

# Bioproduction of lactic acid by lactic acid bacteria exposed to phenobarbital sodium salt

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## Abstract

The efficacy of Phenobarbital sodium salt on bioproduction of lactic acid by lactic acid bacteria such as *Lactobacillus pentosus* NCIM – 2669, *Lactobacillus casei* NCIM – 2732, *Lactobacillus helveticus* NCIM – 2737 and *Lactobacillus plantarum* NCIM –2592 has been assessed. It has been observed that the bacterial strain *Lactobacillus casei* NCIM – 2732 is most effective for the lactic acid fermentation process. It has been found that the compound, i.e., phenobarbital sodium salt at molar concentration  $5.0 \times 10^{-5}$ M enhanced the bioproduction of lactic acid to an extent of 15.300% higher in comparison to control, i.e., 8.503g/100ml while at molar concentration  $7.0 \times 10^{-5}$ M and onwards concentration of phenobarbital sodium salt under trial inhibits and retards the bioproduction of lactic acid when experimental parameters are under optimized condition, viz. 34°C temperature, 6.0 pH, 6 days incubation period with 20% (w/v) molasses solution.

**Key Words:** Lactic acid fermentation, *Lactobacillus casei* NCIM – 2732, and phenobarbital sodium salt.

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## INTRODUCTION

A number of organic molecules and their derivatives are well known to show physiological and pharmacological property. Information regarding their role in biological 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 enzyme 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 active organic compounds. It has been found that a few organic biomolecules are very active and play biological properties of vital importance in the biosynthesis of some useful micro and macro organic molecules. Though

biologically active organic biomolecules are not essentially growth promoter for some microbes yet a few organic biomolecules are utilized by some or all microbes for their nutritional requirements. Although a group of workers<sup>1,2</sup> 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 bioproduction of lactic acid. In view of the scarce knowledge regarding involvement of active organic compounds to any fermentation processes specially lactic acid fermentation the authoress has made an attempt to study the effect of phenobarbital sodium salt on lactic acid fermentation by *Lactobacillus casei* NCIM-2732

## MATERIAL AND METHODS

**Compositions of the production medium:** The composition of the production medium for bioproduction of lactic acid by *Lactobacillus casei* NCIM-2732 exposed to phenobarbital sodium salt is as follows :

**Molasses:** 20% (W/V); Malt extract : 0.80%; yeast extract : 0.75%, Peptone : 0.75%, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> : 0.8%; CaCO<sub>3</sub> : 10%, pH :6.0, (Adjusted by adding requisite amount of phosphate-buffer solution). Distilled water : To make up 100 ml.

**Assay methods:** Evaluation of lactic acid formed and molasses left unfermented was made colorimetrically<sup>7-8</sup>

**Sterilization:** The growth and production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for 30 minutes.

**Strain:** *Lactobacillus casei* NCIM-2732 has been employed in the present study. The strain was procured from NCL - Pune, India

**Age of the inoculum:** 48 hours old.

**Quantum of the inoculum:** 0.5 ml bacterial suspension of *Lactobacillus casei* NCIM-2732

**Incubation period:** 4, 6 and 8 days

**Concentration of phenobarbital sodium salt used:** M/1000 solution of phenobarbital sodium salt under trial has been prepared and  $1.0 \times 10^{-5}M$  to  $10 \times 10^{-5} M$  molar concentration of phenobarbital sodium salt has been employed.

## RESULTS AND DISCUSSION

The influence of phenobarbital sodium salt

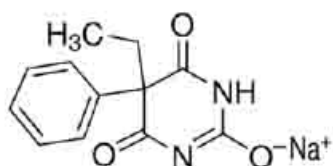


Figure 1: Phenobarbital sodium salt

**Compound I:** The addition of phenobarbital sodium salt vide table -1 in the production medium for bioproduction of lactic acid by *Lactobacillus casei* NCIM-2732 has been found significant. It has been found that there is a gradual increase in the production of lactic acid with stepping up of the compound phenobarbital sodium salt till the maximum yield of □lactic acid, i. e., 9.804g/100 ml was obtained at its molar concentration of  $6.0 \times 10^{-5} M$  which is 15.300% higher in comparison to control fermentor flasks in 6 days of optimum incubation period. The enzymes activities of the phenobarbital sodium salt may be attributed to the presence of  $>C=O$  groups of the nucleus and  $-NH-CO-NH-$  linkage, i.e., peptide linkage, present in the molecule. It has been found that many organic molecules having the  $-NH-CO-NH-$  linkage have been found of great biological significant for the maximum growth and activity of different microbes. The compound taken under trial, i.e., phenobarbital sodium

