Tranquillimonas alkanivorans gen. nov., sp. nov., an alkane-degrading bacterium isolated from Semarang Port in Indonesia

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Strain A34^T, an obligately halophilic, Gram-negative, non-motile, rod-shaped bacterium, was isolated from seawater obtained from Semarang Port in Indonesia. It possesses a pink pigment and degrades short-chain alkanes. It is positive for catalase and oxidase and reduces nitrate to nitrite. Analyses of 16S rRNA gene sequences revealed a clear affiliation of this strain with the family 'Rhodobacteraceae' in the class *Alphaproteobacteria*, with its closest relatives being *Salipiger mucosus* A3^T (94.9 % sequence similarity) and *Palleronia marisminoris* B33^T (93.4 %). The DNA G+C content was 69.1 mol%. The major cellular fatty acids of strain A34^T were C_{18:1}ω7c (56.2 %), C_{19:0} cyclo ω8c (26.0 %) and C_{16:0} (9.1 %), while the predominant respiratory lipoquinone was ubiquinone-10. Based on the physiological and phylogenetic data, it is proposed that strain A34^T should be classified in a new genus and species, for which the name *Tranquillimonas alkanivorans* gen. nov., sp. nov. is proposed. The type strain of *Tranquillimonas alkanivorans* is strain A34^T (=JCM 14836^T =DSM 19547^T).

Contamination of the marine environment with petroleum hydrocarbons is of great public concern because of their toxicity to humans and marine organisms (Malins *et al.*, 1985; Meador *et al.*, 1995). Many studies have therefore been aimed at the isolation of marine hydrocarbon-degrading bacteria (Kasai *et al.*, 2002a, b; Ozaki *et al.*, 2006); however, information regarding tropical hydrocarbon-degrading bacteria is relatively scarce (Chaillan *et al.*, 2004; Zhuang *et al.*, 2003; Zinjarde & Pant, 2002). We have recently isolated 153 marine bacteria from seawater obtained from the Semarang Port in Indonesia and analysed their hydrocarbon-degrading abilities (Harwati *et al.*, 2007). Among them, based on 16S rRNA gene analyses, an alkane-degrading bacterium, strain A34^T, was considered to represent a novel taxon (Harwati *et al.*, 2007). Here, we investigate the taxonomic characteristics of strain A34^T.

Cell morphology was examined by transmission electron microscopy (Beveridge *et al.*, 1994), while motility was checked under a phase-contrast microscope. Gram staining and oxidase and catalase tests were performed using the procedures of Smibert & Krieg (1994). Growth was tested at 30 °C in marine broth 2216 (MB) (Difco) unless

otherwise stated. Salinity requirements were tested at 30 °C using modified MB (Sohn *et al.*, 2004) supplemented with 0–20 % (w/v) NaCl. The pH range and optimum were determined at 30 °C using marine 2216 agar (MA), the pH of which had been adjusted to pH 5.5–11 using HCl and KOH. Detection of poly-β-hydroxyalkanoate (PHA) was done using Sudan black (de Lima *et al.*, 1999). Susceptibility to antibiotics was determined on MA plates in the presence of the following antibiotics at the concentrations given (μg ml⁻¹): ampicillin (50, 100, 150 and 200), chloramphenicol (20), gentamicin (50), kanamycin sulfate (20), nalidixic acid (20, 50, 100 and 200), neomycin (50), streptomycin (20), tetracycline (10) and spectinomycin (7.5, 15 and 20). API ZYM, API 20NE (both from bioMérieux) and Microlog GN2 plates (Biolog) were used for physiological and biochemical characterization according to the manufacturers' instructions.

Cells of strain A34^T were Gram-negative, non-motile, straight rods (1.7–2.8 μm long and 0.2–0.5 μm wide) that lacked flagella. The strain formed pink colonies on MA plates and was capable of growth on decane as a sole source of carbon and energy. It was positive in oxidase, catalase and nitrate reduction (nitrite was the final product) tests. Cells contained PHA. Growth of strain A34^T was observed between 10 and 50 °C, with optimum growth at 43 °C. Strain A34^T grew at pH 6.5–9.5, with optimum growth at

Abbreviation: PHA, poly-β-hydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A34^T is AB302386.

pH 8.5. It showed an absolute requirement for NaCl, as no growth was observed in medium without NaCl. Strain A34^T grew at 1–13 % NaCl, with optimum growth at 2 %. The physiological and biochemical characteristics of strain A34^T are given in the species description. Strain A34^T was susceptible to ampicillin, chloramphenicol, gentamicin, kanamycin, neomycin, spectinomycin, streptomycin and tetracycline, but resistant to nalidixic acid.

