Weissella beninensis sp. nov., a motile lactic acid bacterium from submerged cassava fermentations, and emended description of the genus *Weissella*

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Four Gram-positive, catalase-negative, short rod-shaped or coccoid, heterofermentative lactic acid bacterial strains (2L24P13^T, 1L48P15, 1L24P31 and 1L24P34) with unusual phenotypic and genotypic properties were isolated from submerged fermenting cassava on MRS agar. All strains were motile, grew at 15 °C, produced DL-lactic acid from glucose with gas formation and produced ammonia from arginine. Acid was produced from D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melibiose, D-raffinose, sucrose, N-acetylglucosamine and D-mannitol, but not from D-arabinose or xylose. 16S rRNA gene sequence analysis revealed that the strains belonged to the genus *Weissella* and were most closely related to *Weissella ghanensis* LMG 24286^T. Low DNA–DNA reassociation values were obtained between the isolates and *W. ghanensis* DSM 19935^T. Based on the genetic and phenotypic results, the strains are considered to represent a novel species, for which the name *Weissella beninensis* sp. nov. is proposed. The type strain is 2L24P13^T (=DSM 22752^T=LMG 25373^T).

Cassava, the most widespread root crop in developing countries, is processed in several ways before consumption. A well-known method that is widely practiced in Africa is lactic acid fermentation (Cooke *et al.*, 1987; Nout & Motarjemi, 1997). Cassava fermentation is a complex process that involves the activities of various micro-organisms and leads to the production of various food products such as gari, agbelima, attiéké, fufu and lafun. Several types of micro-organisms have been reported to be associated with such fermentations but lactic acid bacteria (LAB) predominate (Amoa-Awua *et al.*, 1996; Caplice &

Fitzgerald, 1999; Nout & Sarkar, 1999; Kostinek *et al.*, 2005; Coulin *et al.*, 2006). In a previous study, we identified the micro-organisms associated with submerged cassava root fermentation for lafun production to species level, among which four LAB strains, isolated from MRS agar (Merck), had unusual phenotypic and genotypic properties and were tentatively identified as species of the genus *Weissella* (Padonou *et al.* 2009). This study reports the morphological, biochemical and molecular characterization of these strains, designated 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34.

The four isolates and the reference strains *Weissella koreensis* DSM 15830^T, *W. kandleri* DSM 20593^T, *W. soli* DSM 14420^T and *W. ghanensis* DSM 19935^T were maintained in MRS broth at 30 °C for 2–3 days and stored in MRS medium with 20 % (v/v) glycerol at –80 °C.

Abbreviations: LAB, lactic acid bacteria; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 2L24P13^T is EU439435.

After growth for 4 days at 30 °C on MRS agar under anaerobic conditions, all four isolates exhibited colonies that were 1–2 mm in diameter, white to creamish white, smooth, circular and convex. Cell morphology was determined after overnight growth in MRS broth at 30 °C using phase-contrast microscopy and scanning electron microscopy (SEM). A 30 µl sample of the culture was diluted 10-fold with sterile MilliQ-water and filtered through a polycarbonate filter (pore size 0.2 µm). While still attached to the filter, the bacteria were dehydrated with a graded ethanol series, dried with hexamethyldisilazane, fixed with glutaraldehyde and osmium tetroxide, coated with gold/palladium and observed using a FEI Quanta 200 microscope at an acceleration voltage of 30 kV. Cells were short rod-shaped or coccoid, 0.7–0.9 µm wide and 0.9–1.5 µm long, occurring singly, in pairs or in short chains (Fig. 1a, b). Motility was observed for all four isolates under the phase-contrast microscope, although the motility was variable and sometimes only a small fraction of a population was actively moving. Peritrichous flagella were observed using SEM (Fig. 1b, c).

The Gram reaction was determined using the KOH method (Gregersen, 1978). Catalase activity was tested by the standard method using 3 % H₂O₂. Growth was determined at 15 and 45 °C, with 4, 6.5 and 10 % (w/v) NaCl and at pH 3.9 and 8.0 in MRS broth. Gas production from glucose was examined in MRS broth with citrate omitted at 30 °C, using inverted Durham tubes to collect the gas. Production of ammonia from arginine and formation of dextran from sucrose were tested following the protocol of Schillinger & Lücke (1987). The configuration of the lactic acid enantiomer was determined enzymically (Roche Diagnostic) (Schillinger & Lücke, 1987). Carbohydrate fermentation patterns were determined in microtitre plates following the protocol of Jayne-Williams (1976) and using the API 50CH system (bioMérieux). The results of these phenotypic tests are given in Table 1 and the species description.

