

Efficacy of 10-methyl-9(10H)-acridones on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

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Abstract : The efficacy of 10-methyl-9(10H)-acridones on production of ethanol by *Saccharomyces cerevisiae* RK-16 has been assessed. It has been found that the mutagen under trial, i.e; 10-methyl-9(10H)-acridones at 6.0×10^{-5} M molar concentration enhances the production of ethanol by *Saccharomyces cerevisiae* RK-16 to an extent of 20.363% higher in comparison to control fermenter flask, i.e, 5.50 ml/100 in 65 hours of optimum incubation period, 18.5% (w/v) molasses solution 4.5 pH and 30°C temperature alongwith other nutritional ingredients required by the yeast.

(Keywords: Alcoholic fermentation, molasses, mutagen, 10-methyl-9(10H)-acridones, *Saccharomyces cerevisiae* RK-16)

Introduction

Chemical mutagens are standard tools for mutagenesis in a variety of organisms, and they are a primary means of creating mutations in phenotype-based screens in most genetic systems. Although varied in the experimental design, all whole animal screens involve the generation of lines harboring mutated chromosomes followed by the examination of the resulting phenotypes in the heterozygous or homozygous state. In contrast, gene-based screens rely on the identification of lines that carry mutations in specific genes, prior to any phenotypic examination. The ability to perform gene-based screens has been made possible by recent technological advances in the high-throughput detection of subtle mutations.

Chemical mutagens have also been used successfully in the mutagenesis.

A mutagen is defined as any physical or chemical substance that can change the genetic material of an organism, thereby causing a mutation. 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 case¹⁶.

A number of workers¹⁷⁻¹⁹ 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 plants¹⁹. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes²⁰⁻³². Thus, from the above brief review it is evident that chemical mutagens are required for genetic manipulation and exploitation specially for

Table - 1
Efficacy of 10-Methyl-9(10H)-acridones on novel biotechnological method
for production of ethanol by *Saccharomyces cerevisiae* RK-16

Concentration of Mutagen used $A \times 10^{-x} \text{ M}$	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 65 hrs.
Control	65	5.35	1.799	–
$1.0 \times 10^{-5} \text{ M}$	65	5.41	1.736	+ 1.121
$2.0 \times 10^{-5} \text{ M}$	65	5.57	1.578	+ 4.112
$3.0 \times 10^{-5} \text{ M}$	65	5.78	1.366	+ 8.037
$4.0 \times 10^{-5} \text{ M}$	65	5.89	1.258	+ 10.093
$5.0 \times 10^{-5} \text{ M}$	65	6.16	0.989	+ 15.140
$6.0 \times 10^{-5} \text{ M}^{**}$	65	6.32***	0.829	+ 18.130
$7.0 \times 10^{-5} \text{ M}$	65	6.00	1.147	+ 12.149
$8.0 \times 10^{-5} \text{ M}$	65	5.67	1.478	+ 5.981
$9.0 \times 10^{-5} \text{ M}$	65	5.52	1.629	+ 3.177
$10.0 \times 10^{-5} \text{ M}$	65	5.38	1.766	+ 0.560

* Each value represents mean of three observations ** Optimum concentration of mutagen used.

*** Optimum yield of ethanol in 65 hours of optimum incubation period (+) Values indicate % increase in the yield of ethanol after 65 hours. Experimental deviation ($\pm$) 1.5–3%.

