Characterization of strains of *Weissella fabalis* sp. nov. and *Fructobacillus tropaeoli* from spontaneous cocoa bean fermentations

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Six facultatively anaerobic, non-motile lactic acid bacteria were isolated from spontaneous cocoa bean fermentations carried out in Brazil, Ecuador and Malaysia. Phylogenetic analysis revealed that one of these strains, designated M75^T, isolated from a Brazilian cocoa bean fermentation, had the highest 16S rRNA gene sequence similarity towards *Weissella fabaria* LMG 24289^T (97.7 %), *W. ghanensis* LMG 24286^T (93.3 %) and *W. beninensis* LMG 25373^T (93.4 %). The remaining lactic acid bacteria isolates, represented by strain M622, showed the highest 16S rRNA gene sequence similarity towards the type strain of *Fructobacillus tropaeoli* (99.9 %), a recently described species isolated from a flower in South Africa. *pheS* gene sequence analysis indicated that the former strain represented a novel species, whereas *pheS*, *rpoA* and *atpA* gene sequence analysis indicated that the remaining five strains belonged to *F. tropaeoli*; these results were confirmed by DNA–DNA hybridization experiments towards their respective nearest phylogenetic neighbours. Additionally, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry proved successful for the identification of species of the genera *Weissella* and *Fructobacillus* and for the recognition of the novel species. We propose to classify strain M75^T (=LMG 26217^T =CCUG 61472^T) as the type strain of the novel species *Weissella fabalis* sp. nov.

Papalexandratou and colleagues analysed fermenting cocoa pulp-bean samples collected from traditional Brazilian (October–November 2006), Ecuadorian (April 2008) and Malaysian (April 2010) cocoa bean box and platform fermentations (Papalexandratou, 2011; Papalexandratou *et al.*, 2011a, b). In these studies, they isolated a large number of lactic acid bacteria (LAB) that were first screened and identified by $(GTG)_5$-PCR fingerprinting. However, this approach failed to identify one isolate, designated M75^T, from a Brazilian cocoa bean box fermentation, with a unique $(GTG)_5$-PCR fingerprint and another 63 LAB isolates (two, 58 and three isolates from Brazil, Ecuador and Malaysia, respectively) with similar $(GTG)_5$-PCR fingerprints. The precise taxonomic positions of six of these LAB, which belonged to five different rep clusters [clusters I–III, M75^T, M56 and M622 (Brazil); cluster IV, M1588 and M1190 (Malaysia and Ecuador, respectively); cluster V, M710 (Ecuador)] were determined in the present study.

Analysis of the 16S rRNA gene sequences of strains M75^T and M622 was performed as described by De Bruyne *et al.* (2008b), except that sequencing reactions were purified using the BigDye xTerminator purification kit according to the protocol of the supplier (Applied Biosystems). The ARB software package (Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 102 database (Pruesse *et al.*, 2007) were used to align the 16S rRNA gene sequences obtained and those of the type strains of all established species of the genera *Fructobacillus* and *Weissella*, their nearest phylogenetic neighbours (see below). These aligned sequences were imported into the software package MEGA version 5.0 (Tamura *et al.*, 2011) and analysed using the neighbour-joining, maximum-likelihood and maximum-parsimony methods. The statistical reliability of tree topologies was evaluated by bootstrapping analysis based on 1000 tree replicates. The maximum-parsimony and neighbour-joining

Abbreviations: LAB, lactic acid bacteria; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *pheS* gene sequences of strain M75^T are HE576795 and HE576796. Accession numbers of other sequences obtained in this study are detailed in Table 1.

Two supplementary figures are available with the online version of this paper.

trees (not shown) revealed topologies similar to those obtained in the phylogenetic tree reconstructed using the maximum-likelihood approach (Fig. 1). Sequence similarity calculations performed using the ARB software package indicated that the closest relatives of strain M75^T were *Weissella fabaria* LMG 24289^T (97.7 %), *W. ghanensis* LMG 24286^T (93.3 %) and *W. beninensis* 2L24P13^T (93.4 %). Lower sequence similarities (<89 %) were found towards other species of the genus *Weissella* with validly published names. Strain M622 was most closely related to *Fructobacillus tropaeoli* F214-1^T (99.9 %) and *F. pseudoficulneus* LC 51^T (99.2 %). Lower sequence similarities (<98 %) were found towards other species of the genus *Fructobacillus* with validly published names.

