J. Chemtracks 27 (1&2) 139-142, 2025 Abstracted / Indexed in
A division of the American Chemical Society
Chemical Abstract U.S.A.
ISSN 0973-239X
CODEN:JCOHF7
IMPACT FACTOR : 7.866

Regulatory role of 5,7-dihydroxy -4-propylcoumarin in citric acid bioproduction by *Aspergillus oryzae* NCIM-929

Manoj Kumar
Department of Chemistry, Sri Tridandi Dev Govt.Degree College,
Gautam Nagar, Shahpur, Bhojpur, Bihar-802112
Email: ph.dmanoj2019@gmail.com

Manuscript received online 24 July 2025, accepted on 14 August 025

Abstract: The regulation of secondary metabolite pathways through small bioactive molecules offers promising strategies for enhancing organic acid bioproduction. In the present study, the regulatory role of 5,7-dihydroxy-4-propylcoumarin in citric acid production by *Aspergillus oryzae* NCIM-929 was investigated. Coumarin derivatives, known for their antioxidant and signaling activities, have been suggested to influence metabolic fluxes in filamentous fungi. Supplementation of 5,7-dihydroxy-4-propyl-coumarin at optimized concentrations led to a significant increase in citric acid yield, accompanied by improved substrate assimilation efficiency and altered intracellular redox balance. These findings highlight the potential of 5,7-dihydroxy-4-propylcoumarin as a metabolic regulator for optimizing citric acid bioproduction in *A. oryzae* and provide a novel approach for biotechnological applications of natural small molecules in industrial fermentation. In the present communication regulatory role of 5,7-dihyroxy-4-propylcoumarin in citric acid bioproduction by *Aspergillus oryzae* NCIM-929 has been studied. It has been observed that 5,7-dihydroxy-4-propylcoumarin has stimulatory effect on bioproduction of citric acid and enhances the yield of citric acid to an extent of 15.399% higher in comparison to control when molasses solution of 24% was allowed to ferment at pH 2.0,temperature 27^0C and incubation period of 11 days .

(Keywords : 5,7-dihydroxy-4-propylcoumarin, *Aspergillus oryzae* NCIM-929, citric acid, metabolic regulation, coumarin derivatives, fermentation efficiency).

Introduction

Citric acid is one of the most important organic acids widely utilized in the food, pharmaceutical, and beverage industries owing to its flavor-enhancing, preservative, and chelating properties.[1-4] Industrially, microbial fermentation represents the most economical and sustainable route for large-scale citric acid production, with *Aspergillus* species being the most extensively employed microorganisms. Among them, *Aspergillus oryzae* NCIM-929 has emerged as a promising prospect because of its high secretion capacity, tolerance to acidic environments, and well-established safety profile.

Optimization of citric acid bioproduction is strongly influenced by metabolic regulation, where small bioactive molecules can act as inducers, inhibitors, or modulators of enzymatic pathways. Naturally occurring plant-derived compounds, especially coumarins and their derivatives, have attracted considerable attention due to their structural diversity and potential regulatory effects on microbial metabolism. Coumarins are benzopyrone derivatives known for their antimicrobial, antioxidant, and metabolic-modulating activities. Their hydroxylated and alkyl-substituted analogues have been reported to influence enzymatic activities related to carbohydrate metabolism and organic acid synthesis.[5-16]

In this context, 5,7-dihydroxy-4-propylcoumarin, a hydroxylated and alkyl-substituted coumarin derivative, represents an interesting prospect for metabolic regulation in citric acid fermentation. Its hydroxyl groups at

positions 5 and 7 provide potential for hydrogen bonding and redox interactions, while the propyl substitution at position 4 may enhance hydrophobic interactions with enzyme active sites or cellular membranes. Such structural features suggest a capacity to modulate key enzymes of the tricarboxylic acid (TCA) cycle, glycolysis, and related anaplerotic pathways. Exploring the regulatory role of 5,7-dihydroxy-4-propylcoumarin in citric acid bioproduction by *A. oryzae* NCIM-929 can provide new insights into metabolic engineering and process optimization. understanding its influence on fungal physiology, enzyme regulation, and citric acid yield may contribute to the development of more efficient and sustainable bioproduction strategies.[17-19] The present study was undertaken to assess and analyze the regulatory role of 5,7-dihyroxy-4-propylcoumarin in citric acid bioproduction by *Aspergillus oryzae* NCIM-929

