Asian Journal of Chemsitry Vol. 8, No. 4 (1996), 571–574

Lactic Acid Fermentation by *L. leichmannii* Exposed to Some Alkaloids

S.P. SINGH,* G. SANDANI, J.K. DUBEY, A. KUMAR
BIJAY KUMAR and Md. SHAMIM
Department of Chemistry
Magadh University, Bodh-Gaya-824 234, India

The influence of different alkaloids on lactic acid fermentation by *L. leichmannii* was studied. It was found that berberine sulphate, strychnine nitrate and papaverine hydrochloride have been slightly stimulant at all the concentrations used while ricinine was found to be stimulant at lower concentrations only.

INTRODUCTION

The influence of alkaloids on microbes has not been studied extensively[1], due to probably the toxic nature of the alkaloids. Since some alkaloids are known to be produced during some fermentation processes, it is obvious that such alkaloids are not toxic to the organisms involved in the alkaloidal fermentation[2–5]. A review of the literature shows that a very few attempts have been made to study the influence of alkaloids on the enzyme system participating in the process of fermentation. Recently Singh[6] and Singh *et al.*[7,8] also studied the influence of some alkaloids on lactic acid fermentation. The present study was undertaken to assess and analyse the production of lactic acid by *L. leichmannii* exposed to some alkaloids.

EXPERIMENTAL

54 Conical flasks each containing 100 mL of production medium were plugged with non-absorbent cotton and were sterilized in an autoclave for 20–30 min at 15 lbs and were allowed to cool at room temperature. These flasks were then arranged in 3 sets each comprising of 15 flasks. Each set of 15 flasks was rearranged in 5 subsets each comprising of 3 flasks. The remaining 9 flasks out of 54 flasks were kept as control and these were also rearranged in 3 subsets each comprising of 3 flasks. M/1000 Solution of experimental alkaloid in distilled water was prepared and 1.0, 2.0, 3.0, 4.0 and 5.0 mL of this alkaloidal suspension were added to each flask of 1st, 2nd, 3rd, 4th and 5th subsets repectively. The control flasks contained no alkaloid. Thus, the concentrations of alkaloids in 5 subsets were approximately 1.0×10^{-5} M, 2.0×10^{-5} M, 3.0×10^{-5} M, 4.0×10^{-5} M and 5.0×10^{-5} M respectively.

Now, all the flasks were inoculated with 0.05 mL broth culture of *L. leichmannii* and were incubated at 43°C in an incubator. The contents of the flasks were analysed after 3, 5 and 6 days of incubation period for lactic acid formed[9] and sucrose[10] left unfermented.

TABLE-1
LACTIC ACID FERMENTATION BY *L. LEICHMANNII* EXPOSED TO SOME ALKALOIDS

Alkaloids used	Concentration of alkaloids $a \times 10^{-5}$ M	Yield of lactic acid in g/100 mL			Sucrose* left unfermented in g/100 mL			% of lactic acid increase (+)/decrease (–) in 5 days of optimum incubation period
		3 days	5 days	6 days	3 days	5 days	6 days	
Control	—	3.971	6.918	6.305	4.129	1.382	1.379	—
	1.0×10^{-5} M	3.980	6.930	6.307	4.120	1.371	1.368	(+) 0.1734
	2.0×10^{-5} M	3.992	6.950	6.310	4.108	1.351	1.347	(+) 0.4625
Ricinine	3.0×10^{-5} M	3.990	6.945	6.309	4.110	1.354	1.344	(+) 0.3902
	4.0×10^{-5} M	3.962	6.910	6.301	4.138	1.390	1.382	(–) 0.1156
	5.0×10^{-5} M	3.940	6.890	6.279	4.161	1.411	1.402	(–) 0.4047
Control	—	3.972	6.920	6.306	4.128	1.381	1.376	—
	1.0×10^{-5} M	3.975	6.925	6.308	4.124	1.375	1.372	(+) 0.0722
	2.0×10^{-5} M	3.977	6.927	6.310	4.122	1.372	1.365	(+) 0.1011
Berberine sulphate	3.0×10^{-5} M	3.980	6.931	6.313	4.121	1.368	1.361	(+) 0.1589
	4.0×10^{-5} M	3.979	6.929	6.310	4.121	1.370	1.364	(+) 0.1300
	5.0×10^{-5} M	3.976	6.926	6.309	4.125	1.376	1.368	(+) 0.0867

