Pseudolabrys taiwanensis gen. nov., sp. nov., an alphaproteobacterium isolated from soil

Peter Kämpfer,¹ Chiu-Chung Young,² A. B. Arun,² Fo-Ting Shen,² Udo Jäckel,¹ Ramon Rosselló-Mora,³ Wei-An Lai² and P. D. Rekha²

Correspondence

Peter Kämpfer
peter.kaempfer@agrar.uni-
giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig Universität Giessen, IFZ – Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany

²College of Agriculture and Natural Resources, Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung 402, Taiwan, ROC

³Grup d'Oceanografia Interdisciplinari, Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Esporles, Mallorca, Spain

A Gram-negative, short rod-shaped micro-organism (CC-BB4^T) was isolated on nutrient agar from soil from Sinshe in Taichung County, Taiwan. Analysis of the 16S rRNA gene sequence demonstrated that this isolate was novel, as it showed <92% similarity to the sequences of species of the genera *Labrys*, *Beijerinckia* and *Methylocystis*. The micro-organism did not utilize methylamine or methanol as a substrate, but was able to use several organic acids. The fatty acid profile was different from those reported for the genera *Labrys*, *Beijerinckia*, *Methylocystis*, *Angulomicrobium*, *Methylorhabdus* and *Methyloarcula*. On the basis of the 16S rRNA gene sequence analysis, in combination with chemotaxonomic and physiological data, strain CC-BB4^T represents a novel genus, for which the name *Pseudolabrys* gen. nov. is proposed. The type species is *Pseudolabrys taiwanensis* sp. nov., and the type strain of *P. taiwanensis* is CC-BB4^T (= CCUG 51779^T = CIP 108932^T).

During the characterization of organisms in oxisol-type (red) soil samples from a location called Sinshe in Taichung County, Taiwan, strain CC-BB4^T was isolated and maintained on nutrient agar (NA; Oxoid) after incubation at 32 °C for 1 week. Subcultivation was performed on tryptone soy agar (TSA; Oxoid) at 30 °C for between 24 h and 7 days. On this agar, CC-BB4^T was able to grow at 15–36 °C, but not at 10 or 45 °C. The organism was able to grow on NA, TSA and R2A agar (Oxoid). Gram staining was performed as described by Gerhardt *et al.* (1994). Phenotypic tests were performed according to Kämpfer *et al.* (1991), and further physiological tests [API ZYM enzyme profiles (bioMérieux), API 20E (bioMérieux), Biolog GN2 system] were done according to the instructions of the relevant manufacturer. Flexirubin-like pigments were observed by flooding the plates with 20% (w/v) potassium hydroxide (Fautz & Reichenbach, 1980). Fluorescence was tested 48 h after plating on King's B medium. DNA used for the determination of G + C content was isolated by using the UltraClean microbial DNA isolation kit according to the instructions of the manufacturer (MOBIO). The DNA G + C content was determined as described previously (Peña *et al.*, 2005). Cell morphology was observed under a Zeiss light microscope at

× 1000 magnification, using cells that had been grown for 3 days at 30 °C on NA; the results are given in the species description. The 16S rRNA gene sequence was analysed as described previously (Kämpfer *et al.*, 2003; Young *et al.*, 2005).

Analysis of the sequence data was performed by using the software package MEGA, version 2.1 (Kumar *et al.*, 2001), after multiple alignment of the data by CLUSTAL X (Thompson *et al.*, 1997). A distance-matrix method (distance options according to the Kimura two-parameter model), including clustering by the neighbour-joining method (Fig. 1) and a discrete character-based maximum-parsimony method, was used. In each case, bootstrap values were calculated from 1000 replications. The 16S rRNA gene sequence of strain CC-BB4^T was a continuous stretch of 1386 bp. Sequence-similarity calculations indicated that strain CC-BB4^T showed the greatest degree of similarity with *Labrys monachus* VKM B-1479^T (91.3%), *Labrys methylaminiphilus* JLW10^T (91.3%) and *Bradyrhizobium japonicum* DSM 30131^T (91.3%). Lower sequence similarities (<91%) were found with respect to members of all other genera shown in Fig. 1.

