NOTE

***Burkholderia sacchari* sp. nov., a polyhydroxyalkanoate-accumulating bacterium isolated from soil of a sugar-cane plantation in Brazil**

¹ Institut für Mikrobiologie, Westfälische Wilhelms-Universität Münster, Corrensstraße 3, D-48149 Münster, Germany

² Laboratory of Microbiology, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium

³ Agrupamento de Biotecnologia, Instituto de Pesquisas Tecnológicas do Estado de São Paulo – IPT, Av. Professor Almeida Prado, 532, CEP 05508-901, São Paulo, SP, Brazil

Christian O. Brämer,¹ Peter Vandamme,² Luiziana F. da Silva,³ J. Gregório C. Gomez³ and Alexander Steinbüchel¹

Author for correspondence: Alexander Steinbüchel. Tel: +49 251 8339821. Fax: +49 251 8338388. e-mail: steinbu@uni-muenster.de

Strain IPT101^T, isolated from the soil of a sugar-cane plantation in Brazil, was analysed in a polyphasic taxonomic study. The strain produces polyhydroxyalkanoates from sucrose and other carbon sources. Morphological, physiological and biochemical data as well as 16S rDNA, whole-cell protein and fatty acid analyses indicated that strain IPT101^T represents a new species in the genus *Burkholderia*. The name *Burkholderia sacchari* sp. nov. is proposed, with strain IPT101^T (= LMG 19450^T = CCT 6771^T) as the type strain.

Keywords: polyhydroxyalkanoate accumulation, *Burkholderia sacchari* IPT101^T, sucrose utilization, 16S rRNA

Polyhydroxyalkanoic acids (PHAs) are insoluble energy-storage compounds that are synthesized by a wide variety of micro-organisms (Steinbüchel, 1991) and have attracted much interest from industry due to their thermoplastic material properties (Steinbüchel & Fächtenbusch, 1998). The composition of these polyesters depends strongly on the carbon source and on the bacterial strains used for fermentation. Recently, a Gram-negative bacterium designated strain IPT101^T was isolated from the soil of a sugar-cane plantation in Brazil and found to accumulate up to 68 % of the cell dry weight as poly(3-hydroxybutyrate) [poly(3HB)] with sucrose as the sole carbon source and up to 65.9 % of cell dry weight as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [poly(3HB-co-3HV)] with glucose and propionic acid as precursor substrates (Gomez *et al.*, 1996, 1997; Silva *et al.*, 2000).

On the basis of morphological and physiological data, this isolate was previously assigned to the genus *Burkholderia* (Silva *et al.*, 2000). The intention to use this strain for biotechnological production of PHAs from carbon sources, which are abundantly available in Brazil, required a more detailed taxonomic

affiliation and its assignment to a species. The phenotypic and genotypic characteristics of strain IPT101^T reported in the present paper confirm its classification within the genus *Burkholderia* and distinguish it from the presently known species. We therefore classified this organism as a novel *Burkholderia* species, for which the name *Burkholderia sacchari* sp. nov. is proposed.

Phylogenetic analysis based on the 16S rDNA sequence

The 16S rDNA of *Burkholderia* strain IPT101^T, the isolation procedure and growth conditions of which have been described earlier (Gomez *et al.*, 1996), was amplified by PCR from genomic DNA, isolated according to the method of Marmur (1961), using the primers 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AGAAAGGAGGTGATCCAGCC-3') according to Rainey *et al.* (1996). The PCR product was purified by using the Nucleotrap PCR extraction kit (Macherey-Nagel) and sequenced using the following primers (Rainey *et al.*, 1996): 27f (5'-GAGTTTGATCCTGGCTCAG-3'), 343r (5'-CTGCTGCC-TCCCGTA-3'), 357f (5'-TACGGGAGGCAGCAG-3'), 519r [5'-G(T/A)ATTACCGCGGC(T/G)GCTG-3'], 536f [5'-CAGC(C/A)GCCGCGGTAAT(T/A)C-3'], 803f (5'-ATTAGATACCCTGGTAG-3'), 907r (5'-CCGTCAATTCATTTGAGTTT-3'), 1114f (5'-

Abbreviations: PHA, polyhydroxyalkanoates; poly(3HB), poly(3-hydroxybutyrate); poly(3HB-co-3HV), poly(3-hydroxybutyrate-co-3-hydroxyvalerate).