Sequence analysis of the housekeeping genes encoding phenylalanyl-tRNA synthase alpha subunit (*pheS*), RNA polymerase alpha subunit (*rpoA*) and the alpha subunit of ATP synthase (*atpA*) correlates with species delineation as determined by DNA–DNA hybridization in the genus *Fructobacillus* (De Bruyne *et al.*, 2007). Therefore, *pheS*, *rpoA* and *atpA* gene sequences of strains M56, M622, M710, M1190 and M1588 and *F. tropaeoli* LMG 26298^T, for which no *pheS*, *rpoA* or *atpA* sequences were available, were determined as described previously (De Bruyne *et al.*, 2007), except that sequencing reactions were purified using the BigDye xTerminator purification kit as described above. Similarly, sequence analysis of the *pheS* gene has been used successfully to distinguish all established species of the genus *Weissella* (De Bruyne *et al.*, 2007, 2010). Therefore, *pheS* gene sequences were determined for strain M75^T and for *W. beninensis* LMG 25373^T and *W. fabaria* M1160 and M1167, for which no *pheS* gene sequences were available. *pheS*, *rpoA* and *atpA* gene sequences of all remaining *Fructobacillus* and *Weissella* reference strains were available from previous studies (De Bruyne *et al.*, 2007, 2010). Details of strains, depositors and accession numbers are given in Table 1. SeaView version 4 was used to concatenate the *pheS*, *rpoA* and *atpA* gene sequences (Gouy *et al.*, 2010) and the software package MEGA version 5.0 (Tamura *et al.*, 2011) was used to align the translated concatenated gene sequences and to analyse the nucleotide sequences as mentioned above. The maximum-parsimony and neighbour-joining trees (not shown) revealed topologies similar to those obtained in phylogenetic tree reconstructed using the maximum-likelihood approach (Figs 2 and 3) for both analyses. The concatenated *pheS*, *rpoA* and *atpA* gene sequences of strains M56, M622, M710, M1190 and M1588 revealed high similarity to the concatenated sequence of *F. tropaeoli* LMG 26298^T (98.4, 97.4, 99.2, 99.3 and 99.3 % similarity, respectively). Lower gene sequence similarities were found (⩽95 %) towards other species of the genus *Fructobacillus* with validly published names. The phylogenetic tree of the concatenated *pheS*, *rpoA* and *atpA* gene sequences confirmed the discriminatory power of these sequences for species identification within the genus *Fructobacillus*: all species were clearly delineated above 97 % concatenated gene sequence similarity (Fig. 2). Based on these results and the previously established correlation between multilocus sequence analysis (MLSA) of concatenated *pheS*, *rpoA* and *atpA* gene sequences and levels of DNA–DNA hybridization, we concluded that strains M56, M622, M710, M1190 and M1588 belong to *F. tropaeoli*. In addition, pairwise *pheS* gene

