Antonie van Leeuwenhoek (2013) 103:69–77
DOI 10.1007/s10482-012-9787-y

ORIGINAL PAPER

Echinimonas agarilytica gen. nov., sp. nov., a new gammaproteobacterium isolated from the sea urchin *Strongylocentrotus intermedius*

Olga I. Nedashkovskaya · Anna M. Stenkova · Natalia V. Zhukova · Stefanie Van Trappen · Jung-Sook Lee · Seung Bum Kim

Received: 31 May 2012 / Accepted: 27 July 2012 / Published online: 4 September 2012
© Springer Science+Business Media B.V. 2012

Abstract A novel Gram-negative, facultatively anaerobic and motile bacterial strain, designated KMM 6351^T, was isolated from the sea urchin *Strongylocentrotus intermedius* and examined using a polyphasic taxonomic approach. A phylogenetic analysis based on 16S rRNA gene sequencing revealed that the strain formed a distinct phyletic line in the class *Gammaproteobacteria* and was most closely related to the genera *Aliivibrio*, *Photobacterium* and *Vibrio*. Strain KMM 6351^T grows at 4–40 °C and with 0.5–12 % NaCl and decomposes aesculin, agar, gelatin, starch, chitin and DNA. The DNA G+C content of the strain was determined to be 46.1 mol%. The prevalent fatty acids were found to be $C_{16:0}$, $C_{18:1}$ *ω7c*, $C_{12:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH fatty acids). The major polar lipids were determined to be diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid. The predominant ubiquinone was found to be Q-8. The results of the phenotypic, chemotaxonomic and genotypic analyses clearly indicated that the novel strain should be assigned to a new genus and species within the class *γ-Proteobacteria* for which the name *Echinimonas agarilytica* gen. nov., sp. nov. is proposed. The type strain is KMM 6351^T (=KCTC 22996^T = LMG 25420^T).

Keywords *Echinimonas agarilytica* gen. nov.sp. nov. · *γ-Proteobacteria* · Marine bacteria · Phylogeny · Sea urchin *Strongylocentrotus intermedius*

O. I. Nedashkovskaya (✉) · A. M. Stenkova
G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Prospekt 100 Let Vladivostoku 159, 690022 Vladivostok, Russia
e-mail: olganedashkovska@piboc.dvo.ru; olganedashkovska@yahoo.com

N. V. Zhukova
A.V. Zhirmunsky Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, Pal'chevskogo St. 17, 690032 Vladivostok, Russia

N. V. Zhukova
Far Easten Federal University, Sukhanova St. 8, 690950 Vladivostok, Russia

S. Van Trappen
BCCM/LMG Bacteria Collection, and Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium

J.-S. Lee
Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-dong, Yuseong-gu, Daejeon 305-806, Republic of Korea

S. B. Kim
Department of Microbiology and Molecular Biology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

Introduction

The microbial communities associated with sea urchins are characterized by high phylogenetic and functional diversity but they have been very poorly studied until recently. In the course of an exploration of bacteria isolated from the digestive tract of the sea urchin *Paracentrotus lividus* collected from the Aegean Sea using the 16S rRNA gene sequence technique, Meziti et al. (2007) found phylotypes affiliated with the phyla *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Proteobacteria* and *Verrucomicrobia*. Most of the isolates were facultatively anaerobic organisms and belonged to the γ- and δ-classes of the phylum *Proteobacteria*. Phylotypes P151 and P152 were closely related to members of the genus *Vibrio* and phylotype P159 had 97 % 16S rRNA gene sequence similarity to *Photobacterium lipolyticum* isolated from the Yellow Sea (Yoon et al. 2005). During a survey of the taxonomic composition of the bacterial community of the edible sea urchin *Strongylocentrotus intermedius*, a common inhabitant of the Sea of Japan, we isolated several novel *Bacteroidetes* that had yet to be assigned to known taxa. Using 16S rRNA gene sequencing allowed us to identify the isolates as members of the novel genera *Echinicola*, *Gramella* and *Mariniflexile* and a new species of the genus *Roseivirga* (Nedashkovskaya et al. 2005a, b; 2006a, b). Later, representatives of the phyla *Firmicutes* and *Proteobacteria*, *Bacillus berkeleyi* and '*Altererythrobacter troitsensis'*, that were associated with this animal were also proposed (Nedashkovskaya et al. 2012a, b).