The GenBank accession number for the 16S rDNA sequence of strain IPT101^T is AF263278.

Fig. 1. Neighbour-joining phylogenetic tree of *Burkholderia sacchari* and related bacteria based on 16S rRNA sequence comparisons. Accession numbers are given in parentheses. Bar, 5% sequence dissimilarity.

GCAACGAGCGCAACCC-3'), 1385r [5'-CGGTGTGT(A/G)CAAGGCC-3'] and 1525r (5'-AGAAAGGAGGTGATCCAGCC-3').

The consensus sequence of strain IPT101^T and sequences of strains belonging to the same phylogenetic group and of other representatives of the β -*Proteobacteria* (retrieved from the EMBL database) were aligned and a phylogenetic tree was constructed based on the neighbour-joining method by using the GeneCompar 2.0 software package (Applied Maths). The nearly complete sequences (corresponding to positions 63–1453 in the *Escherichia coli* numbering system) of the 16S rRNA genes were used for the calculation of similarity levels and for tree construction; unknown bases were discarded in the calculations. The highest similarity level detected was to the 16S rDNA sequence of *Burkholderia kururiensis* (97.1%); slightly lower values were calculated to 16S rDNA sequences of *Burkholderia stabilis*, *Burkholderia pyrrocinia* and *Burkholderia graminis* (96.0%). The levels of similarity to all other *Burkholderia* species ranged from 93.7 (*Burkholderia andropogonis*) to 95.9% (*Burkholderia cepacia* genomovar III). Similarity levels to other taxa belonging to the β -*Proteobacteria* were below 95.4% (*Pandoraea apista*). The phylogenetic position of strain IPT101^T within the genus *Burkholderia* is shown in Fig. 1.

In order to determine the DNA base composition, DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters Symmetry Shield C18 column at 37 °C. The solvent was 0.02 M $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 4.0) with 1.5% acetonitrile. Non-methylated lambda phage DNA

(Sigma) was used as the calibration reference. The G + C content of strain IPT101^T was 63.7 mol %.

Phylogenetic analysis based on whole-cell protein and cellular fatty acid analysis

After incubation for 24 h at 35 °C on trypticase soy agar (Becton Dickinson), loopfuls of well-grown cells of strain IPT101^T and *B. kururiensis* LMG 19447^T were harvested and fatty acid methyl esters were prepared, separated and identified using the Microbial Identification System (Microbial ID) as described before (Vandamme *et al.*, 1992).

The fatty acid profile of strain IPT101^T consisted of: 14:0 (3.7%), 16:0 (17.7%), 16:0 2OH (1.5%), 16:0 3OH (6.0%), 16:1 2OH (2.5%), 17:0 cyclo (3.7%), 18:1 ω 7c (34.0%) and summed features 2 (7.5%) and 3 (23.4%). The fatty acid profile of strain LMG 19447^T consisted of: 14:0 (5.1%), 16:0 (18.2%), 16:0 2OH (2.6%), 16:0 3OH (6.3%), 16:1 2OH (2.1%), 17:0 cyclo (4.8%), 18:1 ω 7c (33.9%), 18:1 2OH (1.1%), 19:0 cyclo ω 8c (4.4%) and summed features 2 (7.3%) and 3 (13.2%). Summed features 2 and 3 most likely correspond to 14:0 3OH and 16:1 ω 7c, respectively, as these fatty acids have been reported in *Burkholderia* species (Vandamme *et al.*, 1997).

Strain IPT101^T was identified by the Microbial ID system as *B. pyrrocinia* (identification score of 0.547), followed by *B. cepacia* (identification score of 0.492). *B. kururiensis* LMG 19447^T was identified as *B. cepacia* (identification score of 0.531), followed by *Burkholderia caryophylli* (identification score of 0.337) and *Burkholderia glathei* (identification score of 0.267). The fatty acid profiles of strain IPT101^T and *B. kururiensis*

Fig. 2. Dendrogram showing whole-cell protein profiles and the result of a numerical comparison of the protein profiles of IPT101^T and type and reference strains of all presently known *Burkholderia* species and genomovars.

