

Impact of sodium diethyldithiocarbamate on homolactic acid fermentation

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Abstract: The impact of sodium diethyl - dithiocarbamate on homolactic acid fermentation by some lactic acid bacteria such as *Lactobacillus leichmanni*-1569, *Lactobacillus pentosus*-1653, *Lactobacillus casei*-1837, *Lactobacillus delbrueckii* -1685 was assessed. It was found that the strain of *Lactobacillus pentosus*-1653 was much effective and good homolactic acid producer. The compound sodium diethyldithiocarbamate was found toxic when 15% (w/v) molasses solution was allowed to ferment at pH 6.1, temperature 37.0°C and optimum incubation period of 130 hours. It has been found that there is a gradual decrease in the production of homolactic acid with stepping up of the compound sodium diethyl - dithiocarbamate till the maximum yield of homolactic acid, i.e., 5.767 g/100 ml was obtained at its molar concentration of 7.0×10^{-3} M which is 15.912% lower in comparison to control fermentor flasks in 130 hours of optimum incubation period.

(Keywords : Homolactic acid fermentation, molasses, sodium diethyldithiocarbamate and *Lactobacillus pentosus*-1653)

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.

Sodium diethyldithiocarbamate is the organosulfur compound with the formula $\text{NaS}_2\text{CN}(\text{C}_2\text{H}_5)_2$. It is a pale yellow, water soluble salt. When exposed to ultraviolet light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA. Under the name homidium, it has been commonly used since the 1950s in veterinary medicine to treat trypanosomiasis in cattle. The high incidence of antimicrobial resistance makes this treatment impractical in some areas, where the related isometamidium chloride is used instead. Despite its reputation as a mutagen, tests have shown it to have low mutagenicity without metabolic activation¹⁻¹⁰.

In genetics, a mutagen is a physical or chemical agent that permanently changes genetic material, usually DNA, in an organism and thus increase the frequency of mutations above the natural background level. The process of DNA becoming modified is called mutagens¹¹⁻²⁰.

Chemical mutagens induce in the various test systems that have been utilized. Mutation can result in several different types of change in deoxyribo nucleic acid sequences; these can either have no effect, alter the product

Table - 1
Impact of sodium diethyldithiocarbamate on homolactic acid fermentation

Concentration of mutagen used a x 10 ^{-x} M	Incubation period in hours	Yield of homolactic* acid in g/100 ml	Molasses substrate* left unfermented in g/100 ml	%Diff. in the yield of homolactic acid in 130 hours of optimum incubation pd.
Control	50	3.809	7.298	—
(– mutagen)	130	6.858	5.149	—
	150	5.748	5.138	—
1.0 x 10 ⁻⁵ M**	50	3.771	7.336	—
(+ mutagen)	130	6.785***	5.222	(–) 1.071
	150	5.690	5.212	—
2.0 x 10 ⁻⁵ M	50	3.763	7.343	—
(+ mutagen)	130	6.734	5.273	(–) 1.810
	150	5.679	5.263	—
3.0 x 10 ⁻⁵ M	50	3.695	7.412	—
(+ mutagen)	130	6.610	5.397	(–) 3.612
	150	5.575	5.386	—
4.0 x 10 ⁻⁵ M	50	3.618	7.488	—
(+ mutagen)	130	6.459	5.548	(–) 5.821
	150	5.460	5.535	—
5.0 x 10 ⁻⁵ M	50	3.466	7.641	—
(+ mutagen)	130	6.226	5.781	(–) 9.214
	150	5.231	5.773	—

* Each value represents mean of three trials. ** Optimum concentration of mutagen.

*** Optimum yield of homolactic acid

(–) Values indicate % decrease in the yield of homolactic acid Experimental deviation $\pm 2.5 - 3.5\%$

of a gene, or prevent the gene from functioning properly or completely²¹⁻²⁶. Mutations can involve large sections of deoxyribo nucleic acid becoming duplicated, usually through genetic recombination.

The impact of mutagens in fermentation technology specially lactic acid fermentation has been studied extensively. Some mutagens were tested by many research workers²⁷⁻⁴³ for different fermentations process and were found that most of them were found useful for higher production of the desired products. It is obvious from above review of literature that various chemical mutagens and some other mutagenic agents are used to produce mutants. Thus, it is concluded

that a large number of mutagens have been employed to generate the mutants of different microbes and microbial process but still there are some chemical mutagens whose impact on production of lactic acid by *Lactobacillus pentosus* -1653 has not been well studied and established. Moreover, survey of the literature reveals that there has been not enough mention to study the homolactic acid fermentation exposed to mutagens specially chemical mutagens. Therefore, in the present investigation the authoress has made an attempt to study the lactic acid fermentation by *Lactobacillus pentosus* - 1653 exposed to sodium diethyl - dithiocarbamate.

