**Shewanella intestini** sp. nov., isolated from the intestine of abalone, *Haliotis diversicolor*

Yingbao Gai,1,2† Zhaobin Huang,1,2† Qiliang Lai1,2 and Zongze Shao1,2,*

**Abstract**

A Gram-stain-negative, rod-shaped bacterium with a singular polar flagellum, designated as strain XMDDZSB0408<sup>T</sup>, was isolated from the intestine of adult abalone, *Haliotis diversicolor*. Activity of oxidase was positive and catalase activity was negative. It could grow at salinities from 3 to 6 % NaCl (w/v), and pH 7–9. It had the highest sequence similarity of <96.0 % with all previously established species for the complete 16S rRNA gene (1531 bp). The results of phylogenetic analysis indicated that the strain was affiliated to the genus *Shewanella* and closely related to *Shewanella gaetbuli* TF-27<sup>T</sup> and *Shewanella japonica* KMM 3299<sup>T</sup> (95.8 % sequence similarity), *Shewanella electrodiphila* MAR441<sup>T</sup> (95.6 %), *Shewanella pacifica* KMM 3597<sup>T</sup> (95.4 %), *Shewanella donghaensis* LT17<sup>T</sup> (95.3 %) and *Shewanella oleyana* ACEM 9<sup>T</sup> (94.7 %). The respiratory quinones were MK-7, Q-8, Q-7, MK-8, Q-7 (H4) and Q-6. The predominant fatty acids consisted of C<sub>16:0</sub>, summed feature 3 (comprised of C<sub>16:1ω7c</sub>/C<sub>16:1ω6c</sub>), C<sub>18:0</sub>, summed feature 8 (comprised of C<sub>18:1ω7c</sub>/C<sub>18:1ω6c</sub>, C<sub>12:0</sub> and C<sub>14:0</sub>) The polar lipids were identified as phosphatidylethanolamine (PE), a glycolipid (GL), a phospholipid (PL) and one unidentified lipid (L). The DNA G+C content was 41.4 mol% calculated from the draft genome sequence. On the basis of its polyphasic taxonomic properties, strain XMDDZSB0408<sup>T</sup> represented a novel species, for which the name *Shewanella intestini* sp. nov. was proposed, with the type strain XMDDZSB0408<sup>T</sup> (=KCTC 52125<sup>T</sup>=MCCC 1A01895<sup>T</sup>).
Shewanella saini SM2-1T (AB081762)
Shewanella marininterstina IK-1T (AB081757)
Shewanella achelgelana HRRK1T (AB081760)
Shewanella pneumatophori SRCC-2738T (AB204519)
Shewanella pelagia ATCC 700345T (CP000851)
Shewanella haliflexus HAW-EB4T (CP000931)
Shewanella ficta KMM 3562T (AF420212)
Shewanella krasilnikova c911T (AB094598)
Shewanella piezotolerans WP3T (CP000472)
Shewanella psychrophila WP2T (UJ551089)
Shewanella surugensis c962T (AB094597)
Shewanella gelidimarina ACAM 456T (U85907)
Shewanella abyssi c941T (AB201475)
Shewanella violacea DSS12T (AP011177)
Shewanella benthica ATCC 43992T (X82131)
Shewanella haneda CIP 103207T (X82132)
Shewanella marinisediminis DH3T (GC886954)
Shewanella vooodi ATCC 51908T (CP000961)
Shewanella atlantica HAW-EB5T (AY579752)
Shewanella canadensis HAW-EB2T (AY579749)
Shewanella sediminis HAW-EB3T (CP000821)
Shewanella gelidii PV-4T (CP000606)
Shewanella marisflavi SW-117T (AY485224)
Shewanella colevilliana ATCC 39565T (AY653177)
Shewanella algipiscicola 513T (AB205570)
Shewanella litorisediminis SMK1-12T (JQ624139)
Shewanella amazonensis SB2T (CP000507)
Shewanella coronai fav-2-10-05T (FJ041083)
Shewanella mangrovei YQH10T (KJ751544)
Shewanella litorisediminis UDC329T (GQ245918)
Shewanella foidaeae JC10T (FM203122)
Shewanella chikkenis JC6T (FM210003)
Shewanella indica KJW27T (HM016084)
Shewanella haliodis JCM 14758T (B001000107)
Shewanella algae JCM 21037T (B001000089)
Shewanella uponei 20-23RT (GQ260190)
Shewanella japonica KMM 3229T (AF145921)
Shewanella pacifica KMM 3597T (AF500075)
Shewanella electrophilica MAR441T (FR744784)
Shewanella olyaya ACEM 9T (AF295592)
Shewanella donghaensis LT117T (AY326275)
Shewanella intestinis XMDDZSB0408T (KUE663649)
Shewanella decolorationis S12T (AXZL01000039)
Shewanella seohaensis S7-3T (GU944467)
Shewanella gaeubii TF-27T (AY190533)
Shewanella arctica IR12T (GU564402)
Shewanella frigidimarina ACAM 591T (U85903)
Shewanella vesiculosa M7T (AM980877)
Shewanella livingstonensis LMG 19866T (AU300834)
Shewanella aestuarii SC18T (JF751044)
Shewanella dentinificans OS217T (CP000302)
Shewanella algicola ST-6T (FJ093681)
Shewanella basaltis JB8T (EU413381)
Shewanella inventionis KX23T (KT781407)
Shewanella balbica NCTC 10738T (AJ002014)
Shewanella glacialipiscicola T147T (AB205571)
Shewanella morhuae U1411T (AB205576)
Shewanella halnihei P010T (AB205566)
Shewanella profunda DSM 14900T (FR733713)
Shewanella putrefaciens M1228T (X81663)
Shewanella amendenensis S4T (FJ589031)
Shewanella onomoidensis MR-1T (AE14299)
Shewanella marina C4T (EU290154)
Shewanella inccinea UST400317-098T (DQ180743)
Shewanella spongiae HU098T (DQ167234)
Pseudomonas chlororaphis DSM 50083T (Z76673)

