



## The efficacy of 6-bromo-3-pyridyl hydrazine on the bio-production of Lactic acid

**Dr. Archana Kumari**

SRPS Govt. School, Gardanibagh, Patna

Magadh University, Bodh Gaya

Email: archanakumari226@rediffmail.com

### **ABSTRACT**

The efficacy of chemical mutagen, i.e., 6-bromo-3-pyridyl hydrazine on the lactic acid fermentation by the lactic acid bacteria such as *Lactococcus lactis* NCIM-1158, *Lactobacillus brevis* NCIM-1263, *Lactobacillus pentosus* NCIM-1295, *Lactobacillus fructivorans* NCIM-1687 has been assessed. It has been found that the lactic acid bacteria under trial, i.e., *Lactobacillus brevis* NCIM-1263 has been found best lactic acid producer. Therefore, these bacteria has been used throughout the present investigation. The compound, i.e., 6-bromo-3-pyridyl hydrazine as its molar concentration, i.e.,  $6 \times 10^{-5} M$  has been found stimulatory for the bio production of lactic acid and has been found higher in comparison to control to an extent of 8.204% (7.834g/100ml) under optimized conditions of 20% (W/V) molasses, 35°C temperature, 6.0pH and incubation period of 6 days along with other nutritional ingredients required by the Lactic acid bacteria under investigation.

**Keywords:** Lactic acid fermentation, mutagen, 6-bromo-3-pyridyl hydrazine and *Lactobacillus brevis* NCIM-1263

### **INTRODUCTION**

In genetics, a mutation is a permanent change in the nucleotide sequence of an organism. It results from damage to DNA or RNA which cannot be repaired. This damage is caused by radiation or chemical mutagens. Mutations may or may not cause observable changes in the organism. It plays an important role in many biological processes like evolution or development of the immune system<sup>1-10</sup>. When a damage occurs in DNA and the message carried by that gene altered, mutation occurs. A mutagen is that substance which brings permanent change in the gene so that the message carried by that gene is also altered<sup>11-25</sup>.

### **Experimental: Compositions of the production medium:**

The composition of the production medium for facile biosynthesis of  $\alpha$ -hydroxy propionic acid by *Lactobacillus brevis* NCIM-1263 exposed to 6-bromo-3-pyridyl hydrazine is as follows:

Molasses: 20% (W/V); Malt extract : 0.85%; yeast extract: 0.85%,

Peptone: 0.85% (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>: 1.50%; CaCO<sub>3</sub>: 8.5%, pH: 6.0, (Adjusted by adding requisite amount of phosphate-buffer solution). Distilled water: To make up 100ml.

**Assay method:** Evaluation of lactic acid formed and molasses left unfermented was made colorimetrically<sup>26,27</sup>.



**Sterilization:** In an autoclave, the production medium was sterilized. 15 lbs steam pressure was maintained for 30 minutes.

**Strain:** Lactobacillus brevis NCIM-1263 has been employed in the present study. The strain was produced from NCL-Pune, India.

Age of inoculum: 48 hours old.

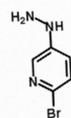
**Quantum of the inoculum:** 0.5 ml bacterial suspension of Lactobacillus brevis NCIM-1263

**Incubation Period:** 4, 6 and 8 days

**Concentration of 6-bromo-3-pyridyl hydrazine used:** M/1000 solution of 6-bromo-3-pyridyl hydrazine under trial has been prepared and 1.0

$\times 10^{-5}$  M to  $10 \times 10^{-5}$  M molar concentration of 6-bromo-3-pyridyl hydrazine has been employed.

**Results and Discussion:** The influence of 6-bromo-3-pyridyl hydrazine



The results obtained in the study of the influence of 6-bromo-3-pyridyl hydrazine is recorded in the table 1

Table 1: Biosynthesis of Lactic Acid by Lactobacillus brevis NCIM-1263 exposed to 6-bromo -3-pyridyl hydrazine

