Ochrobactrum rhizosphaerae sp. nov. and *Ochrobactrum thiophenivorans* sp. nov., isolated from the environment

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Two Gram-negative, rod-shaped, non-spore-forming bacteria, PR17^T and DSM 7216^T, isolated from the potato rhizosphere and an industrial environment, respectively, were studied for their taxonomic allocation. By *rrs* (16S rRNA) gene sequencing, these strains were shown to belong to the *Alphaproteobacteria*, most closely related to *Ochrobactrum pseudogrignonense* (98.4 and 99.3 % similarity to the type strain, respectively). Chemotaxonomic data (major ubiquinone Q-10; major polyamines spermidine, *sym*-homospermidine and putrescine; major polar lipids phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol and phosphatidylcholine and the *Ochrobactrum*-specific unidentified aminolipid AL2; major fatty acids $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$) supported the genus affiliation. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of the isolates from all hitherto-described *Ochrobactrum* species. Hence, both isolates represent novel species of the genus *Ochrobactrum*, for which the names *Ochrobactrum rhizosphaerae* sp. nov. (type strain PR17^T =CCUG 55411^T =CCM 7493^T =DSM 19824^T) and *Ochrobactrum thiophenivorans* sp. nov. (type strain DSM 7216^T =CCUG 55412^T =CCM 7492^T) are proposed.

The genus *Ochrobactrum*, introduced by Holmes *et al.* (1988), is a genus of which several novel species have recently been proposed. At the time of writing, the genus comprises the 11 species *Ochrobactrum anthropi* (the type species; Holmes *et al.*, 1988), *O. intermedium* (Velasco *et al.*, 1998), *O. tritici* and *O. grignonense* (Lebuhn *et al.*, 2000), *O. gallinifaecis* (Kämpfer *et al.*, 2003), *O. lupini* (Trujillo *et al.*, 2005), *O. oryzae* (Tripathi *et al.*, 2006), *O. cytisi* (Zurdo-Piñeiro *et al.*, 2007), *O. pseudintermedium* (Teyssier *et al.*, 2007), *O. haematophilum* and *O. pseudogrignonense* (Kämpfer *et al.*, 2007b).

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PR17^T and DSM 7216^T are respectively AM490632 and AM490617.

Strains PR17^T and DSM 7216^T were both isolated from the environment. Strain PR17^T was isolated in Austria from the potato rhizosphere and strain DSM 7216^T was isolated from wastewater in Germany. The latter strain was able to produce thiophene-2-carboxylate CoA esterase and utilizes thiophene 2-carboxylate as a sole source of carbon and sulfur (Kreimer, 1992). Both strains were presumptively identified as *O. anthropi*-like. They showed beige-coloured colonies on nutrient agar (Oxoid) at 37 °C. Subcultivation was done on tryptone soy agar (TSA) at 28 °C for 48 h.

Gram staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. Partial 16S rRNA gene sequences (both 1387 bp) were studied as described by Kämpfer *et al.* (2003) and subsequent analysis was performed using the

software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining and maximum-parsimony methods was performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequences of these two strains were compared to each other and to entries in the nucleotide collection databases (nr/nt) using the BLASTN search tool available at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. The results of these calculations showed that the closest relative of strain PR17^T was strain DSM 7216^T (98.7 % similarity) and the type strain of *O. pseudogrignonense*, CCUG 30717^T (98.4 % similarity). The closest relatives of strain DSM 7216^T were *O. pseudogrignonense* CCUG 30717^T (99.3 % similarity), strain PR17^T (98.7 % similarity) and the type strain of *O. haematophilum*, CCUG 38531^T (98.6 % similarity). Lower sequence similarities were found with all other species of the genus *Ochrobactrum*. An *rrs*-based tree is shown in Fig. 1.

In *recA*-based phylogenetic analysis including the type strains of some *Ochrobactrum* and *Brucella* species, both strains again grouped close to *O. grignonense* but formed separate branches (Scholz *et al.*, 2006; Fig. 2).

