Shewanella halifaxensis sp. nov., a novel obligately respiratory and denitrifying psychrophile

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Indigenous bacteria found in the sediment of the Emerald Basin (depth of 215 m, Atlantic Ocean) located offshore of Halifax Harbour (Nova Scotia, Canada) were previously found to be able to degrade the explosive compound hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). In the present study, a novel obligately respiratory, denitrifying and RDX-mineralizing bacterium, designated strain HAW-EB4^T, was isolated from the marine sediment. This bacterium utilized peptone, yeast extract, Casamino acids, esters (Tweens 20, 40 and 80), sugars (*N*-acetyl-D-glucosamine, ribose), several C₂ and C₃ acids (acetate, pyruvate, lactate, propionate) and amino acids (serine, proline) as sole carbon and energy sources. Aerobically grown cells (in marine broth 2216 at 10 °C) contained C_{14:0} (6%), iso-C_{15:0} (12%), C_{16:0} (20%), C_{16:1ω7} (37%), C_{18:1ω7} (7%) and C_{20:5ω3} (7%) as major membrane fatty acids, and Q7 (28.1%) and MK-7 (60.9%) as dominant respiratory quinones, consistent with deep-sea species of *Shewanella*. The novel bacterium had a DNA G + C content of 45 mol% and showed similarity to *Shewanella* species in terms of 16S rRNA and *gyrB* gene sequences (93–99 and 67.3–88.4% similarity, respectively), with *Shewanella pealeana* being the most closely related species. Genomic DNA–DNA hybridization between strain HAW-EB4^T and *S. pealeana* revealed a level of relatedness of 17.9%, lower than the 70% species cut-off value, indicating that strain HAW-EB4^T (= NCIMB 14093^T = DSM 17350^T) is the type strain of a novel species of *Shewanella*, for which the name *Shewanella halifaxensis* sp. nov. is proposed.

The genus *Shewanella* was first described by MacDonell & Colwell (1985) to accommodate Gram-negative, oxidase-positive, rod-shaped, aquatic and marine bacteria with genomic DNA G + C contents of 39–53 mol%. Twenty-nine species of *Shewanella* were recognized at the time of writing (Bozal *et al.*, 2002; Brettar & Hofle, 2002; Ivanova *et al.*, 2001, 2003a, b, 2004a, b, c; Leonardo *et al.*, 1999; Skerratt *et al.*, 2002; Satomi *et al.*, 2003; Toffin *et al.*, 2004; Venkateswaran *et al.*, 1999; Xu *et al.*, 2005; Yoon *et al.*, 2004a, b). Deep-sea *Shewanella* species such as *Shewanella violacea*, *S. benthica*, *S. woodyi*, *S. hanedai* and *S. marininitestina* require Na⁺ and low temperature for growth and produce polyunsaturated fatty acids (Bowman *et al.*, 1997;

Deming *et al.*, 1984; Jensen *et al.*, 1980; Kato *et al.*, 1998; Kato & Nogi, 2001; MacDonell & Colwell, 1985; Makemson *et al.*, 1997; Nogi *et al.*, 1998; Russell & Nichols, 1999; Satomi *et al.*, 2003). Mesophilic and non-Na⁺-requiring *Shewanella* species such as *Shewanella algae*, *S. amazonensis*, *S. decolorationis*, *S. japonica*, *S. oneidensis* and *S. putrefaciens* are usually found in coastal regions, estuaries and/or non-marine environments (Ivanova *et al.*, 2001; Long & Hammer, 1941; Nozue *et al.*, 1992; Simidu *et al.*, 1990; Venkateswaran *et al.*, 1998b, 1999; Xu *et al.*, 2005; Zhao *et al.*, 2005). Some species of *Shewanella* are known for their potential to degrade organic pollutants (Petrovskis *et al.*, 1994; Semple & Westlake, 1987; Xu *et al.*, 2005; Zhao *et al.*, 2004b). The strain described herein, designated HAW-EB4^T, was initially isolated as an effective RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)-mineralizing bacterium from marine sediment obtained from a munitions-dumping area located in Emerald Basin, Atlantic Ocean, offshore Halifax Harbour, Nova Scotia, Canada (Zhao *et al.*, 2004b). In the present study, we found that this bacterium represented a novel obligately respiratory and denitrifying species of *Shewanella*.