Alkaloids used	Concentration of alkaloids $a \times 10^{-5}$ M	Yield of lactic acid in g/100 mL			Sucrose* left unfermented in g/100 mL			% of lactic acid increase (+)/decrease (–) in 5 days of optimum incubation period
		3 days	5 days	6 days	3 days	5 days	6 days	
Control	—	3.870	6.919	6.303	4.131	1.380	1.374	—
	1.0×10^{-5} M	3.970	6.920	6.304	4.130	1.380	1.373	(+) 0.0144
	2.0×10^{-5} M	3.981	6.940	6.318	4.119	1.359	1.351	(+) 0.3035
Strychnine nitrate	3.0×10^{-5} M	3.989	6.949	6.320	4.111	1.350	1.347	(+) 0.4335
	4.0×10^{-5} M	3.983	6.937	6.320	4.117	1.363	1.358	(+) 0.2601
	5.0×10^{-5} M	3.982	6.934	6.317	4.118	1.366	1.360	(+) 0.2167
Control	—	3.973	6.921	6.304	4.126	1.379	1.372	—
	1.0×10^{-5} M	3.979	6.940	6.322	4.120	1.361	1.353	(+) 0.2745
	2.0×10^{-5} M	3.985	6.949	6.331	4.115	1.350	1.346	(+) 0.4045
Papaverine hydrochloride	3.0×10^{-5} M	3.993	6.963	6.342	4.107	1.336	1.329	(+) 0.6068
	4.0×10^{-5} M	3.998	6.970	6.350	4.102	1.330	1.323	(+) 0.7079
	5.0×10^{-5} M	3.980	6.959	6.338	4.121	1.340	1.333	(+) 0.5490

*Mean of three trials.

Production medium: Each 100 mL contains 10% sucrose; 0.375% malt-extract; 0.250% $(NH_4)_2HPO_4$; 10% $CaCO_3$; Rest distilled water to make up 100 mL volume. The pH of the medium was adjusted to 6.3 by adding requisite amount of phosphate-buffer solution.

(±) Values indicate % increase/decrease in the yield of lactic acid.

Experimental deviation: ± 2.5–3.0%

(The production of lactic acid usually corresponded with the consumption of sucrose.)

RESULTS AND DISCUSSION

The results obtained in the study of the influence of different alkaloids on lactic acid fermentation is tabulated in Table-1. The results recorded in Table-1 show that the lower concentration of ricinine alkaloid has stimulatory effect on lactic acid fermentation by *Lactobacillus leichmannii*. The maximum yield of lactic acid in presence of ricinine was observed at 2.0×10^{-5} M *i.e.* 6.950 g/100 mL in 5 days which is 80.35% on the basis of fermentable sucrose and 0.4625% more in comparison to control. The higher concentrations of the alkaloid ricinine were not beneficial for the production of lactic acid.

The data recorded in Table-1 shows that the presence of berberine sulphate does not have any marked influence on lactic acid fermentation. At 3.0×10^{-5} M concentration of berberine sulphate the production of lactic acid was maximum in 5 days incubation period *i.e.* 6.931 g/100 mL (80.29% on the basis of fermentable sucrose) and only 0.15% more lactic acid could be produced at this optimum concentration. It is interesting to note that berberine sulphate has been stimulant for lactic acid fermentation at all concentrations used.

The data recorded in Table-1 shows that strychnine nitrate also does not have marked influence on lactic acid fermentation. The maximum lactic acid was found to be 6.949 g/100 mL (80.335% on the basis of fermentable sucrose) at 3.0×10^{-5} M concentration of strychnine nitrate which was just 0.43% more in comparison to control. However, strychnine nitrate has been slightly stimulant at all the concentrations used.

The data recorded in Table-1 shows that the presence of papaverine-hydrochloride has slight stimulating effect on the production of lactic acid by *L. leichmannii* at all the concentrations used. The maximum production of lactic acid was found to be 6.963 g/100 mL (*i.e.* 80.28% on the basis of fermentable sucrose) in 5 days of optimum incubation period, which is 0.60% more in comparison to control. However, at higher concentrations, papaverine hydrochloride has been quite inactive but non-toxic for the lactic acid fermentation.

REFERENCES

1. J.R. Porter, Bacterial Chemistry and Physiology, John Wiley & Sons, New Delhi (1970).
2. T.S. Ram Krishnan and K.M. Thomas, *Madras Agric. J.*, **30**, 441 (1942).
3. D. Muradarajan, T.S. Ram Krishnan, K. Krishnamenon and K.V. Srinivasan, *Proc. Indian Acad. Sci.*, **31(B)**, 103 (1950).
4. J.C. Saha and S.K. Bhattacharjee, *Nature*, **156**, 363 (1945).
5. K.S.M. Shastry, V.R. Pandotra, R.N. Thakur, J.H. Gupta, K.P. Singh and A. Husain, *Proc. Indian Acad. Sci.*, **72(B)**, 99 (1970).
6. R. Singh, Ph.D. Thesis, Magadh Univ., p. 209 (1990).
7. S.P. Singh, L. Kumar and N. Rathor, *Biojournal*, **3**, 367 (1991).
8. S.P. Singh, S.K. Pandey, P.K. Chaurasia, A.K. Brahmachari, B. Pratap and L.D.P. Yadav, *Asian J. Chem.*, **6**, 661 (1994).
9. S.B. Barker and W.H. Summerson, *J. Biol. Chem.*, **138**, 525 (1941).
10. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(Received: 4 March 1995; Accepted: 4 March 1996) AJC-1096