For polar lipid and quinone analyses, cells were grown on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2). Extraction and analyses were carried out as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996; Stolz *et al.*, 2007). Polyamines were extracted and analysed as described by Busse & Auling (1988) and Stolz *et al.* (2007). Detailed results of chemotaxonomic analyses are given in the species descriptions. Analysis of quinones revealed a spot that corresponded to ubiquinone-10 (Q-10). The quinone system supports the affiliation of the two strains to the *Alphaproteobacteria*, where the majority of species have Q-10 as the major quinone (Lechner *et al.*, 1995; Yokota *et al.*, 1992); other *Ochrobactrum* species and unnamed *Ochrobactrum* strains have been reported to exhibit Q-10 as the major quinone (Lechner *et al.*, 1995; Yokota *et al.*, 1992; Kämpfer *et al.*, 2003; Teyssier *et al.*, 2007; B. Huber and H.-J. Busse, unpublished results). The polyamine patterns, with the three predominant compounds putrescine, spermidine and *sym*-homospermidine, are in agreement with patterns reported previously for two species of this genus (Kämpfer *et al.*, 2007b) and with the polyamine patterns of several other species of the genus (B. Huber and H.-J. Busse, unpublished results). Polar lipid profiles of PR7^T and DSM 7216^T exhibited only slight quantitative differences and strongly resembled the profiles of *O. gallinifaecis* (Kämpfer *et al.*, 2003), *O. haematophilum*, *O. pseudogrignonense* (Kämpfer *et al.*, 2007b) and other *Ochrobactrum* strains (B. Huber and H.-J. Busse, unpublished results). Furthermore, the characteristic unknown aminolipid AL2 was detected, which is absent from polar lipid extracts of representatives of the closely related genus *Pseudochrobactrum* (Kämpfer *et al.*, 2006, 2007a).

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI Inc.) (Kämpfer & Kroppenstedt, 1996). The fatty acid profiles of the three strains are shown in Table 1. No significant differences in the fatty acid profiles were found compared with other *Ochrobactrum* species.

Results of physiological and biochemical tests are given in the species descriptions and in Table 2. Tests were performed with methods described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed with strains PR17^T and DSM 7216^T and the type strains of the most closely related *Ochrobactrum* species, *O. grignonense* and *O. pseudogrignonense*, using the method described by Ziemke *et al.* (1998) except that, for nick translation, 2 μg DNA was labelled during a 3 h incubation at 15 °C. The results are shown in Table 3.

Examinations based on almost entire *rrs* and *recA* gene sequences showed the affiliation of the two strains to the genus *Ochrobactrum*. In both trees (Figs 1 and 2), strains PR17^T and DSM 7216^T grouped close to *O. grignonense* OgA9a^T and *O. pseudogrignonense* CCUG 30717^T.

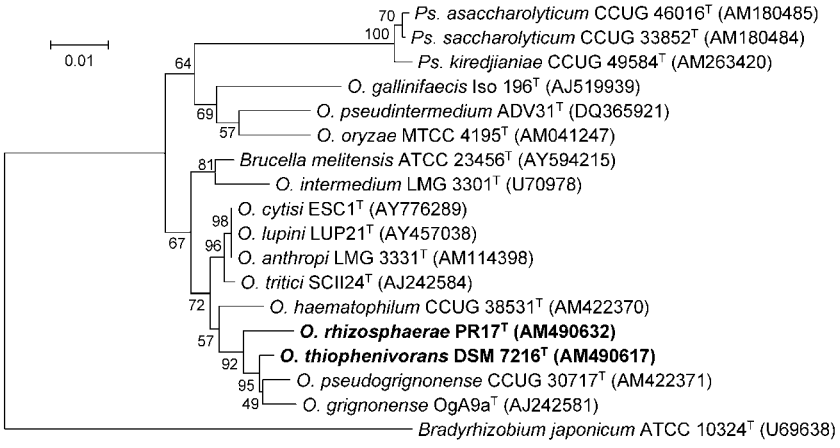

Fig. 1. Phylogenetic analysis based on 16S rRNA gene (1387 bp) sequences available from the EMBL database (accession numbers in parentheses) constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.01 substitutions per nucleotide position. *Ps.*, *Pseudochrobactrum*.

