Ochrobactrum pseudintermedium sp. nov., a novel member of the family *Brucellaceae*, isolated from human clinical samples

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Three novel Gram-negative, non-fermenting aerobic bacilli were isolated from human clinical samples. They shared more than 99.8 % of the 16S rRNA gene nucleotide positions. The strains were related to *Ochrobactrum intermedium* with about 97.48 % 16S rRNA gene sequence similarity. In 16S rRNA gene-, *dnaK*- and *rpoB*-based phylogenies, the strains were grouped in a lineage that was distinct from other *Ochrobactrum* species in the family *Brucellaceae*. Fatty acid composition, polar lipids, quinone system, DNA–DNA relatedness, genome organization, and physiological and biochemical data differentiated these isolates from recognized species of the genus *Ochrobactrum*. The three clinical strains therefore represent a novel species within the genus *Ochrobactrum*, for which the name *Ochrobactrum pseudintermedium* sp. nov., is proposed. The type strain is ADV31^T (= CIP 109116^T = DSM 17490^T). The DNA G + C content of strain ADV31^T was 54.5 mol%.

The genus *Ochrobactrum* belongs to the family *Brucellaceae* in the class *Alphaproteobacteria*. It currently comprises seven species that were isolated from a wide variety of environmental sources (soil, rhizosphere) (Lebuhn *et al.*, 2000), as well as from plants (Trujillo *et al.*, 2005; Tripathi *et al.*, 2006), animals (Kämpfer *et al.*, 2003) and humans (Holmes *et al.*, 1988; Velasco *et al.*, 1998). To date, *Ochrobactrum anthropi* and *Ochrobactrum intermedium* are the only species to be recovered from human clinical samples (Teyssier *et al.*, 2005a). In this study, we characterized three strains of an *Ochrobactrum* sp. that were isolated from two patients hospitalized in intensive-care units of the Montpellier University Hospital, France. We used genetic, phylogenetic, genomic and phenotypic analyses to compare these strains

with known *Ochrobactrum* species. This study led to the description of a novel species in the genus *Ochrobactrum*.

The three isolates were recovered from samples taken during the standard procedure for the detection of multi-resistant bacilli carriage in patients hospitalized in intensive-care units. The strains grew after incubation for 24–48 h at 37 °C on Drigalski agar (Difco), supplemented with 4 mg ceftazidime l⁻¹, a medium that is selective for multi-resistant, non-exigent, Gram-negative bacteria. Strain ADV31^T was isolated in 2002 from an axillary swab from a 20-year-old man. Isolates ADV41 and ADV43 were recovered in 2003 from rectal swabs taken 48 h apart from a 25-year-old man hospitalized in a different intensive-care unit. PFGE of *SpeI*-restricted DNA performed as described previously (Teyssier *et al.*, 2003a) showed that strains ADV41 and ADV43 were not related (data not shown).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *dnaK* and *rpoB* gene sequences of strain ADV31^T are DQ365921, DQ393570 and DQ926698, respectively; those for strain ADV41 are DQ365922, DQ393571 and DQ926699, respectively; and those for strain ADV43 are DQ365923, DQ393572 and DQ926700, respectively.

A phylogenetic tree based on concatenated *dnaK* and *rpoB* gene sequences of strains ADV31^T, AD41 and AD43 and other members of the genera *Ochrobactrum* and *Brucella*, a schematic representation of the *rrn* skeleton of the two chromosomes of strain ADV31^T and a two-dimensional thin-layer chromatogram of the polar lipids of strain ADV31^T are available as supplementary figures in IJSEM Online.

