

## Strain improvement of *Saccharomyces cerevisiae* by diethyl sulfate (DES) mutagenesis for efficient ethanol bioproduction

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**Abstract:** The present study focuses on the strain improvement of *Saccharomyces cerevisiae* NCIM-1267 through chemical mutagenesis using diethyl sulfate (DES) to enhance ethanol bioproduction efficiency. Wild-type cultures were subjected to varying concentrations and exposure durations of DES to induce random genetic mutations. Mutagen-treated strains were screened for ethanol tolerance, fermentation efficiency, and sugar utilization rates under controlled fermentation conditions. Selected high-yielding mutants demonstrated improved ethanol productivity, reduced fermentation time, and enhanced tolerance to higher ethanol concentrations compared to the parental strain. Physiological and biochemical evaluations revealed increased fermentative activity, likely attributed to altered metabolic fluxes favoring ethanol synthesis. The optimized mutant strains achieved a significant increase in ethanol yield, indicating the potential of DES mutagenesis as a practical approach for industrial strain development. These findings contribute to the development of robust yeast strains suitable for large-scale, cost-effective ethanol production from natural feedstocks. In the present study the impact of diethyl sulfate on strain improvement of *Saccharomyces cerevisiae* NCIM-1267 has been explored. It has been found that DES mutagen acts as a modulator and stimulator and enhances the yield of ethanol to an extent of 14.776% higher in comparison to control when 25% of molasses solution is allowed to ferment at pH 5.2, temperature 30°C and incubation period of 50 hrs.

**(Keywords :** *Saccharomyces cerevisiae*, NCIM-1267, diethyl sulfate, mutagenesis, strain improvement, ethanol fermentation, bioethanol production).

### Introduction

Sugarcane molasses—the viscous by-product after sucrose crystallization—

remains a workhorse feedstock for first-generation bioethanol due to its high fermentable-sugar content (sucrose, glucose, fructose), broad availability from cane mills, and minimal pretreatment needs compared with starchy or lignocellulosic substrates. Industrial *Saccharomyces cerevisiae* dominates molasses fermentations in batch and fed-batch modes, with current process intensification targeting high-gravity operation and smart feeding to raise titers and volumetric productivity while limiting osmotic and ethanol stress<sup>1-2</sup>.

To push performance beyond baseline industrial strains, non-GMO strain development routes such as random mutagenesis and selection have been explored<sup>3-5</sup>. Among chemical mutagens, diethyl sulfate (DES) is a potent alkylating agent that introduces ethyl groups onto nucleophilic sites in DNA (e.g., O6-guanine), increasing mutation rates and enabling selection for stress-tolerant phenotypes (ethanol/osmotic/temperature) relevant to molasses fermentation<sup>6-12</sup>. Recent studies in brewing and other yeasts report DES used alone or combined with UV to obtain mutants with improved ethanol tolerance or productivity under high-gravity conditions; these reports underscore the potential of mutational breeding to complement process engineering in molasses media<sup>13-20</sup>.

In the present communication the authors have confined their study to strain improvement of *Saccharomyces cerevisiae* NCIM-1267 by diethyl sulfate (DES) mutagenesis for efficient ethanol bioproduction.