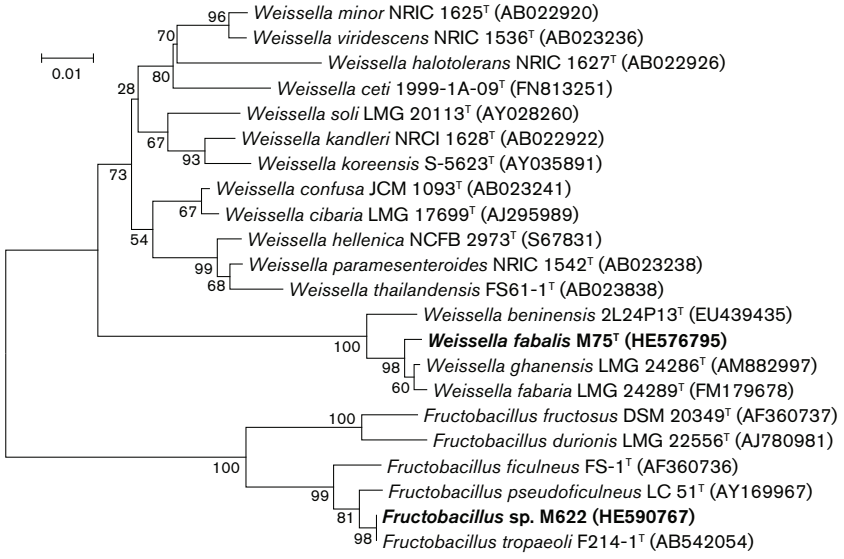

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strains M75^T and M622 among the type strains of all species of the genera *Weissella* and *Fructobacillus*. Bootstrap values (%) based on 1000 replications are shown at branch points. The substitution model used was the general time reversible (GTR) model and the aligned sequence had a length of 1345 bp. Bar, 1 % sequence divergence.

sequence similarity calculations, calculated using the MEGA5 software version 5.0, confirmed that the closest relatives of strain M75^T were *W. fabaria* LMG 24289^T (85.2 %), *W. ghanensis* LMG 24286^T (86.9 %) and *W. beninensis* LMG 25373^T (80 %). Lower *pheS* gene sequence similarities (<78 %) were found towards other species of the genus *Weissella* with validly published names. With the exception of *Weissella viridescens*, De Bruyne *et al.* (2010) demonstrated that strains belonging to the same species of the genus *Weissella* share *pheS* gene sequence similarity of at least 96.8 %. Therefore, the present results suggest that strain M75^T represents a novel species of the genus *Weissella* (Fig. 3).

To confirm these results, DNA–DNA hybridization experiments between strains M75^T and M1190 and their respective nearest neighbours were performed. Genomic DNA of strain M75^T, *W. ghanensis* LMG 24286^T, *W. fabaria* LMG 24289^T, M1190 and *F. tropaeoli* LMG 26298^T was extracted using the guanidine thiocyanate method described by Pitcher *et al.* (1989). DNA–DNA hybridizations were performed using the microplate method, with photobiotin for labelling of the DNA (Ezaki *et al.*, 1989), as modified by Goris *et al.* (1998). The hybridization levels of strain M75^T towards *W. ghanensis* LMG 24286^T and *W. fabaria* LMG 24289^T were 44 and 36 %, respectively, confirming that it represents a distinct species of the genus *Weissella*. The DNA–DNA hybridization between strain M1190 and *F. tropaeoli* LMG 26298^T was 82 %, which confirmed its MLSA-based identification.

DNA G+C content was determined according to the enzymic DNA degradation method described previously (Mesbah & Whitman, 1989) using a Waters Breeze HPLC system and XBridge Shield RP18 column. The solvent used was 0.02 M $NH_4H_2PO_4$ (pH 4.0)/1.5 % (v/v) acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as a calibration reference and *Escherichia coli* LMG 2093 DNA was included as a control. The DNA G+C contents of strains M75^T and M1190 were 37 and 45 mol%, respectively, consistent with the G+C contents found previously in the genera *Weissella* (37–47 mol%; Björkroth *et al.*, 2002; Collins *et al.*, 1993; De Bruyne *et al.*, 2008a, 2010; Lee *et al.*, 2002; Magnusson *et al.*, 2002; Padonou *et al.*, 2010; Tanasupawat *et al.*, 2000; Vela *et al.*, 2011) and *Fructobacillus* (42–45 mol%; Endo & Okada, 2008; Endo *et al.*, 2011).

