

## Novel biotechnological method for production of ethanol exposed to colchicine

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**Abstract** : The efficacy of colchicine on production of ethanol by *Saccharomyces cerevisiae* RK-16 has been assessed. It has been found that the mutagen under trial, i.e; colchicine at  $7.0 \times 10^{-5}$  M molar concentration enhances the production of ethanol by *Saccharomyces cerevisiae* RK-16 to an extent of 16.296% higher in comparison to control fermenter flask, i.e, 5.40mL/100 in 65 hours of optimum incubation period, 18.5% (w/v) molasses solution 4.5 pH and 30°C temperature alongwith other nutritional ingredients required by the yeast.

**(Keywords:** Alcoholic fermentation, molasses, mutagen,colchicine, *Saccharomyces cerevisiae* RK-16)

### Introduction

Colchicine is a chemical mutagen that can induce mutation in organisms. It has been used by scientists in research to study the effect of mutation.

Colchicine is a mutagen that prevents formation of microtubules and which is usually used for doubling the chromosome number. Thus, it is routinely utilized in polyploid plant formation. Colchicine effectively functions as a “mitotic poison,” leading to noticeable mutagenic effects. Many reports highlight the mutagenic effects of colchicine on plant performance (Balkanjiyeva<sup>1</sup>, 1980). Colchicine has been used to induce useful mutations in several economic ornamental plant species, such as *Datura*, *Portulaca*, *Petunia*, *Allium*, and *Cucurbita*. The resulting mutants generally produce larger inflorescences, fruits and pollen grains, and shorter stems<sup>2-5</sup>.

Discovered more than 3,000 years ago, colchicine is one of the oldest drugs still in use today. Like most old remedies, colchicine is a chemical substance found in many plants, most notably in *colchicum autumnale*, known as wild saffron or autumn crocus. It was mentioned in the oldest Egyptian medical text, Ebers Papyrus (circa 1550 B.C.), where it was described for the use of pain As early as the 1st century A.D.,

Chemical mutagens are standard tools for mutagenesis in a variety of organisms, and they are a primary means of creating mutations in phenotype-based screens in most genetic systems. Chemical mutagens have also been used successfully in the mutagenesis.

A mutagen is defined as any physical or chemical substance that can change the genetic material of an organism, thereby causing a mutation. Chemical mutagens are compounds that increase the frequency of some types of mutation.<sup>6-12</sup> Mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens.<sup>13-17</sup> Many chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals.<sup>18</sup> Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. While many researchers found chemical mutagens are to be more effective than physical ones<sup>19,20</sup> and many others researchers found the reverse case<sup>21</sup>.

**Table - 1**  
**Novel biotechnological method for production of ethanol exposed to colchicine**

| Concentration of Mutagen used<br>A X 10 <sup>-x</sup> 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 65 hrs. |
|---------------------------------------------------------|----------------------------|--------------------------------|-----------------------------------------------|---------------------------------------------|
| Control                                                 | 45                         | 2.80                           | 3.334                                         | —                                           |
| (–) Mutagen                                             | 65                         | 5.40                           | 1.753                                         | —                                           |
|                                                         | 75                         | 3.70                           | 1.639                                         | —                                           |
| 1.0 x 10 <sup>-5</sup> M                                | 45                         | 2.82                           | 3.302                                         | —                                           |
| (+) Mutagen                                             | 65                         | 5.42                           | 1.750                                         | + 0.370                                     |
|                                                         | 75                         | 3.72                           | 1.636                                         | —                                           |
| 2.0 x 10 <sup>-5</sup> M                                | 45                         | 2.84                           | 3.281                                         | —                                           |
| (+) Mutagen                                             | 65                         | 5.47                           | 1.683                                         | + 1.296                                     |
|                                                         | 75                         | 3.80                           | 1.574                                         | —                                           |
| 3.0 x 10 <sup>-5</sup> M                                | 45                         | 2.86                           | 3.261                                         | —                                           |
| (+) Mutagen                                             | 65                         | 5.55                           | 1.603                                         | + 2.777                                     |
|                                                         | 75                         | 3.82                           | 0.95                                          | —                                           |
| 4.0 x 10 <sup>-5</sup> M                                | 45                         | 2.88                           | 3.241                                         | —                                           |
| (+) Mutagen                                             | 65                         | 5.63                           | 1.523                                         | + 4.259                                     |
|                                                         | 75                         | 3.84                           | 1.414                                         | —                                           |
| 5.0 x 10 <sup>-5</sup> M                                | 45                         | 2.90                           | 3.221                                         | —                                           |
| (+) Mutagen                                             | 65                         | 5.85                           | 1.304                                         | + 8.333                                     |
|                                                         | 75                         | 3.86                           | 1.201                                         | —                                           |

\* Each value represents mean of three observations \*\* Optimum concentration of mutagen used.