In the present work, we report the phenotypic, genotypic and phylogenetic characterization of a Gram-negative, facultatively anaerobic, whitish, motile and agarolytic bacterium, designated strain KMM 6351^T, which was recovered from the sea urchin *S. intermedius* during a survey of the biodiversity of microbial communities of marine invertebrates. The detail taxonomic analysis based on a polyphasic approach indicated that this marine isolate represents a novel genus and species within the class γ-*Proteobacteria* for which the name *Echinimonas agarilytica* gen. nov., sp. nov. is proposed here.

Materials and methods

Isolation and cultivation

Strain KMM 6351^T was isolated from a sea urchin *S. intermedius* collected in September 2002 at the G.B. Elyakov Pacific Institute of Bioorganic Chemistry Marine Experimental Station, Troitza Bay, Gulf of Peter the Great, Sea of Japan (also known as the East Sea) by a standard dilution plating method. The sample of tissues (5 g) was homogenized in 10 ml sterile seawater in a glass homogenizer and 0.1 ml homogenate was spread onto marine agar 2216 (MA, Difco) plates. The novel isolate was obtained from a single colony after incubation of the plate at 28 °C for 7 days. After primary isolation and purification, the strain was cultivated at 28 °C on the same medium and stored at −80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

16S rRNA gene sequencing and phylogenetic analysis

Bacterial DNA was isolated using a Genomic DNA Purification Kit (Fermentas, EU). The DNA concentration was determined by agarose gel electrophoresis. PCR amplification of the 16S rRNA gene was performed using the universal primers BF-20 (5′-ATC ACGCGTAAAAATCT-3′) and BR2-22 (5′-CCGCA ATATCATTGGTGGT-3′). The expected amplicon size was about 1,500 bp. PCR was done with *GoTaq* DNA polymerase (Promega) in 50 µl at 95 °C for 5 min followed by 25 rounds of thermal cycling (94 °C for 20 s, 55 °C for 20 s and 72 °C for 40 s) in a GeneAmp® PCR System 2700 thermocycler (Applied Biosystems). PCR products were evaluated on a 1.5 % agarose gel stained with ethidium bromide. Unincorporated primers and dNTPs were removed from PCR products with a Silica Bead DNA Gel Extraction Kit (Fermentas, EU). Purified DNA was sequenced on an ABI 3130XL automated sequencer (Applied Biosystems) using the Big Dye v.3.1 sequencing kit. Nucleotide sequence data from forward- and reverse-strand chromatograms were assembled into single contiguous sequences using the Vector NTI Advance 9.1.0 software. The obtained sequences were aligned with those of representative members of selected genera of the class γ-*Proteobacteria* 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 from the Jukes–Cantor model (Jukes and Cantor 1969) and the trees were constructed on the basis of the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood

(Felsenstein 1993) algorithms. A bootstrap analysis was performed with 1,000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package. Pairwise sequence similarities were calculated using EzTaxon-e server (Kim et al. 2012).

Mol% G+C determination

Genomic DNA was prepared from cells cultivated on MA for 24–48 h at 25 °C and extracted by following the DNA-extraction protocol of Pitcher et al. (1989), as modified by Leisner et al. (2002). The DNA G+C content was determined using the HPLC method of Mesbah et al. (1989).

Whole cell polar lipid, fatty acid and lipoquinone composition

For determination of whole-cell fatty acid and polar lipid profiles, strain KMM 6351^T, *Aliivibrio fisheri* LMG 4414^T and *Vibrio proteolyticus* CCUG 20302^T were grown for 24 h on MA. Cellular fatty acid methyl esters were prepared according to the standard protocol of the Microbial Identification System (MIDI, version 3.5; Sasser 1990) and analysed using a GC-17A chromatograph (Shimadzu, Japan) equipped with a fused silica capillary column (30 m × 0.25 mm) coated with Supercowax-10 and SPB-5 phases (Supelco, USA) at 210 °C. FAMEs were identified using equivalent chain length measurements and by comparing of retention times those of authentic standards. FAMEs were also analysed by GC–MS (Shimadzu QP5050A) equipped with an MDN-5S capillary column (30 m × 0.25 mm) at temperature of the injector and detector of 250 °C. Polar lipids were determined by TLC as described by Minnikin et al. (1977).

The isoprenoid quinone composition of strain KMM 6351^T was characterized by HPLC (Shimadzu LC-10A) using a reversed-phase type Supelcosil LC-18 column (15 cm × 4.6 mm) and acetonitrile/2-propanol (65:35, v/v) as a mobile phase at a flow rate of 0.5 ml min^{-1}. The column was kept at 40 °C. Menaquinones were detected by monitoring at 270 nm.