Preparation of peptidoglycan and analysis of peptidoglycan structure were performed according to published protocols (Schumann, 2011). The total hydrolysate (4 M HCl, 100 °C, 16 h) of the peptidoglycan contained the amino acids lysine, alanine, serine, glycine and glutamic acid in an approximate ratio of 1.0 : 1.7 : 0.9 : 0.2 : 2.6. Because the ratio of glutamic acid to lysine was >1 and did not fit the expected A3α peptidoglycan type, the peptidoglycan preparation was subjected to hydrofluoric acid treatment in order to remove contaminating polymers linked to the peptidoglycan via phosphodiester bonds before repeating the analysis. However, the molar ratio of the peptidoglycan amino acids changed only slightly after the hydrofluoric acid treatment. The identity of the amino acids was confirmed by GC-MS. The partial hydrolysate (4 M HCl, 100 °C, 45 min) contained, in addition to the amino acids, the peptides L-Ala–D-Glu–L-Lys–L-Ala, D-Ala–L-Lys and D-Ala–L-Lys–L-Ala. The peptide Ala–Ala could not be found. Dinitrophenylation according to Schleifer (1985) revealed that serine represents the N terminus of the interpeptide bridge. Though the quantitative amino acid ratio contained too much glutamic acid and too little alanine, the peptidoglycan type A3α L-Lys–L-Ala–L-Ser is concluded from the N terminus of the interpeptide bridge and the 2D TLC patterns of peptides and amino acids. The high content of glutamic acid as well as the traces of glycine might be caused by residual polymer contamination.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS has shown to be both rapid and accurate for species and subspecies classification of a broad spectrum of bacteria (Sauer & Kliem, 2010). To test whether MALDI-TOF MS analysis is a suitable tool for the identification of the LAB of the present study, mass spectra were generated from all *Weissella* and *Fructobacillus* strains (Table 1) except for *Weissella hellenica* LMG 15125^T, *W. cibaria* R-31690, *W. confusa* LMG 18480, *W. fabaria* 252 and *F. pseudoficulneus* R-35156 and R-35158, from which good-quality spectra could not be obtained. Bacteria were grown under standardized conditions [28 °C, 24 h, MRS agar (Oxoid) and an aerobic atmosphere] and subcultured twice prior to analysis. For each strain, a spectral profile was generated from three different generations to improve the robustness of the library of mass spectral fingerprints. Harvesting of cells, extract preparation, measuring and data analysis were performed as described previously (Ghyselinck *et al.*, 2011). The similarity between the spectra of strains M75^T, M56, M622, M710, M1190 and M1588 and the reference strains was calculated using Pearson's product moment correlation coefficient and clustering was performed using the unweighted pair group method with arithmetic means (UPGMA) clustering algorithm (Figs S1 and S2, available in IJSEM Online). The MALDI-TOF MS profile of strain M75^T formed a separate cluster that was most similar to the protein profiles of recently described *W. ghanensis* and *W. fabaria* strains originating from Ghanaian cocoa bean fermentations. The remaining cocoa isolates showed high similarity to the recently described *F. tropaeoli*, isolated from a South African flower. For the genus *Fructobacillus*, the intraspecies diversity was consistently smaller than the interspecies divergence towards their nearest neighbours, as was the case for the genus *Weissella*.

Finally, a biochemical analysis of the cocoa isolates was performed to characterize the novel species and to compare the characteristics of the novel *F. tropaeoli* strains (cocoa origin) with those of the type (and currently only known) strain (Chambel *et al.*, 2006; Endo *et al.*, 2011). Cell and colony morphology of strains M56, M622, M710, M1190 and M1588 was verified after growth on MRS agar (Oxoid) supplemented with 1 % (w/v) fructose and 24 h of aerobic

Table 1. *Weissella* and *Fructobacillus* strains examined in this study

Depositors: S. W. Padonou, Université d'Abomey-Calavi, Cotonou, Benin; L. Devriese, Ghent University, Ghent, Belgium; G. Rusul, University Putra, Malaysia; NCFB, National Collection of Food Bacteria (now NCIMB), Aberdeen, UK; KCTC, Korean Collection for Type Cultures, Yusong, Taejon, Republic of Korea; CCMM, Moroccan Coordinated Collections of Microorganisms, Rabat, Morocco; A. Ledeboer, Unilever, Vlaardingen, The Netherlands; ATCC, American Type Culture Collection, Manassas, VA, USA; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; NCIMB, National Collections of Industrial, Food and Marine Bacteria, Aberdeen, UK; S. Roos, Swedish University of Agricultural Sciences, Uppsala, Sweden; JCM, Japan Collection of Microorganisms, RIKEN BRC, Japan; J. Björkroth, University of Helsinki, Helsinki, Finland; J. Leisner, Royal Veterinary and Agricultural University, Frederiksberg, Copenhagen, Denmark; CECT, Colección Española de Cultivos Tipo, Valencia, Spain; R. Tenreiro, University of Lisbon, Lisbon, Portugal; L. Dicks, University of Stellenbosch, South Africa. NK, Not known.

