Kistimonas scapharcae sp. nov., isolated from a dead ark clam (Scapharca broughtonii), and emended description of the genus Kistimonas

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A Gram-negative, motile, facultatively anaerobic rod, designated A36T, was isolated from a dead ark clam found on the south coast of Korea. The isolate was catalase- and oxidase-negative. 16S rRNA gene sequence analysis indicated that strain A36T was most closely related to Kistimonas asteriae KMD 001T, with which it shared 98.2 % 16S rRNA gene sequence similarity. Strain A36T grew optimally at 30–37 °C, with 1 % (w/v) NaCl and at pH 8.0. The major respiratory quinone was ubiquinone-9 (Q-9). The major polar lipids were phosphatidylserine, phosphoethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids were summed feature 3 (comprising C16 : 1v7c and/or iso-C15 : 0) and C16 : 0. The genomic DNA G+C content was 47.3 mol%. DNA–DNA relatedness between the isolate and K. asteriae JCM 15607T was 25 ± 3 %. Strain A36T represents a novel species of the genus Kistimonas, for which the name Kistimonas scapharcae sp. nov. is proposed. The type strain is A36T (=KACC 16204T =JCM 17805T). An emended description of the genus Kistimonas is also provided.

The genus Kistimonas, belonging to the class Gamma-proteobacteria, was first described by Choi et al. (2010) to accommodate a bacterium isolated from the skin of a starfish (Asteria samurensis) found on the coast of the Korean East Sea. The genus Kistimonas is characterized as having ubiquinone-9 as the major quinone and iso-C15 : 0, anteiso-C15 : 0 and iso-C14 : 0 as the major fatty acids. To date, only one species of the genus has been described: Kistimonas asteriae.

During an attempt to isolate and characterize tentative pathogenic bacteria following a mass mortality event within cage-cultured ark clams (Scapharca broughtonii), a dead ark clam was collected at Gang-jin Bay on the south coast of Korea. Homogenized tissue was suspended in PBS, serially diluted and then cultured on marine agar (MA; Difco) at 25 °C for 3 days. A pure culture, strain A36T, was obtained after at least three subcultivations on MA.

Amplification of the 16S rRNA gene was performed by colony PCR using PCR Pre-Mix (Wizbio) with two universal bacteria-specific primers: forward primer 8F (5’T-AGAGTTTGATCCTGGCTCAG-3’T) and reverse primer 1492R (5’T-GGYTACCTTGTTACGACTT-3’T). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis was conducted as described by Roh et al. (2008). Strain A36T was closely related to K. asteriae KMD 001T (98.2 % 16S rRNA gene sequence similarity). The 16S rRNA gene sequences of strain A36T and members of closely related species were aligned using the multiple sequence alignment program CLUSTAL W (Thompson et al., 1994) and the phylogenetic relationships were determined using MEGA5 (Tamura et al., 2011). A phylogenetic consensus tree was constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods, with 1000 random bootstrap replicates. According to the results of the phylogenetic analysis, strain A36T clustered with K. asteriae KMD 001T (Fig. 1).

To obtain a more comprehensive comparison of characteristics, K. asteriae JCM 15607T (=KMD 001T) was obtained from the Japan Collection of Microorganisms and used as the reference strain. All experiments were conducted in triplicate. Growth at 4, 10, 15, 20, 25, 30, 37, 45 and 60 °C was tested in marine broth (MB; Difco). Growth at pH 4.0–11.0 (at intervals of one pH unit) was tested in MB with the pH adjusted using the following...
buffers: 10 mM MES (pH 4–6), 10 mM TAPS (pH 7–9) and 10 mM Na₂HPO₄ (pH 10–11). Growth with 0–11 % (w/v) NaCl at intervals of 1 % was tested in MB that comprised all of the constituents except NaCl and was supplemented with appropriate concentrations of NaCl. Growth of strain A36ᵀ was observed at 10–45 °C at pH 6.0–10.0 and with 0–5 % (w/v) NaCl, with optimum growth at 30–37 °C at pH 8.0 and with 1 % (w/v) NaCl. Unless stated otherwise, all tests were performed with the isolate cultured under optimal growth conditions. Anaerobic growth was assessed in an anaerobic chamber (N₂/H₂/CO₂, 90 : 5 : 5) at 37 °C for 7 days. Strain A36ᵀ and K. asteriae JCM 15607ᵀ were cultured for 72 h at 30 °C in MB or on MA to analyse their physiological and biochemical characteristics. Motility was tested on semi-solid agar medium, as described by Tittsler & Sandholzer (1936). Gram-staining was performed using a Gram-staining kit (bioMérieux), according to the manufacturer’s instructions. Cell morphology of strain A36ᵀ, including cell shape and size, and Gram-staining were observed by light microscopy (Eclipse 50i; Nikon). The catalase and oxidase activities of strain A36ᵀ and the reference strain were tested using the API ZYM system according to the manufacturer’s instructions. Enzyme activities were characterized using the API ZYM system (bioMérieux), according to the manufacturer’s instructions. Acid production from carbohydrates as the sole carbon source was tested using the API 50 CH system with API 50 CHB/E medium (bioMérieux), according to the manufacturer’s instructions. Strain A36ᵀ was facultatively anaerobic, motile, Gram-negative and catalase- and oxidase-negative. Strain A36ᵀ could be distinguished from K. asteriae JCM 15607ᵀ by a comparison of biochemical characteristics (Table 1).