Cellular fatty acids and quinones were analysed by TechnoSuruga Laboratory Co., Ltd, using cells grown in MB for 24 h. The fatty acids (≥ 0.1 %) of strain A34^T were C_{18:1} ω 7c (56.23 %), C_{19:0} cyclo ω 8c (25.95 %), C_{16:0} (9.05 %), C_{13:0} 3-OH (2.33 %), C_{18:0} (1.58 %), 11-methyl C_{18:1} ω 7c (0.80 %), C_{16:1} ω 7c and/or C_{15:0} iso 2-OH (0.71 %), C_{20:2} ω 6,9c (0.70 %), C_{17:0} (0.54 %), C_{10:0} (0.51 %), C_{20:1} ω 7c (0.50 %) and C_{17:0} cyclo (0.40 %) (Table 1). The fatty acid profile did not match any species profile available in the Sherlock Microbial Identification System database (MIDI, Inc.). The presence of C_{18:1} ω 7c as the predominant fatty acid is a feature characteristic of taxa within the class *Alphaproteobacteria*. Nevertheless, the cyclo-substituted fatty acid C_{19:0} cyclo ω 8c is not widely present in the family '*Rhodobacteraceae*'. The only respiratory lipoquinone found in strain A34^T was ubiquinone-10. The presence of ubiquinone-10 as the dominant respiratory lipoquinone is characteristic of members of the class *Alphaproteobacteria*.

The 16S rRNA gene sequence of strain A34^T (1364 bp) was determined previously (Harwati *et al.*, 2007). A sequence similarity search was conducted using the GenBank and RDP (Maidak *et al.*, 1999) databases. Phylogenetic analysis was performed using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997), and a phylogenetic tree was constructed using the neighbour-joining plot program in MEGA version 3.0 (Kumar *et al.*, 2004). Phylogenetic analysis of the 16S rRNA

gene sequence showed that strain A34^T was affiliated with the '*Rhodobacteraceae*' (Fig. 1). The closest relative of strain A34^T was *Salipiger mucosus* A3^T (94.9 % 16S rRNA gene sequence identity), while *Palleronia marisminoris* B33^T (93.4 %) was the second closest relative. The G + C content of strain A34^T as determined by the method of Katayama-Fujimura *et al.* (1984) was 69.1 mol%.

Table 2 shows several phenotypic traits of strain A34^T that can differentiate it from phylogenetically related bacteria in the '*Rhodobacteraceae*'. Based on phylogeny, fatty acid profile, G + C content and phenotypic characteristics (e.g. nitrate reduction), we conclude that strain A34^T should be recognized as a representative of a novel genus and species, for which the name *Tranquillimonas alkanivorans* gen. nov., sp. nov. is proposed.

Description of *Tranquillimonas* gen. nov.

Tranquillimonas (Tran.quil'li.mo'nas. L. adj. *tranquillus* quiet, calm, still; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Tranquillimonas* a still monad).

Cells are Gram-negative, non-motile rods (1.7–2.8 μ m long and 0.2–0.5 μ m wide). Obligately halophilic: Na⁺ ions are required for growth. Form pink colonies when grown on MA plates. Tests for oxidase and catalase are positive. Contain PHA. The major ubiquinone is Q-10. Dominant fatty acids are C_{18:1} ω 7c, C_{19:0} cyclo ω 8c and C_{16:0}. The type species is *Tranquillimonas alkanivorans*.

Description of *Tranquillimonas alkanivorans* sp. nov.

Tranquillimonas alkanivorans (al.ka'ni.vo'rans. N.L. neut. n. *alkanum* alkane; L. part. adj. *vorans* devouring; N.L. part. adj. *alkanivorans* alkane-degrading).

