BIOSYNTHESIS OF ETHYL ALCOHOL BY SUBMEGED FERMENTATION IN PRESENCE OF 8-Acetyl-7-hydroxycoumarin

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ABSTRACT: Coumarin though known for therapeutic activity also indicated their activity as stimulator for fermentation. 8-Acetyl-7-hydroxycoumarin when subjected to alcoholic fermentation by SmF technique yielded very promising results. AHC enhanced the yield to an extent of 4.255319148% in comparison to the control fermentor flasks (7.05 mL/100 mL). During experiments, 24% molasses solution was subjected to incubation for 50 hours at 300 K with pH adjusted to 5.1.

INTRODUCTION

Coumarins are considered as phytoalexins since plants produce them as defence substance when wounded or attacked by other organisms. The antimicrobial effects of methanol extracts prepared from seven plants growing in Finland, namely Aegopodiumpodagraria, Anethumgraveolens, A. archangelica, Levisticumofficinalis, P. crispum, and Peucedanumpalustre, and R graveolens, and pure Coumarins and Furanocoumarins as defensive compounds. The agar-diffusion methods used are suitable for the bioassay-guided isolation of active substances.

Coumarins can be suggested to be beneficial for the plants themselves as natural bio controlling antipathogenic compounds, and for humans as remedy for hyper proliferative skin diseases and as reference compounds in various bioactivity tests. Furthermore, Coumarins containing plants are valuable as dietary supplements on the basis of their mild antimicrobial and anti-inflammatory effects.

Coumarins owe their class name to ‘Coumarou’, the vernacular name of the Tonka bean (DipteyxodorataWilld., Fabaceae), from which Coumarins itself was isolated in 1820 (BRUNETON, 1999). Coumarins belong to a group compounds known as the Benzopyrene, all of which consist of a benzene ring joined to a pyrone. Coumarin and the other members of the Coumarin family are Benzo-a-Pyrones, while the other main members of the Benzopyrene group-the flavonoids-contain the y-pyrone group Coumarins may also be found in nature in combination with sugars, as glycosides.

Like other Phenylpropanoids, Coumarins arise from the metabolism of phenylalanine via a cinnamic acid, p-coumaric acid. The specificity of the process resides in the 2’-hydroxylation, next comes the photo catalysed isomerization of the double bond followed by spontaneous lactonisation. In some rare cases, glycosylation of cinnamic acid occurs, precluding lactonisation.

In such cases, Coumarin only arises after tissue injury and enzymatic hydrolysis. The formation of di-and trihydroxy Coumarins and of their ethers involves the hydroxylation of umbelliferone rather than the lactonisation of the corresponding cinnamic acids.[1-8]

Phytochromes are organic substances which are naturally produced in plants[9-16], control the growth or other physiological functions at a site remote from its place of production and active in extremely minute quantities[17-24].

Growth hormones has been defined as "Substances which are synthesized in particular cells and which are transferred to order cell wherein extremely small quantities influence developmental process.[25-34]However, the term hormone is quite popular and widely used[35-41]. It is meant for an organic substance synthesized in one tissue and migrates to another tissue of the plant wherein very minute quantity affects the growth[42-48].

The common hormones are auxins[49-51], gibberellins[52-56], cytokinins[57-63], ethylene[64-70], durmins[71-81] and etc. But now different categories of substances affecting growth are known which can be broadly classified into growth promoting and growth retarding[71-81]. Substances or into naturally occurring growth substances and synthetic growth substances.

Coumarins in the field of biotechnology has assumed great importance. Some coumarins and its derivative are also used in medicine today and many attempts have been to establish the structure activity relationship of some coumarin derivatives. The correlation of chemical structure with anticoagulant activity of some coumarin derivatives has also been studied by many workers [82].

Literature survey reveals that a few work has been done on efficacy of coumarins on alcoholic fermentation. Therefore, the author have employed 8-Acetyl-7-hyrdoxycoumarin for biosynthesis of alcohol by submerged fermentation technique*Saccharomyces cerevisiae* NCIM-3256

EXPERIMENTAL

The influence of 8-acetyl-7-hydroxycoumarin on alcoholic fermentation by Saccharomyces cerevisiae Rb-39.The compositions of production medium for the alcoholic fermentation by *Saccharomyces cerevisiae* NCIM-3256 prepared as follows:

Molasses	: 24%
Malt extract	: 0.50%
Yeast extract	: 0.50%
Peptone	: 0.40%
$(NH_4)_2 HPO_4$	:0.50%
pH	:5.0%

Distilled water was added to make of the volume up to '100 mL'.
The pH of the medium was adjusted to 5.0 by adding requisite amount of lactic acid.