For all four isolates, the nearly complete 16S rRNA gene sequence was determined as described below. DNA was extracted according to the method of Pitcher *et al.* (1989),

as modified for Gram-positive bacteria (Björkroth & Korkeala, 1996). The 16S rRNA gene was amplified by PCR and amplification products were purified and commercially sequenced at GATC Biotech as described previously (Kostinek *et al.*, 2005). Sequences were manually corrected and aligned using Vector NTI Suite 10. The closest phylogenetic relatives were determined by aligning the corrected sequences with 16S rRNA gene sequences in the GenBank database by using the BLAST algorithm (Altschul *et al.*, 1997). Strains 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34 had identical 16S rRNA gene sequences. The 16S rRNA gene sequences of the four isolates and their closest phylogenetic relatives were aligned and phylogenetic trees were constructed with the neighbour-joining, maximum-likelihood and maximum-parsimony methods using Bionumerics version 4.5 (Applied Maths). Unknown bases were discarded from the analysis and 1450 nucleotides were included. The statistical reliability of the topology of the phylogenetic trees was evaluated using bootstrap calculations with 1000 resamplings. As seen from the maximum-parsimony tree (Fig. 2), the four strains, represented here by strain 2L24P13^T, and *W. ghanensis* LMG 24286^T formed a distinct subgroup within the genus *Weissella*. Similar results were obtained with the neighbour-joining and maximum-likelihood methods (results not shown). Comparison of the 16S rRNA gene sequence of strain 2L24P13^T revealed the highest 16S rRNA gene sequence similarity with *W. ghanensis* LMG 24286^T (97.3 %) and lower similarities with *W. confusa* JCM 1093^T, *W. cibaria* NRIC 0136 and *W. koreensis* S-5623^T (92–94 %).

PCR with genus-specific primers demonstrated that the four isolates belonged to the genus *Weissella*: all of them produced a 1.2 kb fragment with the *Weissella*-specific primer Weissgrp (5'-GATGGTTCTGCTACCACTAAG-3'), using the procedure described by Schillinger *et al.* (2008). The four isolates were further investigated by two genotyping methods: repetitive element palindromic (rep)-PCR, using the primer GTG₅ (5'-GTGGTGGTGGTGGTG-3') and following the method of Gevers *et al.* (2001) for LAB as described by Franz *et al.* (2006), and randomly amplified

Fig. 1. Scanning electron micrographs of cells (a, b and c) and flagella (b and c) of strain 2L24P13^T grown overnight in MRS broth at 30 °C. Bars, 1 µm (a, b), 0.5 µm (c).

Table 1. Differential characteristics of *Weissella beninensis* sp. nov. strains and other *Weissella* species

Taxa: 1, *Weissella beninensis* sp. nov. ($n=4$); 2, *W. cibaria* LMG 17699^T; 3, *W. confusa* JCM 1093^T; 4, *W. ghanensis* LMG 24286^T; 5, *W. halotolerans* LMG 9469^T; 6, *W. hellenica* LMG 15125^T; 7, *W. kandleri* DSM 20593^T; 8, *W. koreensis* JCM 11263^T; 9, *W. minor* LMG 9847^T; 10, *W. paramesenteroides* LMG 9852^T; 11, *W. soli* LMG 20113^T; 12, *W. thailandensis* JCM 10695^T; 13, *W. viridescens* LMG 3507^T. Data were taken from this study and Collins *et al.* (1993), Tanasupawat *et al.* (2000), Björkroth *et al.* (2002), Magnusson *et al.* (2002), Lee *et al.* (2002), Choi *et al.* (2002), Ennahar & Cai (2004) and De Bruyne *et al.* (2008). +, 90 % or more of strains positive; v, 11–89 % of strains positive; –, 10 % or less of strains positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Lactic acid configuration	DL	DL	DL	DL	DL	D	DL	D	DL	D	D	D	DL
NH ₃ from arginine	+	+	+	+	+	–	+	+	+	–	+	–	–
Dextran formation	v	+	+	+	+	–	+	+	–	–	–	–	ND
Acid from:													
Arabinose	–	+	–	–	–	+	–	+	–	v	+	+	–
Cellobiose	v	+	+	–	–	–	–	–	+	v	–	–	–
Galactose	+	–	+	–	–	+	–	–	–	+	–	+	–
Lactose	+	–	–	–	ND	–	–	ND	ND	–	–	ND	ND
Melibiose	+	–	–	–	–	–	–	–	–	+	+	–	–
Raffinose	+	–	–	–	–	–	–	–	–	v	+	–	–
Ribose	v	–	+	–	+	–	+	+	+	v	+	+	–
Salicin	v*	+	+	+	–	–	–	ND	–	–	+	–	–
Sucrose	+	+	+	v	–	+	–	–	+	+	+	v	v
Trehalose	v	–	–	+	–	+	–	–	+	+	+	v	v
Xylose	–	+	+	–	–	–	–	+	–	v	+	–	–
DNA G + C content (mol%)	37	44–48	45–47	40	45	39–40	39	37	44	37–38	43	38–41	41–44

