Lactobacillus fabifermentans sp. nov. and *Lactobacillus cacaonum* sp. nov., isolated from Ghanaian cocoa fermentations

Katrien De Bruyne,¹ Nicholas Camu,² Luc De Vuyst² and Peter Vandamme¹

Correspondence

Katrien De Bruyne
Katrien.DeBruyne@UGent.be

¹Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

²Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Department of Applied Biological Sciences and Engineering, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

Two Gram-positive bacterial strains, LMG 24284^T and LMG 24285^T, were isolated from different spontaneous cocoa bean heap fermentations in Ghana. Analysis of their 16S rRNA gene sequences indicated that they were members of the *Lactobacillus plantarum* and *Lactobacillus salivarius* species groups, respectively. DNA–DNA hybridization experiments with their nearest phylogenetic neighbours demonstrated that both strains represented novel species that could be differentiated from their nearest neighbours by *pheS* sequence analysis, whole-cell protein electrophoresis, fluorescent amplified fragment length polymorphism analysis and biochemical characterization. Therefore, two novel *Lactobacillus* species are proposed, *Lactobacillus fabifermentans* sp. nov. (type strain LMG 24284^T = DSM 21115^T) and *Lactobacillus cacaonum* sp. nov. (type strain LMG 24285^T = DSM 21116^T).

In chocolate manufacture, the desired characteristic flavour is influenced by the cocoa cultivar, cocoa bean fermentation, drying and roasting of the beans and final processing (Camu *et al.*, 2008; Hansen *et al.*, 1998; Schwan & Wheals, 2004; Thompson *et al.*, 2001). Natural cocoa bean fermentation is being studied intensively (Camu *et al.*, 2007, 2008; De Vuyst *et al.*, 2008; Lagunes Gálvez *et al.*, 2007; Nielsen *et al.*, 2007). In this process, lactic acid bacteria are responsible for the conversion of citric acid and sugars into mainly lactic acid. Biodiversity studies report lactobacilli as the dominant species in the fermentation (Camu *et al.*, 2007, 2008); species of the genera *Leuconostoc*, *Pediococcus*, *Weissella* and *Lactococcus* have also been reported (Camu *et al.*, 2008; Carr *et al.*, 1979; Passos *et al.*, 1984a, b; Thompson *et al.*, 2001). The population dynamics of lactic acid bacteria during spontaneous heap fermentations of cocoa beans in Ghana have been studied by Camu *et al.* (2007). In the course of this biodiversity study, two lactobacillus strains that could not be identified at the species level as belonging to any existing species were isolated. In the present study,

additional analyses allocated these strains to two novel *Lactobacillus* species.

Strains LMG 24284^T and LMG 24285^T were isolated during the main crop of 2005 (October–December 2005) in New Tafo, Ghana, from fermenting cocoa beans, which were sampled both for microbiological analysis and for plating, isolation and monitoring of lactic acid bacteria as described by Camu *et al.* (2007). The isolates were checked for purity by microscopy and catalase and oxidase tests were performed. Dereplication and preliminary identification of all isolates was obtained by rep-PCR using the (GTG)₅ primer (Gevers *et al.*, 2001). In the numerical analysis of (GTG)₅ profiles, strains LMG 24284^T and LMG 24285^T occupied distinct positions in the dendrogram.

The phylogenetic position of both strains was investigated by 16S rRNA gene sequence analysis, performed as described by De Bruyne *et al.* (2008). FASTA analysis of both 16S rRNA gene sequences [continuous stretches of 1532 bp (strain LMG 24284^T) and 1527 bp (strain LMG 24285^T)] identified members of the genus *Lactobacillus* as the most closely related bacteria. 16S rRNA gene sequence analysis of strain LMG 24284^T indicated that it belonged to the *Lactobacillus plantarum* species group. 16S rRNA gene sequence similarities between strain LMG 24284^T and the type strains of all species in this group were at least 98.2 %, i.e. similarities of 98.8, 98.7, 98.6 and 98.2 % to the type strains of *Lactobacillus pentosus*, *L. paraplantarum*, *L.*

Abbreviation: FAFLP, fluorescent amplified fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of LMG 24284^T and LMG 24285^T are AM905388 and AM905389, respectively.