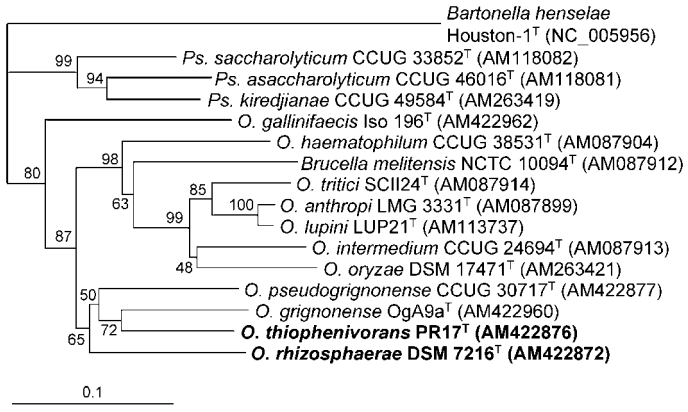

Fig. 2. Phylogenetic analysis based on *recA* (879 bp) gene sequences. See legend to Fig. 1 for further details. Bar, 0.1 substitutions per nucleotide position.

However, DNA–DNA hybridization studies (Table 3) clearly demonstrated that the two strains represent separate species distinct from *O. grignonense* and *O. pseudogrignonense*. On the basis of these results, we describe two novel *Ochrobactrum* species.

Description of *Ochrobactrum rhizosphaerae* sp. nov.

Ochrobactrum rhizosphaerae [rhi.zo.sphae′rae. Gr. fem. n. *rhiza* root; L. fem. n. *sphaera -ae* (from Gr. fem. n. *sphaira -as*)

Table 1. Major fatty acids (%) of the novel strains and type strains of closely related species of the genus *Ochrobactrum*

Strains: 1, PR17^T (*O. rhizosphaerae* sp. nov.); 2, DSM 7216^T (*O. thiophenivorans* sp. nov.); 3, *O. pseudogrignonense* CCUG 30717^T; 4, *O. pseudogrignonense* CCUG 43892^T; 5, *O. haematophilum* CCUG 38531^T; 6, *O. grignonense* DSM 13338^T; 7, *O. gallinifaecis* Iso 196^T; 8, *O. intermedium* LMG 3301^T; 9, *O. anthropi* CIP 14970^T; 10, *O. tritici* LMG 18957^T; 11, *O. lupini* LUP21^T. All strains were grown on TSA at 28 °C for 48 h prior to fatty acid analysis. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Saturated fatty acids											
$C_{14:0}$							0.7				
$C_{15:0}$				0.3							
$C_{16:0}$	8.3	7.3	9.7	9.2	10.8	2.9	8.9	3.7	6.6	3.7	5.8
$C_{17:0}$	2.8	1.4	1.6	2.1	2.5	1.7		3.1	1.4	0.9	1.8
$C_{18:0}$	7.6	2.9	4.9	5.0	7.7	7.2	3.7	4.1	8.8	9.6	3.5
Unsaturated fatty acids											
$C_{13:1}$ at 12–13						0.7			0.6		
$C_{17:1}\omega6c$						0.5		1.1			
$C_{18:1}\omega7c$	61.5	40.9	19.2	24.2	32.7	31.6	28.8	25.8	45.6	77.9	57.3
11-Methyl $C_{18:1}\omega7t$		1.5	1.7	1.3		1.0	1.6	1.5	1.0		2.0
$C_{20:1}\omega7c$				0.2		0.8					
$C_{20:2}\omega6,9c$		1.8	0.9	0.3	1.2	0.8	1.1	0.9	0.5		
Hydroxy fatty acids											
$C_{18:1}$ 2-OH	4.7	1.5	1.8	2.0		0.5	1.5	1.8	0.6	1.4	1.8
$C_{18:0}$ 3-OH			0.5	0.6		0.5					
Summed feature 3*	4.6	5.8	1.4	1.7	1.8	1.0	3.7	0.7	1.1	0.7	2.1
Cyclopropane acids											
$C_{17:0}$ cyclo		2.1	1.0	1.3			2.9		0.8		
$C_{19:0}$ cyclo $\omega8c$	11.2	34.9	57.0	50.8	43.2	50.2	47.2	57.4	32.7	5.9	23.8
Unknown 13.957†			0.3	0.2		0.2					
Unknown 14.959†				0.3		0.7			0.3		2.0

