Lactobacillus vaginalis sp. nov. from the Human Vagina

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Biochemical, chemical, and nucleic acid analyses were carried out on some lactobacilli isolated from the vaginas of patients suffering from trichomoniasis. Phenotypically the strains resembled *Lactobacillus fermentum* and *L. reuteri*. Nucleic acid studies, however, showed that the vaginal strains were distinct from these and all other lactobacilli examined and therefore warrant a new species. The name *Lactobacillus vaginalis* sp. nov. is proposed. The type strain of *L. vaginalis* is NCTC 12197.

In the course of investigations aimed at developing a vaccine (Solco Trichovac/Gynatren; Solco Basle Ltd., Birsfelden, Switzerland) against recurrent vaginitis, a number of lactobacilli were isolated from vaginal swabs from patients with trichomoniasis. These strains showed similarities with *Lactobacillus fermentum*, a species commonly isolated from the vagina. *L. fermentum*, however, exhibits considerable phenotypic diversity (6), and the assignment of the vaginal strains to this species is uncertain. In view of this, we investigated the relationship of the vaginal strains to representatives of *L. fermentum* and other heterofermentative lactobacilli by using biochemical, chemical, and nucleic acid techniques. The results indicated that the vaginal strains form a new species, for which the name *Lactobacillus vaginalis* sp. nov. is proposed.

Cultures. Details of the strains and their sources are given in Table 1.

DNA base composition and DNA-DNA hybridization. Cells for deoxyribonucleic acid (DNA) isolation were grown in MRS broth (CM361; Oxoid Ltd.) at 25 or 37°C to the late exponential phase, harvested by centrifugation, and washed with Tris-EDTA buffer (10 mM Tris, 1 mM EDTA [pH 8.0]). DNA was prepared by a modification of the method of Garvie (2). DNA base composition was estimated from the midpoint of the DNA melting profile (T_m) in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]), with DNA from *Escherichia coli* K-12 as a standard. Preliminary DNA hybridizations were done on Zeta-Probe nylon membranes (Bio-Rad Laboratories Ltd.) with a slot-blot apparatus (Bio-Rad) and probe DNA labeled with biotin-UTP by nick translation (Bethesda Research Laboratories Ltd.). Hybridizations were carried out under semistrict conditions ($T_m - 20^\circ\text{C}$). Quantitative data were generated with radiolabeled DNA and membrane filter hybridizations done under optimum conditions ($T_m - 25^\circ\text{C}$) (2).

Fatty acid analyses. Dried biomass (30 mg) of cells grown in yeast-glucose-phosphate broth (3) was degraded by overnight methanolysis at 55°C. Long-chain fatty acid methyl esters were purified and analyzed by capillary gas chromatography as described previously (1). Capillary gas chromatography-mass spectrometry of picolinyl esters of fatty acids was done as described by Wait and Hudson (7).

Biochemical tests. The API 50CH system was used in accordance with the instructions of the manufacturer (API-BioMerieux Ltd.). Tests were incubated at 30 and 37°C and read after 24 and 48 h and after 7 days. The products of

glucose fermentation were determined by gas chromatography as described in the *Anaerobe Laboratory Manual* (4). The isomer of lactic acid produced by the strains in MRS broth was determined with a commercial test kit (Boehringer Mannheim Biochemicals).

The guanine-plus-cytosine compositions of the five strains of *L. vaginalis* varied between 38 and 41 mol%. The results of preliminary slot blot hybridizations indicated that they were homogeneous and distinct from reference strains of *L. fermentum* and *L. reuteri*. The results of quantitative DNA-DNA hybridizations are shown in Table 1. The strains of *L. vaginalis* formed a single homology group that was 85 to 90% related to the type strain *L. vaginalis* NCTC 12197. Strains of *L. oris* and *L. reuteri* were most closely related to *L. vaginalis* NCTC 12197^T at 26 to 29% and 24 to 35% homology, respectively. *L. fermentum* and the remaining reference strains were only distantly related ($\leq 18\%$) to *L. vaginalis*. If 70% homology under optimum conditions is used as a guideline for species differentiation (5), the DNA data clearly demonstrate that *L. vaginalis* is a distinct taxon worthy of separate specific status. Biochemical features for the differentiation of *L. vaginalis* from *L. fermentum*, *L. oris*, and *L. reuteri* are given in Table 2. A full description of *L. vaginalis* is given below.

