Genetic evidence that *Weissella kimchii* Choi *et al.* 2002 is a later heterotypic synonym of *Weissella cibaria* Björkroth *et al.* 2002

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The taxonomic positions of *Weissella kimchii* and *Weissella cibaria* were examined using phenotypic tests, 16S rRNA gene sequencing and DNA–DNA hybridization. The two species displayed very similar sugar-fermentation patterns and produced the D-form of lactic acid. Phylogenetic analysis showed that *W. cibaria* is the closest neighbour of *W. kimchii*, sharing about 99.7 % similarity in the 16S rRNA gene sequence. DNA–DNA hybridization experiments further reinforced these results, demonstrating a 98.2 % reassociation level between *W. cibaria* LMG 17699^T and *W. kimchii* DSM 14295^T. On the other hand, strain RO5, an isolate from paddy rice silage, exhibited 99.4 % 16S rRNA gene sequence similarity and a DNA reassociation level of more than 97 % with both type strains. Therefore, it is proposed that the species *W. kimchii* should be reclassified as *W. cibaria*.

The genus *Weissella* consists of a group of heterofermentative *Leuconostoc*-like lactic acid bacteria (Collins *et al.*, 1993). It is proposed that the following 12 species be included in the genus: *Weissella confusa*, *Weissella halotolerans*, *Weissella hellenica*, *Weissella kandleri*, *Weissella minor*, *Weissella paramesenteroides*, *Weissella viridescens* (Collins *et al.*, 1993), *Weissella thailandensis* (Tanasupawat *et al.*, 2000), *Weissella cibaria* (Björkroth *et al.*, 2002), *Weissella kimchii* (Choi *et al.*, 2002), *Weissella soli* (Magnusson *et al.*, 2002) and *Weissella koreensis* (Lee *et al.*, 2002).

In a recent study, while investigating lactic acid bacteria inhabiting paddy rice silage in Japan, we reported the presence of isolates belonging to the genus *Weissella* (Ennahar *et al.*, 2003). On the basis of an analysis of the 16S rDNA sequences, strain RO5 appeared to be very closely related to the type strains of *W. kimchii* and *W. cibaria*, suggesting a close phylogenetic affiliation between these species. *W. kimchii* was isolated from a traditional fermented vegetable in Korea (Choi *et al.*, 2002), whereas *W. cibaria* originated from a traditional unfermented food ingredient in Malaysia (Björkroth *et al.*, 2002). Although separate studies clearly distinguished the two species from

other members of the genus *Weissella* on the basis of their phenotypic and phylogenetic characteristics (Björkroth *et al.*, 2002; Choi *et al.*, 2002), a comparative investigation including both species has yet to be carried out.

The present study set out to identify strain RO5, isolated from paddy rice silage, and to investigate whether *W. cibaria* and *W. kimchii* are phylogenetically different. Comparative 16S rDNA sequence and DNA–DNA hybridization analyses as well as a physiological analysis were carried out. Since *Weissella* spp. are relatively common in Japanese rice silage (Cai *et al.*, 1994, 1998; Ennahar *et al.*, 2003), our study is also interesting from an ecological point of view.

The *Weissella* type strains used in the present study are shown in Fig. 1. Strain RO5 was isolated previously from paddy rice silage (Ennahar *et al.*, 2003). Bacterial cultures were grown on MRS agar (Difco) at 30 °C for 24 h, before being transferred to nutrient broth (Difco) with 10 % glycerol and stored as stock cultures at –80 °C for further analysis.

Strain RO5 was compared with type strains of *Weissella* species by using carbohydrate assimilation/fermentation on API 50 CH strips (bioMérieux), as described previously (Ennahar *et al.*, 2003). RO5 displayed fermentation patterns matching those of *W. cibaria* LMG 17699^T and *W. kimchii* DSM 14295^T. The three strains produced acid from D-fructose, D-glucose, D-mannose, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose and sucrose (see Table I, supplementary data), and hydrolysed aesculin. Weak acid production was observed with β -gentiobiose and gluconate. Mannitol, methyl α -D-glucoside, melibiose,

Published online ahead of print on 13 October 2003 as DOI 10.1099/ij.s.0.02783-0.

