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Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples

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A taxonomic study was conducted to clarify the relationships of two bacterial populations belonging to the genus *Weissella*. A total of 39 strains originating mainly from Malaysian foods (22 strains) and clinical samples from humans (9 strains) and animals (6 strains) were analysed using a polyphasic taxonomic approach. The methods included classical phenotyping, whole-cell protein electrophoresis, 16S and 23S rDNA RFLP (ribotyping), determination of 16S rDNA sequence homologies and DNA–DNA reassociation levels. Based on the results, the strains were considered to represent two different species, *Weissella confusa* and a novel *Weissella* species, for which the name *Weissella cibaria* sp. nov. is proposed. *Weisella confusa* possessed the highest 16S rDNA sequence similarity to *Weisella cibaria*, but the DNA–DNA reassociation experiment showed hybridization levels below 49% between the strains studied. The numerical analyses of *Weisella confusa* and *Weisella cibaria* strains did not reveal any specific clustering with respect to the origin of the strains. Based on whole-cell protein electrophoresis, and *Cla*I and *Hin*dIII ribotyping patterns, food and clinical isolates were randomly located in the two species-specific clusters obtained.

Keywords: *Weissella confusa*, *Weissella cibaria*, Malaysian foods, clinical samples

INTRODUCTION

The phylogeny of the bacteria classified currently in the genus *Weissella* was clarified in 1990. Using both 16S and 23S rRNA sequence data, Martinez-Murcia & Collins (1990) and Martinez-Murcia *et al.* (1993) showed that *Leuconostoc paramesenteroides* is phylogenetically distinct from *Leuconostoc mesenteroides* and that it groups together with five heterofermentative lactobacilli, *Lactobacillus confusus*, *Lactobacillus halotolerans*, *Lactobacillus kandleri*, *Lactobacillus minor* and *Lactobacillus viridescens*. In a study of leuconostoc-like organisms originating from fermented sausages (Collins *et al.*, 1993) the taxonomy of these species was further assessed. This resulted in the description of the genus *Weissella* comprising the former *Leuconostoc paramesenteroides*, the five *Lactobacillus* species, and at that time a novel species, *Weissella hellenica*. Recently, a novel species, *Weissella thailandensis*, has been described and suggested to belong to this genus (Tanasupawat *et al.*, 2000). Thus there are currently eight species in the genus *Weissella*, *Weissella confusa*, *Weissella halotolerans*, *Weissella hellenica*, *Weissella kandleri*, *Weissella minor*, *Weissella paramesenteroides*, *Weissella thailandensis* and *Weissella viridescens*.

Weissella strains have been isolated from a variety of sources. *Weisella paramesenteroides* is one of the predominant species in fresh vegetables and it also plays an important role in the first phase of silage fermentation (Dellaglio *et al.*, 1984; Dellaglio & Torriani, 1986). *Weisella halotolerans*, *Weisella hellenica* and *Weisella viridescens* have been commonly associated with meat or meat products (Niven *et al.*, 1957; Milbourne, 1983; Collins *et al.*, 1993), whereas the natural habitat of *Weisella kandleri* is unknown

Abbreviation: LAB, lactic acid bacteria.

The EMBL accession number for the 16S rRNA gene sequence of LMG 17699^T is AJ295989.

