

Submerged fermentative production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 exposed to 7-hydroxycoumarin-3-carboxylic acid

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Abstract : The efficacy of 7-hydroxycoumarin-3-carboxylic acid on fermentative production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 has been assessed. It has been found that 7-hydroxycoumarin-3-carboxylic acid at concentrations 7.0×10^{-5} M enhances the production of lactic acid to an extent of 10.720 g/100ml which 13.367% higher in comparison to control, i.e. 9.456g/100ml while at 8.0×10^{-5} M and onwards concentrations of 7-hydroxy-coumarin-3-carboxylic acid inhibits the fermentative production of lactic acid to a great extent in comparison to control.

(Keywords : Lactic acid fermentation, 7-hydroxycoumarin-3-carboxylic acid and *Lactobacillus delbrueckii* NCIM - 2025).

Introduction

Coumarin was first isolated by Vogel¹ in 1820 from the fruit of *Dipteryx odorata* Wild (Tonka bean) of Munich; he mistook it for benzoic acid.^{1,2} Also in 1820, Nicholas Jean Baptiste Gaston Guibourt (1790-1867) of France independently isolated coumarin; he realized that it was not benzoic acid.³ In a subsequent essay that he read before the pharmacy section of the L'Académie royale de médecine, Guibourt named the new substance "coumarine".⁴ In 1835, the French pharmacist A. Guillemette proved that Vogel and Guibourt had isolated the same substance.⁵ Coumarin was first synthesized in 1868 by the English chemist William Henry Perkin.⁶ More than 1350 coumarins have been isolated till 1997. Coumarin has clinical medical value by itself, as an edema modifier. Coumarin and other benzopyrones, such as 5,6 benzopyrone,^{1,2} benzopyrone, diosmin and others are known to

stimulate macrophages to degrade extracellular albumen, allowing faster resorption of edematous fluids.⁷⁻¹⁵ Certain substances effect the growth and activity quite miraculously. In earlier literature these were referred to as hormones¹⁶⁻²⁶.

The present communication was undertaken to study the submerged fermentative production of lactic acid by *Lactobacillus delbrueckii* NCIM-2025 exposed to 7-Hydroxycoumarin-3-carboxylic acid

Experimental

The influence of 7-hydroxycoumarin-3-carboxylic acid on fermentative production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025.

The composition of the production medium for the production of lactic acid by fermentation was prepared as follows :
Molasses : 22% (w/v) , Malt Extract:1.75%
Yeast Extract : 1.75%, Peptone : 1.75%
(NH₄)₂HPO₄:1.75%, CaCO₃ : 10 %
pH : 5.9 Distilled water, To make up 100 ml.

The pH of the medium was adjusted to 5.9 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 medium for lactic acid fermentation by *Lactobacillus delbrueckii* NCIM-2025 was prepared for 99

Table-1
SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025
exposed to 7-hydroxycoumarin-3-carboxylic acid

Concentration of Coumarin	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 of incubation pd.
Control	5	8.563	1.938	-
(– Coumarin)	6	9.456	1.549	-
	7	7.605	1.458	-
1.0 x 10 ⁻⁵ M	5	8.683	1.818	-
(+ Coumarin)	6	9.603	1.397	-
	7	8.701	1.295	+ 1.554
2.0 x 10 ⁻⁵ M	5	8.785	1.715	-
(+ Coumarin)	6	9.712	1.289	-
	7	8.610	1.198	+ 2.707
3.0 x 10 ⁻⁵ M	5	8.914	1.587	-
(+ Coumarin)	6	9.859	1.143	-
	7	8.745	1.054	+4.261
4.0 x 10 ⁻⁵ M	5	9.093	1.410	-
(+ Coumarin)	6	10.057	0.944	-
	7	9.043	0.856	+ 6.355
5.0 x 10 ⁻⁵ M	5	9.342	1.160	-
(+ Coumarin)	6	10.331	0.669	-
	7	9.229	0.578	+ 9.253
6.0 x 10 ⁻⁵ M	5	9.513	0.989	-
(+ Coumarin)	6	10.521	0.480	-
	7	9.418	0.393	+ 11.262
7.0 x 10 ⁻⁵ M**	5	9.693	0.808	-
(+ Coumarin)	6	10.720***	0.281	-
	7	9.617	0.193	+ 13.367
8.0 x 10 ⁻⁵ M	5	9.307	1.193	-
(+ Coumarin)	6	10.289	0.715	-
	7	9.173	0.626	+ 8.809
9.0 x 10 ⁻⁵ M	5	8.837	1.665	-
(+ Coumarin)	6	9.774	1.228	-
	7	8.663	1.139	+ 3.362
10.0 x 10 ⁻⁵ M	5	8.802	1.699	-
(+ Coumarin)	6	9.733	1.268	-
	7	8.629	1.179	+ 2.929

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

*** Optimum yield of lactic acid in 6 days

Experimental deviation $\pm$ 2.5 – 3.5%

+ve values indicate % increase in the yield of lactic acid.

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-hydroxycoumarin-3-carboxylic acid 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 containing no active organic molecule. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distilled water. Thus, the concentration of 7-hydroxycoumarin-3-carboxylic acid in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-3}M$

$1.0 \times 10^{-5}M$ to $10.0 \times 10^{-5}M$

Where

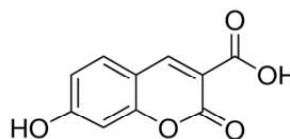
A = amount of active organic molecule in ml,

x = molarity of the solution containing coumarin

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 5, 6 and 7 days for lactic acid²⁸ formed and molasses²⁹ sugars left unfermented.

Results and Discussion

Submerged fermentative production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 exposed to 7-hydroxycoumarin-3-carboxylic acid



7-hydroxycoumarin-3-carboxylic acid

The data recorded in the table-1 shows that the coumarin compound, i.e., 7-hydroxycoumarin-3-carboxylic acid was found to be increasing up to its concentration from $1.0 \times 10^{-5}M$ to $7.0 \times 10^{-5}M$. It has also been observed that gradual addition of 7-hydroxycoumarin-3-carboxylic acid to the fermentation medium gradually increases the production of lactic acid.

It has been observed that higher concentrations of 7-hydroxycoumarin-3-carboxylic acid, i.e.; on $8.0 \times 10^{-5}M$ and onwards has retarded the yield of lactic acid by *Lactobacillus delbrueckii* NCIM-2025.

The maximum yield of lactic acid has been recorded at $7.0 \times 10^{-5}M$ concentration of 7-hydroxycoumarin-3-carboxylic acid, i.e., 10.720g/100 ml in 6 days of optimum incubation period which is 13.367% higher in comparison to the control fermentor flasks, i.e., 9.456g/100 ml in the same set of experimental parameters for submerged fermentative production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025.

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