

Bioproduction of citric acid exposed to some coumarins

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Abstract : The global citric acid market size was valued at USD 2909-59 million in 2021 and is experted to expand at a CAGR of 4.63% during the forecast period reaching USD 3818.2 million by 2027. The efficacy of 7-hydroxy-3-methyl-4-phenyl coumarin on microbial bioproduction of citric acid by *Aspergillus fumigatus* AR - 902, *Aspergillus parasiticus* AR- 904, *Aspergillus awamori* AR - 861 *Aspergillus oryaze* AR - 962 and *Aspergillus niger* AR- 953 has been assessed. It has been observed that the fungal strain *Aspergillus niger* AR - 953 has been found most potential and effective for the citric acid fermentation process. It has been found that the micelles, i.e., sodium hexadecyl sulfate at molar concentration, i.e., 6.0×10^{-5} M has stimulatory effect on production of citric acid by *Aspergillus niger* AR - 953 and enhances the yield of citric acid to an extent of 13.962% higher in comparison to control fermentor flasks, i.e., 4.366 g/100 mL under the optimized conditions, viz. 33°C temperature, 1.8 pH, 11 days of incubation period with 30% (w/v) molasses solution.

(Keywords: Citric acid fermentation, 7-hydroxy-3-methyl-4-phenyl coumarin and *Aspergillus niger* AR - 953)

Introduction

Coumarin is a chemical compound which is found naturally in some plants, although it can be synthetically produced as well. It has a distinctive odor which has led people to use it as a food additive and ingredient in perfume. Coumarins are important class of organic compounds having multiple applications in perfume, cosmetic and pharmaceutical industrial production¹⁻⁷. Many coumarin derivatives are used as photo chemotherapeutic drugs for PUVA

(psoralen plus ultraviolet-A-radiation) therapy⁸. Coumarin compounds show a variety of applications, i.e., coumarin-1-dye/biphenyl-periodic mesoporous organosilica is used as one of the key ingredient in light harvesting materials⁹, furthermore, they are used in the alignment layers of liquid crystal¹⁰, optical brighteners, and dispersed fluorescent and laser dyes¹¹. Perkin, Pechmann, Reformatsky, Wittig Knoevenagel reactions¹² are the most common synthetic methods to produce coumarins. The reactions are catalyzed by weak bases or by suitable combinations of amines and carboxylic or Lewis acids under homogeneous conditions¹³.

Coumarin is a volatile compound that is formed mainly from a nonvolatile glucose derivative upon senescence or injury. This is especially significant in alfalfa and sweet clover, where coumarin causes the characteristic odor of recently mown hay certain sweet clover strains have been developed that contain low amount of coumarins and others that contain it in a bound form. These are of economic importance, because free coumarin can be converted to a toxic product, decumarol, if the clover becomes spoiled during storage Dicumarol is a hemorrhagic and anticoagulant agent responsible for sweet-clover disease (a bleeding disease) in ruminant animals that are fed plants that contain it.

During the recent past commondable development towards citric acid fermentaion exposed to some compounds¹⁴⁻³⁵ like auxins, gibberellins, cytokins, PAOC, mutagens and vitamins has been studied by several workers but

Table - 1
Bioproduction of citric acid exposed to 7-hydroxy-3-methyl-4-phenyl coumarin

Concentration of coumarin used $A \times 10^{-5}M$	Incubation period in days	Yield of citric acid* in g/100mL	Molasses* left Unfermented in g/100 mL	% of citric acid increase after 11 days
Control	8	4.366	3.639	-
(-) Coumarin	11	6.763	1.236	-
	14	5.659	1.133	-
$1.0 \times 10^{-5}M$	8	4.409	3.593	-
(+) Coumarin	11	6.850	1.156	+ 1.286
	14	5.753	1.059	-
$2.0 \times 10^{-5}M$	8	4.483	3.513	-
(+) Coumarin	11	6.959	1.046	+ 2.898
	14	5.856	1.049	-
$3.0 \times 10^{-5}M$	8	4.575	3.423	-
(+) Coumarin	11	7.101	0.896	+ 4.997
	14	6.009	0.794	-
$4.0 \times 10^{-5}M$	8	4.684	3.319	-
(+) Coumarin	11	7.270	0.736	+ 7.496
	14	6.173	0.633	-
$5.0 \times 10^{-5}M$	8	4.872	3.129	-
(+) Coumarin	11	7.561	0.436	+ 11.799
	14	6.463	0.339	-

* 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 ($\pm$) 1.5-3%

no attempt has been made for the class of compounds especially dormins, flowering hormones, miscellaneous natural substances, synthetic growth retardants, miscellaneous synthetic substances and most especially phenolic substance, i. e; coumarins. With the expectation that coumarins might be useful in the citric acid fermentation and its production protocol, the final elucidation of coumarins structure and its reactivity by introducing different groups at 3 and 4 positions into the basic skeleton of coumarins has been taken into consideration for the successful fermentative production of improved quality of citric acid. In view of the importance and good physiological activities of the coumarins, the authors have employed some coumarins on production of citric acid by *Aspergillus niger* AR-953.

