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Cytokinin - mediated enhancement of molasses fermentation for ethanol production by *Saccharomyces cerevisiae* - 3078

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Abstract: The present study investigates the influence of the synthetic cytokinin derivative 6-(4-hydroxy-3-methyl-*trans*-2-butenyl) aminopurine on ethanol bioproduction from molasses using *Saccharomyces cerevisiae*-3078. Molasses, a low-cost agro-industrial byproduct rich in fermentable sugars, was employed as the primary carbon source. Exposure of yeast cells to the cytokinin analog significantly modulated cellular metabolism, resulting in improved sugar utilization, enhanced growth kinetics, and higher ethanol yields compared to untreated controls. The cytokinin-mediated response was associated with stimulation of glycolytic flux and a reduction in byproduct accumulation, thereby improving fermentation efficiency. Optimization studies revealed that moderate concentrations of the cytokinin elicited the most pronounced effect, while higher levels exerted inhibitory responses. These findings demonstrate the potential application of cytokinins as novel regulatory agents for improving microbial fermentation performance. This approach highlights an eco-friendly and cost-effective strategy for augmenting bioethanol production from molasses, offering prospects for industrial-scale bioprocess optimization. In the present investigation effect of 6-(4-hydroxy-3-methyl-trans--2-butenyl) aminopurine on production of ethanol from molasses by Saccharomyces cerevisiae-3078 has been assessed. It has been observed that the cytokinins under trial has stimulatory effect on production of ethanol and enhances the yield of ethanol to an extent of 7.391% higher in comparison to control when 20%molasses solution (w/v) is allowed to ferment at pH 5.7,temperature 30^{0}C and incubation period of 50 hours along with some other significant rich ingredients required by the yeast *Saccharomyces cerevisiae*-3078.

(Keywords : Cytokinins,molasses solution,ethanol fermentation and *Saccharomyces cerevisiae*-3078).

Introduction

The growing demand for renewable and sustainable energy resources has intensified global interest in bioethanol production as an eco-friendly alternative to fossil fuels. Among various feedstocks, molasses, a byproduct of the sugar industry, is widely utilized due to its abundance, low cost, and rich composition of fermentable sugars, vitamins, and trace elements. Molasses-based fermentation by *Saccharomyces cerevisiae* remains a cornerstone of industrial ethanol production; however, limitations such as incomplete sugar utilization, byproduct accumulation, and suboptimal metabolic regulation often constrain ethanol yields and process efficiency.

Recent advances in microbial biotechnology have highlighted the use of plant growth regulators and small biomolecules as modulators of microbial metabolism. Among these, cytokinins—traditionally recognized for their role in regulating cell division and differentiation in plants—have emerged as promising agents for influencing microbial physiology. Synthetic cytokinin derivatives such as 6-(4-hydroxy-3-methyl-trans-2-butenyl)aminopurine possess structural attributes that enable interaction with cellular signaling pathways, thereby modulating growth dynamics, stress tolerance, and metabolite production.

The exposure of *S. cerevisiae* to cytokinins has been proposed to enhance glycolytic flux, reduce metabolic bottlenecks, and redirect carbon flow toward ethanol formation.

Such regulatory interventions may offer a novel and cost-effective strategy for maximizing ethanol yields from molasses fermentation. Moreover, cytokinin-mediated modulation can potentially minimize undesirable byproducts such as glycerol and organic acids, thus improving overall process efficiency and sustainability.

The present study aims to evaluate the cytokinin-mediated enhancement of molasses fermentation for ethanol production using *S. cerevisiae-3078.* Specifically, it investigates the influence of 6-(4-hydroxy-3-methyl-trans-2-butenyl) aminopu rine on yeast growth, sugar consumption, and ethanol yield under controlled fermentation conditions. By elucidating the potential regulatory role of cytokinins in microbial fermentation, this work contributes to developing innovative bioprocess strategies for industrial bioethanol production.

