Screening and Isolation of Higher Yielding Strain for Citric Acid Production

Damu Mokinda Survase
Asst Prof. in Dept. of Botany,
Karmveer Ramraoji Aher Arts, Science & Comm College, Deola,
Tal. Deola, Dist. - Nashik,
Maharashtra- 423102 (India).

Abstract

Microbial production of citric acid carried out by various microorganism especially commercial productions carried out by the Aspergillus niger since twentieth century. Citric acid used as flavouring agent in food, beverages, Pharmaceutical and confectionary industries. Traditional source of citric acid is a citrus fruits. World demand and use of Citric acid in the different industries (food, beverages and pharmaceutical industries) and kitchens is increasing day by day, more than 80 lack tons/year (Pohnerkar and Desai, 2014). Natural source of citric acid was citrus fruits which contain 7-9% of citric acid which is extracted by traditional method. It was obtained by the processing of citrus fruits and precipitation with calcium salt. Now a day citrus fruits could not sufficient for fulfill the required growing demand of citric acid. Hence alternative source of citric acid production is a microbial origin.

In the present study Aspergillus niger considered as more suitable than the other microorganisms for production of citric acid by fermented broth method. Many factors were affecting invitro production of citric acid. It was found that sucrose is suitable carbon source (13-15%). Similar study was carried out by Currie (1917), Wang-Jiaglong (1998). Different factors affecting microbial production of citric acid like methanol, strain of microorganism, temperature, carbon source, P^H etc. were play important role in the production of citric acid. Isolation of Aspergillus niger strain from soil and lemon fruits sample collected from different localities, 2% methanol added into culture medium gives higher yield (Dhankar et al, 1974), 13% sucrose was better carbon source than the glucose and other, 4.5 P^H was suitable for higher production of citric acid in vitro and in 28-30^0 temperature healthy growth was found (Aftab Nadeem et al, 2010).

Keywords: *Citric acid, Fermentation broth, Aspergillus niger, lemon fruit, ascending chromatography*

I. INTRODUCTION

Citric acid, a tricarboxylic acid soluble in water and widely used an acidifying agent and antioxidant in a food, beverages and pharmaceutical industries (Kapoor et. al, 1982). Citric acid is mainly produced by submerged fermentation of Aspergillus niger. The yield depends upon composition of medium (Sing S.P. et al, 1998) and on the microbial strain (Aftab Nadeem et al, 2010). Citric acid production using Aspergillus niger is influenced by the process variables such as initial sucrose concentration, pH, nutrient concentration, additive, incubation period, temperature etc. However demand of Citric acid production is increasing faster than the its production and hence requires more economical process. The fitness of solution would be measured by determining the total weight of the proposed solution. The higher the weight, greater the fitness provided that solution (K. Anand Kishor et al, 2008). Similar Study carried with cane bagasse by Abdullah Al Mahin et al, 2008 and Chaturvedi Madhusudan et al, 2010.

In the present study locally isolated Aspergillus niger employed for the production of Citric acid. In the present study different factors affecting microbial production of citric acid like strain of microorganism, carbon source, P^H and ascending chromatography were considered. Isolation of Aspergillus niger from soil sample collected from different localities, 2% methanol added into culture medium gives higher yield, 13% sucrose (LF1 4.2 mg/ml, FS3 3.9mg/ml and CCG5 3.7mg/ml) was better carbon source than the glucose, Galactose and fructose, 4.5 P^H was suitable for healthy production of citric acid in vitro and in 28-30^0 temperature healthy growth was found.

II. MATERIALS AND METHODS

Aspergillus niger is a fungus recommended for the production of various organic acids. Citric acid is one of the important organic acid synthesized and released on synthetic medium by this fungus. Natural source of Aspergillus niger is a soil, hence soil from

different localities from Nashik District was collected and tested for higher yielding strain of *Aspergillus niger* for the production of citric acid and it also isolated from lemon fruits.

