Vibrio hemicentroti sp. nov., an alginate lyase-producing bacterium, isolated from the gut microflora of sea urchin (Hemicentrotus pulcherrimus)

Duwoon Kim,‡ Keun Sik Baik,‡ Ye Seul Hwang,† Jong-Soon Choi,‡ Joseph Kwon§ and Chi Nam Seong¶

†Department of Food Science and Technology and Functional Food Research Center, Chonnam National University, Gwangju 500-757, Republic of Korea
‡Department of Biological Sciences, Korea Basic Science Institute, Daejeon 305-806, Republic of Korea
§Gwangju Center, Korea Basic Science Institute, Gwangju 500-757, Republic of Korea
¶Department of Biology, Sunchon National University, Suncheon 540-742, Jeonnam, Republic of Korea

An alginate lyase-producing bacterium, designated AlyHP32T, was isolated from the gut of sea urchin (Hemicentrotus pulcherrimus) obtained from the South Sea, Republic of Korea. Cells of strain AlyHP32T were Gram-reaction-negative and motile with a single polar flagellum. The strain grew with 1–6 % (w/v) NaCl (optimum 2–4 %) and at 4–30 °C (optimum 15–25 °C). Phylogenetic analysis based on sequences of the 16S rRNA gene and five housekeeping genes (atpA, pyrH, recA, rpoA and rpoD) revealed that strain AlyHP32T belonged to the genus Vibrio and formed a compact clade with the Vibrio splendidus group. However, DNA–DNA hybridization and fingerprints using the repetitive primers BOX and REP indicated that strain AlyHP32T was distinct from closely related species of the genus Vibrio. The major fatty acids were summed feature 3 (C16:1ω7c and/or C16:1ω6c) and C16:0. The DNA G+C content was 44.1 mol%. The predominant quinone was ubiquinone Q-8. Based on genotypic, phenotypic and DNA–DNA hybridization analysis, strain AlyHP32T represents a novel species of the genus Vibrio; the name Vibrio hemicentroti sp. nov. (type strain AlyHP32T = KCTC 32085T = DSM 26178T) is proposed for this novel taxon.

The genus Vibrio comprises a diverse group of heterotrophic marine bacteria inhabiting aquatic environments, especially marine and estuarine waters, where they are frequently associated with organisms ranging from plankton to fish (Baumann et al., 1984; Farmer et al., 2005; Thompson et al., 2004). At the time of writing, more than 92 species are recognized (http://www.bacterio.net/uw/vibrio.html) and grouped in 14 clades (Sawabe et al., 2007). Among these, the Splendidus clade is the largest group, containing 13 species (Macián et al., 2001; Hedlund & Staley, 2001; Thompson et al., 2003a, b; Faury et al., 2004; Le Roux et al., 2005; Beaz-Hidalgo et al., 2009, 2010; Diéguez et al., 2011), which have been found to be the dominant species of the genus Vibrio in coastal marine sediments, seawater and bivalves in temperate climates (Lambert et al., 1998; Sobecky et al., 1998). Some of these species have been associated with mortality of a wide range of marine animals such as molluscs, crustaceans and fish (Kueh & Chan, 1985; Pujalte et al., 1993; Nicolas et al., 1996; Leaño et al., 1998; Sugumar et al., 1998; Lacoste et al., 2001; Le Roux et al., 2002; Waechter et al., 2002; Farto et al., 2003; Jensen et al., 2003; Gay et al., 2004; Gómez-León et al., 2005). In the course of our study on the microbial degradation of seaweed (Laminaria japonica), a Gram-negative bacterial strain showing alginate lyase activity was isolated from a sea urchin and was the subject of a taxonomic investigation.

Strain AlyHP32T was isolated from the gut microflora of a sea urchin (Hemicentrotus pulcherrimus) collected in the South Sea (34°34’N 127°47’E), Republic of Korea. Isolation was achieved with the standard dilution plating

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA, atpA, gapA, gyrB, recA, rpoA, rpoD and topA gene sequences of strain AlyHP32T are JX204734, JX455825, JX455826, JX455827, JX455828, JX455829, JX455830, JX455831, JX455832 and JX455833, respectively.

