Gas Chromatography Analysis of Cellular Fatty Acids and Neutral Monosaccharides in the Identification of Lactobacilli

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Cellular fatty acids and monosaccharides in a group of 14 lactobacilli were analyzed by gas chromatography and the identity of the components was confirmed by gas chromatography-mass spectrometry. From the same bacterial sample, both monosaccharides and fatty acids were liberated by methanolysis, and in certain experiments, fatty acids alone were released by basic hydrolysis. The results indicate that basic hydrolysis gave more comprehensive information about the fatty acids, but the analysis of monosaccharides was found to be much more useful in distinguishing between different species of lactobacilli. The method described allowed differentiation of 11 of 14 Lactobacillus species, and even single colonies isolated from agar plates could be used for analysis without subculturing.

Gas chromatography has made a considerable contribution to the identification of microorganisms by providing accurate techniques for analyzing bacterial metabolites (6, 15, 17, 20, 24). In the case of lactobacilli, metabolite analyses are of limited value since almost the only metabolite produced by the bacteria is lactic acid (18). Gas chromatography analyses of the cellular fatty acids of some lactobacilli for their identification has been performed with either saponification or methanolysis of the cell wall (4, 21, 23). These two methods give to some extent different results, since acidic methanolysis destroys the cyclopropane fatty acids (7, 8, 11, 12, 16), which are present in large amounts in lactobacilli (21). Alternatively, analysis of the sample by basic hydrolysis degrades the carbohydrates (7). The monosaccharide composition of Lactobacillus species has not been analyzed or used for their identification. The purpose of this study was to develop an analytical method for distinguishing between different species of lactobacilli by analyzing some cellular components obtained from the same sample. We report here a method for analyzing the cellular monosaccharides by methanolysis and the fatty acids by acidic and basic hydrolysis, both performed on the same Lactobacillus species. All the components can be analyzed by gas chromatography with an apolar capillary column. The applicability of the procedure was tested by analyzing single colonies isolated from agar plates to avoid subculturing of the isolated microorganisms.

MATERIALS AND METHODS

Chemicals and solutions. Silver carbonate and acetic acid anhydride were supplied by Fluka AG (Buchs, Switzerland). Trimethylsilylimidazole, hexamethyldisilazane, trimethylchlorosilane, pyridine, glycerol, standard monosaccharides, and solvents were supplied by Merck (Darmstadt, Federal Republic of Germany). Reference standard fatty acid methylesters (FAMEs) were obtained from Applied Science (Oud-Beijerland, The Netherlands).

The 1 N hydrochloric acid in methanol used for the methanolysis was prepared by slowly reacting concentrated

sulfuric acid and sodium chloride and bubbling the evolved dry gas through pure methanol in an ice-cooled glass bottle. The HCl concentration was determined by volumetric titration and corrected to the desired concentration by adding methanol. The reagent was flushed with nitrogen and stored at −20°C.

The internal standard solution used in methanolysis for the analysis of monosaccharides was prepared as follows. myo-Inositol (20 mg) was added to 0.2 ml of water and then diluted to 100 ml with pure methanol. The solution was warmed in a water bath at 50°C for 10 min to completely dissolve inositol, cooled, and stored at −20°C.

Gas chromatography. Both the FAMEs and the monosaccharides were analyzed in the same column. The following capillary columns were used in this study: 35-, 30-, and 25-m glass capillary columns (0.5 mm inner diameter) coated with free fatty acids phase (FFAP), SE30, or OV-1, respectively, and a 25-m fused silica capillary column (0.32 mm inner diameter) coated with OV-1. The temperature program for the analysis of FAMEs was 60°C for 2 min, programmed to rise to 120°C at 40°C/min, remain isothermal for 1 min, and then rise to 235°C at 5°C/min. The temperature program for the monosaccharides was 60°C for 2 min and programmed to rise to 110°C at 40°C/min, remain isothermal for 1 min, and then rise to 215°C at 6°C/min.

The gas chromatograph was a Varian model 3700 equipped with a split-splitless injector (temperature, 250°C) and flame ionization detector (temperature, 260°C). An electronic integrator (Varian model 4270) was connected to the gas chromatograph. The carrier gas was helium at a pressure of 8 lb/in² for the analysis of the monosaccharides and 10 lb/in² for FAMEs. Split flow was 40 ml/min; make-up gas nitrogen was used at a flow rate of 30 ml/min.

