

Efficacy of 7-amino-4-methyl-3-coumarinylacetic acid on LSF production of H₃Cit

Pragati Kiran

Department of Chemistry, Magadh University, Bodh-Gaya (Bihar), India

Email : pragatikiran27@gmail.com

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Abstract: The efficacy of 7-amino-4-methyl-3-coumarinylacetic on liquid substrate fermentative (LSF) production of citric acid (H₃Cit) by *Aspergillus clavatus* NCIM 1679 has been assessed. It has been found that the coumarin, i.e., 7-amino-4-methyl-3-coumarinylacetic under trial has stimulatory effect on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 and enhances the production of citric acid to an extent of 10.512% higher in comparison to control fermentor flasks, i.e., 9.322 g/100mL in 12 days of incubation period, 1.8 pH and 32°C temperature with 32% (w/v) molasses solution.

(Key words : Molasses, coumarin, 7-amino-4-methyl-3-coumarinylacetic and *Aspergillus clavatus* NCIM 1679)

Introduction

Coumarins are an important class of compounds of both natural and synthetic origin. Many compounds which contain the coumarin moiety exhibit useful and diverse pharmaceutical and biological activities, often depending on the substituents they bear in the parent benzopyran moiety^{1,2} and, there has been a growing interest in their synthesis³. Some of these coumarin derivatives have been found useful in photochemotherapy, antitumor⁴, anti-HIV therapy^{5,6}, as CNS-stimulants⁷, antibacterial⁸⁻¹⁰, anticoagulants¹¹⁻¹³, antifungal^{14,15}, antioxidant¹⁶ agents and as dyes¹⁷. Natural, semi-synthetic and synthetic coumarins are useful substances in drug research¹⁸. Coumarins can be used not only to treat cancer, but to treat the side effects caused by radiotherapy^{19,26}.

A group of the compounds closely related to the phenolic acids and also derived from the shikimic acid pathway are the coumarins²⁷. More than 500 coumarins exist in nature, although only a few are usually found in any particular plant family²⁸. Compounds containing two coumarins moieties have been found to be extremely useful as anticoagulants,²⁹ antimicrobials³⁰ and triplet sensitizers³¹. Kiran *et al*³² have reported some coumarin useful for production of H₃Cit.

Thus, from the above brief review it is evident that coumarins are required for genetic manipulation and exploitation specially for citric acid fermentation and in view of this the authoress has studied the influence of 7-amino-4-methyl-3-coumarinylacetic on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679

Experimental

The influence of 7-amino-4-methyl-3-coumarinyl acetic on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679.

The composition of the production medium for LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 has been prepared as follows : Molasses : 32% (w/v), NH₄NO₃ : 0.36%, KH₂PO₄ : 0.36%, MgSO₄.7H₂O : 0.36%, pH : 1.8

The pH of the production medium was adjusted to 1.8 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter.

Table - 1
Efficacy of 7-amino-4-methyl-3-coumarinylacetic acid on LSF production of H₃Cit

Concentration of coumarin used A x 10 ^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 12 days
Control	12	9.322	1.149	-
1.0x10 ⁻⁵ M	12	9.463	1.096	+1.512
2.0x10 ⁻⁵ M	12	9.537	0.973	+2.306
3.0x10 ⁻⁵ M	12	9.822	0.823	+5.363
4.0x10 ⁻⁵ M	12	9.994	4.018	+7.208
5.0x10 ⁻⁵ M**	12	10.302***	3.705	+10.512
6.0x10 ⁻⁵ M	12	10.127	3.879	+8.635
7.0x10 ⁻⁵ M	12	9.896	4.201	+6.157
8.0x10 ⁻⁵ M	12	9.718	4.288	+4.248
9.0x10 ⁻⁵ M	12	9.584	4.425	+2.810
10.0x10 ⁻⁵ M	12	9.438	4.578	+1.244

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

The above composition medium represents volume of a fermentor flask, i.e., "100ml" on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679.

Now, the same production medium for on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 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-amino-4-methyl-3-coumarinylacetic 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-amino-4-methyl-3-coumarinylacetic in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x} M

1.0x10⁻⁵M to 10.0x10⁻⁵M respectively.

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 32°C and analysed after 9, 12 and 15 days for citric acid³³ formed and molasses³¹ left unfermented.

Results and Discussion

The data recorded in the table-1 shows

that the coumarin compound 7-amino-4-methyl-3-coumarinylacetic acid was found to be increasing up to its concentration from 1.0×10^{-5} M to 5.0×10^{-5} M. It has also been observed thus gradual addition of 7-amino-4-methyl-3-coumarinylacetic acid to the fermentation medium gradually increases the production of 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 1.512% to 10.512% only.

It has been observed that higher concentrations of 7-Amino-4-methyl-3-

coumarinylacetic acid, i.e.; on 6.0×10^{-5} M and onwards has been retarded the LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679.

The maximum yield of citric acid has been recorded at 5.0×10^{-5} M concentration of 7-Amino-4-methyl-3-coumarinylacetic acid, i.e., 10.302g/100 ml in 12 days of optimum incubation period which is 10.512% higher in comparison to the control fermentor flasks i.e., 9.322 g/100 ml in the same set of experimental parameters for the LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679.

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