Table 1. Strains used in the study

Species	Strain no.*		Depositor†	Source
	LMG	Other		
Weisella confusa	9497^T	NRRL B-1064^T	NRRL	Sugar cane
Weisella confusa	14040	93/1360R	L. A. Devriese	Dog ear (otitis), Belgium
Weisella confusa	16883	CCUG 30969	E. Falsen	Human drain, Sweden
Weisella confusa	17670	CCUG 36556	E. Falsen	Human blood, Sweden
Weisella confusa	17671	CCUG 30943	E. Falsen	Human gall, Sweden
Weisella confusa	17695	II-I-43	J. Leisner	Chili Bo, Malaysia
Weisella confusa	17696	II-I-48	J. Leisner	Chili Bo, Malaysia
Weisella confusa	17698	II-I-56	J. Leisner	Chili Bo, Malaysia
Weisella confusa	17705	II-2-5	J. Leisner	Chili Bo, Malaysia
Weisella confusa	17709	II-I-67	J. Leisner	Chili Bo, Malaysia
Weisella confusa	17718	II-2-33	J. Leisner	Chili Bo, Malaysia
Weisella confusa	18475	TD35	J. Leisner	Tapai, Malaysia
Weisella confusa	18476	TC10	J. Leisner	Tapai, Malaysia
Weisella confusa	18477	HD5	J. Leisner	Tempeh, Malaysia
Weisella confusa	18478	TC8	J. Leisner	Tapai, Malaysia
Weisella confusa	18479	TD7	J. Leisner	Tapai, Malaysia
Weisella confusa	18480	TD3	J. Leisner	Tapai, Malaysia
Weisella confusa	18500	CCUG 30763	E. Falsen	Dog (autopsy), Sweden
Weisella confusa	18503	CCUG 37938	E. Falsen	Human faeces, Sweden
Weisella confusa	18505	CCUG 39150	E. Falsen	Human faeces, Sweden
Weisella confusa	18815		H. Goossens	Human faeces, Belgium
Weisella confusa	18816		H. Goossens	Human faeces, Belgium
Weisella cibaria	13587	O7	L. A. Devriese	Dog ear (otitis), Belgium
Weisella cibaria	13653	O2	L. A. Devriese	Dog ear (otitis), Belgium
Weisella cibaria	14037	93/1432	L. A. Devriese	Dog ear (otitis), Belgium
Weisella cibaria	16479	95/1815	L. A. Devriese	Canary liver, Belgium
Weisella cibaria	17694	II-I-42	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17697	II-I-52	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17699^T	II-I-59^T, CCUG 41967^T	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17700	II-I-60	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17701	II-I-61	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17704	II-2-32	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17706	II-2-13	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	17708	II-I-68	J. Leisner	Chili Bo, Malaysia
Weisella cibaria	18481	TC24	J. Leisner	Tapai, Malaysia
Weisella cibaria	18482	TD28	J. Leisner	Tapai, Malaysia
Weisella cibaria	18501	CCUG 33604	E. Falsen	Human gall, Sweden
Weisella cibaria	18506	NCFB 889	NCFB	Cheese whey
Weisella cibaria	18507	NCFB 1937	NCFB	Sugar cane
Weisella cibaria	18814		H. Goossens	Human faeces, Belgium
Weisella halotolerans	9469^T	DSM 20190^T	DSM	Sausage
Weisella hellenica	15125^T	NCFB 2973^T	NCFB	Naturally fermented sausage, Greece
Weisella kandleri	14471^T	DSM 20593^T	DSM	Desert spring, South West Africa
Weisella minor	9847^T	NCFB 1973^T	NCFB	Milking machine slime
Weisella paramesenteroides	9852^T	NCFB 803^T	NCFB	Fermented sausages, dry salami
Weisella viridescens	3507^T	NCIB 8965^T	NCIB	Cured meat products

*BCCM/LMG, Belgian Coordinated Collections of Microorganisms, Laboratorium Microbiologie Gent Culture Collection, Universiteit Gent, Belgium; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; DSM, German Collection of Micro-organisms and Cell Cultures, Braunschweig, Germany; NCFB, National Collection of Food Bacteria, Reading, UK; NCIB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NRRL, Agricultural Research Service Culture Collection. Peoria, IL, USA.

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(Hammes & Vogel, 1995). The *Weisella kandleri* type strain originates from a desert spring and desert plants have been suggested as the main habitat of this species (Holzapfel *et al.*, 1982). *Weisella confusa* strains have been detected in sugar cane, carrot juice and occasionally in raw milk and sewage (Hammes & Vogel, 1995). The strains used for the taxonomic description of *Weisella minor* were isolated from the sludge of milking machines (Kandler *et al.*, 1983).

In recent studies, when the lactic acid bacterium (LAB) populations associated with traditionally fermented foods have been characterized (Hancioğlu & Karapinar, 1997; Ampe *et al.*, 1999; Paludan-Müller *et al.*, 1999) many of these foods have been found to contain *Weissella* species. The recently characterized species, *Weisella thailandensis*, was also isolated from fermented fish product in Thailand (Tanasupawat *et al.*, 2000). In non-fermented foods from Southeast Asia, LAB have often been detected as contaminants (Leisner *et al.*, 1997, 1999). Our study set out to identify some LAB originating mainly from the Malaysian foods tapai and chili bo. Tapai is sweet, fermented glutinous rice or cassava, and chili bo is non-fermented chilli and cornstarch containing perishable food ingredients. Initially, when the protein profiles and ribotypes of the LAB strains from tapai and chili bo were analysed, the patterns showed similarity to the patterns of the *Weisella confusa* strains. However, further analysis showed that some of these strains formed a distinct cluster, separate from the cluster containing the *Weisella confusa* type strain. During this stage, some unidentified LAB strains originating from clinical samples were also detected joining both clusters. These findings warranted a taxonomic study clarifying the relationship of the LAB strains within these two groups.