Strain	Depositor	Source	Accession numbers		
			pheS	*rpoA*	*atpA*
W. beninensis					
LMG 25373^T	S. W. Padonou	Submerged fermented cassava (Benin)	HE576797		
W. cibaria					
LMG 13587	L. Devriese	Dog, ear (Belgium)	FM202101		
LMG 17699^T	G. Rusul	Chili bo (Malaysia)	FM202102		
LMG 17704	G. Rusul	Chili bo (Malaysia)	FM202100		
LMG 18507	NCFB	Post-harvest deterioration of sugar cane	FM202098		
LMG 21843	KCTC	Partially fermented kimchi (Korea)	FM202099		
R-31690	CCMM	Fermented skimmed milk (Morocco)	FM202103		
W. confusa					
LMG 9497^T	A. Ledeboer	Sugar cane	FM202105		
LMG 11983	ATCC	Grass silage	FM202104		
LMG 14040	L. Devriese	Dog, ear (Belgium)	FM202107		
LMG 17718	G. Rusul	Chili bo (Malaysia)	FM202108		
LMG 18480	G. Rusul	Tapai (Malaysia)	FM202106		
W. fabaria					
LMG 24289^T	Own isolate	Fermenting cocoa pulp-bean mass (Ghana)	FM202097		
252 (=R-34084)	Own isolate	Fermenting cocoa pulp-bean-mass (Ghana)	FM202096		
M1160 (=R-46910)	Own isolate	Fermenting cocoa pulp-bean-mass (Ecuador)	HE577177		
M1167 (=R-46912)	Own isolate	Fermenting cocoa pulp-bean mass (Ecuador)	HE577176		
W. ghanensis					
LMG 24286^T	Own isolate	Fermenting cocoa pulp-bean mass (Ghana)	FM202095		
194B (=R-27442)	Own isolate	Fermenting cocoa pulp-bean mass (Ghana)	FM202094		
W. halotolerans					
LMG 9469^T	DSMZ	Sausage	FM202114		
W. hellenica					
LMG 15125^T	NCFB	Naturally fermented sausage (Greece)	FM202110		
W. kandleri					
LMG 18979^T	NCIMB	Desert spring (Namibia)	FM202116		
W. koreensis					
LMG 21853^T	KCTC	Kimchi (Korea)	FM202115		
W. minor					
LMG 9847^T	NCFB	Slime from milking machine	FM202117		
W. paramesenteroides					
LMG 9852^T	NCFB	Fermented dry salami	FM202111		
W. soli					
LMG 20113^T	S. Roos	Garden soil (Sweden)	FM202113		
LMG 20114	S. Roos	Garden soil (Sweden)	FM202112		
W. thailandensis					
LMG 19821^T	JCM	Fermented fish (Thailand)	FM202109		
W. viridescens					
LMG 3507^T	NCFB	Cured meat products	FM202120		
LMG 11497	NCFB	NK	FM202122		
LMG 12021	JCM	NK	FM202121		

Table 1. cont.