Fatty acid analyses were performed as described by Kämpfer & Kroppenstedt (1996), and the fatty acid profile of strain CC-BB4^T (given in the species description) was found to be

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-BB4^T is DQ062742.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses), constructed after multiple alignment of the data by CLUSTAL X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering were performed using MEGA, version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are shown as percentages at branching points. Bar, 0.02 substitutions per nucleotide position.

similar to those of the genera listed in Table 1, although significant differences were also found.

The results of the physiological characterization, achieved using methods described previously (Kämpfer *et al.*, 1991; Kämpfer, 1990), are given in the species description and in Table 1. The utilization of methanol, methylamine, formaldehyde and formamide (1 and 5 mM in each case) was tested in the medium described by Widdel & Bak (1992). The presence of the *mxoF* gene (McDonald & Murrell, 1997), which encodes the α -subunit of the methanol dehydrogenase present in all methylotrophs (Hanson & Hanson, 1996; McDonald *et al.*, 1995), was tested with the PCR assay described by McDonald & Murrell (1997); DNA extracted from *Methylosinus trichosporium* OB3b^T was used as the positive control. No positive PCR amplification products were obtained with this test. In conclusion, strain CC-BB4^T is not a methylotrophic bacterium. No flexirubin-like or fluorescent pigments were observed. Strain CC-BB4^T was unable to produce acid from various carbohydrates, but tests with organic acids as the carbon substrates gave positive results. In the API 20E test, strain CC-BB4^T was positive only for the Voges–Proskauer (acetoin) reaction; in the API ZYM enzyme profiling test, CC-BB4^T was positive for esterase, esterase lipase, lipase (weakly), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. On the basis of these results, we propose that strain CC-BB4^T should be classified within a novel genus, *Pseudolabrys* gen. nov., as the type strain of *Pseudolabrys taiwanensis* sp. nov.

Description of *Pseudolabrys* gen. nov.

Pseudolabrys (Pseu.do.la'brys. Gr. adj. *pseudes* false; N.L. masc. n. *Labrys* a bacterial genus name; N.L. masc. n. *Pseudolabrys* the false *Labrys*).

Cells are Gram-negative, aerobic, non-motile and short rod-shaped. Reproduction of cells is by division and not by budding. Methanol, methylamine, formaldehyde and formamide are not used as sole sources of carbon. Growth occurs after 96 h incubation on TSA and NA at 30 °C. The major fatty acids are C_{16:0}, C_{18:1}ω7c and C_{19:0} cyclo ω8c. The DNA G+C content is 67 mol%. The type species is *Pseudolabrys taiwanensis*.

Description of *Pseudolabrys taiwanensis* sp. nov.

Pseudolabrys taiwanensis (tai.wan.en'sis. N.L. masc. adj. *taiwanensis* of Taiwan, where the type strain was isolated).

Shows the following properties in addition to those given in the genus description. Oxidase- and catalase-positive. The fatty acid profile of the type strain comprises iso-C_{15:0} (0.8%), C_{16:0} (4.8%), iso-C_{15:0} 3-OH (2.0%), iso-C_{17:1}ω9c (0.8%), iso-C_{17:0} (6.1%), C_{17:0} (2.9%), C_{18:1}ω7c (24.1%), C_{18:0} (2.9%), 11-methyl C_{18:0}ω7c (1.2%), C_{19:0} cyclo ω8c (51.2%), C_{18:1} 2-OH (1.0%) and C_{20:2}ω6,9c (0.8%). Aesculin is not hydrolysed. Flexirubin-like pigments and fluorescence are absent. Acid production

