Asian Journal of Chemistry Vol. 10, No. 3 (1998), 645–647

NOTE

Submerged Lactic Acid Fermentation by mixed Cultures of *Lactobacilli*

S.P. SINGH*, SHASHIKANT KUMAR and B.P. PANDEY†
Department of Chemistry
Magadh University, Bodh-Gaya-824 234, India

The submerged fermentative production of lactic acid by mixed cultures of *Lactobacillus leishmanni* + *Lactobacillus bulgaricus* and *L. leishmannii L. bulgaricus* + *L. delbrueckii* was studied. It has been forund that association of *L. leishmannii* with *L. bulgaricus* slightly enhances the yield of lactic acid, while the mixed cultures of *L. leishmannii*, *L. bulgaricus* and *L. delbrueckii* is as effective as *L. leishmannii* alone.

Submerged lactic acid fermentation has been studied by many workers[1,6]. Veikko[7,8] has shown symbiotic relationship between different species of lactic acid bacteria. Bautista *et al.*[9] identified some compounds causing symbiotic growth of *S. thermophillus* and *L. bulgaricus*. Branen and Keenan[10] found that all *Lactobacilli* strains with the exception of *L. casei*, were significantly stimulated by a strain of *S. thermophillus*. Growth of mixed populations of *L. casei* and *S. lactis* have also been studied by Oberman and Zdzisalwa[11]. Tiwari *et al.*[12,13] found that association of. *L. bulgaricus* with *L. delbrueckii* and association of *L. bulgaricus* with *L. casei* slightly enhances the yield of lactic acid. The present study has been undertaken to ascertain the impact of mixed cultures of *Lactobacilli* on the submerged lactic acid fermentation.

Production medium: Ingredient composition of production medium for 100 mL is as follows:

Sucrose (10%); $CaCO_3$ (10%); Malt-extract (0.375%); $(NH_4)_2HPO_4$ (0.25%); pH 5.8–6.0 adjusted by phosphate buffer solution. inoculum: 0.05 mL bacterial suspension of *L. delbrueckii*, *L. bulgaricus* and *L. leishmannii* each. Age of the inoculum: 36 h old. Incubation period: 72 h, 144 h and 192 h. Temperature: 45 ± 1°C.

Assay methods: Estimation of lactic acid[14] formed and sucrose[15] left unfermented was made colorimetrically. Sucrose (450 g) mixed with different ingredient constituents was diluted with 2 L of distilled water and total volume was divided into 45 equal parts and poured into 250 mL conical flasks. The

†Department of Chemistry, S.S.J.S. Namdhari College, Garhwa (Ranchi University).

volume of all the 45 flasks containing sucrose solution was made 100 mL with distilled water. These flasks were plugged with non-absorbent cotton and were sterilized in an autoclave at 15 lbs steam pressure for 30 min. These flasks were then allowed to cool at room temperature and divided into five sets, each consisting of nine flasks. Each set was subdivided into three subsets each consisting of three flasks. The first, second and third sets were inoculated with 36 h old cultures of *L. leishmannii*, *L. delbrueckii* and *L. bulgaricus* respectively. The fourth set was inoculated with 36 h old culture of *L. leishmannii* and *L. bulgaricus* and the fifth set with equal amounts of the 36 h old cultures of *L. leishmannii*, *L. bulgaricus* and *L. delbrueckii*. After inoculation, the flasks were incubated at 45°C in an incubator with occasional shaking. The pH of the mash was adjusted between 5.8 to 6.0. The lactic acid formed during the course of fermentation was neutralized by $CaCO_3$. The flasks were analysed colorimetrically for lactic acid[14] formed and sucrose[15] left unfermented after 72, 144 and 192 h of incubation period (Table-1).