Strain	Depositor	Source	Accession numbers		
			pheS	*rpoA*	*atpA*
LMG 13093	NCFB	Frankfurters	FM202119		
LMG 23120	J. Björkroth	Spanish blood sausage (Spain)	FM202118		
W. fabalis sp. nov.					
M75^T	Own isolate	Fermenting cocoa pulp-bean mass (Brazil)	HE576796		
F. durionis					
LMG 22556^T	J. Leisner	Tempoyak made from durian fruit (Malaysia)	AM711166	AM711309	AM711205
LMG 22557	J. Leisner	Tempoyak made from durian fruit (Malaysia)	AM711140	AM711290	AM711171
LMG 22558	J. Leisner	Tempoyak made from durian fruit (Malaysia)	AM711141	AM711291	AM711172
F. ficulneus					
LMG 21928^T	DSMZ	Ripe fig (Portugal)	AM711151	AM711342	AM711183
F. fructosus					
LMG 9498^T	A. Ledeboer	Flower (Japan)	AM711194	AM711321	AM711174
F. pseudoficulneus					
LMG 23899^T	CECT	Ripe fig (Portugal)	AM711281	AM711355	AM711274
R-35156	R. Tenreiro	Ripe fig (Portugal)	AM711235	AM711335	AM711259
R-35157	R. Tenreiro	Ripe fig (Portugal)	AM711236	AM711336	AM711260
R-35158	R. Tenreiro	Ripe fig (Portugal)	AM711237	AM711337	AM711261
R-35159	R. Tenreiro	Ripe fig (Portugal)	AM711238	AM711338	AM711262
R-35160	R. Tenreiro	Ripe fig (Portugal)	AM711239	AM711333	AM711263
F. tropaeoli					
LMG 26298^T	L. Dicks	Flower (South Africa)	HE590678	HE590679	HE590680
M56 (=R-46388)	Own isolate	Fermenting cocoa pulp-bean mass (Brazil)	HE590681	HE590682	HE590683
M622 (=R-46389)	Own isolate	Fermenting cocoa pulp-bean mass (Brazil)	HE590684	HE590685	HE590686
M710 (=R-46397)	Own isolate	Fermenting cocoa pulp-bean mass (Ecuador)	HE590687	HE590688	HE590689
M1190 (=R-46399)	Own isolate	Fermenting cocoa pulp-bean mass (Ecuador)	HE590690	HE590691	HE590692
M1588 (=R-46401)	Own isolate	Fermenting cocoa pulp-bean mass (Malaysia)	HE590693	HE590694	HE590695

incubation at 28 °C. For strain M75^T, MRS agar (Oxoid) was used as the basal medium. Conventional biochemical characteristics and enzyme activities were tested as described previously in triplicate, unless stated otherwise (De Bruyne *et al.*, 2008a). Growth was tested at 4, 15, 20, 37 and 42 °C and in the presence of 6.5, 8 and 10 % NaCl (w/v). The production of gas from glucose was determined using inverted Durham tubes. The API 50 CHL *Lactobacillus* identification system (bioMérieux) proved useful for determining carbohydrate fermentation profiles. The production of D- and L-lactate from glucose was determined enzymically (B-Biopharm). Unlike the *F. tropaeoli* isolates, strain M75^T was able to produce dextran in MRS agar (Oxoid) in which glucose had been replaced with 5 % sucrose. The physiological and biochemical characteristics of the *F. tropaeoli* isolates were identical to those of *F. tropaeoli* LMG 26298^T, with the only difference that the isolates were also able to ferment sucrose, in

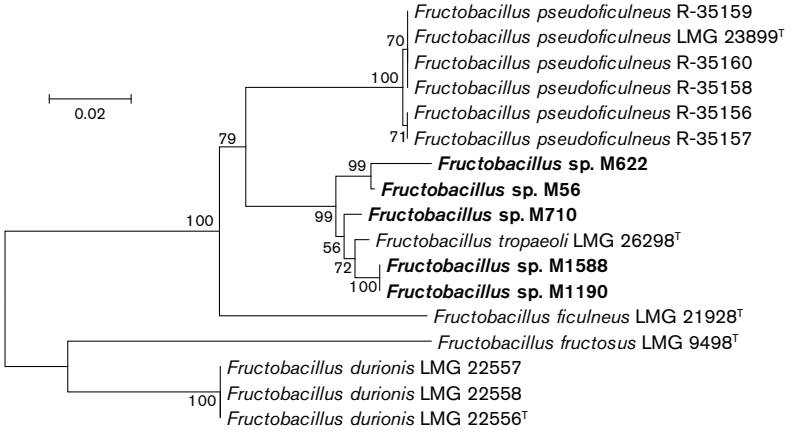

Fig. 2. Maximum-likelihood tree based on concatenated *pheS*, *rpoA* and *atpA* gene sequences showing the phylogenetic relationships of strains M56, M622, M710, M1190 and M1588 among other members of the genus *Fructobacillus.* Bootstrap values (%) based on 1000 replications are shown at branch points. The substitution model used was the GTR model and the aligned concatenated sequence had a length of 903 bp. Bar, 2 % sequence divergence. Accession numbers are given in Table 1.