For chemotaxonomic analysis, strain A36ᵀ and K. asteriae JCM 15607ᵀ were cultivated on MA or in MB at 30 °C for 72 h and Undibacterium pigrum KACC 13403ᵀ was cultured on R2A agar (Difco) at 37 °C for 5 days. Isoprenoid quinones were extracted using chloroform/methanol (2:1, v/v) (Collins & Jones, 1981b) and analysed by one-dimensional TLC on a silica gel 60 F₂₅₄ plate (Merck) using reversed-phase HPLC (Collins & Jones, 1981a) with a Thermo ODS Hypersil column (250 x 4.6 mm). Polar lipids were extracted from strain A36ᵀ, K. asteriae JCM 15607ᵀ and U. pigrum KACC 13403ᵀ using the procedure of Xin et al. (2000) and separated using two-dimensional TLC on silica gel plates (Merck). Each component was detected and identified using appropriate spray reagents (Tindall, 1999). To identify the phospholipids, four standard phospholipid compounds, phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphoethanolamine (PE) and diphosphatidylglycerol (DPG), were obtained (Sigma) and phosphatidylserine (PS) was identified by comparison of PS in U. pigrum KACC 13403ᵀ (Kämpfer et al., 2007). The standard compounds and phospholipids of strain A36ᵀ, K. asteriae JCM 15607ᵀ and U. pigrum KACC 13403ᵀ were separated by one-dimensional TLC on a silica gel plate using chloroform/acetic acid/methanol/water (50 : 6.6 : 1, by vol.) as the solvent and detected by spraying with ninhydrin reagent and Zinzadze reagent. For the analysis of cellular fatty acids, strain A36ᵀ and K. asteriae JCM 15607ᵀ were cultured at 30 °C on MA for 3 days. The physiological ages of strain A36ᵀ and K. asteriae JCM 15607ᵀ were standardized to the exponential phase. The cellular fatty acids were extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 2007).
Table 1. Comparison of the characteristics of strain A36T and its closest phylogenetic relative

Strains: 1, *Kistimonas scapharcae* sp. nov. A36T; 2, *K. asteriae* KMD 001T (Choi et al., 2010). Both strains assimilate Tween 40 and 80, succinic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid and L-serine, produce acid from D-ribose, D-xylose, L-xylose, D-fructose, L-sorbose, aesculin, turanose, D-lyxose, D-tagatose and 5-ketogluconate and are positive for alkaline phosphatase, leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Both strains are negative for catalase and oxidase. +, Positive; W, weakly positive; −, negative.

Table 2. Cellular fatty acid compositions of strain A36T and its closest phylogenetic relative

Strains: 1, *Kistimonas scapharcae* sp. nov. A36T; 2, *K. asteriae* JCM 15607T. All data were taken from this study. ECL, Equivalent chain-length; tr, trace (<0.5%); −, not detected.

*All data from this study.*
between strain A36\textsuperscript{T} and \textit{K. asterae} JCM 15607\textsuperscript{T} was 25±3\% (reciprocal 5±2\%). This value was below the threshold of 70\%, so strain A36\textsuperscript{T} was considered as a distinct genospecies (Wayne et al., 1987).

Based on clearly distinguished phylogenetic, phenotypic and genotypic characteristics, strain A36\textsuperscript{T} represents a novel species of the genus \textit{Kistimonas}, for which the name \textit{Kistimonas scapharcae} sp. nov. is proposed. An emended description of the genus \textit{Kistimonas} is also provided.

**Emended description of the genus \textit{Kistimonas}**

The genus description is based on that given by Choi et al. (2010) with the following amendments. Catalase- and oxidase-negative. The major polar lipids are PS, PG, PE and DPG. The predominant fatty acids are summed feature 3 (comprising C\textsubscript{16:1}ω7c and/or iso-C\textsubscript{15} 2-0H) and C\textsubscript{16:0}.

**Description of \textit{Kistimonas scapharcae} sp. nov.**

\textit{Kistimonas scapharcae} [scapharcae. N.L. gen. n. scapharcae of Scapharca, isolated from Scapharca broughtonii (ark clam)].

Cells are Gram-negative, motile, facultatively anaerobic rods (0.2–0.4 \textmu m long and 0.6–3.9 \textmu m wide). Colonies are beige, circular with entire margins and 0.5–1.5 mm in diameter after incubation for 3 days on MA at 30 °C. Growth occurs at 10–45 °C, with 0–5\% (w/v) NaCl and at pH 6.0–10.0. Optimal growth occurs at 30–37 °C, with 1\% (w/v) NaCl and at pH 8.0. Catalase- and oxidase-negative. With Biolog GN2, utilizes Tweens 40 and 80, succinic acid, bromosuccinic acid, L-alanine, L-alanyl glycine, L-asparagine, l-aspartic acid, L-glutamic acid, L-ornithine and L-serine. With API ZYM, positive for alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and trypsin, but negative for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, \textalpha-chymotrypsin, \textalpha-galactosidase, \beta-galactosidase, \beta-glucuronidase, \alpha-glucosidase, \beta-glucosidase, N-acetyl-\beta-glucosaminidase, \textalpha-mannosidase and \textalpha-fucosidase. With API 50 CHB/E, produces acids from D-ribose, D-xyllose, L-xylene, D-fructose, L-sorbose, arbunitin, aesculine, maltose, xylitol, turanose, D-lyxose, D-tagatose and 5-ketogluconate. The predominant ubiquinone is Q-9. The polar lipid profile contains PS, PE, PG and DPG. The major cellular fatty acids are summed feature 3 (comprising C\textsubscript{16:1}ω7c and/or iso-C\textsubscript{15} 2-0H) and C\textsubscript{16:0}.

The type strain is A36\textsuperscript{T} (\textit{KACC 16204}\textsuperscript{T} = JCM 17805\textsuperscript{T}), which was isolated from a dead ark clam on the south coast of Korea. The DNA G+C content of the type strain is 47.3 mol%.

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**References**