\*\*\* Optimum yield of ethanol in 65 hours of optimum incubation period (+) Values indicate % increase in the yield of ethanols after 65 hours. Experimental deviation (±) 1.5–3%.

A number of workers<sup>22-24</sup> have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants<sup>24</sup>. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes<sup>25-34</sup>. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manipulation and exploitation specially for alcoholic fermentation and in view of this the author has studied the influence of colchicine on production of ethanol by *Saccharomyces cerevisiae* RK-16

### Experimental

The efficacy of chemical mutagens, i.e, colchicine on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

The constitution of bioproduction medium for the novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16 is prepared as follows :

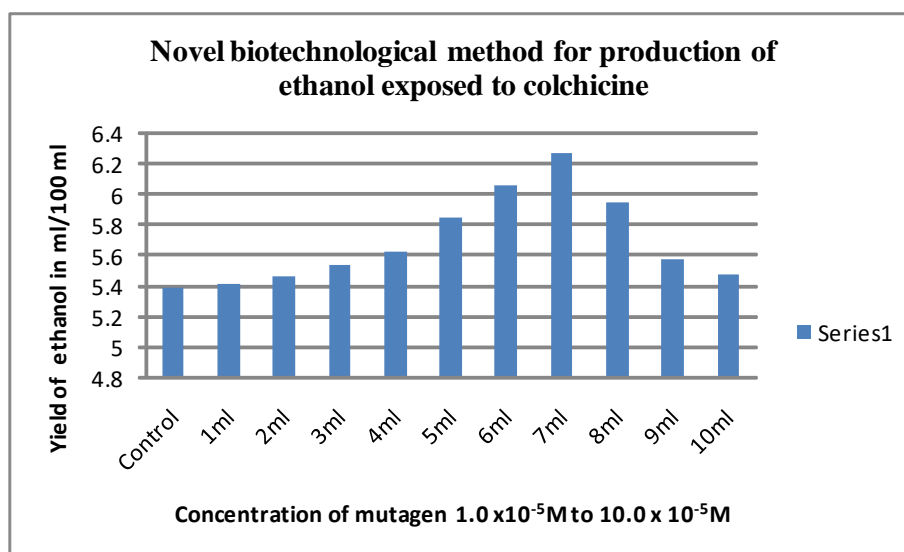
Molasses : 18.5%, Malt extract : 1.80%  
 Yeast extract : 1.80%, Peptone : 1.80%  
 (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> : 1.80%, pH : 4.5

**Table - 1**  
**Novel biotechnological method for production of ethanol exposed to colchicine**

| Concentration of Mutagen used<br>A X 10 <sup>-x</sup> 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 65 hrs. |
|---------------------------------------------------------|----------------------------|--------------------------------|-----------------------------------------------|---------------------------------------------|
| Control                                                 | 45                         | 2.80                           | 3.334                                         | —                                           |
| (-) Mutagen                                             | 65                         | 5.40                           | 1.753                                         | —                                           |
|                                                         | 75                         | 3.70                           | 1.639                                         | —                                           |
| 6.0 x 10 <sup>-5</sup> M                                | 45                         | 3.10                           | 3.021                                         | —                                           |
| (+)Mutagen                                              | 65                         | 6.06                           | 1.093                                         | + 12.222                                    |
|                                                         | 75                         | 4.02                           | 1.045                                         | —                                           |
| 7.0 x 10 <sup>-5</sup> M**                              | 45                         | 3.20                           | 2.91                                          | —                                           |
| (+)Mutagen                                              | 65                         | 6.28***                        | 0.873                                         | + 16.296                                    |
|                                                         | 75                         | 4.15                           | 0.736                                         | —                                           |
| 8.0 x 10 <sup>-5</sup> M                                | 45                         | 3.18                           | 2.941                                         | —                                           |
| (+)Mutagen                                              | 65                         | 5.95                           | 1.203                                         | + 10.185                                    |
|                                                         | 75                         | 3.12                           | 1.108                                         | —                                           |
| 9.0 x 10 <sup>-5</sup> M                                | 45                         | 3.16                           | 2.91                                          | —                                           |
| (+)Mutagen                                              | 65                         | 5.58                           | 1.573                                         | + 3.333                                     |
|                                                         | 75                         | 3.10                           | 1.437                                         | —                                           |
| 10.0 x 10 <sup>-5</sup> M                               | 45                         | 3.11                           | 3.011                                         | —                                           |
| (+)Mutagen                                              | 65                         | 5.48                           | 1.673                                         | + 1.481                                     |
|                                                         | 75                         | 3.00                           | 1.537                                         | —                                           |

