

Production of citric acid by *Aspergillus niger* T-918 exposed to 6-Hydroxy-4-methylcoumarin

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Abstract : The efficacy of some coumarin compound, i.e., 6-Hydroxy-4-methylcoumarin on bioproduction of citric acid by *Aspergillus foetidus* T-501, *Aspergillus fischeri* T-611 6.968, *Aspergillus geganticus* T-715, *Aspergillus oryzae* T-816, *Aspergillus niger* T-918 has been assessed. It has been observed that the fungal strain *Aspergillus niger* T-918 has been found most potential and effective for the citric acid fermentation process. It has been found that the coumarin, i.e., 6-Hydroxy-4-methylcoumarin on at molar concentration, i.e., 6.0×10^{-5} M has stimulatory effect on production of citric acid by *Aspergillus niger* T-918 and enhances the yield of citric acid to an extent of 12.247% higher in comparison to control fermentor flasks, i.e., 9.757 g/100 ml under the optimized conditions, viz. 27°C temperature, 2.0 pH, 13 days of incubation period with 30% (w/v) molasses solution.

(Keywords: Citric acid fermentation process, 6-hydroxy-4-methylcoumarin, coumarin and *Aspergillus niger* T-918)

Introduction

Coumarin belongs to the heterocyclic class of organic compounds, because its molecule contains a ring of atoms of more than one element. The presence of coumarin in the tonka bean, in which it occurs combined with the sugar glucose, was observed in 1820; its synthesis in 1868 by the English chemist Sir William Henry Perkin was the first application of a general chemical reaction now known by his name. Certain substances effect the growth and activity quite miraculously. Coumarin and other benzopyrones, such as 5,6 benzopyrone, 1,2 benzopyrone, diosmin and others are known to stimulate macrophages to degrade

extracellular albumen, allowing faster resorption of edematous fluids.¹⁻⁷ In earlier literature these were referred to as hormones⁸⁻¹². Hormone is a Greek word derived from hormao which means to urge on or to stimulate¹³⁻²⁰. For hormones of plants the term phytochrome has been given. Phytochromes are organic substances which are naturally produced in plants²¹⁻²⁸, control the growth or other physiological functions at a site remote from its place of production and active in extremely minute quantities²⁹⁻³⁶. Growth hormones has been defined as "Substances which are synthesized in particular cells and which are transferred to other cell wherein extremely small quantities influence developmental process".³⁷⁻⁴⁶

Thus, from the above brief review it is evident that coumarin are required for exploitation specially for citric acid fermentation and in view of this the authors have studied the influence of 6-hydroxy-4-methylcoumarin on citric acid production by *Aspergillus niger* T-918.

Experimental

The influence of 6-hydroxy-4-methylcoumarin on production of citric acid by *Aspergillus niger* T-918.

The composition of production medium for the production of citric acid by *Aspergillus niger* T-918 is prepared as follows: Molasses 30% w/v, Ammonium nitrate - 0.55%, Potassium dihydrogen phosphate-0.55%, Magnesium sulphate - 0.55%
Distilled water to make up 100 ml, pH-2.0

Table - 1
Production of citric acid by *Aspergillus niger* T-918 exposed to 6-hydroxy-4-methylcoumarin

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	% difference of citric acid increased after 13 days
Control	11	8.258	5.154	—
(-) Coumarin	13	9.757	4.055	—
	15	6.756	3.550	—
$1.0 \times 10^{-5} M$	11	8.288	5.124	—
$1/10^{-3} \times 10^{-2} M$	13	9.793	4.019	(+) 0.368
(+) Coumarin	15	6.780	3.435	—
$2.0 \times 10^{-5} M$	11	8.419	4.994	—
$2/10^{-3} \times 10^{-2} M$	13	9.948	3.864	(+) 1.957
(+) Coumarin	15	6.884	3.446	—
$3.0 \times 10^{-5} M$	11	8.530	4.883	—
$3/10^{-3} \times 10^{-2} M$	13	10.082	3.734	(+) 3.330
(+) Coumarin	15	6.978	3.426	—
$4.0 \times 10^{-5} M$	11	8.852	4.559	—
$4/10^{-3} \times 10^{-2} M$	13	10.465	3.348	(+) 7.256
(+) Coumarin	15	7.242	3.283	—
$5.0 \times 10^{-5} M$	11	9.059	4.354	—
$5/10^{-3} \times 10^{-2} M$	13	10.720	3.092	(+) 9.869
(+) Coumarin	15	7.418	2.956	—
$6.0 \times 10^{-5} M^{**}$	11	9.265	4.148	—
$6/10^{-3} \times 10^{-2} M$	13	10.952***	2.860	(+) 12.247
(+) Coumarin	15	7.573	2.459	—
$7.0 \times 10^{-5} M$	11	9.017	4.395	—
$7/10^{-3} \times 10^{-2} M$	13	10.661	3.153	(+) 9.265
(+) Coumarin	15	7.370	3.042	—
$8.0 \times 10^{-5} M$	11	8.713	4.699	—
$8/10^{-3} \times 10^{-2} M$	13	10.302	3.510	(+) 5.585
(+) Coumarin	15	7.127	3.317	—
$9.0 \times 10^{-5} M$	11	8.465	4.946	—
$9/10^{-3} \times 10^{-2} M$	13	10.005	3.810	(+) 2.541
(+) Coumarin	15	6.924	3.519	—
$10.0 \times 10^{-5} M$	11	8.267	5.145	—
$10/10^{-3} \times 10^{-2} M$	13	9.769	4.045	(+) 0.122
(+) Coumarin	15	6.764	3.956	—

* Mean of three observations ** Optimum concentration of coumarin *** Optimum yield of citric acid
 (+)ve values indicate % increase in the yield of citric acid, Experimental deviation ($\pm$) 1.5% to 3.5%

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 medium of citric acid by *Aspergillus niger* T-918. Now, the same production medium for citric acid was prepared for 99-fermentor flask, i.e.; each contained '100ml' of production medium.

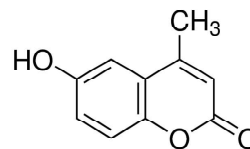
The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermentor flasks. The remaining 9-fermentor flasks out of 99-fermentor flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermentor flasks. After preparing the above sets of fermentor flasks M/1000 solution of 6-hydroxy-4-methylcoumarin 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 6-hydroxy-4-methylcoumarin 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 : A = Amount of coumarin, in ml, i.e. 1.0 ml

to 10 ml. x = Molarity of the coumarin solution and respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 27°C and analysed after 11, 13 and 15 days for citric acid⁴⁷ formed and molasses left unfermented⁴⁸.

Results and Discussion



6-hydroxy-4-methylcoumarin

The data recorded in the table-1 shows that 6-Hydroxy-4-methylcoumarin was found to be significant for higher promotion of citric acid up to its concentration from 1.0×10^{-5} to 6.0×10^{-5} M and above this concentration the bioproduction of H_3Cit by *Aspergillus niger* T-918 was found to be decreasing. However, bioproduction of H_3Cit by *Aspergillus niger* T-918 used were higher than that of control fermentor flasks.

The maximum yield of citric acid was observed at 6.0×10^{-5} M concentration of 6-hydroxy-4-methylcoumarin i.e., 10.952g/ 100 ml in 13 days of optimum incubation period which is 12.247% higher in comparison to control, i.e., 9.757g/100 ml in the same experimental conditions and optimum incubation period.

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