Identification of Lactobacilli from Sourdough and Description of *Lactobacillus pontis* sp. nov.

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The microflora of sourdough preparations was investigated by examining the physiological characteristics, whole-cell protein patterns, and 16S rRNA sequences of *Lactobacillus* isolates. Strains isolated from sourdough were placed in the species *Lactobacillus brevis*, *Lactobacillus sanfrancisco*, and *Lactobacillus reuteri*. 16S rRNA sequences were determined for *L. brevis*, *Lactobacillus fructivorans*, *Lactobacillus fermentum*, *L. sanfrancisco*, and *L. reuteri*, and oligonucleotide probes for fast specific identification of these sourdough lactobacilli were deduced. The physiological characteristics, protein patterns, and 16S rRNA sequences of these organisms were compared with data for other sourdough lactobacilli and additional reference strains. Strains of a *Lactobacillus* species were isolated from rye sourdough; these strains may account for most of the flora in sourdough made from wheat or rye. These organisms were differentiated from other sourdough lactobacilli by their protein pattern, 16S rRNA sequence, G+C content, and physiological properties. The 16S rRNA sequence of this species was determined, and we constructed a phylogenetic tree which reflected the relationships of this species to other lactobacilli. This organism is closely related to *L. reuteri*. A new *Lactobacillus* species, *Lactobacillus pontis*, is proposed. The type strain is *L. pontis* LTH 2587 (= DSM 8475 = LMG 14187). We describe a general strategy in which a polyphasic approach was used to characterize a new species.

Sourdough starter preparations are used to initiate and carry out the essential acidification of rye flour-containing doughs and, in addition, are traditionally used for flavoring wheat doughs. As these doughs are not prepared aseptically, their hygienic safety and high quality must be ensured by the use of well-defined, traditional process conditions. An understanding of the microbial ecology of sourdough is a prerequisite for the development of well-defined single- and multiple-strain preparations, as they are known in the dairy industry. "*Lactobacillus brevis* subsp. *lindneri*" has been identified as the typical organism in rye sourdough (18, 19), whereas the characteristic organism in wheat sourdough was described as the new species *Lactobacillus sanfrancisco* by Kline and Sugihara (10). Subsequently, the "*L. brevis* subsp. *lindneri*" strains were placed in the species *L. sanfrancisco* (21). These organisms account for most of the microbial flora in sourdough (2). *L. sanfrancisco* can be distinguished from *L. brevis* and "*Lactobacillus lindneri*" by the different amino acids in the interpeptide bridge of the peptidoglycan. *L. sanfrancisco* contains L-alanine, whereas *L. brevis* and "*L. lindneri*" contain D-aspartic acid in their interpeptides (15).

In our experience, *L. sanfrancisco*, strains of *L. brevis*, *Lactobacillus fermentum*, and *Lactobacillus fructivorans* are the predominant organisms in sourdough. Taxonomic identification of these organisms by traditional methods, such as sugar fermentation patterns and cell wall analysis, is time consuming and not very reliable. Some of the organisms hardly grow on laboratory media and may escape isolation, and they cannot be identified by standard procedures. Thus, the composition of the microbial flora in these environments is difficult to determine. On the other hand, knowledge concerning the composition of the microflora is an essential prerequisite for ecological and metabolic studies. Finally, these organisms influence the sensory quality of the bread as the metabolic products formed are strongly affected by the ratio of homofermentative lactobacilli to heterofermentative lactobacilli, and the organisms may influence the quality of the sourdough.

In this paper we describe the use of whole-cell protein pattern analysis and 16S rRNA sequence analysis in combination with physiological data to characterize taxonomic relationships among lactobacilli isolated from sourdough. Moreover, we designed 16S rRNA-targeted oligonucleotide probes for rapid identification of the relevant species, including *Lactobacillus pontis* sp. nov.

MATERIALS AND METHODS

Organisms and growth conditions. The bacterial strains investigated and the strains used to construct and evaluate the specificity of the probes are listed in Table 1. The organisms used in the taxonomic evaluation of sourdough lactobacilli are shown in Fig. 1. Strains were grown in sanfrancisco medium, which contained (per liter) 10 g of tryptone, 2 g of meat extract, 7 g of yeast extract, 7 g of glucose, 7 g of fructose, 7 g of maltose, 2 g of sodium gluconate, 5 g of sodium acetate · $3H_2O$, 5 g of citric acid (diammonium salt), 2.5 g of KH_2PO_4 · $3H_2O$, 0.2 g of $MgSO_4$ · $7H_2O$, 0.05 g of $MnSO_4$ · $4H_2O$, 0.01 g of $FeSO_4$ · $7H_2O$, 0.5 g of cysteine HCl, 1 g of Tween 80, 21 g of fresh baker's yeast, and 50 g of rye or wheat bran; the pH was 5.4. When this medium was used as a broth medium, solid compounds were removed after autoclaving and centrifugation twice for 15 min at 5,000 × *g*. Alternatively, cells were grown in modified MRS medium, which contained (per liter) 10 g of tryptone, 5 g of meat extract, 5 g of yeast extract, 7 g of glucose, 7 g of fructose, 7 g of maltose, 2 g of sodium gluconate, 5 g of sodium acetate · $3H_2O$, 2 g of citric acid (diammonium salt),