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Abbreviations: RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine; TMAO, trimethylamine *N*-oxide.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of strain HAW-EB4^T are AY579751 and AY842131.

As we reported previously (Zhao *et al.*, 2004b), strain HAW-EB4^T was a psychrophilic bacterium (optimum growth at 10 °C) and grew well aerobically on Brewer anaerobic agar (Becton Dickinson) supplemented with sea salts (4%; Sigma), producing a slightly dark orange or pinkish pigmentation similar to other species of *Shewanella* (Venkateswaran *et al.*, 1999). Cells harvested after 3 days aerobic incubation in marine broth 2216 (Difco) at 10 °C (spun at 180 r.p.m.) were used for transmission (TEM; Hitachi H7500) or scanning (SEM; Hitachi S3000N) electron microscopic image analyses. For TEM, cells were negatively stained with phosphotungstic acid (0.5%, w/v; pH 6.0) as described by Bozal *et al.* (2002) and Beveridge *et al.* (1994). For SEM, cells were collected on poly-L-lysine-coated coverslips, treated with glutaraldehyde/formaldehyde, tannic acid and osmium tetroxide as described by Prevost *et al.* (1992), dried using hexamethyldisilazane and sputter-coated with gold/palladium. Like those of most *Shewanella* species, cells of strain HAW-EB4^T were Gram-negative, straight or slightly curved rods. Electron microscopic image analyses showed that cells were 1.5–3 µm long with a diameter ranging from 0.55 to 0.65 µm (Fig. 1a) and had a single polar flagellum (Fig. 1b).

To determine the phylogenetic affiliation of strain HAW-EB4^T, we purified its genomic DNA (1–5 mg) using the

Marmur method (Johnson, 1994), and amplified its 16S rRNA and *gyrB* (coding for the β -unit of DNA topoisomerase II) genes using universal primers (Yamamoto & Harayama, 1995; Venkateswaran *et al.*, 1998a) and standard molecular protocols (Sambrook & Russell, 2001). The partial 16S rRNA (1293 bases) and *gyrB* (938 bases) genes were subsequently sequenced and compared with published sequences using BLAST (NCBI GenBank; <http://www.ncbi.nlm.nih.gov/>). The gene sequences of strain HAW-EB4^T and those of closely related species were aligned using CLUSTAL_X (version 1.81). The neighbour-joining method included in the MEGA2 package (Kumar *et al.*, 2001), based on pairwise nucleotide distances with the Kimura two-parameter correction, was used to construct the phylogenetic tree. The number of bootstrap repetitions was 4000.

The genomic DNA G+C content of strain HAW-EB4^T, determined using UV absorbance (A_{269}/A_{280}) at pH 3.0 and its thermal melting profile (T_m) (Johnson, 1985a), was found to be 45 mol%, consistent with reported values for other species of *Shewanella* (39–53 mol%). Phylogenetic analyses of the 16S rRNA gene sequence clearly showed that strain HAW-EB4^T belonged to the genus *Shewanella* (93–99% similarity to recognized *Shewanella* species); it was related most closely to *Shewanella pealeana* (99%), found in Atlantic squid accessory nidamental gland (Leonardo *et al.*, 1999), *S. marinintestina*, *Shewanella schlegeliana* and *Shewanella sairae*, which are marine intestinal species (98.6–99%) (Satomi *et al.*, 2003), and *Shewanella gelidimarina*, found in Antarctic congelation ice (97.9%) (Bowman *et al.*, 1997) (Fig. 2). Among the above related species, *S. pealeana* was found to be phylogenetically most closely related to HAW-EB4^T (99% similarity, bootstrap value of 99%) (Fig. 2). In comparing the *gyrB* gene sequences, strain HAW-EB4^T showed 67.3–88.4% similarities to species of *Shewanella*, and was most similar to *S. pealeana* (Fig. 3). However, *gyrB* gene sequence similarity between HAW-EB4^T and *S. pealeana* was 88.4%, lower than the 90% species cut-off value recommended for *Shewanella* species (Venkateswaran *et al.*, 1999), suggesting that the two bacteria belong to different species.

Strain HAW-EB4^T was also found to be phylogenetically distant from another Halifax sediment *Shewanella* isolate, *Shewanella sediminis* HAW-EB3^T (Zhao *et al.*, 2005), with low 16S rRNA (96.4%; Fig. 2) and *gyrB* (83.3%; Fig. 3) gene sequence similarities.

