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ISOLATION AND PRODUCTION OF CITRIC ACID BY SOLID STATE FERMENTATION FROM ASPERGILLUS NIGER OF LEMON JUICE

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ABSTRACT

Many microorganisms such as bacteria, fungi and yeast can produce citric acid. It is reported that ***Aspergillus niger*** is almost exclusively used for industrial scale production of citric acid due to high citric acid productivity at low pH, without secretion of toxic metabolites, ease of handling and ability to ferment a variety of raw materials .For the isolation and production of citric acid three samples taken. i.e. Lemon juice, potato starch and rice starch.7 strains were observed on potato dextrose agar media and LJ3,PS2, RS1 shows growth of *A.niger*. The isolated strains LJ3,PS2,RS1 were identified by morphological and spore formation to confirm as *A.niger*. The *A.niger* strains (LJ3,PS2,RS1*) were selected for citric acid production on PDB. *A.niger* strain LJ3 gave maximum production of citric acid .i.e.18.2g/ L at 30^{O}c of144hrs incubation and PS2 gave 6.4g/L and RS1 gave 5.2g/L by SSF. The media optimized for three substrates of citric acid production gave maximum yield by LJ3.ie.10.6g/L, PS2.i.e. 8.1 and RS1 i.e.4.4g/L in selective media of basal media in aerobic conditions.

KEY WORDS: citric acid, *Aspergillus niger*, ssf.

INTRODUCTION

Citric acid is a tricarboxylic organic acid, soluble in water with pleasant taste, and is the most important in food and other industries. Citric acid derives its name from the Latin word citrus, the citron tree, the fruit of which resembles a lemon. Refined sugars such as glucose and sucrose are the most commonly used substrates for commercial production of citric acid by fermentation process; however they are expensive and can be replaced by various cheap and abundant substrates like agro -industrial wastes or by-products including starch, cellulose and lignocelluloses material.(Adham 2002).

Surface method and submerged process are widely recognized for the production of citric acid. The surface process though commercially profitable for many years, is labored intensive and inefficient in its use of space. The submerged process has become the method of choice in the industrialized countries (Grewal 1995). because it is less labour intensive, yields higher production rate, and uses less space. Citric acid is used in food, Confectionery, beverages and pharmaceuticals industrial fields. Citric acid forms a wide range of metallic salts including complexes with copper, iron, magnesium, manganese and calcium. These salts are used as sequestering agents in industrial processes and as anticoagulants in blood preservation. Citric acid is used as plasticizer in plastic films and as an antioxidant in oil and fats.

Citric acid fermentation is one of the rare examples of industrial fermentation technology where academic discoveries have worked in tandem with industrial know-how, in spite of an apparent lack of collaboration, to give rise to an efficient fermentation process. The current world market estimates suggest that upwards of 4.0 x 10^{5}tonnes citric acid per year may be produced (Kristiansen et al. 1999). Citric acid is a major product but the upward trend in its use seen over many years is an annual 2-3% increase. The demand for this particular metabolite is increasing day by day which requires a much more efficient fermentation process for higher yield product (Moreira et al. 1996).

When applied to appropriate mass balances, it is possible to predict the utilization of substrates and the yield of individual products. Fermentation media for citric acid biosynthesis should consist of substrates necessary for the growth of microorganism, primarily the carbon, nitrogen. The basic substrates for citric acid fermentation using submerged technique of fermentation are beet or cane-molasses (Pazouki et al. 2000).

Citric acid fermentation is one of the primitive fermentations but still its production is increasing with passage of time. In 2007, its global production has exceeded 1.6 million tons (Pandey et.al.2013). One of the most important fungi used in industrial microbiology, ***Aspergillus niger***, has been employed for many years for the commercial production of citric acid. However, the

worldwide demand for citric acid is increasing faster than its production and more economical processes are required. ***A. niger*** is most commonly used for citric acidproduction.

This is because of the fact that this organism has capacity to utilize varieties of substrates due to its well-developed enzymatic system (Femi &Atere, 2013). Although ***Aspergillus niger*** is the traditional producer of citric acid, during the last 30 years the use of yeasts for citric acid fermentation processes has attracted the interest of researchers. Strains of ***Aspergillus niger*** need a fairly higher initial sugar concentration in the medium.

Many microorganisms have been evaluated for the production of citric acid including bacteria such as *Bacillus licheniformis, B. subtilis, Corynebacterium spp*. fungi such as *A. niger, A. awamori, A. foetidus, Penicillium restrictum* (Kubicek, 1998). Yeast such as *Candida lipolytica, C. intermedia* and *Saccharomyces cerevisiae* (Crolla and Kennedy, 2001; Archer et al., 2001; Kamzolova et al., 2003).

