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Studies on LSF technique for the bioproduction of H_3Cit exposed to carbamate mutagen

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Abstract : Sodium diethyl dithiocarbamate (SDDC) is a chemical compound with various applications. Carbamate compounds can have various biological effects, including potential, mutagenicity. Certain carbamates may cause genetic mutations. Research focuses on understanding the mechanism of carbamate induced mutagenicity and carcinogenecity. Carbamate mutagens could potentially alter the genetic makeup of microorganisms used in citric acid fermentation by *Aspergillus niger*. Mutations induced by carbamates might affect the microorganisms ability to produce citric acid, potentially altering fermentation efficiency or product yield. Researcher might investigate the use of carbamate mutagenes to induce mutations that enhances citric acid production or improve fermentation efficiency.In the pressent communication the impact of carbamate mutagen, i.e., sodium diethyl dithiocarbamate (SDDC) on LSF technique for the bioproduction of H_3Cit by fungal strains of *Aspergillus wentii* SS-63, *Aspergillus carbonarius* SS-65, *Aspergillus foetidus* SS-67 and *Aspergillus niger* SS- 69 has been assessed for their H_3Cit producing capacity. It has been found that fungal strain of *Aspergillus niger* SS-69 has been found most affective and significant as H_3Cit producer. It has been found that the carbamate mutagen, i.e., SDDC has stimulative impact on bioproduction of H_3Cit by *Aspergillus niger* SS-69 and enhances the yield of H_3Cit to an extent of 20.416 % higher in comparison to control fermentation flask, i.e., 9.375gm/100ml under optimised parameters.

(Keywords : Carbamate mutagen SDDC, H_3Cit, LSF technique).

Introduction

Citric acid is represented by the chemical formula H_3Cit because it is a triprotic acid, meaning it can donate three protons (H^+ ions) in aqueous solution.

The chemical formula for citric acid is $C_6H_8O_7$, but it is often represented as H3Cit to emphasize its acidic properties. The "H_3" part of the formula indicates that citric acid has three ionizable hydrogen atoms, which can dissociate to form protons (H^+ ions) in solution.

In aqueous solution, citric acid can dissociate into three different ions:

1. H_3Cit (citric acid) $\rightarrow$ H_2Cit-(hydrogen citrate) + H^+ (proton)
2. H_3Cit (hyrdogen citrate) $\rightarrow$ $HCit_2$-(hydrogen citrate) + H^+ (proton)
2. $HCit_2$-(hyrdogen citrate) $\rightarrow$ Cit_3-(citrate) + H^+ (proton)

The "Cit" part of the formula represents the citrate ion, which is the conjugate base of citric acid. The citrate ion has a negative charge and can accept protons (H^+ ions) to form citric acid.

The representation of citric acid as H_3Cit is useful because it:

1. Emphasizes the acidic properties of citric acid.
2. Indicates the number of ionizable hydrogen atoms
3. Provides a simple and concise way to represent the chemical formula for citric acid.

Overall, the representation of citric acid as H_3Cit is a convenient and informative way to describe its chemical properties and behavior in solution.

The study of chemical mutagenesis is sure to reveal even greater complexities than these, for there are several quite different classes of mutagenic agents. The influence of different

chemical mutagens in different fermentations process has been investigated by a number of workers[1-20]. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes[21-55]. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manupulation and exploitation specially for citric acid fermentation and in view of this the authoress has studied the influence of SDDC on biotic production of citric acid by *Aspergillus niger* SS-69.

Experimental

The influence of SDDC on liquid state fermentative bioproduction of citric acid by *Aspergillus niger* SS-69. The composition of the production medium for liquid state fermentative bioproduction of citric acid by *Aspergillus niger* SS-69 has been prepared as follows: Molasses: 32%(w/v), NH_4NO_3: 0.350% , KH_2PO_4 : 0.375%, $MgSO_4.7H_2O$: 0.400%, pH : 2.1. 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 ascertained by a pH meter.

