

Effect of N-(7-dimethylamino-4-methyl-3-coumarinyl) Maleimide on Fermentation of Citric Acid by *Aspergillus Niger* NCIM-2101.

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Abstract

*The efficacy of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide on production of citric acid by some fungal strains like *Aspergillus-niger* NCIM -2101 has been observed. The coumarin; i.e. N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide under observation has encouraging effect on bioproduction of citric acid and enhances the yield of citric acid to an extent of 8.696% higher in comparison to control fermenter flasks, i.e., 9.199 g/100 ml in 12 days of optimum incubation period, 1.8 pH and 30°C temperature with 28% (w/v) molasses solution along with other nutritional components.*

Key words: *N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide, Citric acid fermentation and *Aspergillus niger* NCIM-2101, Molasses*

Introduction

Coumarin is a flavoring substance which is contained in relatively high concentrations in cinnamon varieties collectively known as "Cassia cinnamon" Coumarins are classified as a member of the benzopyrone family all of which consist of a benzene ring joined to a pyrone ring. The coumarins are of great interest due to their pharmacological properties¹. The benzopyrones can be subdivided into the benzoalpha-pyrones to which the coumarins belong and the benzo-gama-pyrones, of which the flavonoids are principal members. In particular, their physiological, bacteriostatic and anti-tumor activity makes these compounds attractive backbone derivatization and screening as novel therapeutic agents².

Coumarin-containing supramolecular medicinal agents as a new increasing expansion of supramolecular chemistry in pharmaceutical science have also been actively investigated in recent years³. Coumarin-derived artificial ion receptors, fluorescent probes and biological stains are growing quickly and have a variety of potential applications in monitoring timely enzyme activity, complex biological events as well as accurate pharmacological and pharmacokinetic properties⁴. This review provides a systematic summary and insight of the whole range of medicinal chemistry in the current developments of coumarin compounds as anticoagulant, anti-neuro degenerative, anticancer, antioxidative, antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory and analgesic, antidiabetic, anti-depressive and other bioactive agents as well as supramolecular medicinal drugs, diagnostic agents and pathologic probes, and biological stains⁵.

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Experimental Methods

The efficacy of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide on production of citric acid by *Aspergillus niger* NCIM-2101 is observed as : The composition of the production medium for production of citric acid by *Aspergillus niger* NCIM-2101 has been prepared as follows : Molasses: 28% (w/v), NH_4NO_3 : 0.25%, KH_2PO_4 : 0.25%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.25%, pH: 1.8, The pH value of the production medium was adjusted to 1.8 by adding requisite amount of KCl-HCl buffer solution, and this pH was also verified by a pH meter. The above composition medium represents volume of a fermenter flask, i.e., "100ml" production medium for production of citric acid by *Aspergillus niger* NCIM-2101. Now, the same production medium for production of citric acid by *Aspergillus niger* NCIM-2101 was prepared for 99-fermenter flask, i.e; each contained '100ml' of production medium.

Then a 99-fermenter flasks were then arranged to 11-sets, each comprising of 9-fermenter flasks respectively. The 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 N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide was prepared and from the above N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide 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 N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide.

The total volume in each fermenter flasks was made 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x} M, i.e.,

1.0×10 ⁻⁵ M	2.0×10 ⁻⁵ M	3.0 ×10 ⁻⁵ M	4.0 ×10 ⁻⁵ M	5.0×10 ⁻⁵ M	6.0×10 ⁻⁵ M
7.0×10 ⁻⁵ M	8.0 ×10 ⁻⁵ M	9.0 ×10 ⁻⁵ M	10.0×10 ⁻⁵ M		

A = amount of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide, in ml, i.e., 1.0 ml to 10 ml.

x = Molarity of the N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide solution

The fermenter flasks used were then properly sterilized, cooled inoculated and incubated at 30°C and analyzed after 8, 12 and 14 days for citric acid formed.