Genomic DNA used for PCR was obtained using an AquaPure genomic DNA isolation kit (Bio-Rad). Amplification of the 16S rRNA gene was performed using the universal primers 27f and 1492r (Teyssier *et al.*, 2003a) or primers F4 and R2 that are described as being specific for *Brucella* spp. and *O. intermedium* (Romero *et al.*, 1995; Velasco *et al.*, 1998). Partial *dnaK* (encoding the 70 kDa heat-shock protein) and *rpoB* (encoding the DNA-dependent RNA polymerase β -subunit) gene amplifications

were carried out with primers 289f (5'-ATCGTCAAGG-GCGACAATGGC-3')/1142r (5'-CGTCCTTGACGTGCG-CCTGCA-3') and 453f (5'-ATCGTTTCGCAGATGCAC-CG-3')/1232r (5'-CGCATGTTTCATCTTCACGCGGCC-3'), respectively. The PCR reactions were all performed as described previously for 16S rRNA gene amplification (Teyssier *et al.*, 2003a). Sequencing was done in both directions with forward and reverse primers using an Applied Biosystems Automatic Sequencer (Genome Express). Partial 16S rRNA gene sequences of about 1400 bp were compared with sequences deposited in GenBank/EMBL/DDBJ using the standard BLAST program (www.ncbi.nlm.nih.gov/blast) and LALIGN software (www.expasy.org). The three isolates, ADV31^T, ADV41 and ADV43, shared more than 99.8 % of their nucleotide positions. The highest 16S rRNA gene sequence similarity was obtained with the type strain of *O. intermedium* (97.48 %). However, PCR using primers F4 and R2 was negative for strains ADV31^T, ADV41 and ADV43, clearly differentiating these strains from *O. intermedium* (Velasco *et al.*, 1998). The *dnaK* and *rpoB* sequences also distinguished strain ADV31^T from other members of the genus *Ochrobactrum*, as the maximum similarity levels were 95.5 % with *O. intermedium* for *dnaK* and 93.6 % with *O. anthropi* for *rpoB*. The similarity levels among strains ADV31^T, ADV41 and ADV43 were greater than 98.9 and 97.5 % for *dnaK* and *rpoB*, respectively. 16S rRNA gene-, *dnaK*- and *rpoB*-based phylogenies were used

to analyse the relationships between the three clinical isolates and members of the genera *Ochrobactrum* and *Brucella*. A single *dnaK*- and *rpoB*-based tree was reconstructed using a concatenation of *dnaK* and *rpoB* sequences. A distance matrix was calculated using the F84 algorithm (Kishino & Hasegawa, 1989) in the DNADIST program after sequence alignment with CLUSTAL_X (Thompson *et al.*, 1997). The neighbour-joining method (Saitou & Nei, 1987) was used to reconstruct distance trees. The results were compared with trees obtained using parsimony (Kluge & Farris, 1969) and maximum-likelihood (Olsen *et al.*, 1994) with the programs DNAPARS and fastDNAmI, respectively. The robustness of the trees was evaluated by bootstrap analysis of 1000 replicates using the programs SEQBOOT and CONSENSE. All the phylogenetic programs used were from PHYLIP package 3.66 (Felsenstein, 1993). Independent of methods and markers, strains ADV31^T, ADV41 and ADV43 were grouped in a lineage that was distinct from other species of the genus *Ochrobactrum* and supported by high bootstrap values (Fig. 1 and Supplementary Fig. S1 in IJSEM Online). The phylogenetic analysis showed that isolates ADV31^T, ADV41 and ADV43 should be considered as representing a novel species in the genus *Ochrobactrum*. Independent lineages, each corresponding to a single *Ochrobactrum* species, were observed in all the trees reconstructed, except for *Ochrobactrum lupini*, which formed a monophyletic group with *O. anthropi* ATCC 49188^T (Fig. 1 and

Fig. 1. Phylogenetic tree based on partial 16S rRNA gene (1351 bp) sequence comparison showing the relationships between strains ADV31^T, ADV41 and ADV43 (*O. pseudintermedium* sp. nov.) and members of the genera *Ochrobactrum* and *Brucella*. The tree was obtained using the neighbour-joining method with F84 algorithm. GenBank accession numbers are given in parentheses. Numbers at nodes are bootstrap values based on 1000 resamplings. Bar, 0.01 substitutions per nucleotide position. The sequence of *Caulobacter crescentus* CB15 was used as an outgroup.