Description of *Lactobacillus vaginalis* sp. nov. *Lactobacillus vaginalis* (vaginalis, *L.* gen. n. *vaginalis*, of the vagina). Cells are rod shaped, are of variable size (0.5 to 0.8 by 1.5 to 2.5 μm), and occur singly or in pairs or short chains. Colonies are white to grey, small to large (1 to 5 mm), and semirough, often with raised areas. Cells are gram positive. Nonmotile. Grows at 30 and 45°C but not at 22 or 48°C. Facultatively anaerobic. Catalase negative. Heterofermentative, producing D- and L-lactic acid. Acid is produced from D-galactose, D-glucose, lactose, D-levulose, D-mannose, maltose, melibiose, D-raffinose, and sucrose. Most strains produce acid from ribose. Acid is not produced from N-acetylglucosamine, adonitol, D-arabinose, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- and L-fucose, glycerol, glycogen, inositol, inulin, 2- and 5-ketogluconate, D-lyxose, mannitol, melezitose, α -methyl-D-mannoside, rhamnose, sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, xylitol, and D- and L-xylose. Esculin is hydrolyzed slowly by most strains. Produces straight-chain and monounsaturated long-chain fatty acids of the vaccenic series and *cis*-11,12-methylene-octadecenoic (lactobacillic) acid. The guanine-plus-cytosine content of the DNA ranges from 38 to 41 mol% (T_m). Isolated from the vagina of patients suffering from trichomoniasis.

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TABLE 1. Strain histories, DNA base compositions, and DNA homologies of *L. vaginalis* and other lactobacilli

Species and strain	Source or other description	% Relative binding with ³ H-labeled DNA from <i>L. vaginalis</i> NCTC 12197 ^T	Guanine-plus-cytosine content (mol%)
<i>L. vaginalis</i>			
NCTC 12197 ^T	Human vagina	100	39
4	Human vagina	86	38
6	Human vagina	85	38
8	Human vagina	90	39
10	Human vagina	99	41
<i>L. acidophilus</i> NCDO 1748 ^T	ATCC 4356 ^T , DSM 20079 ^T	16	34
<i>L. agilis</i> NCDO 2744 ^T	DSM 20509 ^T , NCIB 11716 ^T ; municipal sewage	13	41
<i>L. brevis</i> NCDO 391	ATCC 4356, DSM 20079	16	34
NCDO 476		12	44
<i>L. buchneri</i> NCDO 110 ^T	ATCC 4005 ^T , DSM 20057 ^T ; tomato pulp	11	45
<i>L. casei</i> NCDO 161 ^T	ATCC 393 ^T , DSM 20011 ^T	10	ND ^a
<i>L. collinoides</i> NCDO 2746 ^T	ATCC 27612 ^T , DSM 20515 ^T , NCIB 10925 ^T	8	47
<i>L. confusus</i> 1635-85	Human	12	ND
<i>L. curvatus</i> NCDO 2739 ^T	DSM 20019 ^T ; milk	13	ND
<i>L. falsus</i> NCDO 151	Originally <i>L. casei</i>	8	ND
<i>L. farciminis</i> NCDO 2330 ^T	ATCC 29644 ^T , DSM 20184 ^T ; sausage	11	36
<i>L. fermentum</i> NCDO 217	M. E. Sharpe (strain F3)	17	ND
NCDO 223	M. E. Sharpe (strain F4)	17	ND
<i>L. gasseri</i> NCDO 2233	ATCC 33323, DSM 20243, NCIB 11718	10	34
<i>L. helveticus</i> J11	M. E. Sharpe	13	ND
<i>L. hilgardii</i> NCDO 264 ^T	ATCC 8290 ^T , DSM 20176 ^T ; wine	11	40
<i>L. intestinalis</i> NCDO 2176	HE1	10	37
<i>L. kefir</i> NCDO 2132	Kefir	12	41
<i>L. malefermentans</i> NCDO 2344	DSM 20177, JCM 1189	13	41
<i>L. minor</i> NCDO 1973	O. Kandler	11	38
<i>L. oris</i> NCDO 2160 ^T	NCIB 8831 ^T ; human saliva	29	49
NCDO 2161	Human saliva	26	49
NCDO 2164	Human saliva	26	50
<i>L. parabuchneri</i> NCDO 2748	Human saliva	12	44
<i>L. pentosus</i> NCDO 363 ^T	Corn silage	10	41
<i>L. plantarum</i> NCDO 1752 ^T	HuATCC 14917 ^T , DSM 20174 ^T ; pickled cabbage	11	44

