

Bioenergetic transformation of molasses pollutant to lactic acid exposed to N-ethyl-N-nitrosourea

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Abstract : The impact of N-ethyl-N-nitrosourea (ENU) on lactic acid fermentation by some lactic acid bacteria such as *Lactobacillus gasseri* AK-126, *Lactobacillus acidophilus* AK-130, *Streptococcus thermophilus* AK-135, and *Lactobacillus lactis* AK-140 was assessed. It was found that the strain of *Lactobacillus lactis* AK-140 was much effective and good lactic acid procedure. The compound N-ethyl-N-nitrosourea was found stimulatory when 15.5% (w/v) molasses solution was allowed to ferment at pH 6.0, temperature 34.5°C and optimum incubation period of 144 hrs. It has been found that there is a gradual increase in the production of lactic acid with stepping up of the compound N-ethyl-N-nitrosourea till the maximum yield of lactic acid, i.e., 6.169 g/100 ml was obtained at its molar concentration of $6.0 \times 10^{-4} / 10000 \times 10M$ which is 15.642% higher in comparison to control fermentor flasks in 144 hours of optimum incubation period.

(Keywords : Lactic acid fermentation, molasses, N-ethyl-N-nitrosourea and *Lactobacillus lactis* AK-140)

Introduction

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 mutagens¹⁻¹⁰.

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 of a gene, or prevent the gene from functioning properly or completely¹¹⁻¹⁶. 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 workers¹⁷⁻³² 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 biotic production of lactic acid by *Lactobacillus lactis* AK-140 has not been well studied and established. Moreover, survey of the literature reveals that there has been not enough mention to study the lactic acid fermentation exposed to mutagens specially chemical mutagens. Therefore, in the present investigation the authors have made an attempt to study lactic acid fermentation by *Lactobacillus lactis* AK-140 exposed to N-ethyl-N-nitrosourea.

Experimental

The influence of succinimide on lactic

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Table - 1
Bioenergetic transformation of molasses pollutant to lactic acid
exposed to N-ethyl-N-nitrosourea

Concentration of mutagens used A × 1/10000 × 10M	Yield of lactic acid* in gm/100 ml			%Diff. in the yield of lactic acid in 144 hrs of optimum incubation pd.
	72 h	144 h	216 h	
Control	3.115	6.169	2.320	—
1×1/10000×10M	3.152	6.255	2.345	1.394
2×1/10000×10M	3.177	6.308	2.366	2.253
3×1/10000×10M	3.239	6.497	2.436	5.316
4×1/10000×10M	3.301	6.552	2.459	6.208
5×1/10000×10M	3.395	6.746	2.528	9.353
6×1/10000×10M**	3.582	7.134***	2.668	15.642
7×1/10000×10M	3.364	6.715	2.505	8.850
8×1/10000×10M	3.267	6.478	2.433	5.008
9×1/10000×10M	3.208	6.361	2.389	3.112
10×1/10000×10M	3.139	6.221	2.338	0.842

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

*** Optimum yield of lactic acid in 144 hours. (+) Values indicate % increase in the yield of lactic acid after 144 hours. Experimental deviation (±) 1.5-3%.

acid fermentation by *Lactobacillus lactis* AK-140. The composition of the production medium for the lactic acid fermentation by *Lactobacillus lactis* AK-140 was prepared as follows : Molasses : 15.5% (w/v) , Malt Extract : 1.95% Yeast Extract : 1.95%, Peptone: 1.95% (NH₄)₂HPO₄ : 1.60%, CaCO₃ : 7.5 %, pH : 6.0 Distilled water To make up 100 ml.

The pH of the medium was adjusted to 6.0 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 lactic acid fermentation by *Lactobacillus lactis* AK-140.

Now, the same production medium for production of lactic acid by *Lactobacillus lactis* AK-140 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 N-ethyl-N-nitrosourea 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 contained no mutagen i.e., N-ethyl-N-nitrosourea Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distilled water. Thus, the concentration of N-ethyl-N-nitrosourea in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A × 1/10000 × 10M

$1.0 \times 1/10000 \times 10M$ to $10.0 \times 1/10000 \times 10M$

Where A = amount of mutagen, i.e. N-ethyl-N-nitrosourea in ml,

$1/10000 \times 10M$ = molarity of the solution.

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 72, 144 and 216 hours for lactic acid³³ formed.

Results and Discussion

The data recorded in the table 1 shows that N-ethyl-N-nitrosourea has stimulatory effect on lactic acid fermentation by *Lactobacillus lactis* AK-140. The maximum yield of lactic acid, i.e.; 7.134g/100 ml in the presence of N-ethyl-N-nitrosourea was observed at $6.0 \times 1/10000 \times 10M$ molar concentration in 144 hours of optimum incubation period which is 15.642% higher in comparison to flat bottom control fermentor flasks, i.e.; 6.169 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of N-ethyl-N-nitrosourea were not much favourable for the lactic acid fermentation by *Lactobacillus*

lactis AK-140. So the gradual addition of the mutagen niacinamide after certain concentrations were not beneficial for the lactic acid fermentation bioprocess.

It has been observed that molar concentration of the mutagen, i.e., N-ethyl-N-nitrosourea from $1.0 \times 1/10000 \times 10M$ to $6.0 \times 1/10000 \times 10M$ enhances the yield of lactic acid to a certain order being 1.394%, 2.253%, 5.316%, 6.208%, 9.353% and 15.642 % higher in comparison to control flat bottom flasks but at $7.0 \times 1/10000 \times 10M$ and $8.0 \times 1/10000 \times 10M$ the yield of lactic acid shifted to be 8.850% and 5.008 % higher in comparison to previous concentrations of N-ethyl-N-nitrosourea taken into experimental trials.

It has been observed further that after optimum concentration, i.e., $6.0 \times 1/10000 \times 10M$, the addition of the same mutagen to the production medium causes fall in the yield of lactic acid gradually and reaches to 0.842%; However, at all the experimental concentrations of N-ethyl-N-nitrosourea used the yield of lactic acid by submerged fermentation has been found higher in comparison to flat bottom control fermentor flasks.

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