Supplementary Fig. S1 in IJSEM Online). The relative branching of *O. intermedium* and *Ochrobactrum grignonense* differed between 16S rRNA- and *dnak-rpoB*-based trees. The *dnak-rpoB*-based tree displayed higher bootstrap values than those obtained in the 16S rRNA gene-based tree. This confirmed that the evolutionary relationships among members of the genus *Ochrobactrum* cannot be resolved using the 16S rRNA gene alone. In addition to *recA* (Scholz *et al.*, 2006), the two markers proposed herein are useful for studying species diversity in the genus *Ochrobactrum*. Additional comparative sequence analysis showed that 16S rRNA gene sequences deposited in databases for strains that have not been identified to the species level matched the sequences of the clinical isolates studied. These sequences were used to reconstruct a 16S rRNA gene-based tree (data not shown). The tree showed that *Ochrobactrum* strain CGL-X (GenBank accession no. DQ305290) might represent another member of the novel species, as well as strain TKW2 (AY631061) deposited as *Rhizobium* sp. and strain YBJCA-1 (DQ305284) deposited as *Brucella* sp.

Strains ADV31^T, ADV41 and ADV43 were compared with the type strains of other *Ochrobactrum* species: *O. anthropi* ATCC 49188^T, *O. intermedium* LMG 3301^T, *O. grignonense* DSM 13338^T, *Ochrobactrum tritici* DSM 13340^T, *Ochrobactrum gallinifaecis* DSM 15295^T and *O. lupini* LMG 22726^T. Data from the literature were used to compare the three strains with the recently described species *Ochrobactrum oryzae* (Tripathi *et al.*, 2006).

DNA–DNA hybridization between *O. intermedium* LMG 3301^T and strain ADV31^T was performed, according to the methods of De Ley *et al.* (1970) with the modifications of Huß *et al.* (1983), at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Germany). The values obtained in duplicate experiments were 37.7 and 48.2 %, confirming that isolate ADV31^T does not belong to the species *O. intermedium*. The DNA G + C content of strain ADV31^T, determined by HPLC (Mesbah *et al.*, 1989), was 54.5 mol%, a value that was different from those reported for other members of the genus *Ochrobactrum* (Table 1).

Intact genomic DNA of strains ADV31^T, ADV41 and ADV43 was subjected to PFGE in order to count and size large replicons (Teyssier *et al.*, 2005b). The *rrn* skeleton was constructed as described previously (Teyssier *et al.*, 2003b, 2005b). The genomes of the three clinical strains harboured two circular chromosomes of about 2.8 and 1.78 Mb. In addition, strain ADV31^T possessed a plasmid of 0.44 Mb. I-*CeuI* digestion led to four restriction fragments, indicating the presence of four *rrn* copies in the genome of the three strains. Two *rrn* copies were located on each chromosome (see Supplementary Fig. S2 in IJSEM Online). These results were in agreement with the affiliation of the isolates to the genus *Ochrobactrum*. Indeed, the bipartite organization of the genome and the *rrn* skeleton of the large chromosome have been described previously as being characteristic of this genus (Teyssier *et al.*, 2005b). The *rrn* organization of the

small chromosome was identical to that of *O. anthropi*, *O. grignonense*, *O. tritici* and *O. lupini*, but was different from that of *O. intermedium* and *O. gallinifaecis* (Teyssier *et al.*, 2005b).

