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Submerged fermentation of lactic acid by *Lactobacillus bulgaricus* NCIM-2095 under 5,5'-Diphenylhydantoin exposure

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Abstract : The present study investigates the influence of 5,5'-diphenylhydantoin exposure on submerged fermentation of lactic acid by *Lactobacillus bulgaricus* NCIM-2095. Hydantoin derivatives are known to interact with microbial regulatory pathways, thereby modulating metabolic fluxes. Fermentation experiments were conducted to evaluate lactic acid yield, cell growth kinetics, and substrate utilization in the presence of varying concentrations of 5,5'-diphenylhydantoin. Results indicated a concentration-dependent response, wherein moderate exposure enhanced lactic acid accumulation, while higher doses exerted inhibitory effects on biomass productivity. The stimulatory effect is attributed to the compound's ability to influence enzymatic activity and cellular redox balance, leading to improved metabolic efficiency. This study highlights the potential of 5,5'-diphenylhydantoin as a regulatory modulator in microbial fermentation processes, opening new avenues for optimizing lactic acid bioproduction through targeted chemical modulation strategies. The influence of 5'5-Diphenylhydantoin on submerged fermentation of lactic acid by *Lactobacillus bulgaricus* NCIM-2095 under optimized conditions has been assessed. It has been found that the compound 5'5-Diphenylhydantoin is stimulatory and enhances the yield of lactic acid by *Lactobacillus bulgaricus* NCIM-2095 to an extent of 10.406% higher in comparison to control , i.e; 6.150 g /100 mL at 5.0×10^{-5}M molar concentration of the compound 5'5-Diphenylhydantoin when 15% molasses solution (w/v)is allowed to ferment at pH 6.0 ,temperature 41^0C and incubation period of 7 days along with some other rich ingredients required by the *Lactobacillus bulgaricus* NCIM-2095.

(**Keywords:** Submerged fermentation,lactic acid, *Lactobacillus bulgaricus* NCIM-2095 and 5'5-Diphenylhydantoin exposure)

Introduction

Lactic acid is an important organic acid with wide applications in the food, pharmaceutical, cosmetic, and biodegradable polymer industries. Microbial fermentation, particularly by lactic acid bacteria (LAB), has emerged as a sustainable and eco-friendly approach for its large-scale production. Among LAB, *Lactobacillus bulgaricus* is extensively studied due to its high fermentative potential, substrate adaptability, and Generally Recognized as Safe (GRAS) status. Submerged fermentation strategies employing *L. bulgaricus* have been widely optimized for yield enhancement through nutrient supplementation, process modifications, and exposure to chemical modulators.

The present study aims to evaluate the effect of 5,5'-diphenylhydantoin exposure on submerged fermentation of lactic acid by *Lactobacillus bulgaricus* NCIM-2095. The work focuses on assessing lactic acid yield, growth kinetics, and substrate utilization under controlled fermentation conditions, with an emphasis on determining concentration-dependent responses. This investigation highlights the potential of hydantoin derivatives as metabolic regulators for improving microbial fermentation efficiency.

Recent advancements in fermentation biotechnology emphasize the role of heterocyclic compounds and hydantoin derivatives in regulating microbial metabolism. 5,5'-Diphenylhydantoin, a well-known

pharmacological compound, possesses structural features capable of influencing cellular enzyme systems, stress responses, and regulatory pathways. Its interaction with microbial cultures is hypothesized to induce metabolic shifts, thereby altering fermentation efficiency and product yield.

Despite extensive reports on the application of chemical modulators in fermentation, the influence of 5,5'-diphenylhydantoin on lactic acid production has not been thoroughly investigated. Understanding its modulatory role may provide new insights into enhancing productivity and tailoring metabolic fluxes during lactic acid biosynthesis.

Experimental

The influence of 5,5-diphenylhydantoin on fermentative production of lactic acid by *Lactobacillus bulgaricus* NCIM-2095.

The composition of the production medium for the production of lactic acid by fermentation was prepared as follows :

Molasses:15% (w/v), Malt Extract : 0.60%
Yeast Extract : 0.60%, Peptone : 0.60%
$(NH_4)_2HPO_4$: 1.75%, $CaCO_3$: 8 %, 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 compostion medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation.

Now, the same produciton medium for fermentation production of lactic acid by *Lactobacillus bulgaricus* NCIM-2095 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 5,5-diphenylhydantoin 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 molecule. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of 5,5-diphenylhydantoin 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 active organic molecule in ml,
x = molarity of the solution containing 5,5-diphenylhydantoin

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 5, 7 and 9 days for lactic acid[7] formed and molasses[8] sugars left unfermented.

Although a group of workers[1-6] have tried to explore the efficacy of some more organic biomolecules and their derivatives on microbial enzyme levels /systems,yet there is no definite reason/opinion regarding its influence on submerged lactic acid fermentation process.