MATERIALS AND METHODS

Collection of Sample: The raw material used for the production of citric acid were lemon juice, potato starch and rice starch collected from local area of Dehradun(U.K). The sample were collected in a sterile container, brought to the laboratory and carried the isolation of ***Aspergillus niger***.

Isolation of Fungal Strain *Aspergillus niger*: The isolation of ***Aspergillus niger***was carried out by direct plate agar method. Take 1ml of lemon juice, potato starch and rice starch in a sterilised petri plate and then pour potato agar media to it. Swirrl the media in clockwise and anti-clockwise direction, solidify and incubated at 27 ^{O}C for 5-6 days . After incubation observe the plates for grayish, black spores of ***Aspergillu sniger***.

Screening ofFungal Strain *Aspergillus niger*: The isolated Fugal culture were screened for pure culture of ***Aspergillus niger*** on sterilized potato dextrose agar media. The isolated fungal strain of ***Aspergillus niger*** was maintained on PDA slants and stored at 4^{O}c in refrigerator.

Identification of Isolated *Aspegillus niger*: The identification of ***Aspergillus niger*** was carried out by staining method. Take a clean glass slide and palce a drop of distilled water. Take aseptically spores of *Aspegillus niger,* tease and place a drop of lacto phenol cotton blue to it. Cover the culture with clean cover slip and observe under microscope at10x, 45x and 100x for the spore formation. Observe for the formation of spore and total number of cells.

Production of Citric acid by Submerged Fermentation: The submerged fermentation of citric acid was carried out by shake flask technique. For the production of citric acid take 100ml of three different substrate i.e. Lemon juice, Potato starch and Rice starch

in a cleaned 250 ml Erlenmeyer flasks. Sterilised the medium at 15 p.s.i. for 15 minute, cool it and inoculate 1% isolated strain of ***Aspergillus niger***. Incubate the flasks at 30 ^{O}c for 5-6 days and calculate the citric acid titrimetrically.

Comparative study of culture media for citric acid production: The isolated strain of ***Aspergillus niger*** were screened for production of citric acid in two media i.e. Potato dextrose broth and Basal media (Appendix-A). The culture media were sterilized at 15 p.s.i. for 15 minute, cool and inoculated with spores of ***Aspergillus niger***. Incubate the culture at 30^{O}c for 5-6 days and calculate the citric acid titrimetrically.

CITRIC ACIDPRODUCTION: $\frac{\text{Titre x100 x10 x 0.064}}{\text{Vol of sample(10ml)}}$

RESULT AND DISCUSSION

Isolation of Aspergillus niger

Towards the goal of identifying fungi various strains were isolated from three substrate i.e. Lemon juice, Potato starch, Rice starch sample collected from local market of Dehradun (U.K). 7 fungal strains were isolated on Potato dextrose Agar media. The isolated strains were named as LJI, LJ2, LJ3, PS1, PS2, RS1, and RS2. The isolated strains were characterized by morphological and identified by Lacto phenol cotton blue staining (Table 1: ,Fig.- 1,).

The isolated strain LJ3, PS2, RS1 were confirmed as ***Aspergillus niger*** on the basis of staining(Fig.-2) ***Aspergillus niger*** has potential application in the food industry. Hence in the present study ***Aspergillus niger***was selected for the citric acid production. For citric acid production the isolated strain LJ3,PS2 and RS1 were screened on Potato dextrose agar media and shows maximum mycelial growth of ***Aspergillus niger*** . Where as LJ2, PS1 and RS2 does not shows growth (Table 1).

Production of Citric acid by *Aspergillus niger*

After screening of***Aspergillus niger*** (LJ3, PS2, RS1) strains were used for production of citric acid by using submerged state fermentation in aerobic conditions. The aerobic fermentation gave maximum production of citric acid by lemon juices at 30°C for 144hrs of 18.2g/L, Potato starch of 6.4g/L and Rice starch of 5.2g/L (Table, 2).

Comparative study of Citric Acid production by *Aspergillus niger* in Submerged state fermentation

The production of citric acid by ***Aspergillus niger*** in submerged state fermentation of two selective media i.e. Potato dextrose broth and Basal media gave maximum production for lemon juice (LJ3)

7.2g/L,10.6g/L, Potato starch (PS2) 5.5g/L, 8.1g/L and Rice starch (RS1) 3.3g/L, 4.4g/L. It has been observed that basal media gave maximum production of citric acid of strain LJ3 10.6g/L then Potato starch PS2 8.1g/L (Table,3).