We conducted genomic DNA–DNA hybridization between strain HAW-EB4^T and *S. pealeana* using the spectrophotometric method as described by Johnson (1985b) and Bowman *et al.* (1998). Strain HAW-EB4^T and *S. pealeana* had a level of genomic DNA relatedness of 17.9%, which was much lower than the 70% species cut-off value accepted for bacteria (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Gillis *et al.*, 2001), demonstrating that strain HAW-EB4^T represents a novel species of *Shewanella*.

Fig. 1. Micrographs of cells of strain HAW-EB4^T: (a) scanning electron micrograph of cells; (b) transmission electron micrograph of a negatively stained cell showing a single polar flagellum. Bars, 2.5 µm (a) and 1 µm (b).

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences of type strains of *Shewanella* species and strain HAW-EB4^T. The phylogenetic tree was generated based on pairwise nucleotide distances with Kimura two-parameter correction using the neighbour-joining method (pairwise deletion) included in the MEGA2 software package (Kumar *et al.*, 2001). Numbers beside the nodes are bootstrap values (percentage of 4000 replications). Bar, 1 % sequence divergence.

To compare the chemotaxonomic properties of strain HAW-EB4^T with those of recognized species of *Shewanella*, we characterized the respiratory quinone and membrane fatty acid composition of strain HAW-EB4^T (grown aerobically in marine broth 2216 at 10 °C) using previously described chromatographic methods (Collins, 1985, 1994; Nishijima *et al.*, 1997; Akagawa-Matsushita *et al.*, 1992; Bowman, 2001; Fay & Richli, 1991; Zhao *et al.*, 2005). Briefly, strain HAW-EB4^T produced mainly straight-chain fatty acids such as palmitic (C_{16:0}, 20 %), myristic (C_{14:0}, 6 %) and palmitoleic (C_{16:1}ω7, 37 %) acid, branched fatty acids (iso-C_{13:0}, 3 %; iso-C_{15:0}, 12 %) and polyunsaturated eicosapentaenoic acid [C_{20:5}ω3 (EPA), 7 %], similar to related species of *Shewanella* (Table 1). The major respiratory quinones of strain HAW-EB4^T were ubiquinones (Q7, 28.1 %) and menaquinones (MK-7, 60.9 %), consistent with *Shewanella* species (Akagawa-Matsushita *et al.*, 1992; Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002; Zhao *et al.*, 2005).

NaCl tolerance was tested on Brewer anaerobic agar that contained 0.5, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 or 8.0 % NaCl (aerobic growth conditions) using a previously described protocol (Zhao *et al.*, 2005). Growth in the absence of Na⁺ was tested in a Na⁺-free agar that contained 0.3 % Bacto beef extract (Difco) and 0.5 % Bacto peptone (Difco). Strain HAW-EB4^T required Na⁺ for growth and was slightly halophilic, with growth at 0.5–4 % NaCl. However, strain HAW-EB4^T appeared to have a lower tolerance to NaCl (optimum growth at 2 % NaCl) than *S. pealeana* (3 %).

We also identified the electron acceptors of strain HAW-EB4^T and compared them with those of recognized *Shewanella* species. Reduction of the following potential electron acceptors by strain HAW-EB4^T was tested in anaerobic jars on Brewer anaerobic agar supplemented with 2 % NaCl and one of the following substrates: MnO₂ (40 mM), ferric citrate (40 mM), amorphous iron oxide (FeOOH, 40 mM)

Fig. 3. Phylogenetic tree based on *gyrB* gene sequences of *Shewanella* species and strain HAW-EB4^T. The phylogenetic tree was generated based on pairwise nucleotide distances with Kimura two-parameter correction using the neighbour-joining method (pairwise deletion) included in the MEGA2 software package (Kumar *et al.*, 2001). Numbers beside nodes are bootstrap values (percentage of 4000 replications; values below 50 % not shown). Bar, 5 % sequence divergence.

Table 1. Fatty acid composition (percentages of total) of strain HAW-EB4^T and related *Shewanella* species

Taxa: 1, HAW-EB4^T; 2, *S. pealeana*; 3, *S. gelidimarina*; 4, *S. schlegeliana*; 5, *S. marinintestina*; 6, *S. sairae*. —, Not detected; ND, no data. Data for strain HAW-EB4^T from this study; data for reference species taken from Bowman *et al.* (1997), Leonardo *et al.* (1999) and Satomi *et al.* (2003).