Gas chromatography-mass spectrometry. A DANI gas chromatograph (model 3800HR) was coupled directly to a mass spectrometer (JEOL model DX-300). The same columns described above were used. The ionization potential was 70 eV, and the ionization current was 100 μA. Identification of the FAMEs was made by comparing the gas chromatographic retention times and sample spectra with those of authentic standards. In the case of the methylglycosides, authentic monosaccharide standards were reacted

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TABLE 1. *Lactobacillus* species examined

Species	Strain no.	Source
L. acidophilus	ATCC 4356	University of Helsinki
L. brevis	ATCC 14869	University of Helsinki
L. buchneri	L858	P. A. Hansen, University of Helsinki
L. bulgaricus	37-12-4	Valio[a]
L. casei subsp. *casei*	ATCC 393	Valio
L. cellobiosus	L872	P. A. Hansen, University of Helsinki
L. delbrueckii	ATCC 9649	University of Helsinki
L. fermentum	ATCC 14931	University of Helsinki
L. helveticus	CasV	Valio
L. lactis	ATCC 12315	University of Helsinki
L. leichmannii	ATCC 4797	University of Helsinki
L. plantarum	ATCC 14917	University of Helsinki
L. salivarius subsp. *salivarius*	ATCC 11741	University of Helsinki
L. viridescens	NCDO 1655	University of Helsinki

[a] Valio Finnish Cooperative Dairies' Association, Helsinki, Finland.

in parallel with the samples, and the gas chromatograms as well as the mass spectra were compared for identification of the sample components (14).

Preparation of bacterial cultures. The bacterial species used in the study are presented in Table 1. The microorganisms were grown anaerobically for 48 h in MRS broth at 35°C as described by Schneitz et al. (19). After being washed twice with distilled sterile water, the cells were lyophilized and kept under nitrogen until analysis. Samples (weighing from 0.1 to 0.5 mg) of each lyophilized bacteria were used for each analysis in three replications. When single colonies were analyzed, they were isolated from lactobacillus selective agar plates (Merck) after incubation in an anaerobic jar at 37°C for 3 days.

Basic hydrolysis. A slight modification of the method of Jantzen et al. (7) was used for the basic hydrolysis. Lyophilized cells (0.1 to 0.5 mg) as well as isolated colonies in some cases were saponified with 0.5 ml of 1 N NaOH in benzene-methanol (4:6, vol/vol) in a 10-ml Teflon-lined screw-cap vial. Two drops of phenolphthalein in methanol were added to the sample tube, and after being flushed with nitrogen, the vial was heated at 100°C for 30 min. After cooling, the sample was acidified by adding 1 N HCl in methanol until colorless (pH <2). Esterification of the fatty acids was performed by adding 0.8 ml of 10% BCl$_3$ in methanol, followed by heating for 15 min at 100°C. A 1.5-ml amount of a saturated aqueous solution of sodium chloride was added, and the FAMEs were extracted twice with 1.5 ml of hexane-chloroform (4:1, vol/vol) by shaking for 1 min. The solvent was evaporated at room temperature, and the residue was dissolved in 25 μl of hexane. A 2-μl amount was injected into the gas chromatograph in the splitless mode; the range of sensitivity was 10^{-11} A/mV at an attenuation of 8.

Methanolysis. For methanolysis (2, 14), 0.25 ml of 1 M HCl in methanol and 50 μl of internal standard solution (*myo*-inositol) were added to 3.5-ml Teflon-lined screw-cap vials over a known amount of lyophilized bacterial sample (0.1 to 0.5 mg). The vials were flushed with nitrogen, sonicated for 5 min, and reacted at 85°C for 17 h.

In the case of single colonies, the bacteria were carefully removed from the plate with a platinum loop and suspended in 1 ml of acetone in a 1.5-ml vial. The samples were rapidly dried under a stream of nitrogen in a microevaporator (Laborexin Oy, Helsinki, Finland) with a three-neck glass flask with a valve interposed between the water pump and

the evaporator to regulate the size of the vacuum. A 0.15-ml amount of 1 M HCl in methanol was added, and methanolysis was performed as described above.