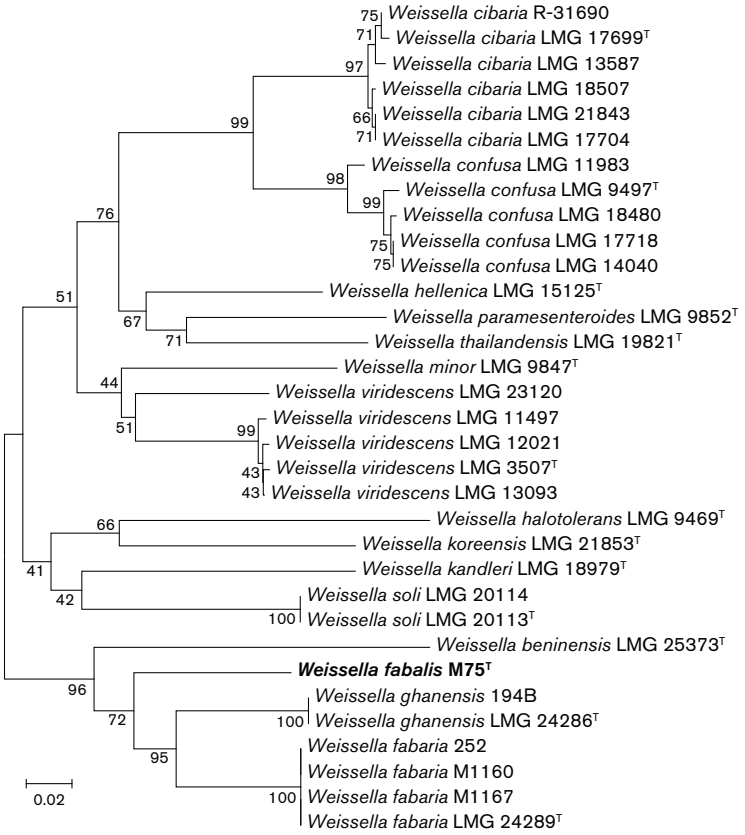

Fig. 3. Maximum-likelihood tree based on *pheS* gene sequences showing the phylogenetic relationships of strain M75^T among other members of the genus *Weissella*. Bootstrap values (%) based on 1000 replications are shown at branch points. The substitution model used was the GTR model and the aligned sequence had a length of 369 bp. Bar, 2 % sequence divergence. Accession numbers are given in Table 1.

addition to D-glucose, D-fructose and D-mannitol. The results for strain M75^T are given in the species description. Characteristics that differentiate strain M75^T from other species of the genus *Weissella* are summarized in Table 2.

In conclusion, the results of the present study demonstrate that strains M56, M622, M710, M1190 and M1588 belong to *F. tropaeoli*. These results and those of Papalexandratou (2011) and Papalexandratou *et al.* (2011a, b) demonstrate that *F. tropaeoli* participates in the fermentation process of cocoa beans in Brazil, Ecuador and Malaysia, which are major cocoa-producing countries. Additionally, the results of the present study demonstrate that strain M75^T represents a novel species of the genus *Weissella* that is closely related to *W. fabaria*, *W. ghanensis* and *W. beninensis* (96.9, 94.9 and 94.2 % 16S rRNA gene sequence similarity towards the respective type strains), but which can be distinguished from these and other species of the genus *Weissella* by DNA–DNA hybridization, *pheS* gene sequence analysis, MALDI-TOF MS-based analysis and biochemical characteristics. Based on these results, we propose to classify strain M75^T as the type strain of the novel species *Weissella fabalis* sp. nov.

Description of *Weissella fabalis* sp. nov.

Weissella fabalis (fa.ba′lis. L. fem. adj. *fabalis* of or belonging to beans).