Concentration of mutagen used $a \times 10^{-x}$ M	Incubation period in days	Yield of $\alpha$ -hydroxy propionic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of $\alpha$ -hydroxy propionic acid increased in 6 days of incubation period.
Control (-mutagen)	4	5.290	5.079	-
	6	7.240	3.130	-
	8	6.217	3.018	-
$1.0 \times 10^{-5}$ M (+mutagen)	4	5.316	5.066	-
	6	7.277	3.096	(+)0.511
	8	6.246	3.013	-
$2.0 \times 10^{-5}$ M (+mutagen)	4	5.385	4.984	-
	6	7.372	2.998	(+)1.823
	8	6.326	2.891	-
$3.0 \times 10^{-5}$ M (+mutagen)	4	5.491	5.878	-
	6	7.516	2.855	(+)3.812
	8	6.451	2.811	-
$4.0 \times 10^{-5}$ M (+mutagen)	4	5.591	4.778	-
	6	7.653	2.718	(+)5.704
	8	6.569	2.611	-
$5.0 \times 10^{-5}$ M (+mutagen)	4	5.612	4.759	-
	6	7.682	2.688	(+)6.104
	8	6.594	2.593	-
$6.0 \times 10^{-5}$ M** (+mutagen)	4	5.723	4.647	-
	6	7.834***	2.539	(+)8.204
	8	6.724	2.468	-
$7.0 \times 10^{-5}$ M	4	5.506	4.863	-

(+mutagen)	6	7.537	2.833	(+)4.102
	8	6.469	2.786	-
8.0 x 10 <sup>-5</sup> M (+mutagen)	4	5.385	4.984	-
	6	7.372	2.996	(+)1.823
	8	6.326	2.886	-
9.0 x 10 <sup>-5</sup> M (+mutagen)	4	****	-	-
	6	****	-	-
	8	****	-	-
10.0 x 10 <sup>-5</sup> M (+mutagen)	4	****	-	-
	6	****	-	-
	8	****	-	-

\*Each value represent mean of three trials.

\*\* Optimum concentration of mutagen.

\*\*\*Optimum yield of  $\alpha$ -hydroxy propionic acid.

\*\*\*\*Insignificant Values

(+)values indicates % increased in the yield of  $\alpha$ -hydroxy propionic acid.

Experimental deviation  $\pm$  2.5 – 3.5%

### **Discussion:**

The data recorded in the table 1 shows that 6-bromo-3-pyridyl hydrazine also has stimulatory effect on facile biosynthesis of  $\alpha$ -hydroxy propionic acid by some pure Lactobacillus species by using the bacterial strain of Lactobacillus brevis NCIM-1263.

The data (in table 1) reveals that the chemical mutagen 6-bromo-3-pyridyl hydrazine stimulates the  $\alpha$ -hydroxy propionic acid fermentation process and enhances the yield of  $\alpha$ -hydroxy propionic acid up to its 6 bromo 3 pyridyl hydrazine concentrations from  $1.0 \times 10^{-5}$  to  $6.0 \times 10^{-5}$  M. The effect of 6-bromo-3-pyridyl hydrazine on the productivity(yield) of  $\alpha$ -hydroxy propionic acid was gradually in increasing order and attains its best role at  $6.0 \times 10^{-5}$  M where maximum yield of  $\alpha$ -hydroxy propionic acid, i.e., 7.834 g/100 ml is fetched in 6 days of optimum incubation period which is 8.204% higher in comparison to control fermentor flask, i.e., 7.240 g/100 ml. In the second phase of mutagenic chemical's effect the molar concentration, i.e., from  $7.0 \times 10^{-5}$  to  $10 \times 10^{-5}$  the production of  $\alpha$ -hydroxy propionic acid has been enhanced but the order of  $\alpha$ -hydroxy propionic acid productivity is reverse

in respect to increasing molar concentration of 6-bromo-3-pyridyl hydrazine. However, the facile biosynthesis of  $\alpha$ -hydroxy propionic acid by some pure Lactobacillus species under the influence of each concentration of 6-bromo-3-pyridyl hydrazine used has been stimulating and the yield of  $\alpha$ -hydroxy propionic acid has been found greater than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of  $\alpha$ -hydroxy propionic acid formed is as below:

#### **Phase-I**

Concentration of 6-bromo-3-pyridyl hydrazine from  $1.0 \times 10^{-5}$  M to  $6.0 \times 10^{-5}$  M.

#### **Productivity of $\alpha$ -hydroxy propionic acid:**

0.511%, 1.823%, 3.812%, 5.704%, 6.104% and 8.204%

#### **Phase-II A**

Concentration of 6-bromo-3-pyridyl hydrazine from  $7.0 \times 10^{-5}$  M to  $8.0 \times 10^{-5}$  M.

