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Studies on citric acid production by *Aspergillus niger* in batch fermentation

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Abstract

Citric acid is one of the very important commercial product with constant demand in the market . Citric acid production by fungi have been reported by many workers since 1913.The present study focused on production of citric acid by Aspergillus niger in batch fermentation. The maximum yield was found to be 1.5mg/ml.

Keywords: Acid production, *Aspergillus niger,* batch fermentation

INTRODUCTION

Citric acid is one of the most common product which has a never ending demand in the global market.It plays a pivotal role of an acidulant in food and beverage industries.Citric acid fermentation is one of the primitive fermentations but still its production is going on increasing with passage of time. In 2007, its global production exceeded 1.6 million tons.(1).

Currie in 1917 performed pioneering work on citric acid producton(2).He worked upon Aspergillus niger for citric acid producton using sucrose as substrate.Kinetics of growth and citric acid production was also investigated by Beherens etal in 1987.(3)Citric acid production by *Candida spp* was investigated under nitrogen limitation by savas et al in 2002(4). Optimised citric acid production by Aspergillus niger using a design model leading to accumulation of the citric acid as major product was investigated by majority of workers.The two most important microbial sources are bacteria and fungi. But fungus remains the preffererd sources for citric acid production.

The present investigation is focused on determining the potential for citric acid production by indegeneously isolated a niger and optimizing the physiochemical cultural parameters for maximum citric acid production.

MATERIALS AND METHOD

Isolation and maintenance of the organisms

The fungi were isolated on potato dextrose agar from soil by serial dilution method .Each isolate was maintained on PDA at 4˚C.Subculturing was done after every 3 months.

Prelimnary screening

The 10 selected isolates were screened qualitatively for citric acid production by plate method on czapeckdox agar containing Bromocresol green as indicator 1% at pH 6.The spore suspension of the isolates were spread on the surface of the medium plates and allowed to grow for 5 days. Yellow zones indicated citric acid production.

Secondary screening

The positive isolates were subjected to secondary screening in the liquid czapeckdox medium containing sucrose as sole source of carbon and $NaNO_3$ as nitrogen source at pH 6 and 30 ˚C room temperature under static conditions.

Identification of the potent strain: The most potent strain was identified morphologically by lactophenol cotton blue staining. The most potent strain was identified to be Aspergillus niger.

Citric acid assay: citric acid was assayed gravimetrically following by Marrier and Boulet method (5)

Effect of incubation period on citric acid production

The citric acid acid production was studied upto 15 days on 1st,3rd , 5th, 7th, 9th, 11th, 13th , and 15th day.The total titrable acidity was also determined by 0.1N NaOH.

Effect of pH on citric acid production

Effect of pH was investigated on citric acid production. The range of pH investigated were 3,4,5,6,7,8 and 9.

Effect of temperature on citric acid production

Effect of temperature on citric acid production was investigated from 20˚C, 30˚C, 40˚C, 50˚C, 60˚C, 70˚C and 80˚C.

RESULTS AND DISCUSSION

Preliminary and secondary screening of the isolates.

All the 10 fungal isolates were screened by plate method and the zone of citric acid acid production was measured in mm as

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indicated in Figure 1. The isolate 1, isolate 6 and isolate 10 were subjected to secondary screening. The isolates yielded 0.13mg/ml ,0.19mg/ml and 0.12mg/ml of citric acid as given in Figure 2 .The isolate number 6 was selected for further studies.The effect of incubation period was determined on the citric acid production.11th day was found to support the optimum production of Citric acid.The optimum pH was found to be 5 and temperature was found to be 30 ˚C.

Reports of very high citric acid production have been registered upto 14.86 mg/ml in presence of prescott salt on 13th day. (6)The isolate would be subjected to strain improvement for increase in citric acid production.

