

Exploring the role of 5,5'-diethyl hydantoin in enhancing efficiency of lactic acid bioproduction by *S. lactis*-2114

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Abstract : The growing demand for sustainable bioproduction of lactic acid has stimulated interest in exploring chemical modulators that can enhance microbial efficiency. In the present study, the role of 5,5'-diethyl hydantoin was investigated in improving lactic acid bioproduction by *Streptococcus lactis* 2114. Controlled fermentations were carried out with sucrose as the carbon source, both in the absence and presence of graded concentrations of 5,5'-diethyl hydantoin. The results demonstrated that the compound exerted a stimulatory influence on microbial growth kinetics, carbohydrate uptake, and lactic acid yield, with a notable reduction in by-product formation. Enhanced enzymatic activity of lactate dehydrogenase (LDH) and improved redox balance were observed, suggesting that 5,5'-diethyl hydantoin facilitated intracellular metabolic regulation. At the optimized concentration, lactic acid productivity increased significantly compared to control conditions, highlighting the compound's potential as an efficiency enhancer in microbial fermentation processes. These findings provide new insights into the application of hydantoin derivatives as modulators of microbial metabolism, opening avenues for cost-effective and scalable lactic acid bioproduction. The compound 5,5'-diethyl hydantoin was found stimulatory when 20% (w/v) molasses solution was allowed to ferment at pH level 6.0, temperature 43°C and optimum incubation period of 144 hrs. The maximum yield of lactic acid was found to be 6.42292 g/100mL at 4.0×10^{-4} M molar concentration of 5,5'-diethyl hydantoin which is 24.924% higher in comparison to control fermentor flasks. In the present communication the authoress has confined her study to explore the role of 5,5'-diethyl hydantoin in enhancing efficiency of lactic acid by *S.lactis*-2114.

(Keywords : 5,5'-diethyl hydantoin, *Streptococcus lactis* 2114, lactic acid, metabolic modulation, fermentation efficiency, LDH activity).

Introduction

Lactic acid is one of the most commercially significant organic acids, widely utilized in the food, pharmaceutical, cosmetic, and biodegradable polymer industries. Its global demand has grown rapidly, particularly due to its role as a precursor for polylactic acid (PLA), a sustainable biopolymer with diverse applications. Microbial fermentation has emerged as the most economical and eco-friendly method for large-scale lactic acid production, with *Streptococcus lactis* strains being among the most efficient producers due to their high lactic acid yield and substrate specificity. However, challenges such as low productivity, metabolic inhibition, and inefficient utilization of carbon sources often limit the industrial feasibility of microbial processes.¹⁻¹⁰

The present work focuses on evaluating the influence of 5,5'-diethyl hydantoin on lactic acid production by *Streptococcus lactis* 2114. By examining its effect on microbial growth kinetics, sugar utilization, and lactic acid yield, this study aims to provide insights into how metabolic enhancers such as hydantoin derivatives could be employed to achieve higher efficiency in bioproduction systems. The findings may contribute to the development of novel, cost-effective strategies for sustainable lactic acid fermentation¹¹⁻¹⁷.

To overcome these limitations, researchers have investigated mutagenesis, nutrient supplementation, and the use of

molecular enhancers that modulate microbial physiology. Hydantoin derivatives, particularly 5,5'-diethyl hydantoin, have attracted attention because of their ability to influence microbial metabolism, enzyme activity, and stress tolerance. These cyclic ureide compounds can act as stabilizers of enzymatic pathways and may reduce feedback inhibition during fermentation. Previous studies have highlighted the potential of hydantoin derivatives in improving microbial growth and metabolite synthesis, though their role in lactic acid bioproduction remains largely unexplored.

Experimental

Influence of 5,5'-diethyl hydantoin on biosynthesis of α -hydroxypropionic acid by *S. lactis* 2114 The composition of the production medium for the biosynthesis of α -hydroxypropionic acid by *S. lactis* 2114 was prepared as follows:

Molasses : 20% (W/V) Malt-extract : 0.378%

$(\text{NH}_4)_2\text{HPO}_4$: 30%, CaCO_3 : 6%, pH : 6.0

Distilled water was added to make up the volume upto 100 ml.

The pH of the production medium was adjusted to 6.0 by adding requisite amount of phosphate buffer solution and the pH value 6.0 was also ascertained by a pH meter.

The above composition medium represents volume of a conical flask, i.e., 100 ml production medium for lactic acid fermentation.

Now, the same production medium for lactic acid fermentation was prepared for 99-conical flask i. e. each flask containing 100 ml of production medium.

The above flasks were then arranged in 10-sets each comprising 9-flasks. Each set was again rearranged in 3-subsets, each comprising of 3-flasks. The remaining 9-flasks out of 99-flasks were kept as control and these were also

rearranged in 3-subsets each consisting of 3-flasks.