Fatty acid	1	2	3	4	5	6
C _{12:0}	—	0.2–4	0.3–0.7	1.1	0.3	0.5
iso-C _{13:0}	3	2.6–13.8	5.4–15.8	2.8	2.8	3.4
C _{13:0}	—	0.9–1.4	0.1–1.3	—	—	1.9
C _{14:0}	6	2.6–5.6	3.8–5.2	4.2	4.4	5.0
C _{14:1}	1	—	0.1–0.8	ND	ND	ND
iso-C _{15:0}	12	8.4–16	8.3–13.2	6.4	9.2	14
anteiso-C _{15:0}	1	1.2	—	0.7	0.1	0.2
C _{15:0}	1	1.2–5	1.6–6.7	0.1	0.6	0.3
C _{16:0}	20	18.6–25	6.4–13.9	22.7	22	18
C _{16:1ω9}	1	0.9	0–0.7	—	—	—
C _{16:1ω7}	37	19.4	27.4–37	26.1	26.1	28.1
C _{17:0}	—	0.7–2	0.4–0.7	—	0.6	0.1
C _{17:1}	—	1–1.2	2.7–5.1	0.5	1.0	0.8
C _{18:0}	2	3–4.1	0.1–0.3	1.2	3.1	1.0
C _{18:1ω9}	—	2.2	0.3–0.9	3.3	2.1	—
C _{18:1ω7}	7	4–6.3	1.0–8.4	6.5	6.9	9.8
C _{20:4ω3}	1	1.6	0.3–0.8	2.2	1.1	0.7
C _{20:5ω3}	7	19.1	11.7–16	18.6	17.5	15.2

or elemental sulfur (40 mM). Anaerobic respiration on trimethylamine *N*-oxide (TMAO) (10 mM), nitrate (20 mM) or nitrite (5 mM) as an electron acceptor (0.1 % Casamino acids as carbon and energy sources) was tested in sea salts mineral medium (5 ml in 20 ml sealed serum bottles, initial biomass of 0.07 OD₆₀₀) under anaerobic conditions (the medium was made anoxic by degassing under vacuum and charging the headspace with argon, and with no reducing agents added). Clear zones around colonies were used to indicate iron(III), manganese(IV) and sulfur reduction (Myers & Nealson, 1988). Enhanced bacterial growth in the presence of electron acceptors (compared with that in their absence) was used as an indicator for dissimilatory reduction of nitrate, nitrite and TMAO. Bacterial growth on agar was estimated by multiplying the mean area of colonies and total number of colony-forming units (Zhao *et al.*, 2005). Similar to *S. pealeana* ATCC 700345^T (Leonardo *et al.*, 1999), strain HAW-EB4^T was a respiratory bacterium that did not grow by fermentation but grew (0.1 % Casamino acids, 6–16 days, 10 or 21 °C) by respiring on oxygen, nitrate, nitrite or TMAO, with a final biomass increase of 0.12–0.6 OD₆₀₀. Strain HAW-EB4^T reduced MnO₂ as was the case with *S. pealeana*, but did not reduce two other electron acceptors of *S. pealeana*, iron(III) and elemental sulfur (Table 2). Strain HAW-EB4^T reduced nitrate and nitrite to nitrous oxide (Casamino acids as carbon source), confirming its identity as a denitrifying

bacterium probably involved in denitrification in the Halifax harbour sediment.

Using previously described protocols (Bowman, 2001; Zhao *et al.*, 2005) and marine broth 2216 as a basal medium, we characterized strain HAW-EB4^T for spore formation, acid production from sugars (in Leifson modified O-F medium; Smibert & Krieg, 1994), H₂S formation from thiosulfate (1 %) and hydrolysis of DNA (in BBL DNA agar plus 4 % sea salts), casein (skimmed milk, 50 %), gelatin (1 %), Tweens 20 (1 %), 40 (1 %) and 80 (1 %), olive oil (1 %), lecithin (5 % egg yolk), pure chitin (0.3 %), alginate (1 %) and starch (1 %). Additional enzyme activities and substrate metabolisms were tested by using API Rapid 20E and ID32A (bioMérieux) test kits (10 and 37 °C) and GN2 microplates (Biolog; cell biomass of 0.9 OD₆₀₀ suspended in sea-salts medium for 7 days at 10 °C). Utilization of carbon substrates (0.1 %) by strain HAW-EB4^T and *S. pealeana* ATCC 700345^T (purchased from the American Type Culture Collection) was tested aerobically at both 10 °C (optimum growth of HAW-EB4^T) and 21 °C (optimum growth of *S. pealeana*) in basic marine salts medium (pH 7.0, 0.1 % NH₄Cl) in the presence or absence of 0.02 % yeast extract or 0.001 % choline chloride. Bacterial growth (OD₆₀₀) was monitored every 3–6 days over 1 month (initial biomass of 0.07 OD₆₀₀). All phenotypic tests were run in triplicate.

