

phage was shaken twice for 5 min with an equal volume of water-saturated phenol together with 1:10 volume each of 0.1 M ethylenediaminetetraacetic acid, saturated NaCl, and 20% Duponol solution. Ribonucleic acid was not detectable in the aqueous phase by the orcinol reaction. The phenol was removed by ether, and the DNA was precipitated by alcohol. The dried DNA was analyzed by the formic acid method of Wyatt (*In Colowock and Kaplan* [ed.], *Methods in enzymology*, vol. 3, p. 715, 1957), and the base ratio was determined by quantitative paper chromatography.

The results of the base analysis are as follows: guanine-adenine-cytosine-thymine = 33.44:17.21:33.44:15.91. The ratio of adenine plus thymine to guanine plus cytosine is 0.495. The value of the above ratio in *M. phlei* was reported to be 0.50 (Laland et al., *J. Chem. Soc.*, p. 3224, 1952) or 0.46 (Jones et al., *J. Chem. Soc.*, p. 2454, 1957). Although the base ratio of *Mycobacterium* sp. Jucho is unknown, it is suspected to be similar to that of *M. phlei*, because both strains are saprophytic mycobacteria. If this is the case, the base ratio of *phagus choremis* may be regarded as quite similar to that of its host.

## CELL-WALL CONSTITUENTS OF *LACTOBACILLUS DELBRUECKII*

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It was previously reported that the cell wall of *Lactobacillus delbrueckii* 730 (ATCC 9649) contained a ribitol teichoic acid (Ikawa and Snell, *J. Biol. Chem.* **235**:1376, 1960). Baddiley and Davison (*J. Gen. Microbiol.* **24**:295, 1961), however, reported that the same strain of this organism contains a glycerol teichoic acid and no ribitol. To clear up this discrepancy, we re-investigated the cell-wall components of several different strains of *L. delbrueckii*.

The strains studied were designated 730-#1 (ATCC 9649, carried in stock for a number of years by Beverly M. Guirard of this department), 730-#2 (ATCC 9649, recently acquired by Dr. Guirard from the American Type Culture Collection, Washington, D.C.), 730, LD-1, and LD-2 (the latter three strains obtained recently from M. Rogosa, National Institute of Dental Research, Bethesda, Md.). Strains 730-#1 and 730-#2 were rods which produced no gas on glucose, did not ferment lactose, did not grow at 15 C, and produced D(-)-lactic acid. These tests were used to confirm their identity as *L. delbrueckii* (Rogosa and Sharpe, *J. Appl. Bacteriol.* **22**:329, 1959). The organisms were grown, the cell walls isolated, and the constituents determined (Table 1) by previously described methods (Ikawa and Snell, *J. Biol. Chem.* **235**:1376, 1960).

Although variations in phosphorus and reduc-

ing-sugar content were observed between batches and strains, most preparations of cell walls showed between 2 and 3% phosphorus, except strain 730-#2, which had consistently higher phosphorus and approximately 10% reducing sugar. Glucose and glycerol were present in all strains. Galactose was detected in appreciable amounts only in strain 730 (Rogosa). In none of the strains was rhamnose or ribitol (as anhydroribitol) detected. The finding of glycerol in place of ribitol is at variance with our previous report, and in agreement with the results of Baddiley and Davison. Upon microscopic examination, it appeared that our former cell-wall preparation was grossly contaminated with a coccus and, therefore, gave misleading results. Glucosamine, muramic acid, and the major cell-wall amino acids, alanine, glutamic acid, aspartic acid, and lysine, were detected in all the strains examined.

The cell walls of these strains differ from that of *L. delbrueckii* NCIB 7473 in that the latter is reported to contain rhamnose, galactose, and galactosamine in addition to glucose, glucosamine, muramic acid, alanine, glutamic acid, aspartic acid, and lysine (Cummins and Harris, *J. Gen. Microbiol.* **14**:583, 1956).

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TABLE 1. Cell-wall constituents of *Lactobacillus delbrueckii* strains

Constituent	Strain				
	730-#1 (BMG)	730-#2* (BMG)	730 (Ro- gosa)	LD-1* (Ro- gosa)	LD-2 (Ro- gosa)
Phosphorus, % . . . . .	2.5- 5.0	5.0- 6.6	2.4- 3.3	1.2- 1.8	1.8- 2.0
Reducing sugar (as glucose), % . . . . .	9-11	11-14	6-10	7-9	6-12
<i>Sugars and polyols</i>					
Glucose . . . . .	+	+	+	+	+
Galactose . . . . .	-	Trace	+	-	-
Rhamnose . . . . .	-	-	-	-	-
Glycerol . . . . .	+	+	+	+	+
Anhydrosorbitol . . . . .	-	-	-	-	-
<i>Aminosugars</i>					
Glucosamine . . . . .	+	+	+	+	+
Galactosamine . . . . .	-	-	-	-	-
Muramic acid . . . . .	+	+	+	+	+
<i>Major amino acids†</i>					
Alanine . . . . .	+	+	+	+	+
Glutamic acid . . . . .	+	+	+	+	+
Aspartic acid . . . . .	+	+	+	+	+
Lysine . . . . .	+	+	+	+	+
Diaminopimelic acid . . . . .	-	-	-	-	-

\* In one cell-wall preparation of these strains, the phosphorus content was low (1% or less), galactose was the only reducing sugar present, neither glycerol nor anhydrosorbitol was detected, and the major amino acids were alanine, glutamic acid, and diaminopimelic acid. The reason for these anomalous batches is not known.

† In addition to the major cell-wall amino acids, trace amounts of glycine and tyrosine were detected, as well as varying amounts of valine and leucine plus isoleucine, especially in the cell-wall preparations from strains LD-1 and LD-2, which were more difficult to rid of these amino acids than the 730 strains.