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Hasllibacter halocynthiae gen. nov., sp. nov., a nutriacholic acid-producing bacterium isolated from the marine ascidian *Halocynthia roretzi*

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A Gram-negative, aerobic, ovoid to rod-shaped bacterial strain, KME 002^T was isolated from a marine ascidian, *Halocynthia roretzi*, off the coast of Gangneung, Korea. Phylogenetic analyses based on 16S rRNA gene sequences showed that this strain belonged to the family *Rhodobacteraceae* in the class *Alphaproteobacteria* and was closely related to the type strains of *Dinoroseobacter shibae*, *Roseovarius crassostreae* and *Pseudoruegeria aquimaris* with 95.0, 94.7 and 94.5 % 16S rRNA gene sequence similarities, respectively. KME 002^T was an obligately halophilic bacterium requiring 1 to 5 % (w/v) NaCl, with an absolute requirement for magnesium chloride for growth. Cells were motile by means of a single polar flagellum and showed budding fission. The predominant cellular fatty acid of the isolate was C_{18:1ω7c} and Q-10 was the major ubiquinone. The DNA G+C content of the strain was 71.6 mol%. The major secondary metabolites from cultures in liquid medium were cholic acid derivatives, including 3α,12α-dihydroxy-7-ketocholanic acid, 12-hydroxy-3-keto-glycocholanic acid, nutriacholic acid and deoxycholic acid. These characteristics determined in this polyphasic study suggest that strain KME 002^T represents a novel species in a new genus of the family *Rhodobacteraceae*. The name *Hasllibacter halocynthiae* gen. nov., sp. nov. is proposed for this isolate, and the type strain is KME 002^T (=JCM 16214^T=KCCM 90082^T).

The family *Rhodobacteraceae* within the class *Alphaproteobacteria* is one of the most abundant marine bacterial groups. It is divided into four subgroups based on phylogenetic analysis of 16S rRNA gene sequences. One of these groups is known as the marine *alpha* group or the *Roseobacter* group (González & Moran, 1997; Macián *et al.*, 2005a). A number of bacteria belonging to the *Roseobacter* clade have important roles in the carbon, sulfur and nitrogen cycles in the ocean (González *et al.*, 1999, 2000; Macián *et al.*, 2005a). They have been isolated from various marine environments, such as seawater, seaweed, sediment, microalgae, invertebrates, vertebrates, polar sea ice, hypersaline microbial mats and coastal biofilms (Giovannoni & Rappe, 2000; Selje *et al.*, 2004; Buchan *et al.*, 2005; Brinkhoff *et al.*, 2008). Their defining characteristics include phototrophy, aerobic sulfite oxidation, organic

sulfur compound degradation, methylotrophy, gas vacuole formation, poly-β-hydroxybutyrate granules and rosette formation (Arahal *et al.*, 2005; Buchan *et al.*, 2005; Choi *et al.*, 2007). In addition to the biodiversity of the *Roseobacter* clade, this group produces diverse biologically active secondary metabolites. For example, *Loktanella* sp. BIO-204 and *Dinoroseobacter shibae* DFL 27 produce (4R,5Z)-dodec-5-en-4-olide, known as buibuilactone, a pheromone of several scarab beetles (Dickschat *et al.*, 2005). *Phaeobacter* species isolated from the German Wadden Sea produce the antibiotic tropodithietic acid, which has strong inhibitory properties against various marine bacteria and algae (Brinkhoff *et al.*, 2004; Rao *et al.*, 2005). Strains of *Oceanibulbus indolifex* produce several indole derivatives, the antibiotic tryptanthrin and cyclic dipeptides, which have mild antiviral and antibiotic properties (Wagner-Döbler *et al.*, 2004).

To investigate the chemical potential of microbes in marine environments, we analysed the microbial diversity associated with marine invertebrates and their production of secondary metabolites. We isolated a novel nutriacholic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KME 002^T is FJ638616.

