

Impact of EtBr on lactic acid fermentation

Manoj Kumar Roy

Department of Chemistry, St. Columba's P.G. College Hazaribagh

E-mail : drmanojkumarroy@gmail.com

Manuscript received 02 December 2019, Revised 12 January 2020

accepted on 04 March 2020

Abstract : The impact of ethidium bromide on lactic acid fermentation by some lactic acid bacteria such as *Lactobacillus gassesi* MKR-23, *Lactobacillus acidophilus* MKR-37, *Streptococcus thermophilus* MKR-42, *Lactobacillus lactis* MKR-48 and *Lactobacillus casei* MKR-56 was assessed. It was found that the strain of *Lactobacillus casei* MKR-56 was much effective and good lactic acid producer. The compound ethidium bromide was found stimulatory when 18.00% (w/v) molasses solution was allowed to ferment at pH 6.2, temperature 37.0°C and optimum incubation period of 6 days. It has been found that there is a gradual increase in the production of lactic acid with stepping up of the compound ethidium bromide till the maximum yield of lactic acid, i.e., 8.125 g/100 ml was obtained at its molar concentration of 6.0×10^{-5} M which is 12.847% higher in comparison to control fermentor flasks in 6 days of optimum incubation period.

(Keywords : Lactic acid fermentation, molasses, ethidium bromide and *Lactobacillus casei* MKR-56)

Introduction

Ethidium bromide (or homidium bromide, chloride salt homidium chloride) is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. It is commonly abbreviated as EtBr, which is also an abbreviation for bromoethane. To avoid confusion, some laboratories have used the abbreviation EthBr for this salt. When exposed to ultraviolet light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA. Under the

name homidium, it has been commonly used since the 1950s in veterinary medicine to treat trypanosomiasis in cattle. The high incidence of antimicrobial resistance makes this treatment impractical in some areas, where the related isometamidium chloride is used instead. Despite its reputation as a mutagen, tests have shown it to have low mutagenicity without metabolic activation¹⁻¹⁰.

In genetics, a mutagen is a physical or chemical agent that permanently changes genetic material, usually DNA, in an organism and thus increase the frequency of mutations above the natural background level. The process of DNA becoming modified is called mutagenesis¹¹⁻²⁰.

Chemical mutagens induce in the various test systems that have been utilized. Mutation can result in several different types of change in deoxyribo nucleic acid sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely²¹⁻²⁶. Mutations can involve large sections of deoxyribo nucleic acid becoming duplicated, usually through genetic recombination.

The impact of mutagens in fermentation technology specially lactic acid fermentation has been studied extensively. Some mutagens were tested by many research workers²⁷⁻⁴³ for different fermentations process and were found that most of them were found useful for higher production of the desired products. It is obvious from above review of literature that various chemical

Table - 1
Impact of EtBr on lactic acid fermentation

Concentration of mutagens used A × 1/10000 × 10M	Yield of lactic acid* in gm/100 ml			%Diff. in the yield of lactic acid in 6 days of optimum incubation pd.
	4 days	6 days	8 days	
Control	3.220	7.200	4.110	—
1×1/10000×10M	3.226	7.203	4.150	+ 0.041%
2×1/10000×10M	3.232	7.254	4.243	+ 0.750%
3×1/10000×10M	3.310	7.306	4.318	+ 1.472%
4×1/10000×10M	3.615	7.507	4.440	+ 4.263%
5×1/10000×10M	3.880	7.808	4.616	+ 8.444%
6×1/10000×10M**	4.115	8.125***	5.213	+ 12.847%
7×1/10000×10M	4.051	8.096	5.014	+ 12.444%
8×1/10000×10M	3.815	7.910	4.986	+ 9.861%
9×1/10000×10M	3.618	7.900	4.866	+ 9.722%
10×1/10000×10M	3.290	7.803	4.673	+ 8.375%

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

*** Optimum yield of lactic acid in 6 days (+) Values indicate % increase in the yield of lactic acid after 6 days. Experimental deviation ($\pm$) 1.5-3%.

mutagens and some other mutagenic agents are used to produce mutants. Thus, it is concluded that a large number of mutagens have been employed to generate the mutants of different microbes and microbial process but still there are some chemical mutagens whose impact on biotic production of lactic acid by *Lactobacillus casei* MKR-56 has not been well studied and established. Moreover, survey of the literature reveals that there has been not enough mention to study the lactic acid fermentation exposed to mutagens specially chemical mutagens. Therefore, in the present investigation the author has made an attempt to study the lactic acid fermentation by *Lactobacillus casei* MKR-56 exposed to ethidium bromide.