The methods used in this polyphasic study included classical phenotyping, whole-cell protein electrophoresis, 16S and 23S rDNA RFLP analysis (Grimont & Grimont, 1986) and the determination of both 16S rDNA sequence and DNA–DNA reassociation levels. Based on the results, the LAB were considered to represent two different species, *Weisella confusa* and a novel *Weissella* species, for which we propose the name *Weissella cibaria* sp. nov.

METHODS

Bacterial strains. Table 1 shows the strains used in this study. Depending on the purpose, the strains were grown either for 3 d on MRS agar (Oxoid) at 30 °C in a microaerophilic atmosphere (approx. 5% O_2, 10% CO_2 and 85% N_2) or overnight in MRS broth (Difco) at 30 °C. All strains were maintained in MRS broth (Difco) at −70 °C.

Phenotypic characterization. All *Weisella cibaria* and *Weisella confusa* strains were Gram-stained, catalase-tested and studied for the production of gas from glucose (Smittle & Cirigcliano, 1992). Further phenotypic characterization of *Weisella cibaria* was done using the strains LMG 17706, LMG 17699^T, LMG 18506, LMG 18507 and LMG 18814. Strains LMG 18477, LMG 17718 and the *Weisella confusa* type strain LMG 9497^T were selected to represent the *Weisella confusa* cluster. Production of ammonia from arginine was determined using the method of Briggs (1953). Dextran formation was studied on 5% sucrose containing agar (Harrigan & McCance, 1976). The API 50 CHL *Lactobacillus* identification system (BioMérieux) was used for the determination of the carbohydrate fermentation profiles. The ability to produce different lactic acid isomers was tested using an enzymic method (Von Krush & Lompe, 1982) utilizing D- and L-lactate dehydrogenases (Roche). The eight strains were also tested for growth in MRS broth (Difco) incubated at 4, 15, 37 and 45 °C until growth was observed or otherwise at least for 21 d. Growth in the presence of 6·5 and 8·0% of NaCl was tested in MRS broth incubated at 30 °C until growth was observed or otherwise at least for 21 d.

Peptidoglycan analysis. Preparation of cell walls and determination of peptidoglycan structure of LMG 17699^T and LMG 18814 strains were carried out by the methods described by Schleifer & Kandler (1972) with the modification of using thin layer chromatography on cellulose sheets instead of paper chromatography. Briefly, 1 mg freeze-dried cell walls was hydrolysed in 0·2 ml 4 M HCl at 100 °C for 16 h (total hydrolysate) or 45 min (partial hydrolysate). Diamino acids were determined from total hydrolysate by one-dimensional chromatography in the solvent system methanol/pyridine/water/10 M HCl (320:40:70:10 by vol.). Amino acids and peptides from total and partial hydrolysates were identified after two-dimensional chromatography in the systems described by Schleifer & Kandler (1972) according to mobility and staining characteristics with ninhydrin spray. The resulting 'fingerprints' were compared with known peptidoglycan structures.

Whole-cell protein analysis. Preparation of cellular protein extracts and PAGE was performed as described by Pot *et al.* (1994). Briefly, discontinuous gels were run overnight at constant current and temperature in a vertical slab apparatus. The separation gel was 12·6 cm long and contained 12% total acrylamide (the monomer solution contained 30% total acrylamide with 2·67% cross-linking in 0·375 M Tris/HCl, pH 8·8, and 0·1% SDS); the stacking gel was 12 mm long and contained 5% total acrylamide (the monomer solution contained 30% total acrylamide with 2·67% cross-linking in 0·125 M Tris/HCl, pH 6·8, and 0·1% SDS). Protein bands were stained with Coomassie blue R-250 in 50% (v/v) methanol and 10% (v/v) acetic acid. These conditions allowed separation of proteins and peptides in the molecular mass range of 14000 to 116000.

Isolation of DNA, restriction endonuclease analysis, and 16S and 23S rDNA RFLP (ribotyping). *Cla*I, *Eco*RI and *Hin*dIII restriction enzymes (New England Biolabs) were used for ribotyping of all strains. DNA was isolated by the guanidium thiocyanate method of Pitcher *et al.* (1989), as modified by Björkroth & Korkeala (1996), by the combined lysozyme and mutanolysin (Sigma) treatment. Restriction endonuclease treatment of 3 μg DNA was done as specified by the manufacturer (New England Biolabs) and electrophoresis was carried out as described previously (Björkroth & Korkeala, 1996). Genomic blots were made using a vacuum device (Vacugene; Pharmacia) and the rDNA probe for