After methanolysis, the vial was cooled for 5 min at 5°C, and 80 mg of silver carbonate (50 mg for single colonies) was added to neutralize the HCl. The vial was then sonicated for 5 min, and 1.25 ml of methanol was added; the vial was then sonicated for another 1 min and centrifuged at 4,500 × *g* for 10 min. The supernatant was transferred to a conical glass-stoppered test tube and evaporated under nitrogen at room temperature. Sterile distilled water (0.25 ml) and 1.25 ml of a mixture of diethylether-hexane (8:2, vol/vol) were added to the residue containing the FAMEs and methylglycosides, mixed for 30 s, and left to stand for 3 min. The upper phase containing the FAMEs was removed, and the lower phase was rewashed with 1 ml of extraction solvent. The washing solvent was combined with the upper phase, carefully evaporated at room temperature, and dissolved in 25 μl of hexane. Sample (2 μl) was injected into the gas chromatograph in the splitless mode. The lower phase containing the monosaccharides methylglycosides was supplemented with 2 ml of acetone, and the solvent mixture was evaporated under nitrogen at 45°C in a water bath with the microevaporator. The acetone treatment and evaporation were repeated three times. The residue was used for the monosaccharide analyses.

Monosaccharide analyses. The methylglycosides were per-*O*-trimethylsilylated for 60 min at room temperature with 150 μl of pyridine-trimethysilylimidazole-hexamethyldisilazane-trimethylchlorosilane (8:2:1:0.5) prepared by adding the components in the order given. After derivatization, portions of the sample were injected into the gas chromatograph in the silylation reagent. The following washing procedure designed to remove the excess reagent was tested. After derivatization, the sample was diluted with 0.5 ml of hexane and washed for 10 s with 0.5 ml of water. The water phase was discarded, the hexane phase was evaporated to dryness, and the residue was dissolved in 150 μl of hexane.

Computer analyses of results. The coefficients of variation for the fatty acids and monosaccharides, obtained from the different lyophilized bacterial species, were analyzed on an IBM personal computer. Similarities between the species were analyzed by a computed cluster analysis with a BAKT-ID program supplied by Scientific Expert System (Helsinki, Finland). The results for monosaccharides were computed by using both the proportional values and the absolute amounts obtained in the analysis. The proportional values were expressed as percentages of the total amounts. The relationships between the species are presented as dendrograms.

RESULTS

Six fatty acids were present in abundant amounts in the gas chromatograms of all of the species: tetradecanoic (*n*-C$_{14}$), hexadecenoic (*n*-C$_{16:1}$), hexadecanoic (*n*-C$_{16}$), octadecenoic (*n*-C$_{18:1}$), octadecanoic (*n*-C$_{18}$) and 11,12-methylenehexadecanoic (C$_{19cyc}$) acids (Table 2). Small amounts of dodecanoic (*n*-C$_{12}$), pentadecanoic (*n*-C$_{15}$), heptadecanoic (*n*-C$_{17}$), and eicosenoic (*n*-C$_{20:1}$) acids were also found. No hydroxy, branched-chain, or polyunsaturated fatty acids were detected. The values for the fatty acids obtained by both saponification and methanolysis are compared in Table 2. The most prominent difference between these two methods was that methanolysis degraded the cyclopropane fatty acids and resulted in by-products. According to tentative

TABLE 2. Fatty acid composition of lyophilized lactobacilli[a]