Morphological, physiological and biochemical tests

Cell morphology was examined by light microscopy (Olympus CX41) and transmission electron microscopy (Libra 120; Carl Zeiss) using cells growing for 24, 48, 72 and 96 h on MA at 28 °C. Gram-staining was done as described by Gerhardt et al. (1994). Oxidative or fermentative utilization of glucose was determined on Hugh and Leifson's medium modified for marine bacteria (Lemos et al. 1985). Catalase activity was tested by addition of 3 % (v/v) H_2O_2 solution to a bacterial colony and observation for the appearance of gas. Oxidase activity was determined by assessing the oxidation of tetramethyl-*p*-phenylenediamine. Degradation of agar, starch, casein, gelatin, chitin, DNA and urea, growth at different pH and production of acid from carbohydrates, hydrolysis of Tweens 20, 40 and 80, nitrate reduction, production of hydrogen sulphide, acetoin (Voges-Proskauer reaction) and indole and presence of β-galactosidase activity were tested according to standard methods (Gerhardt et al. 1994). The temperature range for growth was assessed in MA. Tolerance to NaCl was assessed in medium A containing 2 g Tryptic Soy Broth (Sigma), 1.5 g Bacto Peptone (Difco), 1.5 g Meat Peptone (Difco), 1 g Bacto Yeast Extract (Difco), 5 g $MgSO_4 \cdot 7H_2O$, 1.3 g $CaCl_2 \cdot 2H_2O$, 0.6 g KCl and 0.1 g ferric ammonium citrate prepared with distilled water with 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 15 % (w/v) of NaCl. Physiological and biochemical properties of strain KMM 6351^T were also tested using API 20E, API 20 NE, API 50 CH and API ZYM galleries (bioMérieux) according to the manufacturer's instructions, except that the temperature of incubation was 30 °C. Carbon source utilization was tested (i) using commercial API 20E, API 20 NE, API 32 GN and API 50CH (bioMérieux) identification strips following the instructions of the manufacturer, and (ii) using a medium that contained 0.2 g $NaNO_3$, 0.2 g NH_4Cl, 0.05 g Yeast Extract (Difco) and 0.4 % (w/v) carbon source per liter of artificial seawater as described by Suzuki et al. (2001). Susceptibility to antibiotics was examined by the routine diffusion plate method. Discs were impregnated with the following antibiotics: ampicillin (10 µg), benzylpenicillin (10 U), carbenicillin (100 µg), cefalexin (30 µg), cefazolin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), doxycycline (10 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), nalidixic acid (30 µg), neomycin (30 µg), ofloxacin (5 µg), oxacillin (10 µg), polymyxin B (300 U), rifampicin (5 µg), streptomycin (30 µg), tetracycline (5 µg) and vancomycin (30 µg).

Results and discussion

Molecular phylogenetic analysis

An almost-complete 16S rRNA gene sequence of strain KMM 6351^T was determined (GenBank/EMBL/DDBJ accession number JX072970). Initially, the sequence was compared with those available in GenBank. Phylogenetic analysis revealed that the novel isolate belonged to the phylum *Proteobacteria* and formed a novel and robust evolutionary lineage adjacent to the family *Vibrionaceae* within the class *γ-Proteobacteria* (Fig. 1). The nearest neighbours of the strain were marine bacteria r61 (accession number AB470941) and j221 (accession number EU513001), isolated from the coral *Montipora* and seawater collected from an aquaculture area, respectively, with 96 % 16S rRNA gene sequence identity, according to the BLAST database search. Strain KMM 6351^T was closely related to recognized members of the genera *Aliivibrio* (90.6–91.3 % sequence similarity), *Photobacterium* (90.6–92.3 %) and *Vibrio* (90.6–91.9 %). The values of 16S rRNA gene sequence similarities between the sea urchin isolate and other validly named representatives of the class *γ-Proteobacteria* were <90 %. There is thus sufficient molecular distinctiveness to support a suggestion to classify strain KMM 6351^T in a new genus and species.

Mol% G+C determination

The DNA G+C content of strain KMM 6351^T was determined to be 46.1 mol%.