TABLE-1
PRODUCTION OF LACTIC ACID BY *LACTOBACILLI* ALONE AND IN COMBINATION

S. No.	Name of the strains used	Incubation period in hours	Yields of lactic acid*		Sucrose* left unfermented
			Lactic acid formed in g/100 mL	% yield of lactic acid	
1.	*L. leishmannii*	72	3.980	—	4.120
		144†	6.925	78.29	1.155
		192	6.300	—	1.135
2.	*L. delbrueckii*	72	3.992	—	4.052
		144†	6.988‡	81.03	1.377
		192	6.325	—	1.326
3.	*L. bulgaricus*	72	3.280	—	4.762
		144†	6.635	76.56	1.334
		192	6.002	—	1.313
4.	*L. leishmannii*	72	3.989	—	4.059
		144†	6.935	79.10	1.233
	L. bulgaricus	192	6.325	—	1.159
5.	*L. leishmanii*	72	3.984	—	4.059
	L. bulgaricus	144	6.926	78.28	1.153
	L. delbrueckii	192	6.298	—	1.150

*Each value represents mean of three trials.
Experimental deviation ±1.5–2.5%
†Optimum incubation period
‡Optimum yield of lactic acid

It is evident from Table-1 that *L. leishmannii*, *L. delbrueckii* and *L. bulgaricus* strains of *lactobacilli* individually produced 78.29%, 81.03% and

76.56% lactic acid respectively on the basis of fermentable sugar in 144 h of optimum incubation period. In the associated growth of *L. Leishmannii* and *L. bulgaricus* only a slight increase in the yield of lactic acid was obtained in comparison to the individual capacity of both *Lactobacilli* strains.

However, association of *L. leishmannii* with *L. bulgaricus* and *L. delbrueckii* could produce only 78.28% of lactic acid on the basis of fermentable sucrose which is less than that produced by individual strain of *Lactobacilli.*

The increase in yield of lactic acid by the first combination (*L. leishmannii* + *L. bulgaricus*) and insignificant production of lactic acid by the second combination (*L. leishmannii* + *L. bulgaricus* + *L. delbrueckii*) may be probably due to the fermentative capacity of individual strains of *Lactobacilli* and specific selectivity of sugar substrates by them.

It appears from the results that *L. bulgaricus* stimulated the lactic acid producing activity of *L. leishmannii.* However, it also appears that in the second combination the strains interfered with the activity of each other and suffered a little to give a significant yield of lactic acid.

REFERENCES

1. L. Osipov and F. Osis, *Chem. Abstr.*, **59**, 80 (1963).
2. H.K. Tiwari and S.R. Vyas, *J. Res. Punjab Agric. University*, **8**, 460 (1971).
3. _____, *Indian J. Microbiol.*, **10**, 69 (1976).
4. S.P. Singh, G. Samdani, J.K. Dubey, A. Kumar, B. Kumar and Md. Shamim, *Asian J. Chem.*, **8**, 571 (1996).
5. S.P. Singh, Shashikant Kumar and B. Singh, *Asian J. Chem.*, **10**, 189 (1998).
6. S.P. Singh, Shashikant Kumar, B. Singh and B.K. Singh, *Asian J. Chem.*, **10**, 377 (1998).
7. Veikko and Nurmikko, *Acta Chem. Scand.*, **6**, 1258 (1952).
8. _____, *Biol. Abstr.*, **29**, 8826 (1955).
9. E.S. Bautista, R.S. Dahiya and M.L. Speck, *J. Dairy Res.*, **33**, 299 (1966).
10. A.L. Branen and T.W. Keenan, *Appl. Microbiol.*, **17**, 280 (1969).
11. H. Oberman and L. Zdzislawa, *Acta Microbiol. Polon., Ser. B, Microbiol. Appl.*, **5**, 151 (1973).
12. K.P. Tiwari, A. Pandey and N. Mishra, *Proc. Natl. Acad. Sci. (India)*, **47A**, 130 (1977).
13. _____, *Zbl. Bakt. Il. Abt. Bd.*, **134**, 544 (1979).
14. S.B. Barker and W.H. Summerson, *J. Biol. Chem.*, **138**, 535 (1941).
15. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(*Received:* 30 December 1997; *Accepted:* 17 February 1998) AJC-1464