Three supplementary figures and two supplementary tables are available with the online version of this paper.
technique using marine agar 2216 (MA; Becton Dickinson) at 25 °C for 7 days. The isolate was routinely cultured on MA and preserved at −80 °C as a suspension in distilled water containing 20% (w/v) glycerol. Reference strains Vibrio artabrorum DSM 26480T, V. atlanticus DSM 26479T, V. celticus DSM 26172T, V. chagassi CECT 17138T, V. crassostreae DSM 17220T, V. cyclitrophicus KCTC 12678T, V. gigantis DSM 18531T, V. kanaloe DSM 17181T, V. lentus KCTC 12743T, V. pomeroyi DSM 17180T, V. splendidus KCTC 12679T and V. tasmaniensis KCTC 12831T were purchased from the culture collections.

DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Chun & Goodfellow (1995). Multilocus sequence analysis (MLSA) using the following housekeeping genes was carried out as described by Le Roux et al. (2005), Sawabe et al. (2007) and Thompson et al. (2005, 2007); atpA (encoding the α-subunit of bacterial ATP synthase), gapA (encoding the glyceraldehyde 3-phosphodehydrogenase, glycolysis), gyrB (encoding the β-subunit of DNA gyrase), mreB (encoding a rod-shaping protein, cell cytoskeleton), pyrH (encoding the uridylate kinase, nucleotide biosynthesis), recA (encoding the DNA recombination protein, DNA repair), rpoA (encoding the σ70 of RNA polymerase), rpoD (encoding the sigma factor (σ70) of RNA polymerase) and topA (encoding the topoisomerase I, DNA replication and repair). Sequence similarities of 16S rRNA genes were determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012), and those of housekeeping genes were determined using the BLASTN program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Concatenated sequences of housekeeping genes (atpA, pyrH, recA, rpoA, rpoD and 16S rRNA) were performed with the program PHYDIT (Chun, 1995). Multiple sequence alignments were performed using CLUSTAL X (Thompson et al., 1997). Gaps in the alignments were completely deleted. Phylogenetic analyses were performed by using PAUP* 4.0 (Swofford, 1998). Phylogenetic trees were inferred using neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. Distance matrices for the neighbour-joining method were generated according to the model of Jukes & Cantor (1969). The robustness of the topology in the neighbour-joining phylogenetic tree was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

16S rRNA gene sequence similarities between strain AlyHP32T and members of the Splendidus clade were in the range 99.2–95.4%: V. splendidus ATCC 33125T (99.2%), V. lentus 4OM4T (99.2%), V. cyclitrophicus KP-2P44T (97.9%) and V. tasmaniensis DSM 20012T (98.7%). Sequences of the atpA (1104 bp), gapA (747 bp), gyrB (855 bp), mreB (912 bp), pyrH (444 bp), recA (672 bp), rpoA (657 bp), rpoD (810 bp) and topA (684 bp) genes were obtained for AlyHP32T and compared with those of members of the Splendidus clade (Table S1, available in IJSEM Online). Phylogenetic analyses based on each sequence of the 16S rRNA gene and the housekeeping genes atpA, pyrH, recA, rpoA and rpoD confirmed the position of the novel isolate in the genus Vibrio, and allocated it to the Splendidus clade (Figs 1 and S1). A neighbour-joining tree based on the concatenated

![Phylogenetic tree based on 16S rRNA gene sequences obtained by the neighbour-joining method. Vibrio cholerae CECT 514T was used as an outgroup. Percentage bootstrap values (>50%, 1000 resamplings) are given at branch points and asterisks indicate that the corresponding nodes (groupings) are also recovered in trees generated with the maximum-parsimony and maximum-likelihood method. Bar, 0.01 nucleotide substitution per position.](image-url)
sequences of housekeeping genes (atpA, 1068 bp; pyrH, 417 bp; recA, 567 bp; rpoA, 657 bp; rpoD, 406 bp; 16S rRNA, 1357 bp) supported the phylogenetic position of the isolate, which was revealed by the tree based on 16S rRNA gene sequences alone (Figs 1 and 2).

