Chryseobacterium hispanicum sp. nov., isolated from the drinking water distribution system of Sevilla, Spain

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Strain VP48^T was isolated from drinking water during a screening programme to monitor the bacterial population present in the water distribution system of Sevilla (Spain). A polyphasic taxonomic study of the isolate resulted in its identification as a member of the genus *Chryseobacterium*, members of which are widely distributed in soil, water and clinical sources. However, the 16S rRNA gene sequence similarity values of strain VP48^T to the type strains of *Chryseobacterium* species were 96 % or lower. Furthermore, phenotypic characteristics clearly indicated that the isolate represents a novel *Chryseobacterium* species, for which the name *Chryseobacterium hispanicum* sp. nov. is proposed; strain VP48^T (= CECT 7129^T = CCM 7359^T = JCM 13554^T) is the type strain. The DNA G + C content of this strain is 34.3 mol%.

The genus *Chryseobacterium* was proposed by Vandamme *et al.* (1994). It belongs to the family *Flavobacteriaceae*, phylum *Bacteroidetes* and currently includes 14 species (Table 1). Two previously recognized *Chryseobacterium* species, *Chryseobacterium meningosepticum* (King, 1959; Vandamme *et al.*, 1994) and *Chryseobacterium miricola* (Li *et al.*, 2003), have been transferred recently to the genus *Elizabethkingia* (Kim *et al.*, 2005b). *Chryseobacterium* species are widely distributed in soil, water and clinical sources (Vandamme *et al.*, 1994).

The culturable bacterial population occurring in the drinking water of Sevilla (Spain) was studied in order to monitor the microbiological quality of the water at different points in the distribution system. Four sampling campaigns were performed during a period of 1 year, one each season. Samples of drinking water (25 l) were concentrated using a tangential flow filtration system (Filtron) and plated on plate count agar (PCA; Difco) and R2A (Difco) (Reasoner & Geldreich, 1985). Plates were incubated at 28 °C for 7 days and colonies with different morphologies were subcultivated to obtain pure cultures. Among the organisms isolated in this study (about 600 pure cultures), strain VP48^T was isolated on PCA during the sampling campaign of October 2003 and pure culture was obtained after subcultivation on R2A medium at 28 °C.

The shape and motility of bacterial cells were observed under a phase-contrast microscope ($\times 1000$ magnification) from a 24 h liquid culture in R2A medium. Growth at different temperatures (4–40 °C), pH values (pH 3–12) and NaCl concentrations (0–5 % NaCl) was tested on solid and liquid R2A medium. The isolate was also tested for its ability to grow on MacConkey agar (Difco) and TSA (Difco). H₂S production was determined on Kligler iron agar (Difco). Oxidase activity was detected using a 1 % solution of tetramethyl-*p*-phenylenediamine (Difco) (Kovacs, 1956). Catalase activity was tested by picking up a young colony and smearing it in a drop of H₂O₂. The methyl red and Voges–Proskauer reactions were performed on Clark–Lubs medium (Scharlau). Indole production was determined with Kovacs' reagent on 1 % tryptone broth. Simmons' citrate test was tested on Simmons' citrate agar (Sigma). For determination of acid production from different carbohydrates, a medium containing 0.5 % peptone, 0.5 % NaCl and 0.001 % phenol red was used (Cowan & Steel, 1974). Nitrate reduction was tested on nitrate broth containing 0.2 % KNO₃ (Skerman, 1967). Urease activity was studied on Christensen's medium (Christensen, 1946). Hydrolysis of gelatin, starch and DNA was tested on the corresponding agar media (Scharlau). Tween 80 hydrolysis was tested on R2A medium containing 1 % Tween 80 and 0.02 % CaCl₂. Casein hydrolysis was tested on R2A medium supplemented with 2 % skim milk (Difco). Flexirubin-type pigments were detected as described by Bernardet *et al.* (2002). API 20NE and API ZYM strips (bioMérieux) were inoculated according to the manufacturer's instructions and incubated at 28 °C.

Phenotypic characteristics of strain VP48^T are included in the species description; they clearly support its placement

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain VP48^T is AM159183.

API ZYM profiles of strain VP48^T and other *Chryseobacterium* and *Elizabethkingia* species are available as supplementary material in IJSEM Online.

