Chryseobacterium arothri sp. nov., isolated from the kidneys of a pufferfish

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A Gram-negative, non-flagellated, rod-shaped bacterium, designated strain P2K6^T, was isolated from the kidneys of a pufferfish (*Arothron hispidus*) caught off the coast of Kaneohe Bay, O'ahu, Hawai'i. The strain formed yellowish colonies when grown on marine agar. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain P2K6^T was related most closely to members of the genus *Chryseobacterium*. Levels of 16S rRNA gene sequence similarity between strain P2K6^T and the type strains of recognized species of the genus *Chryseobacterium* were 94–96.6%, suggesting that the strain represents a novel species within this genus. The DNA G + C content of strain P2K6^T was 36.5 mol%, the dominant fatty acids were iso-C_{15:0} (35.3%) and iso-C_{17:0} 3-OH (14.9%), and the most abundant quinone was menaquinone MK6. On the basis of the data from this polyphasic study, it is suggested that strain P2K6^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium arothri* sp. nov. is proposed. The type strain is P2K6^T (=CIP 109575^T=DSM 19326^T).

The genus *Chryseobacterium* was established based on the reclassification of several species of the genus *Flavobacterium* (as *Chryseobacterium balustinum*, *C. gleum*, *C. indologenes*, *C. indoltheticum*, *C. meningosepticum* and *C. scophthalmum*; Vandamme *et al.*, 1994). At the time of writing, the genus comprised 16 recognized species, including *C. hispanicum* (Gallego *et al.*, 2006) and *C. formosense* (Young *et al.*, 2005).

The pufferfish *Arothron hispidus* is commonly found in the warm tropical waters around the Hawaiian islands. Bacterial strain P2K6^T was isolated from the kidneys of one *A. hispidus* caught off the windward side of the island of O'ahu in August 2005. Strain P2K6^T was isolated on half-strength marine agar 2216 (1/2MA; Difco). The yellowish colonies that developed were further purified and maintained in 10% glycerol/90% half-strength marine broth at –80 °C.

A male pufferfish weighing 306 g was dissected into different organs and body parts. Subsamples of each organ were homogenized with sterile artificial seawater (50–100 ml) (Coralife) and serial dilutions were plated onto 1/2MA. Media were subsequently incubated at 30 °C for 1–5 days. Genomic DNA was isolated from pure cultures by using an equal volume ratio of phenol/chloroform (Marmur, 1961). Amplification of the 16S rRNA gene from genomic DNA was done via PCR by using primers 27F and 1492R (Lane, 1991) and Mastermix Taq DNA polymerase (Eppendorf). All PCR products

were purified by using the Ultraclean PCR purification kit (Mo Bio Lab) and sequenced by the Advanced Studies of Genomics, Proteomics, and Bioinformatics Center at the University of Hawai'i at Manoa. Sequences were edited manually and assembled by using Seqman II (Lasergene) and subjected to a BLASTN comparison search against sequences in the public domain (Altschul *et al.*, 1997).

The phylogenetic relationship of strain P2K6^T to recognized species of the genus *Chryseobacterium* was determined based on comparative 16S rRNA gene sequence analyses. These analyses were performed with programs in the PHYLIP 3.63 package (Felsenstein, 2004). Evolutionary distances were calculated by using the maximum-likelihood method with DNADIST. Bootstrap analyses were based on 100 replicates by using the programs SEQBOOT, DNADIST and CONSENSE. Phylogenetic trees were constructed with jumbled orders of the sequences and the neighbour-joining method (Saitou & Nei, 1987).

Fig. 1 shows the phylogenetic tree based on the 16S rRNA gene. The nearly complete (1399 bp) 16S rRNA gene sequence of strain P2K6^T was related most closely to that of the type strain of *C. formosense* (Young *et al.*, 2005; 96.6% similarity). Strain P2K6^T showed a level of 16S gene sequence similarity to the type strains of *C. hispanicum* and *Chryseobacterium caeni* of 96.1%. Based on these low levels of sequence similarity, strain P2K6^T should be considered as being distinct from recognized species of the genus *Chryseobacterium* (Stackebrandt & Goebel, 1994).

