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Studies on biotic production of lactic acid exposed to ethosuximide

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Abstract : The efficacy of ethosuximide on biotic production of lactic acid by *Lactobacillus pentosus* SH -114 was assessed. The compound ethosuximide was found stimulatory when 15% (w/v) molasses solution was allowed to ferment at pH 6.1, temperature 34^{0}C and optimum incubation period of 6 days. It has been found that there is a gradual increase in the biotic production of lactic acid with stepping up of the compound ethosuximide till the maximum yield of lactic acid, i.e., 7.682g/100 ml was obtained at its molar concentration of $7.0 \times 1/10000 \times 10$M which is 20.615% higher in comparison to control fermentor flasks in 6 days of optimum incubation period.

(**Key words :** Lactic acid fermentation, ethosuximide and *Lactobacillus pentosus* SH -114).

Introduction

It has been reported that a few organic biomolecules are very active and play biotic properties of vital importance in the biotic production of some useful micro and macro organic molecules. Though biologically active organic biomolecules are not essentially growth promoter for some microbes and microbial process yet a few organic biomolecules are utilized by some or all microbes for their nutritional requirements. Although a group of workers[1-6] have tried to explore the effect of some organic biomolecules and their derivatives on microbial enzymes systems, yet there is no definite opinion regarding its influence on fermentation processes. Research has shown that there are essential, highly active organic molecules that can, even in extremely small quantities, vastly influence the fermentative actions and interactions. A number of organic molecules and their derivatives are well known to show physiological and pharmacological property. Information regarding their role in biotic system is very much limited and still unsettled. There are large group of some organic molecules which when introduced to the fermentation medium can effect the enzymes responsible for the biosynthesis of micro and macro molecules in the microbial cells as well bioconversion of raw substrate into desired products and such organic compounds may be referred to as physiologically active organic compounds.

In view of the scarce knowledge regarding involvement of active organic biomolecules to any fermentation processes specially lactic acid fermentation the authoress has made an attempt to study the effect of ethosuximide on biotic production of lactic acid by *Lactobacillus pentosus* SH -114.

Experimental

The influence of ethosuximide on biotic production of lactic acid by *Lactobacillus pentosus* SH -114.

The composition of the production medium for the biotic production of lactic acid by *Lactobacillus pentosus* SH -114 was prepared as follows :

Molasses : 15% (w/v) , Malt Extract: 1.85%
Yeast Extract : 1.85%, Peptone : 1.85%
$(NH_4)_2HPO_4$: 1.50%, $CaCO_3$: 7.5 %, 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 compostion medium represents volume of a fermentor flask, i. e., "100ml" production medium for biotic production of lactic acid.

Now, the same produciton medium for biotic production of lactic acid by *Lactobacillus pentosus* SH -114 was prepared for 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 ethosuximide 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 containg no active organic i.e., ethosuximide Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of ethosuximide in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 1/10000×10M

1.0 x 1/10000×10M to 10.0 x 1/10000×10M

Where

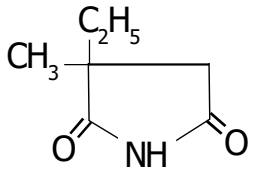
A = amount of active organic molecule, i.e. ethosuximide in ml,

1/10000×10M = molarity of the solution.

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 2, 6 and 10 days for lactic acid[7] formed and molasses[8] sugars left unfermented.

Results and Discussion

Biotic production of lactic acid exposed to ethosuximide



2-ethyl-2-methylsuccinimide (Ethosuximide*) [Compound I]*

The data recorded in the table-1 shows that the addition of 2-ethyl-2-methylsuccinimide (Ethosuximide) into the biotic production of lactic acid fermentation medium enhances the biotic production of lactic acid significantly. It has been observed that there is also a gradual increase in the yield of lactic acid with gradual stepping up of the compound I, i. e., 2-ethyl-2-methyl-succinimide (Ethosuximide) till the maximum yield of lactic acid is reached which is 20.615% higher in comparison to flat bottom control fermentor flasks, i. e., 7.682 g/100 ml at 7.0 x 1/1000×10M molar concentration of the compound 2-ethyl-2-methylsuccinimide (Ethosuximide) in 6 days of optimum incubation period.