Species	Fatty acid (% of total fatty acids)																		
	C_{12}		C_{14}		C_{15}		$C_{16:1}$		C_{16}		C_{17}		$C_{18:1}$		C_{18}		C_{19cyc}		$C_{20:1}$
	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	S
L. acidophilus	0.6	0.7	2.0	2.4	0.1	0.2	4.9	4.6	9.2	6.8	1.5	1.1	41.7	42.5	3.0	1.2	10.4	39.4	0.8
L. brevis	0.4	0.3	3.1	3.5	0.5	0.5	4.5	6.1	27.5	20.5	0.4	0.3	38.3	33.8	5.3	2.0	6.5	32.4	0.4
L. bulgaricus	0.4	0.4	2.2	2.7	0.7	0.6	9.8	11.8	15.0	10.6	1.1	1.0	58.8	57.4	4.5	2.3	2.0	13.0	0.0
L. buchneri	0.3	0.2	1.4	1.7	0.2	0.2	3.4	3.9	45.0	38.0	1.0	0.9	13.2	12.5	4.3	2.4	13.7	40.0	Tr
L. casei subsp. casei	Tr	Tr	3.0	4.1	0.2	0.3	7.5	5.3	22.1	17.2	1.1	0.9	39.2	32.8	4.0	1.4	10.1	37.8	0.1
L. cellobiosus	0.2	0.1	1.7	1.9	0.2	0.1	2.0	3.4	20.5	15.4	1.0	1.0	46.0	50.4	5.2	1.3	8.1	26.2	0.0
L. delbrueckii	0.2	0.2	2.0	1.8	0.3	0.2	2.9	3.0	29.5	23.0	0.5	0.5	43.0	43.6	7.5	3.9	4.6	20.3	3.3
L helveticus	Tr	Tr	4.8	6.0	0.4	0.4	5.3	2.4	28.0	30.6	Tr	Tr	22.5	24.1	4.6	2.4	9.5	27.8	0.2
L. leichmannii	Tr	Tr	1.1	1.3	0.2	0.1	19.4	16.3	28.3	22.3	0.6	0.7	36.0	42.5	3.1	0.7	3.8	15.0	0.5
L. plantarum	0.2	0.1	2.9	3.1	0.4	0.4	4.0	6.2	29.0	22.2	1.0	0.9	34.7	30.4	6.5	1.1	9.3	34.7	0.8
L. salivarius subsp. salivarius	0.1	0.2	0.8	1.0	0.1	0.2	2.5	2.7	35.0	26.1	0.2	0.3	44.8	45.6	6.8	3.2	4.0	17.6	2.0
L. viridescens	0.3	0.3	1.6	1.4	0.7	0.6	3.7	3.4	30.5	25.1	1.0	0.9	34.5	32.1	5.5	1.7	8.4	33.8	0.6
L. lactis	0.3	0.2	5.4	4.0	0.4	0.0	5.1	5.3	38.0	45.3	0.3	0.3	27.5	19.0	5.2	0.7	7.1	25.1	0.3
L. fermentum	Tr	Tr	1.6	1.9	2.0	0.2	2.9	3.7	21.0	21.6	0.7	0.8	48.1	44.1	5.2	2.1	6.0	24.0	1.5
Coefficient of variation	±6.4	±6.1	±3.8	±4.2	±5.3	±5.1	±1.4	±1.5	±3.3	±3.5	±5.6	±5.7	±4.6	±4.9	±1.8	±1.7	±6.7	±4.5	±5.5

[a] Analyzed by methanolysis (M) and saponification (S). Values are averages of three replications.

identification of the degradation products of standard C_{19} cyclopropane fatty acid by mass spectrometry, the main by-products were identified as a mixture of methoxy esters derived from methyl-11,12-methyleneoctadecanoate. Two additional compounds eluted at the beginning of the gas chromatogram proved to be decomposition products of the phenolphthalein used as a pH indicator in the basic hydrolysis. Another difference between the results of the two methods was the larger amount of n-C_{18} and n-C_{16} fatty acids obtained from the sample by acid methanolysis, since a number of them were probably present in amide linkages in the cellular lipidic fraction (Table 2).

The amounts of different monosaccharides in lyophilized and methanolized cells are reported in Table 3. The samples were injected into the gas chromatograph in the silylation reagent, since the washing procedure resulted in decomposition of glycerol and ribitol and appearance of additional, unidentified peaks (Fig. 1). The carbohydrates detected were ribitol, ribose, rhamnose, galactose, and glucose. Glycerol was present in all of the microorganisms.

Ribose and rhamnose appeared as a single peak on the apolar column, but were separated on a 35-m glass capillary column coated with FFAP. The presence of either ribose or rhamnose or both can also be detected with an apolar column, because ribose gives a minor peak before its main peak in the gas chromatogram. In the case of rhamnose, the

TABLE 3. Cellular monosaccharide composition of lyophilized lactobacilli[a]

Species	Glycerol		Ribitol		Ribose		Ribose and rhamnose		Galactose		Glucose	
	%	A	%	A	%	A	%	A	%	A	%	A
L. acidophilus	57.6	94.5	0.0	0.0	8.0	13.5	0.0	0.0	16.5	27.0	17.7	29.0
L. brevis	27.9	105.0	7.0	26.3	18.7	70.3	0.0	0.0	13.6	51.0	32.7	122.6
L. bulgaricus	26.7	235.0	0.0	0.0	4.9	43.0	0.0	0.0	56.1	493.0	12.3	100.0
L. buchneri	49.4	175.0	0.0	0.0	5.4	19.0	0.0	0.0	12.6	44.5	32.4	114.5
L. casei subsp casei	13.8	171.5	3.8	47.5	0.0	0.0	16.3	203.0	27.6	343.5	38.3	475.8
L. cellobiosus	25.4	63.5	0.0	0.0	4.2	10.5	0.0	0.0	38.1	95.0	32.0	80.5
L. delbrueckii	9.8	123.0	0.0	0.0	0.0	0.0	4.7	59.0	16.2	203.0	69.0	868.5
L. fermentum	19.8	112.5	0.0	0.0	0.0	0.0	11.0	63.5	36.0	204.5	33.0	187.5
L. helveticus	47.4	127.0	0.0	0.0	10.4	28.0	0.0	0.0	27.6	74.0	14.5	39.0
L. lactis	56.6	221.6	0.0	0.0	8.6	33.3	0.0	0.0	13.6	53.0	20.9	81.3
L. leichmannii	42.8	131.5	0.0	0.0	8.2	25.2	0.0	0.0	31.7	97.5	17.2	53.0
L. plantarum	26.1	120.0	14.3	66.0	15.9	73.0	0.0	0.0	10.6	49.0	33.0	152.0
L. salivarius subsp. salivarius	10.1	108.0	0.0	0.0	0.0	0.0	4.1	44.2	14.6	156.0	71.0	761.0
L. viridescens	18.2	106.0	0.0	0.0	3.3	19.5	0.0	0.0	43.5	254.4	34.8	202.5
Coefficient of variation	±4.2	±6.2	±6.1	±3.5	±3.7	±4.4	±6.3	±4.6	±4.2	±6.6	±3.5	±6.4