Table 2. Results from optical DNA–DNA reassociation experiment

Species	Strain (LMG)	Optical DNA–DNA reassociation (%) with:							
		17699^T	17706	18506	18507	18814	9497^T	17718	18477
Weisella cibaria	17699^T	ND*	76	90	100	93	49	22	18
Weisella cibaria	17706		ND	ND	ND	ND	46	ND	ND
Weisella cibaria	18506			ND	84	84	42	ND	ND
Weisella cibaria	18507				ND	96	ND	ND	ND
Weisella cibaria	18814					ND	ND	ND	ND
Weisella confusa	9497^T						ND	77	75
Weisella confusa	17718							ND	80
Weisella confusa	18477								ND

* ND, Not determined.

ribotyping was labelled by reverse transcription [AMV-RT (Promega) and Dig DNA Labelling Kit (Roche)] as described by Blumberg *et al*. (1991). Membranes were hybridized at 68 °C as described by Björkroth & Korkeala (1996).

Pattern analysis. Whole-cell protein profiles were scanned using an LKB 2202 UltroScan Laser Densitometer (LKB). The densitometric analysis, normalization and interpolation of the protein profiles were performed using the GelCompar 4.2 software package (Applied Maths). Numerical analysis was performed using the BioNumerics 1.0 software package (Applied Maths). The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage value. For numerical analysis, the *Cla*I, *Eco*RI and *Hin*dIII ribopatterns were scanned using a Hewlett Packard (Boise) ScanJet 4c/T scanner and analysed using the BioNumerics 1.0 software package. The similarity between all pairs was expressed by the Dice coefficient correlation and UPGMA (unweighted pair group method using arithmetic averages) clustering was used for the construction of the dendrogram.

16S rRNA gene sequence analysis. Part of the rDNA operon, comprising the nearly complete 16S DNA, was amplified by PCR. The forward primer was 5′-CTGGCTCAGGAYGAACGCTG-3′, corresponding to positions 19–38 (*Escherichia coli* 16S rRNA numbering). The reverse primer was 5′-AAGGAGGTGATCCAGCCGCA-3′, complementary to positions 1541–1522. PCR-amplified 16S rDNAs were purified by using the QIAquick PCR Purification Kit (Qiagen). Sequence analysis was performed using an Applied Biosystems 377 DNA Sequencer and the protocols of the manufacturer (Perkin-Elmer, Applied Biosystems) using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (with AmpliTaq DNA polymerase). The sequencing primers were those given by Coenye *et al*. (1999). Sequence assembly was performed by using the program AutoAssembler (Applied Biosystems). Phylogenetic analysis of the 16S rDNA sequence of strain LMG 17699^T was performed by using the GeneCompar 2.0 software package (Applied Maths). The consensus sequence and the sequences of strains belonging to the same phylogenetic group of *Weissella* (retrieved from the NCBI GenBank database) were aligned. The accession numbers of the 16S rDNA sequences used are: *Weisella confusa* LMG 9497^T, M23036; *Weisella halotolerans* LMG 9469^T, M23037; *Weisella hellenica* LMG 15125^T, X95981; *Weisella kandleri* LMG 14471^T, M23038; *Weisella minor* LMG 9847^T, M23039; *Weisella paramesenteroides* LMG 9852^T, M23033; *Weisella viridescens* LMG 3507^T, M23040; *Weisella thailandensis* FS61-1^T, AB023838; *Leuconostoc gasicomitatum* LMG 18811^T, AF231131. Calculation of the level of similarity and construction of a phylogenetic tree was based on the neighbour-joining method.

DNA base composition and optical DNA–DNA hybridization analyses. Table 2 shows the pairs selected for DNA–DNA hybridization tests. These organisms were selected based on the clustering observed in the numerical analyses of protein and ribotyping data. The large-scale DNA isolation was performed using the modified (Björkroth & Korkeala, 1996) guanidium thiocyanate method of Pitcher *et al*. (1989). The method was scaled up 10-fold and applied to cells from 200 ml of a well-grown MRS broth culture for each batch of isolation. DNA from one batch was dissolved overnight in 1 ml TE 10:1 (10 mM Tris, 1 mM EDTA, pH 8·0). Ribonuclease A (Sigma) was added to provide a concentration of 125 μg ml^{-1} and the solution was incubated at 37 °C with gentle shaking for 1 h. Following the 1 h incubation, proteinase K (Sigma) was added to a concentration of 0·5 mg ml^{-1} and incubation at 37 °C was continued at least for 6 h. DNA was precipitated as described by Pitcher *et al*. (1989) and dissolved in 1 ml 0·1 × SSC. When dissolved, the SSC concentration of a sample was adjusted with 20 × to 1 × SSC. Purified DNA was dialysed twice overnight at 4 °C using a 12000 to 14000 Da pore-size membrane (Medicell International). The first dialysis was carried out against 1 × SSC/EDTA (10 mM) and the second against 1 × SSC. DNA was fragmented two times in a French pressure cell press (SML Aminco; Colora Messtechnik) at about $1{\cdot}5 \times 10^6$ Pa. Before reassociation, it was dialysed once more overnight at 4 °C against 2 × SSC. The DNA base composition (mol % G+C) was estimated by the thermal denaturation method (De Ley *et al*., 1970) and the DNA homology values were determined from renaturation rates using a Gilford Response spectrophotometer (Ciba Corning Diagnostics).

