Echinicola vietnamensis sp. nov., a member of the phylum *Bacteroidetes* isolated from seawater

Olga I. Nedashkovskaya,[1] Seung Bum Kim,[2] Bart Hoste,[3] Dong Sung Shin,[2] Irina A. Beleneva,[4] Marc Vancanneyt[3] and Valery V. Mikhailov[1]

Correspondence
Olga I. Nedashkovskaya
olganedashkovska@piboc.dvo.ru
or
olganedashkovska@yahoo.com

[1]Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Pr. 100 Let Vladivostoku 159, 690022, Vladivostok, Russia

[2]Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yusong, Daejon 305-764, Republic of Korea

[3]BCCM/LMG Bacteria Collection, and Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

[4]Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, Pal'chevskogo St. 17, 690032, Vladivostok, Russia

The taxonomic position of a novel marine, heterotrophic, gliding, halotolerant and light-pink-pigmented bacterium, designated strain KMM 6221^T, was examined by using a polyphasic approach. 16S rRNA gene sequence analysis indicated that strain KMM 6221^T is affiliated with the genus *Echinicola*, a member of the phylum *Bacteroidetes*, with levels of similarity of 94.7–95.0 % to strains of *Echinicola pacifica*. Growth of strain KMM 6221^T was observed with 0–15 % NaCl and at 6–44 °C. The DNA G+C content of strain KMM 6221^T was 45.9 mol%. On the basis of molecular distinctiveness supported by phenotypic and chemotaxonomic data, strain KMM 6221^T is considered to represent a novel species of the genus *Echinicola*, for which the name *Echinicola vietnamensis* sp. nov. is proposed. The type strain is KMM 6221^T (=DSM 17526^T=LMG 23754^T).

The genus *Echinicola* was proposed to accommodate heterotrophic, Gram-negative, gliding and pigmented bacteria with menaquinone-7 as the major respiratory quinone belonging to the phylum *Bacteroidetes* (Nedashkovskaya *et al.*, 2006). Strains of the only species of the genus recognized so far, *Echinicola pacifica*, were isolated from the sea urchin *Strongylocentrotus intermedius*, and differed from those of their closest relatives *Algoriphagus*, *Hongiella*, *Aquiflexum* and *Belliella* by the ability to ferment D-glucose. In this study we report the isolation and identification of a novel marine bacterium that was affiliated with the genus *Echinicola* on the basis of phylogenetic, phenotypic and chemotaxonomic characteristics.

Strain KMM 6221^T was isolated by direct plating on a medium containing 0.5 % (w/v) Bacto peptone (Difco), 0.2 % (w/v) casein hydrolysate (Merck), 0.2 % (w/v) Bacto yeast extract (Difco), 0.1 % (w/v) glucose, 0.02 % (w/v) KH_2PO_4, 0.005 % (w/v) $MgSO_4$ and 1.5 % (w/v) Bacto agar (Difco) in 50 % (v/v) natural seawater and 50 % (v/v) distilled water, from seawater collected in a mussel farm located in a lagoon of Nha Trang Bay, South China Sea, Vietnam, in January 2005. After primary isolation and purification on marine agar 2216 (Difco), strains were cultivated on the same medium at 25 °C for 48 h and stored at −80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

DNA extraction, PCR and 16S rRNA gene sequencing were carried out as described by Vancanneyt *et al.* (2006). The amplification primers used were MH1 (5′-AGTTTGATCCTGGCTCAG-3′) and MH2 (5′-TACCTTGTTACGACTTCACCCCA-3′), respectively hybridizing at positions 10–27 and 1507–1485 according to the *Escherichia coli* numbering system. Sequence data obtained were aligned with those of representative members of the phylum *Bacteroidetes* by using PHYDIT version 3.2 (http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred by using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated according to the Kimura two-parameter model (Kimura, 1980), and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled data sets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequences revealed that strain KMM 6221^T occupied a distinct lineage within the genus *Echinicola* and possessed

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of KMM 6221^T is AM406795.

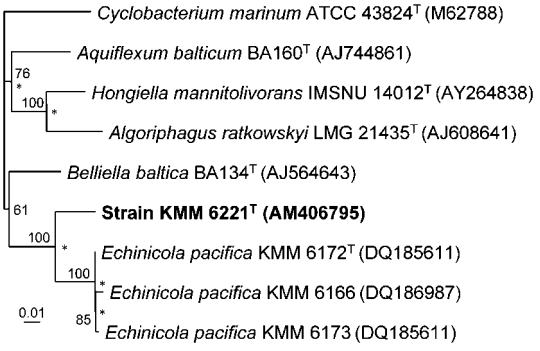

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences of strain KMM 6221^T and members of related genera of the phylum *Bacteroidetes*. The topology of the tree was not changed in trees generated with the least-squares or maximum-likelihood algorithms (not shown; asterisks indicate branches that were also recovered by using the least-squares and maximum-likelihood algorithms). Numbers at nodes indicate bootstrap percentages from 1000 resampled datasets. Bar, 0.01 substitutions per nucleotide position.

sequence similarities of 94.7–95.0 % with strains of *Echinicola pacifica* (Fig. 1).

DNA was isolated according to the method of Marmur (1961) and the G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The G+C content of the DNA of strain KMM 6221^T was 45.9 mol%.