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TABLE 1. Specificity of oligonucleotide probes

Strain	L. sanfrancisco probe	L. brevis probe	L. fructivorans probe	L. fermentum probe	L. pontis probe	L. reuteri probe	Universal probe
L. sanfrancisco ATCC 27651[Tb]	+	−	−	−	−	−	+
L. sanfrancisco ATCC 27652	+	−	−	−	−	−	+
L. sanfrancisco ATCC 27653	+	−	−	−	−	−	+
L. sanfrancisco DSM 20663	+	−	−	−	−	−	+
L. sanfrancisco LTH 671	+	−	−	−	−	−	+
L. sanfrancisco LTH 1728	+	−	−	−	−	−	+
L. sanfrancisco LTH 1729	+	−	−	−	−	−	+
L. sanfrancisco LTH 1730	+	−	−	−	−	−	+
L. sanfrancisco LTH 2582	+	−	−	−	−	−	+
Lactobacillus sp. strain LTH 2590	+	−	−	−	−	−	+
Lactobacillus sp. strain LTH 2594	+	−	−	−	−	−	+
Lactobacillus sp. strain LTH 2595	+	−	−	−	−	−	+
Lactobacillus sp. strain LTH 2598	+	−	−	−	−	−	+
L. reuteri DSM 20016[Tb]	−	−	−	−	−	+	+
Lactobacillus sp. strain LTH 2584	−	−	−	−	−	+	+
L. pontis LTH 2585	−	−	−	−	+	−	+
L. pontis LTH 2586	−	−	−	−	+	−	+
L. pontis LTH 2587[Tb]	−	−	−	−	+	−	+
L. pontis LTH 1731	−	−	−	−	+	−	+
L. pontis LTH 1735	−	−	−	−	+	−	+
L. brevis DSM 20054[Tb]	−	+	−	−	−	−	+
Lactobacillus sp. strain LTH 2588	−	+	−	−	−	−	+
L. fructivorans DSM 20203[Tb]	−	−	+	−	−	−	+
L. fructivorans LTH 669	−	−	+	−	−	−	+
L. homohiochii DSM 20571[T]	−	−	+	−	−	−	+
L. fermentum DSM 20052[Tb]	−	−	−	+	−	−	+

[a] The probes were deduced from the 16S rRNA sequences of the organisms. The following strains did not react with any of the probes: Lactobacillus acidophilus DSM 20079[T], Lactobacillus alimentarius DSM 20249[T], Lactobacillus buchneri DSM 20057[T], Lactobacillus casei subsp. casei DSM 20011[T], Lactobacillus rhamnosus DSM 20021[T], Lactobacillus delbrueckii subsp. bulgaricus DSM 20081[T], Lactobacillus delbrueckii subsp. delbrueckii DSM 20074[T], Lactobacillus delbrueckii subsp. lactis DSM 20072[T], Lactobacillus farciminis DSM 20184[T], Lactobacillus hilgardii DSM 20176[T], "L. lindneri" LTH 2505, "Lactobacillus pastorianus" DSM 20197, Lactobacillus plantarum DSM 20174[T], L. plantarum DSM 20205, Lactobacillus viridescens DSM 20410[T], Leuconostoc mesenteroides subsp. dextranicum DSM 20484[T], and Pediococus acidilactici DSM 20333[T].

[b] Strain used to design a probe.

2.6 g of K₂HPO₄ · 3H₂O, 0.1 g of MgSO₄ · 7H₂O, 0.05 g of MnSO₄ · 4H₂O, 0.5 g of cysteine HCl, and 1 g of Tween 80; the pH was 6.3. Solid media contained agar (15 g/liter).

Physiological characterization. Sugar fermentation patterns were determined by using the API 50 system (API, Biomerieux, Nürtingen, Germany). Cell walls were isolated by the method of Schleifer and Kandler (16). The amino acids of the cell walls were determined by the FMOC method (1). Other physiological properties were determined by the methods described by Kandler and Weiss (8) and Hammes et al. (6). The DNA G+C contents of lactobacilli were determined performed by high-performance liquid chromatography at the Deutsche Sammlung von Mikroorganismen und Zellkulturen,

Braunschweig, Germany. An electron micrograph was taken with Formvar-coated EM grids carrying dried, nonstained cells that were in a solution containing 1 g of tryptone per liter and 8.5 g of NaCl per liter and were fixed with 1% glutaraldehyde and a model CEM 902 energy-filtering transmission electron microscope (Zeiss, Jena, Germany).

