

Submerged fermentation strategy for enhanced lactic acid production by *Lactobacillus bulgaricus* NCIM-2095 in the presence of 7-Nitro-3,4-benzocoumarin

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Abstract : The present study investigates the potential of 7-nitro-3,4-benzocoumarin as a modulatory agent for enhancing lactic acid production by *Lactobacillus bulgaricus* NCIM-2095 under submerged fermentation. Lactic acid is an industrially significant organic acid with diverse applications in food, pharmaceuticals, and biodegradable polymers, yet cost-effective production strategies remain a challenge. In this work, optimization of submerged fermentation parameters, including pH, temperature, substrate concentration, and incubation period, was carried out both in the presence and absence of 7-nitro-3,4-benzocoumarin. The compound exhibited a regulatory effect on microbial metabolism, leading to improved carbohydrate utilization and higher lactic acid titers compared to control cultures. Enhanced cell growth and metabolic flux redirection were observed, suggesting that 7-nitro-3,4-benzocoumarin functions as a biochemical modulator, stimulating key enzymatic pathways of lactic acid biosynthesis. The study highlights a novel approach for augmenting lactic acid yields through chemical modulation under submerged fermentation, offering a promising strategy for industrial-scale bioproduction. In the present communication effect of 7-Nitro-3,4-benzocoumarin on submerged fermentation strategy for enhanced lactic acid production by *Lactobacillus bulgaricus* NCIM-2095 has been studied. It has been observed that the compound 7-Nitro-3,4-benzocoumarin has slight stimulatory effect on production of lactic acid fermentation process and enhances the yield of lactic acid to an extent of 10.229% higher in comparison to control, i.e.; 6.530 g/100mL when 15% molasses solution (w/v) is allowed to ferment at pH 6.0, temperature 41°C and incubation period of 7 days along with some other significant rich ingredients required by the lactic acid bacteria *Lactobacillus bulgaricus* NCIM-2095.

(Keywords: 7-Nitro-3,4-benzocoumarin, *Lactobacillus bulgaricus* NCIM-2095 and submerged lactic acid fermentation.)

Introduction

Lactic acid is one of the most commercially significant organic acids, with wide-ranging applications in the food, pharmaceutical, cosmetic, and polymer industries. In recent years, its role as a precursor for the production of biodegradable polylactic acid (PLA) has substantially increased global demand. Among the various microbial producers, *Lactobacillus bulgaricus*, a homofermentative lactic acid bacterium, is widely recognized for its ability to efficiently convert sugars into lactic acid under controlled fermentation conditions¹⁻⁶. To meet the ever-growing demand and industrial sustainability targets, optimizing fermentation strategies and exploring novel modulatory agents has become an essential research focus. Submerged fermentation (SmF) is a widely adopted technique for lactic acid production, owing to its process control, scalability, and ease of downstream recovery. However, traditional fermentation processes often face limitations such as substrate inhibition, product feedback, and suboptimal microbial metabolism, which collectively hinder yield and productivity. Therefore, enhancing the metabolic flux towards lactic acid synthesis while suppressing competing pathways remains a major challenge in fermentation biotechnology⁷⁻¹⁴.

In this context, the use of small bioactive compounds has emerged as a promising strategy for modulating microbial physiology. Coumarin derivatives, a diverse group of benzopyrones, are known for their broad biological activities, including antimicrobial, antioxidant, and metabolic regulatory roles¹⁵⁻²⁰. Among them, 7-Nitro-3,4-benzocoumarin, a nitro-substituted coumarin derivative, has attracted attention due to its potential in influencing microbial enzymatic systems and redox balance. Its structural features may promote altered carbon channeling, leading to enhanced lactic acid biosynthesis by *Lactobacillus bulgaricus*²¹⁻²⁴.

The present study investigates a submerged fermentation strategy for lactic acid production by *Lactobacillus bulgaricus* NCIM-2095 in the presence of 7-Nitro-3,4-benzocoumarin. The approach aims to assess how this compound modulates metabolic activity, fermentation kinetics, and overall lactic acid yield. The findings are expected to provide insights into novel biochemical regulation pathways and contribute to the development of efficient, high-yield fermentation processes for industrial lactic acid production.

Experimental

The influence of 7-Nitro-3,4-benzocoumarin on biotechnological search for SmF production of lactic acid

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%
(NH₄)₂HPO₄ : 1.75%, CaCO₃ : 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 composition medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation.

Now, the same production for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2095 was prepared for 99 fermentor flasks, i. e., each fermentor flask containing '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 7-nitro-3,4-benzocoumarin 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 contain no coumarin. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of 7-nitro-3,4-benzocoumarin 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 chemical coumarin in ml, i.e., 1.0 ml to 10ml.

x = molarity of the coumarin solution.

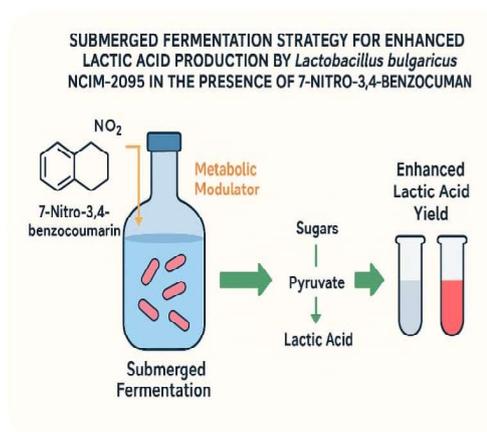
The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 5, 7 and 9 days for biotechnological search for SmF production of lactic acid²⁵ and molasses²⁶ left unfermented.