Supplementary methods, seven supplementary figures and a supplementary table are available with the online version of this paper.

acid-producing Gram-negative bacterium, strain KME 002^T, isolated from an edible ascidian, *Halocynthia roretzi*. NCBI nucleotide BLAST searches using the 16S rRNA gene sequence of the cultured bacterium revealed that the isolate has a close phylogenetic affiliation with members of the family *Rhodobacteraceae*. It was most closely related to the type strains of *Dinoroseobacter shibae*, *Roseovarius crassostreae* and *Pseudoruegeria aquimaris*, with 16S rRNA gene sequence similarities of 95.0, 94.7 and 94.5 %, respectively. Analysis of bioactive secondary metabolites in liquid cultures of strain KME 002^T led to the isolation of 3,12-dihydroxy-7-ketocholanic acid, 12-hydroxy-3-keto-glycocholic acid, nutriacholic acid and deoxycholic acid (Fig. 1). Cholic acid is a major bile acid produced by mammalian liver cells. Currently, only 11 bacterial strains of the genera *Streptococcus*, *Myroides*, *Dokdonia*, *Polaribacter*, *Donghaeana*, *Maribacter*, *Hahella*, *Rhodococcus* and *Psychrobacter* have been reported to produce bile acids. Bile acids produced by these 11 strains include cholic acid, deoxycholic acid, glycocholic acid, glycodeoxycholic acid, cholic acid methyl ester, 3-dimethoxy-12 α -hydroxycholelanic acid (assumed to be an artefact), 3-dimethoxy-7-ketocholanic acid, 12 α -hydroxy-3-ketocholanic acid, 12 α -hydroxy-3,7-diketocholanic acid, 3 α ,12 α -dihydroxy-7-ketocholanic acid, 7 α ,12 α -dihydroxy-3-ketocholanic acid and 3 α ,12 α -dihydroxycholelanic acid (Park *et al.*, 1995; Maneerat *et al.*, 2005; Kim *et al.*, 2007, 2010; Li *et al.*, 2009). However, nutriacholic acid-producing bacteria have not yet been described. In this paper, we describe the characterization of this nutriacholic acid-producing bacterium, strain KME 002^T, using a polyphasic approach.

Strain KME 002^T was isolated from the tissue of a marine ascidian, *Halocynthia roretzi*, collected at a depth of 15 m (water temperature 17 °C) off the coast of Gangneung, Korea, in June 2007. Following collection, the ascidian was washed with autoclaved seawater. Its incurrent and excurrent siphon tissues were finely cut and diluted with autoclaved seawater in a 10:1 ratio and 100 μ l was spread

on an A1+C agar plate. The A1+C agar medium contained 10 g starch (Difco), 4 g peptone (Difco), 2 g yeast extract (Difco), 1 g calcium carbonate (Sigma-Aldrich) and 18 g agar (Difco) in 1 l filtered seawater (pH 7.0). The inoculated plate was incubated for 3 weeks at 25 °C under aerobic conditions. Each bacterial colony on the A1+C agar plate was individually cultured in A1+C liquid medium (25 ml) with shaking at 200 r.p.m. for 5 days at 25 °C. Stocks of each isolated bacterial strain were preserved in A1+C liquid culture medium containing 20 % (v/v) glycerol at -80 °C.

Gram staining was performed according to the method described by Süßmuth *et al.* (1987). Colony morphology and cell motility were observed by light microscopy (Eclipse Te2000 U; Nikon). Cell shape and flagella were observed by scanning electron microscopy (model 515; Philips) and transmission electron microscopy (model CM 20; Philips) after negative staining with 1 % uranyl acetate.

The ability of KME 002^T to grow on various media was evaluated using marine agar (MA) (Difco), A1+C agar, A1 agar (A1+C agar medium without calcium carbonate), R2A agar (Difco) and trypticase soy agar (TSA) (Difco) for 7 days at 25 °C. The requirement for and tolerance to NaCl were tested on MA plates prepared with artificial seawater containing different NaCl concentrations (0 to 8 %, w/v; increments of 1 %) for 7 days at 25 °C. The ability of KME 002^T to grow at various temperatures was examined at 4, 7, 10, 13, 15, 20, 27, 30, 35, 37, 42 and 45 °C on A1+C agar plates. The pH growth range was evaluated in A1+C liquid medium at pH values from 4.0 to 11.0 at unit intervals. Oxidase activity was examined by oxidation of 1 % *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (Sigma-Aldrich). Catalase activity was determined using a 3 % (v/v) hydrogen peroxide solution. The assimilation of 49 sole carbon sources was determined using the API 50CH system (bioMérieux), and the utilization of additional organic substrates was investigated on seawater agar containing single carbon sources (0.2 %). Biochemical properties and enzymic activities were examined using the API 20E and API ZYM kits (bioMérieux) according to the manufacturer's recommendations. Bacterial isoprenoid quinones were extracted from freeze-dried biomass using chloroform/methanol (2:1, v/v) and separated by reverse-phase HPLC with a Spherisorb ODS2 5 μ m column (250 $\times$ 4.6 mm) according to Tamaoka (1986). To determine the fatty acid profile, KME 002^T was grown on MA at 25 °C for 10 days and analysed with a Hewlett Packard 6890 series GC equipped with an HP-1 glass capillary (cross-linked methyl siloxane, 30 m $\times$ 0.32 mm $\times$ 0.25 μ m) column. Fatty acids were analysed with the Microbial Identification System (MIDI; Microbial ID) (Miller, 1982), and the DNA G+C content was determined by HPLC using hydrolysate digested with P1 nuclease (Katayama-Fujimura *et al.*, 1984).