Protein pattern analysis. To cultivate bacteria for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, cultures were grown in Roux flasks containing 120-ml portions of sterile MRS agar (Oxoid) and inoculated with 10-ml portions of a 24-h-old MRS broth culture; the flasks were incubated aerobically for 24 h at 30°C. Then, 30 ml of sodium phosphate-buffered saline (40.5 ml of Na₂HPO₄ · 12H₂O [0.2 mol/liter], 9.5 ml of NaH₂PO₄ · 2H₂O [0.2 mol/liter], 8 g of NaCl, and enough double-distilled water to bring the final volume up to 1 liter) was added to each Roux flask, and the bacterial lawn was harvested with a bent glass rod, filtered through nylon gauze into a centrifuge tube, and centrifuged with a Sorvall model RC-2 centrifuge at 10,000 rpm by using a type SS34 rotor. The resulting pellet was washed twice in the same way.

Preparation of cell extracts. About 70 to 80 mg (wet weight) of bacterial cells was suspended in 0.9 ml of sample treatment buffer (0.75 g of Tris, 5 ml of β-mercaptoethanol, 10 ml of glycerol, and enough double-distilled water to bring the final volume up to 100 ml; pH 6.8) in an Eppendorf tube and sonicated for 3 min on ice with a Labsonic 2000 sonicator (Braun, Melsungen, Germany) by using a needle probe tip (length, 127 mm; diameter, 4 mm) and the "LOW" position at a maximum output of 50 W. Subsequently, 0.1 ml of a 20% (wt/vol) SDS solution was added, and the mixture was vortexed and heated for 10 min at 95°C. After cooling and centrifugation for 10 min at 10,000 rpm, the supernatant was stored at −12°C for immediate use or at −80°C for long-term storage. Electrophoresis was performed as described by Laemmli (11) by using a 12% (wt/vol) polyacrylamide–SDS gel slab. Gels were dried with a Bio-Rad model 224 slab dryer.

Quantitative comparison of the protein electrophoretic patterns and construction of the data base. Protein electropherograms were scanned and digitized by using an Ultroscan laser densitometer (LKB, Bromma, Sweden) linked to a Commodore personal computer equipped with an analog-to-digital converter interface card. Data were registered, processed, and stored in data base format by using the GELCOMPAR software package (version 2.0) (20). The normalized traces (using points 10 to 320 of the 400 points of each interpolated trace) were compared quantitatively by using the techniques described by Kersters and De Ley (9) and Pot et al. (14). The levels of similarity between pairs of traces were expressed by the Pearson product moment correlation coefficient, and clustering was performed by the unweighted pair group method, using average linkage (17).

16S rRNA sequence analysis. Genomic DNA was extracted and purified as described by Lewington et al. (12). In vitro amplification and sequence determinations of almost complete 16S rRNA genes were performed as described previously (22).

Phylogenetic analysis. The 16S rRNA primary structures were added to an alignment of 1,500 homologous bacterial sequences. Distance matrix and parsimony analyses of a set that included all available 16S rRNA sequences from gram-

