Aeromicrobium halocynthiae sp. nov., a taurocholic acid-producing bacterium isolated from the marine ascidian *Halocynthia roretzi*

Sung Hun Kim,^{1,2} Hyun Ok Yang,¹ Young Chang Sohn²
and Hak Cheol Kwon¹

Correspondence

Hak Cheol Kwon
hkwon@kist.re.kr

¹Korea Institute of Science and Technology, Gangneung, Gangwondo, 210-340, Republic of Korea

²Division of Applied Marine Biotechnology and Engineering, Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung, 210-702, Republic of Korea

A marine bacterium, strain KME 001^T, was isolated from the siphon tissue of a marine ascidian, *Halocynthia roretzi*, collected off the coast of Gangneung, Korea. Strain KME 001^T was a Gram-positive, aerobic, non-spore-forming, rod-shaped and non-motile bacterium. Phylogenetic analysis using 16S rRNA gene sequences revealed that strain KME 001^T clustered with the genus *Aeromicrobium* and was closely related to *Aeromicrobium ginsengisoli*, *Aeromicrobium erythreum* and *Aeromicrobium ponti* with 97.7, 97.6 and 97.5 % sequence similarities, respectively. The strain was capable of growth at a variety of temperatures (10–42 °C) and over a broad pH range (5.0–10.0). NaCl was required for robust growth of the strain. The diagnostic diamino acid of the cell-wall peptidoglycan was LL-diaminopimelic acid. The predominant menaquinone was MK-9(H₄). The predominant fatty acids were C_{18:1}ω₉C, C_{16:0} and 10-methyl C_{18:0}. The DNA–DNA hybridization analyses showed that DNA–DNA relatedness values between strain KME 001^T and its nearest neighbours, *A. ginsengisoli* KCTC 19207^T, *A. erythreum* KCCM 41104^T and *A. ponti* KACC 20565^T, were 49.6, 57.1 and 63.5 %, respectively. The DNA G + C content of strain KME 001^T was 75.9 mol%. Chemical investigation of the liquid culture medium of strain KME 001^T led to the isolation of taurocholic acid as a major secondary metabolite. On the basis of phylogenetic and phenotypic data, strain KME 001^T is classified as representing a novel species of the genus *Aeromicrobium*, for which the name *Aeromicrobium halocynthiae* sp. nov. is proposed. The type strain is KME 001^T (=JCM 15749^T=KCCM 90079^T).

In an effort to investigate the chemical potential of microbes growing in marine environments, the microbial diversity associated with marine invertebrates and the production of secondary metabolites was investigated. During this survey, a new bile acid-producing, Gram-positive bacterium was isolated from the ascidian *Halocynthia roretzi*. NCBI nucleotide BLAST searches using the 16S rRNA gene sequence of the cultured bacterium revealed that the new isolate shared a close phylogenetic affiliation with members of the genus *Aeromicrobium*. It was most closely related to *Aeromicrobium ginsengisoli* with 16S rRNA gene sequence similarities of 97.7 %.

Abbreviation: SEM, scanning electron microscope.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KME 001^T is FJ042789.

A table detailing the fatty acid contents of strain KME 001^T and related species of the genus *Aeromicrobium* is available with the online version of this paper. Supplementary figures showing HPLC/MS, ¹H-NMR, ¹³C-NMR spectra and a TLC chromatogram of the secondary metabolites of strain KME 001^T are also available.

The genus *Aeromicrobium*, first proposed by Miller *et al.* (1991), belongs to the family *Nocardioideae* within the order *Actinomycetales*. Currently, nine recognized species of the genus *Aeromicrobium* have been reported including *Aeromicrobium erythreum* (Miller *et al.*, 1991), *A. fastidiosum* (Tamura & Yokota, 1994), *A. alkaliterrae* (Yoon *et al.*, 2005), *A. panaciterrae* (Cui *et al.*, 2007) and *A. ginsengisoli* (Kim *et al.*, 2008) from soil, *A. marinum* (Bruns *et al.*, 2003), *A. tamulense* (Lee & Kim, 2007) and *A. ponti* (Lee & Lee, 2008) from the marine environment, and *A. flavum* (Tang *et al.*, 2008) from air. Erythromycin is a representative secondary metabolite produced by *A. erythreum* (Miller *et al.*, 1991). However, apart from erythromycin, no biologically valuable organic compounds have been reported from members of the genus *Aeromicrobium*.

