

Lactic Acid Fermentation Exposed to Some Alkaloids

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The influence of brucine, berberine, hypaphorine and morphine on production of lactic acid by *L. delbrueckii* has been studied. It has been found that berberine has an inhibitory effect on lactic acid fermentation while brucine, hypaphorine and morphine stimulates the fermentation process.

INTRODUCTION

The influence of alkaloids on microorganisms and fermentation processes have not been studied very extensively¹. Somogli² reported that small amount of quinine and atropine accelerated the growth of yeasts. Kligher³ reported inhibitory action of caffeine on several microbes. Caffeine has been reported as a most effective chemical in various biological processes and significant response has been noted by a number of workers⁴⁻⁷ to produce high quality mutant. Chloro-caffeine⁸⁻¹⁰ and methoxy-caffeine¹¹ has been found much effective in different fermentation processes. Recently Singh¹² also studied the influence of different alkaloids on lactic acid fermentation and noted that strychnine and ephidine enhances the production of lactic acid. The present study was undertaken to assess and analyse the production of lactic acid by *L. delbrueckii* 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 minutes at 15 lb steam pressure 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 were 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 alkaloid suspension were added to each flask of 1st, 2nd, 3rd, 4th and 5th subsets respectively. The control flasks contained no alkaloid. Thus, the concentration of alkaloid 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. delbrueckii* and were incubated at 46°C in an incubator. The contents of the flasks were analysed colorimetrically after 2, 4 and 6 days of incubation period for lactic

TABLE I
FERMENTATIVE PRODUCTION OF LACTIC ACID BY *L. DELBRUECKII* EXPOSED TO SOME ALKALOIDS

Alkaloids used	Concentration of alkaloids $\times 10^{-5}M$	Yield of lactic acid* in g/100 ml.			Sugar left* unfermented in g/100 ml.			% of lactic acid increase (+)/decrease (-) in 6 days (optimum)
		2 days	4 days	6 days	2 days	4 days	6 days	
Control	—	1.40	2.68	3.44	3.00	1.73	0.95	—
Brucine	$1.0 \times 10^{-5}M$	1.55	2.95	3.40	2.89	1.50	0.92	(-) 01.1627
	$2.0 \times 10^{-5}M$	1.62	3.10	3.80	2.73	1.35	0.62	(+) 10.4651
	$3.0 \times 10^{-5}M$	2.00	3.40	4.00	2.53	1.23	0.50	(+) 16.2790
	$4.0 \times 10^{-5}M$	1.85	3.20	3.95	2.65	1.30	0.40	(+) 14.8255
	$5.0 \times 10^{-5}M$	1.78	3.14	3.90	2.71	1.34	0.42	(+) 13.3720
Control	—	1.25	2.50	3.41	3.24	2.18	0.93	—
Berberine	$1.0 \times 10^{-5}M$	1.15	2.34	3.00	3.30	2.26	1.47	(-) 12.0234
	$2.0 \times 10^{-5}M$	1.12	2.44	3.15	3.35	2.05	1.30	(-) 07.6246
	$3.0 \times 10^{-5}M$	1.12	2.40	2.95	3.32	2.00	1.39	(-) 13.4897
	$4.0 \times 10^{-5}M$	1.20	2.35	2.80	3.28	2.20	1.38	(-) 17.8885
	$5.0 \times 10^{-5}M$	1.18	2.60	2.72	3.30	2.05	1.40	(-) 20.2346
Control	—	1.30	2.50	3.52	3.28	2.22	0.91	—
Hypaphorine	$1.0 \times 10^{-5}M$	1.20	2.44	3.50	3.34	2.10	0.81	(-) 0.5681
	$2.0 \times 10^{-5}M$	1.62	2.68	3.92	2.76	1.77	0.71	(+) 11.3636
	$3.0 \times 10^{-5}M$	1.85	3.78	4.50	2.70	0.82	0.26	(+) 27.8409
	$4.0 \times 10^{-5}M$	2.15	3.18	4.68	2.56	0.71	0.20	(+) 32.9545
	$5.0 \times 10^{-5}M$	2.24	4.00	4.60	2.33	0.66	0.24	(+) 30.6818

Control	—	1.18	2.48	3.48	3.40	2.06	0.92	—
Morphine	$1.0 \times 10^{-5}M$	1.18	2.40	3.50	3.34	2.22	0.80	(+)00.5747
	$2.0 \times 10^{-5}M$	1.16	3.00	3.60	3.30	1.48	0.68	(+)03.4482
	$3.0 \times 10^{-5}M$	1.24	2.95	3.80	3.26	1.53	0.52	(+)09.1954
	$4.0 \times 10^{-5}M$	1.18	3.10	3.84	3.36	1.50	0.54	(+)10.3448
	$5.0 \times 10^{-5}M$	1.26	3.14	3.80	3.27	1.37	0.50	(+)09.1954

* Each value represents mean of three observations.

Production Medium: Each 100 ml contains: sucrose 5%, Malt-Extract: 0.375%, $(NH_4)_2HPO_4$: 0.25%, $CaCO_3$: 5%, Distilled Water: to make-up 100 ml. volume.

The pH of the medium was adjusted to 6.2 by adding requisite amount of phosphate-buffer solution.

Experimental Deviation: (\pm) 2.5 to 3.5%.

acid¹³ formed and sucrose¹⁴ left unfermented. The results are recorded in the Table 1.

RESULTS AND DISCUSSION

The data given in the Table 1 indicates that brucine has stimulatory effect on lactic acid fermentation. The higher concentration of brucine 3.0×10^{-5} M favoured the maximum production of lactic acid (4.0 g/100 ml.) which was found to be 16.27% higher in comparison to control (3.44g/100 ml.) in 6 days of incubation period.

The presence of berberine (Table 1) decreases the production of lactic acid. The yield of lactic acid in the control was higher than that obtained from each of the flask containing berberine. The maximum yield of lactic acid (3.15g/100 ml.) was observed at 2.0×10^{-5} M concentration of berberine, but even this yield was much lower (7.62%) than that obtained in the control (3.41 g/100 ml.). It may, therefore, be concluded that berberine has toxic effect on lactic acid fermentation.

It is evident from the results that hypaphorine irrespective of its concentration, increases the fermenting activity of *L. delbrueckii*. The maximum yield of lactic acid was obtained at 4.0×10^{-5} M concentration of hypaphorine which was 32.95% higher in comparison to control in 6 days of incubation period.

The data (Table 1) shows that the presence of morphine does not have any marked influence on lactic acid fermentation. At lower concentrations of morphine, the production of lactic acid was very close to control. The increase in concentration of morphine resulted in gradual increase in lactic acid production although these yields are not much higher than the control. The production of lactic acid usually corresponded with the consumption of sugar.

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