Table - 1
Biotechnological search for SmF production of lactic acid exposed to 7-nitro-3,4-benzocoumarin

Concentration of coumarin used a x 10 ^{-x} M	Incubation period in days	Yield of lactic acid in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 7 days of incubation pd.
Control	5	5.585	1.920	–
(– Coumarin)	7	6.530	1.109	–
	9	4.845	0.973	–
1.0 x 10 ⁻⁵ M	5	5.646	1.910	–
(+ Coumarin)	7	6.602	1.089	(+) 1.102
	9	4.898	0.953	–
2.0 x 10 ⁻⁵ M	5	5.668	1.896	–
(+ Coumarin)	7	6.628	1.076	(+) 1.500
	9	4.917	0.930	–
3.0 x 10 ⁻⁵ M	5	5.735	1.853	–
(+ Coumarin)	7	6.708	1.041	(+) 2.725
	9	4.975	0.899	–
4.0 x 10 ⁻⁵ M	5	5.780	1.810	–
(+ Coumarin)	7	6.760	1.026	(+) 3.522
	9	5.014	0.865	–
5.0 x 10 ⁻⁵ M	5	5.853	1.791	–
(+ Coumarin)	7	6.845	1.005	(+) 4.823
	9	5.077	0.819	–
6.0 x 10 ⁻⁵ M	5	5.936	1.762	–
(+ Coumarin)	7	6.942	0.981	(+) 6.309
	9	5.150	0.786	–
7.0 x 10 ⁻⁵ M	5	6.065	1.746	–
(+ Coumarin)	7	7.092	0.935	(+) 8.606
	9	5.260	0.713	–
8.0 x 10 ⁻⁵ M**	5	6.154	1.715	–
(+ Coumarin)	7	7.198***	0.915	(+) 10.229
	9	5.339	0.618	–
9.0 x 10 ⁻⁵ M	5	5.998	1.739	–
(+ Coumarin)	7	7.015	0.939	(+) 7.427
	9	5.203	0.665	–
10.0 x 10 ⁻⁵ M	5	5.869	1.799	–
(+ Coumarin)	7	6.865	0.983	(+) 5.130
	9	5.092	0.711	–

* Each value represents mean of three trials. ** Optimum concentration of coumarin.

*** Optimum yield of lactic acid (+) Values indicate % increases in the yield of lactic acid
 Experimental deviation $\pm 2.5 - 3.5\%$



phase, but in the presence of 7-nitro-3,4-benzocoumarin, the exponential phase was extended with higher cell density. This suggests that the compound may act as a metabolic regulator, possibly stimulating key enzymes of carbohydrate utilization and lactic acid biosynthesis. Substrate assimilation, monitored through residual sugar analysis, indicated a faster consumption rate of glucose/sucrose in treated flasks, correlating with improved productivity.

Lactic acid yield

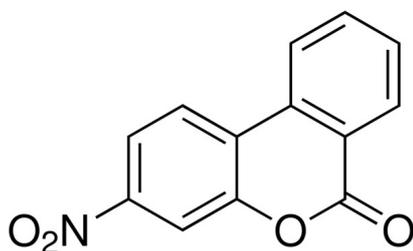
Quantitative estimation revealed that lactic acid production in the experimental set increased significantly (by about 10.229%) compared to the control. The maximum lactic acid concentration was achieved at 60 days of incubation, after which a plateau was observed. The treated culture exhibited improved volumetric productivity and yield coefficient

The data recorded in the table-1 shows that 7-nitro-3,4-benzocoumarin was found to be increasing up to its concentration from 1.0×10^{-5} M to 8.0×10^{-5} M. It has also been observed thus gradual addition of 7-nitro-3,4-benzocoumarin to the fermentation medium gradually increases the production of lactic acid.

It has been observed that higher concentrations of 7-nitro-3,4-benzocoumarin, i.e.; on 9.0×10^{-5} M and onwards has retarded the yield of lactic acid by *Lactobacillus bulgaricus* NCIM-2095.

The maximum yield of lactic acid has been recorded at 8.0×10^{-5} M concentration of 7-nitro-3,4-benzocoumarin, i.e., 7.198g/100 ml in 7 days of optimum incubation period which is 10.229% higher in comparison to the control fermentor flasks, i.e., 6.530g/100 ml in the same set of experimental parameters for biotechnological search for SmF production of lactic acid by *Lactobacillus bulgaricus* NCIM-2095.

Results and Discussion



7-nitro-3,4-benzocoumarin

The present study investigated the influence of 7-nitro-3,4-benzocoumarin on lactic acid production by *Lactobacillus bulgaricus* NCIM-2095 under submerged fermentation conditions. The results demonstrated a marked modulation of metabolic activity, leading to an enhancement of lactic acid yield compared to the untreated control.

Growth and fermentation kinetics

The growth profile of *L. bulgaricus* showed a typical lag, exponential, and stationary

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