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Ethyl carbamate-mediated mutagenesis for enhanced lactic acid production by lactic acid bacteria

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Abstract: Ethyl carbamate (urethane) is a well-known chemical mutagen with established genotoxic and carcinogenic properties, yet its potential application in microbial strain improvement has not been extensively investigated. In this study, we evaluated the efficacy of ethyl carbamate-mediated mutagenesis in enhancing lactic acid production by selected strains of lactic acid bacteria (LABS). Cultures were exposed to varying concentrations and durations of ethyl carbamate to induce random mutagenesis, and resultant mutants were screened for growth kinetics, carbohydrate utilization, and lactic acid yield. These findings highlight the dual nature of ethyl carbamate as both a potent mutagen and a promising tool for strain improvement, offering scope for developing superior LAB strains for industrial lactic acid fermentation. In the present communication EC-mediated mutagenesis for enhanced lactic acid production by lactic acid bacteria *Lactobacillus casei* NCIM-1692 has been explored. It has been observed that ethyl carbamate acts as strong mutagens and stimulatory for lactic acid bacteria *Lactobacillus casei* NCIM-1692 and enhances the yield of lactic acid to an extent of 13.507% higher in comparison to control when 25% molasses solution is allowed to ferment at pH level of 5.9,temperature 38^{0}C and incubation period of 7 days along with some other significant rich ingredients of higher levels.

(Keywords : Ethyl carbamate, mutagenesis, lactic acid bacteria (LAB), strain improvement, lactic acid fermentation).

Introduction

Lactic acid is a value-added organic acid widely utilized in food, pharmaceutical, cosmetic, and biodegradable polymer industries. The growing demand for eco-friendly and sustainable lactic acid production has encouraged the exploration of microbial fermentation using lactic acid bacteria (LABs) as efficient biocatalysts[1-5]. Natural LAB strains, however, often exhibit limitations such as moderate substrate utilization efficiency, suboptimal product yield, and sensitivity to environmental stresses, thereby necessitating strain improvement strategies.

The present study explores ethyl carbamate-mediated mutagenesis of LAB strains with the objective of enhancing lactic acid biosynthesis. By exposing cultures to controlled concentrations of ethyl carbamate and screening resultant mutants, this work aims to identify improved LAB isolates with higher lactic acid productivity. Furthermore, the study discusses the implications of ethyl carbamate-induced genetic modifications on fermentation efficiency, providing insights into the feasibility of employing such mutagenesis as a tool for microbial strain improvement in lactic acid biotechnology[6-11].

Mutagenesis has emerged as a classical yet powerful approach to enhance microbial metabolic potential[12-16]. Both physical (e.g., UV irradiation, gamma rays) and chemical mutagens (e.g., nitrosamines, alkylating agents, carbamates) have been widely used to generate genetic diversity and isolate superior microbial strains. Among these, ethyl carbamate (urethane) represents a potent chemical mutagen known for

its ability to induce base substitutions, chromosomal aberrations, and metabolic pathway alterations. Although ethyl carbamate is primarily recognized as a probable human carcinogen[17-20] (IARC Group 2A), its mutagenic potential can be strategically applied in controlled laboratory settings to develop microbial variants with improved industrial traits.

In the present study of authors have confined their investigation for EC-mediated mutagenesis for enhanced lactic acid production by *Lactobacillus casei* NCIM-1692.

Experimental :

The influence of ethyl carbamate on production of lactic acid by *Lactobacillus casei* NCIM - 1692. The composition of the production medium for the Production of lactic acid by *Lactobacillus casei* NCIM - 1692 was prepared as follows :

Molasses : 25% (w/v), Malt Extract : 1.42%
Yeast Extract : 1.42%, Peptone : 1.42%,
$(NH_4)_2HPO_4$: 1.42%, $CaCO_3$: 9.50 %, pH: 5.9
Distilled water : To make up 100 ml.

The pH of the medium was adjusted to 5.9 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. Now, the same produciton medium for was prepared for production of lactic acid in 99 fermentor flasks, i. e., each fermentor flask containg '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 ethyl carbamate 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 ethyl carbamate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th 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 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 3, 7 and 10 days for lactic acid[20] formed and molasses sugars[21] left unfermented.