Chemotaxonomic characterization

The prevalent fatty acids of strain KMM 6351^T were found to be $C_{16:0}$ (26.5 %), $C_{18:1}$ *ω7c* (18.2 %), $C_{12:0}$ 3-OH (6.7 %) and summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH fatty acids as defined by the MIDI system; 39.2 %). The presence of $C_{16:0}$, $C_{18:1}$ *ω7c* and $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH fatty acids is characteristic for all strains included in this study (Table 1) but strain KMM 6351^T differed from *Photobacterium phosphoreum* LMG 4233^T by the presence of fatty acid $C_{14:1}$ *ω5c* and the absence of $C_{15:0}$ and $C_{18:0}$ fatty acids; the $C_{14:1}$ *ω5c* fatty acid found in the novel isolate was not present in *Vibrio proteolyticus* CCUG 20302^T. Also, there were significant differences in the proportions of some fatty acids between strain KMM 6351^T and its closest phylogenetic neighbours (Table 1). The distinctiveness in the fatty acid composition of strain KMM 6351^T confirmed that it could be considered as the representive of a novel genus and species of the class *γ-Proteobacteria.* The polar lipid profile of the novel strain was found to be composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid which in line with the reference strains (Fig. 2). The sole respiratory quinone of the isolate was determined to be ubiquinone 8, which is in agreement with the ubiquinone patterns reported for the type species of the genus *Photobacterium* (Kim et al. 2010).

Morphological, physiological and biochemical characteristics

The physiological and biochemical characteristics of strain KMM 6351^T are given in the generic and species descriptions and Table 2. The isolate was observed to be a rod-shaped, motile, facultatively anaerobic and oxidase-positive organism. The novel strain can be clearly distinguished from its closest relatives by the ability to grow at up to 12 % NaCl, to hydrolyse agar, to produce acid from L-arabinose, lactose, melibiose, L-rhamnose and xylose and to utilize inositol. It differs from the type strain of *P. phosphoreum* in the absence of *β*-galactosidase and caseinase activities, the hydrolysis of gelatin, Tweens 20, 40 and 80 and DNA and by growth at 40 °C. Other phenotypic characteristics that differentiate the isolate from its nearest neighbours are shown in Table 2.

Polyphasic taxonomic conclusion

Phylogenetic analyses based on the 16S rRNA gene sequences of members of the class *γ-Proteobacteria* indicated that strain KMM 6351^T forms a distinct lineage clearly separated from those of the family *Vibrionaceae* and other related families (Fig. 1). Therefore, it can be proposed that this strain is a family-level taxon in the class *γ-Proteobacteria*. However, a complete polyphasic taxonomic analysis of a single strain is not suitable for the description of a new family. The above-mentioned sufficient molecular distinctiveness taken together with differences in physiological and