plantarum subsp. *plantarum* and *L. plantarum* subsp. *argentoratensis*, respectively. Strain LMG 24285^T belonged to the *Lactobacillus salivarius* group with *Lactobacillus mali* LMG 6899^T (97.5 % 16S rRNA gene sequence similarity) as the nearest neighbour; 16S rRNA gene sequence similarities with the type strains of all other species in this group were below 97 %. The obtained 16S rRNA gene sequences of strains LMG 24284^T and LMG 24285^T and sequences of the type strains of species of both groups (retrieved from GenBank/EMBL) were aligned using CLUSTAL_X. A phylogenetic neighbour-joining tree was constructed using the BioNumerics software package, version 4.61 (Applied Maths). The statistical reliability of the topology of the neighbour-joining tree was evaluated by bootstrap analysis of 500 replicates (Fig. 1). Maximum-parsimony and maximum-likelihood cluster analysis confirmed the tree topology obtained by the neighbour-joining method (data not shown). However, analysis of 16S rRNA gene sequences often does not enable closely related *Lactobacillus* species to be differentiated, e.g. members of the *L. plantarum* species group (Fig. 1), where a maximum interspecies variation of 1.3 % is observed. The usefulness and accuracy of any bacterial species identification system depends on the distinction between intraspecific variation and interspecific divergence in selected loci (Meyer & Paulay, 2005). In the search for an accurate identification system, a higher degree of resolution was obtained when using phenylalanyl-tRNA synthase alpha subunit (*pheS*)

Fig. 1. Phylogenetic neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between *L. fabifermentans* LMG 24284^T, *L. cacaonum* LMG 24285^T and other strains belonging to the *L. plantarum* and *L. salivarius* species groups, respectively. *Lactobacillus delbrueckii* subsp. *delbrueckii* DSM 20074^T was used as the outgroup organism. Bootstrap values (%) based on 500 tree replications are indicated at branching points. Bar, 2 % sequence divergence.

partial gene sequences to differentiate members of the genus *Lactobacillus* (Naser *et al.*, 2007). The conditions for amplification and sequencing of the *pheS* genes from strains LMG 24284^T and LMG 24285^T were as described by Naser *et al.* (2007). The primer combination *pheS*-21-F/*pheS*-23-R was applied to amplify the target genes of both strains. Additional sequence data from type strains within the two species groups under discussion were retrieved from the study of Naser *et al.* (2007). The *pheS* phylogenetic trees are based on the neighbour-joining method and were obtained by importing the external sequence alignments from CLUSTAL_X into the BioNumerics software package. Within the *L. plantarum* species group, strain LMG 24284^T showed a maximum *pheS* gene sequence similarity value of 81.9 % to *L. pentosus* LMG 10755^T. For strain LMG 24285^T, the highest *pheS* gene sequence similarity value was 85.0 % with *L. nagelii* LMG 21593^T. Fig. 2 clearly shows a higher resolution than the 16S rRNA gene sequence analysis and suggests that strains LMG 24284^T (Fig. 2a) and LMG 24285^T (Fig. 2b) both represent novel *Lactobacillus* species.

The relatedness of the two cocoa isolates with their nearest neighbours was further investigated by SDS-PAGE of whole-cell proteins and fluorescent amplified fragment length polymorphism (FAFLP) analysis. SDS-PAGE of cellular proteins was performed as described by Pot *et al.*

Fig. 2. Neighbour-joining analysis of the *pheS* gene sequences of *Lactobacillus* type strains belonging to the *L. plantarum* species group (a) and the *L. salivarius* species group (b). Distance estimations were obtained by the Kimura-2 model. *L. delbrueckii* subsp. *delbrueckii* LMG 6412^T was included as the outgroup organism. Bars, 10 % sequence divergence.

Fig. 3. Cluster analysis of whole-cell protein profiles and dendrogram derived from UPGMA linkage of correlation coefficients of *L. fabifermentans* sp. nov. LMG 24284^T and the type strains of all species in the *L. plantarum* species group and those of *L. cacaonum* sp. nov. LMG 24285^T and its closest phylogenetic neighbours.

(1994). Densitometric analysis, normalization and interpolation of protein profiles of strains LMG 24284^T and LMG 24285^T were performed and the resulting profiles were added to the in-house database. Numerical analysis was performed using the BIO NUMERICS software package, version 4.61 (Applied Maths). The whole-cell protein profiles of strains LMG 24284^T and LMG 24285^T were clearly different from those of the type strains of their nearest phylogenetic neighbours (Fig. 3). Similarly, FAFLP analysis performed as described by Franz *et al.* (2006) confirmed the separate taxonomic status of both strains (Fig. 4).