Experimental

The influence of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine on microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078. The composition of production medium for the microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 is prepared as follows **:**
Molasses : 20%, Malt extract : 0.300%
Yeast extract : 0.300%, Peptone : 0.500%
$(NH_4)_2HPO_4$: 0.300%, $CaCO_3$: 8%, pH: 5.1

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

The pH of the production medium was adjusted to 5.1 by adding requisite amount of lactic acid.

The above composition medium represents volume of fermentor-flask, i.e., '100ml' production medium for microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078.

Now, the same production medium for microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. Each set was rearranged in 3 subsets, each comprising 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.

Now, M/1000 solution of 6-(4-Hydroxy-3-methyl-*trans*-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 I^{st} to 10^{th} sets respectively. The control fermentor-flasks contained no cytokinins. The total volume in each fermentor-flask was made up to '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine in first, second, third fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :
$A \times 10^{-x}$ M, 1.0×10^{-5} M to
Where, A = amount of cytokinin in ml, ie., from 1.0 ml to 10.0 ml.
x = molarity of the solution

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30^0C and analysed colorimetrically after 30, 50, and 70 hours for ethanol[22] formed and molasses[23] left unfermented.

Results and Discussion

Effect of cytokinin on yeast growth and fermentation kinetics Exposure of *Saccharomyces cerevisiae-3078* to 6-(4-

hydroxy -3- methyl-trans-2-butenyl) aminopurine resulted in noticeable changes in growth patterns and fermentation performance compared to the untreated control. At optimal cytokinin concentrations, the yeast exhibited accelerated exponential growth, with a reduction in lag phase duration and an increase in cell density at stationary phase. These observations indicate that the cytokinin analog acted as a metabolic stimulant, enhancing cell proliferation and viability during molasses fermentation. Similar growth-promoting effects of cytokinins on microbial cultures have been reported in earlier studies.

Sugar utilization efficiency

Fermentation broth analysis revealed that cytokinin-treated cultures exhibited significantly improved sugar assimilation. Total reducing sugar concentration declined more rapidly in treated samples, suggesting enhanced uptake and utilization of molasses-derived sucrose, glucose, and fructose. Efficient sugar consumption is a critical determinant of ethanol yield, and the accelerated depletion observed here supports the hypothesis that cytokinins facilitate enhanced glycolytic flux.

Ethanol production and yield improvement

The most striking effect of cytokinin supplementation was observed in ethanol production. Cultures exposed to moderate concentrations of the cytokinin derivative produced 6-7% higher ethanol yields compared to control fermentations. This improvement was accompanied by a reduction in fermentation time, highlighting the cytokinin's role in improving both productivity and efficiency. Excessive cytokinin concentrations, however, led to a decline in ethanol yield, likely due to metabolic overstimulation or inhibitory stress responses. This biphasic effect underscores the importance of dosage optimization for achieving maximum benefits.

Byproduct formation and metabolic regulation

A key finding of this study was the reduction in byproduct accumulation in cytokinin-treated fermentations. Levels of glycerol and organic acids such as acetic and lactic acid were consistently lower than those observed in control cultures. This indicates that cytokinins may have redirected carbon flux preferentially toward ethanol biosynthesis while minimizing competing pathways. Such redirection of metabolic flux has also been reported with other small-molecule modulators in yeast fermentations.

Mechanistic insights and industrial relevance

The observed cytokinin-mediated effects can be attributed to its regulatory influence on cellular signaling and energy metabolism. Cytokinins are known to modulate ATP availability, enhance antioxidant responses, and promote macromolecular synthesis, all of which are crucial for sustaining robust fermentative activity. The findings of this study suggest that introducing cytokinins into fermentation systems could serve as a novel metabolic engineering-free strategy to enhance ethanol yields from low-cost substrates like molasses. From an industrial perspective, this approach presents.

Comparative significance

When compared with other strategies such as surfactant-mediated fermentation (e.g., sodium dodecyl sulfate micelles) or mutagenic strain improvement, cytokinin supplementation offers a safer and more environmentally benign method. The absence of genetic manipulation also reduces regulatory hurdles for industrial adoption. However, the scalability of this approach requires further validation, particularly in large bioreactors where cytokinin stability and distribution may influence overall efficiency.