[a] Obtained after methanolysis. Values are averages of three replications and are expresed as percentage of total monosaccharides and as absolute values (A, area of the peak in square millimeters. The coefficient of variation of the internal standard (myo-inositol) was ±3.2.
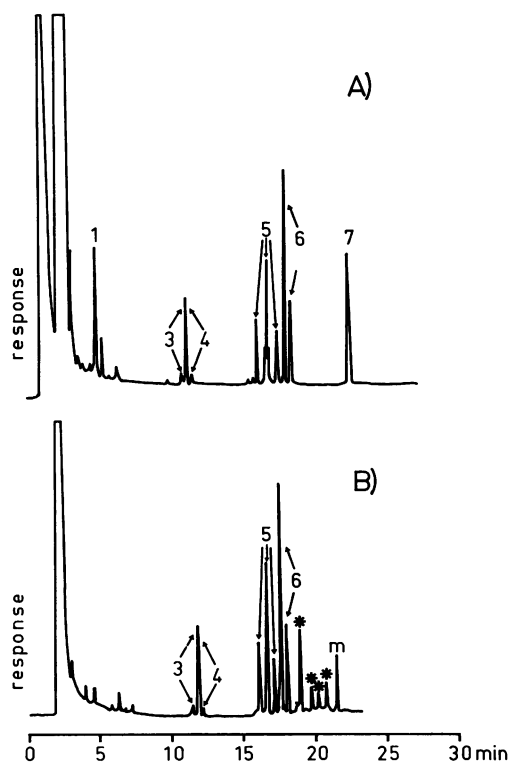
FIG. 1. Gas chromatograms of silylated methylglucosides released form methanolyzed cells of L. fermentum. (A) Lyophilized cells; (B) single colony picked from an agar plate. Sample A was injected with the derivatization reagent. Sample B was washed with water before being injected. In sample B the glycerol was decomposed and artifacts appeared after the glucose peaks. The internal standard in sample A was myo-inositol; in sample B it was mannitol. The major peaks of ribose and rhamnose eluted together, but their presence was detected because the main peak was preceded and followed by a minor peak (see text). The columns used were a 30-m glass capillary coated with SE-30 (A) and 25-m column coated with OV-1 (B). Peaks: 1, glycerol; 3, ribose; 4, rhamnose; 5, galactose; 6, glucose; 7, inositol; m, mannitol; *, artifact.

major peak was followed by the minor one (Fig. 1). Lactobacillus casei, L. delbrueckii, L. fermentum, and L. salivarius contained both ribose and rhamnose that were characterized by minor peaks before and after the main peak. Ribitol was detected only in L. brevis, L. casei, and L. plantarum, while L. salivarius and L. delbrueckii had the largest amount of glucose.

The effect of the agar culture medium on the cell wall carbohydrates was studied by culturing L. fermentum on agar plates and analyzing the monosaccharide composition of isolated colonies. Figure 1 shows gas chromatograms of the monosaccharide patterns of cells cultivated in broth medium and a single colony from an agar plate. The monosaccharide patterns were very similar and were not affected by the culture medium.

