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Production of citric acid by *Aspergillus niger* NCIM -1986 exposed to 7-hydroxy-4-phenyl coumarin

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Abstract : The efficacy of coumarin, i.e., 7-hydroxy-4-phenyl coumarin on production of citric acid by fungal strains such as *Aspergillus aculeatus* NCIM -1636, *Aspergillus awamori* NCIM -1645, *Aspergillus foetidus* NCIM-1765, *Aspergillus wentii* NCIM - 1789 and strain *Aspergillus niger* NCIM-1986 has been assessed. It has been observed that the fungal strain *Aspergillus niger* NCIM-1986 has been found most potential and effective for the citric acid fermentation process. It has been found that the micelles, i.e., 7-hydroxy-4-phenyl coumarin at molar concentration, i.e., 4.0×10^{-5}M has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM-1986 and enhances the yield of citric acid to an extent of 7.909% higher in comparison to control fermentor flasks, i.e., 8.395g/100 ml under the optimized conditions, viz. 30^0C temperature, 1.8 pH, 11 days of incubation period with 32% (w/v) molasses solution.

(Keywords: Citric acid fermentation, 7-hydroxy-4-phenyl coumarin, coumarin and *Aspergillus niger* NCIM-1986)

Introduction

Coumarin has clinical medical value by itself, as an edema modifier. 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] Certain substances effect the growth and activity quite miraculously. In earlier literature these were referred to as hormones[8-12]. Hormone is a Greek word derived from hormao (Upu W) which means to urge on or to stimulate[13-20]. For hormones of plants the term phytochrome has been given.

Phytochromes are organic substances which are naturally produced in plants[21-28], control the growth or other physiological functions at a site remote from its place of production and active in extremely minute quantities[29-36]. 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 7-hydroxy-4-phenyl coumarin on citric acid production by *Aspergillus niger* NCIM-1986.

Experimental

The influence of 7-hydroxy-4-phenyl coumarin on production of citric acid by *Aspergillus niger* NCIM -1986.

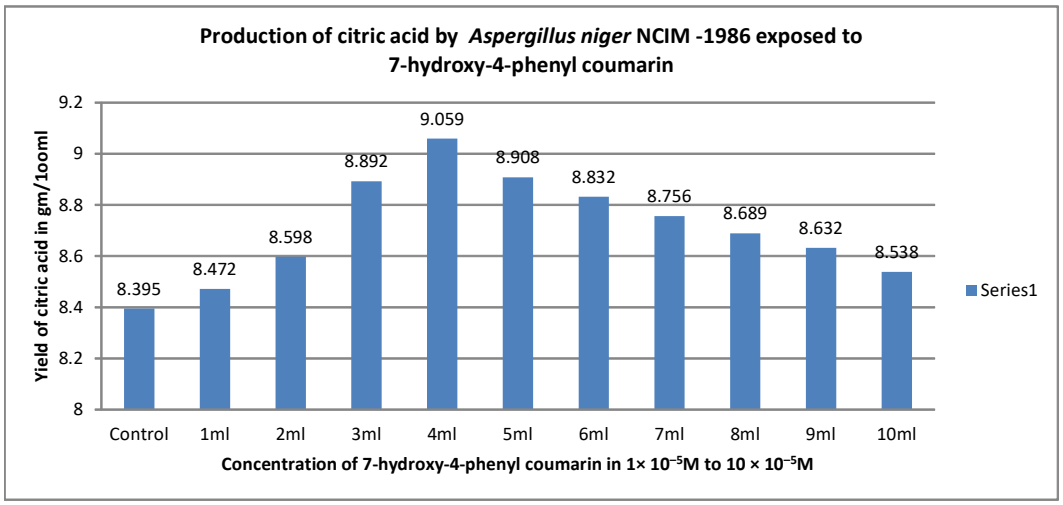
The composition of production medium for the production of citric acid by *Aspergillus niger* NCIM -1986 is prepared as follows:
Molasses : 32% (w/v), NH_4NO_3 : 0.75%
KH_2PO_4 : 0.75%, $MgSO_4.7H_2O$: 0.75%, pH : 1.8

The pH of the production medium was adjusted to1.8 by adding requisite amount of KCI-HCI 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" on production of citric acid by *Aspergillus niger* NCIM -1986. Now, the same production of citric acid by *Aspergillus*

Table - 1
Production of citric acid by *Aspergillus niger* NCIM -1986 exposed to 7-hydroxy-4-phenyl coumarin

Concentration of coumarin used used a x 10^{-5}M	Yield of citric acid* in g/100 ml			% of citric acid increased(+) in 11 days of optimum incubation period.
	10 days	11 days	12 days	
Control	6.410	8.395	7.180	–
1.0 x 10^{-5}M	6.467	8.472	7.244	(+) 0.917
2.0 x 10^{-5}M	6.563	8.598	7.352	(+) 2.418
3.0 x 10^{-5}M	6.788	8.892	7.603	(+) 5.920
4.0 x 10^{-5}M**	6.916	9.059***	7.747	(+) 7.909
5.0 x 10^{-5}M	6.801	8.908	7.617	(+) 6.110
6.0 x 10^{-5}M	6.743	8.832	7.553	(+) 5.205
7.0 x 10^{-5}M	6.685	8.756	7.488	(+) 4.300
8.0 x 10^{-5}M	6.634	8.689	7.431	(+) 3.502
9.0 x 10^{-5}M	6.589	8.632	7.381	(+) 2.823
10 x 10^{-5}M	6.518	8.538	7.302	(+) 1.703

* 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 (±) 1.5 to 3.5%



niger NCIM -1986 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-4-phenyl coumarin was prepared and from the above micell 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 micelle.

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-4-phenyl coumarin 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 30^0C and analysed after 10, 11 and 12 days for citric acid[46] formed.

Results and Discussion

The data recorded in the table-1 shows that the compound, i.e., 7-hydroxy-4-phenylcoumarin was also found to be increasing up to its concentration from 1.0×10^{-5} M to 4.0×10^{-5} M. It has also been observed that gradual addition of 7-hydroxy-4-phenylcoumarin to the fermentation medium gradually increases the production of citric acid by *Aspergillus niger* NCIM -1986. 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 0.917% to 7.909% only.

It has been observed that higher concentrations of 7-Hydroxy-4-phenylcoumarin, i.e.; on 5.0×10^{-5}M and onwards has retarded the yield of citric acid by *Aspergillus niger* NCIM-1986.

The maximum yield of citric acid has been recorded at 4.0×10^{-5} M concentration of 7-hydroxy-4-phenylcoumarin, i.e., 9.059g/100 ml in 11 days of optimum incubation period which is 7.909% higher in comparison to the control fermentor flasks i.e., 8.395g/100 ml in the same set of experimental parameters for production of citric acid by *Aspergillus niger* NCIM -1986.

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