

Metabolic impact of 2-methyl mandelic acid on lactic acid formation by *Lactobacillus casei* NCIM-2732

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Manuscript received online 21 May 2025, accepted on 26 June 2025

Abstract : The present study investigates the influence of 2-methyl mandelic acid, an aromatic α -hydroxy acid derivative, on the bioproduction of lactic acid by *Lactobacillus casei* NCIM-2732. The organism was cultivated under controlled fermentation conditions in the presence of graded concentrations of 2-methyl mandelic acid to evaluate its physiological and metabolic responses. Key parameters such as biomass formation, sugar utilization, lactic acid yield, and process efficiency were monitored to assess the compound's modulatory effects. Exposure to 2-methyl mandelic acid produced measurable changes in fermentation behavior, indicating dose-dependent alterations in microbial growth and lactic acid biosynthesis. Lower concentrations exhibited mild stimulatory effects on metabolic activity, whereas higher concentrations showed inhibitory influence, suggesting that the compound interacts with cellular regulatory pathways associated with carbohydrate metabolism and stress adaptation. Overall, the study provides insights into how substituted mandelic acid analogues affect lactic acid - producing bacteria, offering potential implications for optimizing biotechnological fermentation systems and understanding microbial responses to aromatic α -hydroxy acids. It has been found that the compound 2-MMA at molar concentration 4.0×10^{-5} M enhances the yield of lactic acid by *Lactobacillus casei* NCIM-2732 to an extent of 7.523% higher in comparison to control when molasses solution of 20%(w/v) is allowed to ferment at pH 6.0 temperature 34°C and incubation period of 6 days along with some other rich ingredients required by the LABs.

(Keywords : 2-Methyl mandelic acid 2-MMA, *Lactobacillus casei* NCIM-2732, molasses solution).

Introduction

Lactic acid is an industrially significant organic acid widely utilized in food preservation,

pharmaceutical formulations, biodegradable polymer production, and cosmetic applications. Microbial fermentation, particularly by lactic acid bacteria (LAB), remains the most sustainable method for lactic acid production due to its environmental compatibility, stereospecificity, and high conversion efficiency. Among LAB, *Lactobacillus casei* is recognized for its robust fermentative capability, broad substrate range, and tolerance to diverse environmental conditions, making it a valuable organism for controlled bioproduction processes.

Recent research has focused on understanding how various organic compounds influence microbial metabolism, stress response, and product yield. Aromatic α -hydroxy acids, such as mandelic acid and its structural analogues, are of special interest because they interact with microbial cells through their lipophilic, aromatic, and acidic functionalities. These compounds can modulate membrane integrity, enzyme activity, and redox balance, thereby affecting central metabolic pathways. However, the effects of substituted mandelic acids on LAB physiology and their fermentative behavior remain largely unexplored.

2-Methyl mandelic acid, a methyl-substituted derivative of mandelic acid, presents a distinct structural profile that may influence microbial metabolic networks differently than the parent compound. The presence of both an α -hydroxyl group and an ortho-methyl substituent on the aromatic ring suggests potential interactions with cellular regulatory mechanisms, particularly those governing carbohydrate

uptake, glycolytic flux, and lactate formation. Investigating such interactions can help deepen our understanding of LAB metabolic plasticity and stress adaptation.

This study examines the metabolic impact of 2-methyl mandelic acid on lactic acid formation by *Lactobacillus casei* NCIM-2732. By evaluating changes in microbial growth, sugar utilization, and lactic acid biosynthesis under exposure to this compound, the work aims to elucidate how structural modifications in aromatic α -hydroxy acids influence the fermentation behavior of lactic acid - producing bacteria. The findings may contribute to improved bioprocess optimization and provide insights into microbial tolerance toward functionalized organic acids.

Though some organic biomolecules are not essentially growth promoters for some microbes yet a few organic biomolecules are utilized by some or all microbes for their nutritional benefits¹⁻⁵. Although a group of researchers⁶⁻²³ have tried to explore the impact of some organic molecules on microbial enzymes system, yet there is no definite opinion regarding the impact of such organic biomolecules on biotic production of lactic acid. In view of the insufficient knowledge regarding involvement of organic biomolecules to any fermentation processes specially lactic acid fermentation, the authoresses have made an attempt to study the efficacy of 2-methyl mandelic acid 2-MMA on the yield of lactic acid by *Lactobacillus casei* NCIM-2732

Experimental

Compositions of the production medium :

The composition of the production medium for bioproduction of lactic acid by *Lactobacillus casei* NCIM-2732 exposed to 2-methyl mandelic acid (2-MMA) is as follows : **Molasses** : 20% (W/V); **Malt extract** : 0.80%; **yeast extract** : 0.75% , **Peptone** : 0.75%, **(NH₄)₂HPO₄** : 0.8%; **CaCO₃** : 10%, **pH** :6.0, (Adjusted by adding requisite amount of

phosphate-buffer solution). **Distilled water** : To make up 100 ml.

