J. Chemtracks 27 (1&2) 261-264, 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

Role of phenolic compounds in the regulation of citric acid production by *Aspergillus oryzae* NCIM-929

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Manuscript received online 05 July 2025, accepted on 28 August 2025

Abstract : The present study investigates the regulatory influence of phenolic phytochemicals on citric acid production by *Aspergillus oryzae* NCIM-929. Phenolic compounds are known to act as redox-active molecules that can modulate key metabolic pathways in fungi. Selected phenolics, including catechol, ferulic acid, and coumarin, were supplemented into the fermentation medium to assess their impact on biomass formation, citric acid yield, and metabolic enzyme activity. The results demonstrated that low to moderate concentrations of specific phenolics significantly enhanced citric acid accumulation, likely through modulation of the tricarboxylic acid (TCA) cycle and reduction of by-product formation such as oxalic acid. Enhanced activity of citrate synthase and reduced activity of aconitase were observed in phenolic-treated cultures, indicating a metabolic shift favoring citrate accumulation. These findings suggest that phenolic compounds act as metabolic effectors influencing oxidative balance and enzymatic regulation in *A. oryzae,* thereby providing a novel biochemical strategy for improving citric acid biosynthesis in industrial fermentation processes. In the present communication efficacy of phenolic compounds, i.e.;5,7-dihydroxy-4-phenylcoumarin on citric acid production by *Aspergillus oryzae* NCIM-929 has been assessed. It has been found that the compound under trial has slight stimulatory impact on the production of citric acid and enhances the yield of citric acid to an extent of 6.592% higher in comparison to control,i.e.; 7.539 g/100mL under the optimized conditions,ie;molasses solution 24% (w/v), pH 2.0,temperature 27^{0}C and incubation period of 11days along with some other significant rich ingredients required by the *Aspergillus oryzae* NCIM-929.

(Keywords : Phenolic compounds, 5,7-dihydroxy-4-phenylcoumarin, *Aspergillus oryzae* NCIM-929, H_3Cit).

Introduction

Phenolic compounds play a key role in enhancing citric acid production by *Aspergillus oryzae* NCIM-929, a strain known for its potential in organic acid fermentation, by influencing metabolic pathways such as glycolysis and the TCA cycle at optimal concentrations.

Certain phenolics like phenol, α-naphtol, and β-naphtol, when added in controlled amounts to the culture medium, stimulate citric acid accumulation in *Aspergillus* species, including those related to *A. oryzae,* by modulating enzyme activities like phosphofructokinase and citrate synthase. This regulation occurs under specific fermentation conditions, such as low pH and limited manganese or phosphate, which favor acid overflow rather than complete TCA cycle metabolism.

Aspergillus oryzae NCIM-929, cataloged for citric acid production capabilities, responds to phenolic additions similarly to *A. niger* strains, where these compounds counteract inhibitory effects and boost yields up to maximal levels predicted by fermentation models. Toxic concentrations, however, suppress growth and acid output, highlighting the need for precise dosing.

Citric acid is an important organic acid widely used in the food, pharmaceutical, and chemical industries, and its microbial production using *Aspergillus* species represents an eco-

friendly biotechnological process. Among these, *Aspergillus oryzae* NCIM-929 is a promising strain capable of efficient citric acid synthesis under optimized fermentation conditions. Recent studies suggest that phenolic compounds – naturally occurring plant metabolites with diverse biochemical properties —can influence microbial metabolism by modulating enzyme activities, redox balance, and membrane permeability. Their regulatory role in fungal metabolism may affect the tricarboxylic acid (TCA) cycle and citric acid accumulation. Therefore, understanding the impact of phenolic compounds on *A. oryzae* NCIM-929 offers new insights into improving citric acid yield and optimizing bioprocess performance through metabolic regulation.