Cells of strains ADV31^T, ADV41 and ADV43 were Gram-negative, straight or slightly curved short rods, with one flame-shaped end. The cells appeared to be motile under microscopic observation and after culture on mannitol motility nitrate broth medium (Bio-Rad). Electron microscopy after negative staining, performed as described previously (Marchandin *et al.*, 2003) and observed with a Hitachi H7100 electron microscope, showed the presence of one or two flagella in a subterminal position. Cells were 0.65–0.75 × 1.5–1.7 µm in size. The strains were cultivated on tryptic soy agar (Difco) at 25, 30, 37 and 45 °C. Colonies were non-pigmented, mucoid and opaque. The three strains grew on MacConkey medium (Difco) only at 37 °C and did not grow on cetrimid agar (Difco), independent of the incubation temperature. General metabolic characteristics were common to those of all members of the genus *Ochrobactrum*: aerobic respiration, presence of catalase and cytochrome oxidase, oxidative metabolism and acids not being produced from carbohydrates on the API 20E system (bioMérieux). Eighty-one biochemical characteristics were determined using the API 20E and API 20NE systems and VITEK 2 with ID-GN card version WSVT2-R04.01 (bioMérieux), according to the manufacturer's instructions. A comparison of the data obtained for strains ADV31^T, ADV41 and ADV43 and those for the type strains of other *Ochrobactrum* species is given in Table 1. The antimicrobial susceptibility pattern was determined by using the disc-diffusion assay on Mueller–Hinton agar, according to the recommendations of the SFM antibiogram committee for Gram-negative non-fermenters (Members of the SFM Antibiogram Committee, 2003). Antibiotic susceptibility patterns for strains ADV31^T, ADV41 and ADV43 and other *Ochrobactrum* species are compared in Table 1. As generally observed for the genus *Ochrobactrum*, the three isolates were resistant to all β-lactams except imipenem (10 µg) (Teyssier *et al.*, 2005a). They were also resistant to colistin (50 µg) and susceptible to tobramycin (10 µg) and netilmicin (30 µg). This pattern differentiated strains ADV31^T, ADV41 and ADV43 from the two other *Ochrobactrum* species recovered from human clinical samples (Teyssier *et al.*, 2005a). Indeed, *O. anthropi* strains were susceptible to colistin, tobramycin and netilmicin, whereas *O. intermedium* strains were resistant to these three antibiotics (Teyssier *et al.*, 2005a). Moreover, strains ADV31^T, ADV41 and ADV43 were resistant to tetracycline (30 µg), unlike other *Ochrobactrum* species.

Chemotaxonomic analyses of strain ADV31^T, including fatty acid methyl esters, respiratory quinones and polar lipids, were performed at the Identification Service of the DSMZ, according to Tindall (1990a, b) and Kämpfer *et al.* (1994). Predominant cellular fatty acids were C_{19:0} cyclo ω8c and C_{18:1} ω7c, constituting 39.8 and 29.3 % of the total fatty acid methyl esters, respectively. Other fatty acids were

Table 1. Phenotypic characteristics and DNA base composition of *O. pseudintermedium* sp. nov. (strains ADV31^T, ADV41 and ADV43) and the type strains of other *Ochrobactrum* species

Strains: 1, ADV31^T; 2, ADV41; 3, ADV43; 4, *O. intermedium* LMG 3301^T; 5, *O. anthropi* ATCC 49188^T; 6, *O. grignonense* DSM 13338^T; 7, *O. tritici* DSM 13340^T; 8, *O. gallinifaecis* DSM 15295^T; 9, *O. lupini* LMG 22726^T. +, Positive reaction; –, negative reaction; R, resistant; S, susceptible; I, intermediate; ND, not determined. All strains are positive for activities of cytochrome oxidase, catalase, glycine arylamidase*, L-proline arylamidase* and tyrosine arylamidase*, and assimilation of glucose†, arabinose†, mannose†, malate† and potassium gluconate†. All strains are positive for alkalization of L-lactate* and succinate*. All strains are negative for production of indole†, acetoin† and H₂S†, hydrolysis of gelatin†, assimilation of adipic acid†, phenylacetic acid†, L-histidine and L-lactate, and fermentation of carbohydrates†. All strains are negative for DNase, phenylalanine deaminase, tryptophan deaminase†, arginine dihydrolase†, Ala-Phe-Pro arylamidase*, glutamyl arylamidase pNA*, β-xylosidase*, lipase*, N-acetyl-β-galactosaminidase*, α-galactosidase*, β-galactosidase (ONPG)†, N-acetyl-β-glucosaminidase*, phosphatase*, ornithine decarboxylase*, lysine decarboxylase*, β-glucuronidase* and β-glucosidase (aesculin hydrolysis)†, and for acidification of L-arabitol*, D-cellobiose*, D-mannitol*, D-trehalose*, malonate*, 5-keto-D-gluconate* and coumarate*. All strains are susceptible to vibriostatic compound O/129*. All strains are susceptible to gentamicin, nalidixic acid, pefloxacin, ofloxacin, ciprofloxacin and rifampicin and all are resistant to fosfomycin.