*Delayed reaction.

polymorphic DNA (RAPD)-PCR, using primer M13 (5'-GAGGGTGGCGTTCT-3') or primer AP4 (5'-TCAC-GCTGCA-3'; Andrighetto *et al.*, 2001). A dendrogram was derived using the unweighted pair group method with arithmetic averages linkage of correlation coefficients (Fig. 3). Strains 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34 clustered closely together ($r=90$ –95 %) and clustered with

strains of *W. koreensis*, *W. kandleri*, *W. soli* and *W. ghanensis* with a low correlation ($r=12$ –20 %).

For determination of G + C content and DNA–DNA relatedness, DNA was extracted from the four isolates and *W. ghanensis* DSM 19935^T and purified following the protocol of Marmur (1961) as modified by Stackebrandt &

Fig. 2. Maximum-parsimony phylogenetic tree based on almost-complete 16S rRNA gene sequences showing the phylogenetic positions of strain 2L24P13^T and strains of the species of the genus *Weissella*. Percentages at nodes are bootstrap values based on 1000 resamplings. Bar, 2 % sequence divergence.

Fig. 3. rep-GTG₅ and RAPD PCR fingerprints and a corresponding dendrogram for *Weissella beninensis* sp. nov. and closely related strains of species of the genus *Weissella*.

Kandler (1979). The G + C content of DNA was determined by a modification of the fluorometric method described by Gonzalez & Saiz-Jimenez (2002) using a iQ5 real-time thermocycler (Bio-Rad). Thermal denaturation was performed in 1 × standard saline citrate with 25 µg genomic DNA and 20 × EvaGreen (Biotrend Chemicals) as the fluorescent dye. DNA of six LAB strains with completely sequenced genomes was used to create a calibration curve of G + C values versus the melting temperatures (T_m values) measured with the thermocycler. Regression analysis was performed to derive a formula for the calculation of the G + C content in mol%. DNA–DNA relatedness was determined using the fluorometric method of Jakava-Viljanen *et al.* (2008) with EvaGreen and a real-time thermocycler. Genomic DNA from strains 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34 were hybridized to each other and to *W. ghanensis* DSM 19935^T. The G + C contents of strains 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34 were 37.0–37.2 mol%, which can be compared with the G + C content of 40 mol% for *W. ghanensis* LMG 24286^T (De Bruyne *et al.*, 2008; Table 1). The DNA–DNA relatedness values between strains 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34 were 84–122 %, which indicated that the strains are con-specific, and between the four isolates and *W. ghanensis* DSM 19935^T they were 19–42 %. Thus, DNA–DNA relatedness values between each isolate and its closest relative were well below the 70 % cut-off value recommended to indicate separate species (Wayne *et al.*, 1987).

Based on the morphological, physiological and genetic characteristics described above, strains 2L24P13^T, 1L48P15, 1L24P31 and 1L24P34 clearly formed a homogeneous genetically distinct group that was most closely related to *W. ghanensis*. In addition to their genotypic characteristics, the four isolates could be clearly differentiated from their phenotypic and genotypic closest relatives by their motility and ability to produce acid from raffinose, melibiose and lactose. In conclusion, the isolates represent a novel species in the genus *Weissella*, for which the name *Weissella beninensis* sp. nov. is proposed, with strain 2L24P13^T as the

type strain. *Weissella beninensis* sp. nov. represents the first description of a motile species within the genus *Weissella*. Therefore, the genus *Weissella* described by Collins *et al.* (1993) should be emended.