It has been observed that the compound 2-ethyl-2-methylsuccinimide (Ethosuximide) is a very important physiologically active organic compound and its biological activities may be attributed to the active >C = O groups associated with five membered hetero organic molecule and -CO-NH-CO-linkage. Since no clear evidence could be put forward regarding its activity and stimulating properties of lactic acid fermentation process the compound 2-ethyl-2-methyl-succinimide (Ethosuximide) is considered to influence critically some metabolic enzymatic pathways intimately concerned with the biotic production of lactic acid by using the bacterial strain of *Lactobacillus pentosus* SH-114. It has been discussed earlier that barbiturates and most

Table - 1
Biotic production of lactic acid exposed to ethosuximide

Concentration of AoM 1 × 10000 ×10 M to 10000 ×10 M	Incubation period in days	Yield of lactic acid* in g/100 ml	molasses* left unfermented in g/100 ml	% of lactic acid Increased (+) in 6 days
Control	6	6.369	1.586	–
1.0 x 10000 ×10 M	6	6.490	1.465	(+) 1.899
2.0 x 10000 ×10 M	6	6.646	1.310	(+) 4.349
3.0 x 10000 ×10 M	6	6.911	1.049	(+) 8.509
4.0 x 10000 ×10 M	6	7.220	0.739	(+) 13.361
5.0 x 10000 ×10 M	6	7.404	0.559	(+) 16.250
6.0 x 10000 ×10 M	6	7.531	0.425	(+) 18.244
7.0 x 10000 ×10 M	6	7.682***	0.275	(+) 20.615
8.0 x 10000 ×10 M	6	7.149	0.816	(+) 12.246
9.0 x 10000 ×10 M	6	6.789	1.169	(+) 6.594
10.0 x 10000 ×10 M	6	6.580	1.378	(+) 3.312

* Each value represents mean of three trials. ** Optimum concentration of AOM, i.e., ethosuximide
*** Optimum yield of lactic acid (–) Values indicate % decrease in the yield of lactic acid
Experimental deviation $\pm$ 2.5 – 3.5%

of its derivative compounds possesses most effective >C=O groups and-NH-CO-NH-linkage but the compound II instead bears -CO-NH-CO- and group[9-10]

The compound I bears more than two >C=O groups and -CO-NH-CO- linkage which serves as a most effective energy source and influences significantly the growth and activity of the lactic acid bacteria *Lactobacillus pentosus* SH-114 and thereby enhances the biotic production of lactic acid by *Lactobacillus pentosus* SH-114. It was also interesting to note that almost at all the concentrations of compounds I the % of lactic acid produced was higher than control. The favourable and significant response of the compound I may also be attributed to the fact that in compound I at least at the bonds where oxygen is attached with carbon of compound I there is a chance and probability of accepting protons given by the different enzyme surface area itself thereby getting increased electronegativity. The bacterial activity under the above circumstances is expected to go more exogeneously because the increase of electronegativity more and more of related enzymes are expected to participate and to take the position for reaction with active sites of the compound I and because of this stream of mobility population of the cell enzymes, most of them are expected to occur at the surroundings area of bacterial cells or enzymes elaborated by the strain *Lactobacillus pentosus* SH-114 in the fementation medium.

Although the exact mechanism of the role of compound I is still some what uncertain and at present it is very difficult to predict the real reason of the significant response of the compound I on biotic production of lactic acid by using the bacterial strain of *Lactobacillus pentosus* SH-114 because there may be wide spread probable possibilities in this regard as follows :

Incorporation of compound I in fermentation medium may cause an alternation in the structure and behaviour of enzymes that geometrically fits with the molasses substratum which is vital force as well source of lactic acid production by *Lactobacillus pentosus* SH-114. It may also cause an enhancement of the quantum of the enzymes which may thus increase the efficiency per unit of the elaborated enzyme and hence biotic transformation of molasses pollutant to lactic acid by utilizing a major percent of molasses substrate, i. e., molasses in the fermentation medium[11-29]

It may, therefore, be concluded that addition of 2-ethyl-2-methylsuccinimide (Ethosuximide) to the production medium has stimulatory effect at all its concentrations used, i.e., from 1.0 x 1/1000 × 10M to 10.0 x 1/1000 × 10M and the yield of lactic acid has been found greater in each case in comparison to control fermentor flasks. However, incorporation of 2-ethyl-2-methylsuccinimide (Ethosuximide) at higher concentration level is not encouraging for biotic production of lactic acid by *Lactobacillus pentosus* SH-114.

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