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INFLUENCE OF VITAMINS ON FERMENTATIVE PRODUCTION OF LACTIC ACID BY *LACTOBACILLUS DELBRUECKII*

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The influence of riboflavin, nicotinic acid, folic acid and thiamin on the production of lactic acid by *Lactobacillus delbrueckii* 9649* has been studied. It has been found that thiamin has retarding effect on lactic acid fermentation while riboflavin, nicotinic acid and folic acid stimulate the fermentation process.

INTRODUCTION

William (1919) was the first to observe that only water-soluble vitamins are useful to yeasts, fat-soluble vitamins having no effect on them. It was pointed out by Bachmann (1919) and Friedmann & Funk (1922) that only B-group vitamins have growth-stimulating activity towards the micro-organisms and yeasts. Eastcott (1928) and Wooley (1944) suggested inositol as an essential growth factor for the micro-organisms. Buchmann & Buchmann (1953) observed pyridoxine, folic acid, aneurine and inositol of good use for the bacteria. Snell & Pederson (1952) have shown that pantothenic acid is essential for the normal growth of lactic acid bacteria.

A survey of the literature reveals that much work has been done on the synthesis of several vitamins during fermentation but no light has been thrown on the study of the effect of vitamins on the process of fermentation itself, particularly on lactic acid fermentation. In view of this, present work was undertaken by the authors and it has been found that the presence of some vitamins in small amounts has beneficial effect on the activity of *L. delbrueckii*.

MATERIALS AND METHODS

270.0 g of market sugar, 20.25 g of malt extract, 13.50 g of $(NH_4)_2 HPO_4$, 270.0 g of $CaCO_3$ were dissolved in 2.5 litres of distilled water and a requisite amount of KH_2PO_4-K_2HPO_4 buffer solution was added to the medium to maintain the *p*H at 6.2. The total volume of the medium was divided into 54 equal parts. Each part was taken in a separate 250 ml conical flask. These flasks were then arranged in three sets each comprising of fifteen flasks. Each set of fifteen flasks was rearranged in five subsets each comprising three flasks. The remaining nine out of the 54

* *Lactobacillus delbrueckii* 9649 was obtained from NCL, Poona, India.

flasks were kept as control and these were also arranged in three subsets each of three flasks.

M/1000 solution of experimental vitamin in distilled water was prepared and 1.0, 2.0, 3.0, 4.0 and 5.0 ml of this solution were added to the flasks of 1st, 2nd, 3rd, 4th and 5th subsets respectively. The control flasks contained no vitamin .The total volume of the medium in each flask was made up to 100 ml with distilled water. Thus, the concentration of vitamin in these five subsets was 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. All 54 flasks were then plugged with non-absorbent cotton and sterilized in an autoclave at 15 lb steam pressure for 30 min. After sterilization the flasks were allowed to cool at room temperature, inoculated with a definite quantity of 48 hr old broth culture of *L. delbrueckii*, incubated at 46° (optimum temperature for *L. delbrueckii*) in an incubator and thereafter the contents of lactic acid (Baker & Summerson, 1941) produced and sugar left unfermented were analysed colorimetrically after 2, 4 and 6 days of incubation. The results of colorimetric analysis are recorded in Tables I—IV.

Discussion

The findings in Tables I—IV, show that vitamins in general do not give encouraging results so far as the lactic acid production by *L. delbrueckii* is concerned. However, it appears that lactic acid-producing bacteria have selectivity for certain vitamins.

TABLE I

Production of lactic acid in the presence of riboflavin

Conc. of riboflavin A$\times10^{-5}$M	*Yield of lactic Acid g/100 ml			*Sugar left in g/100 ml		
	2 days	4 days	6 days	2 days	4 days	6 days
1	1.44	2.73	3.40	3.00	2.38	1.10
2	1.51	2.78	3.50	3.00	2.02	1.05
3	1.57	3.11	4.05	3.06	1.36	0.68
4	1.76	3.14	4.02	2.78	1.35	0.62
5	1.77	3.19	4.10	2.83	1.30	0.60
Control	1.62	2.97	3.69	3.10	1.53	0.86

* Each value represents the mean of three trials.

According to results recorded in Table I, it appears that riboflavin which is commonly known as growth factor, appears to be essential for the growth of *L. delbrueckii* and acts as an "activator" for normal developments and activity of this

bacteria. Effective concentration of this vitamin for high production of lactic acid was found to be 5.0×10^{-5}M. At this concentration lactic acid was produced to the extent of 93.18% on the basis of sugar fermented.

TABLE II

Production of lactic acid in the presence of folic acid

Conc. of folic acid A$\times 10^{-5}$M	*Yield of lactic Acid g/100 ml			*Sugar left in g/100 ml		
	2 days	4 days	6 days	2 days	4 days	6 days
1	1·40	3·10	3·57	3·10	2·33	0·93
2	1·48	3·00	3·70	3·05	1·40	0·84
3	1·38	3·14	3·95	3·12	1·31	0·68
4	1·42	3·09	3·39	3·00	1·28	0·59
5	1·45	3·12	3·85	3·06	1·38	0·62
Control	1·53	2·63	3·60	2·96	1·78	0·95

* Each value represents the mean of three trials.

Folic acid (cf. Table II) has no significant effect on lactic acid fermentation, The highest yield of lactic acid in the presence of folic acid was recorded 3.95 g/100 ml (91.4% on the basis of sugar fermented) at 3.0×10^{-5}M concentration of folic acid.

TABLE III

Production of lactic acid in the presence of nicotinic acid

Conc. of nicotinic acid A$\times 10^{-5}$M	*Yield of lactic acid g/100 ml			*Sugar left in g/100 ml		
	2 days	4 days	6 days	2 days	4 days	6 days
1	1·48	3·26	3·60	2·93	1·18	0·89
2	1·62	3·32	3·83	2·82	1·13	0·73
3	1·58	3·40	3·98	3·00	1·20	0·70
4	1·50	3·28	3·70	3·10	1·30	0·77
5	1·64	3·30	3·92	2·96	1·25	0·70
Control	1·48	3·00	3·58	2·97	1·44	0·88

* Each value represents the mean of three trials.

Nicotinic acid (cf. Table III) also could not prove itself effective for the production of lactic acid. Maximum yield of lactic acid (3.98 g/100 ml, 92.5% on the

basis of sugar fermented) was found at 3.0×10^{-5}M concentration of nicotinic acid.

TABLE IV

Production of lactic acid in the presence of thiamin

Conc. of thiamin $A\times10^{-5}$M	* Yield of lactic acid g/100 ml			* Sugar left in g/100 ml		
	2 days	4 days	6 days	2 days	4 days	6 days
1	1·34	1·82	2·80	3·12	2·78	1·85
2	1·30	1·98	2·60	3·20	2·71	2·10
3	1·28	2·15	3·01	3·22	2·45	1·65
4	1·37	2·70	3·50	3·13	2·00	1·16
5	1·26	2·25	2·50	3·25	2·44	1·80
Control	1·32	2·78	3·60	3·18	1·82	0·98

* Each value represents the mean of three trials.

The influence of thiamin (cf. Table IV) was found to be inhibitory for the production of lactic acid. At the optimum concentration of thiamin (4.0×10^{-5}M) only 76.8 % sugar was fermented and the yield of lactic acid was found 3.50 g/100 ml (91.1% on the basis of sugar fermented).

So far as the consumption of sugar during the course of fermentation by *L. delbrueckii* in the presence of vitamins is concerned, it has been observed that the sugar consumption usually corresponded with the production of lactic acid.

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