Emended description of the genus *Weissella* Collins *et al.* 1993

This description is based on that given by Collins *et al.* (1993). Cells are generally short rods with rounded to tapered ends or coccoid in shape occurring singly, in pairs or in short chains. Cells are Gram-positive and non-motile, with the exception of *W. beninensis* where all known strains are motile. Endospores are not formed. Catalase and cytochromes are not produced. Chemo-organotrophs with complex nutritional requirements. Heterofermentative. The configuration of lactic acid produced from glucose is either DL- or (–)-D-, depending on the species. Acidoduric. Growth occurs at 15 °C, but not at 45 °C (with the exception of some strains of *W. confusa*). Strains of some species hydrolyse arginine. The cell-wall peptidoglycan is based upon lysine; the interpeptide bridge contains alanine or serine and alanine as typical constituents. The G + C content of DNA is 37–47 mol%. The type species is *Weissella viridescens*.

Description of *Weissella beninensis* sp. nov.

Weissella beninensis (ben.in.en'sis. N.L. fem. adj. *beninensis* pertaining to Benin, where the type strain was isolated).

Cells are Gram-positive, catalase-negative and short rod-shaped or coccoid (0.7–0.9 × 0.9–1.5 µm). Motile with peritrichous flagella. Non-spore-forming. Occur singly, in pairs or in short chains. Colonies are 1–2 mm in diameter, white to creamish white, smooth, circular and convex after 3–4 days of anaerobic growth. Grows at 15 °C, at pH 3.9–8.0 and with 4 % NaCl. No growth at 45 °C. Ammonia is produced from arginine and gas is produced from glucose. (–)-D- and (+)-L-lactic acid are produced as end products of glucose metabolism. Dextran is formed

from sucrose by most strains. Acid is produced from L-arabinose, D-fructose, D-glucose, D-galactose, β -gentiobiose, lactose, maltose, D-mannose, melibiose, D-raffinose, sucrose, D-mannitol, sorbitol, N-acetylglucosamine, amygdalin, arbutin, aesculin. Acid production from ribose, salicin (delayed reaction), cellobiose and trehalose is strain-dependent. Does not ferment D-arabinose, D- or L-fucose, D-lyxose, rhamnose, L-sorbose, D-tagatose, trehalose, D-turanose, D- or L-xylose, adonitol, D- or L-arabitol, dulcitol, erythritol, glycerol, inositol, xylitol, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -xyloside, inulin, starch, gluconate, glycogen or 2- or 5-ketogluconate. The G+C content is 37.0–37.2 mol%.

The type strain is 2L24P13^T (=DSM 22752^T=LMG 25373^T), isolated from cassava fermentations in Ketou, Benin.