RESULTS AND DISCUSSION

Phenotypic characterization

In basic phenotypic tests *Weisella cibaria* sp. nov. strains produced typical reactions for genus *Weissella* (Collins *et al*., 1993) and all phenotypic reactions of *Weisella confusa* strains LMG 18477, LMG 17718 and LMG 9497 were in agreement with the characteristics

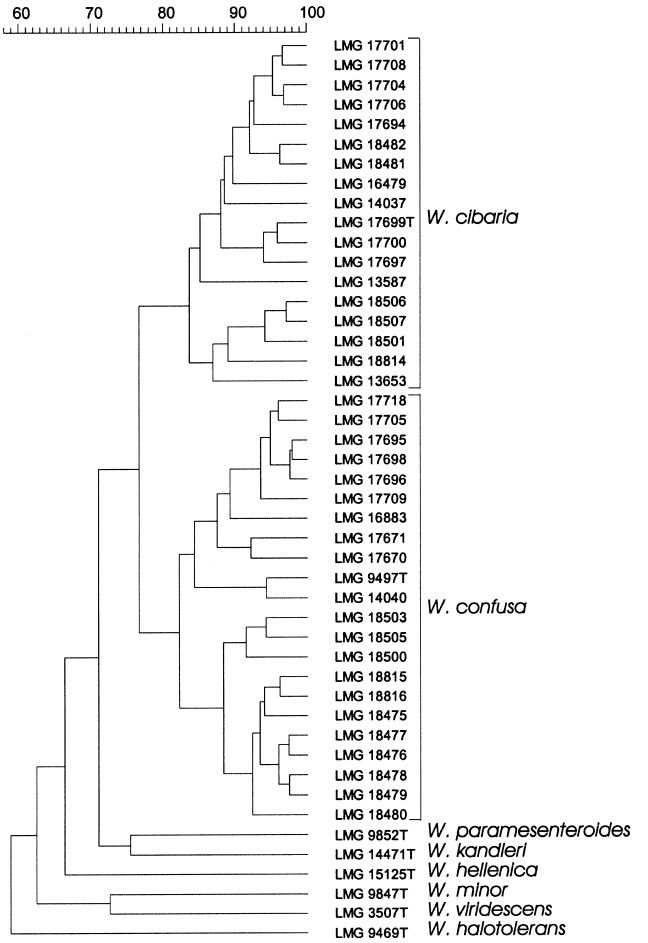

Fig. 1. Numerical analysis of whole-cell protein patterns presented as a dendrogram.

listed by Collins *et al.* (1993). *Weisella cibaria* strains LMG 17706, LMG 17699^T, LMG 18506, LMG 18507 and LMG 18814 were Gram-positive rods, catalase-negative, grew at 15, 37 and 45 °C, and produced gas from glucose. None of the five strains grew at 4 °C, nor in the presence of 8% NaCl, but all grew at 6·5% NaCl. They all produced slime from sucrose, hydrolysed arginine and synthesized D- and L-lactic acid isomers. *Weisella cibaria* strains differed from the majority of weissellas by being able to grow at 45 °C. So far this characteristic has been associated only with some *Weisella confusa* strains (Collins *et al.*, 1993). Production of D- and L-lactic acid isomers, dextran production and arginine hydrolysis are features *Weisella cibaria* also shares with *Weisella confusa*. Carbohydrate fermentation patterns of all five *Weisella cibaria* strains, presented in the description of *Weisella cibaria* below, were identical. *Weisella confusa* strains LMG 9497^T, LMG 17718 and LMG 18477 differed from the five strains of *Weisella cibaria* with respect to a few reactions only. LMG 18477 grew in the presence of 6·5% NaCl, but none of the three *Weisella confusa* strains fermented L-arabinose; strains LMG 17718 and LMG 9497^T fermented ribose and all three fermented galactose.

