

Lactic acid production exposed to some phytochemicals

Pramod Kumar Singh

Department of Chemistry, P.G. Centre Chemistry
College of Commerce, Arts and Science, Patna
E-mail : pksingh19666@gmail.com

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Abstract: The efficacy of 5-nitroindazole on production of lactic acid by *Lactobacillus bulgaricus* PK-2262 has been assessed. It has been observed that 5-nitroindazole is very active at the concentration 8.0×10^{-5} M and enhanced the production of lactic acid to an extent of 6.621% higher in comparison to control. However, higher concentration of 5-nitroindazole has been found insignificant and retards the yield of lactic acid.

(Keywords : Molasses, lactic acid fermentation, 5-nitroindazole and *Lactobacillus bulgaricus* PK-2262)

Introduction

A mutagen is any substance that changes the genetic material of an organism and causes mutation. DNA changes caused by mutagens may harm cells and cause certain diseases, such as cancer. Example of mutagens include radio active substance, X-ray, ultraviolet radiations and certain chemicals, Biological mutagens often consists of viruses and different bacterial species capable of initiating changes in an individual DNA.

Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination.¹⁻⁸ These duplications are a major source of raw material for evolving new genes, with tens to hundreds of genes duplicated in animal genomes every million years.⁹ Most genes belong to larger families of genes of shared ancestry.¹⁰ Novel genes are produced by several methods, commonly through the duplication and mutation of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions.¹¹⁻¹⁴ Another advantage of duplicating a gene (or even an entire genome) is

that this increases redundancy; this allows one gene in the pair to acquire a new function while the other copy performs the original function.^{15,16} Other types of mutation occasionally create new genes from previously noncoding DNA.¹⁷⁻²⁰ The discovery of mutagenic role of chemical initiated a flurry of activities in the field of mutagenesis. Scientists working on different facets of mutagenesis, since then, have been able to accomplish a significant breakthrough in basic understanding of the mechanism of mutagenesis and also its applied value for the benefit of fermentation technology.

The influence of mutagens in fermentation technology specially lactic acid fermentation has been studied extensively²¹⁻³⁷. 5-nitroindazole is one of the few chemical mutagen which has found effective chemical mutagen in fermentations. A large number of chemical mutagens³⁸⁻⁵¹ have been employed to generate the mutants of different microbes but still there are some chemical mutagen such as 5-nitroindazole having mutagenic properties whose influence on lactic acid fermentation by *Lactobacillus bulgaricus* PK-2262 species have not been studied. This communication thus presents a brief account of the action of particular chemical mutagen such as 5-nitroindazole on lactic acid fermentation as carried out for over years in the authors laboratory.

Experimental

The influence of 5-nitroindazole on LSF production of lactic acid by *Lactobacillus bulgaricus* PK-2262 The composition of the production medium on lactic acid fermentation

by *Lactobacillus bulgaricus* PK-2262 has been prepared as follows :

Molasses	:	15% (w/v)
Malt Extract	:	0.65%
Yeast Extract	:	0.65%
Peptone	:	0.65%
(NH ₄) ₂ HPO ₄	:	0.70%
CaCO ₃	:	7.5 %
pH	:	6.2
Distilled water	:	To make up 100 ml.

The pH of the medium was adjusted to 6.2 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 production of lactic acid by *Lactobacillus bulgaricus* PK-2262

Now, the same production medium for production of lactic acid by *Lactobacillus bulgaricus* PK-2262 was prepared for 99-fermentor flask, i. e.; each contained '100ml' of production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting 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.

After preparing the above sets of fermentor flasks M/1000 solution of 5-nitroindazole was prepared and from the above mutagen solution; 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no 5-nitroindazole.

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar

concentration of 5-nitroindazole in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

	$A \times 10^{-x} \text{ M}$	
i.e.,	$1.0 \times 10^{-5} \text{ M}$	Where :
	$2.0 \times 10^{-5} \text{ M}$	A=amount of mutagen
	$3.0 \times 10^{-5} \text{ M}$	in ml, i.e., 1.0 ml .. to 10 ml.
	$4.0 \times 10^{-5} \text{ M}$	x= Molarity of the mutagen
	$5.0 \times 10^{-5} \text{ M}$	solution
	$6.0 \times 10^{-5} \text{ M}$	respectively.
	$7.0 \times 10^{-5} \text{ M}$	
	$8.0 \times 10^{-5} \text{ M}$	
	$9.0 \times 10^{-5} \text{ M}$	and
	$10.0 \times 10^{-5} \text{ M}$	respectively

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 43°C and analysed colorimetrically⁵² after 130, 140 and 150 hrs for lactic acid formed during the course of present investigation.

Results and Discussion

The influence of 5-nitroindazole on production of lactic acid by *Lactobacillus bulgaricus* PK-2262.