Table 1. Differential characteristics of strain CC-BB4^T and related taxa

Taxa: 1, strain CC-BB4^T; 2, *Angulomicrobium* (data from Fritz *et al.*, 2004); 3, *L. monachus* (data from Miller *et al.*, 2005 unless indicated); 4, *L. methylaminiphilus* (data from Miller *et al.*, 2005); 5, *Methylorhabdus* (Doronina *et al.*, 1995); 6, *Methylarcula* (Doronina *et al.*, 2000); 7, *Aminobacter* (Kämpfer *et al.*, 2002); 8, *Methylopila* (Doronina *et al.*, 1998); 9, '*Methylosulfonomonas*' (Holmes *et al.*, 1997). Symbols: +, positive; –, negative/not detectable; W, weak; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9
Morphology (flagella)	–	–	– ^{a*}	–	–	–	+	+	+
Reproduction by:									
Budding	–	+	+ ^a	+	–	–	+	–	–
Division	+	–	– ^a	–	+	+	–	+	+
pH range	4.2–8.5	5.2–8.0	6.0–9.5	4.0–9.5	6.0–7.0	5.5–10	NR	5.0–9.0	6.0–7.6
Temperature range (°C)	15–37	15–40	20–50	10–35	10–45	10–40	NR	NR	20–37
Catalase	+	+	+	+	+	+	+	–	+
Nitrate reduction	NR	–	+	+	+	–	–	+	NR
Utilization of:									
Methanol	–	–	W ^a	–	+	W	–	+	+
Methylamine	–	NR	W ^a	+	+	+	+	+	+
Fatty acids (% of total content)									
C _{16:0}	4.8	5.0	19.7 ^b	17.7	15.4	0.7–1.0	4.9	2.8–5.5	7.8–8.7
C _{18:1ω7c/ω9t/ω12t}	24.2	35.8	2.9 ^b	32.8	53.6	73.4–74.2	46.4	66.0–69.6	72.4–78.7
C _{18:0}	2.9	10.5	13.0 ^b	1.7	4.0	14.7–15.6	1.5	3.6–4.5	2.0–2.3
C _{19:0} cyclo	51.2	24.8	40.4 ^b	49.4	16.7	0.7–1.0	13.7	8.4–15.1	14.2–28
C _{20:1ω9}	–	11.7	11.9 ^b	–	0.7	NR	0.7–1.3	NR	NR
Unknown (ECL 18-081/18-063)	NR	4.1	4.2 ^b	–	NR	NR	10.9–24.5	NR	NR
DNA G+C content (mol%)	67	68	68 ^a	65.7	66.2	57–61	62–64	66–70	57

*Data from other studies as follows: a, Vasil'eva & Semenov (1984); b, Fritz *et al.* (2004).

is negative from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, rhamnose, maltose, trehalose, cellobiose, D-arabitol, D-mannose, sorbitol, L-arabinose, raffinose, D-xylose, methyl D-glucoside, erythritol and melibiose. The following carbon sources are utilized (method according to Kämpfer *et al.*, 1991 and Biolog GN2): acetate, *cis*- and *trans*-aconitate, fumarate, DL-3-hydroxybutyrate, DL-lactate, L-malate (weakly), mesaconate (weakly), 3-hydroxybenzoate, succinic acid, bromosuccinic acid, succinamic acid, formic acid, α -ketoglutaric acid, pyruvic acid methyl ester and succinic acid monomethyl ester. The following tests are negative (Biolog GN2): α -cyclodextrin, dextrin, glycogen, Tweens 40 and 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-glucose, *myo*-inositol, α -D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, itaconate, α -ketobutyric acid, α -ketovaleric acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl

L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D- and L-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate, D-glucose 6-phosphate, salicin, *p*-arbutin, D-xylose, maltitol, adipate, pyruvate, azelate, suberate, L-tryptophan, 4-hydroxybenzoate and phenylacetate. Positive for hydrolysis of L-alanine *p*-nitroanilide (pNA). Negative for hydrolysis of *p*-nitrophenyl (pNP) β -D-glucopyranoside, *o*-nitrophenyl D-galactopyranoside (ONPG), pNP β -D-glucuronide, pNP α -D-glucopyranoside, pNP β -D-xylopyranoside, bis-pNP phosphate, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate, L-glutamate- γ -3-carboxy pNA and L-proline pNA (method according to Kämpfer *et al.*, 1991). Positive results (API ZYM) are seen for activities of esterase, esterase lipase, lipase (weak), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, while results are negative for alkaline phosphatase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Additional physiological features are given in Table 1.

The type strain, CC-BB4^T (=CCUG 51779^T=CIP 108932^T), was isolated from soil from Sinshe in Taichung County, Taiwan.

Acknowledgements

This research work was kindly supported by a grant from the National Science Council and the Council of Agriculture, Executive Yuan, Taiwan, ROC. We thank W. S. Huang for technical assistance. We also thank M. Valens for DNA G+C content determination and Jean Euzéby for his advice regarding the nomenclature.

