV-mutation and immobilization.

MATERIALS AND METHODS

Isolation and identification of citric acid producing fungi

Soil samples from various region of Bangalore, Karnataka, India were serially diluted and plated on Czapeck Dox media containing bromocresol green as a pH indicator. The fungal colonies which changed the medium color (from blue to yellow) were selected for further work.

A total 11 isolate have been isolated and designated as AD1-AD-11. Among 11 isolate AD-2 highest zone of de-colorization which was selected for further work.

The isolate AD-2 was initially identified based microscopic characters, further the isolate was sent to Agarkar Research Institute, Pune, India for further confirmation.

Fermentation and Citric acid assay

For fermentation the inoculum was prepared according to the method suggested by Alam et al. (2004).[9] Czapek Dox medium was used for citric acid production.

The citric acid was determined spectrophotometrically by the acetic anhydride-pyridine method developed by Miller (1958).[10]

Optimization parameters

Effect of initial pH on citric acid production

100 ml of production medium was taken in 250 ml conical flask. The pH of the broth was adjusted to pH ranging (2-8, at interval of 1) with 0.1 N HCl and 0.1 N NaOH, the prepared spore suspension was inoculated and incubated for 8 days at 30 °C.

Effect of temperature on citric acid production

A set of 4 conical flasks containing 100 ml of the production medium was inoculated with spore suspension. The flasks were incubated at different temperature like 30°C, 35°C, 40°C and 45°C. The flasks were incubated for 8 days and the citric acid production was assayed for every 24 h.

Effect of inoculum size on citric acid production

A set of 4 flasks containing 100 ml of the production medium was inoculated with different inoculum (ranging from 1-4%). The flasks were incubated for 8 days and the citric acid production was assayed for every 24 h.

Enhancement of citric acid production by UV mutation

Spore suspension was prepared with concentration of 2 x 10^7 spores/ml. 1 ml of the spore suspension was taken in an empty sterile petri-plate and this plate was exposed to UV light with varying time & length like 6, 31 and 46 cm away from UV light source for various time periods (2, 4 and 8 min). After exposure, the spore was plated on Czapeck Dox agar. The plates were covered by black paper and incubated at room temperature for 3-4 days.[11] The colonies obtained from these were used for estimation of citric acid.

Immobilization

Immobilization was performed by mixing the spore suspension (2x10^7 spores/ml) with 1.3% sodium alginate and then dropped into 0.2 M $CaCl_2$ solution. Conidia were entrapped in Ca-alginate pellets of approximately 2-3mm in diameter. The pellets were left 1hour at 20℃, washed with distilled water and stored in 0.02M $Cacl_2$ solution at 4 ºC.[12] Production medium was prepared and 20g of immobilized conidia was inoculated in 100ml of medium, incubated for 8 days and checked for the production of citric acid at regular intervals.

RESULTS AND DISCUSSION

Isolation and identification of citric acid producing fungi

A total 11 isolate have been isolated among them isolate, AD-2 highest zone of de-colorization (Figure 1 a). The microscopic characters (Figure 1 b) of the isolate AD-2 was similar to that of Aspergillus, further the reports of Agarkar Research Institute, Pune, India suggest that the isolate AD-2 belongs to Aspergillus oryzae AD-2.

Optimization parameters

Effect of initial pH on citric acid production

The effect of initial pH on citric acid production by Aspergillus oryzae AD-2, show the maximum production (1.2 g/L) at pH 3 (Figure 2). The increase in the pH decreased the citric acid production. A similar result was obtained by Aspergillus niger which showed maximum of (1.15 g/L) at pH 2.[13] Further, a low initial pH has the advantage of checking contamination and inhibiting oxalic acid formation.[14]

The yield gradually increased from day 2 and over the time period, the production decreased this maybe due to the fact that, the increased amount of citric acid accumulated over time would be toxic and would start to affect its metabolic activity.[15]

Effect of temperature on citric acid production

Figure 3 shows the effect of temperature on citric acid production. The maximum citric acid was produced at 30 ˚C, above and below this temperature the production decreased. Our result correlates with Steel et al. (1995) where he found the maximum citric acid production from 28 to 32 ˚C.[16] Similarly Pandey et al., (2013) also showed that 30℃ was optimum temperature for the production of citric acid.[17] The report also agrees with the findings of Kareem and Rahman (2013) as they stated that the temperature of fermentation medium is one of the critical factors that have a profound effect on the production of citric acid. When the temperature of the medium was low, the enzyme activity was also low, giving no impact on the citric acid production, but when the temperature of the medium was increased above, the biosynthesis of citric acid decreased. It might be due to the accumulation of byproducts such as oxalic acid.[18]

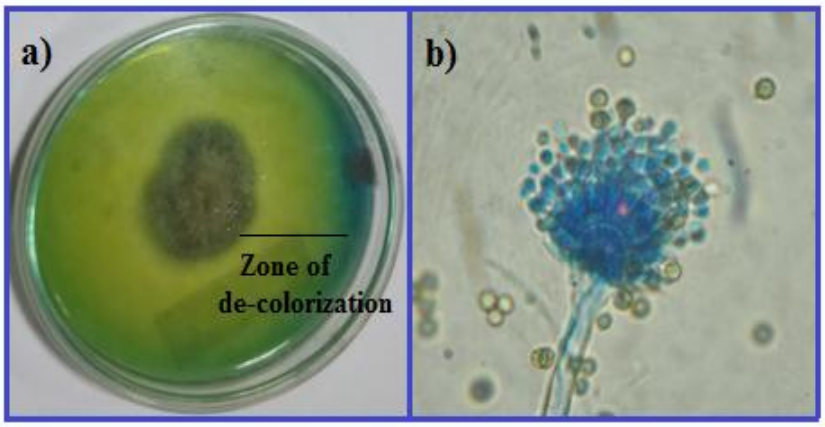

Figure 1: a) Isolate AD-2 showing zone of de-colorization b) microscopic observation of isolate AD-2.