Determination of the DNA G + C content was performed by the Deutsche Sammlung von Mikroorganismen und

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P2K6^T is EF554408.

Table 1. Fatty acid composition of strain P2K6^T and some recognized species of the genus *Chryseobacterium*

Strains: 1, P2K6^T (*Chryseobacterium arothri* sp. nov.); 2, *C. formosense* CCUG 49271^T (data from Young *et al.*, 2005); 3, *C. vrystaatense* LMG 22846^T (De Beer *et al.*, 2005); 4, *C. indoltheticum* ATCC 27950^T; 5, *C. balustinum* LMG 8329^T; 6, *C. gleum* NCTC 11432^T; 7, *C. indologenes* NCTC 10796^T; 8, *C. scophthalmum* LMG 13028^T; 9, *C. joostei* LMG 18212^T (data for 4–9 from Hugo *et al.*, 2003); 10, *C. defluvii* DSM 14219^T (Kämpfer *et al.*, 2003); 11, *C. hispanicum* JCM 13554^T (Gallego *et al.*, 2006); 12, *C. daecheongense* DSM 15235^T (Kim *et al.*, 2005); 13, *C. taichungense* CCUG 50001^T (Shen *et al.*, 2005); 14, *C. wanjuae* DSM 17724^T (Weon *et al.*, 2006); 15, *C. piscium* CCUG 51923^T (De Beer *et al.*, 2006); 16, *C. soldanellicola* NBRC 100864^T (Park *et al.*, 2006); 17, *C. taeanense* NBRC 100863^T (Park *et al.*, 2006). Values are percentages of the total fatty acids. —, Not detected; NA, data not available; tr, trace amount (<1 %).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
iso-C _{13:0}	4.99	3.6	1.1	—	tr	tr	tr	tr	tr	2.8	2.5	1.6	tr	tr	tr	tr	tr
C _{14:0}	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
iso-C _{15:0}	35.30	52.2	41.8	29.4	32.3	35.4	34.3	35.0	34.6	58.5	26.1	51.2	35.4	40.0	38.3	41.8	36.1
anteiso-C _{15:0}	5.45	2.1	1.7	5.9	tr	tr	tr	tr	tr	3.2	3.6	1.0	0.7	tr	2.7	1.9	1.1
C _{16:1} ω5c	2.14	—	—	—	—	—	—	—	—	—	5.3	—	—	—	—	—	—
C _{16:0}	1.53	1.5	1.1	1.0	1.6	1.3	tr	1.2	tr	1.3	2.4	1.8	1.3	—	1.1	1.4	1.8
iso-C _{15:0} 3-OH	4.50	1.8	2.7	2.3	2.7	2.5	2.6	2.7	2.9	2.6	4.0	2.0	4.3	3.7	2.4	2.7	3.1
iso-C _{15:0} 2-OH	tr	SF 3*	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3	SF 3
C _{15:0} 2-OH	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
iso-C _{16:0} 3-OH	tr	1.1	tr	1.3	tr	—	—	—	—	tr	—	tr	1.4	—	1.3	tr	tr
C _{16:0} 3-OH	5.17	tr	1.3	tr	1.4	1.1	1.0	1.0	1.2	tr	4.4	tr	2.6	tr	tr	1.3	tr
C _{18:1} ω9c	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
iso-C _{17:0}	—	2.3	tr	tr	1.0	1.6	tr	tr	tr	2.0	tr	3.0	tr	2.9	1.2	tr	1.1
iso-C _{17:0} 3-OH	14.90	10.9	15.4	14.0	16.8	21.8	19.2	16.3	20.1	14.1	17.6	15.7	22.4	21.9	16.2	17.7	18.8
C _{17:0} 2-OH	0.81	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr
C _{17:1} ω9c	—	4.3	19.7	25.6	27.1	20.2	24.2	24.8	22.9	4.8	—	7.6	8.9	11.7	18.7	14.6	15.8
SF 3*	NA	26.7	9.1	NA	NA	NA	NA	NA	NA	NA	26.7	9.1	—	—	—	—	—
SF 4*	13.89	—	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	—	—	—	—	—
SF 5*	6.23	—	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	—	—	—	—	—
SF 6*	NA	6.5	tr	11.2	9.2	11.8	11.1	11.5	12.1	8.4	NA	8.4	13.8	11.0	10.8	9.7	11.2