Table 1. Differential characteristics of strain VP48^T and related species of the genera *Chryseobacterium* and *Elizabethkingia*

Taxa: 1, strain VP48^T; 2, *C. balustinum*; 3, *C. daecheongense*; 4, *C. defluvi*; 5, *C. formosense*; 6, *C. gleum*; 7, *C. indologenes*; 8, *C. indoltheticum*; 9, *C. jooste*; 10, *C. scopthalmum*; 11, *C. shigense*; 12, *C. soldanellicola*; 13, *C. taeanense*; 14, *C. vrystaatense*; 15, *C. taichungense*; 16, *Elizabethkingia meningoseptica*; 17, *Elizabethkingia miricola*. Symbols: +, positive; –, negative; NA, no data available; w, weakly positive; v, variable; D, delayed. Data for reference species were taken from Kämpfer *et al.* (2003), Li *et al.* (2003), Hugo *et al.* (2003), Shen *et al.* (2005), Kim *et al.* (2005a), Young *et al.* (2005), Shimomura *et al.* (2005), de Beer *et al.* (2005) and Park *et al.* (2006).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Growth on/at:																	
MacConkey agar	–	+	–	–	–	+	+	+	+	–	–	–	–	–*	–	+	+
5 °C	D	D	–	–	–	–	–	+	+	D	+	+	+	+	–	–	–
37 °C	–	+	+	+	–	+	–	+	–	–	–	+	+	–	+	+	+
42 °C	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Enzyme activities:																	
DNase	–	+	NA	NA	NA	+	+	+	+	+	NA	–	–	+	NA	+	+
Urease	–	–	–	–	–	–	–	–	V	+	–	–	–	+	–	D	+
Nitrate reduction	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Hydrolysis of:																	
Aesculin	+	+	+	+	+	+	+	+	+	+	NA	+	+	+	+	+	+
Tween 80	–	+	–	NA	–	+	+	+	+	+	NA	–	+	+	NA	–	+
Production of:																	
H ₂ S	–	–	–	–	–	–	–	+	–	–	–	–	–	V	–	–	+
Indole	w	+	–	+	+	+	+	+	+	–	+	–	–	+	w	D	+
Acid production from:																	
Fructose	+	+	+	+	NA	+	+	–	+	–	+	–	–	NA	NA	+	+
Glycerol	+	–	+	+	NA	+	+	–	V	–	–	–	–	NA	NA	+	–
Lactose	–	–	–	–	NA	–	–	–	–	–	–	–	–	NA	–	+	+
Maltose	+	–	–	+	–	+	+	+	+	–	–	–	–	NA	+	+	+
Mannitol	–	–	–	–	–	–	–	–	V	–	–	–	–	NA	–	+	+
Trehalose	–	–	+	+	+	+	+	–	+	+	–	–	–	NA	+	+	+
D-Xylose	+	–	+	–	w	+	–	–	–	–	–	–	–	NA	+	–	–
G + C content (mol%)	34.3	33.1	36.6	38.8	NA	38.0	38.5	33.8	36.8	34.2	36.6	28.8	32.1	37.1	NA	37.0	34.6

*Most strains are negative.

within the genus *Chryseobacterium*. Phenotypic features that differentiate strain VP48^T from other species of *Chryseobacterium* and *Elizabethkingia* are shown in Table 1. A table showing API ZYM profiles of strain VP48^T and other *Chryseobacterium* and *Elizabethkingia* species is available as supplementary material in IJSEM Online.

The 16S rRNA gene was amplified using the universal primers 16F27 and 16R1488 as described previously by Mellado *et al.* (1995). The almost-complete nucleotide sequence was determined by NBT-Newbiotechnic (Sevilla, Spain) using an automated DNA sequencer model 3100 (Applied Biosystems). Alignment of 16S rRNA gene sequences was carried out using the ARB software program (Ludwig *et al.*, 2004) and phylogenetic trees were inferred using the maximum-parsimony (Kluge & Farris, 1969), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood methods (Felsenstein, 1981).

The almost-complete 16S rRNA gene sequence of strain VP48^T (1421 bp) was compared with sequences of related

organisms retrieved from the GenBank/EMBL/DDBJ databases. Strain VP48^T was most closely related to representatives of the family *Flavobacteriaceae*, especially to members of the genus *Chryseobacterium*. The highest sequence similarity was 96.0 % with *Chryseobacterium indoltheticum* and *Chryseobacterium scopthalmum*, whereas similarity values were ≤96 % with the type strains of other species in the genus *Chryseobacterium* and 93.9–92.7 % with members of the genus *Elizabethkingia*. Moreover, phylogenetic analysis clearly showed that strain VP48^T constitutes a branch that is separate from other *Chryseobacterium* species, although the branching patterns of the different trees were not completely stable (Fig. 1). Therefore, according to phylogenetic data, strain VP48^T could be considered to be a representative of a novel species of the genus *Chryseobacterium* (Stackebrandt & Goebel, 1994).