Peptidoglycan analysis

The interpeptide bridge of the peptidoglycan structure of *Weisella cibaria* is different from the corresponding structure of *Weisella confusa*. The interpeptide bridge of *Weisella cibaria* contains serine, L-Lys–L-Ala(L-Ser)–L-Ala (peptidoglycan type A3α), which is absent from the cell wall of *Weisella confusa*. Purified cell walls of *Weisella cibaria* strains LMG 17699^T and LMG 18814 contain, besides muramic acid and glucosamine, the amino acids lysine, glutamic acid, serine and alanine in a molar ratio of 1:1:0·5:3·5, respectively. As reported before, unique peptidoglycan structures of *Weissella* species are of help in species identification (Collins *et al.*, 1993), whereas carbohydrate fermentation profiles used alone for LAB identification may result in controversy (Milliere *et al.*, 1989; Lyhs *et al.*, 1999; Björkroth *et al.*, 2000). Based on the API 50 CH *Lactobacillus* identification system (BioMérieux) all *Weisella cibaria* strains were identified as '*Lactobacillus coprophilus*'. Unfortunately, '*Lactobacillus coprophilus*' could not be included as a reference strain for this study. The type strain does not exist in any culture collection; therefore it is not a valid species. However, the interpeptide bridge structure in the peptidoglycan of '*Lactobacillus coprophilus*' (Plapp & Kandler, 1967) has been reported to be different from the structures of *Weisella cibaria* and *Weisella confusa*. Since the length and type of the interpeptide bridge is a strong species-specific phenotypic criterion (Schleifer & Kandler, 1972), this finding indicates separate species status between these three species.

Numerical analysis of whole-cell protein and 16S and 23S rDNA RFLP patterns

Numerical analysis of both whole-cell protein and 16S and 23S rDNA RFLP patterns resulted in species-specific clustering. Numerical analyses of whole-cell protein (Fig. 1) patterns, *Cla*I- (Fig. 2a) and, to some extent, *Hin*dIII- (Fig. 2c) based ribotypes were found to provide similar clustering with consistency in strain division between species-specific groups. In the *Eco*RI-based dendrogram (Fig. 2b) the *Weisella hellenica* type strain clustered together with two of the *Weisella cibaria* strains and *Weisella cibaria* LMG 18814 strains clustered together with *Weisella confusa* strains. The patterns generated by *Eco*RI digestion contained mainly a few large molecular mass fragments (Fig. 2b), subjecting the numerical analysis to errors due to the limited differences in the mobility of these fragments. Patterns like this do not provide a good matrix for numerical analysis which should be taken into account when selecting the enzymes. *Eco*RI ribotyping gave similar results also when *Leuconostoc* species were characterized (Björkroth *et al.*, 2000) and its use cannot be recommended for species level identification of these organisms.