*Summed feature (SF) 3 contains iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; summed feature 4 contains C_{16:1}ω7c and/or C_{16:1}ω6c; summed feature 5 contains 10-methyl C_{16:0} and/or iso-C_{17:1}ω9c; and summed feature 6 contains iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain P2K6^T and some recognized members of the genus *Chryseobacterium*. Numbers at nodes are bootstrap percentages based on a maximum-likelihood analysis of 100 resampled datasets. The sequences of *Elizabethkingia meningoseptica* ATCC 13253^T and *Flavobacterium granuli* KCTC 12201^T were used as outgroups. Bar, 0.02 nucleotide substitutions per site.

Table 2. Differential phenotypic characteristics between strain P2K6^T and some recognized species of the genus *Chryseobacterium*

Strains: 1, P2K6^T (*Chryseobacterium arothri* sp. nov.); 2, *C. soldanellicola* NBRC 100864^T; 3, *C. taeanense* NBRC 100863^T; 4, *C. taichungense* CCUG 50001^T; 5, *C. balustinum* LMG 8329^T; 6, *C. daecheongense* DSM 15235^T; 7, *C. defluvii* DSM 14219^T; 8, *C. formosense* CCUG 49271^T; 9, *C. gleum* NCTC 11432^T; 10, *C. indologenes* NCTC 10796^T; 11, *C. indoltheticum* ATCC 27950^T; 12, *C. joostei* LMG 18212^T; 13, *C. scopthalmum* LMG 13028^T; 14, *C. hispanicum* CECT 7129^T. Data for reference species are from Kämpfer *et al.* (2003), Hugo *et al.* (2003), Kim *et al.* (2005), Shen *et al.* (2005), Young *et al.* (2005) and Gallego *et al.* (2006). +, Positive; –, negative; v, variable; D, delayed; NA, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Growth at:														
5 °C	–	–	–	–	+	–	–	–	+	+	+	+	–	D
37 °C	+	+	+	–	D	–	–	–	–	–	+	+	D	–
42 °C	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Enzyme activities														
DNase	NA	–	–	NA	+	NA	NA	NA	+	+	+	+	+	–
Urease	–	–	–	–	–	–	–	–	–	–	–	v	+	–
Nitrate reduction	–	–	–	–	+	+	–	–	+	–	–	–	–	+
Nitrite reduction	–	–	–	NA	–	–	+	NA	+	–	–	–	–	NA
Tween 80 hydrolysis	+	–	+	NA	+	–	NA	–	+	+	+	+	+	–
Acid production from:														
L-Arabinose	–	–	–	–	–	–	–	–	+	–	–	–	–	NA
Fructose	+	–	–	NA	+	+	+	NA	+	+	–	+	–	+
Glycerol	–	–	–	NA	–	+	+	NA	+	+	–	+	–	+
Lactose	–	–	–	–	–	–	–	NA	–	–	–	–	–	–
Maltose	+	–	–	+	–	–	+	–	+	+	+	+	–	+
Mannitol	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Trehalose	–	–	–	+	–	+	+	+	+	+	–	+	+	–
DNA G + C content (mol%)	36.5	28.8	32.1	NA	33.1	36.6	38.8	NA	38.0	38.5	33.8	36.8	34.2	34.3