A. Isolation of Microorganism

The natural source of *Aspergillus niger* is the soil. Collection of soil samples from different locations with respect to P^H of the soil. In this way ten soil samples were collected and diluted 1/100, 1/1000 and 1/10000 of each sample. The diluted samples were observed and they were purified by sub culturing on Czepeck's Dox agar slants. Culture Plates were incubated at 28 ^{0}C and isolated cultures were observed and they were purified by sub culturing on Czepeck's Dox agar slants. Isolation from affected fruits of lemon and pure culture was prepared and maintained. Similar to soil screening lemon strain tested for citric acid production. Similarly fresh and healthy lemon fruits were soaked in the water placed in closed container for a 5- 7 days. After incubation pure cultures of *Aspergillus niger* maintained on culture medium.

B. Aspergillus Niger

Aspergillus niger high yielding strains were LF1, FS3 and CCG5 purified and cultured by streak plate method, loopful of culture at 25 ^{0}C for a week. It was used for further fermentation experiment.

C. Screening for Organic Acid Production

Spore from slant cultures were inoculated on sterile Czepeck's Dox agar medium plates incorporated with Bromo-cresol green dye. Plates were incubated at 28^{0}C for 24 to 28 hrs. And checked colour change blue to yellow indicates organic acid production. To study the effect of media component on citric acid fermentation, all the components except one to be studied are kept constant with respect to the control medium and one component concentration is changed in particular range. Broths were tested for citric acid production by selected strains of Aspergillus niger.

D. Screening of Aspergillus niger for citric acid production

Selection and isolation of micro-organism which produces high amount of citric acid. Primary screening determines which microorganism is able to produce a citric acid followed by secondary screening to determine capacity of that organism producing quantitatively.

Table No.1: Isolation and Selection of Higher Yielding Strain from Soil and Lemon Fruits

Sr. No.	Area	PH	Culture	Yield mg/ml
1.	**Lemon fruits**		**LF1**	**4.0**
			LF2	3.7
			LF3	4.3
			LF4	2.8
			LF5	3.9
2.	**Forest soil**	6.2	FS1	2.5
		6.1	FS2	3.2
		5.9	**FS3**	**3.9**
		5.4	FS4	2.9
		6.1	FS5	3.5
3.	**College Campus Soil**	4.8	CCS1	2.6
		5.2	CCS2	2.7
		4.9	CCS3	1.6
		5.1	CCS4	1.8
		4.8	**CCS5**	**3.7**

Table No.2: Effect of Different Carbon Source % on the Production of CA by *Aspergillus niger*

Carbon source	Yield mg/ml Sucrose			Yield mg/ml Glucose			Yield mg/ml Galactose			Yield mg/ml Fructose		
%	LF1	FS3	CCS5	LF1	FS3	CCS5	LF1	FS3	CCS5	LF1	FS3	CCS5
10	1.6	2.1	1.9	1.4	1.1	1.3	1.1	0.8	0.8	1.2	1.6	1.7
11	1.9	1.8	1.9	1.4	1.6	1.3	0.5	0.8	0.9	1.1	1.5	1.3
12	2.5	2.6	2.8	1.8	1.6	1.9	0.9	0.7	1.0	1.2	1.5	1.4
13	**4.2**	**3.9**	**3.7**	**2.9**	**2.4**	**1.8**	**0.6**	**0.9**	**1.6**	**1.6**	**1.8**	**1.2**
14	3.1	2.9	2.6	2.4	2.1	2.3	1.1	1.5	1.7	1.9	2.0	1.6
15	2.9	2.4	2.1	1.9	1.8	1.8	0.9	0.8	0.7	1.7	1.9	1.4
16	1.8	1.4	1.3	1.0	1.2	1.3	0.5	0.7	0.6	0.9	0.9	0.8

Table No. 3: Effect of pH on the production of CA by Aspergillus niger

Sr. No.	pH	LF1	FS3	CCS5
1.	2.5	2.6	2.8	2.9
2.	3.0	2.8	2.8	3.0
3.	3.5	3.0	3.1	3.3
4.	4.0	2.9	3.5	**3.9**
5.	**4.5**	**3.9**	**3.7**	3.8
6.	5.0	1.8	2.1	1.9
7.	5.5	1.3	1.2	1.5

