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Impact of fluorogenic compounds on biotic production of citric acid

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Abstract: Fluorogenic compounds, i.e., coumarins when used as biotic elicitors or in fermentation media can impact citric acid production by fungi like *Aspergillus niger*. Research on the impact of coumarins on citric acid fermentation is important for developing new strategies to improve the production of this valuable industrial product. The impact of fluorogenic compound, i.e., 7-hydroxy-4-(trifluoromethyl) coumarin on biotic production of citric acid by *Aspergillus fumigatus* AR-902, *Aspergillus parasiticus* AR-904, *Aspergillus awamori* AR-861, *Aspergillus oryzae* AR-962 and *Aspergillus niger* AR-953 has been assessed. It has been found that the fungal strain *Aspergillus niger* AR-953 has been found most effective for the upgradation of citric acid production. It has been found that the flurogenic compound, i.e.,7-hydroxy-4-(trifluoromethyl) coumarin has stimulatory impact at molar concentration of 4×10^{-5}M and enhances the yield of citric acid to an extent of 6.487% higher in comparison to control, i.e., 6.859 gm/100ml under the optimized parameters viz. 29^0C temperature, 1.8 pH, 11 days of incubation period and 20% (w/v) molasses solution.

(Keywords : Citric acid fermentation, fluorogenic compound, 7-Hydroxy-4-(trifluoromethyl) coumarin *Aspergillus niger* AR-953).

Introduction

Coumarins can positively impact citric acid production during fermentation, enhancing yields. Different coumarins can have varying impacts on citric acid yields[1-7]. While the exact mechanism is not fully elucidated, coumarins are thought to influence the metabolic pathways and processes within the microorganism involved in citric acid production, leading to increased yields. The ability of coumarins to enhance citric acid production has potential implications for industrial application allowing for increased production efficiency and cost effectiveness[8-13]

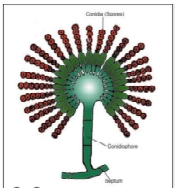
During the recent past commondable development towards citric acid fermentaion exposed to some compounds[14-30] like auxins, gibberellins, cytokins, PAOC, mutagens and vitamins has been studied by several workers but no attempt has been made for the class of compounds especially dormins, flowering hormones, 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

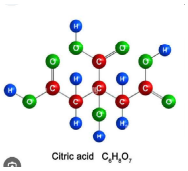
family of compounds.[31-45]

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 authors have studied the influence of 7-Hydroxy-4-(trifluoromethyl)coumarin on production of citric acid by *Aspergillus niger* AR- 953

Experimental

The influence of 7-Hydroxy-4-(trifluoromethyl) coumarin 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%, $MgSO_4.7H_2O$: 0.66%, pH : 2.1





Aspergillus niger

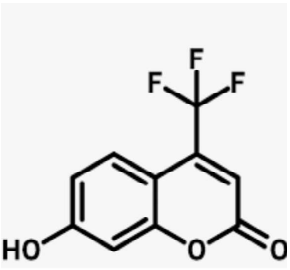
The pH of the production medium was adjusted to 2.1 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter. The above composition medium represents volume of a fermentor flask, i.e., "100ml" production of citric acid by *Aspergillus niger* AR- 953. Now, 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-4-(trifluoromethyl) 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 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-4-(trifluoromethyl) 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

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 29^0C and analysed after 8, 11 and 14 days for citric acid[46] formed and molasses[47] left unfermented.

Results and Discussion



7-hydroxy-4-(trifluoromethyl)coumarin

The data recorded in the table-1 shows that 7-hydroxy-4-(trifluoromethyl)coumarin was 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-(trifluoromethyl) coumarin to the fermentation medium gradually increases the production of

Table - 1
Impact of fluorogenic compounds on biotic production of citric acid

Concentrtion of coumarin used A × 10^{-x}M	Incubation period in days	Yield of citric acid* in g/100mL	Molasses* left Unfermented in g/100 mL	% of citric acid increased after 11 days
Control	11	6.859	1.149	-
1.0 ×10^{-5}M	11	6.948	1.096	+1.297
2.0 ×10^{-5}M	11	7.023	0.973	+2.391
3.0 ×10^{-5}M	11	7.174	0.823	+4.592
4.0 ×10^{-5}M**	11	7.304***	0.693	+6.487
5.0 ×10^{-5}M	11	7.195	0.809	+4.898
6.0 ×10^{-5}M	11	7.105	0.896	+3.586
7.0 ×10^{-5}M	11	7.044	0.959	+2.697
8.0 ×10^{-5}M	11	6.955	1.049	+1.399
9.0 × 10^{-5}M	11	6.920	1.086	+0.889
10.0 ×10^{-5}M	11	6.893	1.106	+0.495

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

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 1.297% to 6.487% only.

It has observed that higher concentrations of 7-hydroxy-4-(trifluoromethyl) coumarin, i.e.; on 5.0 x 10^{-5}M and onwards has been retarded the production of citric acid by *Aspergillus niger* AR- 953.

The maximum yield of citric acid has been recorded at 4.0 x 10^{-5} M concentration of 7-hydroxy -4-(trifluoromethyl)coumarin, i.e., 7.304g/100 ml in 11days of optimum incubation period which is 6.487% higher in comparison to the control fermentor flasks i.e.,6.859 g/100 ml in the same set of experimental parameters for the production of citric acid by *Aspergillus niger* AR-953.

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