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Production of citric acid by *Aspergillus candidus* NCIM-883 exposed to 3- Acetyl-4-hydroxycoumarin

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Abstract- The efficacy of 3-Acetyl- 4-hydroxycoumarin was studied on citric acid production by *Aspergillus candidus* NCIM-883. It has been found that the coumarin i.e. 3-Acetyl-4-hydroxycoumarin at concentration 6.0×10^{-4} M enhances the production of citric acid extent of 2.51383% higher in comparison to control fermentor flasks in 5.58152 g/100ml in 10 day of optimum incubation period.

Keywords : 3- Acetyl-4-hydroxycoumarin, citric acid, sucrose, *Aspergillus candidus* NCIM-883.

INTRODUCTION

Coumarin has a wide spread occurrence in natural products too & is a representative of lactones (where a lactone is an ester group integrated into a carbon ring system. Coumarin is a crystalline white solid when seen pure, with a hay like, sweet aromatic creamy odour with certain nutty shadings much used in synthetic form as a fragrance chemical for perfumes and for fragranced soaps and detergents.

A group of compounds closely related to the phenolic acid and also derived from the shikimic acid pathway are the coumarins.¹ More than 500 coumarins exists in nature, although only a few are usefully found in any particular plant family² Compounds containing two coumarins

moieties have been found to be extremely useful as anticoagulants³, antimicrobials⁴ and triplet sensitizers⁵. Coumarins owe their class name to "Coumarou" the vernacular name of the tonka bean (*Dipteryx odorata* willd, fabaceae), from which coumarin was isolated in 1820.⁶

Thus, from the above review it is evident that coumarins are required for citric acid fermentation & in the view of this, the author has studied the influence of 3-Acetyl-4- hydroxyl coumarin on novel method of citric acid production by *Aspergillus candidus* NCIM-883.

MATERIALS AND METHODS

The influence of 3-Acetyl-4-hydroxy coumarin on citric acid production by *Aspergillus candidus* NCIM-883. The composition of the production medium for the production of citric acid by *Aspergillus candidus* NCIM-

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883 was prepared as follows: sucrose: 15%, NH_4NO_3 : 0.18%, KH_2PO_4 : 0.25%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.35%, pH:2.2

The pH of the production medium was adjusted to 2.2 by adding requisite amount of KCl-HCl buffer solution & this pH was also determined by a pH meter.

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Concentration of coumarin used $\text{a} \times 10^{-x}\text{M}$	Yield of citric acid* in g/100 ml			% of citric acid increase (+) in 10 day of optimum in incubation period
	7 days	10 days	15 days	
Control	3.86131	5.58152	4.82561	-----
$1.0 \times 10^{-4}\text{M}$	3.86903	5.59554	4.81522	(+0.25118
$2.0 \times 10^{-4}\text{M}$	3.87289	5.60292	4.82005	(+)0.38340
$3.0 \times 10^{-4}\text{M}$	3.89989	5.65128	4.85847	(+)1.24983
$4.0 \times 10^{-4}\text{M}$	3.91536	5.66643	4.87725	(+)1.52127
$5.0 \times 10^{-4}\text{M}$	3.93082	5.68315	4.89210	(+)1.82083
$6.0 \times 10^{-4}\text{M}^{**}$	3.95782	5.72183 ^{***}	4.92578	(+)2.51383
$7.0 \times 10^{-4}\text{M}$	3.94625	5.70509	4.91134	(+)2.21391
$8.0 \times 10^{-4}\text{M}$	****	5.69393	****	(+) 2.01378
$9.0 \times 10^{-4}\text{M}$	****	5.58438	****	(+) 0.05124
$10.0 \times 10^{-4}\text{M}$	****	5.58331	****	(+) 0.03207

- * Mean of three observation.
- ** Optimum Concentration of coumarin.
- *** Optimum yield of citric acid
- **** insignificant value (+) ve values indicate increase in the yield of citric acid

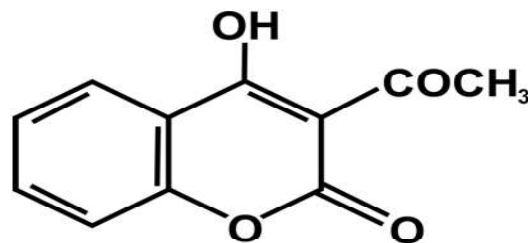
Experimental deviation (\pm) 1.5 to 3.5 %

The above composition medium represents volume of a fermentor flask i.e. "100ml" citric acid production by *Aspergillus candidus* NCIM-883. Now the same production was prepared for 99- fermentor flask ie, 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 3-Acetyl-4-hydroxycoumarin was prepared & form the above coumarin solution 1.0, 2.0,3.0, 4.0, 5.0,6.0,7.0,8.0,9.0, ad 10 ml was added to fermentation flask of above 1st to 10th sets respectively. Thus, the molar concentration of 3-Acetyl-4-hydroxycoumarin in 1st,2nd, 3rd, 4th, 5th, 6th,7th, 8th, 9th, 10th, subsets approximately as given below.

$\text{A} \times 10^{-x} \text{ M}$
 $1.0 \times 10^{-4} \text{ M}$ to $10.0 \times 10^{-4} \text{ M}$
 Where, A = amount of coumarin,
 In ml, i.e. 1.0ml to 10 ml
 x=Molarity of the coumarin solution

The above fermentor flasks were then sterilized, cooled, inoculated, incubated at 28°C and analysed after 7, 10 and 15 days for citric acid formed and sucrose sugar left unfermented.

RESULTS AND DISCUSSION



3- Acetyl-4-hydroxycoumarin

The data recorded in the table-1 shows that 3- Acetyl-4-hydroxycoumarin has stimulatory effect on production of citric acid by *Aspergillus candidus* NCIM-883. The results show that the compound 3-Acetyl-4-hydroxycoumarin

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is not much beneficial and encouraging for bioconversion of sucrose to citric acid by *Aspergillus candidus* NCIM-883 and thus causes slight enhancement in the production of citric acid exposed, to experimental coumarin concentration from 1.0×10^{-4} to 10.0×10^{-4} M.

CONCLUSION

It has been observed that maximum yield of citric acid was found to be at 6.0×10^{-4} M concentration of 3-Acetyl-4-hydroxycoumarin, i.e. 5.72183 g/100 ml in 10 days of optimum incubation period which is 2.51383% higher in comparison to control fermentor flasks, i.e. 5.58152 g/100ml in the same set of experimental parameters.

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