Experimental

The impact of ethidium bromide on lactic acid fermentation by *Lactobacillus casei* MKR-56. The composition of the production medium for the lactic acid fermentation by *Lactobacillus casei* MKR-56 was prepared as

follows : Molasses : 18% (w/v), Malt Extract : 2.00% Yeast Extract : 2.00%, Peptone:2.00% (NH₄)₂HPO₄ : 1.80%, CaCO₃ : 10.00%, pH : 6.0 Distilled water To make up 100 ml.

The pH of the medium was adjusted to 6.0 by adding requisite amount of phosphate-buffer solution and the pH was also ascertained by a pH meter. The above composition medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation by *Lactobacillus casei* MKR-56. Now, the same production medium for production of lactic acid by *Lactobacillus casei* MKR-56 was prepared for 99 fermentor flasks, i. e., each fermentor flask containing '100 ml' of production medium.

The above fermentor flasks were then arranged in ten sets, each comprising 9 fermentor flask. Each set was again rearranged in three subsets, each comprising of 3 fermentor flasks. The remaining nine fermentor flasks out of 99

fermentor flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now M/1000 solution/suspension of ethidium bromide 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 1st to 10th sets respectively. The control fermentor flasks contained no mutagen i.e., ethidium bromide. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distilled water. Thus, the concentration of ethidium bromide in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 1/10000 \times 10M$

$1.0 \times 1/10000 \times 10M$ to $10.0 \times 1/10000 \times 10M$

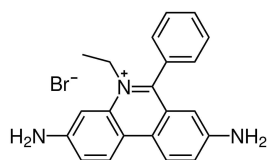
Where A = amount of mutagen, i.e. ethidium bromide in ml,

$1/10000 \times 10M$ = molarity of the solution.

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 4, 6 and 8 days for lactic acid⁴⁴ formed.

Results and Discussion

Impact of EtBr on lactic acid fermentation



The data recorded in the table 1 shows that ethidium bromide has stimulatory effect on lactic acid fermentation by *Lactobacillus casei*

MKR-56. The maximum yield of lactic acid, i.e.; 8.125g/100 ml in the presence of ethidium bromide was observed at $6.0 \times 1/10000 \times 10M$ molar concentration in 6 days of optimum incubation period which is 12.847% higher in comparison to flat bottom control fermentor flasks, i.e.; 7.200 g/100 ml in the same times course and other same experimental parameters. The higher molar concentrations of ethidium bromide were not much favourable for the lactic acid fermentation by *Lactobacillus casei* MKR-56. So the gradual addition of the mutagen ethidium bromide after certain concentrations were not beneficial for the lactic acid fermentation bioprocess.

It has been observed that molar concentration of the mutagen, i.e., ethidium bromide from $1.0 \times 1/10000 \times 10M$ to $6.0 \times 1/10000 \times 10M$ enhances the yield of lactic acid to a certain order being 0.041%, 0.750%, 1.472%, 4.263%, 8.444% and 12.847% higher in comparison to control flat bottom flasks but at $7.0 \times 1/10000 \times 10M$ and $8.0 \times 1/10000 \times 10M$ the yield of lactic acid shifted to be 12.444% and 9.861% higher in comparison to previous concentrations of ethidium bromide taken into experimental trials.

It has been observed further that after optimum concentration, i.e., $6.0 \times 1/10000 \times 10M$, the addition of the same mutagen to the production medium causes fall in the yield of lactic acid gradually and reaches to 8.375%; However, at all the experimental concentrations of ethidium bromide used the yield of lactic acid by submerged fermentation has been found higher in comparison to flat bottom control fermentor flasks.

References

1. P. Stevenson, K.R. Sones, M.M. Gicheru, Mwangi EK *Acta Tropica*. **59** (2): 77 (1995)
2. P. Borst *IUBMB Life*. **57** (11): 745 (2005)
3. J. Sigmon, LL. Larcom. *Electrophoresis*. **17** (10): 1524 (1996)
4. F. Diaz, MP Bayona-Bafaluy, M. Rana, M. Mora H. Hao, CT Moraes *Nucleic Acids Research*. **30** (21): 4626 (2002).
5. A. Roy Chowdhury, R. Bakshi, J. Wang, *Pathogens*. **6** (12): (2010)