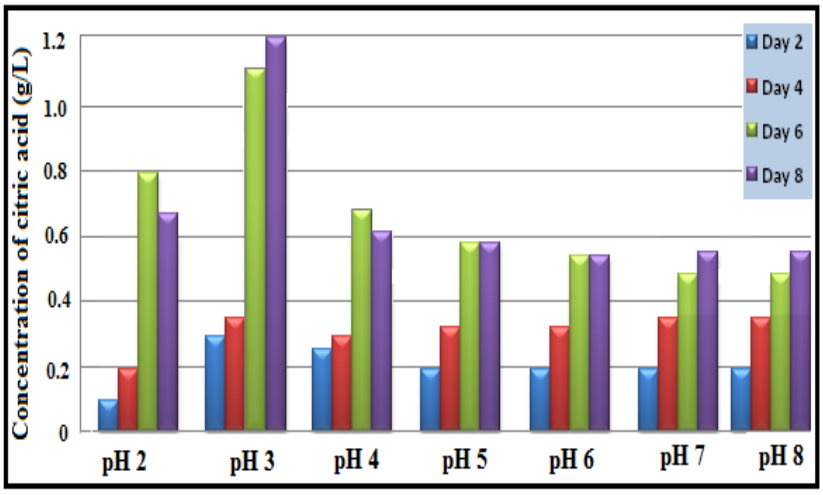

Figure 2: Effect of initial pH on citric acid production

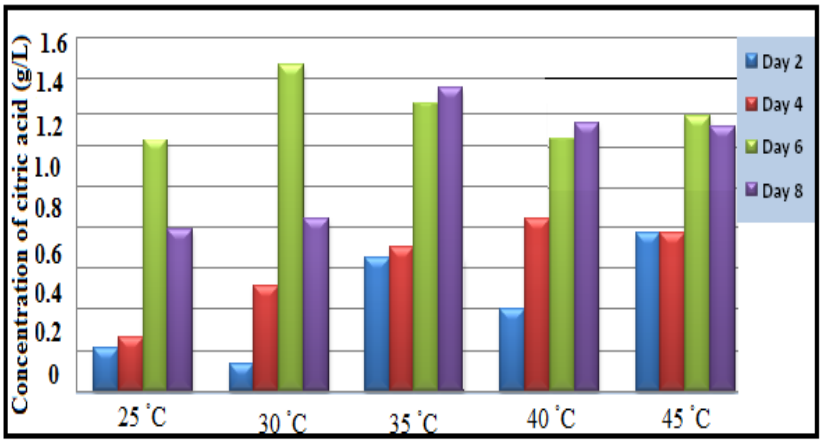

Figure 3: Effect of temperature on citric acid production

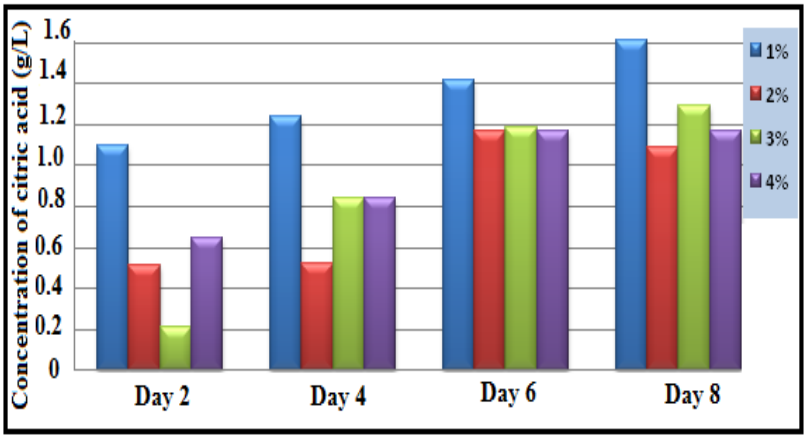

Figure 4: Effect of inoculum size on citric acid production

Effect of inoculum size on citric acid production

1% inoculums size was found to be optimum for the production of citric acid (Figure 4). Our result was in agreement with Sikander Ali et al. (2002) who showed that inoculums size of 1% was optimum for the production of citric acid.[19]

Enhancement of citric acid production by UV mutation

Aspergillus oryzae AD-2 was subjected to mutation with UV radiation. The mutant, which was exposed to UV light 31cm away for 8 minutes showed highest production of citric acid when compared to other mutants. Our results were in agreement with Walid et al., (2012).[20]

Immobilization

The effect of immobilization on citric acid production show highest production of citric acid on day 6, it showed that Aspergillus oryzae has the ability to produce citric acid in immobilized condition as well but free spores showed better citric acid production than the immobilized spores.

CONCLUSION

From the above study it concludes that apart from Apergillus niger other species such as Aspergillus oryzae can be used for citric acid production. The initial acid pH (pH 3) favours the production. The isolate has an optimum temperature (30 ˚C) below and above this, the production decreases. The production of citric acid can be enhanced using UV radiation.

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CONFLICT OF INTERESTS

Declared None

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