LMG 19447^T showed clear qualitative [presence of 18:1 2OH (1.1%) and 19:0 cyclo ω 8c (4.4%) in *B. kururiensis*] and quantitative (percentage of summed feature 3 was 23.4% in the former and 13.2% in the latter) differences.

Preparation of whole-cell proteins of strain IPT101^T grown on nutrient agar (Oxoid CM3) supplemented with 0.04% (w/v) KH_2PO_4 and 0.24% (w/v) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (pH 6.8) for 48 h at 28 °C and SDS-PAGE were performed as described previously (Pot *et al.*, 1994). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis using Pearson's product-moment correlation coefficient were performed using the GelCompar 4.2 software package (Applied Maths). The whole-cell protein profile of strain IPT101^T was compared with over 2000 profiles in a database comprising all presently known *Burkholderia*, *Ralstonia* and *Pandoraea* species (Coenye *et al.*, 1999a, b, 2000, 2001; Vandamme *et al.*, 1997, 1999, 2000; P. Vandamme, unpublished data).

Analysis of the whole-cell protein profile of strain IPT101^T revealed that it was clearly different from those of the presently known *Burkholderia*, *Ralstonia* and *Pandoraea* reference species (data for these reference species were obtained in previous studies). The results of the numerical comparison of the protein profiles of IPT101^T and *Burkholderia* reference strains are shown in Fig. 2. In this dendrogram, strain IPT101^T clusters together with the *B. glathei* type strain at a similarity level below 80%.

Biochemical characterization

The API 20NE system (bioMérieux) was used to determine nitrate reduction, hydrolysis of gelatin and aesculin, urease activity, glucose fermentation and

arginine dihydrolase activity. Carbon substrate assimilation and oxidation tests were performed using the API 50CH system (bioMérieux) according to the instructions provided by the manufacturer by reporting growth and acid production. All tests were run in duplicate at 30 °C. Results are given in the species description below.

Differentiation of strain IPT101^T from other taxa

Strain IPT101^T and *B. kururiensis* LMG 19447^T can be differentiated by the absence of motility in *B. kururiensis* and by several biochemical characteristics, including differences in optimum growth temperature (28–30 °C for the former, 37 °C for the latter; Zhang *et al.*, 2000) and oxidation of carbohydrate sources such as maltose, raffinose, rhamnose, sucrose and xylitol (Table 1).

Description of *Burkholderia sacchari* sp. nov.

Burkholderia sacchari [sac'cha.ri. N.L. adj. *sacchari* of sugar, referring to the location of isolation of the strain, soil of a sugar-cane (*Saccharum officinarum*) plantation].

Colonies of strain IPT101^T on nutrient broth medium (Difco) are white and opaque due to the accumulation of poly(3HB) and poly(3HB-co-3HV). Cells are Gram-negative and are lysed by 3% KOH, motile due to the presence of several polar flagella, rod-shaped (0.5–0.8 μm in width, 1.5–3.0 μm in length) and grow well between 25 and 37 °C with an optimum growth temperature of 28–30 °C. No growth is detectable over a time period of 10 d at temperatures of 7 or 42 °C. Spores are not observed. Oxidase and catalase are produced. Nitrate is reduced to nitrite. Liquefaction of gelatin and hydrolysis of aesculin are not observed.

Table 1. Comparison of the oxidation of carbon sources by strain IPT101^T and type strains of *Burkholderia* species

Taxa are identified as: 1, IPT101^T; 2, *B. kururiensis* KP23^T; 3, *Burkholderia phenazinium*; 4, *B. glathei*; 5, *B. cepacia*; 6, *B. pyrrocinia*; 7, *Burkholderia vietnamiensis*; 8, *Burkholderia glumae*; 9, *Burkholderia plantarii*; 10, *Burkholderia gladioli*; 11, *B. caryophylli*; 12, *B. andropogonis*; 13, *Burkholderia mallei*; 14, *Burkholderia pseudomallei*; 15, *B. graminis*; 16, *Burkholderia caribensis*. The data in columns 2–16 were taken from Zhang *et al.* (2000). The results for type strains are those of Viillard *et al.* (1998) and Achouak *et al.* (1999). Galactose, gluconate, glucose, glycerol, inositol, mannitol and mannose were oxidized by all strains.