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (Microbial ID). The predominant cellular fatty acids of strain KMM 6221^T were iso-$C_{15:0}$, $C_{16:1}\omega5c$, iso-$C_{17:1}\omega9c$, $C_{17:1}\omega6c$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and summed feature 4 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B). *Echinicola pacifica* strains have a similar fatty acid composition (Nedashkovskaya *et al.*, 2006), including the presence of summed feature 4.

Physiological and biochemical properties of strain KMM 6221^T were examined as described by Nedashkovskaya *et al.* (2004, 2006).

The new strain displayed many features similar to those of *Echinicola pacifica* (Table 1). It moved by means of gliding, was positive for oxidase, catalase and alkaline phosphatase activities, and hydrolysed starch, as found previously for strains of *Echinicola pacifica*. However, KMM 6221^T differed in that it could not ferment D-glucose, produce hydrogen sulfide or hydrolyse agar, gelatin or Tween 40. Production of acid from several carbohydrates and susceptibility to antibiotics could also be used to differentiate between strain KMM 6221^T and *Echinicola pacifica* (Table 1). KMM 6221^T also differs from its closest relatives by the ability to grow at up to 44 °C and with 15 % NaCl.

Consequently, in spite of significant differences in 16S rRNA gene sequence similarity between KMM 6221^T and strains of *Echinicola pacifica* (94.7–95 %), similarities in DNA G+C contents, fatty acid compositions and several phenotypic traits support the affiliation of the new strain with the genus *Echinicola* and its description as representing a novel species

Table 1. Differential phenotypic characteristics between strain KMM 6221^T and *Echinicola pacifica*

All strains were positive for the following tests: gliding motility; oxidase, catalase, β-galactosidase and alkaline phosphatase activities; hydrolysis of starch; utilization of L-arabinose, D-glucose, D-lactose, D-mannose and sucrose; susceptibility to lincomycin and resistance to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B, streptomycin and tetracycline. All strains were negative for the following tests: nitrate reduction; hydrolysis of casein, cellulose (carboxymethylcellulose and filter paper), chitin and urea; acid production from D-melibiose, L-raffinose, L-sorbose, glycerol, adonitol, dulcitol, inositol and mannitol; and utilization of inositol, mannitol and sorbitol. Data for *E. pacifica* were taken from Nedashkovskaya *et al.* (2006).

Characteristic	KMM 6221^T	*E. pacifica* ($n=3$)
Fermentation of D-glucose	−	+
Production of H_2S	−	+
Growth with 15 % NaCl	+	−
Growth at 44 °C	+	−
Hydrolysis of agar, gelatin and Tween 40	−	+
Acid production from L-arabinose, D-cellobiose, D-glucose, D-lactose, D-maltose, D-mannose, L-rhamnose and DL-xylose	−	+
Susceptibility to carbenicillin, chloramphenicol, doxycycline, erythromycin and oleandomycin	+	−
DNA G+C content (mol%)	45.9	44–45

of this genus, for which the name *Echinicola vietnamensis* sp. nov. is proposed.

Description of *Echinicola vietnamensis* sp. nov.

Echinicola vietnamensis (vi.et.nam.en′sis. N.L. fem. adj. *vietnamensis* referring to Vietnam, the country of origin of the type strain).

Has the following characteristics in addition to those given for the genus. Cells are 0.4–0.5 × 1.1–2.3 μm. On marine agar, colonies are circular, 2–3 mm in diameter, convex, shiny, smooth and light pink. β-Galactosidase-positive. Does not require Na^+ ions or seawater for growth. Growth occurs at 6–44 °C. Optimal temperature for growth is 30–32 °C. Growth occurs with 0–15 % NaCl. No flexirubin-type pigments are formed. Degrades starch, but not agar, casein, gelatin, Tweens 20, 40 or 80, urea, cellulose (carboxymethylcellulose and filter paper) or chitin. Does not produce acid from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, L-sorbose, sucrose, DL-xylose, *N*-acetylglucosamine, glycerol, adonitol, dulcitol, inositol or mannitol. Does not ferment D-glucose. Nitrate is not reduced to nitrite. Indole and hydrogen sulfide are not produced. Susceptible to carbenicillin, chloramphenicol, doxycycline, erythromycin, lincomycin and oleandomycin. Resistant to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B, streptomycin and tetracycline. Fatty acids accounting for ⩾1 % of the total are anteiso-$C_{15:0}$ (1.4 %), iso-$C_{15:0}$ (20.0 %), $C_{15:1}\omega6c$ (1.2 %), $C_{15:0}$ (1.5 %), $C_{16:1}\omega5c$ (4.9 %), iso-$C_{17:1}\omega9c$ (4.4 %), iso-$C_{17:0}$ (1.0 %), $C_{17:1}\omega6c$ (4.5 %), iso-$C_{15:0}$ 3-OH (3.7 %), $C_{16:0}$ 3-OH (2.3 %), iso-$C_{17:0}$ 3-OH (10.0 %), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso $C_{15:0}$ 2-OH; 34.5 %), and summed feature 4 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{7:1}$ B; 5 %). The G + C content of the DNA is 45.9 mol%.

The type strain, KMM 6221^T (=DSM 17526^T=LMG 23754^T), was isolated from seawater collected in a mussel farm located in a lagoon of Nha Trang Bay, South China Sea, Vietnam.

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