Graph Fig. Showing Effect of pH on Production of Citric Acid

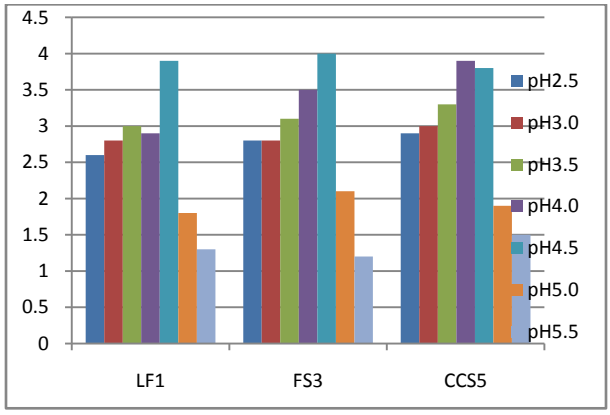
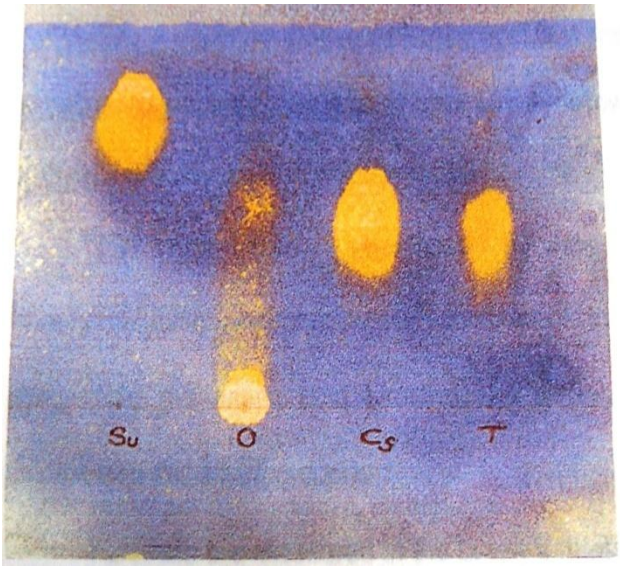


Table No.4: Ascending Chromatography of Organic Acid and Test Solution

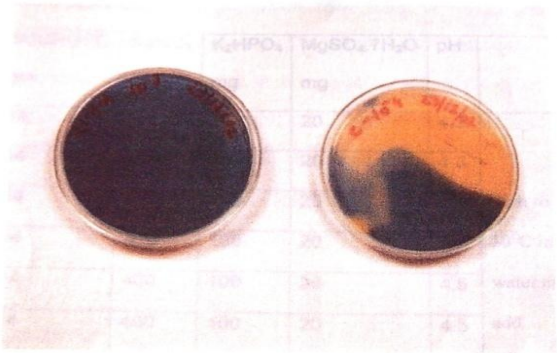
Sr. No.	Sugar	Distance travelled by solvent	Distance travelled by solute	Rf	% Rf
1.	**Citric acid**	**10.0**	**4.0**	**0.4**	**40**
2.	Oxalic acid	10.0	5.0	0.5	50
3.	Succinic acid	10.0	6.0	0.6	60
4.	**Test**	**10.0**	**4.0**	**0.4**	**40**

Photograph No.1: Chromatogram Showing Position of Standard Citric Acid and Fermented Broth



Su-Succinic acid, **O**-Oxalic acid, **Cs**-Standard citric acid, **T**-Fermented broth

Photograph No. 2: Primary Screening of Organic Acid Production on Culture Medium by using Bromocresol Green Dye



III. RESULT AND DISCUSSION

It was clear from Table No.1 Soil from different localities was collected and different concentration cultures prepared and from which Aspergillus niger strains were isolated from it. These were checked for citric acid production. The highest yield was reported from Aspergillus niger strain LF1 (4.0 mg/ml), FS3 (3.9 mg/ml) and CCG5 (3.7 mg/ml). It was found that the highest yield was found when the pH of medium was 4.5, above and below this pH the yield was decrease.

Table no. 2, When all the three cultures were tested for different carbon sources and their concentration (Sucrose, Glucose, Galactose and Fructose), it was found that the at sugar concentration

(Sucrose) 13% all the three cultures LF1 (4.2 mg/ml), FS3 (3.9 mg/ml) and CCS5 (3.7 mg/ml) gave the higher yield. It was also found that sugar concentration (15% to above) the yield found decreased as compared to 13% and residual sugar observed higher concentration of sugar in the culture medium. Kovats (1960) reported that higher sugar concentration (15 to 18%) greater amount of residual sugars remains in the medium and process become uneconomical. Similar study carried out by Gupta et al, 1976.

pH play an important role in the production of citric acid by Aspergillus niger. It was clear from Table no. 3 that pH of 4.5 was found to be suitable for all the three cultures for the yield of citric acid. The culture SG3 showed higher yield at pH 4.0. This showed the contrasts with the finding of Prescott and Dunn (1987) which claims that the initial pH for sucrose.