Coumarins are naturally occurring molecules with a versatile range of activities. Their structural and physicochemical characteristics make them a privileged scaffold in medicinal chemistry and chemical biology. Many research articles and reviews compile information on this important family of compounds.³⁶⁻⁴⁰

Thus, from the above brief review it is evident that coumarins are required for genetic manipulation and exploitation specially for citric acid fermentation and in view of this the authors have studied the influence of 7-Hydroxy-3-methyl-4-phenylcoumarin on production of citric acid by *Aspergillus niger* AR-953

Table - 1
Bioproduction of citric acid exposed to 7-hydroxy-3-methyl-4-phenyl coumarin

Concentration of coumarin used $A \times 10^{-5}M$	Incubation period in days	Yield of citric acid* in g/100mL	Molasses* left Unfermented in g/100 mL	% of citric acid increase after 11 days
Control	8	4.366	3.639	-
(-) Coumarin	11	6.763	1.236	-
	14	5.659	1.133	-
$6.0 \times 10^{-5}M^{**}$	8	4.955	3.043	-
(+) Coumarin	11	7.689***	0.316	+ 13.692
	14	6.583	0.219	-
$7.0 \times 10^{-5}M$	8	4.732	3.263	-
(+) Coumarin	11	7.344	0.659	+ 8.590
	14	6.246	0.556	-
$8.0 \times 10^{-5}M$	8	4.562	3.436	-
(+) Coumarin	11	7.080	0.923	+ 4.687
	14	6.089	0.829	-
$9.0 \times 10^{-5}M$	8	4.475	3.529	-
(+) Coumarin	11	6.945	1.053	+ 2.691
	14	5.843	1.056	-
$10.0 \times 10^{-5}M$	8	4.418	3.586	-
(+) Coumarin	11	6.857	1.149	+ 1.389
	14	5.759	1.043	-

* 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 ($\pm$) 1.5-3%

Experimental

The influence of 7-Hydroxy-3-methyl-4-phenylcoumarin on production of citric acid by *Aspergillus niger* AR- 953. The composition of the production medium for production of citric acid by *Aspergillus niger* AR- 953 has been prepared as follows :

Molasses : 20% (w/v), NH_4NO_3 : 0.66% KH_2PO_4 : 0.66%, $MgSO_4 \cdot 7H_2O$: 0.66%, pH : 2.1

The pH of the production medium was adjusted to 2.1 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 niger* AR- 953.

Now, the same production medium of citric acid by *Aspergillus niger* AR- 953 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 7-Hydroxy-3-methyl-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 7-Hydroxy-3-methyl-4-phenylcoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x} \text{ M}$

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

Where :

A = amount of coumarin, in ml, i.e. 1.0 ml to 10 ml. x = Molarity of the coumarin solution

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 29°C and analysed after 8, 11 and 14 days for citric acid⁴¹ formed and molasses⁴² left unfermented.

Results and Discussion

The efficacy of 7-Hydroxy-3-methyl-4-phenylcoumarin

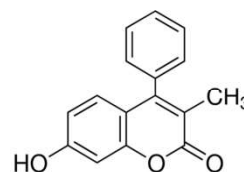


Table-1 shows that 7-Hydroxy-3-methyl-4-phenylcoumarin was found to be significant for higher promotion of citric acid up to its concentration from 1.0×10^{-5} to $6.0 \times 10^{-5} \text{ M}$ and above this concentration the production of citric acid by *Aspergillus niger* AR-953 was found to be decreasing. However, production of citric acid by *Aspergillus niger* AR-953 used were higher than that of control flasks.

The maximum yield of citric acid was observed at $6.0 \times 10^{-5} \text{ M}$ concentration of 7-Hydroxy-3-methyl-4-phenylcoumarin i.e., 7.689 g/100 ml in 11 days of optimum incubation period which is 13.692% higher in comparison to control, i.e., 6.763g/100 ml in the same experimental conditions and optimum incubation period.

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