BIOENERGETIC TRANSFORMATION OF MOLASSES POLLUTANT TO ETHANOL BY SACCHAROMYCES CEREVISIAE NCIM-2086 EXPOSED TO 3-AMINOCOUMARIN

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Abstract : The efficacy of 3-aminocoumarin on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 has been assessed. It has been found that the coumarin i.e 3-aminocoumarin under trial has stimulatory effect on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 and enhances the yield of ethanol to an extent of 9.10652% higher in comparison to control fermentor flasks i.e, 6.35ml/100ml in 46 hours of optimum incubation period 4.8 p^H and 32^0C temperature with 16% (W/V) molasses solution

(Key words : Molasses, coumarin, alcoholic fermentation, 3-aminocoumarin and Saccharomyces cerevisiae NCIM- 2086)

Introduction

Coumarin is a phytochemical with a vanilla like flavour. coumarin is a oxygen heterocycle. Coumarin can occur either free or combined with the sugar glucose(coumarin glycoside).

Coumarins are a group of important natural compounds, and have been found to have multi- biological activities such as anti- HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-osteoporosis, pain relief, preventing asthma and antisepsis. Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognised as inhibitors not only of the lipoxygenase and cycloxygenase enzymic system, but also of the neutrophil-dependent superoxide anion generation. Such derivatives also possess anti-inflammatory as well as antioxidant activities.

Coumarins, an old class of compounds, are naturally occurring benzopyrene derivatives. A lot of coumarins have been identified from natural sources, especially green plants. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Among these properties, their cytotoxic effects were most extensively examined. In this review, their broad range of effects on the tumors as shown by various in vitro and in vivo experiments and clinical studies are discussed. Hence, these cytotoxic coumarins represent an exploitable source of new anticancer agents, which might also help addressing side-toxicity and resistance phenomenan . These natural compounds have served as valuable leads for further design and synthesis of more active analogoues. In this review, plant derived coumarins and their synthetic analogues were systematically evaluated based on their plant origin, structureactivity relationship and anticancer efficacy. Owing the their diverse effects and inconclusive results from different in vitro studies, the mechanism of their action is not yet fully understood and correlation of effects with chemical structures is not conclusive at the moment. It is the objective of this communication to summarize experimental data for different coumarins, used as cytotoxic agents, because promising data have been reported for a series of these agents. Yet, the result form different coumarins with various tumor lines are contradictory in part.We therefore conclude that there is still a long way to go until we know which cytotoxic agent will clinically be suitable for what tumor entity for treatment. Their ability to bind metal ions represents an additional means of modulating their Pharmacological responses .

Coumarins in the field of biotechnology has assumed great importance. Some coumarins and its derivatives are also used in medicine today, and many attempts have been to be establish the structure- activity relationship of some coumarin derivatives. The corelation of chemical structure with anticoagulant activity of some coumarin derivatives has also been studied by many workers[1-31].

Literature survey reveals that a few work has been done on efficiency of coumarins on alcoholic fermentation. Therefore, the authors have employed, 3-aminocoumarin for biosynthesis of alcohol by submerged fermentation technique by Saccharomyces cerevisiae NCIM- 2086.

EXPERIMENTAL

The influence of 3-aminocoumarin on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086

The composition of production medium for the bioenergetic transformation of molasses pollutant to ethanol. *Saccharomyces cerevisiae* NCIM- 2086 is prepared as follows :

Molasses	:	16 % (w/v)
Malt-Extract	:	1.25%
Yeast-Extract	:	1.25%
Peptone	:	1.25%
Distilled water	:	To make up 100 ml
pH	:	4.8

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

The pH of the medium was adjusted to 4.8 by adding requisite amount of lactic acid.

The same production medium for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 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. 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 3-aminocoumarin 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 coumarin. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 3-aminocoumarin 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,
2.0×10^{-5} M,
3.0×10^{-5} M,
4.0×10^{-5} M,
5.0×10^{-5} M,
6.0×10^{-5} M,
7.0×10^{-5} M,
8.0×10^{-5} M,
9.0×10^{-5} M, and
10.0×10^{-5} M respectively.