References

- Doronina, N. V., Braus-Strohmeyer, S. A., Leisinger, T. & Trotsenko, Y. A. (1995). Isolation and characterization of a new facultatively methylotrophic bacterium: description of *Methylophaga multi-vorans* gen. nov., sp. nov. *Syst Appl Microbiol* **18**, 92–98.
- Doronina, N. V., Trotsenko, Y. A., Krausova, V. I., Boulygina, E. S. & Tourova, T. P. (1998). *Methylophaga capsulata* gen. nov., sp. nov., a novel non-pigmented aerobic facultatively methylotrophic bacterium. *Int J Syst Bacteriol* **48**, 1313–1321.
- Doronina, N. V., Trotsenko, Y. A. & Tourova, T. P. (2000). *Methylophaga marina* gen. nov., sp. nov. and *Methylophaga terricola* sp. nov.: novel aerobic, moderately halophilic, facultatively methylotrophic bacteria from coastal saline environments. *Int J Syst Evol Microbiol* **50**, 1849–1859.
- Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.
- Fritz, I., Strömpl, C. & Abraham, W. R. (2004). Phylogenetic relationships of the genera *Stella*, *Labrys* and *Angulomicrobium* within the ‘Alphaproteobacteria’ and description of *Angulomicrobium amanitiforme* sp. nov. *Int J Syst Evol Microbiol* **54**, 651–657.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Hanson, R. S. & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiol Rev* **60**, 439–471.
- Holmes, A. J., Kelly, D. P., Baker, S. C., Thompson, A. S., De Marco, P., Kenna, E. M. & Murrell, J. C. (1997). *Methylosulfonomonas methylovora* gen. nov., sp. nov., and *Marinosulfonomonas methylo-tropha* gen. nov., sp. nov.: novel methylotrophs able to grow on methanesulfonic acid. *Arch Microbiol* **167**, 46–53.
- Kämpfer, P. (1990). Evaluation of the Titertek-Enterobac-Automated System (TTE-AS) for identification of members of the family Enterobacteriaceae. *Zentralbl Bakteriell* **273**, 164–172.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterisation of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Neef, A., Salkinoja-Salonen, M. S. & Busse, H.-J. (2002). *Chelatobacter heintzii* (Auling *et al.* 1993) is a later subjective synonym of *Aminobacter aminovorans* (Urakami *et al.* 1992). *Int J Syst Evol Microbiol* **52**, 835–839.
- Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.
- Kumar, S., Tamura, K., Jakobsen, I.-B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- McDonald, I. R. & Murrell, J. C. (1997). The methanol dehydrogenase structural gene *mxhA* and its use as a functional gene probe for methanotrophs and methylotrophs. *Appl Environ Microbiol* **63**, 3218–3224.
- McDonald, I. R., Kenna, E. M. & Murrell, J. C. (1995). Detection of methanotrophic bacteria in environmental samples with the PCR. *Appl Environ Microbiol* **61**, 116–121.
- Miller, J. A., Kalyuzhnaya, M. G., Noyes, E., Lara, J. C., Lidstrom, M. E. & Chistoserdova, L. (2005). *Labrys methylaminiphilus* sp. nov., a novel facultatively methylotrophic bacterium from a freshwater lake sediment. *Int J Syst Evol Microbiol* **55**, 1247–1253.
- Peña, A., Valens, M., Santos, F., Buczolits, S., Antón, J., Kämpfer, P., Busse, H.-J., Amann, R. & Rosselló-Mora, R. (2005). Intraspecific comparative analysis of the species *Salinibacter ruber*. *Extremophiles* **9**, 151–161.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Vasil’eva, L. V. & Semenov, A. M. (1984). New budding prosthecate bacterium *Labrys monahos* with radial cell symmetry. *Microbiology* (English translation of *Mikrobiologiya*) **53**, 68–75.
- Widdel, F. & Bak, F. (1992). Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Young, C.-C., Kämpfer, P., Shen, F.-T., Lai, W.-A. & Arun, A. B. (2005). *Chryseobacterium formosense* sp. nov., isolated from the rhizosphere of *Lactuca sativa* L. (garden lettuce). *Int J Syst Evol Microbiol* **55**, 423–426.