A taxonomic study was conducted on two Gram-reaction-positive, catalase-negative, irregular short-rod-shaped or coccoid lactic acid bacteria, designated strains SG25^T and SG23, that were isolated from grains of fermented Japanese rice (*Oryza sativa* L. subsp. *japonica*). A phylogenetic analysis based on 16S rRNA gene sequence data clearly showed that the strains belonged to the genus *Weissella* and were most closely related to *Weissella solii* LMG 20113^T^ (with a sequence similarity of 96.9 % for each novel strain). The peptidoglycan of each strain contained the amino acids glutamic acid, lysine, serine and alanine in a molar ratio of 1.0 : 1.2 : 0.5 : 3.0, respectively. On the basis of the unusual phenotypic characteristics of the novel strains and the low levels of DNA–DNA relatedness recorded between each novel strain and *Weissella solii* JCM 12536^T^, strains SG25^T^ and SG23 represent a single novel species in the genus *Weissella*, for which the name *Weissella oryzae* sp. nov. is proposed. The type strain is SG25^T^ (=JCM 18191^T^ =DSM 25784^T^).

Japanese rice (*Oryza sativa* L. subsp. *japonica*) has been a dietary staple in Japan for over 2000 years. Recently, rice has garnered much interest in Japan, not only as a dish for human consumption but also as a valuable forage crop. During a study on certain isolates of lactic acid bacteria from rice grains that had been fermented to feed to livestock, two unidentified *Weissella*-like isolates, designated SG25^T^ and SG23, were discovered in Tochigi, Japan.

The taxonomic positions of these strains, which were isolated on plates of de Man, Rogosa and Sharpe (MRS) agar (Difco) after 72 h at 30 °C and under the anaerobic conditions produced in an ANX-1 TE-HER Hard Anaerobox (Hirosawa), have now been determined by using a polyphasic approach.

The genus *Weissella* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Leuconostocaceae* (Collins *et al.*, 1993). The natural habitats of members of the genus *Weissella* are widely distributed in meat and meat products, soil or fermented vegetable food (Björkroth *et al.*, 2002; De Bruyne *et al.*, 2008, 2010; Lee *et al.*, 2002; Magnusson *et al.*, 2002; Padonou *et al.*, 2010; Vela *et al.*, 2011). In the present study, two unique *Weissella*-like micro-organisms isolated from fermented rice grains were phylogenetically and phenotypically characterized.

In order to determine the taxonomic position of strains SG25^T^ and SG23, approximately 1,500 bases of the 16S rRNA gene sequences of the strains were analysed as described below. DNA was extracted and purified from cells harvested from MRS broth, as described by Saito & Miura (1963). Amplification of the 16S rRNA gene was performed with the prokaryotic 16S rRNA gene universal primers: 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GGTTACCTTGTTACGACTT-3’) (Suzuki *et al.*, 1996). Sequencing was carried out in triplicate on both strands by the dideoxy method (Suzuki *et al.*, 1996), using an ABI PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) in combination with an ABI 3730 sequencer (Applied Biosystems). We also confirmed the sequences using a rapid-PCR method without DNA preparation to amplify the 16S rRNA gene (Tohno *et al.*, 2012a, b). Comparative sequence analysis indicated that, in terms of the sequences analysed (each of about 1500 nt), strains SG25^T^ and SG23, were phylogenetically identical. A *BLAST* search and phylogenetic analysis revealed that the

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*Weissella oryzae* sp. nov., isolated from fermented rice grains

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In all three phylogenetic trees, both novel strains forming a cluster with the closest relatives of the novel strains were members of the genus Weissella. After 16S rRNA gene sequences were compared within version 2.0.12 of the CLUSTAL_X program (Larkin et al., 2007), the novel strains’ closest relatives were identified as Weissella soli LMG 20113^T (96.9 % sequence similarity), Weissella confusa JCM 1993^T (96.8 %) and Weissella cibaria LMG 17699^T (96.7 %). The member of the genus Weissella that showed the lowest 16S rRNA gene sequence similarity to the novel strains (92.0 %) was Weissella fabaria 257^T. An amplicon of 725 bp was produced when DNA from either novel strain was run in a Weissella-specific PCR (Jang et al., 2002) using the genus-specific primers produced a 725 bp band from DNA templates of the isolates (data not shown), suggesting that strains SG25^T and SG23 belong in the genus Weissella. However, the highest 16S rRNA gene sequence similarity recorded between each novel strain and an established species (96.9 %) was below the cut-off value of <98.7–99.0 % for species discrimination and therefore was too low to indicate that the novel strains belonged to any established species (Stackebrandt & Ebers, 2006).