The search for bioactive secondary metabolites in the culture broth of strain KME 001^T led to the isolation of taurocholic acid, a major secondary metabolite of this bacterium. Cholic acid is a bile acid produced by

mammalian liver cells. The acid is synthesized in the liver from cholesterol and is generally found in a conjugated form with taurine and glycine. Taurocholic acid, taurine-conjugated cholic acid and its sodium salt have been used as cholagogues (agents which promote the flow of bile) and these compounds are currently manufactured commercially by purification from animal bile (Dewick, 2001). Currently, only 10 bacterial strains have been reported as bile acid-producing prokaryotes. Bile acids produced by these strains include cholic acid, deoxycholic acid, glycocholic acid, glycodeoxycholic acid and cholic acid methyl ester (Park *et al.*, 1995; Maneerat *et al.*, 2005; Kim *et al.*, 2007). To date, a taurocholic acid-producing bacterium has not been reported.

This paper describes the characterization of strain KME 001^T by means of a polyphasic taxonomic approach.

Strain KME 001^T was isolated from a marine ascidian, *Halocynthia roretzi*, collected at a depth of 15 m (water temperature: 17 °C) near Kyung-Po beach, Korea (37° 48' 36.33"N, 128° 54' 46.19"E), in June 2007. As soon as the ascidian was collected, it was washed with autoclaved seawater. The incurrent and excurrent siphon tissues were ground and diluted with autoclaved seawater (ratio of ground tissue to seawater 1:10). The diluted suspension (100 µl) was spread on an A1+C agar plate. The A1+C medium contained 10 g starch (Difco), 4 g peptone (Difco), 2 g yeast extract (Difco), 1 g calcium carbonate (Aldrich) and 18 g agar (Difco) in 1 l filtered seawater (pH 7.0). The plate was incubated for 3 weeks at 25 °C under aerobic conditions. Individual colonies were subcultured on A1+C agar medium before each pure bacterial strain was cultured in A1+C liquid medium (25 ml) while shaking at 200 r.p.m. for 7 days at 25 °C. Stocks of each isolated bacterial strain were generated and stored at -80 °C in liquid culture medium containing 20 % (v/v) glycerol.

Gram-staining was performed according to the method described by Süßmuth *et al.* (1987). Cultures of strain KME 001^T grown at 25 °C for 5 days in A1+C medium were used to determine phenotypic characteristics. The ability of strain KME 001^T to grow on various agar 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 5 days at 25 °C. Colony morphology and cell motility were investigated using a light microscope (Eclipse Te2000-U; Nikon). Cell morphology and the presence of flagella were observed using a scanning electron microscope (SEM; S-3500N, Hitachi). The requirement for and tolerance of NaCl was examined on A1+C agar plates that were prepared by using artificial seawater containing different concentrations of NaCl (0–10%; w/v with intervals of 1%). The ability of strain KME 001^T to grow at various temperatures was examined at 4, 7, 10, 15, 20, 25, 30, 37, 42 and 45 °C on A1+C agar plates. Growth at different pH values was evaluated using A1+C liquid medium for which the pH values were adjusted from pH 3.0 to pH 12.0

(at intervals of 1.0 pH unit). Oxidase activity was examined using 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 utilization of carbon sources was investigated using the API 50 CHE system (bioMérieux) according to the manufacturer's recommendations. API 20E and API ZYM tests (bioMérieux) were used to determine additional biochemical properties.

The diaminopimelic acid stereoisomers in the cell-wall peptidoglycan were determined by using the TLC method as described by Stanek & Roberts (1974). The bacterial menaquinones were extracted from freeze-dried biomass by using chloroform-methanol (2:1, v/v), and analysed by reverse-phase HPLC with a Spherisorb 5 µm ODS2 (250 × 4.6 mm) column as described previously (Tamaoka, 1986). The analysis of fatty acid methyl esters was carried out by GC (6890; Hewlett Packard) with an HP-1 (cross-linked methyl siloxane, 30 m × 0.32 mm × 0.25 µm) column using the MIDI Microbial Identification System (MIS; Microbial ID) (Miller, 1982).

