

Impact of succinimide on lactic acid fermentation

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Abstract : The impact of succinimide on lactic acid fermentation by some lactic acid bacteria such as *Lactobacillus gasserii* 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 producer. The compound succinimide 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 hours. It has been found that there is a gradual increase in the production of lactic acid with stepping up of the compound succinimide till the maximum yield of lactic acid, i.e., 7.736g/100 ml was obtained at its molar concentration of $7.0 \times 1/10000 \times 10M$ which is 21.249% higher in comparison to control fermentor flasks in 144 hours of optimum incubation period.

(Keywords : Lactic acid fermentation, succinimide and *Lactobacillus lactis* AK-140)

Introduction

Biologically active compounds from aromatic and medicinal herbs are relatively unstable, have strong odors, can have low water solubility, and degrade quickly during the processing and storage. Encapsulation technology offers protection, controlled and targeted release of encapsulated biologically active compounds. Nanoencapsulation technology is always evolving, providing thus new methodology in preparation and application of delivery systems loaded with bioactive compounds. This review aims to summarize the most important information for the impact of active organic molecules on lactic acid fermentation.

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 workers¹⁻⁷ 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 influence 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 molecules.

In view of the scarce knowledge regarding involvement of active organic biomolecules to any fermentation processes specially lactic acid fermentation the authors

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have made an attempt to study the effect of succinimide on lactic acid fermentation by *Lactobacillus lactis* AK-140.

Experimental

The influence of succinimide on lactic 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 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 lactic acid fermentation by *Lactobacillus lactis* AK-140 was prepared for 99 fermentor flasks, i. e., each fermentor flask containing '100 ml' of

Table - 1
Impact of succinimide on lactic acid fermentation

Concentration of active organic molecules used A × 1/10000 × 10M	Incubation Period in hours	Yield of lactic acid* in gm/100 ml	Molasses* Pollutant left unfermented in g/100 ml	%Diff. in the yield of lactic acid in 144 hrs of optimum incubation pd.
Control	144	6.375	1.590	—
1×1/10000×10M	144	6.462	1.503	+1.364
2×1/10000×10M	144	6.650	1.315	+4.313
3×1/10000×10M	144	6.925	1.041	+8.627
4×1/10000×10M	144	7.156	0.808	+12.250
5×1/10000×10M	144	7.140	0.556	+16.235
6×1/10000×10M	144	7.522	0.443	+17.992
7×1/10000×10M	144	7.736***	0.229	+21.249
8×1/10000×10M	144	7.099	0.867	+11.356
9×1/10000×10M	144	6.771	1.194	+6.211
10×1/10000×10M	144	6.556	1.409	+2.839

* Each value represents mean of three trials. ** Optimum concentration of AOM 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%.

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 succinimide 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 active organic molecules i.e., succinimide. Now the total volume in each fermentor flask was made up to 100ml by adding requisite amount of distilled water. Thus, the concentration of succinimide in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below:

$A \times 1/10000 \times 10M$

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

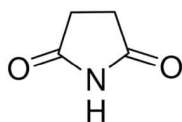
Where A = amount of active organic molecule, i.e. succinimide in ml,

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

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 72, 144 and 216 hours for lactic acid⁸ formed and molasses⁹ sugars left unfermented.

Results and Discussion

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Succinimide Compound I

The data recorded in the table-1 shows that the addition of succinimide into the lactic acid fermentation medium enhances the production of lactic acid significantly. It has been observed that there is a gradual increase in the yield of lactic acid with gradual stepping up of the compound I, i.e., succinimide till the maximum yield of lactic acid is reached which is 21.249% higher in comparison to flat bottom control fermentor flasks, i.e., 6.375 g/100 ml at $7.0 \times 1/1000 \times 10M$ molar concentration of the compound succinimide in 144 hours of optimum incubation period.

It has been observed that the compound succinimide 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 succinimide is considered to influence critically some metabolic enzymatic pathways intimately concerned with the lactic acid fermentation by *Lactobacillus lactis* AK-140. It has been discussed earlier that barbiturates and most of its derivative compounds possess most effective $>C=O$ groups and $-NH-CO-NH-$ linkage but the compound II instead bears $-CO-NH-CO-$ and group¹⁰⁻¹¹

The compound I bears 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 lactis* AK-140 and thereby enhances the production of lactic acid by *Lactobacillus lactis* AK-140. 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 lactis* AK-140 in the fermentation medium.

Although the exact mechanism of the role of compound I is still somewhat uncertain and at present it is very difficult to predict the real reason of the significant response of the

compound I on lactic acid fermentation by using the bacterial strain of *Lactobacillus lactis* AK-140 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 lactis* AK-140. 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 medium¹²⁻²⁶

It may, therefore, be concluded that addition of succinimide to the production medium has stimulatory effect at all its concentrations used, i.e., from $1.0 \times 1/10000 \times 10M$ to $10.0 \times 1/1000 \times 10M$ and the yield of lactic acid has been found greater in each case in comparison to control fermentor flasks. However, incorporation of succinimide at higher concentration level is not encouraging for lactic acid fermentation by *Lactobacillus lactis* AK-140.

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