Cluster analyses of phenotypic properties (enzyme activities, growth substrates, salt and temperature tolerance) of strain HAW-EB4^T and recognized species of *Shewanella* using the method of Zhao *et al.* (2005) also indicated that strain HAW-EB4^T was similar to deep-sea species of *Shewanella*. Like *S. pealeana* (Leonardo *et al.*, 1999), strain HAW-EB4^T grew better in the presence of choline chloride (peptone, Casamino acids or yeast extract as carbon source; 1000–1300 % increase in OD₆₀₀) than in its absence (600–900 % increase in OD₆₀₀) (10 °C, 16 days). However, strain HAW-EB4^T differed from *S. pealeana* (Table 2) and three other known marine fish or scallop intestinal *Shewanella* species, *S. marinintestina*, *S. schlegeliana* and *S. sairae*, on activities of some enzymes and utilization of several carbon sources (Satomi *et al.*, 2003). For example, strain HAW-EB4^T produced much less biomass (15–150 % increase in OD₆₀₀) than *S. pealeana* (150–700 % increase in OD₆₀₀) at both 10 and 21 °C when growing on single compounds as sole carbon sources (Table 2). Yeast extract appeared to improve utilization of several carbon sources by strain HAW-EB4^T (Table 2).

Finally, strain HAW-EB4^T was able to utilize the biodegradation product of RDX, formate, as sole carbon and energy source for growth (at 21 °C) (Table 2), suggesting that RDX can be utilized by strain HAW-EB4^T during potential occurrence of *in-situ* natural attenuation of RDX at the contaminated site (Zhao *et al.*, 2004a, b).

The phenotypic, chemotaxonomic and genetic data presented here demonstrate that strain HAW-EB4^T is a distinct marine bacterium, representing a novel species of

Table 2. Phenotypic properties of strain HAW-EB4^T and related species of *Shewanella*

Taxa: 1, HAW-EB4^T; 2, *S. pealeana*; 3, *S. gelidimarina*; 4, *S. schlegeliana*; 5, *S. marinintestina*; 6, *S. sairae*. All data for strain HAW-EB4^T and growth substrate data for *S. pealeana* were from the present study. Other data were from Bowman *et al.* (1997), Satomi *et al.* (2003), Leonardo *et al.* (1999) and Kato & Nogi (2001). All listed species grew on *N*-acetyl-D-glucosamine, ribose and valerate as sole carbon sources. +, Positive; w+, weakly positive; –, negative; ND, no data. Values in parentheses for HAW-EB4^T and *S. pealeana* indicate the increase in biomass (OD₆₀₀; as a percentage relative to initial value of 0.07) after 6–16 days incubation in basic sea-salts medium at 10 or 21 °C.

Characteristic	1	2	3	4	5	6
Habitat	Atlantic sediment	Atlantic squid accessory nidamental gland	Antarctica sea ice	Black porgy intestine	Squid intestine	Pacific saury intestine
Pigmentation	Orange/pink	Slightly pink	Tan	Ivory	Ivory	Ivory
Fe(III) reduction	–	+	+	ND	ND	ND
Sulfur (S ⁰) reduction	–	+	ND	ND	ND	ND
Temperature optimum (°C)	10	25	15–17	20–25	20–25	20–25
Growth at 30 °C	–	+	–	+	–	–
Growth at 6 % NaCl	–	–	+	–	–	–
Gelatinase	+	–	+	+	+	+
Caseinase	–	ND	+	ND	+	+
Chitinase	+	–	+	–	–	–
Growth with sole carbon sources:						
Glucose	w+ (15–50)	+	–	+	+	+
Acetate	w+* (15–50)	+	+	–	+	–
Butyrate	– (<15)	+	+	–	–	–
Leucine	– (<15)	+	–	–	+	–
Lactate	+* (50–150)	+	+	–	–	–
Galactose	– (<15)	w+ (15–50)	–	–	–	–

*Yeast extract enhanced growth.