Carbon source	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Adonitol	+	+	+	+	+	+	–	+	–	+	+	+	–	–	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+
Arabitol	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Cellobiose	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Fucose	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+
Lactose	–	–	–	+	–	–	–	–	–	–	–	+	–	–	+	+
Maltose	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Melibiose	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Raffinose	+	–	–	–	–	–	+	+	–	–	+	–	–	–	+	–
Rhamnose	–	+	+	+	–	–	–	–	+	–	+	–	–	–	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Sucrose	+	–	+	–	+	+	+	–	–	–	+	–	+	+	+	–
Trehalose	–	–	–	–	+	+	+	+	+	+	+	–	+	–	+	+
Xylitol	–	+	+	+	+	+	–	–	–	–	+	–	–	–	+	+

The following substrates are assimilated: acetate, *N*-acetylglucosamine, adipate, adonitol, DL-arabinose, D-arabitol, citrate, D-fructose, DL-fucose, galactose, gluconate, D-glucose, glycerol, inositol, 2-ketogluconate, lactate, D-lyxose, malate, mannitol, D-mannose, phenylacetate, propionate, pyruvate, D-raffinose, sorbitol, sucrose and D-xylose. The following carbon sources are not used: starch, amygdalin, L-arabitol, arbutin, caprate, cellobiose, dulcitol, erythritol, methyl α -D-glucoside, methyl α -D-mannoside, β -gentiobiose, glycogen, inulin, 5-ketogluconate, lactose, maltose, melezitose, melibiose, methyl β -xyloside, rhamnose, ribose, salicin, L-sorbose, D-tagatose, trehalose, D-turanose, xylitol and L-xylose. The following carbohydrates are oxidized: adonitol, DL-arabinose, D-arabitol, D-fructose, DL-fucose, galactose, gluconate, D-glucose, glycerol, inositol, 2-ketogluconate, D-lyxose, mannitol, D-mannose, D-raffinose, ribose, sorbitol, sucrose and D-xylose. The following carbohydrates are not oxidized: *N*-acetylglucosamine, starch, amygdalin, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, β -gentiobiose, glycogen, inulin, 5-ketogluconate, lactose, maltose, melezitose, melibiose, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -xyloside, rhamnose, salicin, L-sorbose, D-tagatose, trehalose, D-turanose, xylitol and L-xylose. Strain IPT101^T is susceptible to the antibiotics tetracycline (100 μ g ml^{–1}), kanamycin (50 μ g ml^{–1}), chloramphenicol (100 μ g ml^{–1}) and ampicillin (15 μ g ml^{–1}) (Silva *et al.*, 2000).

The type strain is IPT101^T, which has been deposited in the BCCM/LMG Bacteria Collection as LMG

19450^T and in the Tropical Culture Collection as CCT 6771^T.

Acknowledgements

P. V. is indebted to the Fund for Scientific Research-Flanders (Belgium) for a position as a post-doctoral fellow. We wish to thank Severine Laevens for excellent technical assistance. This study was supported by the Fonds der Chemischen Industrie (Frankfurt, Germany). The isolation of strain IPT101^T was supported by grants from PADCT/MCT (Brazil).

References

- Achouak, W., Christen, R., Barakat, M., Martel, M.-H. & Heulin, T. (1999). *Burkholderia caribensis* sp. nov., an exopolysaccharide-producing bacterium isolated from vertisol microaggregates in Martinique. *Int J Syst Bacteriol* **49**, 787–794.
- Coenye, T., Holmes, B., Kersters, K., Govan, J. R. W. & Vandamme, P. (1999a). *Burkholderia cocovenenans* (van Damme *et al.* 1960) Gillis *et al.* 1995 and *Burkholderia vandii* Urakami *et al.* 1994 are junior synonyms of *Burkholderia gladioli* (Severini 1913) Yabuuchi *et al.* 1993 and *Burkholderia plantarii* (Azegami *et al.* 1987) Urakami *et al.* 1994, respectively. *Int J Syst Bacteriol* **49**, 37–42.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K. & Vandamme, P. (1999b). Classification of some *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.
- Coenye, T., Falsen, E., Hoste, B., Ohlén, M., Goris, J., Govan, J. R. W., Gillis, M. & Vandamme, P. (2000). Description of *Pandoraea* gen. nov. with *Pandoraea apista* sp. nov., *Pandoraea*