The similarity between the Lactobacillus species according to the fatty acid composition is shown as dendrograms in Fig. 2. The similarity level obtained from the results based on the saponification procedure was 72%. Only four clusters could be distinguished at the 90% similarity level. The values obtained by the methanolysis procedure were very similar. L. acidophilus differed from all the other lactobacilli studied on the basis of fatty acid composition. L. leichmannii and L.
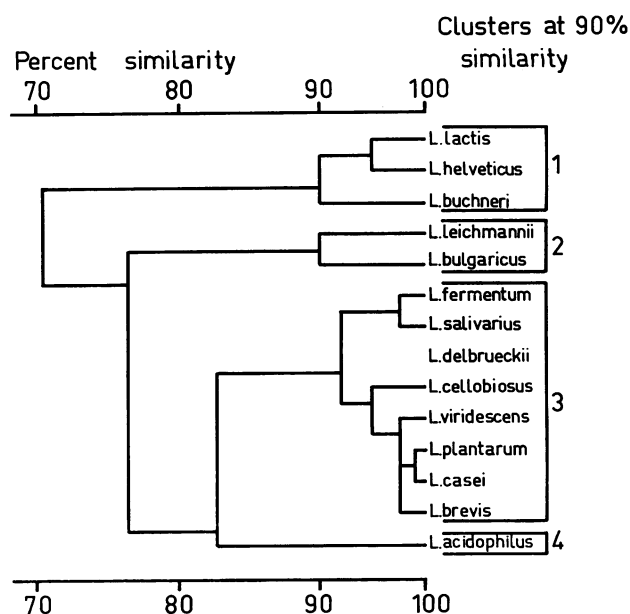

FIG. 2. Dendrogram generated by analysis of the cellular fatty acids obtained by saponification of various species of Lactobacillus. Clusters 1 to 4 were formed at 90% similarity.

bulgaricus also formed a group of their own. L. buchneri produced a single cluster after methanolysis (data not shown) but showed a close relationship with L. lactis and L. helveticus when saponification was performed.

The relationship between the species according to the monosaccharide composition is shown in Fig. 3 and 4. The level of similarity was 42% when the proportional values (Table 3) were used (Fig. 3) and 24% when the absolute values (Table 3) were considered (Fig. 4). At the 90% similarity level, six clusters were distinguished by the pro-
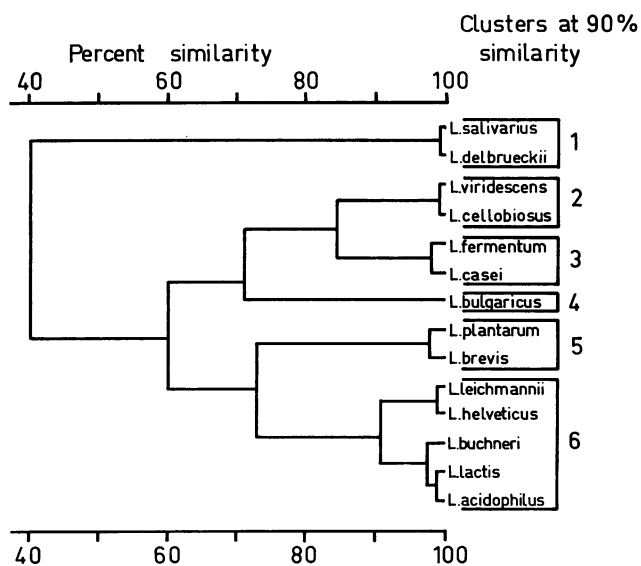

FIG. 3. Dendrogram obtained by cluster analysis of the monosaccharide composition of 14 species of Lactobacillus. The cells were methanolyzed, and the relative percentages of the monosaccharides were taken into account. Clusters 1 to 6 were formed at 90% similarity.

Percent similarity

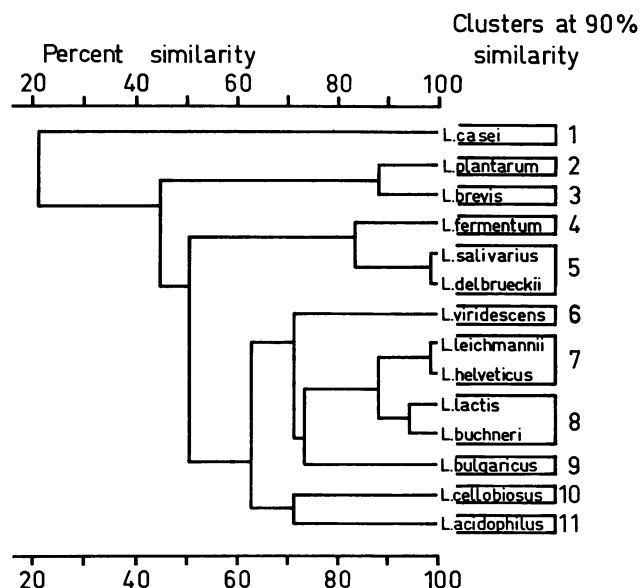Clusters at 90% similarity

20　40　60　80　100

FIG. 4. Dendrogram generated by cluster analysis of the monosaccharide composition of the methanolyzed bacterial cells. The absolute values obtained by gas chromatography analysis of the carbohydrates were taken into account. Clusters 1 to 11 were formed at 90% similarity.