Results and Discussion
The influence of ethyl carbamate

The data given in the table -1 shows that the mutagen ethyl carbamate has been found stimulatory for production of lactic acid by *Lactobacillus casei* NCIM - 1692. From the data given in the table it is obvious that ethyl carbamate influences the lactic acid fermentation process in different phases. The main characteristics of the ethyl carbamate is as follows :

(i) Ethyl carbamate is stimulatory at its molar concentrations used during course of the production of lactic acid by *Lactobacillus casei* NCIM - 1692, i.e. from $1.0 \times 10^{-5}M$ to $6.0 \times 10^{-5}M$.

Table - 1
Production of lactic acid by *Lactobacillus casei* NCIM - 1692 exposed to ethyl carbamate

Concentation of mutagen used a x 10^{-x} M	Incubation period in days	Yield of lactic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 7 days of incubation pd.
Control	3	5.996	4.337	–
(– mutagen)	7	9.365	0.986	–
	10	6.820	0.925	–
1.0 x 10^{-5} M	3	6.109	4.225	–
(+ mutagen)	7	9.550	0.953	(+) 1.975
	10	6.949	0.905	–
2.0 x 10^{-5} M	3	6.211	4.139	–
(+ mutagen)	7	9.705	0.918	(+) 3.630
	10	7.066	0.876	–
3.0 x 10^{-5} M	3	6.379	3.954	–
(+ mutagen)	7	9.965	0.863	(+) 6.406
	10	7.256	0.814	–
4.0 x 10^{-5} M	3	6.583	3.759	–
(+ mutagen)	7	10.285	0.813	(+) 9.823
	10	7.488	0.768	–
5.0 x 10^{-5} M	3	6.697	3.636	–
(+ mutagen)	7	10.462	0.778	(+) 11.713
	10	7.618	0.733	–
6.0 x 10^{-5} M**	3	6.805	3.530	–
(+ mutagen)	7	10.630***	0.710	(+) 13.507
	10	7.740	0.691	–
7.0 x 10^{-5} M	3	6.643	3.695	–
(+ mutagen)	7	10.380	0.805	(+) 10.838
	10	7.556	0.717	–
8.0 x 10^{-5} M	3	6.433	3.915	–
(+ mutagen)	7	10.050	0.888	(+) 7.314
	10	7.316	0.793	–
9.0 x 10^{-5} M	3	6.313	4.112	–
(+ mutagen)	7	9.863	0.915	(+) 5.317
	10	7.182	0.886	–
10.0 x 10^{-5} M	3	6.163	4.178	–
(+ mutagen)	7	9.629	0.993	(+) 2.819
	10	7.010	0.927	–

* Each value represents mean of three trials.
** Optimum concentration of mutagen.
*** Optimum yield of lactic acid (+) Values indicate % increase in the yield of lactic acid
Experimental deviation $\pm$ 2.5 – 3.5%

(ii) The molar concentration 1.0x10^{-5}M, 2.0 x10^{-5} M and 3.0 x 10^{-5} of ethyl carbamate influence the yield of lactic acid in a approximately regular doubling order after each state i. e. 1.975%, 3.630% and 6.406%.

(iii) The molor concentration 4.0 x 10^{-5}M, 5.0 × 10^{-5}M and 6.0 x10^{-5} of ethyl carbamate now influence the productivity of lactic acid in a regular manner enhancing the yield from X to 2 + X and 3+ X approx. respectively where X is the % increase in the yield of lactic acid. The % increase in the yield of lactic acid at respective molar concentration of ethyl carbamate has been found to be as follows : 9.823%,11.713%, and 13.507% approximataly (X, 2 + X, and 4 + X)

(iv) The higher molar concentrations, i.e., 7.0 x 10^{-5}M, to 10.0 x 10^{-5} of ethyl carbamate influences the yield of lactic acid in decreasing order and therefore, the % difference in the yield of lactic acid has been found to be almost discouraging as : 10.838%, 7.314%, 5.317% and 2.819%.

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