Cells are Gram-stain-positive, catalase-negative, facultatively anaerobic and non-motile. Cells are coccoid, approximately 1.0 μm wide and 1.5 μm long, and occur singly, in pairs or in short chains. Colonies grown for 2 days on MRS agar at 28 °C are approximately 1 mm in diameter, beige, opaque, smooth and circular, with a low-convex elevation. Gas is produced from glucose, indicating the heterofermentative character of the type strain. Produces D-lactic acid. Growth occurs at 15–37 °C and in the presence of 5–6 % NaCl but not in the presence of 7–8 % NaCl. Arginine is hydrolysed. Acid is produced from glucose, fructose, mannose, *N*-acetylglucosamine, aesculin, cellobiose, maltose, trehalose and gentiobiose. Acid is not produced from glycerol, erythritol, D- or L-arabinose, ribose, D- or L-xylose, adonitol, methyl β-D-xylopyranoside, galactose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium gluconate, potassium 2-ketogluonate or potassium 5-ketogluconate. The DNA G+C content of the type strain is 37 mol%.

The type strain, M75^T (=LMG 26217^T =CCUG 61217^T), was isolated from a Brazilian cocoa bean box fermentation carried out in Ilhéus, Bahia, Brazil, in 2007.

Table 2. Differential characteristics of strain M75^T (*Weissella fabalis* sp. nov.) and other species of the genus *Weissella*

Species: 1, *W. fabalis* sp. nov.; 2, *W. fabaria*; 3, *W. ghanensis*; 4, *W. halotolerans*; 5, *W. minor*; 6, *W. viridescens*; 7, *W. soli*; 8, *W. kandleri*; 9, *W. koreensis*; 10, *W. cibaria*; 11, *W. confusa*; 12, *W. thailandensis*; 13, *W. hellenica*; 14, *W. paramesenteroides*; 15, *W. beninensis*. +, 90 % or more strains positive; −, 90 % or more strains negative; d, 11–89 % of strains positive; ND, no data available. Data partially adapted from Collins *et al.* (1993), Tanasupawat *et al.* (2000), Björkroth *et al.* (2002), Lee *et al.* (2002), Magnusson *et al.* (2002), De Bruyne *et al.* (2008a, 2010) and Padonou *et al.* (2010).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acid from:															
Arabinose	−	−	−	−	−	−	+	−	+	+	−	+	+	d	−
Cellobiose	+	+	+	−	+	−	−	−	−	+	+	−	−	d	d
Fructose	+	+	+	+	+	+	−	+	ND	+	+	+	+	+	+
Galactose	−	−	−	−	−	−	−	+	−	−	+	+	−	+	+
Maltose	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+
Melibiose	−	−	−	−	−	−	+	−	−	−	−	+	−	+	+
Raffinose	−	−	−	−	−	−	+	−	−	−	−	+	−	d	+
Ribose	−	−	−	+	+	−	+	+	+	−	+	+	−	d	d
Salicin	−	−	+	−	−	−	+	−	ND	+	+	−	−	−	d
Sucrose	−	−	d	−	+	d	+	−	−	+	+	d	+	+	+
Trehalose	+	+	+	−	+	d	+	−	−	−	−	d	+	+	d
Xylose	−	−	−	−	−	−	+	−	+	+	+	−	−	d	−
Hydrolysis of aesculin	+	+	+	−	+	−	+	−	−	+	+	−	ND	d	+
NH_3 from arginine	+	+	+	+	+	−	+	+	+	+	+	−	−	−	+
Dextran formation	+	+	+	ND	−	ND	−	+	+	+	+	−	−	−	ND
Lactic acid configuration	D	DL	DL	DL	DL	DL	D	DL	D	DL	DL	D	D	D	DL
Growth at/in:															
15 °C	+	+	+	ND	ND	ND	+	ND	+	+	ND	ND	ND	ND	+
37 °C	+	+	+	ND	ND	ND	+	ND	+	+	ND	+	ND	ND	ND
42 °C	−	−	−	ND	ND	ND	−	ND	−	+	ND	−	ND	ND	−
6.5 % NaCl	−	−	−	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
8.0 % NaCl	−	−	−	ND	ND	ND	ND	ND	−	−	ND	ND	ND	ND	ND
10.0 % NaCl	−	−	−	ND	ND	ND	ND	ND	−	−	ND	+	ND	ND	ND
DNA G+C content (mol%)	37	38	40	44	44	41–44	43	39	37	44–45	45–47	38–41	39–40	37–38	37

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