\* Each value represents mean of three observations \*\* Optimum concentration of mutagen used.

\*\*\* Optimum yield of ethanol in 65 hours of optimum incubation period (+)Values indicate % increase in the yield of ethanols after 65 hours. Experimental deviation ( $\pm$ ) 1.5–3%.



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

The pH of the medium was adjusted to 4.5 by adding desired quantity of lactic acid.

Now, the same production medium for novel biotechnological method for production of ethanol by a fungal yeast strain of *Saccharomyces cerevisiae* RK-16 was prepared for ninety nine flat bottom fermentor-flasks, i.e., each flat bottom fermentor flask containing 100 ml of production medium. These flat bottom fermentor-flasks, i.e., each containing 100 ml of bioproduction medium. These flat bottom fermentor-flasks were then well arranged in ten sets each comprising nine flat bottom fermentor-flasks. The remaining nine flat bottom fermentor-flasks out of ninety nine flat bottom fermentor-flasks were kept as control and these were also rearranged in three subsets each consisting of three flat bottom fermentor flasks.

Now, M/1000 solutions of chemical mutagen, i.e., colchicine was made 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 mutagenic solution was added to the flat bottom fermentor-flasks of first ten sets respectively. The control flat bottom fermentor-flask contained no chemical mutagens. The total volume in each flat bottom fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of colchicine 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 chemical mutagens in ml, ie; from 1.0 mL to 10.0 mL.

x = molarity of the solution.

The flat bottom fermentor-flasks were then carefully steam sterilized, cooled, inoculated and incubated at temperature 30°C and then carefully analysed colorimetrically after 45, 65, and 75 hours for alcohol formed<sup>35</sup> and molasses<sup>36</sup> solution sugars left unused.

## Results and Discussion

### The influence of chemical mutagen, i.e., colchicine on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

The data recorded in the table-1 shows that chemical mutagen, i.e., colchicine has stimulatory effect on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16.

The maximum bioproduction of  $\text{C}_2\text{H}_5\text{OH}$ , i.e., 6.28 mL/100 ml in the presence of chemical mutagen, i.e., colchicine was observed at  $7.0 \times 10^{-5}$  M molar concentration in 65 hours of optimum incubation period which is 16.296% higher in comparison to control, i.e., flat bottom fermentor flasks, i.e; 5.40 mL/100mL in the same times course and other same experimental parameters.

The higher molar concentrations of chemical mutagen, i.e., colchicine were not much favourable for the novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16. So the gradual addition of the mutagen colchicine after certain concentrations were not beneficial for the novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

It has been observed that molar concentration of the chemical mutagen, i.e., colchicine from  $1.0 \times 10^{-5}$  M to  $7.0 \times 10^{-5}$  M enhances the yield of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) to a certain order being 0.370%, 1.296%, 2.77%, 4.259%, 8.333%, 12.222% and 16.296% higher in comparison to flat bottom control fermentor flasks.

It has been found further that after optimum concentration, i.e;  $7.0 \times 10^{-5}$  M, the addition of the same chemical mutagen to the production medium causes fall in the yield of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) gradually and well reached to

1.481%. However, at all the experimental concentrations of colchicine exploited, the yield of ethanol ( $C_2H_5OH$ ) by alcoholic fermentation

has been found higher in comparison to control flat bottom fermentor flasks.