In view of the above knowledge regarding involvement of some physiologically active organic molecules to any fermentation process specifically submerged lactic acid fermentation

Table - 1
SmF bioproduction of lactic acid exposed to 5,5-diphenylhydantoin

Concentration of Mutagen 1×10^{-5}M to 10×10^{-5}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 7 days
Control	5	5.135	2.225	—
(– mutagen)	7	6.150	1.259	—
	9	4.285	1.215	—
1.0 x 10^{-5} M	5	5.400	2.205	—
(+ mutagen)	7	6.390	1.240	+3.902
	9	4.520	1.200	—
2.0 x 10^{-5} M	5	5.516	2.190	—
(+ mutagen)	7	6.526	1.231	+6.113
	9	4.618	1.196	—
3.0 x 10^{-5} M	5	5.553	2.181	—
(+ mutagen)	7	6.570	1.227	+6.829
	9	4.648	1.183	—
4.0 x 10^{-5} M	5	5.610	2.170	—
(+ mutagen)	7	6.639	1.204	+7.951
	9	4.697	1.180	—
5.0 x 10^{-5} M**	5	5.738	2.159	—
(+ mutagen)	7	6.790***	1.193	+10.406
	9	4.803	1.162	—
6.0 x 10^{-5} M	5	5.637	2.169	—
(+ mutagen)	7	6.670	1.214	+8.455
	9	4.719	1.170	—
7.0 x 10^{-5} M	5	5.552	2.174	—
(+ mutagen)	7	6.570	1.219	+6.829
	9	4.648	1.182	—
8.0 x 10^{-5} M	5	5.473	2.189	—
(+ mutagen)	7	6.477	1.228	+5.317
	9	4.582	1.203	—
9.0 x 10^{-5} M	5	5.405	2.210	—
(+ mutagen)	7	6.395	1.239	+3.983
	9	4.525	1.208	—
10.0 x 10^{-5} M	5	5.337	2.219	—
(+ mutagen)	7	6.315	1.48	+2.682
	9	4.467	1.210	—

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

process an attempt has been made by author to study the efficacy of 5'5-Diphenylhydantoin on lactic acid fermentation process by *Lactobacillus bulgaricus* NCIM-2095.

Results and Discussion

The submerged fermentation of lactic acid by *Lactobacillus bulgaricus* NCIM-2095 was evaluated under varying concentrations of 5,5'-diphenylhydantoin (DPH) to investigate its modulatory influence on microbial metabolism. The results revealed that exposure to DPH altered both the growth kinetics and lactic acid biosynthesis profile of the organism.

Fermentation Kinetics :

The specific productivity (qP) and yield coefficient (Yp/s) were enhanced at lower DPH concentrations, demonstrating a favorable metabolic shift. A significant reduction in residual sugars in low-dose DPH fermentations further confirmed improved substrate utilization efficiency. In contrast, higher concentration caused delayed substrate uptake and increased residual sugars, highlighting inhibitory stress.

Mechanistic Insight:

The biphasic effect of DPH may be explained by its structural similarity to hydantoin derivatives known to interact with cellular enzymes and redox systems. At sub-lethal levels, DPH possibly triggers stress-response pathways, leading to enhanced energy flux toward lactic acid synthesis. Beyond a threshold, however, the compound exerts toxic effects on membrane integrity, enzyme activity, and interacselly redox balance, thereby impairing fermentation.

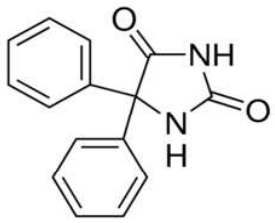
Comparative Context:

Similar biphasic modulatory patterns have been reported with other xenobiotics in lactic acid bacteria, where low-level exposure stimulates secondary metabolism, while higher concentrations exert inhibitory or mutagenic effects. This finding emphasizes the potential of controlled xenobiotic stress as a tool to fine-tune industrial fermentations.

Summary:

The results demonstrate that 5,5'-diphenylhydantoin has a concentration-dependent influence on lactic acid fermentation by *L. bulgaricus* NCIM-2095. Low doses act as positive modulators, enhancing lactic acid yields, whereas higher concentrations are inhibitory. These insights provide a foundation for exploring xenobiotic-mediated metabolic regulation in lactic acid biotechnology.

The influence of 5,5-diphenylhydantoin



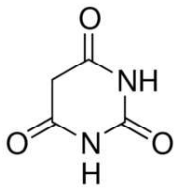
5,5-Diphenylhydantoin
Compound - I

The addition of 5,5-diphenylhydantoin vide table -1 in the production medium for SmF production of lactic acid by *Lactobacillus bulgaricus* NCIM-2095 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, 5,5-diphenylhydantoin till the maximum yield of lactic acid, i. e., 6.790 g/100 ml was obtained at its molar concentration of 5.0×10^{-5}M which is 10.406% higher in comparison to control fermentor flasks in 7 days of optimum incubation period.

The enzymes activities of the compound I, i. e., 5,5-diphenylhydantoin 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 significance for the maximum growth and activity of different microbes and microbial process. The compound taken under trial, i.e., 5,5-diphenylhydantoin 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 bulgaricus* NCIM-2095

Margalith and Pagani[9-10] 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.[11-13] 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 bulgaricus* NCIM-2095.



Barbituric acid

Further, a group of researchers[14-21] have reported stimulatory effect of barbituric acid and its derivatives possessing barbiturate nucleus. Since the organic molecule, i. e., 5,5-diphenyl-hydantoin also possess part structure combination of barbiturate nucleus, it may influence critically the outcome of lactic acid by the bacterial strain of *Lactobacillus bulgaricus* NCIM-2095. Rizvi[22] also studied effect of such type of compound on biosynthesis of citric acid by LSCF process and found it very significant for higher production of citric acid. Poonam[23] also studied the effect of 5, 5-diethylhydantoin and 1,3-dimethyl-2-thiobarbituric acid (both having barbiturate nucleus in their structure) on lactic acid fermentation and observed that both the compounds are very effective and stimulating for lactic acid fermentation process. Singh *et al*[24-25] also found 5-phenyl hydantoin stimulatory for lactic acid and citric acid fermentation respectively. The efficacy of 3-ethyl-3-phenyl piperidine-2,6-dione on lactic acid fermentation has been assessed and has been found stimulatory for higher yield of lactic acid[26-29].

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