Fig. 1. Phylogenetic tree, reconstructed using neighbour-joining method based on the 16S rRNA gene sequences, indicating the relationship of XMDDZSB0408T with the type strains of species of the genus Shewanella. Bootstraping was carried out with 1000 replicates. Branch node values below 50 are not shown. Pseudomonas chlororaphis DSM 50083T was chosen as the outgroup. Bar, 0.01 nucleotide substitutions per position.
The draft genome of XMDDZSB0408\textsuperscript{T} was obtained using a HiSeq 2000 platform (Illumina) by Shanghai Majorbio BioPharm Technology (Shanghai, PR China) according to the manufacturer's instructions. About 1 gigabase of paired-end reads (2×150 bp) were retrieved. The raw reads were assembled using SPAdes version 3.6.0 with parameter k to 21, 33, 55, 77 [4]. The genome size of XMDDZSB0408\textsuperscript{T} was about 4.0 Mb, slightly smaller than those of the species within the genus *Shewanella* for which genomes had been sequenced (www.ncbi.nlm.nih.gov-genome/browser/). The genomic DNA G+C content of the contigs was determined to be 41.4 mol%, calculated using QUAST [5]. The complete 16S rRNA gene sequence was annotated from the assembled genome via RNAmmer 1.2 Server (www.cbs.dtu.dk/services/RNAmmer/). The G+C content of the contigs was determined to be 41.4 mol%, calculated using QUAST [5]. The complete 16S rRNA gene sequence was obtained via PCR clone sequencing (1411 bp), was used in the following close relative search and phylogenetic analysis.