Chromosomal DNA of strain KME 001^T was extracted using a G-spin Genomic DNA Extraction kit (iNtRON Biotechnology, Inc.). PCR amplification of the 16S rRNA gene was performed with the universal primers 27F and 1492R (Lane, 1991). The DNA sequencing reaction was carried out with an ABI Prism BigDye terminator cycle sequencing ready reaction kit v3.1 (Applied Biosystems). The sequencing product was purified using a Montage dye removal kit (Millipore) according to the manufacturer's protocol. Gene sequences were determined on a Perkin-Elmer capillary DNA Sequencer (model ABI 3730XL; Applied Biosystems). The 16S rRNA gene sequence of strain KME 001^T was edited using the BioEdit program (Hall, 1999) and compared with primary sequence information within the GenBank/DBJ/EMBL nucleotide sequence database using BLAST (Altschul *et al.*, 1997). Pairwise nucleotide sequence similarity values were determined by using the EzTaxon server 2.1 (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Multiple alignments were performed with the CLUSTAL W program 1.83 (Thompson *et al.*, 1994) and evolutionary distances calculated by using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed by means of the neighbour-joining method (Saitou & Nei, 1987) with bootstrap values based on 1000 resamplings (Felsenstein, 1985) with the MEGA software package 4.0 (Kumar *et al.*, 2008).

DNA-DNA hybridization was determined by the membrane filter method using the DIG High Prime DNA labelling and detection starter kit II (Roche Applied Science) according to the manufacturer's instructions. The DNA G+C content of strain KME 001^T was determined by HPLC of the P1 nuclease hydrolysate (Katayama-Fujimura *et al.*, 1984).

The optical rotation of the taurocholic acid was measured on a Perkin-Elmer polarimeter (model 343; Perkin-Elmer). NMR spectra were obtained in CD₃OD on a Varian UNITY Plus 500 MHz spectrometer. NMR chemical shifts were referenced to the residual solvent peaks (δ_{H} 3.30 and δ_{C} 45.3 for CD₃OD). Low-resolution electrospray ionisation mass spectroscopy (ESI-MS) was measured on an Agilent Technologies VS/Agilent 1100 system and TLC was performed by using Merck silica gel 60 F254. Silica gel 60 (Merck, 0.063–0.200 mm) was also used for flash column chromatography. Preparative HPLC separations were performed using a Gilson 321 HPLC system with a Phenomenex Luna C18(2) 10 μm column (10 $\times$ 250 mm) at a flow rate of 4 ml min⁻¹. HPLC-MS was performed with an Agilent 1100 LC-MS system using a Phenomenex Luna C18(2) 5 μm column (4.6 $\times$ 150 mm) at a flow rate of 0.7 ml min⁻¹. A Waters 1525 HPLC-PDA system with a Phenomenex Luna C18(2) 5 μm column (4.6 $\times$ 150 mm) at a flow rate of 1.0 ml min⁻¹ was used for the analysis of extracts and fractions.

Strain KME 001^T was a Gram-positive, aerobic, non-spore-forming and non-motile bacterium on A1 + C medium at 25 °C. SEM observations of strain KME 001^T revealed rod-shaped bacterial cells. Cells were 4.1–6.0 μm in length and 0.4–0.5 μm in diameter (Fig. 1). Colonies were circular, convex, smooth, light yellowish and 0.6–1 mm in diameter on A1 + C agar plates after 5 days at 25 °C. Strain KME 001^T grew between 10 and 42 °C, with an optimal temperature for growth of 25 °C on A1 + C agar plates. Strain KME 001^T also grew on marine agar, R2A agar and A1 agar plates at 25 °C. However, no growth was observed on TSA medium. Previous studies have shown that the type strains of all recognized species of the genus *Aeromicrobium* are capable of growth on TSA, apart from *A. panaciterrae*. The phenotypic characteristics of strain KME 001^T were compared with those of related species of the genus *Aeromicrobium* (Table 1). Interestingly, only strain KME

001^T and the type strain of *A. marinum* were capable of using mannitol as a sole carbon source.