The data given in the table 1 shows that the mutagen 5-nitroindazole has been found stimulatory for production of lactic acid by *Lactobacillus bulgaricus* PK-2262. From the data given in the table it is obvious that 5-nitroindazole influences the lactic acid fermentation process in different phases. The main characteristics of the 5-nitroindazole is as follows:

- 5-nitroindazole is stimulatory at its all molar concentrations used during course of the lactic acid fermentation, i.e., from $1.0 \times 10^{-5} \text{ M}$ to $10.0 \times 10^{-5} \text{ M}$.
- The molar concentration $1.0 \times 10^{-5} \text{ M}$, $2.0 \times 10^{-5} \text{ M}$ and $3.0 \times 10^{-5} \text{ M}$ of 5-nitroindazole influence the yield of lactic acid in a approximately regular doubling order after each state, i.e., 0.224%, 0.298% and 1.345%.

- (iii) The molar concentration $4.0 \times 10^{-5}\text{M}$, $5.0 \times 10^{-5}\text{M}$ and 6.0×10^{-5} of 5-nitroindazole now influence the productivity of lactic acid in a regular manner enhancing the yield of lactic acid. The % increase in the yield of lactic acid at respective molar concentration of 5-nitroindazole has been found to be as follows : 2.585%, 3.363% and 4.245% .
- (iv) The higher molar concentrations, i.e., $7.0 \times 10^{-5}\text{M}$, 8.0×10^{-5} , 9.0×10^{-5} and $10 \times 10^{-5}\text{M}$ of 5-nitroindazole influences the yield of lactic acid without any distinction and therefore, the % difference in the yield of lactic acid has been found to be almost same as mentioned below : 5.575%, 6.621%, 4.977% and 3.692% respectively.

Table- 1
Production of lactic acid exposed to some phytochemicals

Concentration of mutagen used $a \times 10^{-5}\text{M}$	Yield of lactic acid* in g/100 ml			% of lactic acid increase/(+) in 140 hrs of optimum incubation period.
	130 hrs	140 hrs	150hrs	
Control	5.430	6.690	6.625	–
$1.0 \times 10^{-5}\text{M}$	5.440	6.705	6.638	(+) 0.224
$2.0 \times 10^{-5}\text{M}$	5.445	6.710	6.644	(+) 0.298
$3.0 \times 10^{-5}\text{M}$	5.480	6.780	6.691	(+) 1.345
$4.0 \times 10^{-5}\text{M}$	5.538	6.863	6.758	(+) 2.585
$5.0 \times 10^{-5}\text{M}$	5.593	6.915	6.823	(+) 3.363
$6.0 \times 10^{-5}\text{M}$	5.648	6.974	6.889	(+) 4.245
$7.0 \times 10^{-5}\text{M}$	5.701	7.063	6.956	(+) 5.575
$8.0 \times 10^{-5}\text{M}$	5.755	7.133***	7.022	(+) 6.621
$9.0 \times 10^{-5}\text{M}$	5.646	7.023	6.900	(+) 4.977
$10.0 \times 10^{-5}\text{M}$	5.592	6.937	6.826	(+) 3.692

* Mean of three observatins; ** Optimum concentration of mutagen *** Optimum Yield of lactic acid; (+) ve values indicate % increase in the yield of lactic acid. Experimental deviation ($\pm$) 1.5 to 3.5%

References

1. J. Holland, K. Spindler, F. Horodyski, E. Grabau Nichol, S. Vande Pol *Science* **215** (4540): 1577 (1982)
2. J. W. Drake, J. J. Holland :*Proc. Natl. Acad. Sci. U.S.A.* **96** (24): 13910 (1999)
3. P. Sniegowski, P. Gerrish, T. Johnson, A. Shaver *Bioessays* **22** (12): 1057 (2000).
4. J. Bertram *Mol. Aspects Med.* **21** (6): 167 (2000)
5. V. Burrus, M. Waldor *Res. Microbiol.* **155** (5): 376 (2004)
6. Y. T. Aminetzach, J. M. Macpherson, D. A. Petrov *Science* **309** (5735):764 (2005).
7. S. A Sawyer, J. Parsch, Z. Zhang, DL Hartl *Proc. Natl. Acad. Sci. U.S.A.* **104**, (16): 6504 (2007)
8. P. J. Hastings, J. R. Lupski, M. Rosenberg, ; G. Ira *Nature Reviews. Genetics* **10** (8): S, 551 (2009)
9. S. B. Carroll, J. Grenier, S. D Weatherbee *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. Second Edition.* Oxford: Blackwell Publishing. (2005).
10. P. Harrison, M. Gerstein, *J Mol Biol* **318** (5): 1155 (2002).