16S rRNA gene sequences of the novel strains and the type strains of all members of the genus Weissella were aligned using CLUSTAL-X, and then a phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987), using evolutionary genetic distances that had been calculated by the Kimura two-parameter model (Kimura, 1980). The topology of the tree was evaluated by bootstrap analysis with 1000 replicates, again using CLUSTAL-X. Maximum-likelihood and minimum-evolution trees were also constructed, within version 5.0 of the MEGA software package (Tamura et al., 2011). DNA–DNA hybridization was conducted using biotinylated DNA according to the method of Ezaki et al. (1989). The genomic DNA G+C contents of the novel strains were determined as previously described (Kitahara et al., 2010).

In all three phylogenetic trees, both novel strains forming a distinct cluster with W. soli LMG 20113^T, (Fig. 1). On the basis of the phylogenetic trees, the phylogenetically closest strain, W. soli JCM 12536^T, was chosen as a reference strain for DNA–DNA hybridization experiments with strains SG25^T and SG23. As the DNA–DNA relatedness values recorded between strains SG25^T and SG23 exceeded 99 %, the strains were considered to be conspecific. The relatively low DNA–DNA relatedness values recorded between each novel strain and W. soli JCM 12536^T (20–22 %) indicated that the novel strains belonged to a single novel species. The genomic DNA G+C contents of SG25^T and SG23 were 40.6 mol% and 40.5 mol%, respectively, which are within the range of values previously reported for members of the genus Weissella (37–47 mol%; Padonou et al., 2010).

The two isolates were analysed by ribotyping as described previously (Kitahara et al., 2010). The dendrogram of ribotyping patterns revealed that strains SG25^T and SG23 clustered closely together with a high correlation, and were clearly different from other type strains of all established members of the genus Weissella (Fig. S1). In addition, the slightly different ribotyping patterns of strains SG25^T and SG23 indicated that they are two distinct strains (Fig. S1).

Cell and colony morphology were observed after growth on MRS agar plates at 30 °C for 48 h under anaerobic conditions. The Gram reaction was determined using a Favor G kit (Nissui Pharmaceuticals). Gas production from glucose was investigated by growing the bacteria in MRS broth that contained inverted Durham tubes. The production of dextran was observed on plates of glucose-free MRS agar in which glucose had been replaced with 5 % sucrose (Hitchener et al., 1982). Catalase activity was determined by transferring fresh colonies to a 3 % (v/v) H₂O₂ solution. Cell-wall peptidoglycan was prepared and hydrolysed as described previously (Komagata & Suzuki, 1987). The peptidoglycan’s amino-acid composition was then determined by ultraperformance liquid chromatography on an ACQUITY chromatography system (Waters) using the method described by Minamida et al. (2008). Lactic acid configuration was determined enzymically using the DL-lactate test kit (Roche Diagnostic). Physiological and biochemical characteristics were analysed using API 50 CH strips (bioMérieux) and API 20 Strep (bioMérieux) in duplicate at 30 °C, according to the manufacturer’s instructions. The API 50 CH strips were read after incubation for up to 7 days. The peptidoglycan of each strain contained glutamic acid, lysine, serine and alanine in a molar ratio of 1.0 : 1.2 : 0.5 : 3.0, respectively. The other results of these phenotypic characterizations, which can be useful for differentiating strains SG25^T and SG23 from closely related members of the genus Weissella, are given in Table S1 and the species description.

In conclusion, based on the phenotypic and phylogenetic evidence presented in this study, strains SG25^T and SG23 are considered to represent a single novel species of the genus Weissella, for which the name Weissella oryzae sp. nov. is proposed.

**Description of Weissella oryzae sp. nov.**

Weissella oryzae (o.ry’za.e. L. gen. n. oryzae of rice, from which the type strain was isolated).

Cells cultivated on MRS agar plates are Gram-reaction-positive, non-motile, non-spore-forming and catalase-negative irregular short rod-shaped or coccoid (0.7–0.9 × 0.9–3.0 μm) and occur singly or in pairs or short chains. After 2 days of anaerobic growth on MRS agar at 30 °C, colonies are 1 mm in diameter, greyish white, smooth, circular and convex. The cell-wall peptidoglycan contains glutamic acid, lysine, serine and alanine. Cells grow at 10–42 °C (but not at 4 or 50 °C), at pH 3.9–9.0, and with 4.0–6.5 % (w/v) NaCl. No growth was observed with 8 % (w/v) NaCl. Dextran is not produced from sucrose. Facultatively anaerobic, produces gas from glucose, and produces D-lactic acid heterofermentatively. Acid is produced from L-arabinose, D-ribose, D-xyllose, D-galactose (delayed reaction), D-glucose, D-fructose,
D-mannose, N-acetyl-D-glucosamine, maltose, melibiose (delayed reaction), trehalose and gluconate. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, 2-ketogluconate or 5-ketogluconate. Aesculin is not hydrolysed and arginine is cleaved.

The type strain, SG25T (=JCM 18191T =DSM 25784T), was isolated from fermented rice grain in Tochigi, Japan.
The genomic DNA G+C content of the type strain is 40.6 mol%.

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