Finally, genomic DNA was prepared as described by Stackebrandt & Kandler (1979). DNA–DNA hybridizations were performed according to a modification (Goris *et al.*, 1998) of the microplate method described by Ezaki *et al.* (1989). Reciprocal hybridization experiments were performed for each pair of strains. DNA–DNA hybridization

values between strain LMG 24285^T and *L. mali* LMG 6899^T were $20 \pm 2\%$, confirming that strain LMG 24285^T represents a novel species. For strain LMG 24284^T, hybridizations were performed against representatives of all members of the *L. plantarum* group. Hybridization values between strain LMG 24284^T and *L. pentosus* LMG 10755^T, *L. paraplantarum* LMG 16673^T, *L. plantarum* subsp. *plantarum* LMG 6907^T and *L. plantarum* subsp. *argenteratensis* LMG 9205^T were 16, 16, 26 and 26 %, respectively, again confirming that strain LMG 24284^T represents a novel species within the genus *Lactobacillus*.

The DNA G+C content was determined according to the enzymic DNA degradation method of Mesbah *et al.* (1989). The DNA nucleotide mixture was analysed chromatographically using a Waters Breeze HPLC system. A thermostable XBridge Shield RP18 column was used at 37 °C with 0.02 M NH₄H₂PO₄ (pH 4.0)/1.5 % (v/v) acetonitrile as solvent. Non-methylated lambda phage

Fig. 4. FAFLP patterns and dendrograms based on the UPGMA linkage of Dice coefficients of *L. cacaonum* sp. nov. LMG 24285^T and its closest neighbours from the *L. salivarius* species group (a) and of *L. fabifermentans* sp. nov. LMG 24284^T and representatives of the *L. plantarum* species group (b).

DNA (Sigma) was used as calibration reference and *Escherichia coli* LMG 2093 DNA was used as control. The G+C content of strain LMG 24285^T was 34.5 mol%, which was comparable to that of *L. mali* (32–34 mol%; Kato *et al.*, 2000). The G+C content of strain LMG 24284^T was 44.9 mol%, which was consistent with the G+C contents determined for members of the *L. plantarum* group (44–47 mol%; Bringel *et al.*, 2005; Curk *et al.*, 1996; Zanoni *et al.*, 1987).

Cell and colony morphology were investigated after growth on MRS agar (pH 5.4; Oxoid) for 48 h aerobic incubation at 37 °C, except where stated otherwise. Conventional biochemical tests and growth characteristics were determined as described by De Bruyne *et al.* (2008). Carbohydrate fermentation tests were performed in duplicate using the API 50 CHL system (bioMérieux) and enzyme activities were tested in duplicate using the API ZYM system (bioMérieux), both following the manufacturer's instructions. For the detection of glucose metabolites and the proportion of D- and L-lactate, strains were grown for 24 h at 30 °C in MRS broth (pH 5.4; Oxoid). Detailed phenotypic descriptions are given in the species descriptions. Characteristics that differentiate LMG 24284^T and LMG 24285^T from their closest relatives are summarized in Table 1.

Based on the discussed polyphasic analysis, it is proposed that these strains should be classified as representatives of

two novel *Lactobacillus* species: *Lactobacillus fabifermentans* sp. nov. (LMG 24284^T) and *Lactobacillus cacaonum* sp. nov. (LMG 24285^T).

Description of *Lactobacillus fabifermentans* sp. nov.

Lactobacillus fabifermentans (fa.bi.fer.men'tans. L. n. *faba* a bean; L. part. adj. *fermentans* fermenting; N.L. part. adj. *fabifermentans* of fermenting beans).

Cells are Gram-positive, catalase-negative, facultatively anaerobic and non-motile. Cells are long rods (1.0–3.0 µm wide and 10.0 µm long) that appear singly, in pairs or in short chains. Colonies are greyish white, opaque, smooth and circular with a convex elevation and an entire margin (diameter approx. 1.0 mm). Growth is observed at temperatures from 10 °C (from day 8 of incubation) up to 37 °C (immediate growth from day 1 of incubation). At 37 °C, growth is observed in MRS broth (pH 3.9). Growth occurs in MRS broth supplemented with 6% NaCl. The type strain (LMG 24284^T) produces lactic acid only as a metabolite from glucose. The ratio of production of D- and L-lactic acid isomers is 80:20. No gas production is observed. Arginine is deaminated. Acid is produced from L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose,

Table 1. Differential phenotypic features of strains LMG 24284^T and LMG 24285^T and closely related species

Strains: 1, *L. cacaonum* sp. nov. LMG 24285^T; 2, *L. mali* (n=15); 3, *L. fabifermentans* sp. nov. LMG 24284^T; 4, *L. pentosus* (n=3); 5, *L. paraplantarum* (n=4); 6, *L. plantarum* subsp. *plantarum* (n=15); 7, *L. plantarum* subsp. *argenteratensis* (n=14). Data were adapted partially from Bringel *et al.* (2005), Carr & Davies (1970), Curk *et al.* (1996) and Zanoni *et al.* (1987). +, 90% or more strains positive; –, 90% or more strains negative; d, 11–89% of strains positive; NA, no data available. All taxa were negative for acid production from D-arabinose and L-xylose and positive for acid production from salicin (i.e. 90% or more strains negative or positive for reference species).

