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Studies on Bioenergetic Transformation of Molasses Pollutant to Ethanol by *Saccharomyces Cerevisiae* Ncim- 2086 Exposed To 4-Acetyl-1-Methylnaphthalene

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ABSTRACT

The efficacy of 4-acetyl-1-methylnaphthalene on bioenergetic transformation of molasses pollutant to ethanol by saccharomyces cerevisiae NCIM-2086 has been assessed. It has been found that the mutagen i.e; 4-acetyl-1- methylnaphthalene under trial has stimulatory effect on bioenergetic transformation of molasses pollutant to ethanol by saccharomyces cerevisiae NCIM-2086 and enhances the yield of ethanol to an extent of 9.72222% higher in comparison to control fermentor flasks i.e; 6.32 ml/100 ml in 46 hours of incubation period, 4.8 pH and 32ºC temperature with 16%(W/V) molasses solution.

Keywords : Molasses, Mutagen, Ethanol, Fermentation, 4-acetyl -1-methylnaphthalene, saccharomyces cerevisiae NCIM-2086)

I. INTRODUCTION

In genetics and molecular biology, mutagens are physical or chemical agent that changes the genetic material, usually DNA of an organism and thus increases the frequency of mutations above the natural background level. Mutation can result in several different types of change in sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly on completely.

Studies on genetic variations between different species of Drosophila suggests that if a mutation changes a protein produced by a gene, the result is likely to be harmful, with an estimated 70 percent of amino acid polymorphisms having damaging effects, and the remainder being either neutral or weakly beneficial. Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as DNA repair to prevent mutations.

Mutation breeding is the process of exposing seeds to chemicals or radiation in order to generate mutants with desirable traits to be bred with other cultivars. Plants created using mutagenesis are sometimes called mutagenic plants or mutagenic seeds. These varieties are currently used in agricultural production around the world, as these seeds are not always identified or labeled as being mutagenic or having a mutagenic provenance. There are different kind of mutagenic breeding such as using chemical mutagens like EMS and DMS, radiation and transposons are used to generate mutants. Mutation breeding is commonly used to produce traits in crops such as larger seeds, new colors, or sweeter fruits.

Genetically modified foods, the use of transgenic processes is often compared and contrasted with mutagenic processes.1 Mutagenic varieties tend to be made freely available for plant breeding, in contrast to many commercial plant varieties or germplasm that increasingly have restrictions on their use. Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination.2 These duplications are a major source of raw material for evolving new genes, with tens to hundreds of genes duplicated in animal genomes every million years.3 Most genes belong to larger families of genes of shared ancestry. Novel genes are produced by several methods, commonly through the duplication and mutation of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions.4-11

The influence of mutagens in fermentation technology specially ethanol fermentation has been studied extensively, 4-acetyl-1-methylnapthalene is one of the few chemical mutagen which has been found effective chemical mutagens in fermentations. A large number of chemical mutagens have been employed to generate the mutants of different microbes but still there are some chemical mutagen such as 4-acetyl-1- methylnaphthalene having mutagenic properties whose influence on ethanol fermentation by Saccharomyces cerevisiae NCIM-2086.

This communication thus presents a brief account of the action of particular chemical mutagen on bioenergetic transformation of molasses pollutant to ethanol by saccharomyces cerevisiae NCIM-2086 as carried out for over years in the authors laboratory.

II. EXPERIMENTAL

The influence of 4-acetyl-1-methylnaphthalene on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 .

The composition of production medium for the bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 is prepared as follows :

Molasses	:	16% (w/v)
Malt extract	:	1.25%
Yeast extract	:	1.25%
Peptone	:	1.25%
$(NH_4)_2HPO_4$	:	1.25%
pH	:	4.8

Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 4.8 by adding requisite amount of lactic acid. Now, the same production medium for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM-2086 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 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.

Now, M/1000 solutions of 4-acetyl-1-methylnaphthalene 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 fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no chemical mutagens. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 4-acetyl-1-methylnaphthalene in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

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

1.0×10^{-5} M,

2.0×10^{-5} M,

3.0×10^{-5} M,

4.0×10^{-5} M,

5.0×10^{-5} M,

6.0×10^{-5} M,

7.0×10^{-5} M,

8.0×10^{-5} M,

9.0×10^{-5} M,

10.0×10^{-5} M

Where, A = amount of mutagens in ml, ie; from

1.0 ml to 10.0 ml.