Continued

TABLE 1.—Continued

Species and strain	Source or other description	% Relative binding with ³ H-labeled DNA from <i>L. vaginalis</i> NCTC 12197 ^T	Guanine-plus-cytosine content (mol%)
<i>L. reuteri</i> NCDO 2589 ^T	ATCC 23272 ^T , DSM 20016 ^T ; adult human intestine		
NCDO 1089	M. E. Sharpe	33	38
NCDO 219	M. E. Sharpe	24	38
NCDO 230	M. E. Sharpe	27	39
NCDO 2655	Rat	28	40
<i>L. rhamnosus</i> NCDO 86	ATCC 11977, NCIB 76, NCTC 76	9	ND
<i>L. viridescens</i> NCDO 1655 ^T	ATCC 12706 ^T , DSM 20410 ^T	15	43
NCDO 403	C. F. Niven	14	43

^a ND, Not determined.TABLE 2. Differential features of *L. vaginalis*, *L. fermentum*, *L. oris*, and *L. reuteri*^a

Acid produced from ^b :	No. of the following strains positive ^c :			
	<i>L. vaginalis</i> (n = 6)	<i>L. fermentum</i> (n = 13)	<i>L. oris</i> (n = 5)	<i>L. reuteri</i> (n = 8)
Amygdalin	—	—	+	—
L-Arabinose	—	3	+	7
D-Arabitol	—	—	+	—
Esculin	4 ^D	—	3	1
D-Fructose	+	11	+	—
D-Furanose	—	—	3	—
Gentiobiose	—	—	+	—
Gluconate	—	+ ^D	4	5
D-Mannose	+	6	1	—
α-Methylglucoside	—	4	4	2
α-Methylxyloside	—	1	3 ^D	—
Salicin	—	—	3	—
Sucrose	+	10	+	+

^a The guanine-plus-cytosine contents for *L. vaginalis*, *L. fermentum*, *L. oris*, and *L. reuteri* were 38 to 41, 42 to 53, 49 to 51, and 37 to 41 mol%, respectively.^b All *L. vaginalis* strains produced acid from ribose (after 7 days), D-galactose, D-glucose, lactose, maltose, melibiose, D-raffinose, and saccharose; all *L. vaginalis* strains failed to produce acid from N-acetylglucosamine, adonitol, D-arabinose, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- and L-fucose, glycerol, glycogen, inositol, inulin, 2- and 5-ketogluconate, D-lyxose, mannitol, melezitose, α-methyl-D-mannoside, rhamnose, sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, xylitol, and D- and L-xylose.^c +, All strains positive; —, all strains negative; D, delayed reaction.

The type strain is NCTC 12197. In most respects the description of the type strain resembles the species description. The type strain does not produce acid from ribose and does not hydrolyze esculin.

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