

Efficacy of cyclopentanone phenyl hydrazone on fermentative bioproduction of ethanol

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Abstract: The efficacy of cyclopentanone phenyl hydrazone on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* DK-18 has been assessed. It has been found that the mutagen, i.e., cyclopentanone phenyl hydrazone under observation has stimulatory effect on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* DK-18 and enhances the yield of ethanol to an extent of 20.289% higher in comparison to control fermentor flasks, i.e., 6.90 ml/100mL in 56 hrs of incubation period, 4.7 pH and 33°C temperature with 22% (w/v) molasses solution.

(Keywords: Molasses, ethanol, mutagens, cyclopentanone phenyl hydrazone, *Saccharomyces cerevisiae* DK-18)

Introduction

In genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually deoxyribonucleic acid, of an organism and thus increases the frequency of mutations above the natural background level. As many mutations cause cancer, mutagens are therefore also likely to be carcinogens. Not all mutations are caused by mutagens: so-called "spontaneous mutations" occur due to spontaneous hydrolysis, errors in deoxyribonucleic acid replication, repair and recombination.

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.

Mutagens are simultaneously both required and avoided substances. They are "substances of transformation," but also "genetic poisons." Their transformative qualities destined mutagens to become unavoidable instruments within genetics and molecular biology. Since the 1960s, however, mutagens have defined a transdisciplinary problem of risk policy. Substances such as radioactive particles from fall-out and the nuclear industries, pharmaceuticals, chemical supplements in the foodstuffs industry or pesticides were silent, efficient and ubiquitous.¹⁻⁴ The precarious status between efficiency and (dangerous) autonomy formed the key characteristics of mutagens that nurtured the ambivalent career of mutants. Daily life became populated by horrifying, but also superhuman creatures.⁵⁻¹⁷

Literature survey reveals that a little work has been done on fermentative production of ethanol production by *Saccharomyces cerevisiae* DK-18 exposed to cyclopentanone phenyl hydrazone, therefore, the authors have employed cyclopentanone phenyl hydrazone on fermentative production of ethanol production by *Saccharomyces cerevisiae* DK-18.

Experimental

The influence of cyclopentanone phenyl hydrazone on fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 The constitution of production medium for the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 is prepared as follows : Molasses Solution : 22 % (w/v), Malt-Extract : 0.40%, Yeast-Extract : 0.40%, Peptone : 0.40% Distilled water : 100 ml, , pH : 4.7, Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 4.7 by adding requisite amount of lactic acid. Now, the same production medium for fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 was prepared for ninety nine fermentor-flasks, i.e., each containing hundred ml of production medium.

These fermentor-flasks, i.e., each containing hundred ml of production medium. These fermentor-flasks were then arranged in ten sets each comprising nine fermentor-flasks. The remaining nine fermentor-flasks out of ninety nine fermentor-flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now, M/1000 solutions of cyclopentanone phenyl hydrazon 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 solution was added to the fermentor-flasks of first ten 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 cyclopentanone phenyl hydrazon 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 mutagen in ml, ie; from 1.0 ml to 10.0 ml.
and x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 33^o C and analysed colorimetrically after 46, 56, and 62 hours for ethanol¹⁸ formed and molasses sugars¹⁹ left unfermented.

Results and Discussion

The influence of cyclopentanone phenyl hydrazon

The data recorded in the table-1 shows that cyclopentanone phenyl hydrazone has stimulatory effect on fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18. The maximum bioproduction of ethanol, i.e., 8.30 ml/100 ml in the presence of cyclopentanone phenyl hydrazone was observed at $6.0 \times 10^{-5} M$ molar concentration in 56 hours of optimum incubation period which is 20.289% higher in comparison to control fermentor flasks, ie; 6.90 ml/100ml in the same times course and other same experimental parameters.

The higher molar concentrations of cyclopentanone phenyl hydrazone were not much favourable for the fermentative bioproduction of ethanol from molasses. So the gradual addition of the mutagen cyclopentanone phenyl hydrazone after certain concentrations were not beneficial for the fermentative bioproduction of from molasses. It has been observed that molar concentration of the mutagen, i.e., cyclopentanone phenyl hydrazone from $1.0 \times 10^{-5} M$ to $6.0 \times 10^{-5} M$ enhances the production of ethanol to a certain order being 0.724%, 2.173%, 5.217%, 10.289%, 15.217% and 20.289% higher in comparison to control flasks. It has been observed further that after optimum concentration, i.e; $6.0 \times 10^{-5} M$, the addition of the same mutagen to the production medium causes fall in the yield of alcohol gradually and reached to 0.869%. However, at all the experimental concentrations of cyclopentanone phenyl hydrazone used, the yield of alcohol by fermentation has been found higher in comparison to control fermentor flasks.

Table - 1
Efficacy of cyclopentanone phenyl hydrazone on
fermentative bioproduction of ethanol

Concentration of mutagen used $A \times 10^{-x}$ 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 56 hrs.
Control	46	4.20	4.948	—
(-) Mutagen	56	6.90	3.842	—
	62	5.88	3.725	—
1.0×10^{-5} M	46	4.22	4.929	—
(+) Mutagen	56	6.95	3.793	+0.724
	62	5.90	3.635	—
2.0×10^{-5} M	46	4.28	4.867	—
(+) Mutagen	56	7.05	3.693	+2.173
	62	6.10	3.539	—
3.0×10^{-5} M	46	4.41	4.738	—
(+) Mutagen	56	7.26	3.482	+5.217
	62	6.15	3.375	—
4.0×10^{-5} M	46	4.62	4.527	—
(+) Mutagen	56	7.61	3.132	+10.289
	62	6.36	3.029	—
5.0×10^{-5} M	46	4.83	4.318	—
(+) Mutagen	56	7.95	2.794	+15.217
	62	6.46	2.649	—
6.0×10^{-5} M**	46	5.04	4.110	—
(+) Mutagen	56	8.30***	2.444	+20.289
	62	7.15	2.350	—
7.0×10^{-5} M	46	4.71	4.439	—
(+) Mutagen	56	7.75	2.995	+12.318
	62	6.41	2.859	—
8.0×10^{-5} M	46	4.45	4.697	—
(+) Mutagen	56	7.75	2.995	+12.318
	62	6.41	2.859	—
9.0×10^{-5} M	46	4.29	4.857	—
(+) Mutagen	56	7.06	3.682	+2.318
	62	6.01	3.575	—
10.0×10^{-5} M	46	4.23	4.918	—
(+) Mutagen	56	6.96	3.781	+0.869
	62	5.91	3.679	—

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

*** Optimum yield of ethanol in 56 hours. (+) Values indicate % increase in the yield of ethanol after 56 hours. Experimental deviation ($\pm$) 1.5–3%.

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