The above composition medium represents volume of a fermenter flask, i.e., "100ml" production medium of citric acid by *Aspergillus niger* SS-69. Now, the same production medium for bioproduction of citric acid by *Aspergillus niger* SS-69 was prepared for 99-fermenter flask, i.e.; each contained '100ml' of production medium. The above 99-fermenter flasks were then arranged to 11-sets each comprising of 9-fermenter flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermenter flasks. The remaining 9-fermenter flasks out of 99-fermenter flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermenter flasks.

After preparing the above sets of fermenter flasks M/1000 solution of SDDC was prepared and from the above mutagenic solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml

Table - 1
Efficacy of SDDC on LSF technique for biotic production of H_3Cit

Concentration of SDDC used A X 10^{-x} M	Incubation Period in days	Yield of H_3Cit* in g/100ml	Molasses* left unfermented in g/100 ml	%Diff. in the yield of H_3Cit in 11 days
Control	11	9.375	4.520	-
1.0 x 10^{-5}M	11	9.498	4.398	(+) 1.312
2.0 x 10^{-5}M	11	9.690	4.206	(+) 3.360
3.0 x 10^{-5}M	11	9.867	4.029	(+) 5.248
4.0 x 10^{-5}M	11	9.996	3.897	(+) 6.624
5.0 x 10^{-5}M	11	10.205	3.690	(+) 8.853
6.0 x 10^{-5}M	11	10.748	3.146	(+) 14.645
7.0 x 10^{-5}M**	11	11.289***	2.605	(+) 20.416
8.0 x 10^{-5}M	11	10.300	3.593	(+) 9.866
9.0 x 10^{-5}M	11	9.686	4.210	(+) 3.317
10.0 x 10^{-5}M	11	9.550	4.345	(+) 1.866

* Each value represents mean of three trials.**Optimum concentration of mutagens used. *** Optimum yield of H_3Cit in 11 days. (+) Values indicate % increase in the yield of citric acid after 11 days. Experimental deviation ($\pm$) 1.5-3%.

was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermenter flasks contained no mutagen. Now, the total volume in each fermenter flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of SDDC in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x}M$

$1.0 \times 10^{-5}M$ to $10.0 \times 10^{-5}M$ respectively.

i.e., Where A = amount of mutagen, in ml, i.e., 1.0 ml ..... to 10 ml, x =Molarity of the mutagen solution

The above fermenter flasks were then sterilized, cooled inoculated, incubated at 28^0C and analysed after 10, 11 and 12 days for citric acid[56] formed and molasses left unfermented[57].

Results and Discussion

The influence of carbamate mutagen

The data given in the table -1 shows that the SDDC has been found stimulatory for the bioproduction of H_3Cit by *Aspergillus niger* SS-69. From the data given in the table-1 it is obvious that SDDC influences the citric acid fermentation process in different phases. The main characteristics of the carbamate mutagen is as follows :

(i) Carbamate mutagen is stimulatory at its all molar concentrations used during the course of fermentation for bioproduction of H_3Cit by *Aspergillus niger* SS-69 i. e., from $1.0 \times 10^{-5}M$ to $10.0 \times 10^{-5}M$.

(ii) The molar concentration $1.0 \times 10^{-5}M$ to 7.0×10^{-5} M of carbamate mutagen influence the yield of citric acid (H_3Cit) in a approximately regular increasing order after each state, i. e., 1.312%, 3.360%, 5.248%, 2.624%, 8.53%, 14.645% and 20.416%

(iii) The molor concentration $8.0 \times 10^{-5}M$ to $10.0 \times 10^{-5}M$ of carbamate mutagen also influences the productivity of citric acid in a regular decreasing manner. The % increase in the yield of citric acid (H_3Cit) at respective molar concentration of SDDC has been found to be as follows : 9.866% , 3.317%, and 1.866%

(iv) The maximum yield of citric acid, (H_3Cit) i,e; 11.289g/100 ml in the presence of carbamate mutagen was observed at $7.0 \times 10^{-5}M$ molar concentration in 11 days of optimum incubation period which is 20.416% higher in comparison to control fermentor flasks, i.e.; 9.376g/100 ml in the same times course and other same experimental parameters.

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