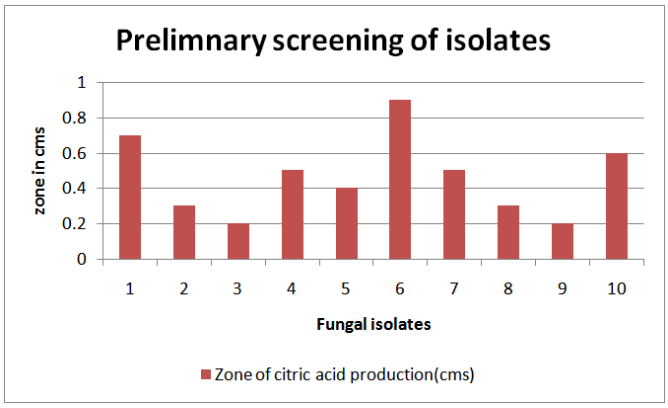

Fig 1. Preliminary Screening of the isolates by plate method

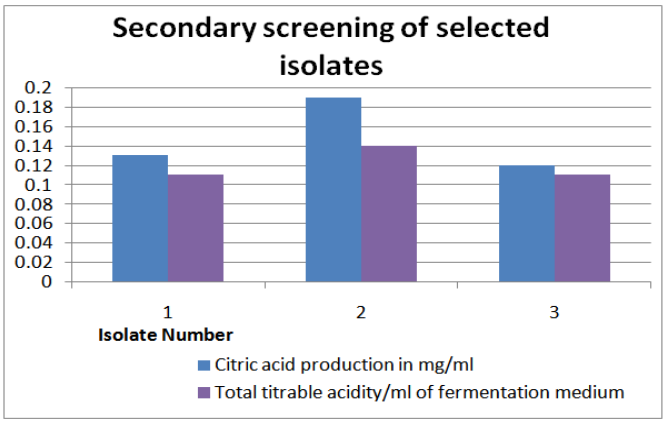

Fig 2. Secondary screening of the selected isolates

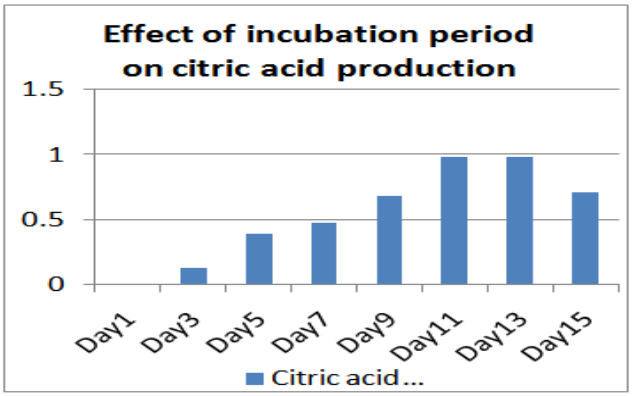

Fig 3. Effect of incubation period on citric acid production

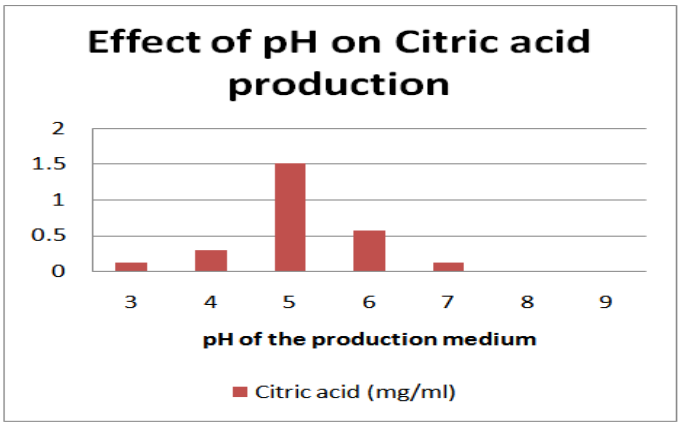

Fig 4. Effect of pH on citric acid production

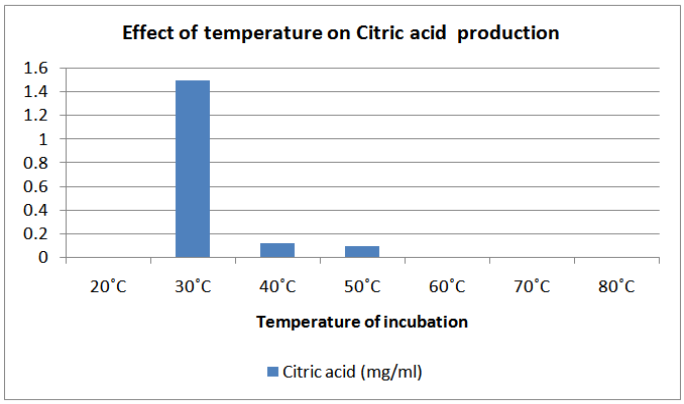

Fig 5. Effect of temperature on citric acid production

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