

## Biotransformation of molasses to $\alpha$ -hydroxy propionic acid by *S. lactis* 2114 exposed to 4-hydrazinobenzoic acid

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**Abstract:** The influence of mutagen, i.e., 4-hydrazinobenzoic acid on biotransformation of molasses to  $\alpha$ -hydroxy propionic acid by *S. lactis*-2114 has been studied. It has been found that 4-hydrazinobenzoic acid was stimulatory upto its concentration from  $1.0 \times 10^{-5}$  M to  $2 \times 10^{-5}$  M and onwards concentration of the mutagen under trial decreases the yield of  $\alpha$ -hydroxy propionic acid, i.e., lactic acid.

**(Keywords:** Fermentation,  $\alpha$ -hydroxy propionic acid, 4-hydrazinobenzoic acid and *S. lactis* 2114).

### Introduction

“Mutagens are the known agents either physical, chemical or biological causes mutations by altering the genotype or gene expression which results in genetic abnormality.” Anything that causes a mutation (a change in the DNA of a cell). DNA changes caused by mutagens may harm cells and cause certain diseases, such as cancer. Examples of mutagens include radioactive substances, x-rays, ultraviolet radiation, and certain chemicals.<sup>1-5</sup> There has been a considerable interest in the chemistry of mutagenic chemicals and related compounds, because of their biological importance in fermentation process.<sup>6-14</sup> The discovery of chemical mutagens initiated a flurry of activities in the field of mutagenesis. Therefore, one of the very interesting and exciting fields of biochemical research to-day is that of mutagenic effect on different fermentation processes<sup>15-33</sup>. The survey of the literature reveals that much work has been done related to mutagenesis of plant breeding but not to fermentation technology. The

present communication deals with the influence of chemical mutagen 4-hydrazinobenzoic acid on biotransformation of molasses to  $\alpha$ -hydroxy propionic acid by *S. lactis* 2114. The pH of the medium was adjusted to 6.0 by adding requisite amount of phosphat - buffer solution and the pH was ascertained by a pH meter.

### Experimental

#### Compositions of the production medium :

Molasses : 20% (W/V), Malt extract : 0.3780%  
(NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> : 0.30%, CaCO<sub>3</sub> : 6%, pH : 6.0

**Assay methods :** Evaluation of  $\alpha$ -hydroxy propionic acid (lactic acid) formed and molasses left unfermented was made colorimetrically<sup>34-35</sup>.

#### Sterilization :

The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

**Strain :** *S. lactis* 2114 was used in the present study. The strain was procured from NCL Pune, India.

**Age of the inoculum:** 50 hours old.

**Quantum of the inoculum:** 0.5 ml bacterial suspension of *S. lactis* 2114

**Molasses concentration :** 20%,

**Temperature (in °C):** 43°C

**Incubation period:** 120, 144, and 178 hours

**Optimum incubation period:** 144 hours

**pH :** 6.0

**Table -1**  
**Biotransformation of molasses to  $\alpha$ -hydroxy propionic acid by**  
***S. lactis* 2114 exposed to 4-hydrazinobenzoic acid**