Characteristic	1	2	3	4	5	6	7
Acid from:							
Cellobiose	+	d	+	+	+	+	+
Galactose	–	d	+	+	+	+	+
Lactose	–	–	–	+	+	+	+
Maltose	+	–	+	+	+	+	+
Mannitol	–	d	+	+	+	+	+
Melibiose	–	NA	–	+	+	+	+
Methyl α-D-mannopyranoside	–	NA	–	–	–	+	–
Melezitose	–	–	–	d	+	+	–
Raffinose	–	–	–	+	–	NA	NA
Ribose	–	–	+	+	+	NA	NA
Sucrose	–	+	+	+	+	+	+
Trehalose	–	+	+	+	+	+	+
D-Xylose	–	–	+	+	–	–	–
Growth at 45 °C	–	NA	–	–	–	–	–
Lactic acid configuration	DL	L	DL	DL	DL	DL	DL
Maximum NaCl concentration for growth (%)	–*	NA	6	NA	8	NA	NA
DNA G+C content (mol%)	34.5	32–34	44.9	46–47	44–45	44–46	44–46

*No growth with added NaCl in MRS broth.

sucrose, trehalose and gentiobiose. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, methyl β -D-xylopyranoside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate.

The type strain is LMG 24284^T (=DSM 21115^T), isolated from a cocoa bean heap fermentation in Ghana. The DNA G + C content of the type strain is 44.9 mol%.

Description of *Lactobacillus cacaonum* sp. nov.

Lactobacillus cacaonum (ca.ca'o.num. N.L. n. *cacao* -onis a cacao bean; N.L. gen. pl. n. *cacaonum* of cacao beans).

Cells are Gram-positive, catalase-negative and facultatively anaerobic. Motility is not observed. Cells are small rods (0.8–1.0 μ m wide and 2.0–3.0 μ m long) that appear singly, in pairs or short chains. Colonies are beige, opaque, smooth and circular with a convex elevation and an entire margin (diameter approx. 0.5 mm). Growth is observed at temperatures from 10 °C (from day 6 of incubation) up to 37 °C (immediate growth from day 1 of incubation). At 37 °C, growth is observed in MRS broth (pH 3.9). No growth is observed in MRS broth supplemented with NaCl. Besides the production of (mainly) lactic acid, acetic acid is also observed as an end product of glucose metabolism. The type strain (LMG 24285^T) produces both D- and L-lactate in a 10:90 ratio. No gas production is observed. Arginine is deaminated. Acid is produced from glucose, fructose, mannose, N-acetylglucosamine, aesculin, salicin, cellobiose and maltose. 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, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate.

The type strain is LMG 24285^T (=DSM 21116^T), isolated from a cocoa bean heap fermentation in Ghana. The DNA G + C content of the type strain is 34.5 mol%.

Acknowledgements

This work was supported by the Federal Research Policy [Action for the promotion of and Cooperation with the Belgian Coordinated Collections of Microorganisms (C3/00/17)], the Research Council of the Vrije Universiteit Brussel (GOA project), the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT Project 040043) and Barry Callebaut NV. The cooperation of the Ghanaian Cocoa Producers' Alliance (COCOBOD, Accra, Ghana) and the Cocoa Research Institute of Ghana is highly appreciated. Approval was obtained by COCOBOD to cooperate with local farmers.