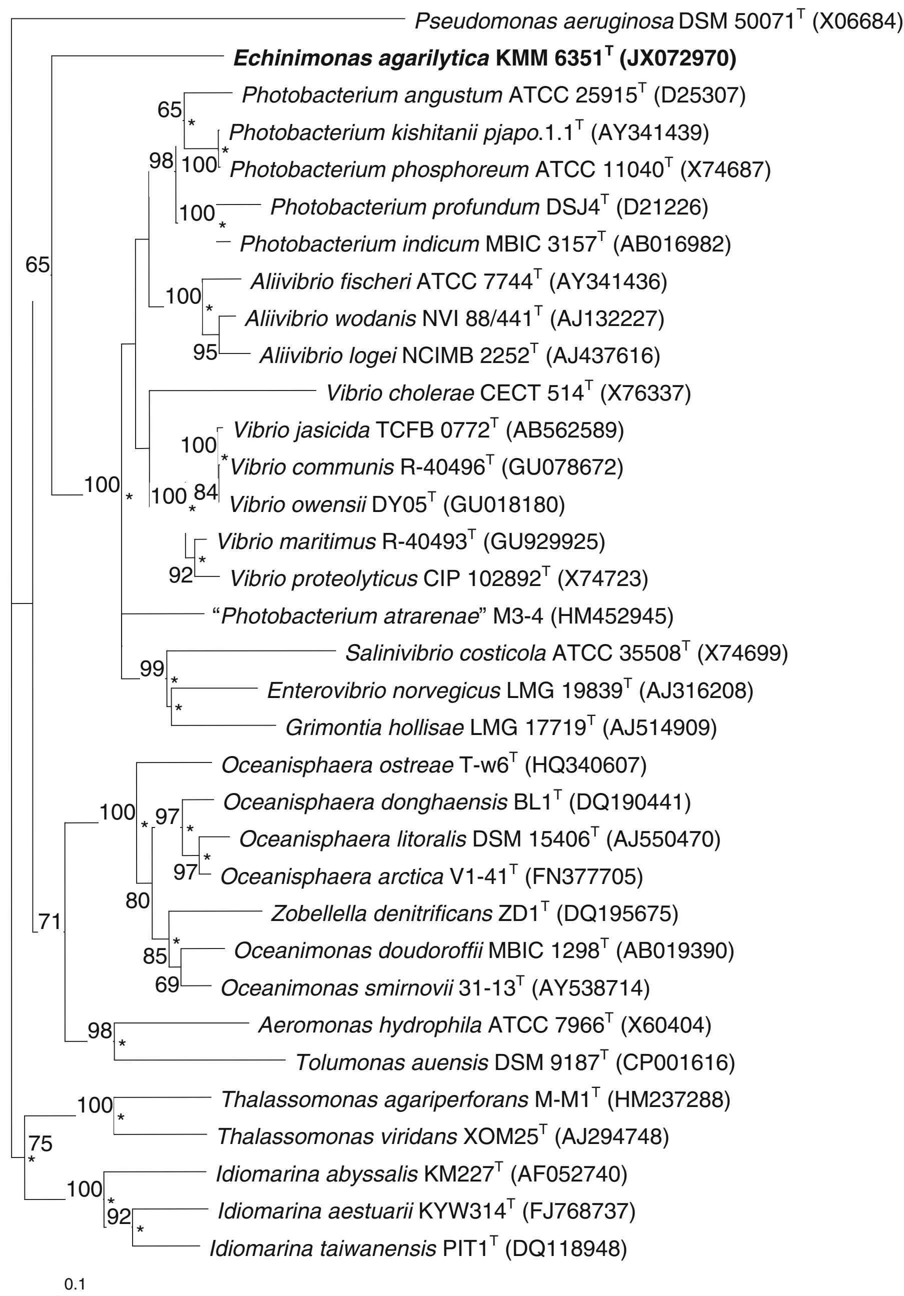

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of *E. agarilytica* gen. nov., sp. nov. KMM 6351^T and related members of the class *γ-Proteobacteria*. Bootstrap values is based on 1,000 replications. *Bar* 0.01 substitutions per nucleotide position

biochemical characteristics and in polar lipid and fatty acid compositions strongly suggest the separate taxonomic status of the novel strain. On the basis of the combined phylogenetic, genotypic, chemotaxonomic and phenotypic data presented here, we suggest that strain KMM 6351^T should be classified as a representative of a novel genus and species within the class *γ-Proteobacteria*.

Table 1 Cellular fatty acid contents of *E. agarilytica* gen. nov., sp. nov. and related taxa of the family *Vibrionaceae*

Fatty acid	1	2	3	4
$C_{12:0}$	tr	2.0	**4.8**	1.2
$C_{14:0}$	2.1	3.8	4.3	4.0
$C_{14:1}$ *ω5c*	3.3	tr	–	–
iso-$C_{15:0}$	tr	–	–	1.0
$C_{15:0}$	–	1.0	2.6	1.7
iso-$C_{16:0}$	–	–	–	2.0
$C_{16:0}$	**26.5**	**21.7**	**17.6**	**30.8**
$C_{16:1}$ *ω5c*	1.2	**6.8**	–	–
iso-$C_{17:0}$	–	–	–	1.5
$C_{17:0}$	tr	tr	–	1.6
$C_{18:0}$	tr	tr	3.0	1.3
$C_{18:1}$ *ω7c*	**18.2**	**14.6**	4.0	**19.1**
$C_{12:0}$-3OH	**6.7**	**4.8**	2.7	3.0
$C_{14:0}$-3OH	1.0	**5.0**	3.1	**5.0**
Summed feature 3	**39.2**	**44.4**	**50.7**	**26.9**

Strains: 1, *Echinimonas agarilytica* gen. nov., sp. nov. KMM 6351^T; 2, *Aliivibrio fisheri* LMG 4414^T; 3. *Photobacterium phosphoreum* LMG 4233^T; 4, *Vibrio proteolyticum* CCUG 20302^T. All data from this study except for 3 (data from Yoshizawa et al. 2009)

Values are percentages of total fatty acids; those fatty acids for which the mean amount in all taxa was <1 % are not given. An amount of the predominant fatty acids are shown by a bold font

Summed feature 3 consists of the following fatty acids which could not be separated by the microbial identification system: one or more of $C_{16:1}$*ω7c* and iso-$C_{15:0}$ 2-OH

– Not detected, *tr* trace amount (<1 %)

Description of *Echinimonas* gen. nov

Echinimonas (E.chi.ni.mo'nas. L. masc. n. *echinus*, sea urchin; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Echinimonas*, monad isolated from a sea urchin).

