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Production of citric acid by *Aspergillus niger* NCIM-2101 exposed to 7-diethylamino-3-[N-(2-maleimidoethyl) carbamoyl] coumarin

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Abstract : The efficacy of 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin on bioproduction of citric acid by some fungal strains such as *Aspergillus carbonarius* NCIM -2097, *Aspergillus saitoi* NCIM -2056, *Aspergillus usumii* NCIM - 2045, *Aspergillus wentii* NCIM-2020 and *Aspergillus niger* NCIM -2101 has been assessed. It has been found that the fungal strain *Aspergillus-niger* NCIM -2101 has been found most suitable to give higher yield of citric acid. The coumarin; i.e. 7-diethylamino-3-[N-(2-maleimidoethyl) carbamoyl] coumarin under trial has stimulatory effect on bioproduction of citric acid and enhances the yield of citric acid to an extent of 14.697% higher in comparison to control fermenter flasks, i.e., 8.559 g/100 ml in 12 days of optimum incubation period, 1.8 pH and 30^0C temperature with 28% (w/v) molasses solution alongwith other nutritional ingredients.

(**Key words :** Molasses, citric acid fermentation, 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin and *Aspergillus niger* NCIM-2101)

Introduction

Coumarin, an organic compound having the characteristic odour of new-mown hay, obtainable from the tonka tree (native to Guyana) or by chemical synthesis. It is used in perfumes and flavourings and for the preparation of other chemicals.

Coumarins in the field of biotechnology has assumed great importance. Some coumarins and its derivative are also used in medicine today. The corelation of chemical structure with anticoagulant activity of some coumarin derivatives has also been studied by many workers[1-34]. Literature survey reveals that a little work has been done on different fermentative products with respective microorganism[35-38] exposed to coumarins, therefore, the author has employed 7-diethylamino-3-[N-(2-maleimidoethyl) carbamoyl] coumarin on production of citric acid by *Aspergillus niger* NCIM-2101.

Experimental

The influence of 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin on bioproduction of citric acid by *Aspergillus niger* NCIM-2101. 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%, $MgSO_4.7H_2O$: 0.25%, 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. The above composition medium represents volume of a fermenter flask, i.e., "100ml" production medium for bioproduction of citric acid by *Aspergillus niger* NCIM-2101. Now, the same production medium for bioproduction of citric acid by *Aspergillus niger* NCIM-2101 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.

Table - 1
Production of citric acid by *Aspergillus niger* NCIM-2101 exposed to 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin

Concentration of coumarin used A × 10^{-x}M	Incubation period in days	Yields of citric acid* in g/100 ml	Molasses* left unfermented in g/100 ml	% of Citric acid increased after 12 days
Control	8	6.411	5.589	-
(-) Coumarin	12	8.559	3.443	-
	14	7.458	3.341	-
1.0x10-5M	8	6.487	5.515	-
(+) Coumarin	12	8.678	3.329	+1.390
	14	7.569	3.239	-
2.0x10-5M	8	6.577	5.423	-
(+) Coumarin	12	8.798	3.202	+2.792
	14	7.689	3.105	-
3.0x10-5M	8	6.673	5.327	-
(+) Coumarin	12	8.924	3.076	+4.264
	14	7.814	3.033	-
4.0x10-5M	8	6.668	5.119	-
(+) Coumarin	12	9.209	2.795	+7.594
	14	8.108	2.693	-
5.0x10-5M	8	7.064	4.939	-
(+) Coumarin	12	9.449	2.554	+10.398
	14	8.340	2.468	-
6.0x10-5M	8	7.180	4.825	-
(+) Coumarin	12	9.603	2.398	+12.197
	14	8.500	2.289	-
7.0x10-5M**	8	7.340	4.669	-
(+) Coumarin	12	9.817***	2.189	+14.697
	14	8.711	2.086	-
8.0x10-5M	8	6.955	5.045	-
(+) Coumarin	12	9.303	2.698	+8.692
	14	8.200	2.593	-
9.0x10-5M	8	6.750	5.259	-
(+) Coumarin	12	9.029	2.978	+5.491
	14	8.013	2.889	-
10.0x10-5M	8	6.545	5.455	-
(+) Coumarin	12	8.755	3.249	+2.289
	14	7.651	3.150	-

* 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%

After preparing the above sets of fermenter flasks M/1000 solution of 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin was prepared and from the above 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] 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 lithium dodecyl sulfate.

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 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin 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.,

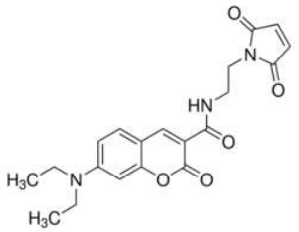
1.0×10^{-5}M	6.0×10^{-5}M
2.0×10^{-5}M	7.0×10^{-5}M
3.0×10^{-5}M	8.0×10^{-5}M
4.0×10^{-5}M	9.0×10^{-5}M
5.0×10^{-5}M	10.0×10^{-5}M

A= Amount of 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin , in ml, i.e., 1.0 ml ..... to 10 ml.

x= Molarity of the 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin solution

The above fermenter flasks were then sterilized, cooled inoculated and incubated at 32^0C and analysed after 8,12 and 14 days for citric acid[39] formed and molasses[40] left unfermented.

Results and Discussion



7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin

The data recorded in the table-1 shows the influence of 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin on production of citric acid by *Aspergillus niger NCIM*-2101. The results show that the compound 7-diethylamino-3-[N-(2-maleimidoethyl) carbamoyl] coumarin is also benificial and encouraging for production of citric acid by *Aspergillus niger NCIM*-2101 and thus causes enhancement in the production of citric acid exposed to experimental coumarin concentrations from 1.0×10^{-5} to 10.0×10^{-5} M.

The maximum yield of citric acid was found to be at 7.0×10^{-5} M concentration of 7-diethylamino-3-[N-(2-maleimidoethyl)carbamoyl] coumarin, i.e., 9.817g/100 ml in 12 days of optimum incubation period which is 14.697% higher in comparison to control fermentor flasks, i.e., 8.559g/ 100 ml in the same set of experimental parameters.

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