

Microbial production of lactic acid by *Lactobacillus delbrueckii* NCIM-2025 exposed to coumarin

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Abstract: The impact of 7-amino-4-methylcoumarin on bioproduction of lactic acid by some lactic acid producing bacteria such as *Lactobacillus jugrti* NCIM-2367, *Lactobacillus lactis* NCIM-2369, *Lactobacillus plantarum* NCIM-2373, *Lactobacillus brevis* NCIM-2436 and *Lactobacillus delbrueckii* NCIM-2025 has been studied. The bacterial strain *Lactobacillus delbrueckii* NCIM-2025 has been found quite effective and useful for maximum bioproduction of lactic acid, i.e., 11.588 g/100 ml of lactic acid which is 20.834% higher in comparison to control fermentor flasks, i.e., 9.590 g/100ml at 6.0×10^{-5} M molar concentration of 7-amino-4-methylcoumarin mutagene. It has been found that bioproduction of lactic acid in the presence of 7-amino-4-methylcoumarin at optimum molar concentration, i.e; at 6.0×10^{-5} M attains its best activity when 24% (w/v) molasses solution is allowed to ferment for 5 days of optimum incubation period, at 38°C temperature by maintaining the pH value of the fermentation medium at 5.9 along with other nutritional ingredients required by the lactic acid bacteria.

(Keywords: Lactic acid fermentation, 7-amino-4-methylcoumarin, molasses, *Lactobacillus delbrueckii* NCIM-2025, pH, temperature and incubation period.)

Introduction

Coumarin is a fragrant organic chemical compound in the benzopyrone chemical class, which is a colorless crystalline substance in its standard state. Coumarin is a naturally occurring constituent of many plants and essential oils, including tonka beans, sweet clover, woodruff, oil of cassia, and lavender. Coumarin is a member of a class of compounds called benzopyrones. Coumarin compounds have been used to treat

such diverse ailments as cancer, bums, brucellosis, and rheumatic disease, and they have been used as antispasmodics. Although coumarin itself has no anticoagulant properties, it is transformed into the natural anticoagulant dicoumarol by a number of species of fungi. This occurs as the result of the production of 4-hydroxycoumarin, then further into the actual anticoagulant dicoumarol, a fermentation product and mycotoxin. This substance is responsible for the bleeding disease known historically as "sweet clover disease" in cattle eating moldy sweet clover silage.¹⁻⁷

Many compounds containing coumarin moiety, exhibit useful and diverse biological activity and, in recent years, there has been a growing interest in their synthesis.⁸⁻⁹ Some of the coumarin derivatives have been found to be useful as dyes,¹⁰ CNS-stimulants,¹¹ antibacterials,^{12,13} anticoagulants^{14,15} and in photochemotherapy, antitumor and anti-HIV therapy.^{16,17} Coumarins are widely distributed throughout the plant kingdom, with the vast majority carrying an oxygen substituent at the C-7 position. 7-Hydroxycoumarin (umbelliferone) is often regarded as the parent (in a structural and biogenetic sense) of a large number of structurally more complex coumarins.¹⁸⁻¹⁹

Experimental

Compositions of the production medium :

The composition of the production medium for the bioproduction of lactic acid by *Lactobacillus delbrueckii* NCIM-2025 exposed to 7-amino-4-methylcoumarin is as follows :

Molasses : 24% (w/v), Malt Extract : 1.60%

Table - 1
Microbial production of lactic acid by *Lactobacillus delbrueckii*
NCIM-2025 exposed to coumarin

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 5 days of incubation pd.
Control	5	9.590	2.499	—
1.0 x 10 ⁻⁵ M	5	9.735	2.280	(+) 1.511
2.0 x 10 ⁻⁵ M	5	9.913	2.146	(+) 3.368
3.0 x 10 ⁻⁵ M	5	10.205	1.859	(+) 6.412
4.0 x 10 ⁻⁵ M	5	10.584	1.474	(+) 10.364
5.0 x 10 ⁻⁵ M	5	11.053	0.962	(+) 15.255
6.0 x 10 ⁻⁵ M**	5	11.588***	0.426	(+) 20.834
7.0 x 10 ⁻⁵ M	5	10.957	1.105	(+) 14.254
8.0 x 10 ⁻⁵ M	5	10.381	1.634	(+) 8.248
9.0 x 10 ⁻⁵ M	5	10.041	2.016	(+) 4.702
10.0 x 10 ⁻⁵ M	5	9.816	2.198	(+) 2.356

* 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 $\pm 2.5 - 3.5\%$

Yeast Extract : 1.60%, Peptone : 1.60%, (NH₄)₂HPO₄ : 1.60%, CaCO₃ : 11.0%, pH : 5.9

(The pH was Adjusted by adding requisite amount of phosphate-buffer solution). Distilled water : To make up 100 ml.

Assay methods :

Evaluation of lactic acid²⁰ formed and molasses²¹ left unfermented was made colorimetrically

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

Strain : *Lactobacillus delbrueckii* NCIM-2025 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 delbrueckii* NCIM-2025.

Incubation period : 4, 5 and 6 days

Concentration of 7-amino-4-methylcoumarin used :

M/1000 solution of 7-amino-4-methylcoumarin under trial has been prepared and 1.0 x 10⁻⁵ M to 10 x 10⁻⁵ M molar concentration of 7-amino-4-methylcoumarin has been employed.

Results and Discussion

The data recorded in the table-1 shows that 7-amino-4-methylcoumarin was found to be significant for higher production of lactic acid up to its concentration from 1.0 x 10⁻⁵ to 6.0 x 10⁻⁵ M and above this concentration the production of microbial lactic acid by *Lactobacillus delbrueckii* NCIM-2025 was found to be decreasing. However, production of microbial lactic acid by *Lactobacillus delbrueckii* NCIM-2025 used were higher than that of control flasks.

The maximum bioproduction of $\text{CH}_3\text{CHOHCOOH}$ was found at molar concentration 6.0×10^{-5} M concentration of coumarin 7-amino-4-methylcoumarin, i.e., 11.588 g/ 100 ml in 5 (Five) days of optimum (maximum) incubation period which is 20.834% higher in

comparison to control, i.e., 9.590g/100 ml in the same experimental parametric conditions and optimum (maximum) incubation time.

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