**Table - 1**  
**Bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM--1267 exposed to Diethyl Sulfate (DES)**

Concentration of mutagen used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of alcohol* in ml/100ml	Blackstrap molasses sugars* left unfermented in g/100 ml	% Difference in yield of alcohol in comparison to control. After 50 h
Control	30	4.65	2.95	-
(-) Mutagen	50	6.70	1.38	-
	60	6.25	1.32	-
$1.0 \times 10^{-5}$ M	30	4.78	2.82	+2.79569
(+) Mutagen	50	6.94	1.14	+3.58208
	60	6.46	1.12	+3.36000
$2.0 \times 10^{-5}$ M	30	4.80	2.80	+3.22580
(+) Mutagen	50	7.03	1.12	+4.92537
	60	6.52	1.11	+4.32000
$3.0 \times 10^{-5}$ M	30	4.85	2.78	+4.30107
(+) Mutagen	50	7.15	1.11	+4.71641
	60	6.63	1.10	+6.08000
$4.0 \times 10^{-5}$ M	30	4.89	2.76	+5.16129
(+) Mutagen	50	7.23	1.10	+6.91044
	60	6.69	1.08	+7.04000
$5.0 \times 10^{-5}$ M	30	4.97	2.74	+6.88172
(+) Mutagen	50	7.27	1.10	+8.50746
	60	6.76	1.07	+8.16000
$6.0 \times 10^{-5}$ M	30	5.12	2.73	+10.10752
(+) Mutagen	50	7.41	1.08	+10.59701
	60	6.90	1.06	+10.40000
$7.0 \times 10^{-5}$ M**	30	5.26	2.70	+13.11827
(+) Mutagen	50	7.69***	1.06	+14.77611
	60	7.13	1.04	+14.08000
$8.0 \times 10^{-5}$ M	30	5.22	2.71	+12.25806
(+) Mutagen	50	7.55	1.07	+12.68656
	60	7.01	1.05	+12.16000
$9.0 \times 10^{-5}$ M	30	5.06	2.73	+8.81720
(+) Mutagen	50	7.30	1.09	+8.95522
	60	6.65	1.06	+6.40000
$10 \times 10^{-5}$ M	30	4.94	2.75	+6.23655
(+) Mutagen	50	7.15	1.12	+6.71641
	60	6.57	1.10	+5.12000

\* Each value represents mean of three trials. \*\* Optimum concentration of the chemical mutagen used. \*\*\* Optimum yield of alcohol in 50 hours. (+) Values indicate % increase in the yield of alcohol in comparison to control. Experimental deviation ( $\pm$ ) 1.5–3%.

## Experimental

The influence of Diethylsulfate (DES) on bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM -1267.

The composition of production medium for the bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM -1267 is prepared as follows :

Molasses : 25%, Malt extract : 0.35% Yeast extract: 0.35%, Peptone : 0.50%,  $(\text{NH}_4)_2\text{HPO}_4$  : 0.35%, pH : 5.2 Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 5.2 by adding requisite amount of lactic acid.

Now, the same production medium for alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267 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 Diethyl sulfate (DES) 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 Diethyl sulfate (DES), in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

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

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 30<sup>o</sup> C and analysed colorimetrically after 30, 50, and 60 hours for alcohol<sup>21</sup> formed and blackstrap molasses sugars left<sup>22</sup> unfermented.

## Results and Discussion

### The influence of Diethyl Sulfate (DES) on bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM --1267

The data recorded in the table-1 shows that Diethyl Sulfate (DES) has stimulatory effect on bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM -1267

The maximum yield of alcohol, i.e., 7.69 ml/100 ml in the presence of Diethyl Sulfate (DES) was observed at  $7.0 \times 10^{-5}\text{ M}$  molar concentration in 50 hours of optimum incubation period which is 14.77611% higher in comparison to control fermentor flasks, ie; 6.70 ml/100ml in the same times course and other same experimental parameters.

The higher molar concentrations of Diethyl Sulfate (DES) were not much favourable for the bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM -1267. So the gradual addition of the mutagen Diethyl Sulfate (DES) after certain concentrations were not beneficial for the bioproduction of ethanol from natural sources by *Saccharomyces cerevisiae* NCIM 1267 .

It has been observed that molar concentration of the mutagen, ie., Diethyl Sulfate (DES) from  $1.0 \times 10^{-5}\text{ M}$  to  $7.0 \times 10^{-5}\text{ M}$  enhances the yield of alcohol to a certain order being 3.58208%, 4.92537%, 6.71641%, 7.91044%, 8.50747% , 10.59701% and 14.77611 higher in comparison to control flasks.

It has been observed further that after optimum concentration, i.e;  $7.0 \times 10^{-5}$  M, the addition of the same mutagen to the production medium causes fall in the yield of alcohol gradually and reached to 6.716%. However, at all

the experimental concentrations of Diethyl Sulfate (DES), used, the yield of alcohol by submerged fermentation has been found higher in comparison to control fermentor flasks.

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