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Influence of Some Nitrogenous Compounds on Microbial Production of Lactic Acid by *Lactobacillus delbrueckii*†

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The influence of ammonium sulphate, urea, potassium nitrate and ammonium nitrate on the production of lactic acid by *Lactobacillus delbrueckii* 9649* has been studied. It has been found that urea, ammonium sulphate and ammonium nitrate stimulate the fermentative production of lactic acid whereas potassium nitrate retards the fermentation process.

INTRODUCTION

Nitrogen in different forms has been used for microbial culture. Ammonium salts, such as NH_4Cl, $(NH_4)_2SO_4$ and NH_4NO_3 are employed as the source of nitrogen for microbial culture media, while nitrogen in the form of nitrates of potassium, sodium, barium, strontium and nickel has also been used in various microbial investigations. Hacskaylo[1] observed that nitrogen in the form of nitrate increases the growth of many organisms. The importance of nitrates as a source of nitrogen for microorganisms was also recognized by Agnihotri[2]. Nitrogen compounds have been widely used in alcoholic[3], lactic[4–12] and citric acid[13] frementation. Wenck, Peterson and Fred[14] studied in detail the nitrogen metabolism of *Aspergillus fischeri*. Steinberg[15] studied nitrogen metabolism of *A. niger*. It is evident from the results of earlier investigation that all nitrogen sources are not equally suitable for different species and that microorganisms have got different specificity in respect of the utilization of nitrogen compounds.

In the present paper, the authors have reported the results of the study of the influence of some nitrogenous compounds *viz.*, ammonium sulphate, urea, potassium nitrate and ammonium nitrate on lactic acid fermentation.

EXPERIMENTAL

270.0 g of market sugar, 20.25 g of malt extract, 13.50 g of $(NH_4)_2HPO_4$ and 270.0 g of $CaCO_3$ were dissolved in 2.5 L of distilled water nad requisite amount of KH_2PO_4-K_2HPO_4 buffer solution was added to the medium to maintain the pH at 6.2. The total volume of the medium was divided into 54 equal parts. Each

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part was taken in a separate 250 mL conical flask. These flasks were than arranged in three sets each comprising fifteen flasks. Each set of fifteen flasks was rearranged in five subsets each of three flasks. The remaining nine out of 54 flasks were kept as control and these were also rearranged in three sets of three flasks each.

M/10 solution of experimental nitrogen compound 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 experimental nitrogen compounds. The total volume of the medium in each flask was made up to 100 mL by adding requisite amount of distilled water. The flasks were sterilised, cooled and inoculated with 0.05 mL inoculum of *L. delbrueckii* and analysed after 2, 4 and 6 days of incubation for lactic acid produced[16] and sugar left unfermented[17]. The results obtained are shown in Tables 1–4. Table-5 shows comparative assessment of the influence of various experimental nitrogenous compounds.

RESULTS AND DISCUSSION

The presence of ammonium sulphate was found to be favourable to the microorganism. In this case, it was observed (cf. Table-1) that in all 2, 4 and 6 days of incubation period there was a gradual increase in the lactic acid production with the increase in the concentration of ammonium sulphate. Maximum production of lactic acid, 90.45% (on the basis of sugar fermented) was observed at 4.0×10^{-3} M concentration and 5.0×10^{-3} M concentration of the salt.

TABLE-1
FERMENTATIVE PRODUCTION OF LACTIC ACID IN THE PRESENCE OF AMMONIUM SULPHATE

Conc. of ammonium sulphate $a \times 10^{-3}$ M	*Yield of lactic acid g/100 mL			*Sugar left unfermented g/100 mL		
	2 days	4 days	6 days	2 days	4 days	6 days
Control 0.0	1.30	2.90	3.80	3.00	1.69	0.82
1.0	1.60	2.95	3.90	2.98	1.60	0.68
2.0	1.50	3.00	3.82	3.02	1.55	0.78
3.0	1.52	3.18	3.10	3.00	1.50	1.76
4.0	1.48	3.10	3.98	2.95	1.44	0.60
5.0	1.70	3.15	3.98	2.75	1.50	0.62

*Each value represents the mean of three trials.

In can be seen from Table-2 that the presence of urea increases the production of lactic acid. Maximum lactic acid production, 92.44% (on the basis of sugar fermented) was obtained at 3.0×10^{-3} M concentration of urea.

