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Bioproduction of citric acid by *Aspergillus oryzae* NCIM-944 exposed to 4-hydroxy-7-methoxy-3-phenyl coumarin

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Abstract: The efficacy of 4-hydroxy-7-methoxy-3-phenyl coumarin on bioproduction of citric acid by fungal strains such as *Aspergillus fischeri* NCIM – 508, *Aspergillus foetidus* NCIM–510, *Aspergillus giganticus* NCIM – 568, *Aspergillus luchuensis* NCIM – 991 and *Aspergillus oryzae* NCIM - 944 has been assessed. It has been observed that the fungal strain *Aspergillus oryzae* NCIM - 944 has been found most significant and effective for the citric acid fermentation process. It has been found that the compound, i.e., 4-hydroxy-7-methoxy-3-phenyl coumarin has stimulatory effect on bioproduction of citric acid by *Aspergillus oryzae* NCIM - 944 and enhances the yield of citric acid to an extent of 18.587% higher in comparison to control fermenter flasks, i.e., 7.715g/ 100 ml under the optimized conditions.

(**Keywords:** Citric acid fermentation, 4-hydroxy-7-methoxy-3-phenyl coumarin and *Aspergillus oryzae* NCIM - 944)

Introduction

Many compounds which contain the coumarin moiety exhibit useful and diverse pharmaceutical and biological activities[1-3].

A group of the compounds closely related to the phenolic acids and also derived from the shikimic acid pathway are the coumarins[4-14]. More than 500 coumarins exist in nature, although only a few are usually found in any particular plant family[15-21]. Compounds containing two coumarins moieties have been found to be extremely useful as anticoagulants[22] antimicrobials[23] and triplet sensitizers[24]. Coumarins owe their class name to 'Coumarou', the vernacular name of the tonka bean (Dipteryx odorata Willd, Fabaceae), from which coumarin, it was isolated in 1820 (Bruneton[25], 1999). Furanocoumarins consist of a five-membered furan ring attached to the coumarin nucleus, divided into linear or angular types with substitution at one or both of the remaining benzoid positions (Ojala T.,[26] 2001). Pyranocoumarin members are analogous to the furanocoumarins, but contain a six-membered ring. Coumarins substituted in the pyrone ring include 4-hydroxycoumarin (Keating *et al*.[27], 1997).The synthetic compound, warfarin, belongs to this coumarin subtype. By virtue of its structural the benzo-alpha-pyrone, although it is generally accepted that 7-hydroxycoumarin be regarded as the parent compound of the more complex coumarins (Murray et al.[28], 1982 and others[29-36])

Thus, from the above brief review it is evident that coumarins are required for genetic manupulation and exploitation specially for citric acid fermentation and in view of this the author has studied the influence of 4-hydroxy-7-methoxy-3-phenyl coumarin on production of citric acid by *Aspergillus oryzae* NCIM - 944

Experimental

The influence of 4-hydroxy-7-methoxy-3-phenyl coumarin on production of citric acid by *Aspergillus oryzae* NCIM - 944.

The composition of the production medium for production of citric acid by

Aspergillus oryzae NCIM - 944 has been prepared as follows: Molasses:24.5%(w/v), NH_4NO_3: 0.65% , KH_2PO_4 : 0.65%, $MgSO_4.7H_2O$:0.65%, pH : 2.2

The pH of the production medium was adjusted to 2.2 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 fermenter flask, i.e., "100ml" production of citric acid by *Aspergillus oryzae* NCIM-944. Now, the same production medium for production of citric acid by *Aspergillus oryzae* NCIM-944 was prepared for 99-fermenter flask, i. e; each contained '100ml' of production medium.

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

After preparing the above sets of fermenter flasks M/1000 solution of 4-hydroxy-7-methoxy-3-phenyl coumarin 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 fermenter flasks contained no coumarin. Now, the total volume in each fermenter flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of 4-hydroxy-7-methoxy-3-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 respectively.

Table - 1
Bioproduction of citric acid by *Aspergillus oryzae* NCIM-944 exposed to 4-hydroxy-7-methoxy-3-phenyl coumarin

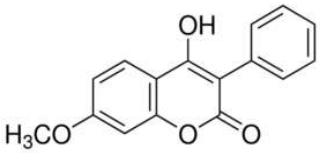
Concentration of coumarins used A X 10^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of citric acid in comparison to control.
Control	10	7.715	2.589	-
1.0×10^{-5}M	10	7.915	2.386	+2.592%
2.0×10^{-5}M	10	8.247	2.058	+6.895%
3.0×10^{-5}M	10	8.471	1.826	+9.799%
4.0×10^{-5}M	10	8.841	1.453	+14.594%
5.0×10^{-5}M**	10	9.149***	1.153	+18.587%
6.0×10^{-5}M	10	8.710	1.593	+12.896%
7.0×10^{-5}M	10	8.363	1.936	+8.399%
8.0×10^{-5}M	10	8.062	2.236	+4.497%
9.0×10^{-5}M	10	7.923	2.376	+2.696%
10.0×10^{-5}M	10	7.823	2.476	+1.399%

* 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 10 days.
Experimental deviation (±) 1.5-3%

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

The above fermenter flasks were then sterilized, cooled inoculated, incubated at 30^{0}C and analysed after 8, 10 and 12 days for citric acid[37] formed and molasses left unfermented[38].

Results and Discussion



4-hydroxy-7-methoxy-3-phenyl coumarin

The data recorded in the table-1 shows the influence of 4-hydroxy-7-methoxy-3-phenyl coumarin on production of citric acid by *Aspergillus oryzae* NCIM-944. The results show that the compound 4-hydroxy-7-methoxy-3-phenyl coumarin is also benificial and encouraging for the production of citric acid by *Aspergillus oryzae* NCIM-944 and thus causes enhancement in the production of citric acid by *Aspergillus oryzae* NCIM-944 exposed to experimental coumarin concentrations from 1.0 x 10^{-5} to 10.0 x 10^{-5} M.

The maximum yield of citric acid was found to be at 5.0 x 10^{-5} M concentration of 4-hydroxy-7-methoxy-3-phenyl coumarin i.e., 9.149 g/100 ml in 10 days of optimum incubation period which is 18.587% higher in comparison to control fermenter flasks, i.e., 7.715g/ 100 ml in the same set of experimental parameters.

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