6. R. Caesar, J. Warringer, A. Blomberg. *Eukaryotic Cell*. **5** (2): 368 (2006)
7. H. Liang, B. Peng, C. Dong, L. Liu, *Nature Communications*. **9**(1): 4291. (2018)
8. J. Olmsted, DR Kearns *Biochemistry*. **16** (16): 3647 (1977)
9. VL Singer, TE Lawlor, S. Yue *Mutation Research*. **439** (1): 37 (1999)
10. T. Ohta, S. Tokishita, H. Yamagata *Mutation Research*. **492** (1–2): 91 (2001)
11. J. Bertram: *Mol. Aspects Med.* **21** (6): 167, (2000)
12. J. Holland, K Spindler, F. Horodyski, E. Grabau, S. Nichol, S. VandePol : *Science* **215** (4540): 1577 (1982).
13. B. N. Ames, J. McCann, and E. Yamasaki “*Mutation Research* **31**, 347 (1975).
14. T. Sugimura et al., in *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, and J. A. Winsten, Eds., (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1561 (1977)
15. J. W. Drake, J. J. Holland : *Proc. Natl. Acad. Sci. U.S.A.* **96** (24): 13910 (1999).
16. J. S. Felton et al., *Food Chemical Toxicology* **32** (10), 897 (1994).
17. P. Sniegowski, P. Gerrish, T. Johnson, A. Shaver: *Bioessays* **22** (12): 1057 (2000).
18. SA Sawyer, J. Parsch, Z. Zhang, DL. Hartl : *Proc. Natl. Acad. Sci. U.S.A.* **104** (16): 6504 (2007).
19. B. Commoner et al., *Science* **210**, 913 (1978).
20. G. A. Gross, *Carcinogenesis* **11**, 1597 (1990).
21. Y.P. Lai, J. Huang, L. Li and Z.R. Wu, *Biotechnology and Bioengineering*, **86**(6), 622 (2004).
22. P.J. Hastings, J. R. Lupski, S.M. Rosenberg, ; G. Ira, *Nature Reviews. Genetics* **10** (8): 551 (2009)
23. SB. Carroll, J. Grenier, SD Weatherbee : *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design*. Second Edition. Oxford: Blackwell Publishing. (2005).
24. P. Harrison, M. Gerstein : *J Mol Biol* **318** (5): 1155 (2002).
25. CA Orengo, JM Thornton : *Annu. Rev. Biochem.* **74**: 867 (2005).
26. M. Long, E. Betrán, K. Thornton, W. Wang : *Nat. Rev. Genet.* **4** (11): 865 (2003).
27. Bireshwar Mitra, M. Ziauddin Shahzada, Md. Shahabuddin, Afsar Nasim, Akhouri Prashant Kumar and Manjushree Prasad *J. Chemtracks*, **17** (1), 47 (2015)
28. H. K. Satapathy, V. Mandal, J. P. Kumar, Sanjit K. Anand, Jyotimala and S.P. Singh *J. Chemtracks*, **17** (1), 141 (2015)
29. S. Kumar, Ejaz Jawaid, Nagmani Kumar, Md. Shahabuddin, Afsar Nasim and S. P. Singh *J. Chemtracks*, **17** (1), 181 (2015)
30. R. Mishra, L. Kumari, M. Prasad, Mona Kumari, P. K. Sinha and S. P. Singh, *J. Chemtracks*, **17** (2), 247 (2015)
31. P. Singh, J. P. Kumar, Sheela Kumari, D. K. Singh, Vijay Kumar, U. S. Singh, and S. P. Singh *J. Chemtracks*, **17** (2), 333 (2015)
32. R. Kumar, J. Prakash Kumar, S. Kumari, Vijay Kumar, U.S. Singh and S. P. Singh *J. Chemtracks*, **17** (2), 369 (2015)
33. M. Kumari, A. Singh, L. Kumari, P.K. Sinha, S. S. Kumari and S. P. Singh *J. Chemtracks* **18**(1), 85(2016).
34. R.S. Sharma, B.K. Choudhary, Neeta Sinha, and B.B. Sharma *J. Chemtracks* **18**(2), 319 (2016).
35. R. Kumari, S. Singh and S. P. Singh *J. Chemtracks* **18**(2), 329 (2016).
36. P. Singh, F. Qmar, B.K. Choudhary, B.B. Sharma and S.P. Singh *J. Chemtracks* **18**(1), (2016).
37. K. Sangita, Lalan Kumar and S.P. Singh *J. Chemtracks* **19**(1), 71 (2017)
38. Shivankari, Madhurendra, Bijay Kumar and S. P. Singh *J. Chemtracks* **19**(2), 237 (2017)
39. Shivankari, A. Suraiya and S. P. Singh *J. Chemtracks* **21**(1&2), 5(2019)
40. Shivankari, S. Singh and S. P. Singh *Biotech Today* **10**(1), 44 (2020)
41. Shivankari, *J. Chemtracks* **22**(1&2), 279 (2020)
42. Sheela Kumari and Vinod Mandal, *J. Chemtracks* **23**(1&2), 177 (2019)
43. A. K. Singh, A. K. Gautam and Vidya Singh *J. Chemtracks* **24**(1&2) 39 (2022)
44. S. B. Barker, and W. H. Summerson, *J. Biol. Chem.* **138**, 534 (1941)