Now, M/100 solution of 5,5'-diethyl hydantoin 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 flasks of 1st to 10th sets respectively. The control flasks contained no physiologically active organic compound. Now, the total volume in each conical flask was made upto 100 ml by adding requisite amount of distilled water. Thus, the concentration of 5,5'-diethyl hydantoin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$a \times 10^{-x} \text{ M}$

i. e. $1.0 \times 10^{-4} \text{ M}$ to $10.0 \times 10^{-4} \text{ M}$

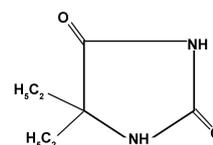
Where a=amount of physiologically active organic compound in ml.

x = molarity of the solution

The flasks were then sterilized, cooled, inoculated, incubated and analysed after 120, 144 and 168 hours for lactic acid¹⁸ formed and sugar¹⁹ left unfermented.

Results and Discussion

The influence of 5,5'-diethyl hydantoin ;



5,5'-diethyl hydantoin (Compound - I)

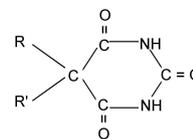
The presence of 5,5'-diethyl hydantoin (-vide table -1) was found to be most significant. It was observed that there is a gradual increase in lactic acid production with stepping up of 5,5'-diethyl hydantoin till the maximum yield of lactic acid 6.64656 g/100 ml was obtained at $4.0 \times 10^{-4} \text{ M}$ concentration of compound I which is 24.92% higher in comparison to control in 144 hours of optimum incubation period. The biological activity of the compound 5,5'-diethyl hydantoin may be attributed to the unsaturated $>\text{C}=\text{O}$ groups and -NH-CO-NH- peptide linkage. Many organic

Table-1
Biosynthesis of α -hydroxy propionic acid by *S. lactis* 2114 exposed to 5,5'- diethyl hydantoin

Concentration of PAOC used	Incubation period in hrs.	Yield of lactic acid* in g/100 ml	Sugar left* unfermented in g/100 ml	% of lactic acid increased in 144 hrs. of optimum incubation period
Control – PAOC	120	4.40268	2.36028	—
	144	5.32046	1.42531	—
	168	4.80530	1.41673	—
1.0 x 10 ⁻⁴ M + PAOC	120	5.01902	1.74853	—
	144	6.11481	0.63279	(+)14.93010
	168	5.42995	0.59601	—
2.0 x 10 ⁻⁴ M + PAOC	120	5.10709	1.66100	—
	144	6.27433	0.47481	(+) 17.92833
	168	5.62218	0.46399	—
3.0 x 10 ⁻⁴ M + PAOC	120	5.19508	1.57309	—
	144	6.38068	0.36848	(+) 19.92722
	168	5.71830	0.35792	—
4.0 x 10 ⁻⁴ M** + PAOC	120	5.36966	1.39848	—
	144	6.64656	0.10438	(+) 24.92453
	168	5.90867	0.10327	—
5.0 x 10 ⁻⁴ M + PAOC	120	***	—	—
	144	6.59338	0.15722	(+) 23.92499
	168	***	—	—
6.0 x 10 ⁻⁴ M + PAOC	120	***	—	—
	144	6.42292	0.32659	(+) 20.72114
	168	***	—	—
7.0 x 10 ⁻⁴ M + PAOC	120	***	—	—
	144	6.38746	0.36121	(+) 20.05465
	168	***	—	—
8.0 x 10 ⁻⁴ M + PAOC	120	***	—	—
	144	6.20682	0.54236	(+) 16.65946
	168	***	—	—
9.0 x 10 ⁻⁴ M + PAOC	120	***	—	—
	144	5.49818	1.24495	(+) 3.34031
	168	***	—	—
10.0 x 10 ⁻⁴ M + PAOC	120	***	—	—
	144	5.49809	1.24556	(+) 3.33862
	168	***	—	—

* Each value represents mean of three observations. (+) values indicate % increase in the yield of lactic acid. ** Optimum concentraion of PAOC and yield of lactic acid. *** Insignificant yield of lactic acid but more than control. Experimental deviation (\pm) 1.5 to 2.5%

compounds with the -NH-CO-NH-peptide linkage have been found of great biological importance for the growth and activity of different microbes. The -NH-CO-NH- linkage serves as a more efficient energy source and influences the growth promoting activity of microbes. The compound 5,5'-diethyl hydantoin possesses unsaturated $>C=O$ groups which probably activates the enzyme systems of lactic acid bacteria *S. lactis* 2114. Margalith and Pagani²⁰⁻²¹ during their investigations compared various derivatives of the barbituric nucleus and found that the compounds of general formula given below were the most useful and effective on industrial fermentation process.



(Barbiturate nucleus)

Singh and Tiwari²² have also reported stimulatory effect of barbituric acid, alloxan etc. having combination of barbiturate nucleus. Since 5,5'-diethyl hydantoin also possesses part structure combination of barbiturate nucleus, it influences critically the outcome of lactic acid production.

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