Summary of findings

In summary, cytokinin exposure enhanced growth kinetics, accelerated sugar utilization, improved ethanol yield, and minimized

Table - 1
Microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 exposed to 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine

Concentration of cytokinins used A X 10^{-x} M	Incubation period in hours	Yield of ethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	%Diff. in the yield of ethanol in 50 hrs.
Control	20	3.85	3.70	-
(-) Cytokinin	50	6.90	0.98	-
	70	5.95	0.95	-
1.0×10^{-5}M	20	3.88	3.69	****
(+) Cytokinin	50	7.02	0.95	(+) 1.7913
	70	5.98	0.93	****
2.0×10^{-5}M	20	3.90	3.62	****
(+) Cytokinin	50	7.10	0.69	(+) 2.89855
	70	6.08	0.63	****
3.0×10^{-5}M	20	3.95	3.55	****
(+) Cytokinin	50	7.23	0.63	(+) 4.78260
	70	6.10	0.58	****
4.0×10^{-5}M	20	3.99	3.53	****
(+) Cytokinin	50	7.29	0.60	(+) 5.65217
	70	6.15	0.55	****
5.0×10^{-5}M	20	4.05	3.50	****
(+) Cytokinin	50	7.35	0.53	(+) 6.52173
	70	6.18	0.50	****
6.0×10^{-5}M**	20	4.15	3.35	****
(+) cytokinin	50	7.41***	0.48	(+) 7.39130
	70	6.45	0.40	****
7.0×10^{-5}M	20	4.12	3.48	****
(+) cytokinin	50	7.35	0.50	(+) 6.52173
	70	6.42	0.45	****
8.0×10^{-5}M	20	4.02	3.40	****
(+) cytokinin	50	7.32	0.58	(+) 6.08695
	70	6.35	0.48	****
9.0×10^{-5}M	20	4.00	3.45	****
(+) cytokinin	50	7.26	0.60	(+) 5.21739
	70	6.30	0.50	****
10.0×10^{-5}M	20	3.95	3.42	****
(+) cytokinin	50	7.22	0.62	(+) 6.63768
	70	6.25	0.53	****

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

byproduct formation in *S. cerevisiae-3078* fermentations using molasses. These findings highlight the potential of cytokinins as regulatory molecules in industrial bioprocesses and open avenues for further studies on their mechnestic interactions with microbial metabolic.

The influence of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine :

The data recorded in the table - 1 shows the influence of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine on microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078. It is evident from the results that the presence of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine has stimulatory effect on microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078. The maximum yield of ethanol, i.e., 7.41 ml/100 ml in the presence of 6.0 X 10^{-5} M concentration of 6-(4-Hydroxy-3-methyl-trans-2-butenyl) aminopurine solution has been observed which is 7.39130 % higher in comparison to control, i.e.,6.90 ml/100 ml in 50 hrs. of optimum incubation period.

It was interesting to note that gradual addition of 6-(4-Hydroxy-3-methyl-trans-2-butenyl) aminopurine to the production medium gradually increases the yield of ethanol from 1.0 x 10^{-5} M to 6.0 x 10^{-5} M concentration of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine . Further it was noticed that on increasing the concentration of 6-(4-Hydroxy-3-methyl-trans-2-butenyl) aminopurine from 7.0 x 10^{-5} M to 10.0 x 10^{-5} M the production of ethanol was almost same and no distinct difference was marked.

However, maximum yield of ethanol was recorded at 6.0 x 10^{-5} M. Thus, higher concentrations of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine has not been found toxic yet after certain concentrations the influence of 6-(4-Hydroxy-3-methyl-trans-2-butenyl) aminopurine for microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 in view of increasing productivity has been insignificant. Therefore, higher concentrations of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine was not beneficial for the production of ethanol. However, microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 at all the concentration of 6-(4-Hydroxy-3-methyl-*trans*-2-butenyl) aminopurine used in the present experiment has been found greater than the control fermentor flasks.