FIG. 1. Mean correlation coefficient dendrogram. The mean correlation coefficients were calculated by the unweighted average pair group method for all strains investigated. Points 10 to 320 of the 400-point traces were used to calculate levels of similarity for individual pairs of traces. For every species or subspecies the number of strains used is indicated in parentheses. The protein pattern of the type strain of each of the species falls into its respective cluster.
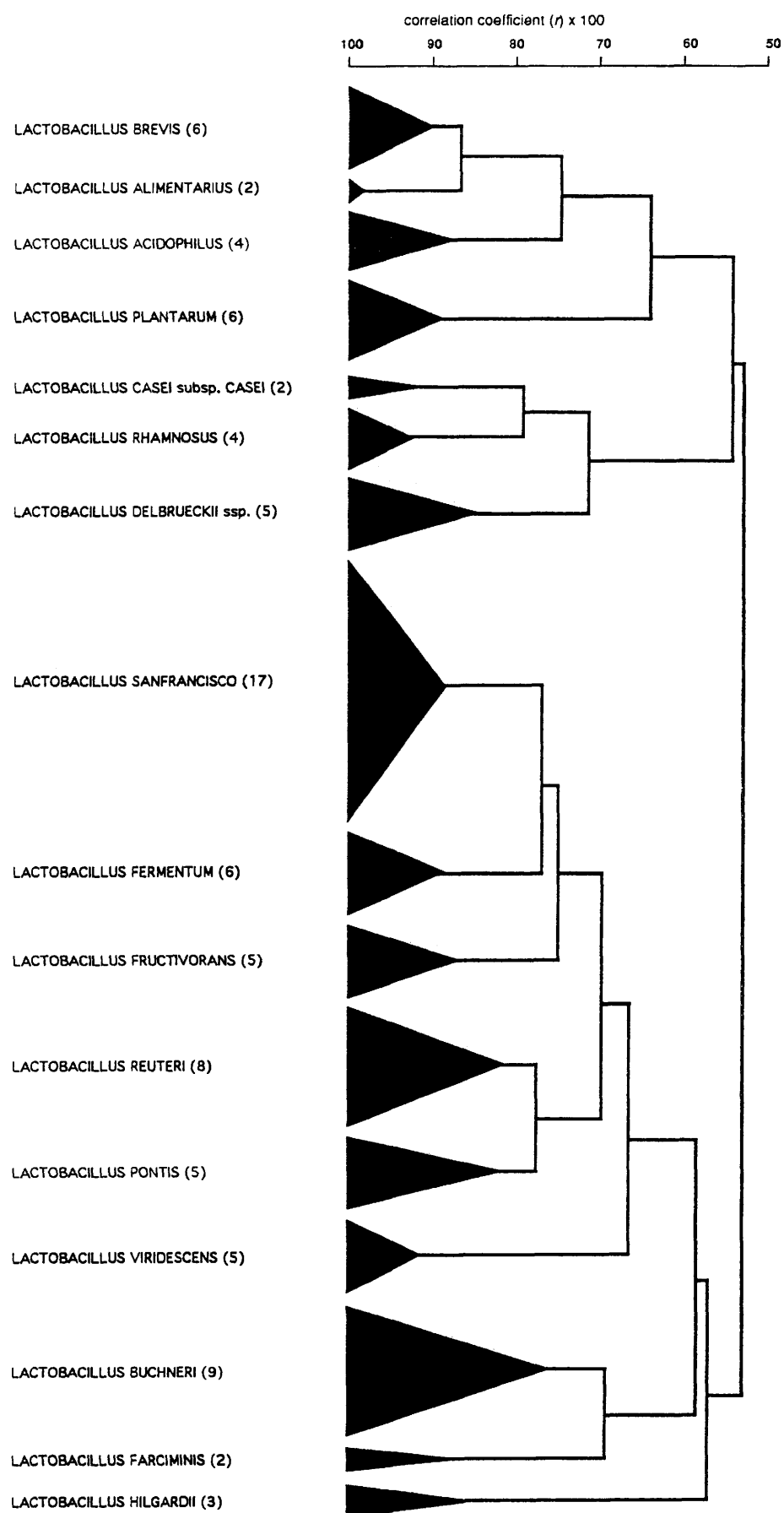
correlation coefficient (r) x 100

100   90   80   70   60   50

LACTOBACILLUS BREVIS (6)

LACTOBACILLUS ALIMENTARIUS (2)

LACTOBACILLUS ACIDOPHILUS (4)

LACTOBACILLUS PLANTARUM (6)

LACTOBACILLUS CASEI subsp. CASEI (2)

LACTOBACILLUS RHAMNOSUS (4)

LACTOBACILLUS DELBRUECKII ssp. (5)

LACTOBACILLUS SANFRANCISCO (17)

LACTOBACILLUS FERMENTUM (6)

LACTOBACILLUS FRUCTIVORANS (5)

LACTOBACILLUS REUTERI (8)

LACTOBACILLUS PONTIS (5)

LACTOBACILLUS VIRIDESCENS (5)

LACTOBACILLUS BUCHNERI (9)

LACTOBACILLUS FARCIMINIS (2)

LACTOBACILLUS HILGARDII (3)

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TABLE 2. Matrix of overall 16S rRNA sequence similarity values for *Lactobacillus* spp. and *Escherichia coli*

Taxon	% rRNA sequence similarity with:															
	L. oris	*L. vaginalis*	*L. reuteri*	*L. fermentum*	*L. plantarum*	*L. brevis*	*L. buchneri*	*L. hilgardii*	*L. fructivorans*	*L. sanfrancisco* ATCC 27651^T	*L. sanfrancisco* DSM 20663	*L. alimentarius*	*L. sake*	*L. casei*	*L. salivarius*	*E. coli*
L. pontis	96.32	96.79	96.29	92.72	90.15	90.92	90.63	90.36	90.86	89.51	89.51	88.94	90.53	90.37	88.12	77.73
L. oris		97.11	96.08	92.86	90.23	90.67	92.09	92.04	91.25	89.56	89.49	89.42	91.26	91.17	89.50	78.36
L. vaginalis			96.42	92.83	90.94	90.79	91.33	91.61	91.15	89.44	89.44	89.65	91.26	90.71	88.78	77.12
L. reuteri				93.97	90.45	90.69	90.32	90.71	90.91	89.49	89.49	88.99	91.27	90.86	88.51	78.03
L. fermentum					90.85	91.19	90.68	89.92	90.85	89.48	89.48	89.99	90.69	89.75	89.25	77.08
L. plantarum						93.61	91.99	92.05	92.41	91.28	91.28	93.29	92.30	91.49	90.03	77.21
L. brevis							94.12	93.80	92.99	91.11	91.24	91.47	91.57	91.12	90.58	76.50
L. buchneri								97.42	94.05	92.08	91.93	90.71	91.58	91.90	91.27	77.10
L. hilgardii									93.28	91.55	91.32	90.91	91.69	92.42	90.88	76.85
L. fructivorans										94.64	94.64	90.54	91.36	91.01	90.28	77.82
L. sanfrancisco ATCC 27651^T											99.75	89.50	89.21	90.18	89.71	77.20
L. sanfrancisco DSM 20663												89.43	89.14	90.05	89.57	77.13
L. alimentarius													90.70	90.06	89.80	76.59
L. sake														92.52	90.45	77.08
L. casei															90.55	78.07
L. salivarius																77.22

positive bacteria with low DNA G+C contents (3, 13) were performed by using Felsenstein's (4) PHYLIP program package. The fastDNAml program (13) was used for maximum likelihood analysis of a smaller data set that included 16S rRNA sequences of selected representatives of lactobacilli.