The closely related species were searched through the EzTaxon database [6]. Our strain showed the greatest sequence similarity of 95.8 % with both *Shewanella gaetubuli* TF-27\textsuperscript{T} and *Shewanella japonica* KMM 3299\textsuperscript{T}, followed by *Shewanella electrophiloda* MAR441\textsuperscript{T}, *Shewanella aestuarii* SC18\textsuperscript{T} and *Shewanella decolorationis* S12\textsuperscript{T}, all of which showed the same similarity of 95.6 % with the novel strain. The closest sequence was meanwhile searched against nucleotide collection (nr/nt) databases using online Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to check other type strains not deposited in the EzTaxon database. Three different phylogenetic trees were reconstructed using MEGA 6.0 [7], neighbour joining (NJ), maximum likelihood (ML) and minimum evolution (ME). The node support of each tree topology was evaluated using bootstrapping estimation of 1000 replicates. The best substitution model T92+G+I for the ML tree was determined under the lowest Bayesian information criterion (BIC) selection scores using the 24 different nucleotide substitution models. The results of phylogenetic analysis indicated that XMDDZSB0408\textsuperscript{T} was affiliated to the genus *Shewanella*, but formed a unique clade separated from other species, indicating that this strain represents a novel species (Figs 1, S1 and S2). The topology of the ML tree disagreed with that of the NJ tree but with low bootstrapping support (Fig. S2).

XMDDZSB0408\textsuperscript{T} was cultured on MS agar plates at 25 °C for 2 days. The cell morphology was observed using transmission electron microscopy (TEM-1230, JEOL) after negative staining. The cells were rod-shaped, 1.7–4 µm long and 0.5–0.7 µm wide with a singular polar flagellum (Fig. S3). The salinity for growth was determined using the basic medium Luria–Bertani broth supplemented with NaCl [0, 0.5, 1, 2 and 3 % (w/v)]; and using MB supplemented with NaCl [4, 5, 6, 7, 8, 9, 10, 12, 15, 18 and 20 % (w/v)]. The pH for growth was tested in MB medium ranging at pH 3 to 11 at intervals of 1 pH unit, adjusted with citrate/phosphate buffer (pH 3–7), Tris/HCl buffer (pH 8–9) and sodium carbonate/sodium bicarbonate buffer (pH 10–11). Growth was observed at salinities from 3 to 6 % (NaCl, W/V) with an optimal salinity of 5 %, and pH 7–9 with an optimum of pH 7–8.

On the basis of the phylogenetic relationship of XMDDZSB0408\textsuperscript{T} with other species of the genus *Shewanella*, three representative type strains, *S. japonica* KMM 3299\textsuperscript{T} and closely related type strains within the genus *Shewanella*.

**Table 1.** Physiological and biochemical properties of XMDDZSB0408\textsuperscript{T} and closely related type strains within the genus *Shewanella*

<table>
<thead>
<tr>
<th>Isolation source</th>
<th>Abalone</th>
<th>Seawater*</th>
<th>Seawater*</th>
<th>Saline water*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S production</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Enzymatic tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>w</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>−</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>−</td>
<td>+</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Gellanase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Fermentation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>w</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>4-Nitrophenyl-β-D-galactopyranoside</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>N-acetylgalactosamine</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Potassium gluconate</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content</td>
<td>41.4</td>
<td>43–44*</td>
<td>39.5–40.5*</td>
<td>44*</td>
</tr>
</tbody>
</table>

*Data for *S. japonica* KMM 3299\textsuperscript{T} from [10]; for *S. pacifica* KMM 3597\textsuperscript{T} from [9]; for *S. alleyana* ACEM 9\textsuperscript{T} from [11].
Isoprenoid quinones were measured using reversed-phase HPLC as previously described [8]. The respiratory quinones were MK-7 (52.3 %), Q-8 (23.2 %), Q-7 (8.9 %), and Q-6 (2.1 %), which were different from those of the closely related *S. pacifica* [9]. To examine the fatty acid profiles of cells, XMDHZSB0408\(^T\) and the three reference strains were cultured on MS agar plates at 28 °C for 48 h, and the cellular fatty acids were extracted from the cells and identified following the protocol of the standard MIDI (Sherlock Microbial Identification System, version 6B). The predominant fatty acids were \(C_{16:0}\) (18.8 %), summed feature 3 (comprised of \(C_{16:1ω7c/C_{16:1ω6c}}\) (16.3 %), \(C_{18:0}\) (10.7 %), summed feature 8 (comprised of \(C_{18:1ω7c/C_{18:1ω6c}}\) (10.3 %), \(C_{12:0}\) (7.2 %) and \(C_{14:0}\) (6.0 %). The detailed fatty acids composition is listed in Table S2.