The description is identical to that of the genus, with the following additions. Growth occurs at 10–50 °C (optimum 43 °C), pH 6.5–9.5 (optimum pH 8.5) and 1–13 % (w/v) NaCl (optimum 2 %). Susceptible to (μ g per disc) ampicillin (50), chloramphenicol (20), gentamicin (50), kanamycin (20), neomycin (50), spectinomycin (7.5), streptomycin (20) and tetracycline (10), but resistant to nalidixic acid (200). Tests for nitrate reduction, β -glucosidase, protease, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase, β -glucosidase and ONPG are positive. Tests for indole production, glucose fermentation, urease, arginase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are negative. The following compounds are utilized as carbon sources in the Biolog GN2 plate: Tweens 40 and 80, L-arabinose, D-fructose, L-fructose, D-galactose, gentiobiose, α -D-glucose, *myo*-inositol, α -D-lactose, lactulose, maltose, D-mannitol, methyl β -D-glucose, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose,

Table 1. Cellular fatty acid compositions of strain A34^T and related members of the family '*Rhodobacteraceae*'

Strains: 1, strain A34^T; 2, *Salipiger mucosus* A3^T (data from Martínez-Cánovas *et al.*, 2004); 3, *Palleronia marisminoris* B33^T (Martínez-Checa *et al.*, 2005). Values are percentages of total fatty acids; fatty acids that accounted for less than 1 % of the total in all strains are not shown. —, Not detected/not reported.

Fatty acid	1	2	3
C _{16:0}	9.1	12.4	4.3
C _{18:0}	1.6	2.0	3.4
C _{16:1} ω 7c	—	1.3	—
C _{18:1} ω 7c	56.2	78.0	68.9
C _{10:0} 3-OH	—	—	5.0
C _{12:0} 3-OH	2.3	—	—
C _{12:1} 3-OH	—	2.3	—
11-Methyl C _{18:1} ω 7c	—	1.9	—
C _{19:0} cyclo ω 8c	26.0	12.8	2.3

Fig. 1. Phylogenetic relationships between strain A34^T and members of other genera in the family 'Rhodobacteraceae' based on 16S rRNA gene sequences. The tree was constructed using the neighbour-joining algorithm. Only bootstrap values above 50 (from 100 replications) are shown. Bar, 1% estimated sequence divergence.

turanose, xylitol, pyruvic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, α -ketoglutaric acid, D- and L-lactic acid, succinamic acid, glucuronamide, aspartic acid, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine,

L-proline, L-pyroglyutamic acid, D- and L-serine and L-threonine. Degrades alkanes (C_{10–13}). Dominant fatty acids (%) are C_{18:1} ω 7c (56.2), C_{19:0} cyclo ω 8c (26.0), C_{16:0} (9.1) and C_{13:0} 3-OH (2.3). The DNA G + C content of the type strain is 69.1 mol%.

The type strain, A34^T (=JCM 14836^T =DSM 19547^T), was isolated from seawater in Semarang Port, Indonesia.

Table 2. Characteristics that distinguish strain A34^T from related members of the family 'Rhodobacteraceae'

Strains: 1, strain A34^T; 2, *Salipiger mucosus* A3^T (data from Martínez-Cánovas *et al.*, 2004); 3, *Palleronia marisminoris* B33^T (Martínez-Checa *et al.*, 2005); 4, *Roseivivax halotolerans* JCM 10271^T (Suzuki *et al.*, 1999; Nishimura *et al.*, 1994). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4
Source	Seawater	Hypersaline soil	Hypersaline soil	Cyanobacterial mat in a saline lake
Pigmentation	Pink	–	Pink	Pink
Flagella	–	–	–	Single, subpolar
PHA	+	+	+	ND
Oxidase	+	+	–	+
Temperature range (°C)	10–50	20–40	20–37	27–30*
NaCl concentration for growth (% w/v)				
Range	1–13	0.5–20	0.5–15	0.5–20
Optimum	2	9–10	5	ND
Nitrate to nitrite	+	–	–	–
Acid from glucose	–	–	–	+
Indole	–	–	–	+
ONPG	+	–	+	+
Urease	–	+	–	+
Phosphatase	+	+	+	–
DNA G + C content (mol%)	69.1	64.5	64.2	59.7

*Only the optimum temperature has been published.

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