DNA–DNA hybridization was performed by the membrane filter technique using the DIG High Prime DNA Labelling and Detection Starter kit II (Roche Molecular Biochemicals) according to the method described in detail by Lee et al. (2003), with the modification that the hybridization temperature was 55 °C. Genomic DNA–DNA relatedness values for strain AlyHP32T and the type strains of members of the Splendidus clade tested were always below 58 % (see Table S2). Thus, levels of genetic relatedness according to DNA–DNA hybridization experiments were less than 70 %, which led to the conclusion that strain AlyHP32T represents a novel and distinct species (Wayne et al., 1987).

We performed repetitive primer BOX-PCR to fingerprint the genomes of strain AlyHP32T and reference strains using TaKaRa LA Taq polymerase (TAKARA BIO) and primer BOXA1R (Louws et al., 1994). The resultant fingerprints were analysed using the GelCompar II software package (version 4.5; Applied Maths). A similarity matrix was calculated with the Jaccard coefficient (Kosman & Leonard, 2005) with a band position tolerance of 0.59 %, and a dendrogram was reconstructed with the Ward algorithm (Ward, 1963). The banding patterns from these PCR experiments clearly showed the distinctiveness of strain AlyHP32T from the members of the Splendidus clade (Fig. S2).

Physiological and biochemical tests of strain AlyHP32T and five reference strains were carried out under the same conditions; i.e. cells grown on MA at 25 °C for 3 days were used for inocula. Alginate lyase activity was measured using the cetylpyridinium chloride (CPC) assay (Kawamoto et al., 2006). Cells were spread on ZoBell medium (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 1 l distilled water) containing 0.1 % sodium alginate and incubated for 3 days. After 1 h of incubation at 20 °C with 10 % CPC solution, the samples were examined for the presence of clear zones around the colonies. Growth on various standard bacteriological media was tested by using nutrient agar (NA), R2A agar, plate-count agar (PCA), MA, thiosulfate-citrate-bile salts sucrose agar (TCBS) and tryptic soy agar (TSA) according to the instructions of the manufacturer (Becton Dickinson). All media were supplemented with 1 % (w/v) NaCl when required. The Gram reaction test of cells grown on MA at 25 °C for 2 to 14 days was performed by using the bioMérieux Gram stain kit according to the manufacturer’s instructions and the Ryu non-staining KOH method (Powers, 1995). Motility was examined by observing the cells grown in wet mounts using phase-contrast microscopy (TMS-F; Nikon). Flagellation was determined with a transmission electron microscope.
(CM-20; Philips) using cells cultured for 48 h in marine broth (MB; Becton Dickinson). Requirement for and tolerance to NaCl [final concentration (w/v) 0–10%, increments of 1%] for growth was tested in ZoBell broth. The growth experiment at pH 4–11 (increments of 1 pH unit) was performed using MB containing 100 mM acetate buffer, 100 mM phosphate buffer or 100 mM NaHCO₃/Na₂CO₃ buffer, at pH 4–5, 6–8 and 9–11, respectively. The optimal temperature and temperature range for growth were tested on MB at 4 °C and 10–50 °C (at 5 °C intervals). Anaerobic growth was tested on MA in a jar containing the AnaeroPack-Anaero (Mitsubishi Gas Chemical), which works as an oxygen absorber and CO₂ generator, for up to 10 days. Catalase and oxidase activities were tested in 3% (v/v) hydrogen peroxide solution (Hanker & Rabin, 1975) and 1% (w/v) p-tetramethyl phenylenediamine (bioMérieux), respectively. Acid production from sugars was described as being positive by Yamaguchi & Yokoe (2000). Nitrate reduction was tested according to Smibert & Krieg (1994). DNase activity was determined with DNase test agar (Becton Dickinson). Other biochemical tests and enzyme activities were determined using API 20NE, API 20E and API ZYM kits (bioMérieux) and GN2 MicroPlate (Biolog) prepared according to the manufacturers instructions.

