

Bioethanol production by *S. cerevisiae* AK-22 exposed to 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine

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Abstract : The influence of cytokinin, i.e., 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine on bioethanol production by the yeast *Saccharomyces cerevisiae* AK-22 has been assessed. It has been found that the cytokinin, i.e., 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine at its molar concentration of 6.0×10^{-5} M has stimulatory effect on bioproduction of ethyl alcohol and enhances the yield of bioethanol to an extent of 8.159% higher in comparison to control fermenter flask, i.e. 5.27mL/100 mL while molar concentration of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine under trial at 6.0×10^{-5} M and onwards inhibits and retards the bioethanol production. The molar concentration of cytokinin, i.e., 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine has been employed in between 1.0×10^{-5} M to 10×10^{-5} M and has been found that at initial concentration, i.e., 1.0×10^{-5} M it is least effective and at higher concentrations it gives insignificant yield of ethyl alcohol. Experimental parameters has been optimized viz. : 32°C temperature, 4.6 pH, 50 hrs incubation period with 16.5% (w/v) molasses solution alongwith other nutritional ingredients required by the yeast *Saccharomyces cerevisiae* AK-22 .

(Key words: Molasses, 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine and *S. cerevisiae* AK-22)

Introduction

Cytokinins are hormones involved in all aspects of plant growth and development and are essential for in vitro manipulation of plant cells and tissues. Much information has been gathered regarding the chemistry and biology of cytokinins, while recent studies have focused on the genetics and cytokinin-related genes.¹⁻⁵

Cytokinin is a generic name for substances that promote cytokinesis in cultured plant cells and also serve other regulatory functions similar to those of kinetin, the first chemically defined cytokinin⁶⁻¹⁰ Virtually all the known naturally-occurring cytokinins are substituted purine.

A large number of compounds have been synthesized with substituents at different position on the purine nucleus and with alterations in the nucleus itself. Substituents at different positions on the purine nucleus generally result in lower biological activity. Alteration in the nucleus usually lead to compounds with much reduced activity and often result in complete loss of cytokinin activity.

Although cytokinin research is not yet at a stage to be able to recognize physiologically active pools, knowledge of the biochemical mechanisms controlling cytokinin turnover can provide considerable insight into the way in which processes which modify growth and development may modulate pool sizes of cytokinins.¹¹⁻¹³

Thus, from the above brief review it is evident that cytokinins are required for genetic manipulation and exploitation specially for alcoholic fermentation and in view of this the author has studied the influence of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine on alcoholic fermentation by *S. cerevisiae* AK-22

Table - 1
Bioethanol production by *S. cerevisiae* AK-22 exposed to
6-(4-hydroxy-3-methyl-cis-2-butenyl) aminopurine

Concentration of cytokinins used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of bioethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	% Diff. in the yield of bioethanol in 50 hrs
Control	48	3.29	3.835	-
	50	5.27	2.881	-
	52	4.12	2.769	-
1.0×10^{-5} M	48	3.30	3.825	-
	50	5.30	2.852	(+)0.569
	52	4.18	2.729	-
2.0×10^{-5} M	48	3.34	3.789	-
	50	5.37	2.788	(+)1.897
	52	4.20	2.667	-
3.0×10^{-5} M	48	3.38	3.746	-
	50	5.43	2.725	(+)3.036
	52	4.25	2.656	-
4.0×10^{-5} M	48	3.48	3.645	-
	50	5.59	2.563	(+)6.072
	52	4.31	2.538	-
5.0×10^{-5} M**	48	3.55	3.579	-
	50	5.70**	2.459	(+)8.159
	52	4.39	2.393	-
6.0×10^{-5} M	48	3.52	3.606	-
	50	5.64	2.515	(+) 7.020
	52	3.33	2.452	-
7.0×10^{-5} M	48	3.47	3.659	-
	50	5.56	2.589	(+)5.502
	52	4.32	2.497	-
8.0×10^{-5} M	48	3.43	3.696	-
	50	5.50	2.656	(+)4.364
	52	4.29	2.566	-
9.0×10^{-5} M	48	3.35	3.778	-
	50	5.38	2.769	(+) 2.087
	52	4.22	2.698	-
10.0×10^{-5} M	48	3.31	3.816	-
	50	5.29	2.859	(+)0.379
	52	4.16	2.765	-

* Each value represents mean of three trials. ** Optimum concentration of cytokinins used and bioethanol formed. (+) Values indicate % increase in the yield of alcohol after 50 hours. Experimental deviation ($\pm$) 1.5-3%.

Experimental

The influence of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine on bioethanol production by *S. cerevisiae* AK-22. The composition of production medium for the bioethanol production by *S. cerevisiae* AK-22 is prepared as follows :

Molasses : 16.5 % (w/v), Malt-Extract: 0.300%
Yeast-Extract : 0.300%, Peptone : 0.300%, Distilled water : To make up 100 ml, pH : 4.6

Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 4.6 by adding requisite amount of lactic acid.

Now, the same production medium for bioproduction of bioethanol by *Saccharomyces cerevisiae* AK-22 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of the 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/1000 solutions of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no cytokinins. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$$A \times 10^{-x} \text{M}, 1.0 \times 10^{-5} \text{ M to } 10.0 \times 10^{-5} \text{ M}$$

Where, A = amount of cytokinin in ml, i.e.; from 1.0 ml to 10.0 ml.

x = molarity of the solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 32^o C and analysed colorimetrically after 48, 50 and 52 hours for bioethanol formed¹⁴ and molasses¹⁵ pollutant left unfermented.

Results and Discussion

The influence of 6-(4-Hydroxy-3-methyl-*cis*-2-butenyl) aminopurine on bioethanol production by *Saccharomyces cerevisiae* AK-22

The data recorded in the table - 1 shows results of bioethanol produced in the presence of cytokinin 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine in concentration between 1.0×10^{-5} M to 10.0×10^{-5} M. It is evident from the results that like 6-(4-hydroxy-3-methyl-*trans*-2-butenyl) aminopurine¹⁶, the 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine was also found stimulatory for the bioethanol production by *S. cerevisiae* AK-22 and enhances the yield of bioethanol upto 1.0×10^{-5} M to 5.0×10^{-5} M. The maximum yield of alcohol, i.e., 5.70 ml/100 ml was observed at molar concentration 5.0×10^{-5} M of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine which is 8.159% higher in comparison to control fermentor flask, i.e., 5.27 ml/100ml in comparison to control in 50 hrs. of optimum incubation period.

However, addition of 6-(4-hydroxy-3-methyl-*cis*-2-butenyl) aminopurine to the production medium in concentration between 1.0×10^{-5} M to 10.0×10^{-5} M enhances the bioethanol production and thus yield of bioethanol was found to be higher in each case in comparison to control in 50 hrs. of optimum incubation period and same experimental conditions.

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