Table - 1
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Concentration of mutagen used a x 10 ^{-x} M	Incubation period in hours	Yield of homolactic* acid in g/100 ml	Molasses substrate* left unfermented in g/100 ml	%Diff. in the yield of homolactic acid in 130 hours of optimum incubation pd.
Control	50	3.809	7.298	—
(– mutagen)	130	6.858	5.149	—
	150	5.748	5.138	—
6.0 x 10 ⁻⁵ M	50	3.390	7.717	—
(+ mutagen)	130	6.053	5.954	(–) 11.741
	150	5.116	5.946	—
7.0 x 10 ⁻⁵ M	50	3.238	7.869	—
(+ mutagen)	130	5.767	6.240	(–) 15.912
	150	4.886	6.237	—
8.0 x 10 ⁻⁵ M	50	****	—	—
(+ mutagen)	130	****	—	—
	150	****	—	—
9.0 x 10 ⁻⁵ M	50	****	—	—
(+ mutagen)	130	****	—	—
	150	****	—	—
10.0 x 10 ⁻⁵ M	50	****	—	—
(+ mutagen)	130	****	—	—
	150	****	—	—

* Each value represents mean of three trials. ** Optimum concentration of mutagen.

*** Optimum yield of homolactic acid

(–) Values indicate % decrease in the yield of homolactic acid Experimental deviation $\pm 2.5 - 3.5\%$

Experimental

The influence of sodium diethyl - dithiocarbamate on SmF biotransformation of molasses pollutant to homolactic acid by using the bacterial strain of *Lactobacillus pentosus*-1653

The composition of the production medium for the production of homolactic acid by submerged fermentation was prepared as follows:

Molasses :15% (w/v), Malt Extract :0.25 %
 Yeast Extract :0.25 %, Peptone:0.60%
 (NH₄)₂HPO₄:0.25 %, CaCO₃:8 %, pH:6.1
 Distilled water :To make up 100 ml.

The pH of the medium was adjusted to 6.1 by adding requisite amount of phosphate-buffer solution, and the pH was also ascertained by a pH meter.

The above composition medium represents volume of a fermentor flask, i. e., "100ml" production medium for homolactic acid fermentation.

Now, the same production for lactic acid fermentation by *Lactobacillus pentosus*-1653 was prepared for 99 fermentor flasks, i. e., each fermentor flask containing '100 ml' of production medium.

The above fermentor flasks were then arranged in ten sets, each comprising 9 fermentor flask. Each set was again rearranged in three subsets, each comprising of 3 fermentor flasks. The remaining nine fermentor flasks out of 99 fermentor flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now M/1000 solution/suspension of niacinamide in 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 1st to 10th sets respectively. The control fermentor flasks contain no chemical mutagens. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of niacinamide in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x}M$ to $10.0 \times 10^{-4}M$, Where

A = amount of chemical mutagen in ml
i.e., 1.0 ml to 10ml.

x = molarity of the solution containing chemical mutagen

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 50, 130 and 150 hours for homolactic acid formed and molasses sugars left unfermented as described in the experimental portion, i. e., chapter II of this thesis. The experimental procedure for the study of influence of other chemical mutagen were exactly the same as described above with the only difference that in place of M/1000 solution of niacinamide other chemical mutagen under trials were added to the lactic acid fermentation medium respectively.

Results and Discussion

The influence of sodium diethyldithio carbamate :

The data recorded in the table-1 shows that the chemical mutagen sodium diethyldithio-

carbamate has detrimental effect on SmF biosynthesis of homolactic acid using bacterial strain of *Lactobacillus pentosus* - 1653. The yields of homolactic acid obtained in the control fermenter flasks has been found to be higher than that obtained from each of the fermentor flasks containing chemical mutagen, i.e., sodium diethyldithiocarbamate.

The maximum yield of homolactic acid, i.e., 5.89285 g/100 ml. in the presence of sodium diethyldithiocarbamate, i.e., at $1.0 \times 10^{-5} M$ was found 130 hours of optimum incubation period, even this optimum yield has been found to be 0.98014% less in comparison to control fermentor flasks, i.e., 5.95118 g/100ml.

However, at higher concentrations of the chemical mutagen, i.e., sodium diethyldithiocarbamate the production of homolactic acid was found almost negligible, and insignificant. Thus, it is obvious from the results that the chemical mutagen sodium diethyldithiocarbamate under trial is much detrimental and inhibitory for SmF biotransformation of molasses pollutant to homolactic acid by *Lactobacillus pentosus*-1653.

It is interesting to note that the chemical mutagens used in the present investigation except sodium diethyldithiocarbamate in molar concentrations, i.e., from $1.0 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$ has given better yield of homolactic acid in each case in comparison to respective controls. So far as effectiveness of mutagenic chemicals used in the present investigation is concerned niacinamide is most effective and sodium diethyldithiocarbamate is least effective for SmF biotransformation of molasses pollutant to homolactic acid by *Lactobacillus pentosus*-1653. The mutagens used, i.e., niacinamide, nimorazole and d,l-diepoxybutane could produce the maximum yield of homolactic acid at their respective optimum concentration as follows : % increase in the yield of homolactic acid in comparison to control for each is. 20.31456%, 12.50095% and 11.43264% respectively.

From the present investigation it is obvious that the chemical mutagens used niacinamide, nimorazole and d,l-diepoxybutane are all useful for SmF biotransformation of

molasses pollutant to homolactic acid by *Lactobacillus pentosus*-1653 and, therefore, can be employed for the improved yield of homolactic acid.

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