portional values. When the absolute values were taken into account, 11 clusters were obtained at the same similarity level. It is important to note that analysis of the absolute values of the monosaccharides provided better identification of the *Lactobacillus* species than their percentage values, as shown in Fig. 4. The combined results indicate that the differences in the cell wall monosaccharide composition of *Lactobacillus* species are much more pronounced than those of the fatty acid composition. Thus, cell wall monosaccharide analyses are more useful than fatty acid analyses in identifying different species.

DISCUSSION

Two procedures for fatty acid analysis—basic hydrolysis (saponification) and methanolysis—and one for monosaccharides were used in this study to investigate and identify 14 lactobacilli species. Saponification is fast, can be carried out in a single test tube, and does not result in degradation of the cyclopropane fatty acids, but the data provided by the procedure are not sufficient for distinguishing between the lactobacilli. Methanolysis gives results similar to the saponification procedure for the composition of the fatty acids, except for lactobacillic acid (C_{19cyc}), which is partially degraded during the procedure. The advantage of acid methanolysis is that the monosaccharide and fatty acid compositions can be analyzed from the same sample following a single treatment.

Gas chromatographic analysis of the monosaccharides (Fig. 3 and 4) essentially increased the possibility of identifying and distinguishing among *Lactobacillus* species. To test the applicability of the method for single colonies isolated from agar plates, *L. fermentum* was cultured on two different culture media. The monosaccharide pattern was not found to be affected by the type of culture medium. This is an important finding, since it is well known that the composition of the medium has a marked influence on the fatty acid composition of bacterial cells (5, 9, 10, 13, 22).

Methylglycosides of some sugars appear as multiple peaks in gas chromatograms as a result of anomerization and ring isomerization. This pattern of multiple peaks was found to be useful in some cases for confirming the identity of the carbohydrates. The methanolysis conditions were optimized as described by Chambers and Clamp (2) and Mononen (14) by using 1 M HCl in methanol in the procedure and removing HCl with silver carbonate to avoid loss of monosaccharides. We found that the amount of water present in methanol-HCl increased the strength of the acid and influenced the chromatogram patterns both qualitatively and quantitatively (data not shown). The same was true for the reaction time and temperature. This means that it is necessary to standardize the analysis conditions and the reagents to obtain good reproducibility of the results. Trimethylsilylation was preferred over other possible carbohydrate derivatization techniques in this work because it gives stable derivatives and is easy to perform. The reagent does not damage apolar chromatographic columns and is not very sensitive to atmospheric moisture, as opposed, for instance, to trifluoroacetic anhydride (1), which is also used for derivatizing carbohydrates.

Although the washing procedure of the silylation mixture resulted in degradation of glycerol and ribitol, it proved to be useful when a column coated with polar phase was used or interfering compounds, such as carboxylic acids, amino acids, etc., were present in the sample. In fact, polyhydroxy trimethylsilyl ethers such as saccharides will not be hydrolyzed by brief exposure to water but remain undecomposed if they are first diluted in an apolar nonaqueous solvent (3). The other components and the excess reagent will be hydrolyzed and washed away.

The results of the composition of bacterial fatty acids and monosaccharides were subject to cluster analysis to evaluate their applicability to the identification of *Lactobacillus* species. It turned out that at the 90% similarity level, fatty acid patterns resulted in 4 clusters, monosaccharide patterns in 6 clusters, and absolute monosaccharide amounts in 11 clusters. These results indicate that in addition to monosaccharide proportions, their absolute amounts in the cell walls are informative in differentiating among the *Lactobacillus* species. The combined evidence demonstrates that it was possible by using the procedure described to identify 11 of 14 *Lactobacillus* species even when they were single colonies grown on agar plates. This indicates that the method may be useful in laboratories working with lactobacilli and their identification.