Assay methods : Evaluation of lactic acid formed and molasses left unfermented was made colorimetrically²⁴⁻²⁵

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

Strain : *Lactobacillus casei* NCIM-2732 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 casei* NCIM-2732

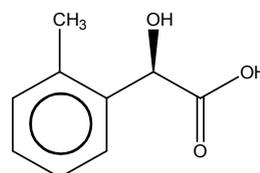
Incubation period : 4, 6 and 8 days

Concentration of 2-methyl mandelic acid (2-MMA) used:

M/1000 solution of 2-methyl mandelic acid (2-MMA) under trial has been prepared and 1.0 x 10⁻⁵M to 10x10⁻⁵ M molar concentration of 2-methyl mandelic acid (2-MMA) has been employed.

Results and Discussion

The influence of 2-methyl mandelic acid



**2-methyl mandelic acid (2-MMA)
(Compound I)**

The introduction of 2-methyl mandelic acid (2-MMA) into the fermentation medium produced a concentration-dependent effect on the growth kinetics of *L. casei* NCIM-2732.

At low concentrations, no significant inhibition of biomass formation was observed, indicating that *L. casei* tolerates 2-MMA at sub-inhibitory levels.

However, at higher concentrations, a gradual decline in optical density and viable

Table – 1
Impact of 2-methyl mandelic acid on bioproduction of lactic acid by
***Lactobacillus casei* NCIM-2732**

Concentration of active organic compound	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
Control	6	8.400	1.615	–
1×10^{-5} M	6	8.503	1.513	(+) 1.226
2×10^{-5} M	6	8.589	1.428	(+) 2.250
3×10^{-5} M	6	8.840	1.176	(+) 5.238
4×10^{-5} M**	6	9.032***	0.985	(+) 7.523
5×10^{-5} M	6	8.925	1.089	(+) 6.250
6×10^{-5} M	6	8.776	1.240	(+) 4.476
7×10^{-5} M	6	8.727	1.286	(+) 3.892
8×10^{-5} M	6	8.585	1.429	(+) 2.202
9×10^{-5} M	6	8.489	1.530	(+) 1.059
10×10^{-5} M	6	8.447	1.567	(+) 0.559

*Each value represents mean of three trials. ** Optimum concentration of 2-methyl mandelic acid under trial. *** Optimum yield of lactic acid in 6 days Experimental deviation $\pm 1.5 - 3.0\%$ +ve values indicate % increase in the yield of lactic acid.

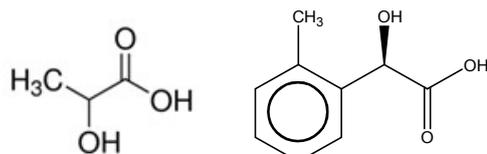
counts suggested partial growth suppression. This inhibitory trend is consistent with the known antimicrobial and membrane-active nature of α -hydroxy aromatic acids.

The data given in the table-1 shows that the compound 2-methyl mandelic acid (2-MMA) has inhibitory effect on bioproduction of lactic acid by *Lactobacillus casei* NCIM–2732.

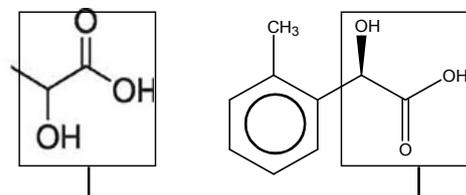
It has been observed that there is a gradual increase in the yield of lactic acid with the stepping up of 2-methyl mandelic acid (2-MMA) up to the molar concentration of 4.0×10^{-5} M but after this concentration a big fall in the production of lactic acid has been noticed. It has been found that maximum yield of lactic acid, i.e., 9.032 g/100ml has been obtained at 4.0×10^{-5} M molar concentration of 2-methyl mandelic acid (2-MMA) which is 7.523% higher in comparison to control, i.e., 8.400g/100 ml in 6 days of optimum incubation period. It has been observed that at concentrations in between 5.0×10^{-5} M to 6.0×10^{-5} M the yield of lactic acid has been recorded to be 6.250% and 4.476%

respectively in comparison to control fermentor flasks, i. e., 8.400g/100ml in 6 days of optimum incubation period. It is interesting to note that the higher concentrations of 2-methyl mandelic acid (2-MMA), i.e., 7.0×10^{-5} M and onwards for the production of lactic acid has been found much inhibitory and yield of lactic acid on these concentrations has been found insignificant in comparison to control. The inhibitory action of 2-methyl mandelic acid (2-MMA) may be explained from the fact that 2-methyl mandelic acid (2-MMA) is partially structurally similar to lactic acid Therefore, 2-methyl mandelic acid (2-MMA) happens to be biological metabolite structural antagonist and the enzyme catalysed activity of *Lactobacillus casei* NCIM–2732 is expected and supposed to be inhibited in the fermentation process. The 2-methyl mandelic acid (2-MMA) which happens to be structural metabolite antagonist has one end similar to the - lactic acid. Thus, the compound 2-methyl mandelic acid (2-MMA) a structural biological metabolite antagonist may perhaps prevent the proper functioning of enzyme catalysed reactions of *Lactobacillus casei* NCIM–2732

but probably remains firmly attached to it by its one end. The probable structurally similar combination of 2-methyl mandelic acid (2-MMA) with lactic acid may be illustrated as given under :



Lactic acid (LA) 2-methyl mandelic acid



Lactic acid (LA) 2-methyl mandelic acid

***Structural antagonist combination have one common encircled upper end.**

Therefore, 2-methyl mandelic acid antagonises with one upper end of the lactic acid and thus retards the bioproduction of lactic acid by *Lactobacillus casei* NCIM-2732.

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