Novel method of citric acid production by *Aspergillus candidus* NCIM-883 exposed to Acridine

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Abstract: The efficacy of Acridine on novel method of citric acid production by *Asperillus candidus* NCIM-883 has been assessed. It has been found that mutagen i.e. Acridine under trial has stimulatory effect on novel method of citric acid production by *Aspergillus candidus* NCIM-883 and enhances the yield of citric acid to an extent of 14.82% higher in comparison to control fermentor flasks i.e, 5.46348g/100ml in 10 days of incbation period 2.2pH and 28^0 C citric acid.

Key words: molasses, mutagen, Acridine and Aspergillus candidus NCIM-883

Introduction

Mutations or changes in DNA sequences are credibly important in biology, from evolution and diversity to cancer and genetic diseases. Irradiation is exposure to radiation and chemical mutagens are chemicals that cause changes to DNA sequences [1]. Mutation can result in several different types of change in DNA sequences. These can either have no effect or prevent the gene from functioning properly or completely[2] . Mutations can involve large sections of DNA becoming duplicated usually through genetic recombination[3]. These duplications are a major source of raw material for evolving new genes, with tends to hundreds of genes duplicated in animal genomes every million years[4].

Most genes belong to larger families of genes of shared ancestry[5]. Novel genes are produces by several methods, commonly through duplication acid mutation of ancestral, by recombining parts of different genes to form new combinations with new functions [6.] .There domains that can be mixed together to produce genes encoding new proteins with novel properties[7]. For example, human eye user four genes to make structures that sense light, three for color vision and one for night vision: all four arose from a single ancestral gene.[8]

It is obvious that various chemical mutagens and some other mutagenic agents are used to produce mutants. The present study was undertaken for the production of citric acid by *Aspergillus candidus* NCIM-883 exposed to Acridine.

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Materials and Methods:

The influence of Acridine on production of citric acid by *Aspergillus candidus* NCIM-883. The composition of the production medium for the production of citric acid by *Aspergillus candidus* NCIM-883 was prepared as follows.

Molasses	: 14.85 g [15%]
NH_4NO_3	: 24.75 g [0.25%]
KH_2PO_4	: 24.75 g [0.25%]
$MgSO_4$, $7H_2O$	: 34.65 g [0.35]
pH	: 2.2

The above compositon mediyum represents volume of a fermentor flask ie " 100ml" citric acid production by *Aspergillus candidus* NCIM-883. Now the same production was prepared for 99- fermentor flask ie, each contained '100ml' of production medium. The above 99- fermentor flasks were then arranged to 11- sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3- subsets, each consisting of 3- fermentor flasks. The remaining 9-fermentor flasks out of 99- fermentor flasks were kept as control and these were also rearranged in 3- subsets each consisting of 3- fermentor flasks. After preparing the above sets of fermentor flasks M/1000 solution of Acridine was prepared & form the above mutagen solution 1.0, 2.0,3.0, 4.0, 5.0,6.0,7.0,8.0,9.0, ad 10 ml was added to fermentation flask of above 1^{st} to 10^{th} sets respectively. Thus, the molar concentration of Acridine in 1st,2^{nd} , 3^{rd}, 4^{th}, 5^{th}, 6^{th},7^{th}, 8^{th}, 9^{th}, 10^{th}, subsets approximately as given below.

$Ax10^{-x}$ M

$1.0x10^{-5}$M to $10.0x10^{-5}$ M

Where, A = amount of mutagen,

In ml, i.e. 1.0ml ..... to 10 ml

x=Molarity of the mutagen solution

The above fermentor flasks were then sterilized, cooled, inoculated, incubated at 28^0C and analysed after 7, 10, and 15 days for citric acid formed and sucrose sugar left unfermented.

The pH of the production medium was adjusted to 2.2 by adding requisite amount of KCl-HCl buffer solution and this pH was also determined by a pH meter. The above composition medium represents volume of a fermentor flask i,e. 100ml citric acid production by *Aspergillus candidus* NClM-883.

Results and Discussion:

The data recorded in the Table-1 shows that Acridine has stimulatory effect on Novel Method of citric acid production by *Aspergillus candidus* NCIM-883.

Table-1

Novel method of citric acid production by *Aspergillus candidus* NCIM-883 exposed to Acridine

Concentration of Mutagen	Incubation period in days	Yield of citric acid* in g/100 ml	Molasses Left unfermented in g/100 ml	% of citric acid increased in 10 days
Control	10	5.46348	3.19861	-------
$1.0x10^{-5}$ M	10	5.53222	3.12986	(+) 1.25817
$2.0x10^{-5}$ M	10	5.66310	2.99888	(+) 3.65271
$3.0x10^{-5}$ M	10	5.75074	2.91131	(+) 5.25782
$4.0x10^{-5}$ M	10	5.87878	2.78329	(+) 7.60138
$5.0x10^{-5}$ M	10	5.96988	2.69218	(+) 9.26881
$6.0x10^{-5}$ M	10	6.03489	2.62718	(+) 10.45871
$7.0x10^{-5}$ M**	10	6.27350***	2.38849	(+) 14.82608
$8.0x10^{-5}$ M	10	6.15564	2.50644	(+) 12.66884
$9.0x10^{-5}$ M	10	6.02397	2.63808	(+) 10.25884
$10.0x10^{-5}$ M	10	6.02216	2.63990	(+) 10.22571

* Each value represents mean of three trials

** Optimum concentration of mutagen used

*** Optimum yield of citric acid

(+ve) values indicate % increase in the yield of citric acid after 10 days

Experimental deviation (±) 1.5- 3%

The data recorded in table-1 represents that Acridine has stimulatory effect on production of citric acid by *Aspergillus candidus* NCIM-883. The maximum yield of citric acid i.e, 6.27350 g/100ml in the presence of Acridine was observed at $7.0x10^{-5}$M molar concentration in 10 days of optimum incubation period which is 14.82% higher in comparison to control fermentor flasks i.e, 5.46348 g /100 ml in the same times course and other some experimental parameters.

It has been observed that molar concentration of the mutagen, i.e, Acridine from $1.0x10^{-5}$M enhances the yield of citric acid to a certain order being 1.25%, 3.65%, 5.25%, 7.60% and 9.26% higher in comparison to control flasks but at 6.010^{-5}M & $7.0X10^{-5}$M the yield of citric acid shifted to be 10.45% and 14.82% higher in comparison to previous

concentrations of Acridine taken into experimental trials. It has been observed further that after optimum concentration, i.e, $7.0x10^{-5}$M, the addition of the same mutagen to the production medium causes fall in the yield of citric acid gradually & reaches to 14.82%

Conclusion:

The higher molar concentration of Acridine were not much favourable for the production of citric acid by *Aspergillus candidus* NCIM-883. So, the gradual addition of the mutagen. Acridine after certain concentration were not beneficial for the acid fermentation process. However, at all the experimental concentrations of Acridine used the yield of citric acid by submerged fermentation has been found higher in comparison to control fermentor flasks.

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