### Table 1. Differential phenotypic characteristics of strain AlyHP32ᵀ and related species of the genus Vibrio

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth at/with:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio hemicentroti sp. nov. AlyHP32ᵀ</td>
<td>35 °C</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>6% NaCl</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>β-galactosidase (API 20NE)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td>Alginate</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tween 20</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fermentation of:</td>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Melibiose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>D-Mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td>Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>α-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Esterase (C4)</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>Esterase lipase (C8)</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Species: 1, Vibrio hemicentroti sp. nov. AlyHP32ᵀ; 2, V. splendidus KCTC 12679ᵀ; 3, V. lentus KCTC 12743ᵀ; 4, V. cyclitrophicus KCTC 12678ᵀ; 5, V. tasmaniensis KCTC 12831ᵀ; 6, V. kanaloe DSM 17181ᵀ; 7, V. atlanticus DSM 26479ᵀ; 8, V. celticus DSM 26172ᵀ; 9, V. gigantis DSM 18531ᵀ; 10, V. artuborum DSM 26480ᵀ; 11, V. crassostreae DSM 17220ᵀ; 12, V. pomeroyi DSM 17180ᵀ; 13, V. chagasii DSM 17138ᵀ. +, Positive; –, negative; w, weakly positive. All strains are positive for fermentation of glucose and mannitol, and enzyme activity of oxidase, catalase and alkaline phosphatase. All strains are negative for enzyme activity of N-acetyl-β-glucosaminidase, lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase and valine arylamidase, and fermentation of myo-inositol, lactose and sorbitol.
except that bacterial strains were suspended in distilled water supplemented with 2% (w/v) NaCl. Antibiotic resistance was determined using the disc diffusion method (Bauer et al., 1966) with commercial antibiotic-impregnated discs (Becton Dickinson). After 3 days of incubation at 25 °C on Mueller Hinton II agar (Becton Dickinson) supplemented with 1% (w/v) NaCl, the results were interpreted according to the guidelines set forth by the CLSI (2009).

Strain AlyHP32T could be distinguished from closely related species by alginate lyase activity and the inability to grow at 35 °C. The detailed results of physiological and biochemical analyses are given in Table 1 and the species description. Strain AlyHP32T was sensitive to (µg per disc, unless otherwise indicated): chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), polymyxin B (300 IU), streptomycin (10) and tetracycline (30), but resistant to amikacin (30), ampicillin (30), polymyxin B (300 IU), streptomycin (10) and tetracycline (30).

For cellular fatty acid analysis, strain AlyHP32T and V. splendidus KCTC 12679T, V. cyclitrophicus KCTC 12678T, V. lentus KCTC 12743T and V. tasmaniensis KCTC 12831T were grown on MA and harvested at late exponential phase, i.e. after 3 days at 25 °C. Extraction of fatty acid methyl esters and separation by GC were performed by using the instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database. For G+C content calculations, DNA samples were prepared in triplicate and G+C content was determined by the thermal denaturation method of Marmur & Doty (1962). Isoprenoid quinones were extracted and purified according to the method of Minnikin et al. (1984) and analysed by TLC as described by Collins (1994).

The predominant fatty acids (>10.0% of total fatty acids) of strain AlyHP32T were C16:0 (25.7%) and summed feature 3 (C16:1ω7c and/or C16:1ω6c; 28.2%), as in the type strains of V. splendidus and V. tasmaniensis. A comparison of the cellular fatty acid compositions of strain AlyHP32T and some related species of the genus Vibrio is given in Table 2. Ubiquinone 8 (Q-8) was the major respiratory quinone of strain AlyHP32T. The DNA G+C content was 44.1 ± 0.5 mol% (mean ± SD of three determinations).