Design and application of rRNA-targeted specific probes. A comparative analysis of aligned 16S rRNA sequences revealed diagnostic regions which were used as target sites for species-specific oligonucleotide probes. The oligonucleotides were obtained from MG W, Ebersberg, Germany. The hybridization procedures used have been described previously (7).

Nucleotide sequence accession numbers. The EMBL accession numbers for the 16S rRNA sequences of *L. sanfrancisco* ATCC 27651^T (T = type strain) and DSM 20663, *L. fructivorans* DSM 20203^T, *L. reuteri* DSM 20016^T, and *Lactobacillus* sp. strain LTH 2587^T are X76327, X76331, X76330, X76328, and X76329, respectively.

RESULTS

To taxonomically evaluate the microbial populations in sourdough starter preparations, we used two groups of organisms. Group 1 contained the type strains of *L. sanfrancisco*, *L. brevis*, *L. fermentum*, and *L. fructivorans*, which were used to design the probes. For hybridization studies and protein pattern analyses additional well-characterized (type) strains of bacteria were included as reference organisms. The second group consisted of original isolates obtained from sourdough and sourdough starter preparations. Prior to this work some of these isolates were taxonomically identified to the species level (2) by using the discriminatory schemes described by Kandler and Weiss (8) and Hammes et al. (6). Additional isolates were initially characterized as *Lactobacillus* species only. These strains were typical organisms that were found in sourdough preparations and accounted for 30 to 80% of the microbial

flora; they were used to check the effectiveness of probing and protein pattern analysis for strain identification.

Phylogenetic relationships of sourdough lactobacilli. The 16S rRNA sequences of *L. sanfrancisco* ATCC 27651^T and DSM 20663, *L. fructivorans* DSM 20203^T, *L. reuteri* DSM 20016^T, and *Lactobacillus* sp. strain LTH 2587^T were determined and deposited in the EMBL sequence data bank.
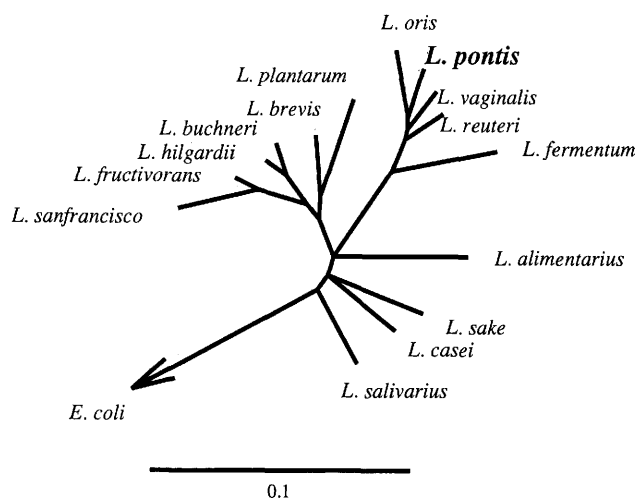

FIG. 2. Phylogenetic tree showing the phylogenetic relationships of *L. pontis* LTH 2587^T and other lactobacilli. The 16S rRNA sequence of *Escherichia coli* was included as an outgroup reference rRNA. The tree was constructed by using a maximum-likelihood approach as implemented in the fastDNAml program of Olsen et al. (13). The tree was based on a data set that included only positions which are present in at least 50% of all available *Lactobacillus* 16S rRNA sequences. Bar = 0.1 estimated substitution per sequence position.

TABLE 3. Nucleotide sequences of and optimum hybridization conditions used for the 16S rRNA
probes designed for identification of sourdough lactobacilli

Probe	Sequence	Specificity	Temp (°C) used for: Hybridization	Washing
L. sanfrancisco	5'-TAAGAATCAATTGGGCGAC-3'	L. sanfrancisco	40	47
L. farciminis	5'-CTCGCTGCTAACTTAAGTC-3'	L. fructivorans	40	50
L. fermentum	5'-GCGACCAAAATCAATCAGG-3'	L. fermentum	40	50
L. brevis	5'-TGTTGAAATCAGTGCAAG-3'	L. brevis	40	48
L. reuteri	5'-GATCCATCGTCAATCAGG-3'	L. reuteri	40	50
L. pontis	5'-GGTAATCCATCGTCAAATC-3'	L. pontis	40	49
1037	5'-CGACAAGGAATTTCGCTAC-3'	Universal	40	48