LL-Diaminopimelic acid was found to be the diagnostic diamino acid of the cell-wall peptidoglycan of strain KME 001^T. The predominant menaquinone was MK-9(H₄). These characteristics were similar to those of other type strains of species of the genus *Aeromicrobium*. The predominant fatty acids of KME 001^T ($\geq 1.00\%$) were C_{18:1} ω 9c (35.24%), C_{16:0} (31.50%), 10-methyl C_{18:0} (21.05%), C_{18:0} (5.47%), C_{17:0} (1.47%) and C_{16:1} ω 9c (1.71%) (see Supplementary Table S1 in IJSEM Online). The fatty acid composition of strain KME 001^T was similar to those of other species of the genus *Aeromicrobium*. However, strain KME 001^T was unique in that it lacked C_{16:0} 2-OH, a fatty acid detected in all other recognized species of the genus *Aeromicrobium*.

Phylogenetic analyses using the 16S rRNA gene sequence (1358 nt) of strain KME 001^T revealed that it was associated with the family *Nocardioidaceae* and was most closely related to members of the genus *Aeromicrobium* (Fig. 2). Comparisons of the 16S rRNA gene sequence of strain KME 001^T with sequences from related genera, including *Aeromicrobium*, *Marmoricola*, *Pimelobacter*, *Nocardioides* and *Lechevalieria*, showed that strain KME 001^T was most closely related to members of the genus *Aeromicrobium*. Strain KME 001^T shared relatively high sequence similarity (96.5–97.7%) with all other species of the genus *Aeromicrobium* species depicted on the phylogenetic tree (Fig. 2). The 16S rRNA gene sequence similarity values between strain KME 001^T and the type strains of related *Aeromicrobium* species were: *A. ginsengisoli* (97.7%), *A. erythreum* (97.6%), *A. ponti* (97.5%), *A. alkaliterrae* (97.4%), *A. marinum* (97.4%), *A. panaciterrae* (97.1%), *A. tamulense* (96.5%), *A. fastidiosum* (96.5%) and *A. flavum* (96.2%). Although the type strain of *A. ginsengisoli* appeared to have the highest gene sequence similarity with strain KME 001^T, it was located on another branch belonging to *A. marinum* and *A. panaciterrae* in the phylogenetic tree due to the higher levels of 16S rRNA sequence similarity with these species, 99.3% and 99.0%, respectively.

DNA–DNA hybridization was performed to evaluate the genomic DNA relatedness between strain KME 001^T and its nearest neighbours, *A. ginsengisoli* KCTC 19207^T, *A. erythreum* KCCM 41104^T and *A. ponti* KACC 20565^T. Strains with more than 70% DNA–DNA relatedness are generally assumed to be members of the same species (Wayne *et al.*, 1987). The DNA–DNA relatedness values between strain KME 001^T and *A. ginsengisoli* KCTC 19207^T, *A. erythreum* KCCM 41104^T and *A. ponti* KACC 20565^T were 49.6%, 57.1% and 63.5%, respectively. The DNA G + C content of strain KME 001^T was 75.9 mol%. This value appeared to be slightly higher than that found for other members of the genus *Aeromicrobium*, which range from 65.5 to 74.0 mol%.

On the basis of these phenotypic and phylogenetic data, it is proposed that strain KME 001^T represents a new

Fig. 1. Scanning electron micrograph of cells of strain KME 001^T cultivated at 25 °C for 5 days in A1 + C liquid medium. Bar, 2 μm .