| Concentration of mutagen used | Incubation period in hrs | Yield of lactic acid* in g/100 ml | molasses* left unfermented in g/100 ml | % of lactic acid increase (+) in 144 hrs. |
|-------------------------------|--------------------------|-----------------------------------|----------------------------------------|-------------------------------------------|
| Control                       | 120                      | 4.56425                           | 2.14853                                | —                                         |
| – Mutagen                     | 144                      | 5.29369                           | 1.48752                                | —                                         |
|                               | 168                      | 4.90861                           | 1.47556                                | —                                         |
| 1.0 x 10 <sup>-5</sup> M      | 120                      | 4.99649                           | 1.71882                                | —                                         |
| + Mutagen                     | 144                      | 5.72374                           | 1.05801                                | (+) 8.12382                               |
|                               | 168                      | 5.34065                           | 1.04615                                | —                                         |
| 2.0 x 10 <sup>-5</sup> M      | 120                      | 5.50707                           | 1.21135                                | —                                         |
| + Mutagen                     | 144                      | 6.23442                           | 0.55044                                | (+) 17.77077                              |
|                               | 168                      | 5.85134                           | 1.04615                                | —                                         |
| 3.0 x 10 <sup>-5</sup> M      | 120                      | 5.84291                           | 0.87765                                | —                                         |
| + Mutagen                     | 144                      | 6.57057                           | 0.21634                                | (+) 24.12079                              |
|                               | 168                      | 6.18718                           | 0.20478                                | —                                         |
| 4.0 x 10 <sup>-5</sup> M**    | 120                      | 6.75143                           | 0.02532                                | —                                         |
| + Mutagen                     | 144                      | 7.47928**                         | 0.68683                                | (+) 41.28670                              |
|                               | 168                      | 7.09610                           | 0.69849                                | —                                         |
| 5.0 x 10 <sup>-5</sup> M      | 120                      | 5.52673                           | 1.19191                                | —                                         |
| + Mutagen                     | 144                      | 6.25398                           | 0.53100                                | (+) 18.14027                              |
|                               | 168                      | 5.87070                           | 0.51933                                | —                                         |
| 6.0 x 10 <sup>-5</sup> M      | 120                      | 4.66757                           | 2.04594                                | —                                         |
| + Mutagen                     | 144                      | 5.39472                           | 1.38503                                | (+) 1.90849                               |
|                               | 168                      | 5.01113                           | 1.37366                                | —                                         |
| 7.0 x 10 <sup>-5</sup> M      | 120                      | 5.50707                           | 2.13308                                | —                                         |
| + Mutagen                     | 144                      | 6.23442                           | 1.47217                                | (+) 0.25048                               |
|                               | 168                      | 5.85134                           | 1.46080                                | —                                         |
| 8.0 x 10 <sup>-5</sup> M      | 120                      | ***                               | —                                      | —                                         |
| + Mutagen                     | 144                      | ***                               | —                                      | —                                         |
|                               | 168                      | ***                               | —                                      | —                                         |
| 9.0 x 10 <sup>-5</sup> M      | 120                      | ***                               | —                                      | —                                         |
| + Mutagen                     | 144                      | ***                               | —                                      | —                                         |
|                               | 168                      | ***                               | —                                      | —                                         |
| 10.0 x 10 <sup>-5</sup> M     | 120                      | ***                               | —                                      | —                                         |
| + Mutagen                     | 144                      | ***                               | —                                      | —                                         |
|                               | 168                      | ***                               | —                                      | —                                         |

\* Each value represents mean of three observations.

(+) values indicate % increase in the yield of lactic acid.

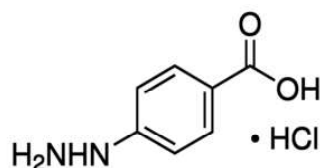
\*\* Optimum concentraion of mutagens and yield of lactic acid.

\*\*\* Insignificant yield of lactic acid but more than control.  
 Experimental deviation ( $\pm$ ) 1.5 to 2.5%

**Concentration of 4-hydrazinobenzoic acid used :** M/1000 solution of 4-hydrazinobenzoic acid has been prepared and  $1.0 \times 10^{-5}$  M to  $10 \times 10^{-5}$  molar concentration of benzoyl peroxide has been employed.

## Results and Discussion

### The influence of 4- hydrazinobenzoic acid :



The data given in the table 1 shows that 4-hydrazinobenzoic acid was found to be stimulating and increasing upto its concentration from  $1.0 \times 10^{-5}$  M to  $4.0 \times 10^{-5}$  M and above this concentration the bioconversion of molasses to

lactic acid by *S. lactis* 2114 was found to be decreasing. However, bioconversion of molasses to lactic acid at all the experimental concentrations of 4-hydrazinobenzoic acid used were higher than that of control flasks.

Many other workers have studied the influence of hydrazine and its derivatives in different fermentation system and have reported these compounds to be detrimental for the microbes and production of corresponding acids, alcohol, and ergot alkaloids. In the case of hydrazine compounds it was interestingly found that this compound effect adverse and favours functioning of enzyme systems.

The maximum yield of lactic acid was observed at  $4.0 \times 10^{-5}$  M concentration of 4-hydrazinobenzoic acid, i. e., 7.47928 g/100 ml in 144 hours of optimum incubation period which is 41.28% higher in comparison to the control flasks i. e. 5.29369 g/100 ml in the same experimental condition and optimum incubation period.