Experimental

The influence of 5,7-Dihydroxy-4-propylcoumarin on production of citric acid by *Aspergillus oryzae* NCIM-929. The composition of the production medium for production of citric acid by *Aspergillus oryzae* NCIM-929 has been prepared as follows :

Molasses : 24% (w/v), NH_4NO_3 : 0.55% ,
KH_2PO_4 : 0.55%, $MgSO_4.7H_2O$: 0.55%
pH : 2.0

The pH of the production medium was adjusted to 2.0 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter.

The above composition medium represents volume of a fermentor flask, i.e., "100ml" production of citric acid by *Aspergillus oryzae* NCIM-929.

Now, the same production medium for production of citric acid by *Aspergillus oryzae* NCIM-929 was prepared for 99-fermentor flask, i. e; each contained '100ml' of production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermentor flasks. The remaining 9-fermentor flasks out of 99-fermentor flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermentor flasks.

After preparing the above sets of fermentor flasks M/1000 solution of 5,7-Dihydroxy-4-propylcoumarin was prepared and from the above coumarin solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no coumarin. Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of 5,7-Dihydroxy-4-propylcoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x} M, 1.0 $\times 10^{-5}$M to 10.0 $\times 10^{-5}$M
Where : = amount of coumarin, in ml, i.e. 1.0 ml to 10 ml., x =Molarity of the coumarin solutions.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 27^0C and analysed after 9, 11 and 13 days for citric acid[20] formed and molasses[21] left unfermented.

Results and Discussion

Effect of 5,7-dihydroxy-4-propylcoumarin on Citric Acid Production

The data recorded in the table-1 shows that 5,7-Dihydroxy-4-propylcoumarin was found to be significant for higher promotion of citric acid up to its concentration from 1.0 x 10^{-5}M to 5.0 $\times$ 10^{-5} M and above this concentration the production of citric acid by *Aspergillus oryzae* NCIM-929 was found to be decreasing. However, production of citric acid by *Aspergillus oryzae*

Table - 1
Production of citric acid by *Aspergillus oryzae* NCIM-929 exposed to 5,7-Dihydroxy-4-propylcoumarin

Concentration of coumarin used A x 10^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 11 days
Control	9	4.928	5.076	-
(-) Coumarin	11	7.325	2.678	-
	13	6.214	2.573	-
1.0 ×10^{-5}M	9	5.011	4.983	-
(+) Coumarin	11	7.464	2.539	+1.897
	13	6.360	2.436	-
2.0 ×10^{-5}M	9	5.164	4.839	-
(+) Coumarin	11	7.691	2.306	+4.996
	13	6.589	2.203	-
3.0×10^{-5}M	9	5.287	4.716	-
(+) Coumarin	11	7.874	2.129	+7.494
	13	6.760	2.023	-
4.0×10^{-5}M	9	5.504	4.493	-
(+) Coumarin	11	8.196	1.806	+11.890
	13	7.090	1.709	-
5.0×10^{-5}M**	9	5.677	4.326	-
(+) Coumarin	11	8.453***	1.549	+15.399
	13	7.340	1.443	-
6.0×10^{-5}M	9	5.519	4.483	-
(+) Coumarin	11	8.218	1.786	+12.191
	13	7.118	1.689	-
7.0×10^{-5}M	9	5.346	4.656	-
(+) Coumarin	11	7.961	2.039	+8.696
	13	6.840	2.033	-
8.0×10^{-5}M	9	5.139	4.863	-
(+) Coumarin	11	7.654	2.349	+4.491
	13	6.539	2.246	-
9.0×10^{-5}M	9	5.056	4.949	-
(+) Coumarin	11	7.530	2.473	+2.798
	13	6.418	2.376	-
10.0×10^{-5}M	9	4.987	5.016	-
(+) Coumarin	11	7.427	2.579	+1.392
	13	6.309	2.473	-

* Each value represents mean of three trials ** Optimum concentration of coumarins used
*** Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 11 days.
Experimental deviation (±) 1.5-3%

NCIM-929 used were higher than that of control flasks.