Various parts of 16S rRNA genes of *Lactobacillus* sp. strains LTH 1731, LTH 1735, LTH 2585, and LTH 2586 were sequenced. The partial 16S rRNA sequences of *Lactobacillus* sp. strains LTH 1731, LTH 1735, LTH 2585, and LTH 2586 were identical to the corresponding regions of the complete 16S rRNA primary structure of *Lactobacillus* sp. strain LTH 2587^T and different from the sequences of all other strains. rRNA sequence similarity values are shown in Table 2. Figure 2 shows a phylogenetic tree that was based on the 16S rRNA sequence data and reflects the phylogenetic position of *L. pontis* sp. nov.

Design of oligonucleotide probes. A comparison of the 16S rRNA sequences of lactobacilli and other reference organisms revealed diagnostic regions which could be used to design oligonucleotide probes for rapid specific identification of lactobacilli isolated from sourdough. The sequences and specificities of these probes, as well as the hybridization and washing temperatures used to achieve selective hybridization, are summarized in Table 3.

Hybridization studies. The specificities of the oligonucleotide probes were evaluated in dot blot hybridizations experiments performed with nucleic acids isolated from the bacterial strains listed in Table 1. The type strains used to design the probes served as controls. In addition, nucleic acids obtained from well-characterized sourdough lactobacilli and strains preliminarily characterized as *Lactobacillus* sp. were hybridized. The results are summarized in Table 1. The probes hybridized to the type strains and other strains their respective

species. No cross-reactions were observed with members of other *Lactobacillus* species isolated from sourdough. However, *Lactobacillus homohiochii* DSM 20571^T hybridized with the probe derived from the rRNA of *L. fructivorans*. *Lactobacillus* spp. strains LTH 2588 and LTH 2584 were identified as *L. brevis* and *L. reuteri* strains, respectively, whereas *Lactobacillus* sp. strains LTH 2590, LTH 2594, LTH 2595, and LTH 2598 were identified as strains of *L. sanfrancisco*. These findings were subsequently confirmed by physiological data. The probe designed for *L. pontis* specifically detected *L. pontis* LTH 1731, LTH 1735, LTH 2585, LTH 2586, and LTH 2587^T and did not react with the nucleic acids of the other strains investigated.

Protein pattern analysis. The soluble proteins patterns were determined for selected strains and compared with the patterns for strains in a data base containing normalized whole-cell protein patterns of representative strains belonging to most species of lactic acid bacteria. *Lactobacillus* spp. strains LTH 2588 and LTH 2584 were confirmed to be *L. brevis* and *L. reuteri* strains, respectively, and the patterns of *Lactobacillus* sp. strains LTH 2590, LTH 2594, and LTH 2595 were similar to the pattern of one of the strains of *L. sanfrancisco*. The protein patterns of all *L. pontis* strains were similar but differed from those of other lactobacilli. The position of these strains is shown in the dendrogram in Fig. 1.

Physiological characterization of *L. pontis*. The taxonomically relevant physiological features of *L. pontis* strains are summarized in Table 4. *L. pontis* LTH 2585 and LTH 2586 fermented only fructose and ribose, and thus this pattern did

TABLE 4. Differential characteristics of *L. pontis* sp. nov. and other sourdough lactobacilli

Strain(s)	D,L-Lactate	NH₃ from arginine	Growth at 15°C	Gas from glucose	L-Arabinose	Ribose	D-Xylose	Galactose	D-Glucose	D-Fructose	D-Mannose	Mannitol	Esculin	Maltose	Lactose	Melibiose	Sucrose	D-Raffinose	Gluconate	Peptidoglycan type	G+C content of DNA (mol%)
L. sanfrancisco ATCC 27651^T	D,L	–	+	+	–	+	–	+^{a}	+	–	–	–	–	+	–	–	–	–	–	Lys-Ala	36-38
L. fructivorans DSM 20203^T	D,L	+	+	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	+	Lys-D-Asp	38-41
L. fermentum DSM 20052^T	D,L	+	–	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	Orn-D-Asp	52-54
L. brevis DSM 20054^T	D,L	+	+	+	+	+	+	+	+	+	–	–	–	+	–	+	–	–	–	Lys-D-Asp	44-47
L. pontis LTH 1731 and LTH 1735	D,L	+	+	+	–	+	–	+	+	+	–	–	–	+	+	+	–	+	–	Orn-D-Asp	53-56
L. pontis LTH 2585 and LTH 2586	D,L	+	+	–^{b}	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	Orn-D-Asp	53
L. pontis LTH 2587^T	D,L	+	+	–^{b}	–	+	–	–	–	+	–	–	–	+	–	–	+	+	+	Orn-D-Asp	ND^c
L. reuteri LTH 2584	D,L	+	+	+	–	+	–	–	+	–	–	–	–	+	+	+	–	+	–	Lys-D-Asp	ND
L. reuteri DSM 20016^T	D,L	+	–	+	+	+	–	+	+	+	–	–	–	+	+	+	+	+	ND	Lys-D-Asp	40-42