KME 002^T chromosomal DNA was extracted with a G-spin genomic DNA extraction kit (iNtRON). Fragments of the

Fig. 1. Secondary metabolites isolated from strain KME 002^T: (a) 3 α ,12 α -dihydroxy-7-ketocholanic acid; (b) 12-hydroxy-3-keto-glycocholic acid; (c) nutriacholic acid; (d) deoxycholic acid.

16S rRNA gene were PCR-amplified using the universal primers 27F and 1492R (Lane, 1991) and purified using a PCR purification kit (Solgent). The product was purified using the Montage dye removal kit (Millipore) according to the manufacturer's protocol. Sequencing was performed with an ABI 3730XL DNA sequencer (50 cm capillary) with an ABI PRISM BigDye terminator cycle sequencing ready reaction kit V3.1 (Applied Biosystems). The 16S rRNA gene sequence was assembled with the program BioEdit (Hall, 1999) and compared to available sequences from GenBank/DBJ/EMBL using BLAST (Altschul *et al.*, 1997). Pairwise nucleotide sequence similarity values were determined using the EzTaxon server 2.1 (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The obtained 16S rRNA sequence (1429 nt) and 30 reference sequences from GenBank were aligned using the CLUSTAL W (1.83) multiple alignment program (Thompson *et al.*, 1994). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods were used to construct phylogenetic trees with bootstrap values based on 1000 replicates (Felsenstein, 1985) in MEGA version 4.0 (Kumar *et al.*, 2008). The evolutionary distance matrix for the neighbour-joining method was calculated with the Kimura two-parameter model (Kimura, 1983).

To investigate production of secondary metabolites, strain KME 002^T was cultured in ten 1 l Erlenmeyer flasks containing 500 ml A1+C liquid medium under aerobic conditions. The flasks were incubated at 25 °C for 7 days with shaking at 200 r.p.m. Secondary metabolites in the culture broth were analysed by an analytical RP HPLC using a Phenomenex Luna C18(2) analytical column (4.6 × 150 mm, 5 µm) and a linear aqueous acetonitrile (including 0.05 % trifluoroacetic acid) gradient from 10 to 100 % for 30 min as eluent (flow rate 0.7 ml min⁻¹) (Supplementary Fig. S1, available in IJSEM Online). The ethyl acetate extract (245.7 mg) of the liquid culture broth was fractionated and purified using a C18 RP flash column chromatography and RP prep-HPLC to obtain 3,12-dihydroxy-7-ketocholanic acid (1.4 mg), 12-hydroxy-3-keto-glyocholanic acid (0.2 mg), nutriacholic acid (0.9 mg) and deoxycholic acid (3 mg) (see Supplementary methods available in IJSEM Online). The structures of cholic acid derivatives isolated from the KME 002^T liquid culture broth were determined by comparing their NMR data (Supplementary Figs S2–S7, available in IJSEM Online) with previously reported data (Li *et al.*, 2009; Ijare *et al.*, 2005; Bertolasi *et al.*, 2007; Waterhous *et al.*, 1985).

KME 002^T was a Gram-negative, motile, aerobic, marine bacterium. Scanning and transmission electron microscopic observations revealed ovoid to rod-shaped cells and a single polar flagellum. Cells were 1.0–2.3 µm in length and 0.5 to 1.0 µm in width (Fig. 2). This investigation demonstrated that cells of strain KME 002^T showed budding fission and irregular binary division (Fig. 2) (Nedashkovskaya *et al.*, 2006; Labrenz *et al.*, 1998, 1999). The budding property of the cell division was distinct, enabling the strain to be differentiated from close phylogenetic relatives in the genera *Dinoroseobacter*,

Fig. 2. Morphological features of *Haslibacter halocynthiae* KME 002^T. (a, b) Scanning electron micrographs of strain KME 002^T; cells show budding fission and irregular binary division (indicated by arrows). (c, d) Transmission electron micrographs of negatively stained KME 002^T: (c) cells show budding fission; (d) cell with a single polar flagellum. Bars: a, 5 µm; b, 2 µm; c, d, 1 µm.