LITERATURE CITED

1. Alvin, C., L. Larsson, M. Magnusson, P. A. Mårdh, G. Odham, and G. Westerdahl. 1983. Determination of fatty acids and carbohydrate monomers in micro-organisms by means of glass capillary gas chromatography. J. Gen. Microbiol. 129:1–5.
2. Chambers, R. E., and J. R. Clamp. 1971. An assessment of methanolysis and other factors used in the analysis of carbohydrate-containing materials. Biochem. J. 125:1009–1018.
3. Clay, K. L., and R. C. Murphy. 1979. New procedure for isolation of amino acids based on selective hydrolysis of amino acids trimethylsilyl derivatives. J. Chromatogr. 164:417–426.
4. Dainty, R. H., C. M. Hibbard, and R. A. Edwards. 1984. Cellular fatty acids of streptobacteria isolated from vacuum packaged meats. Syst. Appl. Microbiol. 5:233–240.
5. Farshtchi, D., and N. M. McClung. 1970. Effect of substrate on fatty acid production in *Nocardia asteroides*. Can. J. Microbiol. 16:213–217.
6. Holdeman, L. V., and W. E. C. Moore (ed.). 1972. Anaerobe laboratory manual, 2nd ed., Virginia Polytechnic Institute and State University, Blacksburg, Va.

7. Jantzen, E., K. Bryn, N. Hagen, T. Bergan, and K. Bøvre. 1978. Fatty acids and monosaccharides of *Neisseriaceae* in relation to established taxonomy. Natl. Inst. Public Health Ann. (Oslo) 1:59–71.

8. Jantzen, E., and J. Lassen. 1980. Characterization of *Yersinia* species by analysis of whole-cell fatty acids. Int. J. Syst. Bacteriol. 30:421–428.

9. Kamio, Y., Y. Kanegasaki, and H. Takahashi. 1970. Fatty acid and aldehyde compositions in phospholipids of *Selenomonas ruminantium* with reference to growth conditions. J. Gen. Appl. Microbiol. 16:29–37.

10. Knivett, V. A., and J. Cullen. 1965. Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem. J. 96:771–776.

11. Lambert, M. A., and C. W. Moss. 1977. Degradation of bacterial cyclopropane acids with boron trihalide reagents. Microbios 18: 51–58.

12. Lambert, M. A., and C. W. Moss. 1983. Comparison of the effects of acid and base hydrolyses on hydroxy and cyclopropane fatty acids in bacteria. J. Clin. Microbiol. 18:1370–1377.

13. Marr, A. G., and J. L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. 84:1260–1267.

14. Mononen, I. 1981. Quantitative analysis, by gas-liquid chromatography and mass fragmentography, of monosaccharides after methanolysis and deamination. Carbohydr. Res. 88:39–50.

15. Moore, W. E. C. 1970. Relationships of metabolic products to taxonomy of anaerobic bacteria. Int. J. Syst. Bacteriol. 20: 535–538.

16. Moss, C. W. 1978. New methodology for the identification of nonfermenters: gas-liquid chromatographic chemotaxonomy, p. 182–195. *In* G. L. Gilardi (ed.), Glucose nonfermenting gram-negative bacteria in clinical microbiology. CRC Press, Inc., West Palm Beach, Fla.

17. Rizzo, A. F. 1980. Rapid gas chromatographic method for identification of metabolic products of anaerobic bacteria. J. Clin. Microbiol. 11:418–421.

18. Rogosa, M. 1974. Genus *Lactobacillus*, p. 576–593. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.

19. Schneitz, C., E. Seuna, and A. Rizzo. 1981. The anaerobically cultured cecal flora of adult fowls that protects chickens from *Salmonella* infections. Acta Pathol. Microbiol. Scand. B 89: 109–116.

20. Sutter, V. L., V. L. Vargo, and S. M. Finegold. 1975. Wadsworth anaerobic bacteriology manual, 2nd ed. Wadsworth Hospital Center Department of Medicine, Los Angeles.

21. Uchida, K., and K. Mogi. 1973. Cellular fatty acid spectra of *Sporolactobacillus* and some other *Bacillus-Lactobacillus* intermediates as a guide to their taxonomy. J. Gen. Appl. Microbiol. 19:129–140.

22. Vaczi, I., I. Redai, and A. Rathy. 1967. Changes in the fatty acid composition of *Staphylococcus aureus* under various cultural conditions. Acta Microbiol. Acad. Sci. Hung. 14:293–298.

23. Veerkamp, J. H. 1971. Fatty acids composition of *Bifidobacterium* and *Lactobacillus* strains. J. Bacteriol. 108:861–867.

24. Willis, A. T. 1977. Anaerobic bacteriology, clinical and laboratory practice, 3rd ed. Butterworths, London.