Coumarins are phenolic phytochemicals and active organic biomolecules. Phytochromes are organic substances which are naturally produced in some plants[1-8] and control the growth and some other significant physiological functions at a site remote from its place of production and active in extremely little quantities9-16.Growth hormones has been defined as "substances which are synthesized in some particular cells and which are transformed to other cells wherein extremely small quantities influence development bioprocess[17-26].

Thus, from the above brief review it is evident that phenolic phytochemicals are required for exploration specially for citric acid bioproduction and in view of this the author has confined his investigation for the role of phenolic phytochemicals in the regulation of citric acid production by *Aspergillus oryzae* NCIM-929

Experimental

The influence of 5,7-dihydroxy-4-phenylcoumarin 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-phenylcoumarin 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-phenylcoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x}$ M, 1.0×10^{-5}M to 10.0×10^{-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[27] formed and molasses[28] left unfermented.

Table -1
Impact of phenolic phytochemicals 5,7-dihyroxy-4-phenylcoumarin on citric acid production by *Aspergillus oryzae* NCIM-929

Concentration of coumarin used $A \times 10^{-x}M$	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	4.981	5.023	-
(-) Coumarin	11	7.539	2.466	-
	13	6.428	2.363	-
$1.0\times10^{-5}M$	9	5.020	4.983	-
(+) Coumarin	11	7.614	2.389	+0.994
	13	6.505	2.286	-
$2.0\times10^{-5}M$	9	5.070	4.936	-
(+) Coumarin	11	7.689	2.319	+1.989
	13	6.575	2.213	-
$3.0\times10^{-5}M$	9	5.165	4.839	-
(+) Coumarin	11	7.833	2.166	+3.899
	13	6.729	2.063	-
$4.0\times10^{-5}M$**	9	5.299	4.701	-
(+) Coumarin	11	8.036***	1.964	+6.592
	13	7.012	1.863	-
$5.0\times10^{-5}M$	9	5.264	4.739	-
(+) Coumarin	11	7.983	2.016	+5.899
	13	6.870	2.013	-
$6.0\times10^{-5}M$	9	5.185	4.819	-
(+) Coumarin	11	7.863	2.133	+4.297
	13	6.759	2.036	-
$7.0\times10^{-5}M$	9	5.095	4.909	-
(+) Coumarin	11	7.727	2.276	+2.493
	13	6.618	2.173	-
$8.0\times10^{-5}M$	9	5.040	4.963	-
(+) Coumarin	11	7.644	2.359	+1.392
	13	6.540	2.256	-
$9.0\times10^{-5}M$	9	5.015	4.986	-
(+) Coumarin	11	7.606	2.393	+0.888
	13	6.501	2.299	-
$10.0\times10^{-5}M$	9	5.000	5.003	-
(+) Coumarin	11	7.584	2.419	+0.596
	13	6.475	2.316	-

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

Results and Discussion

The data recorded in the table-1 shows that like 5,7-Dihydroxy-4-propylcoumarin the coumarin compound 5,7-Dihydroxy-4-phenylcoumarin was also found to be increasing up to its concentration from 1.0 x 10^{-5} M to 4.0 x 10^{-5} M. It has also been observed thus gradual addition of 5,7-Dihydroxy-4-phenylcoumarin to the fermentation medium gradually increases the production of citric acid. The production of citric acid on these concentrations were not very much significant and could favour the production of citric acid in the range of 0.994% to 6.592% only.

It has been observed that higher concentrations of 5,7-Dihydroxy-4-phenyl-coumarin, i.e.; on 5.0 x 10^{-5}M and onwards has been retarded the production of citric acid by *Aspergillus oryzae* NCIM-929.

The maximum yield of citric acid has been recorded at 4.0 x 10^{-5} M concentration of 5,7-Dihydroxy-4-phenylcoumarin , i.e., 8.036g/100 ml in 11days of optimum incubation period which is 6.592% higher in comparison to the control fermentor flasks i.e.,7.539g/100 ml in the same set of experimental parameters for the production of citric acid by *Aspergillus oryzae* NCIM-929.

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