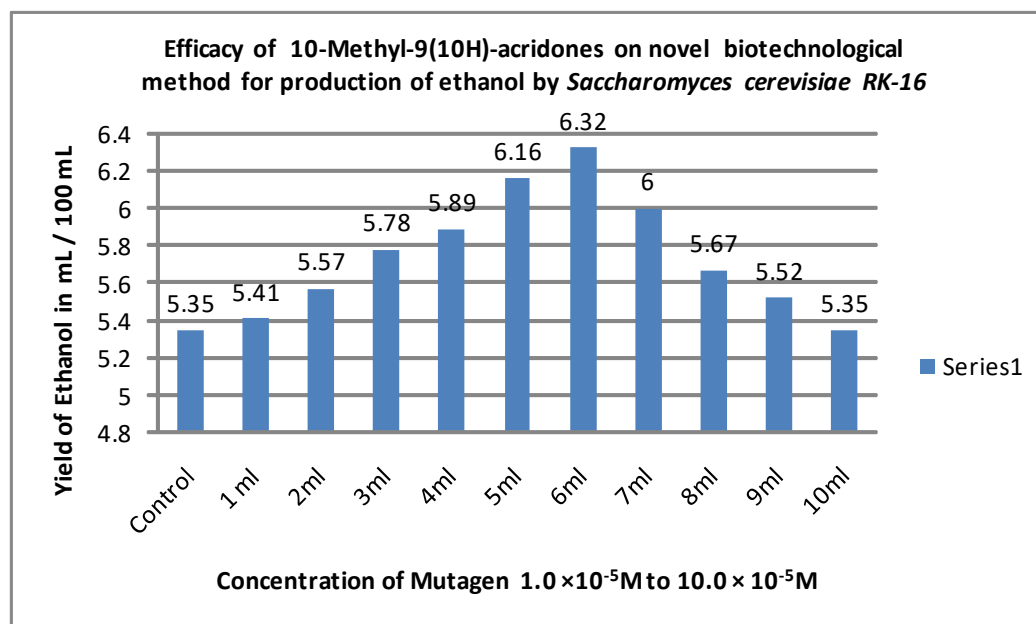


Figure - 1

alcoholic fermentation and in view of this the author has studied the influence of 10-methyl-9(10H)-acridones on production of ethanol by *Saccharomyces cerevisiae* RK-16

Experimental

The efficacy of chemical mutagens, i.e., 10-Methyl-9(10H)-acridones on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

The constitution of bioproduction medium for the novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16 is prepared as follows :

Molasses : 18.5%, Malt extract : 1.80%
Yeast extract : 1.80%, Peptone : 1.80%
(NH₄)₂HPO₄ : 1.80%, pH : 4.5
Distilled water was well added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 4.5 by adding desired quantity of lactic acid.

Now, the same production medium for novel biotechnological method for production of ethanol by a fungal yeast strain of *Saccharomyces cerevisiae* RK-16 was prepared for ninety nine flat bottom fermentor-flasks, i.e., each flat bottom fermentor flask containing 100 ml of production medium. These flat bottom fermentor-flasks, i.e., each containing 100 ml of bioproduction medium. These flat bottom fermentor-flasks were then well arranged in ten sets each comprising nine flat bottom fermentor-flasks. The remaining nine flat bottom fermentor-flasks out of ninety nine flat bottom fermentor-flasks were kept as control and these were also rearranged in three subsets each consisting of three flat bottom fermentor flasks.

Now, M/1000 solutions of chemical mutagen, i.e., 10-methyl-9(10H)-acridones was made 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 mutagenic solution was added to the flat bottom fermentor-flasks of first ten sets

respectively. The control flat bottom fermentor-flask contained no chemical mutagens. The total volume in each flat bottom fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 10-methyl-9(10H)-acridones in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below:

$a \times 10^{-x}M$, $1.0 \times 10^{-5}M$ to $10.0 \times 10^{-5}M$

Where, a = amount of chemical mutagens in ml, ie; from 1.0 mL to 10.0 mL.

x = molarity of the solution.

The flat bottom fermentor-flasks were then carefully steam sterilized, cooled, inoculated and incubated at temperature 30°C and then carefully analysed colorimetrically after 45, 65, and 75 hours for alcohol formed³⁸ and molasses³⁹ solution sugars left unused.

Results and Discussion

The influence of the chemical mutagen, i.e., 10-methyl-9 (10H)-acridones on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

The maximum yield of alcohol (C₂H₅OH), i.e., 6.32 mL/100 ml in the presence of 10-methyl-9(10H)-acridones was observed at 6.0×10^{-5} M molar concentration in 65 hours of optimum incubation period which is 18.130% higher in comparison to control flat water fermentor flasks, i.e; 5.35 ml/100ml in the same times course and some other same lab experimental parameters.

The data given in the table-1 shows that the chemical mutagen 10-methyl-9(10H)-acridones has been found quite stimulatory for the novel biotechnological method for production of ethanol (C₂H₅OH) by *Saccharomyces cerevisiae* RK-16. From the data given in the table -3 it is clear that the chemical mutagen, i.e., 10-methyl-

9(10H)-acridones influences the ethanolic fermentation bioprocess in different phases. The main well characteristics of the chemical mutagen, i.e., 10-methyl-9(10H)-acridones is as follows:

- (i) 10-methyl-9(10H)-acridones is quite stimulatory at its all molar concentrations used during the course of the ethanolic fermentation bioprocess, i.e; from 1.0×10^{-5} M to 10.0×10^{-5} M.
- (ii) The molar concentration 1.0×10^{-5} M, 2.0×10^{-5} M and 3.0×10^{-5} M of chemical

mutagen, i.e., 10-Methyl-9(10H)-acridones influences the yield of ethanol (C_2H_5OH) in a approximately regular doubling order after each stage; i.e., 1.121%, 4.12% and 8.037%.

- (iii) The higher molar concentrations, i.e.; 4.0×10^{-5} M to 10.0×10^{-5} M of chemical mutagen, i.e., 10-Methyl-9(10H)-acridones influences the yield of ethanol (C_2H_5OH) is as follows : 10.093%, 15.140%, 18.130%, 12.148%, 5.981%, 3.187% and 0.560% respectively.

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