Table 1: Isolation of *Aspergillus niger* from different substrate: Lemon juice, Potato Starch and Rice Starch

SR.NO.	Substrate	Strain No.	Spore colour	LCB Staining
1.	Lemon Juice	LJ1	White cottony growth	No conidia present
2.	Lemon Jiuce	LJ2	Dark grayish spore	No conidia present
3.	Lemon Juice	LJ3	Greenish blue, black spores	Conidia present from a foot cell on vesicles
4.	Potato Starch	PS1	White mycelia	No conidia present
5.	Potato Starch	PS2	Greenish blue, black spores	Conidia present from a foot cell on vesicles
6.	Rice Starch	RS1	Dark grayish spore	Conidia present from a foot cell on vesicles
7.	Rice Starch	RS2	White cottony growth	No conidia present

Fig 1 (c): Lemon Juice.

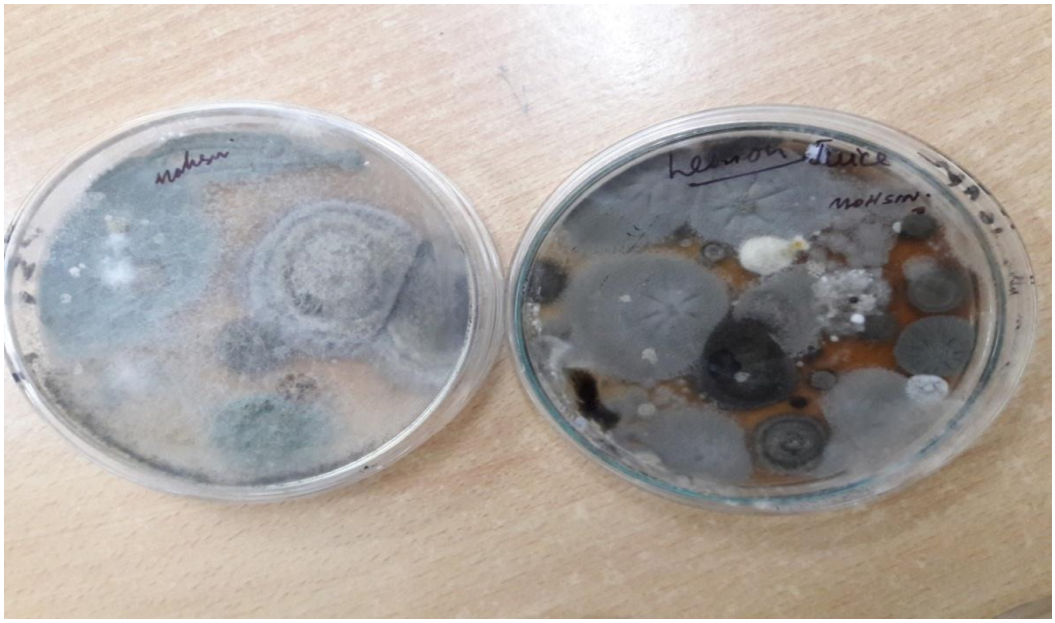
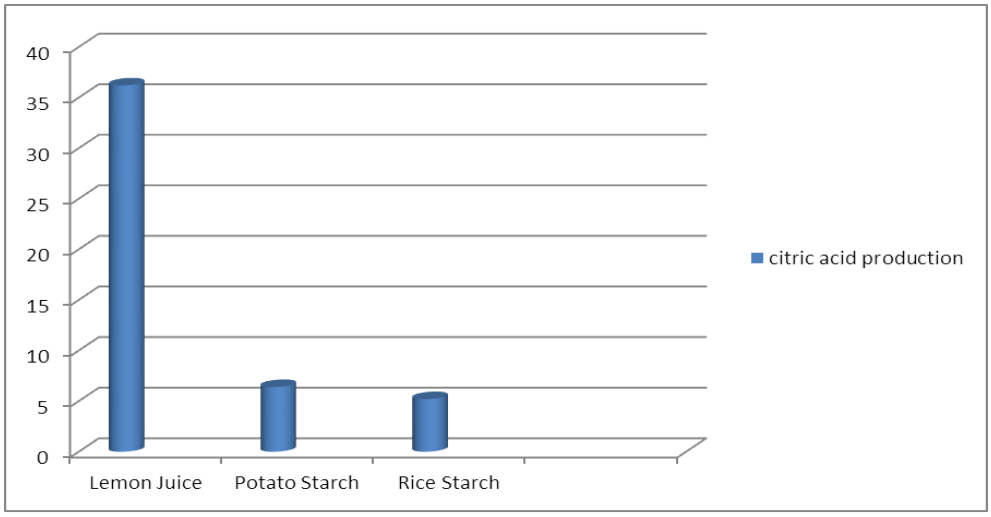