The GenBank accession number for the 16S rDNA sequence of strain RO5 is AF515221.

A phylogenetic tree constructed using a larger data set than shown in Fig. 1, details of acid production from carbohydrates (Table I) and DNA–DNA reassociation data (Table II) are available in IJSEM Online.

Fig. 1. Phylogenetic tree showing the relative positions of strain RO5 and *Weissella* species as inferred by the neighbour-joining method for 16S rDNA sequences. Bootstrap values for a total of 100 replicates are shown at the nodes of the tree. References of the type strains used for comparison are given, as well as the accession numbers for all 16S rDNA sequences (in parentheses). *Leuconostoc mesenteroides* was used as an outgroup. Bar, 1% sequence divergence. A phylogenetic tree constructed using a larger data set can be found in IJSEM Online.

trehalose, melezitose, D-turanose and D-arabitol were not fermented. Variable reactions were obtained with L-arabinose, ribose, D-galactose and D-xylose, which revealed differences involving weak reactions.

The lactic acid configuration was determined enzymically using a test kit from Boehringer, as described previously (Cai *et al.*, 1998). Strain RO5, *W. cibaria* LMG 17699^T and *W. kimchii* DSM 14295^T produced more than 90% of their lactic acid in the D-isomer form.

Amplification, purification and sequencing were performed as described previously (Ennahar *et al.*, 2003). The virtually complete RO5 16S rDNA sequence was used to search Ribosomal Database Project II. The highest level of sequence similarity, 99.4%, was found with *W. kimchii* and *W. cibaria*. Additionally, type strains from these two species showed an overall level of sequence similarity of 99.7% (1491 out of 1496 bases were identical). On the basis of the 16S rDNA sequences, phylogenetic trees were constructed by using the neighbour-joining method, as described previously (Ennahar *et al.*, 2003). This analysis placed strain RO5 in the same cluster as *W. kimchii* and *W. cibaria*, and confirmed its equally close relationship to both species (Fig. 1).

W. cibaria LMG 17699^T and *W. kimchii* DSM 14295^T, as well as type strains from somewhat more distant species, were chosen as reference strains for DNA–DNA hybridization experiments with strain RO5 (see Table II, supplementary data). Chromosomal DNA was purified by using standard methods (Sambrook *et al.*, 1989), as modified by Satomi *et al.* (1997). DNA–DNA relatedness was studied by the method of Ezaki *et al.* (1989) using photobiotin labelling and colorimetric detection. The results for strain RO5 showed reassociation levels of 97.3 and 98.6% with *W. cibaria* LMG 17699^T and *W. kimchii* DSM 14295^T, respectively. In addition, the level of DNA relatedness between the type strains of *W. cibaria* and *W. kimchii* was 98.2%. This is far higher than the DNA reassociation threshold value recommended for species delineation (70%) (Wayne *et al.*, 1987). Both species, however, showed less than 40% relatedness with other members of the genus *Weissella*, with the exception of *W. confusa* (43–53%).

DNA base content was determined by the method of Tamaoka & Komagata (1984) using HPLC, as described previously (Ennahar *et al.*, 2003). The mean G + C content of the DNA of strain RO5 was 48.5 mol%, which is comparable to the corresponding published values for *W. kimchii* and *W. cibaria*, i.e. 48.2 and 44–45 mol%, respectively (Björkroth *et al.*, 2002; Choi *et al.*, 2002).

As described above, phenotypic characterization, 16S rDNA sequence analysis, DNA–DNA hybridization and G + C-content determination showed that strain RO5, isolated from paddy rice silage, should be classified within the genus *Weissella*, with *W. cibaria* and *W. kimchii* as its nearest phylogenetic relatives. More importantly, on the basis of the detailed phenotypic and genetic characteristics, we could find no significant differences to justify separation of the type strains *W. kimchii* DSM 14295^T and *W. cibaria* LMG 17699^T. Therefore, given the earlier publication of the *W. cibaria* description, we propose that *W. kimchii* (Choi *et al.*, 2002) is a later heterotypic synonym of *W. cibaria* (Björkroth *et al.*, 2002) and consequently should be renamed.

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