Table 1. Phenotypic characteristics of strain KME 001^T and related species of the genus *Aeromicrobium*

Taxa: 1, strain KME 001^T (data from this study); 2, *A. ginsengisoli* Gsoil 098^T (Kim *et al.*, 2008); 3, *A. erythreum* NRRL B-3381^T (Miller *et al.*, 1991; Cui *et al.*, 2007); 4, *A. ponti* HSW-1^T (Lee & Lee, 2008); 5, *A. alkaliterrae* KSL-107^T (Yoon *et al.*, 2005; Kim *et al.*, 2008); 6, *A. marinum* T2^T (Bruns *et al.*, 2003; Yoon *et al.*, 2005; Kim *et al.*, 2008); 7, *A. panaciterrae* Gsoil 161^T (Cui *et al.*, 2007); 8, *A. tamense* SSW1-57^T (Lee & Kim, 2007); 9, *A. fastidiosum* DSM 10552^T (Collins & Stackebrandt, 1989; Tamura & Yokota, 1994; Yoon *et al.*, 2005; Cui *et al.*, 2007); 10, *A. flavum* TYLN1^T (Tang *et al.*, 2008). All strains were positive for utilization of trehalose. +, Positive reaction or growth; w, weakly positive; –, negative reaction or no growth; ND, not determined.

Characteristic	1	2*	3*	4*	5†	6†	7†	8†	9†	10†
Cell morphology	Rods	Cocci	Irregular rods, cocci	Rods	Rods, cocci	Rods	Rods	Irregular rods	Rods, cocci	Irregular rods
Motility	–	–	–	–	–	–	–	–	+	–
Temperature range (°C)	10–42	ND	21–40	4–42	4–35	4–35	15–30	10–42	5–30	25–37
Optimum temperature for growth (°C)	25	30	35	30–37	25	25	ND	30	25	30
pH range	5.0–10.0	5.0–8.5	5.0–9.0	4.1–12.1	6.0–11.0	5.5–9.5	5.0–8.5	5.1–10.1	5.0–8.0	5.0–10.0
Oxidase	–	+	+	–	–	–	–	–	+	+
Catalase	+	–	+	+	+	+	–	+	+	+
Urease	–	–	–	–	–	–‡	–	–	–	w
Utilization of:										
Acetate	+	+	+	+	–	–‡	+	+	+	ND
L-Arabinose	+	+	+	+	+	–	–	–	+	–
Citrate	–	–	ND	+	–	–	–	–	+	ND
D-Fructose	+	+	+	+	–	–	–	+	+	+
D-Glucose	+	+	+	+	+	–	+	+	+	+
D-Mannose	+	+	–	+	–	–	+	+	+	–
Mannitol	+	–	–	–	w§	+	–	–	–	ND
Maltose	+	+	–	+	+	–	+	+	–	+
Rhamnose	–	+	–	–	–§	–	–	–	–	–
Salicin	–	–	–	w	+	–	+	–	–	–
Sucrose	+	+	+	+	+	–	+	+	+	+
D-Xylose	+	+	+	+	–	–	–	–	+	–
Enzyme activities (API ZYM):										
Acid phosphatase	–	+	+	+	+	–	+	+	+	–
Alkaline phosphatase	–	–	+	+	–	–	+	+	+	–
Esterase (C4)	+	+	w	+	+	+	w	–	+	+
α-Glucosidase	w	+	+	+	+	–	–	+	+	+
Naphthol-AS-BI-phosphohydrolase	w	+	–	–	+	w	+	w	w	–
Trypsin	–	–	w	–	–	–	–	w	–	–
DNA G + C content (mol%)	75.9	66.8	71–73	74.0	71.5	70.6	65.5	72.7	71–72	73.3

*The results of API ZYM tests for taxa 2, 3 and 4 were derived from our experiments, while other data were from previously reported literature (Kim *et al.*, 2008; Miller *et al.*, 1991; Cui *et al.*, 2007; Lee & Lee, 2008).

†All data were from the above-mentioned literature for each type strain.

‡Data from Kim *et al.* (2008). The API ZYM data for taxon 6 were from Yoon *et al.* (2005). Other data in the same column were from Bruns *et al.* (2003).

§Data from Kim *et al.* (2008). Other data in the same column were from Yoon *et al.* (2005).

member of the genus *Aeromicrobium*. The name *Aeromicrobium halocynthiae* sp. nov. is proposed.