Cells are Gram-negative, facultatively anaerobic, chemoheterotrophic, non-endospore-forming, rod-shaped and motile. Moderately halophilic. Positive for catalase and oxidase activities. The prevalent fatty acids are $C_{16:0}$, $C_{18:1}$ *ω7c*, $C_{12:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH fatty acids as defined by the MIDI system). The predomonant ubiquinone is Q-8. The polar lipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid. The DNA G+C content of the type strain of the type species is 46.1 mol% (as determined by HPLC). Phylogenetically, the genus belongs to the phylum *Proteobacteria*, the class *γ-Proteobacteria*. The type species is *E. agarilytica*.

Description of *Echinimonas agarilytica* sp. nov

Echinimonas agarilytica (a.ga.ri.ly'ti.ca. N.L. neut. n. *agarum*, agar; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*), able to loosen, able to dissolve; N.L. masc. adj. *agarilytica*, agar-dissolving).

Exhibits the following properties in addition to those given for the genus. Cells are 0.4–0.6 μm in

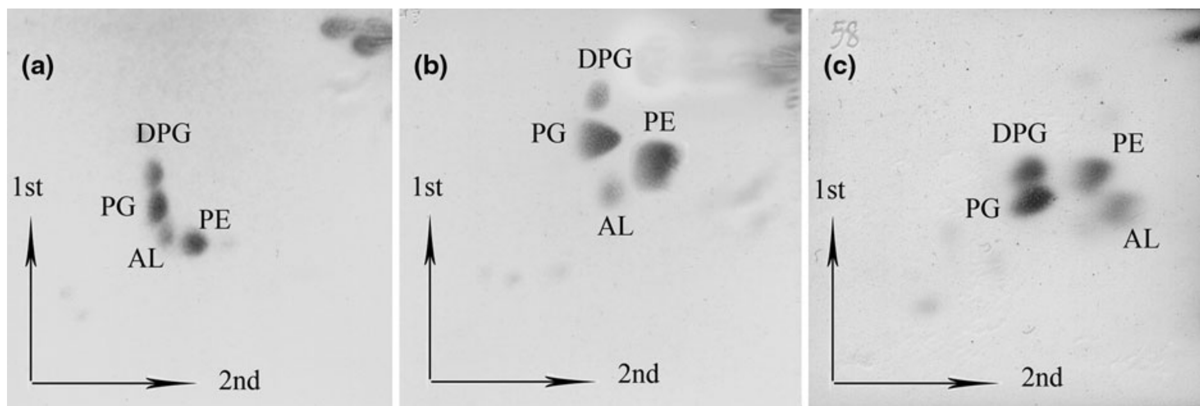

Fig. 2 Thin-layer chromatogram sprayed with molybdatophosphoric acid reagent showing the polar lipid profiles of strain KMM 6351^T (**a**), *Aliivibrio fisheri* LMG 4414^T (**b**), and *Vibrio proteolyticum* CCUG 20302^T (**c**) following separation by two-dimensional TLC. Solvent systems: (I) chloroform–methanol–water (65:25:4, v/v/v.); (II) chloroform–acetic acid–methanol–water (80:15:12:4, v/v/v/v.). For detection of the polar lipids, phosphomolybdic acid (for *PG*, *DPG*, *PE* and *AL*) and ninhydrin (*PE* and *AL*) were applied. *AL* unidentified aminolipid, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *AL* aminolipid

Table 2 Phenotypic characteristics, differentiating strain KMM 6351^T from its closest relatives

Characteristic	1	2	3	4
β-Galactosidase	−	−	+	−
H_2S production	+	+	+	−
Indole production	−	−	−	+
Acetoin production	+	−	+	+
Growth at 40 °C	+	−	−	+
With 12 % NaCl	+	−	−	−
Hydrolysis of				
Agar	+	−	−	−
Casein	−	−	+	+
Gelatin	+	−	−	+
Starch	+	+	+	+
Tween 20	+	+	−	+
Tween 40	+	−	−	+
Tween 80	+	−	−	+
Chitin	+	−	+	−
Urea	−	+	−	−
DNA	+	+	−	+
Acid production from				
Arabinose, lactose, melibiose, rhamnose, xylose	+	−	−	−
Cellobiose	+	+	−	+
Galactose	+	+	+	−
Fructose, glucose, maltose, mannose, *N*-acetylglucosamine	+	+	+	+
Ribose, glycerol	−	+	+	+
Sucrose	−	+	+	−
Mannitol	+	−	−	+
Utilization of				
Arabinose	+	+	−	−
Lactose, sucrose	+	+	+	−
Glucose, mannose, rhamnose	+	+	+	+
Citrate, malonate	−	−	−	−
Inositol	+	−	−	−
Mannitol, sorbitol	+	−	−	+
Enzyme activity				
Naphthol-AS-BI-phosphohydrolase	+	+	+	+
Valine arylamidase	+	−	−	+
Cysteine arylamidase	−	+	−	+
N-acetylglucosaminidase	−	+	+	+
α-Galactosidase	−	+	−	−
Lipase (C14), trypsin, *α*-chymotrypsin	−	−	−	+
Susceptibility to				
Ampicillin	+	−	+	+
Lincomycin	+	+	+	+
Benzylpenicillin	+	−	−	−
Oxacillin	+	−	−	+
Doxycycline, kanamycin, neomycin, streptomycin	−	+	+	+
Nalidixic acid	+	−	+	+
Oleandomycin	−	−	+	−
Tetracycline	−	−	+	+
Cefazolin	+	−	+	−
Vancomycin	+	+	+	−

