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% reassocation level between W. cibaria DSM 17699T and W. kimchii DSM 14295T. On the other hand, strain RO5, an isolate from paddy rice silage, exhibited 99.4% 16S rRNA gene sequence similarity and a DNA reassocation 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 DSM 17699T and W. kimchii DSM 14295T. 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,
trehalose, melezitose, D-turanose and D-arabitol were not fermented. Variable reactions were obtained with L-arabinose, ribose, D-galactose and D-xylene, 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, Weissella cibaria DSM 14295T and Weissella kimchii DSM 14295T 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 Weissella 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 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 DSM 17699T and W. kimchii DSM 14295T, 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 DSM 17699T and W. kimchii DSM 14295T, 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 & Komaga (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 14295T and W. cibaria DSM 17699T. 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.

References