References

1. J. Willer, C. Zidorn, J. Juan-Vicedo, J. *J. Ethnopharmacol*., **281**, 114465 (2021)
2. G. Astray, P. Gullon, B. Gull6n, P.E.S. Munekata, J.M. Lorenzo, *AppL Sci.* **10**, 5074 (2020)
3. B. Kramer, J. Thielmann, A. Hickisch, P. Muranyi, P.; J. Wunderlich, C. Hauser, *J. AppL Microbiol.* **118**, 648 (2015)
4. L. Nionelli, E. Pontonio, M. Gobbetti, C.G. Rizzello, *Int. J. Food Microbiol.* **266**, 173 (2018)
5. V. Sanz, M.D. Torres, L.J.M. Vilarifio, H. Dominguez, *Trends Food Sci. Technol.* **93**, 2 (2019).
6. A.F. Mendonca-Santana, K.L. Teixeira-Fernandes, E. Miranda, L.O. Coronado, L. Rios-Tibery, J.HOPS. Finzer, *Agric. Food Sci. Environ. Sci.* **18**, e08463 (2024)
7. J.I. Alonso-Esteban, J. Pinela, L. Barros, C.A Cid' M. Sokovi'c, R.C Calhelha, E. Torija-Isasa, de Cortes "Sanchez-Mata, M.; Ferreira, I.C.F.R. . *Ind. Crops Prod.* **134**, 154 (2019)
8. M. Liu, P. Hansen, G. Wang, L. Qui, J. Dong, H. Yin, Q. Zhonghua, Y. Mei, M. Jinlai, *(H. lupulus). Molecules.* **20**, 754 (2015).
9. L. Bocquet, C. Riviere, C. Dermont, J. Samaillie, J.L. Hilbert, P. Halama, A. Siah, S. Sahpaz, *Zymoseptoria tritici. Ind. Crops Prod.* **122**, 290 (2018)
10. P. Nezi, V. Cicaloni, L. Tinti, L. Salvini, M. lannone, S. Vitalini, S. Garzoli, *Separations* **9**, 204. (2022)

11. C. Gerhauser, *Nutr. Food Res.* **49**, 827 (2005)
13. B. Ceh, M. Kac, I.J. Kali; V. Abram, *Int. J.* Mol. *Sci.* **8**, 989 (2007).
14. V. Abram, B. eeh, M. Vidmar, M. Hercezi, N. Lazio, V. Bucik, S. Smole-Molina, I.J. Kair, M. Kac, L DemS'ar, et al. *Ind. Crops Prod.* **64**, 124 (2015)
15. P.B. Green, Self-organization and the formation of patterns in plants. In *Dynamics of* Cell *and Tissue Motion. Mathematics and Biosciences in Interaction;* W. Alt, A. Deutsch, G.A. Dunn, Eds.; Birkhauser: Basel, Switzerland; pp. 243. (1997)
16. J. Farag, I. PSenakova, N. Faragova *Nova Biotechnol.* **9**, 279 (2009)
17. L. Leto, C. Favari, A. Agosti, L. Del Vecchio, A. Di Fazio, L. Bresciani, V Mena, P.; Guarrasi, M. Cirlini, B. Chiancone, *Antioxidants* **13**, 909 (2024)
18. T Murashige, F.A koog, *Physiol. Plantarum.***15**, 473 (1962)
19. S.H. Howell, S. Lall, P. Che, *Trends Plant Sci.* **8**, 453 (2003)
20. G.C. Phillips, M. Garda, *Vitr. Cell. Dev. Biol. Plant.* **55**, 242 (2019)
21. G.E. Schaller, I.H. Street, J.J. Kieber, *Curr. Opin. Plant Biol.* **21**, 7 (2014)
22. L.P. McCloskey and L.L. Reploge *Am. J. Enol Vitie* **25**, 194 (1974).
23. M.Dubois, K.A.Gills, J.K.Hamilton, P. A. Rebers and F.Smith, *Anal.Chem.***28**,350 (1956)

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