References

- Bringel, F., Castioni, A., Olukoya, D. K., Felis, G. E., Torriani, S. & Dellaglio, F. (2005). *Lactobacillus plantarum* subsp. *argenteratensis* subsp. nov., isolated from vegetable matrices. *Int J Syst Evol Microbiol* **55**, 1629–1634.
- Camu, N., De Winter, T., Verbrugghe, K., Cleenwerck, I., Vandamme, P., Takrama, J. S., Vancanneyt, M. & De Vuyst, L. (2007). Dynamics and biodiversity of populations of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. *Appl Environ Microbiol* **73**, 1809–1824.
- Camu, N., González, Á., De Winter, T., Van Schoor, A., De Bruyne, K., Vandamme, P., Takrama, J. S., Addo, S. K. & De Vuyst, L. (2008). Influence of turning and environmental contamination on the dynamics of populations of lactic acid and acetic acid bacteria involved in spontaneous cocoa bean heap fermentation in Ghana. *Appl Environ Microbiol* **74**, 86–98.
- Carr, J. G. & Davies, P. A. (1970). Homofermentative lactobacilli of ciders including *Lactobacillus mali* nov. spec. *J Appl Bacteriol* **33**, 768–774.
- Carr, J. G., Davies, P. A. & Dougan, J. (1979). *Cocoa fermentation in Ghana and Malaysia*. I. Chatham, UK: Natural Resources Institute.
- Curk, M. C., Hubert, J. C. & Bringel, F. (1996). *Lactobacillus paraplantarum* sp. nov., a new species related to *Lactobacillus plantarum*. *Int J Syst Bacteriol* **46**, 595–598.
- De Bruyne, K., Camu, N., Lefebvre, K., De Vuyst, L. & Vandamme, P. (2008). *Weissella ghanensis* sp. nov., isolated from a Ghanaian cocoa fermentation. *Int J Syst Evol Microbiol* **58**, 2721–2725.
- De Vuyst, L., Camu, N., De Winter, T., Vandemeulebroecke, K., Van de Perre, V., Vancanneyt, M., De Vos, P. & Cleenwerck, I. (2008). Validation of the (GTG)₅-rep-PCR fingerprinting technique for rapid classification and identification of acetic acid bacteria, with a focus on isolates from Ghanaian fermented cocoa beans. *Int J Food Microbiol* **125**, 79–90.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- 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.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Hansen, C. E., del Olmo, M. & Burri, C. (1998). Enzyme activities in cocoa beans during fermentation. *J Sci Food Agric* **77**, 273–281.
- Kato, Y., Sakala, R. M., Hayashidani, H., Kiuchi, A., Kaneuchi, C. & Ogawa, M. (2000). *Lactobacillus algidus* sp. nov., a psychrophilic lactic acid bacterium isolated from vacuum-packaged refrigerated beef. *Int J Syst Evol Microbiol* **50**, 1143–1149.
- Lagunes Gálvez, S., Loiseau, G., Paredes, J. L., Barel, M. & Guiraud, J.-P. (2007). Study on the microflora and biochemistry of cocoa fermentation in the Dominican Republic. *Int J Food Microbiol* **114**, 124–130.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

- Meyer, C. P. & Paulay, G. (2005).** DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol* **3**, e422.
- Naser, S. M., Dawyndt, P., Hoste, B., Gevers, D., Vandemeulebroecke, K., Cleenwerck, I., Vancanneyt, M. & Swings, J. (2007).** Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. *Int J Syst Evol Microbiol* **57**, 2777–2789.
- Nielsen, D. S., Teniola, O. D., Ban-Koffi, L., Owusu, M., Andersson, T. S. & Holzapfel, W. H. (2007).** The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. *Int J Food Microbiol* **114**, 168–186.
- Passos, F. M. L., Lopez, A. S. & Silva, D. O. (1984a).** Aeration and its influence on the microbial sequence in cacao fermentations in Bahia, with emphasis on lactic acid bacteria. *J Food Sci* **49**, 1470–1474.
- Passos, F. M. L., Silva, D. O., Lopez, A., Ferreira, C. L. L. F. & Guimarães, W. V. (1984b).** Characterization and distribution of lactic acid bacteria from traditional cocoa bean fermentations in Bahia. *J Food Sci* **49**, 205–208.
- Pot, B., Vandamme, P. & Kersters, K. (1994).** Analysis of electrophoretic whole-organism protein fingerprints. In *Chemical Methods in Prokaryotic Systematics*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Schwan, R. F. & Wheals, A. E. (2004).** The microbiology of cocoa fermentation and its role in chocolate quality. *Crit Rev Food Sci Nutr* **44**, 205–221.
- 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.
- Thompson, S. S., Miller, K. B. & Lopez, A. S. (2001).** Cocoa and coffee. In *Food Microbiology: Fundamentals and Frontiers*, 2nd edn, pp. 721–733. Edited by M. P. Doyle, L. R. Beuchat & T. J. Montville. Washington, DC: American Society for Microbiology.
- Zanoni, P., Farrow, J. A. E., Phillips, B. A. & Collins, M. D. (1987).** *Lactobacillus pentosus* (Fred, Peterson, and Anderson) sp. nov., nom. rev. *Int J Syst Bacteriol* **37**, 339–341.