Short Communication

Pak. J. Sci. Ind. Res. 2004 ***47****(5) 403-405*

COMPARISON OF HYPER PRODUCER *ASPERGILLUS NIGER* CULTURES (IFS-5, IFS-6 AND IFS-17) FOR CITRIC ACID FERMENTATION IN SURFACE CULTURE

Ikram-ul-Haq [a]*, ***Sikander Ali*** [a] ***and M A Qadeer*** [b]

[a]*Biotechnology Research Centre, Department of Botany, G C University, Lahore, Pakistan*

[b]*Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan*

(Received March 17, 2002; accepted August 20, 2004)

Citric acid fermentation by *Aspergillus niger* is an aerobic process and the organism needs a fairly high and constant oxygen supply for its growth (Hang 1988; Haq *et al* 2000). Surface culture technique (SCT) is a conventional method of citric acid production. Most of the pilot plants are using this technique due to low energy consumption and manpower involved (Singh *et al* 1998). In SCT, the substrate remains stationary and organism form mycelial mat on the surface of medium. The relation between constitution of the fermentation medium and rate of citric acid production has been investigated (Elimer and Ewaryst 1995). Sucrose salt medium as synthetic fermentation medium while cane or beet molasses as natural fermentation medium have long been employed as usual routine basal media (Ali *et al* 2001). Clark *et al* (1965) obtained 80% conversion of available sugar, 8 days after incubation. Farouk *et al* (1977) pointed out that the age of culture also affect the yield of citric acid. Both of these authors used synthetic medium in their study on citric acid fermentation. The mutant strains have greater ability to produce citric acid. The present investigation deals with the time course study during citric acid production by surface culture technique using three different mutant cultures of *A. niger* (IFS-5, IFS-6 and IFS-17) and their comparison on kinetic basis.

Organism. In the present study, 3 different mutant strains of *A. niger* (hyper producers of citric acid) were used. These strains (IFS-5, IFS-6 and IFS-17), have already been developed by mutation (Ali *et al* 2001) in Biotechnology Laboratories, Government College University, Lahore and maintained on potato dextrose agar medium. The cultures were stored at 4°C in a refrigerator.

Culture medium. Sucrose salt medium containing (g/l); sucrose 150.0, $MgSO_4.7H_2O$ 0.25, KH_2PO_4 2.5, NH_4NO_3 2.5 at pH 3.5 was used as the basal fermentation medium.

*Author for correspondence; E-mail:ikrhaq@yahoo.com

Fermentation technique. Citric acid was produced by surface culture technique, following the method of Singh *et al* (1998). Conidial inoculum ($1x10^7$ conidia/ml), prepared in sterilized distilled water was used. The optimum conditions for citric acid production were investigated in 250 ml Erlenmeyer cotton wool plugged conical flasks, containing 25 ml fermentation medium. The flasks were incubated at 30°C for 7 days. The results are sum mean of three parallel replicates.

Analysis. Dry cell mass was determined according to Kirimura *et al* (1992). Residual sugar was estimated by DNS method (Ghose and Ghen 1970) while pyridine acetic anhydride method was employed for the determination of citric acid as reported by Marrier and Boulet (1958). The kinetics of time course was also undertaken (Pirt 1975).

Time course study is one of the most critical factors, which determines the efficacy of the process along with product formation (Elimer and Ewaryst 1995). The data of Table 1 shows the biosynthesis of citric acid at different intervals of time. Three different mutant strains of *A. niger* (IFS-5, IFS-6 and IFS-17) were used for their time course comparison. These cultures were incubated at 30°C for 1-11 days. The maximum production of citric acid (48.14 g/l) with mutant IFS-5 was obtained 10 days after incubation, which seems to be uneconomical due to longer fermentation period. When IFS-6 was used for inoculating the culture medium, a maximum citric acid production of 31.52 g/l was obtained with a high degree of consumable sugars (96.0 g/l). Although the fermentation period became very short (5 days) as compared to mutant IFS-5 but the yield of product was too low for an economical process.

The maximum production of citric acid (46.22 g/l) by mutant strain of *A. niger* IFS-17, was achieved 7 days after the inoculation. The dry cell mass and sugar consumed were 23.20 and 94.0 g/l, respectively. Further, increase in the incubation period did not enhance the production of citric acid, rather it was decreased. It might be due to the reduction of sugar contents in the fermentation medium and accumulation of other by-products. Thus incubation period of 7 days was found to be optimum for maximal citric acid biosynthesis. Our results are in agreement with the findings of many workers (Jaszwry *et al* 1971; Singh *et al* 1998). For maximum citric acid production, the optimum time of incubation varies from organism to organism depending on fermentation medium provided (Elimer and Ewaryst 1995).

The kinetic study of time course during citric acid fermentation by mutant strains of *A. niger* was also worked out. There was a marked difference of product yield coefficients (Yp/s and Yp/x) among different mutant strains i.e., maximum Yp/s value in case of IFS-17 (0.492 g/g) was much higher as compared to mutants IFS-5 and IFS-17 (0.384 and 0.328 g/g)