Chromosomal DNA was isolated and purified as described by Marmur (1961). The G + C content of genomic DNA was determined by the method of Marmur & Doty (1962) using the equation of Owen & Hill (1979). The DNA G + C

Fig. 1. Maximum-parsimony tree based on the comparison of 16S rRNA gene sequences showing the position of strain VP48^T and type strains of *Chryseobacterium* species and related species in the family *Flavobacteriaceae*. GenBank accession numbers are shown in parentheses. Bootstrap values greater than 50 (from 100 resamplings) are indicated at branching points. Bar, 2% sequence divergence.

content of strain VP48^T was 34.3 mol%, a value that falls within the range described for the genus *Chryseobacterium* (Vandamme *et al.*, 1994).

The whole-cell fatty acid composition was determined using the Microbial Identification System (MIDI, Inc.). Cells were cultured for 24 h at 28 °C on TSA medium at pH 7.0 and cellular fatty acids were analysed using GC at the Belgian Co-ordinated Collections of Microorganisms (Laboratory of Microbiology, University of Gent, Gent, Belgium). The predominant fatty acids of strain VP48^T were summed feature 3 (15:0 iso 2-OH and/or 16:1 ω 7c, 26.7%), 15:0 iso (26.1%) and 17:0 iso 3-OH (17.6%). Significant amounts of these fatty acids are characteristic of members of the genus *Chryseobacterium* (Vandamme *et al.*, 1994). However, the fatty acid profile of strain VP48^T differs from those of other *Chryseobacterium* species in its larger amount of summed feature 3 and smaller amounts of 15:0 iso and 17:1 iso ω 9c and in the presence of 16:1 ω 5c. A detailed fatty acid composition of strain VP48^T is given in the species description.

Phylogenetic data and several phenotypic features enabled strain VP48^T to be differentiated from other members of the genus *Chryseobacterium*. A novel species, *Chryseobacterium hispanicum* sp. nov., is therefore proposed to accommodate this strain.

Description of *Chryseobacterium hispanicum* sp. nov.

Chryseobacterium hispanicum (his.pa'ni.cum. L. neut. adj. *hispanicum* from Spain).

Gram-negative rods, 0.9 × 1.5–4.0 µm, occurring singly, in pairs or in short chains. Cells are non-motile and

non-spore-forming. Colonies on TSA plates are white–yellow, opaque, round, convex and 2–3 mm in diameter after 48 h at 28 °C. Strictly aerobic. Flexirubin reaction is weakly positive. Growth occurs between 5 and 30 °C (optimal growth at 25–28 °C) and from pH 5 to 10 (optimal growth at pH 7.0). Growth occurs with 0–2% NaCl (optimal growth without NaCl). Good growth occurs on R2A, PCA and TSA, but not on MacConkey agar. Positive for catalase and weakly positive for oxidase; urease activity is absent. Indole is weakly positive. Methyl red and Voges–Proskauer reactions are negative. Does not produce H₂S. Casein, starch, aesculin and gelatin are hydrolysed, but Tween 80 and DNA are not. Simmons' citrate test is negative. Nitrate is reduced to nitrite. Acid is produced oxidatively from D-arabinose, D-fructose, D-glucose, glycerol, D-maltose, D-mannose and D-xylose, but not from D-galactose, lactose, D-mannitol or D-trehalose. Glucose fermentation is negative. D-Glucose, L-arabinose, D-mannose and D-maltose are used as sole carbon sources. N-Acetylglucosamine, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not used as sole carbon sources. Alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -glucosidase and β -glucosidase activities are present. Arginine dihydrolase, lipase (C14), α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities are absent. Cellular fatty acids amounting to more than 1% are: 13:0 iso, 2.5%; unknown 13:565, 1.0%; 15:0 iso, 26.1%; 15:0 anteiso, 3.6%; summed feature 3 (16:1 ω 7c and/or 15:0 iso 2-OH), 26.7%; 16:1 ω 5c, 5.3%; 16:0, 2.4%; 15:0 iso 3-OH, 4.0%; 17:1 iso ω 9c, 1.3%; unknown 16:582, 1.9%; 16:0 3-OH, 4.4%; and 17:0 iso 3-OH,

17.6%. The DNA G+C content of the type strain is 34.3 mol% (T_m method).

The type strain is VP48^T (=CECT 7129^T=CCM 7359^T=JCM 13554^T), isolated from drinking water in Sevilla, Spain.

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