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References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Amoa-Awua, W. K. A., Appoh, F. E. & Jakobsen, M. (1996). Lactic acid fermentation of cassava dough into agbelima. *Int J Food Microbiol* **31**, 87–98.
- Andrighetto, C., Knijff, E., Lombardi, A., Torraini, S., Vancanneyt, M., Kersters, K., Swings, J. & Dellaglio, F. (2001). Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. *J Dairy Res* **68**, 303–316.
- Björkroth, J. & Korkeala, H. J. (1996). Evaluation of *Lactobacillus sake* contamination in vacuum-packaged sliced cooked meat products by ribotyping. *J Food Prot* **59**, 398–401.
- Björkroth, K. J., Schillinger, U., Geisen, R., Weiss, N., Hoste, B., Holzapfel, W. H., Korkeala, H. J. & Vandamme, P. (2002). Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. *Int J Syst Evol Microbiol* **52**, 141–148.
- Caplice, E. & Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. *Int J Food Microbiol* **50**, 131–149.
- Choi, H.-J., Cheigh, C.-I., Kim, S.-B., Lee, J.-C., Lee, D.-W., Choi, S.-W., Park, J.-M. & Pyun, Y.-R. (2002). *Weissella kimchii* sp. nov., a novel lactic acid bacterium from kimchi. *Int J Syst Evol Microbiol* **52**, 507–511.
- Collins, M. D., Samelis, J., Metaxopoulos, J. & Wallbanks, S. (1993). Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *J Appl Bacteriol* **75**, 595–603.
- Cooke, R. D., Twiddy, D. R. & Reilly, P. J. A. (1987). Lactic-acid fermentation as a low-cost means of food preservation in tropical countries. *FEMS Microbiol Rev* **46**, 369–379.
- Coulin, P., Farah, Z., Assanvo, J., Spillmann, H. & Puhon, Z. (2006). Characterisation of the microflora of attiéké, a fermented cassava product, during traditional small-scale preparation. *Int J Food Microbiol* **106**, 131–136.
- De Bruyne, K., Camu, N., Lefebvre, K., De Vuyst, L. & Vandamme, P. (2008). *Weissella ghanensis* sp. nov., isolated from Ghanaian cocoa fermentation. *Int J Syst Evol Microbiol* **58**, 2721–2725.
- Ennahar, S. & Cai, Y. (2004). Genetic evidence that *Weissella kimchii* Choi et al. 2002 is a later heterotypic synonym of *Weissella cibaria* Björkroth et al. 2002. *Int J Syst Evol Microbiol* **54**, 463–465.
- Franz, C. M. A. P., Vancanneyt, M., Vandemeulebroecke, K., De Wachter, M., Cleenwerck, I., Hoste, B., Schillinger, U., Holzapfel, W. H. & Swings, J. (2006). *Pediococcus stilesii* sp. nov., isolated from maize grains. *Int J Syst Evol Microbiol* **56**, 329–333.
- Gevers, D., Huys, G. & Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol Lett* **205**, 31–36.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.
- Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.
- Jakava-Viljanen, M., Murros, A., Palva, A. & Björkroth, K. J. (2008). *Lactobacillus sobrius* Konstantinov et al. 2006 is a later synonym of *Lactobacillus amylovorus* Nakamura 1981. *Int J Syst Evol Microbiol* **58**, 910–913.
- Jayne-Williams, D. J. (1976). The application of miniaturized methods for the characterization of various organisms isolated from the animal gut. *J Appl Bacteriol* **40**, 189–200.
- Kostinek, M., Specht, I., Edward, V. A., Schillinger, U., Hertel, C., Holzapfel, W. H. & Franz, C. M. (2005). Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. *Syst Appl Microbiol* **28**, 527–540.
- Lee, J.-S., Lee, K. C., Ahn, J.-S., Mheen, T.-I., Pyun, Y.-R. & Park, Y.-H. (2002). *Weissella koreensis* sp. nov., isolated from kimchi. *Int J Syst Evol Microbiol* **52**, 1257–1261.
- Magnusson, J., Jonsson, H., Schnürer, J. & Roos, S. (2002). *Weissella soli* sp. nov., a lactic acid bacterium isolated from soil. *Int J Syst Evol Microbiol* **52**, 831–834.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Nout, M. J. R. & Motarjemi, Y. (1997). Assessment of fermentation as household technology for improving food safety: a joint FAO/WHO workshop. *Food Contr* **8**, 221–228.
- Nout, M. J. R. & Sarkar, P. K. (1999). Lactic acid food fermentation in tropical climates. *Antonie van Leeuwenhoek* **76**, 395–401.
- Padonou, S. W., Nielsen, D. S., Hounhouigan, J. D., Thorsen, L., Nago, M. C. & Jakobsen, M. (2009). The microbiota of Lafun, an African traditional cassava food product. *Int J Food Microbiol* **133**, 22–30.
- Pitcher, D. G., Saunters, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Schillinger, U. & Lücke, F.-K. (1987). Identification of lactobacilli from meat and meat products. *Food Microbiol* **4**, 199–208.
- Schillinger, U., Boehringer, B., Wallbaum, S., Caroline, L., Gonfa, A., Holzapfel, W. H. & Franz, C. M. A. P. (2008). A genus-specific PCR method for differentiation between *Leuconostoc* and *Weissella* and its

application in identification of heterofermentative lactic acid bacteria. *FEMS Microbiol Lett* **286**, 222–226.

Stackebrandt, E. & Kandler, O. (1979). Taxonomy of the genus *Cellulomonas*, based on phenotypic characters and deoxyribonucleic acid-deoxyribonucleic acid homology, and proposal of seven neotype strains. *Int J Syst Bacteriol* **29**, 273–282.

Tanasupawat, S., Shida, O., Okada, S. & Komagata, K. (2000). *Lactobacillus acidipiscis* sp. nov. and *Weissella thailandensis* sp. nov.,

isolated from fermented fish in Thailand. *Int J Syst Evol Microbiol* **50**, 1479–1485.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.