Characteristic	1	2	3	4‡	5‡	6‡	7‡	8‡	9
Growth at 45 °C	+	+	+	+	–	–	–	–	–
Urease activity†	–	–	–	–	+	–	+	+	+
Nitrate reduction†	+	+	+	+	+	+	+	+	–
Assimilation of:									
D-Mannitol†	+	+	+	+	+	+	+	–	+
N-Acetylglucosamine†	+	+	+	+	+	+	+	–	+
D-Maltose†	+	+	+	+	+	–	+	–	+
Capric acid†	+	+	+	+	+	+	+	–	+
Trisodium citrate†	–	+	+	+	+	+	+	–	+
Activity of:									
L-Pyrrolidonyl arylamidase*	+	+	+	+	+	+	+	–	+
Glu-Gly-Arg arylamidase*	–	–	–	–	–	+	–	–	–
β-Alanine arylamidase pNA*	–	–	–	–	+	–	–	–	+
γ-Glutamyl transferase*	–	+	–	–	–	–	–	–	–
α-Glucosidase*	–	+	–	+	+	–	+	–	–
Acidification of:									
Adonitol*	+	+	+	+	+	–	+	–	+
D-Glucose*	–	–	–	–	–	+	–	+	–
D-Maltose*	+	+	–	+	–	–	–	–	–
D-Mannose*	–	–	–	–	–	+	–	–	–
Palatinose*	+	–	–	+	–	–	–	–	–
D-Sorbitol*	+	–	–	–	–	–	–	–	–
Sucrose*	+	+	–	+	–	–	–	–	–
D-Tagatose*	+	+	+	+	+	+	+	–	+
Resistance to antibiotics:									
Amoxicillin (25 µg)	R	S	R	R	R	R	R	S	R
Amoxicillin/clavulanic acid (20 µg/10 µg)	R	S	R	R	R	S	R	S	R
Other β-lactams§II	R	R	R	R	R	R	R	S	R
Tobramycin (10 µg)	S	S	S	R	S	S	S	S	S
Netilmicin (30 µg)	S	S	S	R	S	S	S	S	S
Amikacin (30 µg)	S	S	S	R	S	S	S	S	S
Isepamicin (30 µg)	S	S	S	R	S	S	S	S	S
Colistin (50 µg)	R	R	R	R	S	R	S	R	S
Chloramphenicol (30 µg)	R	R	R	R	R	I	R	S	R
Trimethoprim-sulfamethoxazole (1.25 µg/23.75 µg)	S	S	S	S	S	R	S	S	S
Tetracycline (30 µg)	R	R	R	S	S	S	S	I	S
DNA G + C content (mol%)¶	54.5	ND	ND	57.7 ^a	57.0 ^b	58.0 ^c	59.0 ^c	ND	56.8 ^d

*Data obtained using VITEK 2 with ID-GN card (bioMérieux).

†Data obtained using 20E and 20NE API systems (bioMérieux) after 48 h of incubation.

‡Data for API systems and antibiotic susceptibility are from Teyssier *et al.* (2005a).

§Other β -lactams: aztreonam (30 μ g), cefalotin (30 μ g), cefepime (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), imipenem (10 μ g), piperacillin (75 μ g), piperacillin/tazobactam (75 μ g/10 μ g), ticarcillin (75 μ g), ticarcillin/clavulanic acid (75 μ g/10 μ g).

||All strains are susceptible to imipenem and resistant to aztreonam.