Table 1
Time course study during citric acid fermentation by *Aspergillus niger*

Fermentation period (h)	Dry cell mass (g/l)			Sugar consumption (g/l)			Citric acid (g/l)		
	IFS-5	IFS-6	IFS-17	IFS-5	IFS-6	IFS-17	IFS-5	IFS-6	IFS-17
24	2.50	3.72	4.00	46.5	24.5	35.0	1.56	2.13	0.67
48	5.05	9.10	7.50	54.2	56.6	49.5	2.10	6.25	1.26
72	6.55	16.25	10.61	69.5	79.1	56.2	6.26	9.55	4.50
96	9.00	24.80	12.31	79.5	88.5	73.0	9.55	16.12	16.80
120	13.25	26.45	17.22	87.4	96.0	86.0	13.18	31.52	20.04
144	16.40	29.05	20.11	96.0	100.8	89.5	16.52	26.27	38.55
168	21.15	32.46	23.20	98.5	114.5	94.0	23.55	21.05	46.22
192	24.52	36.62	26.19	104.6	121.0	99.5	32.16	18.66	37.50
216	28.90	37.15	29.27	121.0	126.2	100.6	39.25	18.50	29.53
240	34.62	40.65	31.12	126.5	132.5	106.5	48.14	17.16	27.62
264	39.46	41.20	31.50	128.5	135.6	118.0	42.66	17.05	19.40

Sugar added 150 g/l, Initial pH 3.5, Temperature 30°C

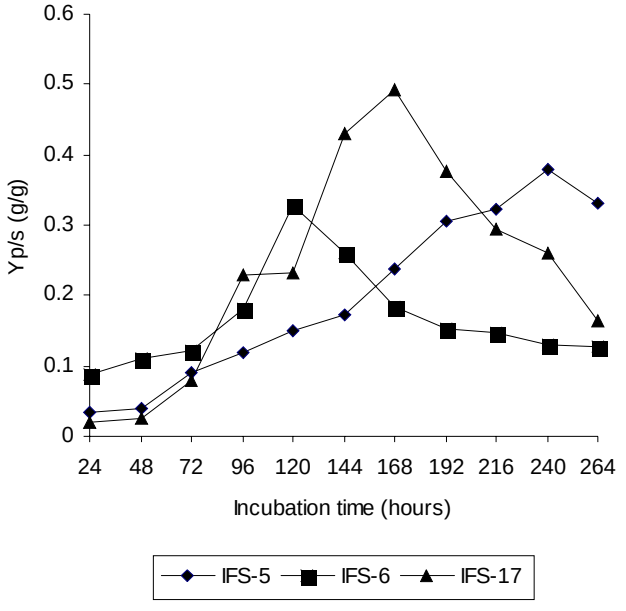

Fig 1. Comparative study of product yield coefficients (Yp/s, g/g) during citric acid fermentation by *A. niger* strains (IFS-5, IFS-6 and IFS-17). The value of Yp/s was determined by product (g/l)/substrate utilized (g/l).

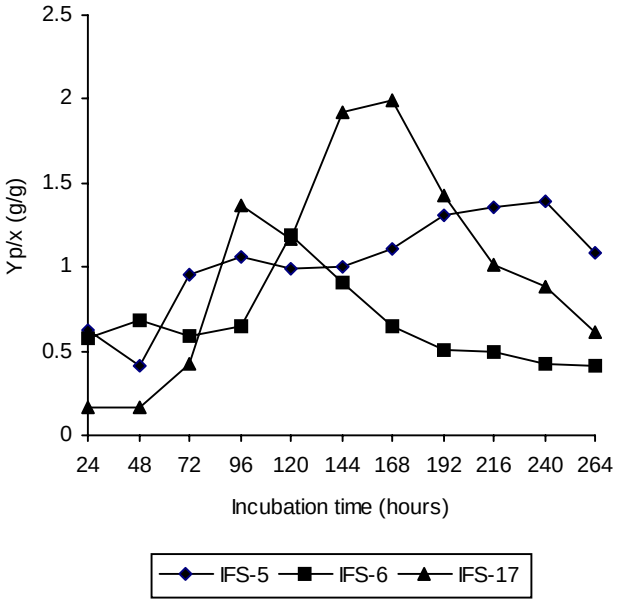

Fig 2. Comparative study of product yield coefficients (Yp/x, g/g) during citric acid fermentation by *A. niger* strains (IFS-5, IFS-6 and IFS-17). The value of Yp/x was determined by product (g/l)/cell mass (g/l).

and the maximum Yp/x value in case of mutants IFS-5 and IFS-6 (1.402 and 1.192 g/g) was lower in comparison with IFS-17. So, the mutant *A. niger* IFS-17 is better producer of citric acid as compared to others. Meyrath and Ahmed (1989) have attempted to reduce the fermentation time by vermiculite addition and found that time is reduced from 0-9 days. Lakshminarayan *et al* (1975) incubated cultures of *A. niger* for 6 days and achieved good results. Shamrai and Orlow (1986) described that the optimum period of fermentation was dependent on the intensity of fermentation and got better citric acid production (28.65 g/l), 8 days after incubation. So, our finding (46.22 g/l) citric acid, 7 days after inoculation by mutant IFS-17 is more encouraging and significant as compared to Shamrai and Orlow (1986).

The mutant strain of *A. niger* IFS-17 is a better citric acid producer (i.e. Yp/s=0.492 g/g) due to faster growth rate. Time

required for maximal citric acid production depends mainly on the fermentation design, type of the strain and composition of basal medium. Mutation can raise the status of fermentation process. The mutant IFS-17 is D-glc-resistant and has been preserved in paraffin oil as a master culture. Proper aeration, suitable depth and Cu^{++} ions addition in the fermentation medium may increase the mycelial branching level and subsequently citric acid productivity. Better performances can be obtained by more quantitative analysis and mathematical modelling, which are left for further study.

***Key words*:** Citric acid, Biosynthesis, Fermentation, *Aspergillus niger*.

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