11. C. A. Orengo, JM Thornton *Annu. Rev. Biochem.* **74**: 867(2005)
12. M. Long, E. Betrán, K. Thornton, W. Wang *Nat. Rev. Genet.* **4** (11): 865 (November 2003).
13. M. Wang, G. Caetano-Anollés : *Structure* **17** (1): 66 (2009)
14. JK Bowmaker *Eye (London, England)* **12** (Pt 3b): 541 (1998). (2009)
15. TR Gregory, PD Hebert : *Genome Res.* **9** (4): 317 (1999).
16. M. Hurles *PLoS Biol.* **2** (7): E206 (July 2004)
17. N. Liu, K. Okamura, D. M. Tyler *Cell Res.* **18** (10): 985(2008)
18. A. Siepel *Genome Res.* **19** (10): 1693–5(October 2009)
19. J. Zhang, X. Wang, O. Podlaha *Genome Res.* **14** (5): 845 (2004)
20. F. J. Ayala, M. Coluzzi : *Proc. Natl. Acad. Sci. U.S.A.* **102** (Suppl 1): 6535–42 (2005)
21. R.W. Thoma *Folia Microbiol.* **16**.197 (1971)
22. V.I. Kalinina, Bogatyreva I.G. and Lysenko A.M. *Prikl. Biokhim. Microbiol.* **8**,29 (1977)
23. K.P. Tiwari and S. P. Singh *Zbl. Bakt., II Abt.*, **135**,328 (1979).
24. S.K. Mahna *Indian J. Expt. Biol.*, **22**,338 (1984)
25. H. Miller and W. D. McElcoy *Science*, **107**, 193 (1948).
26. S.P. Singh and S.K. Roy *Acta Botanica Indica*, **12**,185(1985)
27. S. P. Singh, B. Pratap, B. K. Ambasta, R. K. Pandey, A. C. Sinha and A. Prasad, *Asian. J. Chem.* **6**, 753 (1994).
28. S. P. Singh, C. K. Singh, D. Narayan, M. A. Khan and R. Kumar *J. Chemtracks*, **5**, 51 (2003).
29. S. P. Singh, F. R. Faizi, M. A. Khan, Md. Irfan A. P. Kumar and P. K. Chaurasia *J. Chemtracks* **5**, 37, (2003)
30. Shalini Singh, *J. Chemtracks* **7**, 125, (2005)
31. E. Freese and E.B. Freese : *Radiation Res. Suppl.* **6**,97,(1966)
32. E. Freese In " Molecular Genetics" J. M. Taylor Ed. pp.207 New York (1963)
33. Savita Kumari, F. R. Faizi, G. K. Mishra, K. Ahmad, and S.P. Singh *J. Chemtracks* **8**, 103 (2006)
34. Savita Kumari, Dharmendra Kumar ; G. K. Mishra Khursheed Ahmad and S. P. Singh *J. Chemtracks* **9**, 177 (2007)
35. Anurag Singh, and Shalini Singh, *J. Chemtracks* **10**, 49 (2007)
36. Shalini Singh, and Anurag Singh, *J. Chemtracks* **11**, 165 (2009)
37. S. P. Singh, S. Kumar, V. Kumar and B. P. Pandey *Vijanan Parishad Anushandahn Patrika* **41**, 205 (1998).
38. D. R. McCalla and D. Voutsions *Mutation Research* **26**, 3 (1974).
39. Y. Tazima, T. Kada and A. Murakami, *Mutation Research* **32**, 55 (1975).
40. D. R. Goodman, P. J. Hakkinen, J. H. Nemenzo and Mary Vove *Mutation Research* **48**, 295 (1977).
41. G. K. Mishra, Snigdha, A. Suraiya, Km Sinha, Ranjan Kumar and S. P. Singh *J. Chemtracks* **12**(1) 17 (2010)
42. R. Kumar, D. Kumar, K. Ahmad, L. K. Jha, B. P. Sinha, K. Singh and S. P. Singh *J. Chemtracks* **12**(2) 417 (2010)
43. R. Kumar, N. Rathor, B. B. Sharma, Km S. Sinha, B. Kumar and S.P. Singh *J. Chemtracks* **12**(2) 495 (2010)
44. S. Kumar, R. K. Bharti, S. Ranjan, S. Akhtar, S. D. Yadav, S. P. Singh *J. Chemtracks* **12**(2) 331 (2010)
45. Shalini Singh and Anurag Singh *J. Chemtracks* **13**(1) 185 (2011)
46. M. Prasad, S. K. Srivastava, R. Prasad, M. Imam, R. Kumar and S. P. Singh *J. Chemtracks* **13**(1) 231 (2011)
47. S. K. Yadav *International Journal of Current Science* **12**(2) 1-4, (2022)
48. J. Zhao, B. Zhang, Y. Meng, Y. Han, Y. Wang *J. Biotechnol* **10**(5) 1-11 (2020)
49. V. Yadav, S. Kundu, R. Sen. : *Proc. Biochem.* **111**, 4-18 (2021)
50. Q. Wang, M. Ma, Y. Zhang, L. LIV, X. Liv. C. Jiang *Appl. Microbiol Biotechnol* **102**(7) 3269-3281 (2018)
51. M.B. Osha, O.E. Sobande *J. Biotechnol* **36**(1), 186-193 (2019)
52. S.B. Barker and W.H. Summerson *J. Biol. Chem.* **138**,535 (1941)