Shewanella, for which the name *Shewanella halifaxensis* sp. nov. is proposed.

Description of *Shewanella halifaxensis* sp. nov.

Shewanella halifaxensis (ha.li.fax.en'sis. N.L. fem. adj. *halifaxensis* from Halifax, Nova Scotia, a harbour city near the sediment sampling site, Emerald Basin, where the type strain was isolated).

Cells are Gram-negative, non-spore-forming, straight or slightly curved rods generally 2–3 µm long and 0.55–0.65 µm in diameter. Cells 6 µm long also occur. Motile by a single polar flagellum. Biomass is slightly dark yellow and non-bioluminescent. Psychrophilic growth at temperatures of 4–25 °C (optimum at 10 °C). No growth at 30 °C. Na⁺-requiring and slightly halophilic (0.5–4 % NaCl; optimum at 2 %). Growth at 4 % but not at 6 % NaCl. MnO₂, nitrate, nitrite, TMAO, thiosulfate and RDX are reduced. No reduction of Fe³⁺ (iron oxide or ferric citrate) or elemental sulfur. Strong production of H₂S from thiosulfate. Anaerobic growth by reducing nitrate (to N₂O), nitrite or TMAO, but not by fermentation. Positive for catalase, oxidase, nitroreductase, chitinase, DNase, gelatinase, urease, arginine

dihydrolase, ornithine decarboxylase, lysine decarboxylase, glutamic acid decarboxylase, *N*-acetyl-β-D-glucosaminidase, β-galactosidase, β-galactosidase-6-phosphate, alkaline phosphatase, leucine arylamidase and alanine arylamidase; weakly positive for hydrolysis of Tweens 20, 40 and 80, proline arylamidase and arginine arylamidase; negative for caseinase, alginase, amylase, α-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, histidine arylamidase, serine arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycylarylamidase, leucine glycine arylamidase, α-fucosidase and glutamyl glutamic acid arylamidase. On GN2 microplate (10 °C, 7 days), positive for metabolism of *N*-acetyl-D-glucosamine, α-D-glucose, methylpyruvate, DL-lactic acid, L-alanine, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-serine, inosine, uridine and thymidine; weakly positive for Tweens 40 and 80, acetic acid, β-hydroxybutyric acid, α-ketobutyric acid, L-leucine and L-threonine; negative for α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, D-fructose, D-fucose, D-galactose, gentiobiose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose,

D-trehalose, turanose, xylitol, succinic acid monomethyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α - or γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, phenylethylamine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. Weak aerobic acid formation from *N*-acetyl-D-glucosamine and glucose. No anaerobic acid formation from *N*-acetyl-D-glucosamine, fructose, sucrose, galactose, lactose, mannose or glucose. Peptone, yeast extract, Casamino acid, Tweens (20, 40 and 80), propionate, pyruvate, serine and proline are sole carbon and energy sources for growth. *N*-Acetyl-D-glucosamine, ribose, lactate, acetate, valerate, glutamate, formate and glycine are weak growth substrates. Alanine, threonine, leucine, butyrate and glucose are poorly utilized. Succinate, citrate, malate and fumarate are not utilized. Choline and yeast extract are not required but improve growth on certain substrates. Fatty acids iso-C_{13:0} (3%), C_{14:0} (6%), C_{14:1} (1%), anteiso-C_{15:0} (1%), C_{15:0} (1%), iso-C_{15:0} (12%), C_{16:0} (20%), C_{16:1} ω 7 (37%), C_{16:1} ω 9 (1%), C_{18:0} (2%), C_{18:1} ω 7 (7%), C_{20:5} ω 3 (7%) and C_{20:4} ω 3 (1%) are produced. Quinone composition is Q7 (28.1%), Q8 (4.0%), MK-7 (60.9%) and MMK-7 (6.9%). The DNA G + C content is 45 mol%.

The type strain is HAW-EB4^T (=NCIMB 14093^T=DSM 17350^T), isolated from marine sediment.

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