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Studies on effect of bergapten on bioproduction of itaconic acid

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Abstract : Itaconic acid is an organic compound derived from the fermentation of sugars. The compound appears as a white crystalline solid. The global itaconic acid market size was \$112 million in 2023 and is expected to reach \$164.33 million by 2032. The present communication deals the efficacy of bergapten on bioproduction of itaconic acid by *Aspergillus terreus* A-22. It has been observed and found that the bergapten under trial has stimulatory effect on itaconic acid fermentation process and enhances the yield of itaconic acid to an extent of 14.757% higher in comparison to control.

(Keywords : Bergapten / 5 – methoxypsoralene, itaconic acid, *Aspergillus terreus* A-22).

Introduction

Bergapten, is a natural compound found in certain plants such as citrus fruits and bergamot essential oil. Bergapten is a furanocoumarin which is type of coumarin derivatives. Coumarins are a class of organic compounds with a benzopyrone structure. Furanocoumarins, like bergapten have a furan ring fused to the coumarin structure. With a renewed interest in sustainable development, the chemical industry is making many efforts to replace petrochemical-based monomers with natural ones.[1-3] Itaconic acid is an organic acid that is used as a platform chemical for the production of various value added chemicals such as poly-itaconic acid, resins biofuel components, ionomer cements etc. Itaconic acid and its derivatives have wide applications in the textile, chemical and pharmaceutical industries. Recent trends in itaconic acid research summarized in this paper indicate that itaconic acid can be produced cost effectively from sustainable raw materials and have the potential to replace petro-based chemicals in various applications. Itaconic acid, or methylenesuccinic acid, is an organic compound. Itaconic acid is a white crystalline powder[4-10]. Itaconic acid is a naturally occurring compound, non-toxic, and readily biodegradable. The name itaconic was devised as an anagram of aconitic.

The variety of physiological effects of natural coumarins is extensive. More than 500 coumarins exist in nature, although only a few are usefully found in any particular plant family[11–15]. Numerous physiological effect of coumarins are known but clear functions of these compounds generally remains to be unknown[16-20]. Literature survey reveals that a very few work has been done on efficacy of coumarins on fermentation especially itaconic acid fermentation[21-29]. Therefore, the author has employed some coumarins on itaconic acid production by *Aspergillus terreus* - A-22

Experimental

The influence of bergapten on itaconic acid production by *Aspergillus terreus* A-22. The composition of the production medium for itaconic acid production by *Aspergillus terreus* A-22 has been prepared as follows :

Glucose : 20.0 g ; $MgSO_4.7H_2O$: 0.25 g; NH_4NO_3 :0.80 g ; KCl : 0.05 g ; NaCl :0.05g ; KH_2PO_4 : 0.25 g ; pH : 2.2.

The pH of the production medium was

adjusted to 2.2 by adding requisite amount of NH_4OH buffer solution. The production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for about 15 minutes and cooled to room temperature.

The above composition medium represents volume of a fermentor flask, i.e., "100mL" production medium for itaconic acid production by *Aspergillus terreus* A-22 Now, the same production medium for itaconic acid production by *Aspergillus terreus* A-22 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/100 solution of bergapten 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 bergapten in 1^{st},2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th} and 10^{th} subsets were approximately as given below :

Table - 1
Studies on effect of bergapten on bioproduction of itaconic acid

Concentration of coumarin used	*Yield of itaconic acidin g/100mL			% Difference in the yield of Itaconic acid after 8 days
	7 days	8 days	9 days	
Control – coumarin	2.485	4.540	4.990	–
1.0×10^{-4} M	2.518	4.678	4.999	(+) 3.039
2.0×10^{-4} M	2.577	4.749	5.115	(+) 4.603
3.0×10^{-4} M	2.584	4.760	5.000	(+) 4.845
4.0×10^{-4} M	2.593	5.115	5.108	(+) 12.665
5.0×10^{-4} M**	2.650	5.210	5.115	(+) 14.757
6.0×10^{-4} M	2.620	4.920	4.802	(+) 8.370
7.0×10^{-4} M	2.603	4.850	4.750	(+) 5.726
8.0×10^{-4} M	2.569***	4.755	4.700	(+) 4.735
9.0×10^{-4} M	****	****	****	****
10.0×10^{-4} M	****	****	****	*****

* Mean of three observations, ** Optimum concentration of coumarin
*** Optimum yield of itaconic acid, (+)ve values indicate % increase in the yield of itaconic acid
Experimental deviation ($\pm$) 1.5% to 3.5% **** Insignificant value

A x 10^{-x} M i.e., 1.0x10^{-4}M : 2.0x10^{-4}M, 3.0x10^{-4}M, 4.0x10^{-4}M,5.0x10^{-4}M, 6.0x10^{-4}M, 7.0x10^{-4}M, 8.0 x 10^{-4}M, 9.0x10^{-4}M and 10.0x 10^{-4}M.
A = amount of chemical coumarin, i.e., 1.0 ml to 10 ml, x = Molarity of the coumarin soln.

The above fermentor flasks were then sterilized, cooled inoculated, incubated and analysed after 7, 8 and 9 days for itaconic acid formed[30] by *A. terreus* A-22

Results and Discussion

The data recorded in the table-1 shows the influence of bergapten on itaconic acid production by *Aspergillus terreus* A-22. The results show that the compound bergapten is not much benificial and encouraging for the itaconic acid production by *Aspergillus terreus* A-22 and thus causes slight enhancement in the production of itaconic acid exposed to coumarin concentrations from 1.0 x 10^{-4} to 10.0 x 10^{-4} M.

The maximum yield of itaconic acid was found to be at 5.0×10^{-4} M concentration of bergapten, i.e., 5.210g/100mL in 8 days of optimum incubation period which is 14.757% higher in comparison to control fermentor flasks, i.e.,4.540g/ 100 mL in the same set of experimental parameters.

It has been noticed that the presence of above coumarin compound, i.e., bergapten, was found to be beneficial and encouraging for the improved yield of itaconic acid up to its concentration from 1.0x10^{-4}M to 5.0×10^{-4} M but not so significant and above this concentration the coumarin under trial has retarded the yield of itaconic acid. However, it is interesting to note that itaconic acid production by *Aspergillus terreus* A-22 exposed to all the experimental coumarin concentration, ie., from 1.0 x 10^{-4} M to 10.0x10^{-4} M of coumarin under trial has been found to be slight better than the control fermentor flask.

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