J. Chemtracks 20(1&2) 49-52, 2018

Abstracted / Indexed in
A division of the American Chemical Society
Chemical Abstract U.S.A.

ISSN 0973-239X
CODEN:JCOHF7
IMPACT FACTOR : 4.950

Ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-1989 exposed to N-acetoxy-2-acetamidophenanthrene

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Manuscript received online 21 November 2017, accepted on 03 January 2018

Abstract : The efficacy of N-acetoxy-2-acetamidophenanthrene on production of ethanol by *Saccharomyces cerevisiae* NCIM-1989 has been assessed. It has been found that the mutagen under trial, i,e; N-acetoxy-2-acetamidophenanthrene at 4.0×10^{-5} M molar concentration enhances the production of ethanol by *Saccharomyces cerevisiae* NCIM-1989 to an extent of 5.513% higher in comparison to control fermenter flask, i.e, 7.18 ml/ 100 in 48 hours of optimum incubation period, 24% (w/v) molasses solution 5.1 pH and 28^0C temperature alongwith other nutritional ingredients required by the yeast.

(Keywords: Alcoholic fermentation, molasses, *Saccharomyces cerevisiae* NCIM-1989, mutagen, N-acetoxy-2-acetamidophenanthrene)

Introduction

Chemical mutagens are compounds that increase the frequency of some types of mutation.[1-7] Mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens.[8-12] Many chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals.[13] Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. While many researchers found chemical mutagens are to be more effective than physical ones[14,15] and many others researchers found the reverse case[16].

A number of workers[17-18] have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants[19]. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes[20-47]. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manupulation and exploitation specially for alcoholic fermentation and in view of this the authoress has studied the influence of N-acetoxy-2-acetamidophenanthrene on production of ethanol by *Saccharomyces cerevisiae* NCIM-1989

Experimental

The influence of N-acetoxy-2-acetamido-phenanthrene on production of ethanol by *Saccharomyces cerevisiae* NCIM-1989

The composition of the production medium for production of ethanol by *Saccharomyces cerevisiae* NCIM-1989 is prepared as follows: Molasses : 24%, (w/v) Malt Extract : 1.25%, Yeast Extract :1.25%, Peptone: 1.25% Distilled water to make up : 100 ml, pH: 5.1 Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 5.1 by adding requisite amount of lactic acid. Now, the same production medium for production of

ethanol by *Saccharomyces cerevisiae* NCIM-1989 was prepared for 99 fermenter-flasks, i.e., each containing 100 ml of production medium. These fermenter-flasks were then arranged in 10 sets each comprising 9 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.

Now, M/1000 solution of N-acetoxy-2-acetamidophenanthrene was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml of this solution was added to the fermenter-flasks of first 10 sets respectively. The control fermenter-flask contained no chemical mutagens. The total volume in each fermenter-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of N-acetoxy-2-acetamidophenanthrene in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :
$A\times10^{-x}$M, 1.0×10^{-5}Mto 10.0×10^{-5} M respectively.
Where, A = amount of mutagens in ml, ie; from 1.0 ml to 10.0 ml.
x = molarity of the solution.

The fermenter-flasks were then steam sterilized, cooled, inoculated, incubated at 28^0 C and analysed colorimetrically after 45, 48, and 50 hours for alcohol[48] formed and molasses[49] sugars left unfermented.

Results and Discussion

The influence of N-acetoxy-2-acetamido-phenanthrene on ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-1989

The data recorded in the table-1 shows

Table - 1
Ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-1989 exposed to N-Acetoxy-2-acetamidophenanthrene

Concentration of mutagen used A X 10^{-x} M	Incubation Period in hours	Yield of alcohol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of alcohol in comparison to control.
Control	48	7.18	1.13654639	—
1.0×10^{-5}M	48	7.25	1.14642353	+0.974930362
2.0×10^{-5}M	48	7.35	1.13090812	+2.367688022
3.0×10^{-5}M	48	7.40	1.12911353	+3.064066852
4.0×10^{-5}M**	48	7.55***	1.11413596	+5.513203342
5.0×10^{-5}M	48	7.50	1.12886714	+4.456824512
6.0×10^{-5}M	48	7.45	1.13765941	+3.760445682
7.0×10^{-5}M	48	7.30	1.13986887	+1.671309192
8.0×10^{-5}M	48	7.28	1.14515941	+1.392757660
9.0×10^{-5}M	48	7.26	1.14626886	+1.114206128
10.0×10^{-5}M	48	7.22	1.14754982	+0.557103064

* Each value represents mean of three trials.
** Optimum concentration of the chemical mutagen used.
*** Optimum yield of alcohol in 48 hours.
(+)Values indicate % increase in the yield of alcohol in comparison to control.
Experimental deviation (±) 1.5–3%.

that N-acetoxy-2-acetamidophenanthrene has stimulatory effect on ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-1989

The maximum yield of alcohol, i.e., 7.55 ml/100 ml in the presence of N-acetoxy-2-acetamidophenanthrene was observed at 4.0 x 10^{-5} M molar concentration in 48 hours of optimum incubation period which is 5.153203342% higher in comparison to control fermenter flasks, ie; 7.18ml/100ml in the same times course and other same experimental parameters.

The higher molar concentrations of N-acetoxy-2-acetamidophenanthrene were not much favourable for the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-1989. So the gradual addition of the mutagen N-acetoxy-2-acetamidophenanthrene after certain concentrations were not beneficial for the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-1989 .

It has been observed that molar concentration of the mutagen, ie., N-acetoxy-2-acetamidophenanthrene from 1.0 x 10^{-5} M to 4.0 x 10^{-5} M enhances the yield of alcohol to an order being 0.9749303%, 2.367688022%, 3.064066852%, and 5.153203342% higher in comparison to control flasks.

It has been observed further that after optimum concentration, i.e; 4.0 x 10^{-5} M, the addition of the same mutagen (N-acetoxy-2-acetamidophenanthrene) to the production medium causes fall in the yield of alcohol gradually and reached to 0.557103064%. However, at all the experimental concentrations of mutagen (N-acetoxy-2-acetamidophenanthrene) used, the yield of alcohol by *Saccharomyces cerevisiae* NCIM-1989 has been found higher in comparison to control fermenter flasks.

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Abstracted / Indexed in : Chemical Abstract U.S.A.
JC- 1277/2K18 (ISSN:0973-239X), INDIA
Journal Chemtracks Vol. 20 (1&2), 49-52, January to December 2018