Where, A= amount of coumarins in ml, ie; from1.0 ml to 10.0 ml.
x = molarity of the solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 32^0C and analysed colorimetrically[32] after 40, 46, and 50 hours for ethanol formed and molasses sugars left unfermented

Table-1

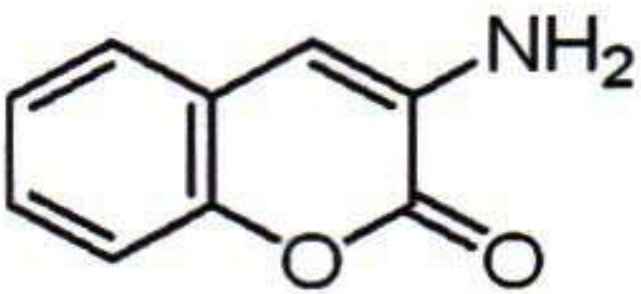
Bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086 exposed to 3-aminocoumarin

Concentration Of coumarin Used A X 10* M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses Sugars* left unfermented in g/100 ml	% Difference in yield of ethanol in comparison to control
Control (-) Coumarin	46	5.82	2.31631	
1.0 x10^{-5}M (+) Coumarin	46	5.92	2.21627	+ 1.71821
2.0 x10^{-5}M (+) Coumarin	46	5.99	2.14634	+ 2.92096
3.0 x10^{-5}M (+) Coumarin	46	6.10	2.03638	+ 4.81099
4.0 x10^{-5}M (+) Coumarin	46	6.20	1.93628	+ 6.52920
5.0 x10^{-5}M (+) Coumarin	46	6.35***	1.78630	+ 9.10652
6.0 x10^{-5}M (+) Coumarin	46	6.22	1.91633	+ 6.87285
7.0 x10^{-5}M (+) Coumarin	46	6.03	2.10629	+ 3.60824
8.0 x10^{-5}M (+) Coumarin	46	5.93	2.20636	+1.89003
9.0 x10^{-5}M (+) Coumarin	46	****	–	–
10.0 x10^{-5}M (+) Coumarin	46	****	–	–

* Each value represents mean of three trials.
** Optimum concentration of the chemical coumarin used.
*** Optimum yield of ethanol in 46 hours.
**** Insignificant Value
(+)Values indicate % increase in the yield of ethanol in comparison to control.
Experimental deviation (±) 1.5–3%.

RESULTS AND DISCUSSION

3-Aminocoumarin



The data recorded in the table-1 shows that 3-aminocoumarin has also stimulatory effect on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086

The data (table-1) reveals that the coumarin, i.e., 3-aminocoumarin stimulates the bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM-2086 enhances the yield of ethanol upto its (3-aminocoumarin) concentrations from 1.0×10^{-5} M to 8.0×10^{-5} M in two phases :

In the first phase, ie; from 1.0×10^{-5} M to 5.0×10^{-5} M the effect of 3-aminocoumarin on the productivity (the yield) of ethanol was gradually in increasing order and achieves its best function at 5.0×10^{-5} M where maximum yield of ethanol, i.e; 6.35ml/100 ml is obtained in 46 hours of optimum incubation period which is 9.10652% higher in comparison to control fermentor flasks (5.82 ml/100 ml).

In the second phase of coumarins effect the molar concentrations, ie; from 6.0×10^{-5} M to 8.0×10^{-5} M the production of ethanol has been enhanced but the order of ethanol productivity is found reversed in respect to increasing molar concentrations of 3-aminocoumarin . However, the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 under the influence of each concentration of 3-aminocoumarin used has been stimulating and the yield of

ethanol has been found greater than that obtained in the control fermentor flasks. In three phases the order of productivity and % of ethanol formed after 46 hrs is as under :

Concentration of 3-aminocoumarin
from 1.0 x 10^{-5} M to 5.0 x 10^{-5} M .
Productivity of ethanol: 1.71821%, 2.92096%, 4.81099%, 6.52920% and 9.10652%.

Concentration of 3-aminocoumarin
from 6.0 x 10^{-5} M to 8 x 10^{-5} M .
Productivity of ethanol : 6.87285%, 3.60824% and 1.89003%.

Concentration of 3-aminocoumarin
from 9.0 x 10^{-5} M to 10 x 10^{-5} M .
Productivity of ethanol : insignificant values

Thus, it is concluded that 3-aminocoumarin at lower concentrations is found stimulatory and at higher concentrations it is deterioratory for the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086.

REFERENCES

1. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. Second Edition, Hampshire UK, Intercept Ltd 1999; pp 263-277.
2. Ojala T. Biological Screening of Plant Coumarins. PhD Thesis, University of Helsinki, Helsinki, Finland, 2001.
3. Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT, Owens IS (Mar 1992). "A novel complex locus UGT1 encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini". J Biol Chem 267 (5): 3257–61. PMID 1339448.
4. F. P. Schäfer (Ed.), Dye Lasers, 3rd Ed. (Springer-Verlag, Berlin, (1990).
5. F. J. Duarte, Tunable Laser Optics (Elsevier-Academic, New York, (2003)
6. U.S. Pat. No. 4175982 to Loutfy et al, issued Nov 27 (1978)