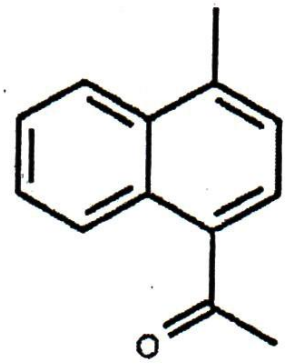
x = molarity of the solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 32^0 C and analysed colorimetrically after 40,46, and 50 hours for alcohol formed and molasses sugars left unfermented.

III. RESULTS AND DISCUSSION

The influence of 4-acetyl-1-methylnaphthalene on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086

4-Acetyl-1-methylnaphthalene



The data given in the table-1 shows that the mutagen , i.e ; 4-acetyl-1-methylnaphthalene has been found stimulatory for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086. From the data given in the table-1, it is obvious that 4-acetyl-1-methylnaphthalene influences the ethanolic fermentation process in different phases. The main characteristics of the ethanol formation exposed to 4-acetyl-1-methylnaphthalene is as follows:

(i) 4-acetyl-1-methylnaphthalene is stimulatory at its all molar concentrations used during course of the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086, i.e; from 1.0×10^{-5} M to 10.0×10^{-5} M.

(ii) The molar concentration of chemical mutagen, i.e.; 4-acetyl-1-methylnaphthalene from 1.0×10^{-5} M to 7.0×10^{-5}M influences the yield of ethanol in approximately regular increasing order after each stage; ie: 1.21527%, 1.73611%,2.95138%, 4.86111%, 6.77083 %, 8.85416% and 9.72222%.

(iii) The other molar concentrations of 4-acetyl-1- methylnaphthalene i.e; 8.0×10^{-5}M to 10.0×10^{-5}M influences the yield of ethanol in almost fast decreasing manner and could give the yield of ethanol with major differnce, i.e., 6.59722%, 4.68750% and 2.95138% respectively. However, the maximum yield of ethanol has been found at 7.0×10^{-5}M concentration of 4-acetyl-1-

methylnaphthalene, i.e., 6.32 ml/100 ml which is 9.72222 % higher in comparison to control fermentor flasks.

It has been observed further that after optimum concentration i.e; 7.0×10^{-5}M the addition of the same mutagen 4-acetyl-1-methylnaphthalene to the production medium causes fall in the yield of ethanol gradually and reached to 2.95138%. However, at all the experimental concentrations of mutagen (4-acetyl-1-methylnaphthalene) used, the yield of ethanol by *Saccharomyces cerevisiae* NCIM- 2086 has been found slight higher in comparison to control fermentor flasks.

Table-1. Bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086 exposed to 4-acetyl-1-methylnaphthalene

Concentration Of Mutagen Used A X 10* M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses Sugars* left unfermented in g/100 ml	% Difference in yield of ethanol in 46 hours.
Control (-) Mutagen	46	5.76	2.30118	-
1.0 x10^{-5}M (+) Mutagen	46	5.83	2.23181	+ 1.21527
2.0 x10^{-5}M (+) Mutagen	46	5.86	2.20116	+ 1.73611
3.0 x10^{-5}M (+) Mutagen	46	5.93	2.13124	+ 2.95138
4.0 x10^{-5}M (+) Mutagen	46	6.04	2.02124	+ 4.86111
5.0 x10^{-5}M (+) Mutagen	46	6.15	1.91127	+ 6.77083
6.0 x10^{-5}M (+) Mutagen	46	6.27	1.79120	+ 8.85416
7.0 x10^{-5}M (+) Mutagen	46	6.32***	1.74117	+ 9.72222
8.0 x10^{-5}M (+) Mutagen	46	6.14	1.92119	+ 6.59722
9.0 x10^{-5}M (+) Mutagen	46	6.03	2.03122	+ 4.68750
10.0 x10^{-5}M (+) Mutagen	46	5.93	2.13123	+ 2.95138

* Each value represents mean of three trials.

** Optimum concentration of the chemical mutagen used.

*** Optimum yield of ethanol in 46 hours.

(+)Values indicate % increase in the yield of ethanol in comparison to control.

Experimental deviation (±) 1.5–3%.

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