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

Table 2. Physiological characteristics of the novel strains and type strains of closely related *Ochrobactrum* species

Strains: 1, PR17^T (*O. rhizosphaerae* sp. nov.); 2, DSM 7216^T (*O. thiophenivorans* sp. nov.); 3, *O. pseudogrignonense* CCUG 30717^T and CCUG 43892; 4, *O. haematophilum* CCUG 38531^T; 5, *O. grignonense* DSM 13338^T; 6, *O. anthropi* CIP 14970^T; 7, *Ochrobactrum gallinifaecis* Iso 196^T; 8, *O. intermedium* LMG 3301^T; 9, *O. tritici* LMG 18957^T; 10, *O. lupini* LUP21^T; 11, *O. oryzae* DSM 17471^T (data from Tripathi *et al.*, 2006). +, Positive; −, negative; (+), weakly positive; ND, no data available. All strains were positive for hydrolysis of L-alanine *p*-nitroanilide (pNA) and weak hydrolysis of bis-*p*-nitrophenyl (pNP) phosphate. All strains (except *O. oryzae* DSM 17471^T, for which not all tests were performed) were negative for hydrolysis of aesculin[a*], pNP *β*-D-galactopyranoside[a], pNP *β*-D-glucuronide, pNP α-D-glucopyranoside, pNP *β*-D-glucopyranoside, pNP phosphorylcholine and 2-deoxythymidine-5′-pNP phosphate. All strains were also positive for assimilation of L-arabinose[a], D-galactose[a], D-glucose, D-mannose, D-ribose[a], D-xylose, acetate, propionate[a], fumarate[a], glutarate, DL-lactate, L-malate[a], oxoglutarate[a], pyruvate[a], L-alanine, L-proline, L-serine[a] and ornithine[a]. All strains were negative for assimilation of *p*-arbutin, salicin, putrescine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, adipate[b], itaconate, mesaconate, phenylacetate[a], melibiose[a] and azelate.

Test	1	2	3	4	5	6	7	8	9	10	11
Hydrolysis of:											
pNP phenylphosphonate	+	(+)	(+)	−	−	−	−	−	(+)	−	ND
L-Glutamate-γ-3-carboxy pNA	−	−	−	−	−	(+)	−	+	(+)	+	ND
L-Proline pNA[b*]	+	+	+	+	+	+	+	(+)	(+)	+	ND
Assimilation of:											
D-Fructose, *myo*-inositol[a], D-sorbitol[a], DL-3-hydroxybutyrate[a]	+	+	+	+	+	+	−	+	+	+	+
L-Rhamnose[a]	+	−	+	+	+	+	−	+	+	+	+
cis-Aconitate	+	−	+	+	+	+	−	+	+	−	+
Citrate[ab]	+	−	+	+	+	+	−	+	+	+	−
4-Aminobutyrate, *β*-alanine[a]	+	+	+	+	+	+	(+)	+	+	+	ND
Maltose[ab]	+	−	−	+	−	+	−	+	+	+	+
D-Gluconate	−	+	+	+	+	+	+	+	+	(+)	+
N-Acetyl-D-glucosamine[a]	(+)	(+)	−	+	−	+	−	+	+	+	+
Adonitol[ac]	+	−	+	+	−	+	−	+	+	+	+
Sucrose[ab], trehalose	+	−	+†	+	−	+	−	−	+	+	+
Maltitol	+	−	−	+	−	+	−	−	−	+	ND
trans-Aconitate[a]	+	−	+	+	−	−	−	(+)	−	−	ND
Cellobiose[ac]	+	−	−	−	−	+	−	+	−	−	ND
N-Acetyl-D-galactosamine	+	+	+	−	+	(+)	−	+	+	+	ND
Suberate[a]	−	−	−	−	−	−	−	−	+	−	ND
L-Aspartate[a]	+	+	+	+	+	+	+	+	−	+	+
4-Hydroxybenzoate	−	−	−	+	−	(+)	−	(+)	+	+	ND
L-Histidine[a]	+	−	+	+	+	+	+	+	(+)	+	+
L-Leucine[a]	+	−	+	+	+	+	−	+	(+)	+	+

*Test (based on a different method) was also performed as follows and gave congruent results: *a*, Holmes *et al.* (1988) with *O. anthropi*; *b*, Velasco *et al.* (1998) with *O. intermedium*; *c*, Lebuhn *et al.* (2000) with type strains of all four previously described species.
†Strain CCUG 30717^T showed only weakly positive results.

ball, any globe, sphere; N.L. gen. fem. n. *rhizosphaerae* of the rhizosphere].