Now the same production medium for alcoholic fermentation by *Saccharomyces cerevisiae* NCIM-3256was prepared for 99 fermentorflasks, i.e., each containing 100ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 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.

Now, M/100 solutions of 8-acetyl-7-hydroxycoumarinwas prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no coumarin. The total volume in each fermentor-flask was made up to '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 8-acetyl-7-hydroxycoumarinin first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below:

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

Where, A = amount of mutagens in ml, ie, from 1.0ml to 10.0ml

The fermentor-flasks were then steam sterilized, cooled inoculated, incubated at 30^0C and analyzed colorimetrically[35] after 25, 50 and 75 hours for alcohol formed.

Table-1
Alcoholic fermentation by Saccharomyces cerevisiae NCIM 3256 in presence of 8-Acetyl-7-hydroxycoumarin

Concentration of mutagens used A X 10^{-x}M	Yield of ethanol* in ml/100ml			% of ethanol increases/(+) in 50 hrs of optimum incubation period
	40 hrs	50hrs	60 hrs	
control	4.66	7.05	7.02	--
1x 10^{-5}M	4.70	7.10	7.08	+0.709219858
2x 10^{-5}M	4.75	7.15	7.12	+1.418439716
3x 10^{-5}M	4.80	7.18	7.15	+1.834397163
4x 10^{-5}M	5.00	7.25	7.21	+2.836879432
5x 10^{-5}M**	5.20	7.35***	7.30	+4.255319148
6 x 10^{-5}M	5.18	7.30	7.26	+3.546099290
7x 10^{-5}M	5.16	7.28	7.25	+3.262411347
8x 10^{-5}M	5.12	7.25	7.20	+2.836879432
9x 10^{-5}M	5.10	7.20	7.15	+2.127659574
10x 10^{-5}M	5.06	7.17	7.10	+1.702127659

**** Each value represents mean of three trials. **Optimum concentration of mutagen used. *** Optimum yield of bioalcohol in 40 hours.(+) values indicate % increase in the yield of bioalcohol after 50 hours. Experimental deviation (±) 1.5-3%.***

The influence of 8-acetyl-7-hydroxycoumarin on biosynthesis of alcohol by submerged fermentation by saccharomyces cerevisiae NCIM- 3256

The data recorded in the table-1 shows that The data recorded in the table-1 shows that The data recorded in the table-1 shows that 8-Acetyl-7-hydroxycoumarin has stimulatory effect on alcoholic fermentation by Saccharomyces cerevisiae NCIM-3256 at all its molar concentration i.e.; from 1 x 10 $^{-5}$M to 10.0 x 10 $^{-5}$M in two phases

In the first phase the molar concentration of the coumarin i.e. 8-Acetyl-7-hydroxycoumarin from 1 x 10 $^{-5}$M to 10.0 x 10 $^{-5}$M is significant and the production of alcohol gradually increases till a maximum value is reached at 5.0 x 10 $^{-5}$M. i.e. 7.35/100mL in 50 hours of optimum incubation period which is **4.255319148%** higher in comparison to control i.e.7.05/100mL.

In the second phase of the coumarin effect i.e. 8-Acetyl-7-hydroxycoumarin at 6.0 x 10 $^{-5}$M and onwards the molar concentration of 8-Acetyl-7-hydroxycoumarin on biosynthesis of alcohol by submerged fermentation technique by Saccharomyces cerevisiae NCIM-3256 has been increased but in lesser amount in the presence of 8-Acetyl-7-hydroxycoumarin.So, the gradual addition of 8-Acetyl-7-hydroxycoumarin after the optimum concentration of 5.0 x 10 $^{-5}$M slow down the biosynthesis od alcohol by submerged fermentation technique by Saccharomyces cerevisiae NCIM-3256and the part of % increase at 6 x 10^{-5}M,7 x 10^{-5}M,8 x 10^{-5}M and 9 x 10^{-5}M molar concentration of 8-Acetyl-7-hudroxycoumarin is follow

3.546099290, 3.262411347, 2.836879432, 2.127659574 and 1.702127659% respectively.

Fromthe present investigation it is obvious that the coumarins under trial i.e.8-Acetyl-7-hudroxycoumarin is effective and useful for biosynthesis of alcohol by submerged fermentation technique by Saccharomyces cerevisiae NCIM-3256 and therefore, can be employed for the improved and higher yield of alcohol.

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