Strains: 1, *Echinimonas agarilytica* gen. nov., sp. nov. KMM 6351^T; 2, *Aliivibrio fisheri* LMG 4414^T; 3, *Photobacterium phosphoreum* LMG 4233^T; 4, *Vibrio proteolyticus* CCUG 20302^T. All strains were positive for the following tests: motility; flagellation; presence of oxidase, catalase, nitrate reductase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase activities; susceptibility to carbenicillin, cefalexin, chloramphenicol, erythromycin, gentamicin, ofloxacin, polymixin and rifampicin. All strains were negative for the following tests: presence of *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *α*-mannosidase and *α*-fucosidase activities; acid production from raffinose

+ Positive reaction, − negative reaction

diameter and 1.2–2.4 µm in length, motile by means of subpolar flagella. On MA, colonies are 2–3 mm in diameter, circular, with entire edges, shiny, whitish and sunken into the agar. Production of diffusible dark-grey pigment is observed after 72 h of incubation on MA agar at 28 °C. The salinity optimum is 4 % NaCl. Growth occurs at 4–40 °C (optimum, 28–32 °C), at pH 5.5–10.0 (optimum, pH 7.5), and with 0.5–12 % NaCl (optimum, 4 %). Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are absent. D-Glucose is fermented without production of gas. Aesculin, agar, gelatin, starch, Tweens 20, 40 and 80, chitin and DNA are hydrolysed but casein and urea are not. Acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, mannose, melibiose, L-rhamnose, xylose, *N*-acetylglucosamine and mannitol but not from raffinose, ribose, sucrose, glycerol or amygdalin. Sucrose, inositol, mannitol and sorbitol are utilized but adipate, caprate, citrate, gluconate, malate, malonate or phenylacetate are not utilized. In the API 50 CH gallery, only aesculin, salicin and potassium 5-ketogluconate are utilized. None of substrates of the API 32GN gallery are utilized.

In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphtol-AS-BI-phosphohydrolase activities are present; but lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α- glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Nitrate is reduced. Hydrogen sulphide and acetoin are produced but indole is not. The DNA G+C content of the type strain is 46.1 mol%.

The type strain, KMM 6351^T (=KCTC 22996^T = LMG 25420^T), was isolated from the sea urchin *S. intermedius* collected from the Troitsa Bay, Sea of Japan. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain KMM 6351^T is JX072970.

Acknowledgments We thank Dr. S. Van Trappen and C. Vereecke (Ghent, Belgium) for providing us with the type strains *Aliivibrio fisheri* LMG 4414^T and *Photobacterium phosphoreum* LMG 4233^T, Dr. Jung-Sook Lee (Daejeon, Republic of Korea) for the type strain *Oceanisphaera donghaensis* KCTC 12522^T and Dr. E. R. B. Moore (Göteborg, Sweden) for the type strain *Vibrio proteolyticum* CCUG 20302^T. Dr. D. V. Fomin (Cooperative Far Eastern Center of Electron Microscopy, Vladivostok, Russia) is gratefully acknowledged for his excellent technical assistance. This research was supported by grants of the Presidium of the Russian Academy of Sciences “Molecular and Cell Biology”, the Presidium of the Far-Eastern Branch of the Russian Academy of Sciences no. 12-III-A-06-105, the government of Russian Federation for the state support of scientific investigations conducting under the guidance of the leading researchers at the Russian education institutions of the high professional education, agreement no. 11.G34.31.0010 and the Russian Foundation for Basic Research (RFBR) no. 11-04-00781.

