Asian Journal of Chemistry Vol. 8, No. 1 (1996) 165–168

NOTE

Lactic Acid Fermentation by *Lactobacillus leishmanii* Exposed to Some Organic Compounds

S.P. SINGH*, MD. SHAMIM, RABINDRA K. SINGH,
AWADH K. SINGH, M.K. NIRALA and K.P. KAMAL
Department of Chemistry
Magadh University, Bodh Gaya-824 234, India

The influence of 3-chloro-4-hydroxy mandelic acid, *ortho*-nitrophenol, *para*-nitrophenol and chloro-theophylline on the fermentative production of lactic acid by *Lactobacillus leishmanii* has been studied and it has been found that all the compounds have been toxic at all the concentrations used for lactic acid production.

Microbial requirements of physiologically active organic compounds are not well established. In recent years a few attempts have been made to study the effect of organic compounds on the enzyme system participating in the process of different fermentations[1–8]. The present paper was undertaken to assess and analyse the fermentative production of lactic acid to some physiologically active organic compounds by *L. leishmanii*.

54 Conical flasks, each containing 100 mL of production medium, were plugged with non-absorbent cotton and were sterilized in an autoclave for 30 min at 15 lb steam pressure and allowed to cool at room temperature. Then, the total flasks were divided into 3 sets each comprising 18 flasks. The 1st to 3rd set of 18 flasks each were again divided into 6 subsets each comprising 3 flasks and were taken for the study of different concentrations of organic compounds. Now, 1st to 5th set, each comprising 3-flasks, were inoculated with 0.05 mL inoculum of *L. leishmanii* in presence of different concentrations of organic compounds and the 6th set was kept as control. All the experimental flasks were kept in an incubator maintained at 43°C. The contents of the flasks were analysed colorimetrically for lactic acid formed[9] and sucrose left[10] unfermented after 3, 5 and 6 days of incubation period. The results are recorded in Table-1.

Production medium

For 100 mL volume, sucrose: 10% (w/v); Malt-extract: 0.375%; $(NH_4)_2HPO_4$. 0.25%; $CaCO_3$: 10%; pH: 6.3 adjusted by adding requisite amount of phosphate-buffer solution and rest distilled water to make up the volume to 100 mL.

The data recorded in Table-1 shows that 3-chloro-4-hydroxy mandelic acid, *ortho*-nitrophenol, *para*-nitrophenol and chloro-theophylline have inhibitory

TABLE-1
THE INFLUENCE OF DIFFERENT PHYSIOLOGICALLY ACTIVE ORGANIC COMPOUNDS (PAOC) ON LACTIC ACID FERMENTATION BY *L. LEISHMANII*

Physiologically active organic compounds used (PAOC)	Concentration of PAOC	Yield of lactic acid* in g/100 mL			Sucrose left unfermented* 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-chloro-4-hydroxy mandelic acid	—	3.970	6.915	6.300	4.301	1.285	1.280	—
	1.0×10^{-4} M	3.965	6.910	6.290	4.035	1.290	1.280	(–) 0.0723
	2.0×10^{-4} M	3.545	6.492	5.870	4.456	1.708	1.693	(–) 6.1171
	3.0×10^{-4} M	3.116	6.062	5.451	4.883	2.138	2.129	(–) 12.3355
	4.0×10^{-4} M	2.235	5.181	4.571	5.763	3.018	3.009	(–) 25.0759
	5.0×10^{-4} M	1.758	4.733	4.125	6.241	3.465	3.459	(–) 31.5545
Control *ortho*-nitrophenol	—	3.975	6.921	6.301	4.024	1.279	1.263	—
	1.0×10^{-4} M	3.923	6.709	5.789	4.078	1.491	1.483	(–) 3.0631
	2.0×10^{-4} M	3.801	6.584	5.666	4.616	1.609	1.609	(–) 4.8692
	3.0×10^{-4} M	3.210	6.365	5.448	4.790	1.836	1.831	(–) 8.0335
	4.0×10^{-4} M	1.984	5.139	4.222	6.015	3.062	3.059	(–) 25.7477
	5.0×10^{-4} M	1.235	4.390	3.473	6.765	3.810	3.804	(–) 30.5698