In order to investigate secondary metabolite production by strain KME 001^T, the strain was cultured in ten 1 l Erlenmeyer flasks each containing 500 ml A1 + C broth. 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 every day (from days 3–7) by using an Agilent 1100 LC-MS system using a Phenomenex Luna 5 µm C-18(2) analytical column (4.6 × 150 mm) (see Supplementary Fig. S1 in IJSEM Online). At the end of the culture period (day 7), 20 g l^{–1} Amberlite XAD-7 adsorbent resin was added to each flask, followed by

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain KME 001^T, recognized species of the genus *Aeromicrobium* and some species of related taxa. Bootstrap values (>50 %) obtained with 1000 resamplings are given at the branch-points. Bar, 0.01 substitutions per nucleotide position.

shaking for two additional hours. The resin was then collected by filtration through cheesecloth, washed with deionized water and eluted twice with acetone. The acetone solution was concentrated under reduced pressure to yield 1.2 g crude extract. The acetone extract was fractionated using silica gel flash column chromatography and a step gradient elution with methylene chloride and methanol (20:1, 10:1, 3:1 and methanol) to give four subfractions. The 100 % methanol fraction (374 mg) was dried *in vacuo* and fractionated by reverse-phase HPLC using an isocratic elution of 23 % acetonitrile in water [flow rate 4 ml min⁻¹, Phenomenex Luna C18(2) 10 µm 10 × 250 mm]. Taurocholic acid (3 mg) was purified by repeated HPLC in the same manner as the 100 % methanol fraction. Taurocholic acid was obtained as an optically active, pale yellowish powder. The structure of the taurocholic acid was characterized by comparing the physicochemical properties and spectroscopic data (see Supplementary Figs S2 and S3) with values previously reported in the literature (Gowda *et al.*, 2006; Ijare *et al.*, 2005). Cholic acid is synthesized from cholesterol in the mammalian liver. To determine whether cholesterol, the precursor of many cholic acids, was present in the A1 + C liquid medium used to cultivate strain KME 001^T, TLC analysis of the A1 + C liquid medium alone were performed and compared with authentic cholesterol (see Supplementary Figure S4). No cholesterol was detected in the medium, suggesting that strain KME 001^T was capable of producing taurocholic acid *de novo*. To the authors' knowledge, strain KME 001^T is the first example of a taurocholic acid-producing bacterium.

Description of *Aeromicrobium halocynthiae* sp. nov.

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

Cells are Gram-positive, aerobic, non-spore-forming, rod-shaped and non-motile. Cells are approximately 0.4–0.5 µm in diameter and 4.1–6.0 µm in length. When grown on A1 + C agar plates at 25 °C for 5 days, colonies

are circular, convex, smooth, light yellowish and 0.6–1 mm in diameter. Growth is observed at 10–42 °C, with optimal growth at 25 °C. Growth occurs at pH 5–10. Grows in culture media containing 0–6 % (w/v) NaCl. No growth on TSA medium. Oxidase-negative and catalase-positive. Produces acetoin and oxidizes arabinose. Does not utilize citrate, does not produce H₂S or indole and does not oxidize glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose or amygdalin. The following carbon sources are utilized: acetate, glycerol, L-arabinose, ribose, D-xylose, D-glucose, D-fructose, D-mannose, inositol, mannitol, maltose, sucrose, trehalose, turanose, D-tagatose and 5-ketogluconate. Erythritol, D-arabinose, L-xylose, adonitol, methyl β-xyloside, galactose, L-sorbose, rhamnose, dulcitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, β-gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate and 2-ketogluconate are not utilized as sole carbon and energy sources. With API ZYM and API 20E systems, esterase (C4), esterase lipase (C8) and leucine arylamidase activities are detected. α-Glucosidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase are weakly active. The following enzyme activities are not detected: alkaline phosphatase, lipase (C14), cystine arylamidase, trypsin, acid phosphatase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, cytochrome-oxidase and gelatinase. LL-Diaminopimelic acid is the diagnostic diamino acid of the cell-wall peptidoglycan. The predominant menaquinone is MK-9(H₄). The major fatty acids are C_{18:1}ω₉C, C_{16:0} and 10-methyl C_{18:0}. The type strain produces taurocholic acid, a bile acid, as a major secondary metabolite.

The type strain, KME 001^T (=JCM 15749^T=KCCM 90079^T), was isolated from a marine ascidian, *Halocynthia roretzi*, collected off the coast of Gangneung,

Korea. The DNA G+C content of the type strain is 75.89 mol%.

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