## References

1. Alghamdi SS, Khan AM, Ammar MH, El-Harty EH, et al. *Int. J. Mol. Sci.* 15: 277 (2014).
2. H.N. Ananthaswamy, U. K. Vakil UK and A. Sreenivasan *Radiat. Bot.* **11**: 1 (1971)
3. SA Ansari, C. Narayanan, S.A. Wali, R. Kumar *Ann. For. Res* **55**: 11 (2012)
4. P. Archana, S.P. Taware and V.M. Raut *Mutat. Res.* **334**: 49 (2004)
5. J. Balkanjieva *Barley Genet. Newslett.* (10/11): 7-10. (2010)
6. M. Milovanovic, et al. Nanoparticles in antiviral therapy. *Antimicrobial Nanoarchitectonics*, pp. 383 (2017)
7. K. Kataoka, et al. *J. Controll. Release.* **24**(1-3), 119 2 (1993)
8. Y. Matsumura, H. Maeda, *Cancer Res.* **46**, 6387 (1986)
9. S. Nagayama, et al. *Int. J. Pharm.* **342**(1-2), 215(2007)
10. K.R. Vega-Villa, et al. *Adv. Drug. Deliv. Rev.* **60**(8), 929(2008)
11. Y.M. Ning, et al.: (Williston Park). 21(12), 1503–1508 (2007)
12. K.I. Ogawara, .: *Int. J. Pharm.* **359**(1-2), 234 (2008)
13. J.T. Davis, "Advances in Catalysis" Vol. VI, Academic press inc. New York, N.Y. PP 1-65 (1951).
14. A.K. Panigrahi, S. Misra, M. Patra and B.K. Sinha : *J. Indian Chem. Soc.* **76**, 395 (1999).
15. A.C. Dash, and R.K. Nanda, : *Inorg. Chem.* **18**, 655 (1974).
16. E.F.J. Duynstee, and E. Grunwald, : *Journal American Chemical Society* **81**, 4540 (1959).
17. E.F.J. Duynstee, and E. Grunwald, : *Journal American Chemical Society* **81**, 4542 (1959)
18. F.R. Faizi Ph.D. Thesis Chemistry Magadh Univ. Bodh-Gaya Unpublished (2002).
19. Lalan Kumar, S.N. Prasad and S.P. Singh : *J. Chemtracks* **2**, 79 (2000)
20. F. R. Faizi, M.A. Khan, V. Kumar, P.K. Chaurasia and S.P. Singh : *J. Chemtracks* **6**, 59 (2004).
21. F. R. Faizi, K. Ahmad, O.P. Srivastava, Vinita and S.P. Singh, *J. Chemtracks* **7**, 117 (2005).
22. U.P. Chaurasia *J. Chemtracks* **15**(2), 589 (2013).
23. K. Ahmad, S.K. Srivastava, B. Kumar, Kumar, P. Srivastava, and S.P. Singh : *J. Chemtracks*, **12** (I) 147 (2010)
24. G. Kumari, R. K. Bharti, K. Ahmad, S.K. Srivastava, A.K. Ojha, and S.P. Singh : *J. Chemtracks*, **11** (2), 401 (2009)
25. R. Kumar, R.K. Sinha, S. Kumar, D. Kumar, K. Ahmad, and S.P. Singh : *J. Chemtracks* **10**, 183 (2008)
26. Ravindra Kumar : Ph.D. Thesis Chemistry Magadh Univ. Bodh-Gaya PP. 119-139(2002).
27. F.R. Faizi, S. N. Prasad, A. K. Sinha, R. Kumar, S. Equbal and S.P. Singh *J. Chemtracks* **8**, 91 (2006).
28. J. P. Kumar and S. P. Singh *J. Chemtracks* **18**(1) 57 (2016)
29. Pragati Kiran, S.R.K. Singh and S.P. Singh *J. Chemtracks* **18**(2), 353 (2016)
30. S.K. Sahay & M.K. Roy *J. Chemtracks* **19**(1), 103 (2017)
31. S. K. Sahay and K. K. Seth *J. Chemtracks* **19**(2), 201 (2017)
32. Gaytri Kumari, Ramesh Prasad and Manjushree Prasad *J. Chemtracks* **20**(1&2) 79 (2018)
33. Rohini Singh, B.B. Sharma *J. Chemtracks* **21**(1&2) 47 (2019)
34. Rohini Singh *J. Chemtracks* **22**(1&2) 181 (2022)
35. J. R. Mirror and M. Boulet *J. Dairy Science*, **41**, 1683 (1983).
36. M. Dubois, K.A. Gilles, J. K. Hamilton and F. Smith, *Anal. Chem.* **28**, 350 (1956)