- pulmonicola* sp. nov., *Pandoraea pnomenusa* sp. nov., *Pandoraea sputorum* sp. nov. and *Pandoraea norimbergensis* comb. nov. *Int J Syst Evol Microbiol* **50**, 887–899.
- Coenye, T., LiPuma, J. J., Henry, D., Hoste, B., Vandamme, P., Gillis, M., Speert, D. P. & Vandamme, P. (2001). *Burkholderia cepacia* genomovar VI, a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients. *Int J Syst Evol Microbiol* **51**, 271–279.
- Gomez, J. G. C., Rodrigues, M. F. A., Alli, R. C. P., Torres, B. B., Bueno Netto, C. L., Oliveira, M. S. & da Silva, L. F. (1996). Evaluation of soil Gram-negative bacteria yielding polyhydroxyalkanoic acids from carbohydrates and propionic acid. *Appl Microbiol Biotechnol* **45**, 785–791.
- Gomez, J. G. C., Fontolan, V., Alli, R. C. P., Rodrigues, M. F. A., Bueno Netto, C. L., Silva, L. F. & Simões, D. A. (1997). Production of P3HB-co-3HV by soil isolated bacteria able to use sucrose. *Rev Microbiol* **28** (Suppl. 1), 43–48.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acids from microorganisms. *J Mol Biol* **3**, 208–218.
- 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.
- 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.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Silva, L. F., Gomez, J. G. C., Oliveira, M. S. & Torres, B. B. (2000). Propionic acid metabolism and poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P3HB-co-3HV) production by *Burkholderia* sp. *J Biotechnol* **76**, 165–174.
- Steinbüchel, A. (1991). Polyhydroxyalkanoic acids. In *Biomaterials. Novel Materials from Biological Sources*, pp. 123–213. Edited by D. Byrom. Basingstoke: Macmillan.
- Steinbüchel, A. & Fächtenbusch, B. (1998). Bacterial and other biological systems for polyester production. *Trends Biotechnol* **16**, 419–427.
- Vandamme, P., Vancanneyt, M., Pot, B. & 10 other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.
- Vandamme, P., Holmes, B., Vancanneyt, M. & 8 other authors (1997). Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. *Int J Syst Bacteriol* **47**, 1188–1200.
- Vandamme, P., Goris, J., Coenye, T., Hoste, B., Janssens, D., Kersters, K., De Vos, P. & Falsen, E. (1999). Assignment of Centers for Disease Control group IVc-2 to the genus *Ralstonia* as *Ralstonia paucula* sp. nov. *Int J Syst Bacteriol* **49**, 663–669.
- Vandamme, P., Mahenthiralingam, E., Holmes, B., Coenye, T., Hoste, B., De Vos, P., Henry, D. & Speert, D. P. (2000). Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). *J Clin Microbiol* **38**, 1042–1047.
- Viallard, V., Poirier, I., Cournoyer, B., Haurat, J., Wiebkin, S., Ophel-Keller, K. & Balandreau, J. (1998). *Burkholderia graminis* sp. nov., a rhizospheric *Burkholderia* species, and reassessment of [*Pseudomonas*] *phenazinum*, [*Pseudomonas*] *pyrrocinia* and [*Pseudomonas*] *glathei* as *Burkholderia*. *Int J Syst Bacteriol* **48**, 549–563.
- Zhang, H., Hanada, S., Shigematsu, T., Shibuya, K., Kamagata, Y., Kanagawa, T. & Kurane, R. (2000). *Burkholderia kururiensis* sp. nov., a trichloroethylene (TCE)-degrading bacterium isolated from an aquifer polluted with TCE. *Int J Syst Evol Microbiol* **50**, 743–749.