Pseudoruegeria and *Jannaschia*. The morphology of colonies grown on MA plates after 5 days at 25 °C was circular, convex with clear margins, light red in colour and 0.4 to 1.3 mm in diameter. The growth temperature ranged from 13 to 35 °C, with optimal growth at 25 °C on A1 + C agar plates. Growth occurred between pH 6 and 10, with optimum growth at pH 8. The strain had a strict requirement for 1–5 % NaCl (optimum of 4%). Magnesium chloride, a major inorganic component of seawater, was necessary for growth. The isolate grew on A1 + C agar, A1 agar and MA but not on R2A or TSA at 25 °C. The phenotypic and genetic characteristics of KME 002^T were compared with those of its phylogenetic neighbours within the *Roseobacter* clade (Table 1).

Strain KME 002^T differed from its relatives in that it was not able to utilize succinate as a sole carbon source. Additionally, it was not able to assimilate pyruvate or citrate, whereas its relatives assimilated pyruvate, except for *Jannaschia helgolandensis* Hel 10^T, and citrate, except for *Thalassobacter stenotrophicus* CECT 5294^T (Table 1). The results of the API ZYM test for this strain also differed from those of closely related strains (Table 1). Only KME 002^T displayed β -glucuronidase and β -glucosidase activities, but it did not possess alkaline phosphatase activity. Analyses of the predominant isoprenoid quinones of KME 002^T revealed a characteristic peak corresponding to ubiquinone Q-10, the predominant respiratory lipoquinone of members of the *Alphaproteobacteria*.

Table 1. Phenotypic characteristics of strain KME 002^T and its close phylogenetic relatives in the *Roseobacter* clade

Strains: 1, KME 002^T; 2, *Dinoroseobacter shibae* DFL 12^T (Biebl *et al.*, 2005a, except where marked); 3, *Roseovarius crassostreae* CV919-312^T (Boettcher *et al.*, 2005; Yoon *et al.*, 2008); 4, *Pseudoruegeria aquimaris* SW-255^T (Yoon *et al.*, 2007a); 5, *Jannaschia rubra* 4SM3^T (Macián *et al.*, 2005b; Choi *et al.*, 2006); 6, *Jannaschia helgolandensis* Hel 10^T (Wagner-Döbler *et al.*, 2003; Macián *et al.*, 2005b; Choi *et al.*, 2006); 7, *Thalassobacter stenotrophicus* CECT 5294^T (Macián *et al.*, 2005a; Pujalte *et al.*, 2005; Kim *et al.*, 2009). +, Positive reaction or growth; w, weakly positive; –, negative reaction or no growth; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Cell shape	Rod to ovoid	Cocci, ovoid rod	Rod to ovoid	Rod	Rod	Irregular rod	Ovoid rod
Motility	+	+	+	–	+	–	+
Colony colour	Light red	Deep wine-red	Pink-beige*	Greyish yellow	Dark red	Whitish	Salmon-pink/deep brown
Growth at							
4 °C	–	–†	–	–	+	–	–
37 °C	–	+	+	+	–	–	+
Tolerance to NaCl (%)	1–5	1–7	1.0–1.5‡	2‡	0.34–9.0	0.68–8.0	0.85–70
pH range for growth	6–10	6.2–8.9	6.5–8.0‡	7–8‡	7–9	7–8	ND
Assimilation of:							
Acetate	+	+	+	+	+	–	+
L-Arabinose	+	+	–	+	–	–	–
Cellobiose	–	–†	–	+	–	+	–
Citrate	–	+	+	+	w	+	–
D-Fructose	+	+	–	+	+	+	–
Galactose	–	–†	–	+	+	+	–
D-Glucose	–	–†	–	+	+	+	–
Glycerol	+	–†	+	ND	+	+	–
Maltose	–	–†	–	+	w	–	–
Mannitol	–	–†	–	ND	+	+	–
D-Mannose	–	–†	–	+	+	+	–
Pyruvate	–	+	+	+	+	–	+
Sorbitol	+	–†	–	ND	+	+	–
Succinate	–	+	+	+	+	+	+
Sucrose	–	w	–	+	–	–	–
Trehalose	–	–†	–	+	–	–	–
Enzyme activity (API ZYM):							
Alkaline phosphatase	–	+	+	+	+	+	+
Lipase (C14)	w	–†	w	–	–	–	–
Acid phosphatase	–	+	+	+	+	w	–
Naphthol-AS-BI-phosphohydrolase	+	w†	–	w	+	–	+
α-Galactosidase	–	–†	–	–	+	w	–
β-Glucuronidase	+	–†	–	–	–	–	–
α-Glucosidase	+	–†	–	–	+	–	–
β-Glucosidase	+	–†	–	–	–	–	–
N-Acetyl-β-glucosaminidase	w	–†	–	+	–	–	–
DNA G + C content (mol%)	71.6	64.8	59.0	67.0	64.6	63.0–63.1	59.1