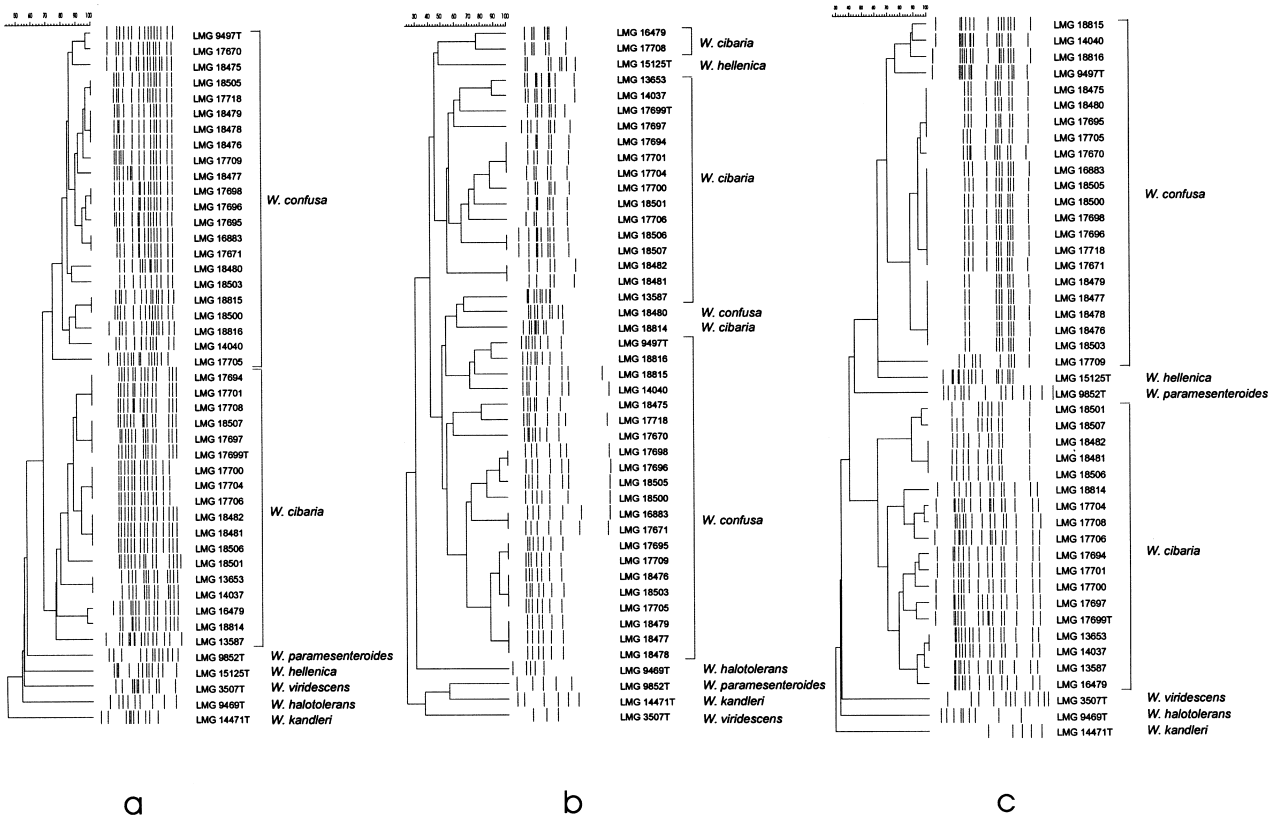

Fig. 2. (a), (b) and (c) present *Cla*I, *Eco*RI and *Hin*dIII ribopatterns, respectively, and the numerical analysis of the patterns is presented as a dendrogram. Left side of the banding patterns, high molecular masses, < 23 kbp in all patterns; right side of the banding patterns, low molecular masses, > 1000 bp in *Cla*I and *Hin*dIII patterns and > 4000 bp in *Eco*RI patterns.

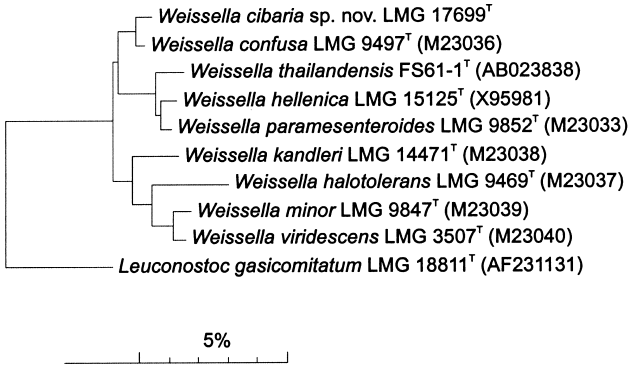

Fig. 3. Phylogenetic tree based on homologies of 1320 bp sequences in the 16S rDNA of *Weissella* type strains; *Weisella cibaria* is included.

16S rDNA sequence analysis

Fig. 3 shows the phylogenetic tree of genus *Weissella* type strains; *Weisella cibaria* is included, and is based on a comparison of a set of 1320 common nucleotide positions in the sequences analysed. According to the 16S rDNA sequence data, the *Weisella confusa* type strain possessed the highest similarity, 99·2 %, to *Weisella cibaria* LMG 17699^T (unknown bases were excluded from the calculations). Only these two species were located in this branch of the phylogenetic tree. In our study, the degree of 16S rDNA sequence similarities ranged between 89·2 and 99·2 % among the different *Weissella* species (data not shown). Some pairs of species, in particular *Weisella confusa* and *Weisella cibaria*, *Weisella hellenica* and *Weisella paramesenteroides*, and *Weisella minor* and *Weisella viridescens*, share 99·0 % or more of their 16S rDNA sequences, excluding the comparison of (near) entire 16S rDNA sequences for setting a similarity cut-off value between the different species. Our study also shows that the topology of the trees based on protein pattern or ribopattern similarity differed from that of the phylogenetic tree, and therefore that the former methods should be used for species level identification and not phylogeny. This was also observed in a taxonomic study of *Leuconostoc gasicomitatum* and other *Leuconostoc* species (Björkroth *et al*., 2000).