7. "Extraction and Isolation of compounds from Herbal medicines" in 'Traditional Herbal Medicine Research Methods', ed by Willow JH Liu John Wiley and Sons, Inc (2011)
8. Floc'h F ,Mauger F, Desmurs J R." coumarin in plants and fruits ;27: 32-37(2002)
9. Tornoe, C.W.; Christensen, C.; Meldal, M.J. Org. Chem. 2002, 67, 3057-3064 (2002)
10. Wang H.X., Ng T.B. Planta Med., 67,669-672 (2001)
11. Lake B. Coumarin metabolism, toxicity, and carcinogenicity: relevance for human risk assessment. Food and Chemical Toxicology:37:423- 453 (1999)
12. D I Brahmbhatt, B R Hirani, Shashi U Pandya Indian journal of chemistry, vol. 39B, March 2000, pp, 233-235 Canadian Journal on Chemical Engineering & Technology Vol. 1, No. 2, April (2010)
13. Felter S.P., Vassallo J.D., Carlton B.D. & Daston G.P. . "A safetyassessment of coumarin taking into account species specificity oftoxiokinetics". Food & Chem. Toxicol. 44, 462-475 (2006)
14. Vocanson M., Goujon C., Chabeau G., Castelain M., Valeyrie M., Floc'h F., Maliverney C., Gard A. & Nicolas J.F. "The skin allergenic properties of chemicals may depend on contaminants" Int Arch Allergy Immunol 140, 231-238. (2006)
15. F. J. Duarte and L. W. Hillman (Eds.) :*Dye Laser Principles* (Academic, New York, 1990).
16. F. J. Duarte, : *Tunable Laser Optics* (Elsevier-Academic, New York, Appendix of Laser Dyes. (2003)
17. Link KP : "The discovery of dicumarol and its sequels". Circulation 19 (1): 97–107. (1959)
18. Farinola, Nicholas; Piller, Neil : "Pharmacogenomics: Its Role in Re-establishing Coumarin as Treatment for Lymphedema". Lymphatic Research and Biology 3 (2): 81–86. (2005).
19. V.N. Dholakia and K.N. Trivedi, : J. Indian Chem. Soc., 47, 1058 (1970)
20. Y.A.Shaikh and K.N. Trivedi, : J. Indian Chem. Soc., 48, 1041 (1971)
21. Pardanani Y. A. , Y.A. Shaikh and K.N. Trivedi, : J. Indian Chem. Soc., 52,45 (1979)
22. Martinez R. , E. Cortes, R.A. Toscano, L. J. Alfaro and J. G. Avila,: J. Heterocycl. Chem., 28, 589, (1991)
23. Cervello, M. Gil, P. de March, J. Marquet, M. Moreno-Manas,

J.L. Roca and F. Sanchez-Ferrando, Tetrahedron, 43, 2381 (1987)

24. S. K. Talapatra, R. Chakrabarti, P. K. Mukhopadhayay, P. K. Das and B. Talapatra, Heterocycles,22, 519 (1984)
25. Ivanov, I. Manolov and L. A. Alexandrova,: Arch. Phram. (Weinheim), 330, 521 (1990)
26. M. Darbarwar, V. Sundermurty and N. V. S. Rao, : Proc. Indian Acad. Sci., Sect. A, 80, 93 (1974)
27. A. Patra, A. K.Mukhopadhayay and A. K. Mitra, : Indian J. Chem., Sect. B, 17, 638, (1979)
28. Hismat H. , A. M. Gohar, M. E. Wassef, N. M. A., El-El-rashi and A. A. Nawar, : Justus Liebigs Ann. Chem., 692, 166 (1966)
29. Steinfuhrer T. , A.Hantschmann, M. Pietcsch and M. Weissonfells, : Justus Liebigs. Ann. Chem., 23 (1992)
30. Haywood J. , R.G. Hunt, C. J. Potter and S. T. Reid, : J. Chem. Soc., Perkin Trans. 1., 2458 (1977)
31. H.Suginome, C. F. Liu, S. Seko, K. Kobayashi and A. Furusaki, :J. Org. Chem., 53, 5952 (1988)
32. De, S. K. and Srivastava, A. S.: Zbl. Bakt. II Abst. 135, 408 (1980).
33. Kumari Mukul, Sinha A. K. , T. Alam, G.K. Mishra, S. K. Verma and S. P. Singh J. Chemtracks 13(1) 21-24 (2011).
34. Singh Akhileshwar Prasad, Z. Shahzada, Narendra Kumar Singh, N.P. Singh, Akhouri Prasant Kumar and S. P. Singh J. Chemtracks 14(1) 179-181 (2012).