Physiologically active organic compounds used (PAOC)	Concentration of PAOC	Yield of lactic acid* in g/100 mL			Sucrose left unfermented* 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 *para*-nitrophenol	—	3.972	6.918	6.295	4.029	1.283	1.279	—
	1.0×10^{-4} M	3.693	6.639	5.719	4.307	1.562	1.555	(–) 4.0329
	2.0×10^{-4} M	3.485	6.432	5.512	4.516	1.768	1.758	(–) 7.0251
	3.0×10^{-4} M	2.263	5.210	4.290	5.738	2.991	2.983	(–) 24.6892
	4.0×10^{-4} M	1.428	4.375	3.456	6.572	3.824	3.819	(–) 36.7591
	5.0×10^{-4} M	0.281	3.228	2.309	7.719	4.973	4.967	(–) 53.3391
Control chloro-theophylline	—	3.977	6.922	6.308	4.022	1.279	1.268	—
	1.0×10^{-4} M	3.969	6.915	6.000	4.032	1.285	1.276	(–) 0.1011
	2.0×10^{-4} M	3.900	6.850	5.935	4.101	1.352	1.343	(–) 1.0401
	3.0×10^{-4} M	3.776	6.725	5.811	4.223	1.476	1.468	(–) 2.8459
	4.0×10^{-4} M	3.591	6.540	5.625	4.409	1.661	1.654	(–) 5.5186
	5.0×10^{-4} M	3.284	6.234	5.318	4.716	1.967	1.956	(–) 9.9393

* Each value represents mean of three trials.
(–) Values indicate % decrease in lactic acid production. Experimental deviation ±2.5–3%.
(The production of lactic acid usually corresponded with the consumption of sucrose).

effect on the production of lactic acid by *Lactobacillus leishmanii*. 3-Chloro-4-hydroxy mandelic acid which is metabolite antagonist has one end similar to structural end of lactic acid and prevents proper functioning of the enzyme and thus retards lactic acid production.

Ortho- and *para*-nitrophenol have inhibitory effect on lactic acid fermentation. It is interesting to note that in comparison to *ortho*-nitrophenol, *para*-nitrophenol has greater inhibitory action on lactic acid fermentation as it suffers inter-molecular hydrogen-bonding, resulting in the formation of high polymer by the combination of n molecules of *p*-nitrophenol which adds its antagonising properties due to additive value and is less depressed; thus it is a comparatively more active inhibitor than *ortho*-nitrophenol.

Chloro-theophylline bears a certain structural analogy to the barbiturates[11, 12] but has physiological activity of opposite type. It has got typical carbonyl groups like alloxan[13, 14] and develops an envelope of proton round it that may retard the conversion of pyruvic acid into lactic acid.

REFERENCES

1. K.P. Tiwari and S.P. Singh, *Nat. Acad. Sci. Letters*, **1**, 146 (1978).
2. T. Elizabeth, *Physiol. Plant*, **50**, 158 (1980).
3. K.P. Tiwari and A. Pandey, *Zbl. Bakt. II Abt.*, **136**, 70 (1981).
4. S.P. Singh, N. Rathor, G. Samdani, A.K. Singh and L. Kumar, *Indian J. Agric. Chem.*, **283**, 191 (1990).
5. S.P. Singh, L. Kumar and N. Rathor, *Biojournal*, **3**, 367 (1991).
6. S.P. Singh, M. Prasad, A.P. Sinha, L. Kumar and S.K. Yadav, *Biojournal*, **4**, 241 (1992).
7. S.P. Singh, B. Kumar, A.K. Pandey and S.K. Srivastava, *Proc. VIII Carbohydrate Conf., Trivandrum*, 173–175, 18–20 Nov. (1992).
8. S.P. Singh, B. Kumar and R. Kant, *Nat. Acad. Sci. Letters*, **15**, 11 (1992).
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. Robers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
11. P. Margalith and H. Pagani, *J. Appl. Microbiol.*, **9**, 325 (1961 b).
12. P. Margalith, *Adv. Appl. Microbiol.*, **6**, 85 (1964).
13. S.P. Singh and K.P. Tiwari, *Zbl. Bakt II Abt.*, **135**, 328 (1980).
14. S.P. Singh, B.K. Singh, C.D. Prasad and A. Suraiya, *Columban J. Life Sci.*, **1**, 31 (1993).

(*Received*:31 December 1994; *Accepted*: 13 September 1995) AJC-1010