

# Technical Papers

## Nitrate Reduction by Certain Strains of *Lactobacillus plantarum*\*

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During a taxonomic study of lactic acid bacteria from cucumber fermentations conducted in this laboratory, it was noted that certain isolates of acid-forming bacteria were able to reduce nitrate. These cultures were found to be similar to *Lactobacillus plantarum* in all other respects. Therefore, a number of pure cultures of this species from various sources were tested for nitrate reduction.

Thirty-eight cultures of *L. plantarum* were tested by growing in indole-nitrite medium (Baltimore Biological Laboratory) for 3 days and by testing for the presence of nitrites with sulphanilic acid and  $\alpha$ -naphthylamine (1). Of these cultures, 18 were able to reduce nitrate in this medium (Table 1). The addition

Table 1. Reduction of nitrate by strains of *Lactobacillus plantarum*.

Source	No. of cultures	No. of cultures	
		Reduced nitrate	Nitrate not reduced
A.T.C.C.*	6	3 (8014)†	3 (8292)
N.R.R.L.‡	1	0	1 (B-227)
C. S. Pederson	3	0	3 (7-4)
Cucumber fermentations	28	15 (L-20)	13 (A-91-3)
Totals	38	18	20

\* American-type culture collection.

† Numbers in parenthesis are representative strains.

‡ Northern Regional Research Laboratory.

of zinc to the other cultures indicated that no reduction had occurred. The results of replications of this experiment were identical.

It has been established by a number of investigators (2) that oxygen tension is a very important factor in the ability of bacteria to reduce nitrates. Also, the composition of the medium used may influence the results obtained. Thus, an experiment was conducted using two strains of *L. plantarum* that were found to reduce nitrates in indole-nitrite medium and one strain that did not. These strains were grown in five mediums, each containing 0.1-percent  $\text{KNO}_3$ , and tested over a period of 4 days for nitrate reduction.

The results of this experiment are given in Table 2. It is obvious that the oxygen tension of the mediums was the most important factor in nitrate reduction by strains A.T.C.C. 8014 and L-20. These cultures were unable to reduce nitrates in either nitrate broth or indole-nitrite medium with the 0.1-percent agar

Table 2. Relation of medium to nitrate reduction by *Lactobacillus plantarum*.

Medium	Incubation time (days)	Strain of <i>L. plantarum</i>		
		A.T.C.C. 8014	L-20	B-227
Nitrate broth (Difco)	1	—*	—	—
	2	—	—	—
	3	—	—	—
	4	—	—	—
Nitrate broth + 0.1%-agar	1	—	—	—
	2	—	—	—
	3	+	+	—
	4	+	+	—
Nitrate broth + 0.1%-agar + 0.3%-yeast extract	1	—	—	—
	2	+	+	—
	3	+	+	—
	4	+	+	—
Indole-nitrite medium (BBL)	1	—	—	—
	2	+	+	—
	3	+	+	—
	4	+	+	—
Indole-nitrite medium without agar	1	—	—	—
	2	—	—	—
	3	—	—	—
	4	—	—	—

\* All tests run in duplicate: —, nitrate not reduced; +, nitrate reduced.

omitted. They reduced nitrates in all mediums containing 0.1-percent agar. However, the addition of yeast extract and agar to nitrate broth resulted in a positive test for nitrite 1 day earlier than when only agar was added. Strain B-227 failed to reduce nitrate in any medium. This experiment was repeated with five additional cultures of *L. plantarum* (three positive and two negative for nitrate reduction) with the same results.

It is apparent from these data that the descriptions of the family Lactobacteriaceae and of the genus *Lactobacillus*, as now given in *Bergey's Manual* (3), should be changed in order to permit the inclusion of certain species that reduce nitrates. Further work is in progress in this laboratory to determine the advisability of making a separate species of those cultures that reduce nitrates.

### References and Notes

- \* Journal Article No. 1672.
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