*Some strains of this species produce colonies with a greenish-yellow appearance.

†Data for growth at 4 °C, utilization of carbon sources and API ZYM tests were derived from our experiments.

‡Optimal values.

The major fatty acid of KME 002^T was C_{18:1}ω7c (84.1 %) and the minor fatty acids (≥0.5 %) included the following: C_{18:0} (5.6 %), C_{10:0} 3-OH (4.3 %), C_{19:0} cyclo ω8c (3.6 %), C_{20:1}ω7c (1.4 %), C_{17:0} (0.5 %) and C_{19:0}

(0.5 %) (Supplementary Table S1, available in IJSEM Online). The major fatty acid of KME 002^T, C_{18:1}ω7c, was the same as that of relatives within the *Rhodobacteraceae*, but KME 002^T contained a higher relative level of this fatty acid.

C_{19:0} cyclo ω 8c fatty acid was present in KME 002^T but absent in its closest neighbour, *D. shibae*. The general fatty acid profile was similar to those of its relatives, but only KME 002^T had C_{19:0}, albeit in a small amount.

The fatty acid profiles of KME 002^T and its phylogenetic neighbours are described in Supplementary Table S1 (available in IJSEM Online). The 1429 nt 16S rRNA gene sequence of KME 002^T was most similar to those of *D. shibae* DFL 12^T (95 %) (Biebl *et al.*, 2005a), *R. crassostreae* CV919-312^T (94.7 %) (Boettcher *et al.*, 2005) and *P. aquimaris* SW-255^T (94.5 %) (Yoon *et al.*, 2007a). A comparison of 16S rRNA gene sequences between KME 002^T and strains of related genera revealed sequence similarities ranging from 92.7 to 95.0 %: *Dinoroseobacter* (95.0 %) (Biebl *et al.*, 2005a); *Roseovarius* (92.7 to 94.7 %) (Labrenz *et al.*, 1999; González *et al.*, 2003; Biebl *et al.*, 2005b; Boettcher *et al.*, 2005; Yoon *et al.*, 2008; Oh *et al.*, 2009; Wang *et al.*, 2009, 2010; Jung *et al.*, 2011); *Pseudoruegeria* (94.1 to 94.5 %) (Yoon *et al.*, 2007a; Jung *et al.*, 2010); *Jannaschia* (93.4 to 94.2 %) (Wagner-Döbler *et al.*, 2003; Macián *et al.*, 2005b; Choi *et al.*,

2006; Yoon *et al.*, 2007b; Kim *et al.*, 2008; Yoon *et al.*, 2010); and *Thalassobacter* (93.3 to 93.8 %) (Macián *et al.*, 2005a; Kim *et al.*, 2009). 16S rRNA gene-based phylogenetic analysis clustered strain KME 002^T tightly with members of the *Rhodobacteraceae*. However, KME 002^T formed an independent clade with *D. shibae* DFL 12^T within the *Roseobacter* group (Fig. 3). This phylogenetic analysis and low 16S rRNA gene sequence similarities (92.7 to 95.0 %) revealed that KME 002^T belongs to a new genus of the family *Rhodobacteraceae*. The DNA G + C content of strain KME 002^T was 71.6 mol%, a relatively high value. The DNA G + C contents of members of related genera were: 64.8 mol% in *Dinoroseobacter*; 58.3 to 66.0 mol% in *Roseovarius*; 67.0 to 73.5 mol% in *Pseudoruegeria*; 63.0 to 68.4 mol% in *Jannaschia*; and 56.0 to 59.1 mol% in *Thalassobacter*.