References

Felsenstein J (1993) PHYLIP (phylogenetic inference package), version 3.5c. Department of Genetic, University of Washington, Seattle

Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) (1994) Methods for general and molecular bacteriology. American Society for Microbiology, Washington DC

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mamalian protein metabolism, vol 3. Academic Press, New York, pp 21–213

Kim YO, Kim KK, Park S, Kang SJ, Lee JH, Lee SJ, Oh TK, Yoon JH (2010) *Photobacterium gaetbulicola* sp. nov., a lipolytic bacterium isolated from a tidal flat sediment. Int J Syst Evol Microbiol 60:2587–2591

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Leisner JJ, Vancanneyt M, Lefebvre K, Vandemeulebroecke K, Hoste B, Vilalta NE, Rusul G, Swings J (2002) *Lactobacillus durianis* sp. nov., isolated from an acid-fermented condiment (tempoyak) in Malaysia. Int J Syst Evol Microbiol 52:927–931

Lemos ML, Toranzo AE, Barja JL (1985) Modified medium for oxidation-fermentation test in the identification of marine bacteria. Appl Environ Microbiol 40:541–1543

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 38:159–167

Meziti A, Kormas KA, Pancucci-Papadopoulou MA, Thessalou-Legaki M (2007) Bacterial phylotypes assiciated with the digestive tract of the sea urchin *Paracentrotus lividus* and the ascidian *Microcosmus* sp. Russ J Mar Biol 33:84–91

Minnikin DE, Patel PV, Alshamaony L, Goodfellow M (1977) Polar lipid composition in the classification of Nocardia and related bacteria. Int J Syst Bacteriol 27:104–117

Nedashkovskaya OI, Kim SB, Lysenko AM, Frolova GM, Mikhailov VV, Bae KS, Lee DH, Kim IS (2005a) *Gramella echinicola* gen. nov., sp. nov., a novel halophilic bacterium of the family *Flavobacteriaceae* isolated from the sea urchin *Strongylocentrotus intermedius*. Int J Syst Evol Microbiol 55:391–394

Nedashkovskaya OI, Kim SB, Lysenko AM, Park MS, Mikhailov VV, Bae KS, Park H-Y (2005b) *Roseivirga echinicomitans* sp. nov., a novel marine bacterium isolated from the sea urchin *Strongylocentrotus intermedius*, and emended description of the genus *Roseivirga*. Int J Syst Evol Microbiol 55:1797–1800

Nedashkovskaya OI, Kim SB, Vancanneyt M, Lysenko AM, Shin DS, Park MS, Lee KH, Jung WJ, Mikhailov VV, Bae KS, Swings J (2006a) *Echinicola pacifica* gen. nov., sp. nov., a novel flexibacterium isolated from the sea urchin *Strongylocentrotus intermedius*. Int J Syst Evol Microbiol 56:953–958

Nedashkovskaya OI, Kim SB, Mikhailov VV, Bae KS (2006b) *Mariniflexile gromovii* gen. nov., sp. nov., a new gliding bacterium isolated from the sea urchin *Strongylocentrotus intermedius*. Int J Syst Evol Microbiol 56:1635–1638

Nedashkovskaya OI, Van Trappen S, Frolova GM, De Vos P (2012a) *Bacillus berkeleyi* sp. nov., isolated from the sea urchin *Strongylocentrotus intermedius*. Arch Microbiol 194:215–221. doi:10.1007/s00203-011-0771-0pmid:22102083

Nedashkovskaya OI, Cho SH, Joung Y, Joh K, Kim MN, Shin KS, Oh HW, Bae KS, Mikhailov VV, Kim SB (2012b) *Altererythrobacter troitsensis* sp. nov., isolated from the sea urchin *Strongylocentrotus intermedius*. Int J Syst Evol Microbiol. doi:10.1099/ijs.0.038836-0

Pitcher DG, Saunders NA, Owen RJ (1989) Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 8:151–156

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20:16

Suzuki M, Nakagawa Y, Harayama S, Yamamoto S (2001) Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov., and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. Int J Syst Evol Microbiol 51:1639–1652

Yoon JH, Lee JK, Kim YO, Oh TK (2005) *Photobacterium lipolyticum* sp. nov., a bacterium with lipolytic activity isolated from the Yellow Sea in Korea. Int J Syst Evol Microbiol 55:335–339

Yoshizawa S, Wada M, Kita-Tsukamoto K, Yokota A, Kogure K (2009) *Photobacterium aquimaris* sp. nov., a luminous marine bacterium isolated from seawater. Int J Syst Evol Microbiol 59:1438–1447