DNA–DNA hybridization results and DNA base composition

Table 2 presents the DNA–DNA hybridization results. The results show that *Weisella cibaria* strains shared DNA–DNA hybridization levels of 22–49 % with the

strains of their closest phylogenetic neighbour, *Weisella confusa*. The DNA G+C content of *Weisella cibaria* strains LMG 17699^T, LMG 17706, LMG 18506, LMG 18507, LMG 18814 ranged between 44 and 45 mol% (43·9–44·9 mol%). In an earlier DNA–DNA hybridization study (Vescovo *et al.*, 1979) among LAB species belonging to Orla–Jensen's *Betabacterium* group, DNA hybridization levels of *Weisella confusa* (*Lactobacillus confusus*) strains LMG 18506 (NCFB 889) and LMG 18507 (NCFB 1937) were assessed. As in the present study, strain LMG 18506 (NCFB 889) was found to have a low hybridization level (44%) with the *Weisella confusa* type strain. However, strain LMG 18507 (NCFB 1937) was reported to have a hybridization level of 93% with the *Weisella confusa* type strain, which is not in agreement with any of our results. We detected 84% hybridization between strains LMG 18506 and LMG 18507 and these strains had a similar hybridization level (90 and 100%, respectively) towards the *Weisella cibaria* type strain. Our reassociation experiment results are also in agreement with the results obtained in the polyphasic study.

Polyphasic approach

By means of the polyphasic taxonomic approach, *Weisella cibaria* strains were clearly distinguished from the *Weisella confusa* strains as well as from the other *Weissella* species. The phylogenetic study showed that *Weisella confusa* is the closest neighbour of *Weisella cibaria*, as reflected in the results obtained in the biochemical analyses, and in the numerical analysis of whole-cell protein patterns and *Hin*dIII and *Cla*I ribotypes. However, the low hybridization values (Table 2) in the DNA–DNA reassociation experiments clearly indicated that both taxa represent distinct species.

Exploitation of polyphasic taxonomic approaches has clarified bacterial classification in controversial situations. Additionally, the use of numerical analysis for large bacterial populations originating from various sources gives information on the sources and habitats of bacteria. In the case of LAB, the difficulties with phenotypic identification schemes may have resulted in poor knowledge of the occurrence of food-associated LAB in other ecosystems. The present study shows surprisingly diverse habitats for *Weisella confusa* and *Weisella cibaria* ranging from fermented foods to canary liver, from an otitis sample from a dog to human faeces. There is so far no evidence of pathogenicity of *Weissella* species. The majority of strains originating from clinical samples in this study had been growing as mixed cultures and the depositors had not considered them to be highly infectious. While working with diverse bacterial populations occurring in various habitats, such as *Weisella cibaria* and *Weisella confusa*, care must be taken in the selection of the identification methods. A polyphasic approach including a good set of reference strains is likely to give the best results.

Description of *Weissella cibaria* sp. nov.

Weissella cibaria (ci.ba′ri.a. L. adj. *cibarius* pertaining to food).

Cells are Gram-positive, short rods growing in pairs, 0·8–1·2 μm wide and 1·5–2 μm long. Non-motile. Peptidoglycan type is A3α, L-Lys–L-Ala(L-Ser)–L-Ala. Colonies are small and greyish white and catalase-negative. Growth occurs at 15, 37 and 45 °C, but not at 4 °C. Heterofermentative, producing CO_2 from glucose. D- and L-lactic acid isomers are synthesized. Arginine is hydrolysed. Slime is produced from sucrose. None of the strains grew in the presence of 8% NaCl but all grew in 6·5% NaCl. The following carbohydrates were fermented: L-arabinose, D-xylose, glucose, fructose, mannose, *N*-acetylglucosamine, amygdaline, arbutine, aesculin, salicin, cellobiose, maltose, saccharose and gentiobiose. Glycerol, erythritol, D-arabinose, ribose, L-xylose, adonitol, β-methyl-D-xyloside, galactose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, lactose, melibiose, trehalose, inulin, melezitose, raffinose, amidon, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate were not fermented. The DNA base ratio is 44–45 mol% G+C (thermal denaturation method). *Weisella cibaria* strains have been isolated from fermented food products and from clinical samples from humans and animals. The type strain is LMG 17699^T which was isolated from chili bo in Malaysia. Its DNA base ratio is 44 mol% and its phenotypic characteristics are as described above. The EMBL accession no. of the 16S rRNA gene sequence of LMG 17699^T is AJ295989. The type strain has been deposited in the BCCM/LMG and CCUG culture collections as LMG 17699^T = CCUG 41967^T; all other strains are available from the BCCM/LMG collection.

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