^{a} Galactose utilization was not observed in this study but has been described by Kandler and Weiss (8).
^{b} *L. pontis* LTH 2585, LTH 2586, and LTH 2587^T do not utilize glucose but produce gas from fructose.
^{c} ND, not determined.

not correspond to the sugar fermentation pattern of any previously described *Lactobacillus* species. *L. pontis* LTH 1731, LTH 1735, and LTH 2587^T fermented additional carbohydrates. Analysis of the cell wall revealed the presence of ornithine and aspartic acid in *L. pontis* LTH 1731, LTH 1735, LTH 2585, LTH 2586, and LTH 2587^T, which is consistent with an Orn-D-Asp peptidoglycan type. The DNA G+C contents of *L. pontis* LTH 1735 and LTH 2587^T were determined to be 54.7 ± 1.4 and 53.3 ± 0.2 mol%, respectively. *L. pontis* can clearly be separated physiologically from the closely related taxon *L. reuteri* and other lactobacilli on the basis of its sugar fermentation pattern, high DNA G+C content, and peptidoglycan type. As determined by light microscopy, *L. pontis* cells are slender and occur in long chains which may be bent or may even resemble vine tendrils. Figure 3 shows phase-contrast photomicrographs of cells of *L. pontis* LTH 1735 and LTH 2587^T. Electron micrographs of *L. pontis* LTH 2587^T revealed that the tendril-like forms are associations of bent cells which stick together because of surface layers or polysaccharides (Fig. 4). This "matrix" may be responsible for the difficulties encountered in obtaining pure cultures of *L. pontis* and physically separating *L. pontis* from strains of *L. sanfrancisco*.

DISCUSSION

The genus *Lactobacillus* includes 55 species (5), and, therefore, a polyphasic approach and thorough taxonomic research are necessary before a new species can be described. We confirmed that the major fermenting organisms in the preparations which we examined were strains of *L. brevis*, *L. fructivorans*, *L. fermentum*, and *L. sanfrancisco*, and we also found that strains of *L. pontis* accounted for a large percentage of the microbial flora. In some sourdough preparations the concentration of *L. pontis* LTH 2585 or *L. pontis* LTH 2586 was 2.7 × 10^9 CFU/g, and the concentration of *L. pontis* LTH 2587^T was 2.2 × 10^9 CFU/g; these organisms accounting for approximately 80% of the lactobacilli. Some of these strains utilize only a very limited number of carbohydrates and live in close association with *L. sanfrancisco*, from which they are difficult to separate physically.

We used a combination of DNA data, physiological characteristics, and protein patterns in a general strategy to describe a microbial population (in this case a sourdough population), which resulted in a reliable taxonomic classification of the microorganisms and a determination of the phylogenetic relationships of the bacteria. In addition to identification of a previously unknown species, the development and use of oligonucleotide probes allowed us to quantitatively analyze the microflora of sourdough at all stages of fermentation reliably.

Description of *Lactobacillus pontis* sp. nov. Vogel, Böcker, Stolz, Ehrmann, Fanta, Ludwig, Pot, Kersters, Schleifer, and Hammes. Stolz, Ehrmann, Fanta, Ludwig, Pot, Kersters, Schleifer, and *Lactobacillus pontis* Vogel, Böcker, Hammes (pon'tis. L. gen. n. *pons*, bridge, referring to BRIDGE, which is the abbreviation for the Commission of European Communities Research Programme entitled Biotechnology Research for Innovation, Development and Growth in Europe; during this program the organism was isolated and characterised by the joint efforts of workers in three laboratories).