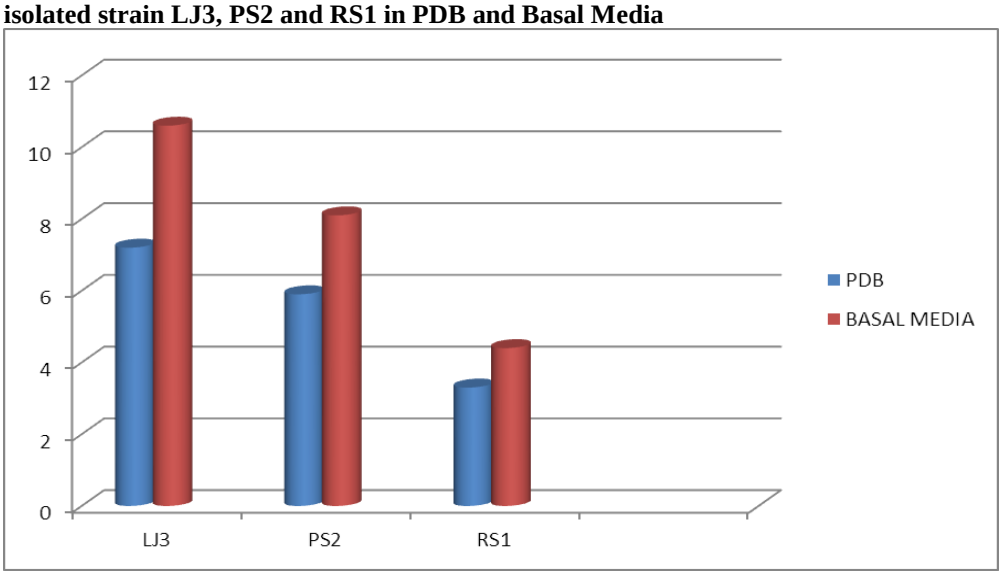
Figure 1: Isolation of *Aspergillus niger* from different substrate: Lemon juice, Potato Starch and Rice Starch.

Table 2: Submerged fermentation (SMF) by *Aspergillus niger* for the production of citric acid from Isolated Strain LJ3, PS2 and RS1 in aerobic condition.



Graph 1. Submerged fermentation (SMF) by *Aspergillus niger* for the production of citric acid from Isolated Strain LJ3, PS2 and RS1 in aerobic condition.

Table 3: Comparative study of Citric Acid production by *Aspergillus niger* in Submerged state fermentation (SMF) from isolated strain LJ3, PS2 and RS1 in PDB and Basal Media



Graph 2: Comparative study of Citric Acid production by *Aspergillus niger* in Submerged state fermentation (SMF) from isolated strain LJ3, PS2 and RS1 in PDB and Basal Media.

CONCLUSION

Citric acid is an important multi functional organic acid with a broad range of versatile uses in household and industrial applications. It is widely used in food, pharmaceutical, biomedicine, biopolymer synthesis, bioremediation and agriculture industries. It has application in beverages, cosmetics, textiles, chemical, bioleaching and detergent industries.

Commercially, Citric acid is produced by large scale fermentation using Fungal / Yeast strain in aerobic fermentation. Citric acid production can be improved by optimizing the fermentation parameter such as substrate conc., pH, nutrient conc., temp. , oxygen and nitrogen supply.

The aerobic fermentation of ***Aspergillus niger*** gave the maximum citric acid production of LJ3 i.e. 36.2g/L and least byRS1 i.e.5.2g/Lin aerobic conditions.

The best citric acid production by ***Aspergillu sniger*** using Lemon juice as a subs trate shown in Basal media at 30^{O} C for 144 hour s i.e.10.6g/L and least at Potato dextrose broth at 30^{O} C for 144 hour s i.e. 4.4 g/L. The other substrate such as Potato starch and Rice starch gave 8.1g/L and 5.9g/L in Basal media and Potato dextrose broth. Hence144hrs incubation of ***Aspergillus niger*** the Basal media used as a selective media for the production of citric acid.

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