Polar lipids were extracted using a chloroform/methanol system and analyzed using one- and two-dimensional TLC using Merck silica gel 60 F254 aluminium-backed thin-layer plates. Total lipids were detected by spraying the plates with 10 % ethanolic molybdophosphoric acid. The polar lipids were identified as phosphatidylethanolamine (PE), a glycolipid (GL), a phospholipid (PL) and one unidentified lipid (L) (Fig. S4).

Combining the above results, XMDHZSB0408\(^T\) represents a novel species within the genus *Shewanella* affiliated with the family *Shewanellaceae* belonging to the order *Alteromonadales* in the class *Gammaproteobacteria*. Thus, *Shewanella intestinii* sp. nov. is proposed, with the type strain XMDHZSB0408\(^T\) (\(=KCTC 52125^T\)=MCCC 1A01895\(^T\)) isolated from the intestine of adult abalone, *Haliotis diversicolor*. The genomic DNA G+C content of the type strain is 41.4 mol%, calculated from the assembly draft genome sequence.

**DESCRIPTION OF SHEWANELLA INTESITINI SP. NOV.**

*Shewanella intestinii* sp. nov. (in. tst. ti’ni. L. gen. n. intestini of the intestine of abalone, where the type strain was isolated).

The bacterial cells are Gram-stain-negative, rod-shaped, 1.7–4 μm long and 0.5–0.7 μm wide with a single polar flagellum. Oxidase activity is positive and catalase activity is negative. Growth is observed at salinities from 3 to 6 % with an optimal salinity of 5 %, and at pH values from 7 to 9 with an optimum of 7–8. Positive for alkaline phosphatase, leucine arylamidase, trypsin, \(α\)-chymotrypsin, acid phosphatase, and \(N\)-acetyl-\(β\)-glucosaminidase; weakly positive for esterase (C4), esterase lipase (C8) and valine arylamidase; and negative for lipase (C14), cysteine arylamidase, naphthol-AS-BI-phosphohydrolase, \(α\)-galactosidase, \(β\)-galactosidase, \(β\)-glucuronidase, \(α\)-glucosidase, \(β\)-glucosidase, \(α\)-mannosidase, \(β\)-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase. Weakly positive for glucose fermentation. Utilization of citrate and Voges–Proskauer reaction are negative. Cannot produce \(H_2S\) or indole. Fermentation of mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose is negative. Nitrate can be reduced to nitrite. Hydrolysis of aesculin is positive and hydrolysis of gelatin is weakly positive. Cannot produce arginine dihydrolase, urease or 4-nitrophenyl-\(β\)-D-galactopyranoside. No growth occurs with \(D\)-glucose, \(L\)-arabinose, \(D\)-mannose, \(D\)-mannitol, \(N\)-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid as a sole carbon source. The predominant fatty acids are \(C_{16:0}\), summed feature 3 (comprised of \(C_{16:1ω7c/C_{16:1ω6c}}\) \(C_{18:1ω7c/C_{18:1ω6c}}\) \(C_{18:0}\) summed feature 8 (comprised of \(C_{18:1ω7c/C_{18:1ω6c}}\) \(C_{12:0}\) \(C_{14:0}\) \(C_{15:0}\). The respiratory quinones are MK-7, Q-8, Q-7, MK-8, Q-7 (H4) and Q-6. The polar lipids are phosphatidylethanolamine (PE), aglycolipid (GL), a phospholipid (PL) and one unidentified lipid (L).

The type strain XMDHZSB0408\(^T\) (\(=KCTC 52125^T\)=MCCC 1A01895\(^T\)) was isolated from the intestine of adult abalone, *Haliotis diversicolor*. The genomic DNA G+C content of the type strain is 41.4 mol%, calculated from the assembly draft genome sequence.

**Funding information**

This work was financially supported by the project of Xiamen Southern Oceanographic Center (14CZP034HJ08) and the National Infrastructure of Microbial Resources of China (NIMR 2016–9).

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


---

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.