salt possesses active unsaturated  $>C=O$  groups directly attached with ring system which may serve as a more efficient source of energy and influences the growth and activity of the enzyme system associated with *Lactobacillus casei* NCIM-2732. Margalith and Pagani<sup>9,10</sup> during their industrial investigations successfully studied and compared different derivatives of barbituric acid, i.e., barbiturates and reported that the organic molecules, i.e., barbiturates has been found to be most effective and useful for various industrial fermentations process. Barbiturates in general has been found most effective and useful in different biological processes and a lot of questions are still unsettled and open concerning the mode of action of these barbiturate molecules on the enzymes catalysed systems involved in the pathways leading to the mode of enzyme functions. However, whatever their biological functions may be, these organic molecules should be incorporated in to the fermentation medium for the better functioning of the process and improved yield of the desired products. It is a secondary factor that influences the fermentation technique associated with enzymes of *Lactobacillus casei* NCIM-2732

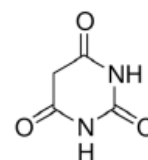


Figure 2: Barbituric acid

Further, a group of researchers<sup>11-17</sup> have reported stimulatory effect of barbituric acid and its derivatives possessing barbiturate nucleus and also some active organic molecules on microbes and microbial process<sup>18-25</sup>. Since the organic molecule, i.e., phenobarbital sodium salt also possess part structure combination of barbiturate nucleus, it may influence critically the outcome of lactic acid by the bacterial strain of *Lactobacillus casei* NCIM-2732. Singh *et al*<sup>26-27</sup> also found 5,5'-diphenylhydantoin and 5-phenyl hydantoin stimulatory for lactic acid and citric acid fermentation respectively. Leena<sup>28</sup> has reported Phenobarbital as a most effective organic compound for the higher production of lactic acid

Table 1: Bioproduction of latic acid by *Lactobacillus casei* NCIM-2732 exposed to phenobarbital sodium salt

Concentration of active organic compound	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	8.503	0.497	-
$1 \times 10^{-5}M$	6	8.691	1.309	(+) 2.210
$2 \times 10^{-5}M$	6	8.865	1.135	(+) 4.257
$3 \times 10^{-5}M$	6	9.104	0.896	(+) 7.068
$4 \times 10^{-5}M$	6	9.371	0.629	(+) 10.208
$5 \times 10^{-5}M$	6	9.524	0.480	(+) 12.007
$6 \times 10^{-5}M$	6	9.804***	0.196	(+) 15.300

$7 \times 10^{-5}M$	6	9.459	0.541	(+) 11.243
$8 \times 10^{-5}M$	6	9.226	0.774	(+) 8.502
$9 \times 10^{-5}M$	6	8.951	1.049	(-) 5.268
$10 \times 10^{-5}M$	6	8.689	1.311	(-) 2.187

\*Each value represents mean of three trials. \*\* Optimum concentration of phenobarbital sodium salt under trial. \*\*\* Optimum yield of lactic acid in 6 days Experimental deviation  $\pm 1.5 - 3.0\%$  +ve values indicate % increase in the yield of lactic acid.

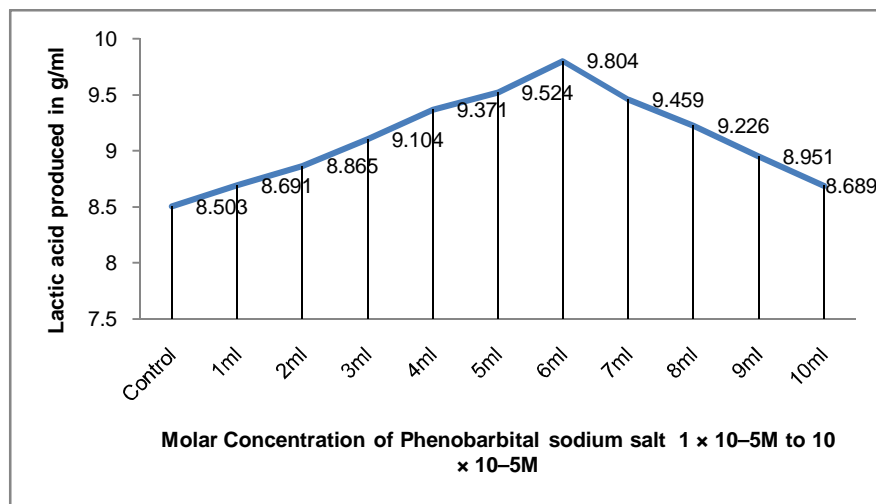


Figure 1:

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