It was confirmed from the Table no. 4 that ascending chromatography was done by using the solvent system n-Butanol, formic acid and water in the proportion of 10:2:5 respectively. It was prepared by separating funnel and out of the two layers upper organic layer was used. The fermented broth concentrated by evaporating it in a Petri dish. Standard Succinic acid, oxalic acid and citric acid with test fermented sample were loaded. Chromatogram removed, dried and sprayed with 0.4% Bromo-Cresol Green prepared in ethanol (pH is equal to 6.7) and Rf values were calculated. Test sample and standard citric acid Rf value are matched. Rf value of samples compared with the organic acid and from this it was confirmed that fermentation broth contains citric acid

ACKNOWLEDGEMENT

Author is thankful to Head department of Botany and our Principal for providing constant support, facility and encouragement during this experiment.

REFERENCES

[1] Abdullah – Al –Mahin, Shek Mehdi Hasan MahboobHossain Khan and Rehan Begum : Citric Acid production by Aspergillus niger through solid state cane bagasse, Banglades Journ Microbiology, Vol 25 ,Number 1, June 2008 , pp-9-12.

[2] Aftab Nadeem, Quratulain Sayed, Shahjahan Baig, Muhammed Irfan and Muhammed Nadeem : Enhanced production of citric acid by Aspergillus niger M-101 using lower alcohols, Turk Biyokimya Dergisi (Turkish Journal of Biochemistry), 2010, 35; 10; 7-13

[3] Ashish Kumar and V.K. Jain : Solid state fermentation studies of citric acid production, African Journal of Biotech, 2008, Vol. 7 (5)pp. 644- 650.

[4] Chaturvedi Madhusudan ,Singh Manoj, Chugh M Rishi : Citric acid production from cane molasses using submerged fermentation by Aspergillus niger. ATCC9142, Journal of Pharmacy Research 2010, 3(6), 1215-1222.

[5] Currie J.N. : The citric acid fermentation of Aspergillus niger, Can. Journal of microbiology, 1917, 7, 447-453.

[6] Dhankar H.S., Ethiraj S. and Vyas S.R. (1974): Effect of methanol on citric acid production from sugar cane molasses by Aspergillus niger, Indian Journ. Technology,12 316-317.

[7] Gupta J.K., Heding L.G. &Jargensen O.B. : Effect of sugars, pH and Ammonium nitrate on formation of Citric acid by Aspergillus niger, Acta. microbiology Acad. Science Hung., 1976, 23, 63-67.

[8] J.K. Pohnerkar and S.A. Desai : Selective synthesis of citric acid from molasses using Aspergillus niger, International Journ. of Engg. trends and technology,2014, Vol.9 pp 7-9

[9] Jianlong Wang: Improvement of citric acid production by Aspergillus niger with addition of phytate to beet molasses, Bioresource Technology, Sept 1998, Vol. 65 Issue3, pp 243-245

[10] K. Anand Kishor, M. Praveen Kumar, V. Ravi Krishna and G. Venkat Reddy: Optimization of process variables of citric acid production using Aspergillus niger in a batch fermenter, Engineering Letter, 2008, 16:4, EL_4_17.

[11] Kapoor K.K., Chaudhary K. and Tauro P: Citric acid in Prescott and Dunns Industrial microbiology, 1982, 4th ed Greed (ed). AVI Publ. Company West port, Connectcut.

[12] Kovats J : Studies on submerged citric acid fermentation, Acta microbiology Pat 9,1960, 275-287.

[13] Prescott and Dunn : A Industrial microbiology, 4Th edition, CBS publisher and distributor, New Dehli, India, August, 1987, p 710-715.

[14] Sing, S.P.; Verma, U.N.; Kishor, M. And Samdani, H.K. : Effect of medium concentration on citric acid production by submerged fermentation. Orient Journal of Chemistry, March 1998, vol. 14, no. 1, p. 133-135