In our study, KME 002^T was clearly distinguishable from its phylogenetic neighbours by 16S rRNA gene phylogeny, the presence of C_{19:0} fatty acids, β -glucuronidase and β -glucosidase activities, and its inability to metabolize citrate, succinate and pyruvate. Based on these phenotypic,

Fig. 3. Neighbour-joining and maximum-parsimony tree based on 16S rRNA gene sequences showing the phylogenetic position of KME 002^T among related members of the family *Rhodobacteraceae*. Filled circles indicate that the corresponding nodes were recovered in both methods. Bootstrap values (>50 %) based on 1000 replicates from the neighbour-joining method (above branch) and the maximum-parsimony method (below branch) are shown at nodes. GenBank accession numbers are indicated in parentheses. Bar, 0.01 nt substitutions per site.

chemotaxonomic and genotypic features, KME 002^T should be assigned to a new genus in the family *Rhodobacteraceae* as a representative of a novel species, *Hasllibacter halocynthiae* gen. nov., sp. nov.

Description of *Hasllibacter* gen. nov.

Hasllibacter (Has.li.bac'ter. N.L. n. *Haslla* Haslla, ancient name of the city of Gangneung, located on the coast of the East Sea in Korea, from which the organism was collected; N.L. masc. n. *bacter* rod; N.L. masc. n. *Hasllibacter* rod isolated from marine ascidian).

Cells are ovoid or rod-shaped. Gram-negative, aerobic and motile by a single polar flagellum. Buds may be produced. Irregular cell division may be observed. The isoprenoid quinone Q-10 is the major ubiquinone. The predominant fatty acid is C_{18:1}ω7c. The genus *Hasllibacter* is a member of the family *Rhodobacteraceae* within the class *Alphaproteobacteria*. The type species is *Hasllibacter halocynthiae*.

Description of *Hasllibacter halocynthiae* sp. nov.

Hasllibacter halocynthiae (ha.lo.cyn'thi.ae N.L. gen. n. *halocynthiae* of *Halocynthia roretzi* the ascidian from which the type strain was isolated).

Gram-negative, aerobic, motile by a single polar flagellum and ovoid or rod-shaped. Budding morphology may be observed. Light red, circular, convex colonies are formed on MA, A1 + C agar and A1 agar media. NaCl and MgCl₂ are necessary for growth. Grows at 13–35 °C, with optimal growth at 25 °C. Growth occurs between pH 6 and 10, with optimum growth at about pH 8. H₂S and indole are not produced. Metabolizes the following carbon sources in the API 50CH test and in additional organic substrate assimilation tests: acetate, glycerol, L-arabinose, ribose, D-xylose, L-xylose, D-fructose, L-sorbose, inositol, sorbitol, amygdalin, aesculin, sucrose, melezitose, starch, β-gentiobiose, turanose, D-lyxose, D-tagatose and 5-ketogluconate. Does not utilize erythritol, D-arabinose, adonitol, methyl β-xyloside, galactose, D-glucose, D-mannose, rhamnose, dulcitol, mannitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, arbutin, salicin, sucrose, succinate, pyruvate, cellobiose, maltose, lactose, melibiose, trehalose, inulin, raffinose, glycogen, xylitol, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-ketogluconate. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, citrate utilization, tryptophan deaminase, acetoin production and gelatinase in the API 20E test. API ZYM tests indicate positive reactions for esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase and β-glucosidase activities. Weak lipase (C14), β-galactosidase and N-acetyl-β-glucosaminidase activities are present, but negative for alkaline phosphatase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, α-mannosidase and α-fucosidase. Produces cholic acid derivatives as major secondary metabolites including

3,12-dihydroxy-7-ketocholanic acid, 12-hydroxy-3-keto-glycocholic acid, nutriacholic acid and deoxycholic acid.

The type strain is KME 002^T (=JCM 16214^T=KCCM 90082^T), isolated from an edible marine ascidian, *Halocynthia roretzi*, collected off the coast of Gangneung, in the East Sea of Korea. The DNA G+C content of the type strain is 71.6 mol%.

Acknowledgements

This study was funded by a grant from the National Fisheries Research and Development Institute (RP-2011-BT-001) and in part by the Korea Institute of Science and Technology (KIST) institutional program, grant number 2Z03470, Republic of Korea.

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