Results and Discussion

Table 1: Production of citric acid by *Aspergillus niger* NCIM-2101 exposed to N-(7-dimethylamino-4-methyl-3-coumarinyl) maleimide

Control	8	6.315	5.685	-
(-) Coumarin	12	8.463	3.537	-
	14	7.362	3.432	-
1.0x10 ⁻⁵ M (+) Coumarin	8	6.397	5.615	-
	12	8.589	3.412	+ 1.488
	14	7.479	3.309	-

2.0x10 ⁻⁵ M	8	6.491	5.513	-
(+) Coumarin	12	8.714	3.284	+ 2.989
	14	7.603	3.193	-
3.0x10 ⁻⁵ M	8	6.637	5.369	-
(+) Coumarin	12	8.911	3.089	+ 5.293
	14	7.801	3.045	-
4.0x10 ⁻⁵ M**	8	6.851	5.149	-
(+) Coumarin	12	9.199***	2.812	+ 8.696
	14	8.085	2.716	-
5.0x10 ⁻⁵ M	8	6.700	5.301	-
(+) Coumarin	11	8.996	3.004	+ 6.298
	14	7.884	2.915	-
6.0x10 ⁻⁵ M	8	6.580	5.426	-
(+) Coumarin	12	8.835	3.166	+ 4.395
	14	7.725	3.059	-
7.0x10 ⁻⁵ M	8	6.460	5.539	-
(+) Coumarin	12	8.674	3.328	+ 2.493
	14	7.563	3.239	-
8.0x10 ⁻⁵ M	8	6.422	5.578	-
(+) Coumarin	12	8.623	3.380	+ 1.890
	14	7.520	3.291	-
9.0x10 ⁻⁵ M	8	6.365	5.639	-
(+) Coumarin	12	8.540	3.458	+ 0.908
	14	7.439	3.369	-
10.0x10 ⁻⁵ M	8	6.340	5.660	-
(+) Coumarin	12	8.507	3.498	+ 0.519
	14	7.400	3.395	-

* Each value represents mean of three trials

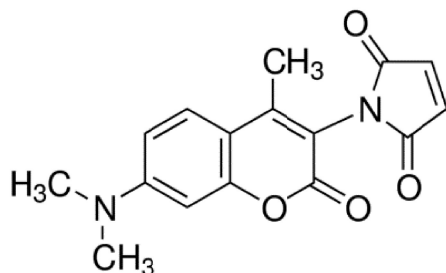
** Optimum concentration of coumarin used

*** Optimum yield of citric acid

(+) values indicate % increase in the yield of citric acid after 12 days.

Experimental deviation (\pm) 1.5-3%

The influence of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide



N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide

As per the recorded observation and above mentioned data in table-1 shows that N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide also has inspiratory effect on citric acid production by *Aspergillus niger* NCIM-2101.

The data (table-1) reveals that the coumarin N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide stimulates the citric acid fermentation process and enhances the yield of citric acid upto its N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide concentrations from 1.0×10^{-5} to 4.0×10^{-5} M. The effect of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide on the productivity (yield) of citric acid was gradually in increasing order and attains its best role at 4.0×10^{-5} M where maximum yield of citric acid, i.e., 9.199g/100 ml is fetched in 12 days of optimum incubation period which is 8.696% higher in comparison to control fermenter flask, i.e., 9.199 g/100 mL.

The effect of above coumarin on the molar concentration, i. e., from 5.0×10^{-5} M to 10×10^{-5} M the production of citric acid has been bit enhanced but the order of citric acid productivity is reverse in respect to increasing molar concentrations of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide. However, the citric acid production by *Aspergillus niger* NCIM-2101 under the influence of each concentration of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide used has been inspiring and the yield of citric acid has been found greater than that obtained in the control fermenter flasks. In order of productivity and % of citric acid formed is as below:

Concentration of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide from 1.0×10^{-5} M to 4.0×10^{-5} M.

Productivity of citric acid:

1.488%, 2.989%, 5.293%, 8.696%

Concentration of N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide from 7.0×10^{-5} M to 10.0×10^{-5} M.

Productivity of citric acid:

6.298%, 4.395%, 2.493%, 1.890%, 0.908%, 0.519%

The openness of fungal strain *Aspergillus niger* NCIM-2101 to N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide may produce a variety of effects.

Conclusions

Hence, it is concluded that the N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide maintained at lower concentrations is stimulatory and at higher concentrations is detrimental for citric acid production by *Aspergillus niger* NCIM-2101.

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