Cells are nonmotile, nonsporing, gram-positive, slender rods (diameter, 0.3 to 0.6 μm; length, 4 to 6 μm) that occur singly, in pairs, and in chains. Some strains have a strong tendency to form chains of long bent rods; the chains may even resemble a vine tendril. After 2 to 5 days of anaerobic incubation at 30°C colonies on sanfrancisco agar are 1 to 2 mm in diameter; these
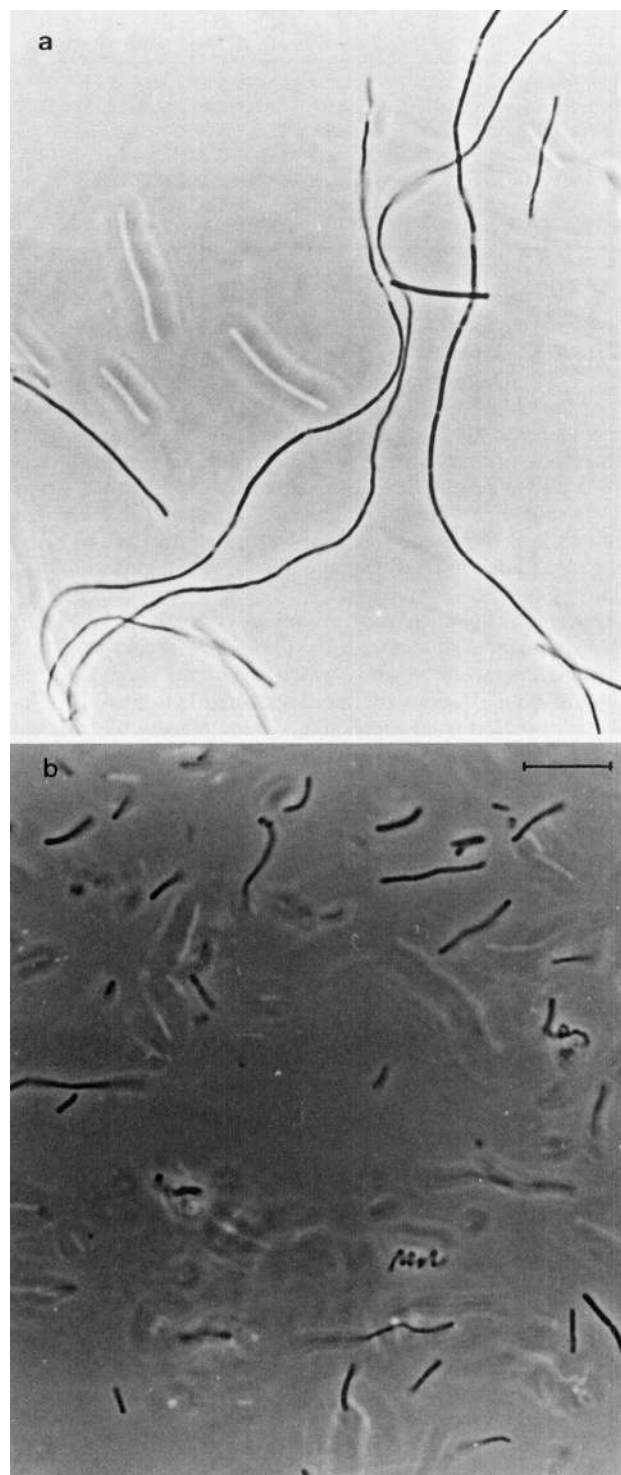

FIG. 3. Phase-contrast photomicrographs of cells of *L. pontis* LTH 1735 (a) and LTH 2587^T (b). The cells were grown in sanfrancisco medium. Magnification, ×800. Bar = 10 μm. *L. pontis* LTH 1735 forms long chains of slender cells (a), whereas strain LTH 2587^T also forms vine tendril-like associations (b) (see Fig. 4).
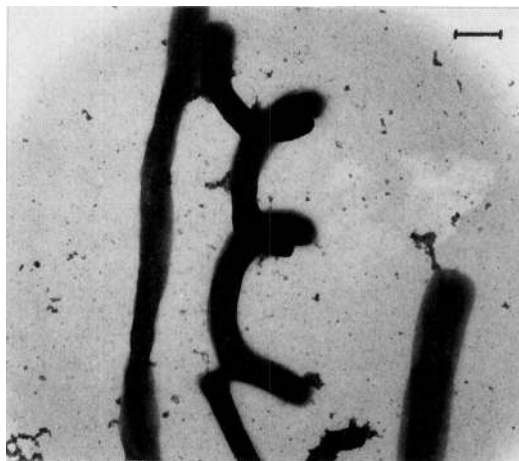
FIG. 4. Electron micrograph of *L. pontis* LTH 2587^T. Magnification, ×12,000. Bar = 0.6 μm. Formvar-coated EM grids were used with dried, nonstained cells that had been suspended in a solution containing 1 g of tryptone per liter and 8.5 g of NaCl per liter and fixed with 1% glutaraldehyde. The micrograph was taken with an energy-filtering model CEM 902 transmission electron microscope (Zeiss). The vine tendril-like appearance is due to chain formation by single bent cells.

colonies are rough circular plateaus that have irregular borders and smooth convex centers and are translucent and greyish. All strains grow at 15 and 45°C. The main fermentation products from maltose or fructose are lactate, acetate, ethanol, glycerol, and CO_2. Catalase activity is not detected.

The terminal pH in sanfrancisco medium ranges from 3.9 to 4.2. All strains ferment ribose and fructose. *L. pontis* LTH 2587^T also ferments maltose. In addition, *L. pontis* LTH 1731 and LTH 1735 ferment galactose, glucose, lactose, melibiose, and raffinose. All strains cleave arginine. The type strain is strain LTH 2587 (= DSM 8475 = LMG 14187). Strain LTH 1735 (= DSM 8476 = LMG 14188) is a reference strain that exhibits different characteristics. The DNA G+C contents of *L. pontis* LTH 1735 and LTH 2587^T are 54.7 ± 1.4 and 53.3 ± 0.2 mol%, respectively. Both strains were originally isolated from rye sourdough and can be predominant in some batches.

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