Cells from the early exponential growth phase are stongly motile, but may become less motile or non-motile when approaching the stationary growth phase. Cells are non-spore-forming rods (approx. 2 μm long). Gram-negative and oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, nutrient agar, PYE agar and MacConkey agar at 25–30 °C. Colonies are beige, translucent and shiny. Colonies with entire edges are formed within 24 h, with a diameter of

Table 3. DNA–DNA reassociation (%) between the novel strains and closely related *Ochrobactrum* type strains

Strain	PR17^T	DSM 7216^T	*O. grignonense* DSM 13338^T	*O. pseudogrignonense* CCUG 30717^T
PR17^T	100	–	40.0	27.3
DSM 7216^T	32.1	100	33.3	24.2

approximately 2 mm. The quinone system consists of Q-10 (99 %) and Q-9 (1 %). The polyamine pattern consists of the major compounds spermidine [28 μmol (g dry weight)$^{-1}$], putrescine [41 μmol (g dry weight)$^{-1}$] and *sym*-homospermidine [10 μmol (g dry weight)$^{-1}$] and minor amounts of 1,3-diaminopropane [1 μmol (g dry weight)$^{-1}$] and spermine [<1 μmol (g dry weight)$^{-1}$]. Predominant polar lipids are phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, phosphatidylcholine and diphosphatidylglycerol. Moderate to minor amounts of phosphatidyldimethylethanolamine and two unidentified aminolipids AL1 and the *Ochrobactrum*-specific AL2 and traces of several unknown lipids are also present. The fatty acid profile is composed mainly of $C_{18:1}\omega7c$ (61.5 %) and $C_{19:0}$ cyclo $\omega8c$ (11.2 %). Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Ochrobactrum* species) are indicated in Table 1.

The type strain PR17^T (=CCUG 55411^T =CCM 7493^T =DSM 19824^T) was isolated from the potato rhizosphere in Austria.

Description of *Ochrobactrum thiophenivorans* sp. nov.

Ochrobactrum thiophenivorans (thi′o.phe.ni.vo′rans. N.L. n. *thiophenum* thiophene; L. part. adj. *vorans* devouring; N.L. part. adj. *thiophenivorans* thiophene-devouring, referring to the ability to utilize thiophene 2-carboxylate as a sole source of carbon and sulfur).

Cells are non-motile, non-spore-forming rods (approx. 2 μm long). Gram-negative and oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar, nutrient agar and MacConkey agar at 25–30 °C. Forms beige, translucent and shiny colonies with entire edges within 24 h, with a diameter of approximately 2 mm. The quinone system consists of Q-10 (99 %) and Q-9 (1 %). The polyamine pattern consists of the major compounds spermidine [28 μmol (g dry weight)$^{-1}$], putrescine [19 μmol (g dry weight)$^{-1}$] and *sym*-homospermidine [9 μmol (g dry weight)$^{-1}$] and minor amounts of 1,3-diaminopropane [4 μmol (g dry weight)$^{-1}$] and spermine [2 μmol (g dry weight)$^{-1}$]. Predominant polar lipids are phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol and the unknown *Ochrobactrum*-specific aminolipid AL2. Moderate amounts of phosphatidyldimethylethanolamine and another unidentified aminolipid AL1 and small to trace amounts of several unknown lipids are also present. The fatty acid profile is composed mainly of $C_{18:1}\omega7c$ (19.2 %) and $C_{19:0}$ cyclo $\omega8c$ (57.0 %). Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Ochrobactrum* species) are indicated in Table 2.

The type strain DSM 7216^T (=CCUG 55412^T =CCM 7492^T) was isolated from wastewater in Germany.

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