## References

1. Kalinina VI Prikl, Biokhim and A. M. Lysenko *Microbiol, Bogatyreva I G* **135**, 715 7 (1977).
2. R. W. Thoma *Folia Microbiol*, **16**, 197 (1971)
3. E M Wetkin *Annual Rev. Microbiol*, **23**, 487 (1969 a)
4. S I Alikhanian *Adv. Appl. Microbiol*, **4**, 1 (1962)
5. OL Daview *Bionnetries*, **20**, 576 (1964)
6. Sermoniti Genetics of Antibiotics Producing Organisms, Wiley Inter Sciences, London (1969)
7. S. I. Alikhanian Yugoslav and Zarcreb Arts , *Academy of science* **69**, (1969)
8. S G Bradley *Adv. appl. Microbiol* **8**, 29 (1966)
9. C T Calm *Progr. Ind. microbiol* **5**, 1 (1964)
10. CT Calm "Methods in microbiology" Vol. 3A, Chapt. VII, Norrie JR & Ribbons Nw, PP. 435; Academic Press, New-York (1969)
11. E L Tatum *J. Cellular Comp. Physiol.* **35**, Suupl. **1**, 119 (1950)
12. C M Stevens and A Myloric *Am. J. Bot.* **40**, 424 (1953)
13. K Arima and S Abe *J. Antibiotics* **4**, 342 (1951)
14. H Miller and W D McElcoy *Sci* **107**, 193 (1948)
15. H. Miller and W.D. McElcoy, *Science* , **107**, 193 (1948).
16. S.K. Mahna , *Indian J. Expt. Biol.*, **22**, 338 (1984)
17. R.W. Thoma, *Folia Microbiol*, **16**, 197 (1971)
18. V.I. Kalinana, I.G. Bogatyreva and A.M. Lysenko, *Prikl. Biokhim. Microbiol*, **8**, 29 (1977)
19. K.P. Tiwari and S.P. Singh, *Zbl. Bakt.* II Abt., **135**, 328 (1979).
20. S.P. Singh and S.K. Roy, *Acta Botanica Indica*, **12**, 185 (1985)
21. S. P. Singh, B. Pratap, B. K. Ambasta, R. K. Pandey, A. C. Sinha and A. Prasad , *Asian J. Chem.* **6**, 753 (1994).
22. S. P. Singh, C. K. Singh, D. Narayan, M. A. Khan and R. Kumar *J. Chemtracks*, **5**, 51 (2003).
23. S. P. Singh, F. R. Faizi, M. A. Khan, Md. Irfan, A. P. Kumar and P. K. Chaurasia, *J. Chemtracks* **5**, 37 (2003).
24. E. Freese and E.B. Freese : *Radiation Res. Suppl.* **6**, 97 (1966).

25. Shalini Singh *J. Chemtracks* **7**, 125 (2005).
26. Anurag Singh, Parul Jhunjhunwala and A. K. Srivastava, *J. Chemtracks* **7**, 63 (2005).
27. E. Freese In " Molecular Genetics" J. M. Taylor Ed. pp.207 New York (1963).
28. S. Kumari, F.R. Faizi, G.K. Mishra, K. Ahmad and S.P. Singh, *J. Chemtracks* **8**, 103 (2006).
29. S. Singh *J. Chemtracks* **8**, 53 (2006).
30. Anurag Singh and A.K. Srivastava *J. Chemtracks* **8**, 87 (2006).
31. Anurag Singh and A.K. Srivastava *J. Chemtracks* **9**, 257 (2007).
32. Kumari C, Prasad M., Kumari S, Narayan D and Singh S. P. *J. Chemtracks* **9**, 115 (2007).
33. Poonam Kumari, Vinay Kumar, Dharmendra Kumar, S. R. Sharma, K. Ahmad and S. P. Singh *Journal Chemtracks Vol. 10, (1&2)*, 333 (2008)
34. S.B. Barker and W.H. Summerson *J. Biol. Chem.* **138**, 535 (1941).
35. M. Dubois, K. A. Gilles, J. K. Hamilton and F. Smith, *Anal. Chem.* **28**, 350 (1956).