¶Data from: a, Velasco *et al.* (1998); b, Holmes *et al.* (1988); c, Lebuhn *et al.* (2000); d, Trujillo *et al.* (2005).

present at moderate or low amounts (Table 2). This profile was most similar to that of *O. gallinifaecis*, but differed from those of the type strains of other *Ochrobactrum* species (Kämpfer *et al.*, 2003; Trujillo *et al.*, 2005; Tripathi *et al.*, 2006). Two respiratory lipoquinones, ubiquinone 10 and ubiquinone 9, were detected, at peak area ratios of 96 and 4%, respectively. The presence of ubiquinone 10 as the dominant respiratory lipoquinone is characteristic of members of the *Alphaproteobacteria* (Lechner *et al.*, 1995). The major polar lipids were phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. In addition, diphosphatidylglycerol, aminophospholipid and an unidentified aminolipid were detected in moderate amounts (see Supplementary Fig. S3 in IJSEM Online).

Table 2. Fatty acid methyl ester compositions (%) of the type strains of *Ochrobactrum* species

Strains: 1, ADV31^T (*O. pseudintermedium* sp. nov.; data from this study); 2, *O. intermedium* LMG 3301^T (Kämpfer *et al.*, 2003); 3, *O. anthropi* ATCC 49188^T (Kämpfer *et al.*, 2003); 4, *O. grignonense* DSM 13338^T (Kämpfer *et al.*, 2003); 5, *O. tritici* DSM 13340^T (Kämpfer *et al.*, 2003); 6, *O. gallinifaecis* DSM 15295^T (Kämpfer *et al.*, 2003); 7, *O. lupini* LMG 22726^T (Trujillo *et al.*, 2005); 8, *O. oryzae* MTCC 4195^T (Tripathi *et al.*, 2006). –, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
C _{13:1} at 12–13	–	–	0.6	0.7	–	–	–	–
C _{14:0}	–	–	–	–	–	0.7	–	0.1
C _{16:0}	12.0	3.7	6.6	2.9	3.7	8.9	4.2	5.1
C _{17:0}	3.1	3.1	1.4	1.7	0.9	–	1.6	0.3
C _{17:0} cyclo	1.4	–	0.8	–	–	2.9	–	<0.1
C _{17:1} ω 6c	–	1.1	–	0.5	–	–	1.4	–
C _{18:0}	4.2	4.1	8.8	7.2	9.6	3.7	3.0	5.6
C _{18:1} ω 7c	29.3	25.8	45.6	31.6	77.9	28.8	70.8	65.2
C _{18:1} 2-OH	3.5	1.8	0.6	0.5	1.4	1.5	6.2	–
C _{18:0} 3-OH	1.1	–	–	0.5	–	–	1.0	–
C _{19:0} cyclo ω 8c	39.8	57.4	32.7	50.2	5.9	47.2	4.3	–
C _{20:1} ω 7c	–	–	–	0.8	–	–	–	–
C _{20:2} ω 6,9c	1.4	0.9	0.5	0.8	–	1.1	–	–
Summed feature 2*	0.4	–	–	–	–	–	1.9	–
Summed feature 3*	1.6	0.7	1.1	1.0	0.7	3.7	2.3	–

*Summed feature 2 contains C_{12:0} unknown aldehyde with ECL 10.928/C_{16:1} isoI/C_{14:0} 3-OH. Summed feature 3 contains C_{16:1} ω 7cI/C_{15:0} iso 2-OH.

The unidentified aminophospholipid in the profile of strain ADV31^T exhibited the same R_F value as that of phosphatidylmonomethylethanolamine in the polar lipid profile of *O. gallinifaecis* (Kämpfer *et al.*, 2003). Consequently, we could not show any obvious differences between the polar lipid profiles of strain ADV31^T and *O. gallinifaecis*.