The data given in Table-3 show that the presence of potassium nitrate is not favourable to lactic acid fermentation. It is clear from the fact that even in the presence of optimum amounts of potassium nitrate (3.0×10^{-3} M) the lactic acid production was less than that in control.

TABLE-2
FERMENTATIVE PRODUCTION OF LACTIC ACID IN THE PRESENCE OF UREA

Conc. of urea $a \times 10^{-3}$ M	*Yield of lactic acid g/100 mL			*Sugar left unfermented g/100 mL		
	2 days	4 days	6 days	2 days	4 days	6 days
Control 0.0	1.40	2.80	3.79	3.18	1.70	0.78
1.0	1.65	3.22	3.60	3.02	1.50	0.92
2.0	1.72	3.24	4.00	2.90	1.48	0.56
3.0	2.00	3.20	4.16	2.70	1.55	0.50
4.0	1.90	3.15	3.70	2.58	1.40	0.82
5.0	1.85	3.15	4.15	2.54	1.52	0.50

*Each value represents the mean of three trials.

Experimental deviation ± 1.5–2.5%.

TABLE-3
FERMENTATIVE PRODUCTION OF LACTIC ACID IN THE PRESENCE OF POTASSIUM NITRATE

Conc. of potassium nitrate $a \times 10^{-3}$ M	*Yield of lactic acid g/100 mL			*Sugar left unfermented g/100 mL		
	2 days	4 days	6 days	2 days	4 days	6 days
Control 0.0	1.50	2.98	3.80	3.10	1.64	0.78
1.0	1.54	3.00	3.50	3.10	1.62	1.08
2.0	1.50	2.85	3.20	3.00	1.38	1.00
3.0	1.68	3.25	3.70	2.95	1.58	0.90
4.0	1.60	3.05	3.45	3.00	1.62	1.00
5.0	1.55	3.15	3.68	2.98	1.55	0.98

*Each value represents the mean of three trials.

Experimental deviation ± 1.5–2.5%.

The presence of ammonium nitrate does not favour lactic acid fermentation considerably. It was found (cf. Table-4) that in all 2, 4 and 6 days of incubation periods there was slight increase in the lactic acid production with the increase in the concentration of ammonium nitrate till the optimum production of lactic acid, 90.76% (on the basis of sugar fermented) was obtained at 4.0×10^{-3} M concentration.

TABLE-4
FERMENTATIVE PRODUCTION OF LACTIC ACID IN THE PRESENCE OF AMMONIUM NITRATE

Conc. of ammonium nitrate $a \times 10^{-3}$ M	*Yield of lactic acid g/100 mL			*Sugar left unfermented g/100 mL		
	2 days	4 days	6 days	2 days	4 days	6 days
Control 0.0	1.35	2.68	3.76	3.10	1.77	0.80
1.0	1.50	3.10	3.70	3.00	1.38	0.70
2.0	1.50	3.12	3.60	3.02	1.30	0.80
3.0	1.65	2.90	3.52	2.68	1.70	1.00
4.0	1.54	3.16	3.85	2.93	1.54	0.70
5.0	1.60	3.12	3.85	2.90	1.32	0.80

*Each value represents the mean of three trials.

Experimental deviation ± 1.5–2.5%.

TABLE-5
COMPARATIVE ASSESSMENT OF THE INFLUENCE OF VARIOUS NITROGENOUS COMPOUNDS ON LACTIC ACID PRODUCTION IN 6 DAYS OF INCUBATION

Nitrogen source	Yield of lactic acid in control flasks g/100 mL	Max. yield of lactic acid in presence of nitrogen sources	% difference in the yield of lactic acid
Amonium sulphate	3.80	3.98	+ 4.73
Urea	3.79	4.16	+ 9.75
Potassium nitrate	3.80	3.70	– 2.63
Ammonium nitrate	3.76	3.85	+ 2.39

(+)ve value indicates the % increase in the yield of lactic acid.
(–)ve value indicates the % decrease in the yield of lactic acid.

So far as the consumption of sugar during the course of fermentation in the presence of experimental nitrogen compounds is concerned, it has been observed that the sugar consumption corresponded with the production of lactic acid.

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