#### **Productivity of $\alpha$ -hydroxy propionic acid:**

4.102% and 1.823%

#### **Phase-III A**



Concentration of 6-bromo-3-pyridyl hydrazine from  $9.0 \times 10^{-5}$  M to  $10.0 \times 10^{-5}$  M.

### **Productivity of $\alpha$ -hydroxy propionic acid:**

#### **Insignificant**

Exposure of bacterial strain to 6-bromo-3-pyridyl hydrazine may produce a variety of effects. Depending upon the concentration 6-bromo-3-pyridyl hydrazine to which bacterial strain *Lactobacillus brevis* NCIM- 1263 were exposed may influence disruption of cells, precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells. Although the special mode of action is not very clear, there is a consensus that the lethal effect is associated with physical damage of the membrane structure of the cell surface, which initiates further deterioration.

#### **Conclusion:**

From the experimental observations, it is revealed that the chemical mutagen 6-bromo-3-pyridyl hydrazine first increases the yield of lactic acid when the concentration of mutagen varies from  $1.0 \times 10^{-5}$  M to  $6.0 \times 10^{-5}$  M. After that the yield of lactic acid decreases when concentration of the mutagen varies from  $7.0 \times 10^{-5}$  M to  $10 \times 10^{-5}$  M. The maximum yield of lactic acid that is 7.834g/100ml is at concentration  $6.0 \times 10^{-5}$  M.

#### **References**

[1] TR Gregory, PD Hebert: *Genome Res.* 9 (4): 317 (1999).

[2] M Hurles: *PLoS Biol.* 2 (7):206 (2004)

[3] N Liu, K, Okamura and DM. Tyler *Cell Res* 18 (10): 985(2008)

[4] A. Siebel: *Genome Res.* 19 (10): 1693 (2009)

[5] J. Zhang, X Wang, O Podlaha: *Genome Res.* 14 (5): 845 (2004)

[6] FJ Ayala, M Coluzzi: *Proc. Natl. Acad. Sci. U.S.A.* 102 (Suppl 1): 6535 (2005)

[7] GD Hurst, JH Werren *Nat. Rev Genet* 2 (8): 597 (2001)

[8] JHäsler, K. Strub *Nucleic Acids Res.* 34 (19): 5491 (2006).

[9] Eyre-Walker, A.; Keightley, : *Nature reviews.Genetics* 8 (8): 610 (2007).

[10] Freese, Ernst: *Proc. Natl. Acad. Sci. U.S.A.* 45 (4): 622 (April 1959)

[11] Freese, Ernst: *J. Mol. Biol.* 1: 87 (1959).

[12] Elis NA, Ciocci S, German J *Hum Genet* 108 (2): 167 (2001)

[13] Pfohl-Leskowicz A, Mandeville RA: *Mol Nutr Food Res* 51 (1): 61 (2007).

[14] S. Kozmin, G Slezak, A. Reynaud-Angelin, C. Elie, Y. De Rycke, S. Boiteux, E. Sage: *Proc. Natl. Acad. Sci. U.S.A.* 102 (38): 13538 (2005).

[15] L. Pilon, Y. Langelier, A. Royal: *Mol. Cell. Biol.* 6 (8): 2977 (1986)

[16] SA. Sawyer, J. Parsch, Z. Zhang, DL. Hartl *Proc. Natl. Acad. Sci. U.S.A.* 104 (16): 6504 (2007)

[17] S. W. Doniger, H. S. Kim, and D. Swain: *PLoS Genet.* 4 (8): e1000183. (2008).

[18] J. Bertram: *Mol. Aspects Med.* 21 (6): 167 (2000).

[19] YT. Aminetzach, JM. Macpherson and DA Petrov: *Science* 309 (5735): 764 (2000)

[20] V. Burrus and M. Waldor: *Res. Microbiol.* 155(5): 376 (2004)

[21] S. A. Sawyer, J. Parsch, Z. Zhang, DL. Hartl *Proc. Natl. Acad. Sci. U.S.A.* 104 (16): 6504(2007)

[22] P. Sniegowski, P. Gerrish, T. Johnson, A. Shaver: *Bioessays* 22 (12): 1057 (2000).