Based on data derived using a polyphasic taxonomic approach, it is proposed that strain AlyHP32T represents a novel species belonging to the genus Vibrio, for which the name Vibrio hemicentroti sp. nov. is proposed.

**Description of Vibrio hemicentroti sp. nov.**

*Vibrio hemicentroti* [he.mi.cen.tro’ti. N.L. gen. n. hemicentroti of *Hemicentrotus (Hemicentrotus pulcherrimus, a sea urchin), the isolation source of the type strain*.]

Cells are Gram-reaction-negative, facultatively anaerobic, oxidase- and catalase-positive, motile and rod-shaped (0.6–0.8 × 1.0–2.5 µm in size; Fig. S3). Cells grow best on media such as MA and TCBS; slowly on TSA; not on NA, PCA or R2A agar. Colonies on MA are convex, circular, smooth, opaque with entire margins, non-pigmented and approxi-

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>C12:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>C14:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>C15:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>C17:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>C18:0</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Branched</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>1.7</td>
<td>4.1</td>
<td>–</td>
<td>0.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>2.0</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
<td>2.0</td>
</tr>
<tr>
<td>C20:1ω7c</td>
<td>–</td>
<td>0.5</td>
<td>–</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Summed features*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0 3-OH/iso-C16:1</td>
<td>3.1</td>
<td>2.8</td>
<td>–</td>
<td>2.8</td>
<td>2.4</td>
</tr>
<tr>
<td>C16:1ω7c/C16:1ω6c</td>
<td>28.2</td>
<td>33.5</td>
<td>8.2</td>
<td>39.0</td>
<td>37.0</td>
</tr>
<tr>
<td>iso-C17:1 H/antisepio-C17:1</td>
<td>0.7</td>
<td>0.4</td>
<td>0.8</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>C18:1ω7c/C16:1ω6c</td>
<td>7.4</td>
<td>9.9</td>
<td>67.1</td>
<td>19.6</td>
<td>6.8</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the Microbial Identification System.

Species: 1, *Vibrio hemicentroti* sp. nov. AlyHP32T; 2, *V. splendidus* KCTC 12679T; 3, *V. lentus* KCTC 12743T; 4, *V. cyclitrophicus* KCTC 12678T; 5, *V. tasmaniensis* KCTC 12831T. All data were obtained in this study. Values are percentages of total fatty acids; fatty acids that represented <1.0% in all strains were omitted. –, Not detected.
assayed on the Biolog GN2 Microplate system: dextrin, Tween 80, 3-d-glucose, 3-d-mannitol and inosine; the following substrates are weakly assimilated: Tween 40, N-acetyl-D-glucosamine, L-arabinose, D-fructose, D-raffinose and glucose 6-phosphate; other substrates are not assimilated. In the API ZYM gallery, positive for acid phosphatase, alkaline phosphatase, leucine arylamidase and naphthol-AS-Bl-phosphohydrolase activities; weakly positive for esterase (C4) and esterase lipase (C8) activities; negative for N-acetyl-β-glucosaminidase, 3-chymotrypsin, cystine arylamidase, 3-fucosidase, 3-galactosidase, 3-galactosidase, 3-glucosidase, 3-glucosidase, 3-glucuronidase, lipase (C14), 3-mannosidase, trypsin activity and valine arylamidase activities. Major fatty acids are C16:0 and summed feature 3 (C16:1ω7c and/or C16:1ω6c); the complete fatty acid composition is given in Table 2. Ubiquinone Q-8 is the predominant quinone type.

The type strain is AlyHP32T (=KCTC 32085T=DSM 26178T), isolated from the gut of sea urchin (Hemicentrotus pulcherrimus) from the South Sea, Republic of Korea. The DNA G+C content of the type strain is 44.1 mol%.

Acknowledgements

This work was financially supported by the Korea Fisheries Resources Agency of the Ministry for Food, Agriculture, Forestry and Fisheries (20120631251-00), the project fund to J.S.C. from the Center for Analytical Research of Disaster Science of Korea Basic Science Institute (C32730), Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (project no. 2010-0020141) and the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea.

References