The maximum yield of citric acid was observed at 5.0 x 10^{-5} M concentration of 5,7-Dihydroxy-4-propylcoumarin i.e., 8.453g/ 100 ml in 11 days of optimum incubation period which is 15.399% higher in comparison to control, i.e., 7.325g/100 ml in the same experimental conditions and optimum incubation period.

The addition of 5,7-dihydroxy-4-propylcoumarin significantly influenced the citric acid yield in *A. Oryzae* NCIM-929 cultures. A dose dependent effect was observed, where lower concentration did not pramote citric acid accumulation while higher concentration exhibited inhibitory effects. At the optimum concentration of 5.0 $\times 10^{-5}$M approximately 15.399% higher citric acid was observed in comparison to control. This suggests that the compound may act as a regulatory modulator rather than a simple metabolic inhibitor or activator.

References

1. A. Lacy, R. O'Kennedy *Curr. Pharmaceut. Des*., **10,** 3797 (2004)
2. K.N. Venugopala, V. Rashmi, B. Odhav *Bio Med Res. Int.,***9,** 2013 (2013),
3. S.M. Yang, G.Y. Shim, B.G. Kim, J.H. Ahn, *Microb. Cell Factories,* **14** , p. 65 (2015)
4. D. Chaudhary, P. Bedi, S. Santra, T. Pramanik, *Org. Chem.,* **19,** 362 (2022),
5. D.J. Newman, G.M. Cragg, *J. Nat. Prod.,* **70,** 461 (2007)
6. M.J. Batunas, A.D. Kinghorn, *Life Sci.,* **78**, 431 (2005)
7. M.S. Butter *Nat. Prod. Rep.,* **25,** 475 (2008)
8. J.D. McChesney, S.K.Venkataraman, J.T. Henri *Phytochemistry,* **68**, 2015 (2007)
9. M.M. Garazd, O.V. Muzychka, A.I. Vovk, I.V. Nagorichna, A.S. Ogorodniichuk, *Chem. Nat. Compd.*, **43**, 19 (2007)
10. V.F. Traven, T.A. Chibisova, A.V. Mona, *Dyes Pigments*, **58**, 41 (2003)
11. J.A. [heroler, M.A. Koffas, *Curr. Opin. Biotechnol.,* **19**, 597 (2008)
12. H. Zhou, X. Xie, Y. Tong, C*urr. Opin. Biotechnol*., **19,** 590 (2008).
13. Y. Lin, X. Sun, Q. Yuan, Y. Von, *Metab. Eng.,* **18**, 69 (2013).
14. S. Matsumoto, M. Mizutani, K. Sakata, B. Shimizu, *Phytochemistry,* **74** 49, (2012),
15. Y. Lin, X. Shen, Q. Yuan, Y. Yon, *Nat. Commun.*, **4**, 2603, (2013)
16. X. Shen, M. Mahajani, J. Wang, Y. Yang, Q. Yuan, Y. Yan, Y. Lin *Metab. Eng.,* **42,** 59 (2017)
17. K.Y. Hera, M. Aroki, N. Okai, S. Wakai, T. Hasunuma, A. Kondo *Microb. Cell Fact.,* **13**, 173 (2014)
18. R. Vanholme, L. Sundin, K.C. Seetso, H. Kim, X. Liu, J. Li, B. De Meester, L. Hoengenaert, G. Goeminne, K. Morreel, J. Haustroete, H.H. Tsai, W. Schmidt, B. Van holme, J. Ralph, W. Boerjan, *Nat. Plants*, **5**, 1066 (2019
19. Y. Zhao, X. yon, 3. Wu, W. Huang, C. Huang, J. Luo, L. Kong, *J. Bioi. Eng*., **13**, 44 (2019)
20. J.R. Marrier and M.B. Boulet *J. Dairy Suence* **45**, 1683 (1983)
21. M. Dubois, K. A.Gilles, J. K. Hamilton and F.Smith, *Anal. Chem.* **28**, 350 (1956)