Some characteristics that are useful for the routine identification of *Ochrobactrum* spp. (Teyssier *et al.*, 2005a) were common to strains ADV31^T, ADV41 and ADV43 and *O. intermedium* (mucoid colonies, ability to grow on tryptic soy agar at 45 °C, absence of urease activity and resistance to colistin). In addition, genotyping data and phylogeny showed that the novel species was related to *O. intermedium*, supporting its denomination as *Ochrobactrum pseudintermedium* sp. nov. However, the 16S rRNA gene-, *dnaK*- and *rpoB*-phylogenies, DNA–DNA hybridization, G + C content, *rrn* skeleton of the small chromosome, PCR specific for *Brucella* spp. and *O. intermedium*, phenotype and chemotaxonomy clearly differentiated strains ADV31^T, ADV41 and ADV43 from *O. intermedium*. Together, the results support the creation of a novel species to accommodate isolates ADV31^T, ADV41 and ADV43 and its denomination as *Ochrobactrum pseudintermedium* sp. nov. is proposed.

Description of *Ochrobactrum pseudintermedium* sp. nov.

Ochrobactrum pseudintermedium (pseud.in'ter.med'i.um. Gr. adj. *pseudos* false; L. neut. adj. *intermedium* intermediate, and a specific epithet of the genus *Ochrobactrum*; N.L. neut. adj. *pseudintermedium* a false *Ochrobactrum intermedium*).

Cells are Gram-negative, non-spore forming, short rods, and motile by subpolar flagella. Cells are 0.65–0.75 $\times$ 1.5–1.7 μ m in size. Growth occurs at 25–45 °C on tryptic soy agar. Grows on MacConkey agar and R2A agar at 37 °C. Colonies are non-pigmented, mucoid and opaque. Aerobic respiration, oxidative metabolism, nitrate reduction and catalase and oxidase are positive. Positive for glycine arylamidase, L-proline arylamidase, tyrosine arylamidase and L-pyrrolidonyl arylamidase activities, assimilation of glucose, arabinose, mannose, malate, potassium gluconate, D-mannitol, *N*-acetylglucosamine, D-maltose and capric acid, acidification of adonitol and D-tagatose, and alkalization of L-lactate and succinate. Negative for activities of DNase, urease, phenylalanine deaminase, tryptophan deaminase, Glu-Gly-Arg arylamidase and β -alanine arylamidase *p*-nitroanilide (pNA), arginine dihydrolase, Ala-Phe-Pro arylamidase, glutamyl arylamidase pNA,

β -xylosidase, lipase, *N*-acetyl- β -galactosaminidase, α -galactosidase, β -galactosidase (ONPG), *N*-acetyl- β -glucosaminidase, phosphatase, ornithine decarboxylase, lysine decarboxylase, β -glucuronidase and β -glucosidase (aesculin hydrolysis). Negative for acidification of D-glucose, D-mannose, L-arabitol, D-cellobiose, D-mannitol, D-trehalose, malonate, 5-keto-D-gluconate and coumarate, production of indole, acetoin and H₂S, hydrolysis of gelatin, assimilation of adipic acid, phenylacetic acid, L-histidine and L-lactate, and fermentation of carbohydrates. Resistant to β -lactams except imipenem, and to chloramphenicol, tetracycline, fosfomycin and colistin. Susceptible to aminoglycosides (gentamicin, tobramycin, netilmicin, isepamicin, amikacin), fluoroquinolones (pefloxacin, ofloxacin, ciprofloxacin), nalidixic acid, rifampicin and trimethoprim-sulfamethoxazole. Major fatty acids are C_{19:0} cyclo ω 8c and C_{18:1} ω 7c. Ubiquinone 10 is the dominant respiratory lipoquinone. Polar lipids mainly comprise phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. 16S rRNA gene amplification is not obtained with primers F4 and R2 (Velasco *et al.*, 1998). Genome is constituted of two chromosomes of about 2.8 and 1.8 Mb comprising four *rrn* copies with two *rrn* operons in divergent orientation on the small chromosome. The G+C content of the DNA of the type strain is 54.5 mol%. Can be differentiated from other species of the genus *Ochrobactrum* by 16S rRNA, *dnaK* and *rpoB* gene sequencing.

The type strain is ADV31^T (= CIP 109116^T = DSM 17490^T), which was isolated from a human clinical sample.

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