"Free" Diaminopimelic Acid and Deoxyribonucleic Acid Homology of *Lactobacillus helveticus*

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Thirty-eight strains of *Lactobacillus helveticus*, including 26 strains of *L. helveticus* biotype *jugurti*, were isolated from natural starters for Grana cheese. Seventeen strains had diaminopimelic acid (DAP) as a cytoplasmic (but not cell wall) component; 21 strains did not contain DAP. DAP-positive and galactose-negative strains may constitute new biotypes of *L. helveticus*. Despite these variations, all of the strains studied displayed high deoxyribonucleic acid (DNA)-DNA homologies to the reference DNA, and the presence or absence of DAP is not considered to be of great taxonomic importance in *L. helveticus*.

During the past 10 years, detailed investigations on the homofermentative, thermophilic lactobacilli normally present in natural whey starters for Grana cheese have been undertaken at our Institute. It has been found (3) that *Lactobacillus helveticus* (including its biotypes) constitutes the species responsible for the lactic acid fermentation in Grana cheese, although small numbers of strains of *L. bulgaricus*, *L. lactis*, and *Streptococcus thermophilus* are also present. The most frequently occurring strains have unusual properties such as: (i) high proteolytic activity (2); (ii) sensitivity to xanthine and guanine (1); (iii) inability to produce normal amounts of lactic acid in milk (3, 13); (iv) presence of "free" diaminopimelic acid (DAP) as a cytoplasmic, but not as a cell-wall, component (4); (v) inability to ferment galactose; (vi) cell morphology variation (18); and (vii) optimal growth temperatures different from those of previously described strains.

Despite wide differences in the quantities of lactic acid produced by strains of *L. jugurti* from Grana cheese starters, Matteuzzi (14) demonstrated high deoxyribonucleic acid (DNA)-DNA homologies (89 to 100%) in all strains. The purpose of this investigation was to determine the extent of DNA-DNA homology among phenotypically different strains of *L. helveticus*, especially those that differ with respect to DAP production and galactose fermentation.

MATERIALS AND METHODS

Bacterial strains. The strains are listed in Table 1. They were isolated from whey cheese starters obtained from different dairies producing Grana cheese. All of the organisms were cultured in MRS broth (7).

Methods. The identities of the strains were determined on the basis of the criteria established by the Subcommittee on the Taxonomy of the Lactobacilli and Closely Related Organisms of the International Committee on Systematic Bacteriology.

Cells were treated with acetic acid (10% vol/vol), and the extracted "free" DAP was determined by the paper chromatography method of Hoare and Work (10).

Cells for DNA extraction were washed twice with saline-ethylenediaminetetraacetic acid (EDTA) (0.15 M NaCl plus 0.1 M EDTA, pH 8) and, since it was difficult to lyse the organisms, a dual system of lysozyme and Pronase was used (6). The isolation and purification of DNA was performed according to the procedure described by Marmur (12). The mole percent of guanine plus cytosine (% G+C) in the DNA of duplicate samples was determined by the thermal melting procedure using equation 9 or 10 of De Ley (5) for calculation. DNA-DNA hybridization was carried out with the single-point competition version of Johnson and Ordal (11) as described in our previous paper (6).

RESULTS AND DISCUSSION

The 38 strains of *Lactobacillus* studied had the physiological characters of *L. helveticus*; 26 of them were considered to be maltose-negative

TABLE 1. Fermentation pattern, free DAP, % G+C, and DNA homology among DAP-positive and -negative strains of *Lactobacillus helveticus*

Bacterial strain	Fermentation of		Free DAP	Mol/G+C	DNA homology relative to strains:	
	maltose	galactose			Mb 66 (DAP+)	Mb 68 (DAP-)
<i>L. helveticus</i>						
biotype <i>jugurti</i>						
Mb 66	—	—	+	38.2	100	98
Mb 83	—	—	+	38.4	81	91
Mb 88	—	—	+	39.8	86	80
Mb 136	—	—	+	39.1	80	96
Mcb 6	—	+	+	38.4	93	95
Mcb 7	—	+	+	38.7	81	81
Mcb 27	—	+	+	38.5	88	98
Mcb 33	—	—	+	38.4	82	90
Mco 4	—	—	+	37.9	74	78
Mco 44	—	—	+	38.3	82	89
Mco 46	—	+	+	38.9	99	96
W 86	—	+	+	38.6	82	94
i 160	—	+	+	40.1	89	86
M 699	—	+	+	39.2	87	88
M 481	—	+	+	38.8	92	85
Mb 68	—	+	—	39.1	97	100
Mb 64	—	—	—	38.6	92	91
Mcb 2	—	+	—	39.0	96	94
Mcb 12	—	—	—	39.3	83	94
Mcb 26	—	+	—	37.9	81	91
Mco 33	—	—	—	38.7	79	84
Mco 42	—	—	—	39.2	81	76
Ms 5	—	+	—	38.2	78	81
Mau 121	—	—	—	38.5	86	94
Mca 56	—	—	—	38.1	80	86
Mca 61	—	—	—	38.6	87	80
<i>L. helveticus</i>						
AH 5	+	+	+	40.4	90	85
WL 1	+	+	+	39.2	92	87
Mcb 35	+	+	—	38.1	84	91
Mb 91	+	—	—	38.9	82	95
H 1	+	+	—	37.5	96	90
H 4	+	+	—	38.6	97	95
H 5	+	+	—	37.4	100	99
H 18	+	+	—	37.5	95	89
M 46	+	+	—	38.1	92	98
P 72	+	+	—	38.4	99	95
V 89	+	+	—	37.9	88	95
V 90	+	+	—	38.7	97	98

biotypes of *L. jugurti*. The maltose and galactose fermentation results, the presence of free DAP, the % G+C, and the genetic homology values among DAP-positive and -negative strains are reported in Table 1. Surprisingly, 15 strains were atypical in that they did not ferment galactose.

The presence of free, i.e., cytoplasmic, DAP in *L. helveticus* strains is thought to result from a disturbance in the regulation of lysine biosynthesis (3). DAP is frequently present in strains of this species isolated from Grana

cheese starters (17 of 38 strains). Moreover, the presence of DAP-positive strains in starters varies from dairy to dairy (4).

The G+C values (37.4 to 40.4 mol%) are in good agreement with those previously reported by Gasser and Mandel (8), Miller et al. (15, 16), Simonds et al. (17), and Dellaglio et al. (6).

The DNA-DNA hybridization experiments show that all of the strains, regardless of DAP content, display high genetic homology to the reference organisms. For instance, *L. helveticus* biotype *jugurti* Mb 66 was DAP positive and

Mb 68 was DAP negative, yet the DNA-DNA homologies ranged from 74 to 100% and from 76 to 100%, respectively. Hence, the presence or absence of cytoplasmic DAP, although possibly of some taxonomic significance, does not reflect any important genetic difference among the organisms studied.

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REPRINT REQUESTS

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