Zellkulturen (Braunschweig, Germany) by using an HPLC method (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). The DNA G + C content of strain P2K6^T was 36.5 mol%. Fatty acid methyl ester analysis for strain P2K6^T was performed by MIDI Laboratories by using whole cells grown on trypticase soy agar (TSA) for 24 h at 28 °C (Sasser, 1990). The fatty acid compositions of strain P2K6^T and of other recognized species of the genus *Chryseobacterium* are summarized in Table 1. The major fatty acids for *C. formosense* were iso-C_{15:0} (52.2 %) and iso-C_{17:0} 3-OH (10.9 %). These were the main two fatty acids found in strain P2K6^T (35.3 and 14.9 %, respectively). C_{16:1}ω5c and C_{16:0} 3-OH were present (2.14 and 5.17 %, respectively) in strain P2K6^T but were not detected or were present in trace amounts in *C. formosense*.

Quinone analysis was performed by using fresh whole cells of strain P2K6^T grown in marine broth for 2–3 days. Cells were extracted with a mixture of chloroform/methanol (2:1, v/v) (Hiraishi, 1988); after filtration and dilution in acetone, the extract was analysed via HPLC on an Agilent 1100 series chromatograph coupled to a diode array detector and an Agilent model SL single quadrupole mass spectrometer. The HPLC column was a Vydac C18 Mass Spec. Strain P2K6^T had menaquinone MK6 as the most abundant quinone.

Cells of strain P2K6^T were non-motile, Gram-negative rods. Motility was checked by the hanging drop method by using

light microscopy under a 100 × oil-immersion objective, and by stab inoculation into modified motility test agar (per litre distilled water: 10 g tryptone, 20 g NaCl and 5 g agar). After 2 days growth on MA at 30 °C, cells were about 0.5 µm in diameter. They required salt for growth, which was observed over the range 0.5–3.0 % NaCl (w/v), with optimum growth at 2 % NaCl. Growth was observed over a temperature range from 20 °C (slow growth, 2–3 days) to 37 °C. Growth was tested at temperatures of 5, 20, 30, 37 and 43 °C on MA plates over 1 week. Strain P2K6^T grew anaerobically on TSA (in a Gas-Pak Pouch; Becton Dickinson).

Physiological and biochemical test results are summarized in Table 2. The presence or absence of constitutive enzymes and the substrate profile were determined by using the API ZYM and API 20NE systems (bioMérieux). Oxidation of carbon substrates was determined with Biolog GN microplates (Table 2).

On the basis of 16S rRNA gene sequence data, and distinct morphology, physiology and fatty acid composition, strain P2K6^T is considered to represent a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium arothri* sp. nov. is proposed.

Description of *Chryseobacterium arothri* sp. nov.

Chryseobacterium arothri (a.ro'thri. N.L. n. *Arothron* scientific genus name of a genus of fish; N.L. gen. n.

arothri of *Arothron*, isolated from the pufferfish *Arothron hispidus*).

Cells are aerobic, facultatively anaerobic, non-motile, Gram-negative rods ($1 \times 0.5 \mu\text{m}$ in size). Circular, yellowish colonies are formed on TSA at 30°C after 1 day. Optimum temperature range for growth is $20\text{--}37^\circ\text{C}$. NaCl is required for growth, in the range $0.5\text{--}3.0\%$ (w/v), with optimum growth at 2.0% NaCl. Utilizes α -cyclodextrin, dextrin, glycogen, Tweens 40 and 80, D-cellobiose, D-fructose, gentiobiose, α -D-glucose, maltose, D-mannose, D-psicose, sucrose, turanose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, α -ketobutyric acid, α -ketovaleric acid, propionic acid, quinic acid, D-saccharic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, α -D-glucose 1-phosphate, and D-glucose 6-phosphate as carbon source in Biolog GN2 tests. Produces H_2S on triple-sugar iron + 2% NaCl agar. The predominant fatty acids are iso-C_{15:0} (35.3 %) and iso-C_{17:0} 3-OH (14.9 %). The DNA G + C content of the type strain is 36.5 mol%.

The type strain, P2K6^T (=CIP 109575^T=DSM 19326^T), was isolated from the kidneys of a pufferfish *Arothron hispidus* caught off the coast of the windward side of the island of O'ahu, Hawai'i.

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