Shewanella carassii sp. nov., isolated from surface swabs of crucian carp and faeces of a diarrhoea patient

Yujie Fang,1,2,3 Yonglu Wang,4 Zongdong Liu,5 Binghuai Lu,6 Hang Dai,1,2,3 Biao Kan1,2 and Duochun Wang1,2,3,*

Abstract

Two strains, 08MAS2251T and LZ2016-166, were isolated from diverse samples in China collected from the surface of crucian carp and the faeces of a diarrhoea patient, respectively. Both strains were pink-orange coloured, gram-negative, oxidase- and catalase-positive, facultative anaerobic and motile bacteria, produced H2S and reduced nitrates to nitrites. Growth occurred in the presence of 0–9 % (w/v) NaCl and at 10–42 °C. The optimum conditions were with 1 % (w/v) NaCl and at 35 °C. The phylogenetic tree of 16S rRNA gene demonstrated that strains 08MAS2251T and LZ2016-166 clustered in a distinctive clade next to the species Shewanella chilikensis JC5T within the genus Shewanella. Meanwhile, gyrB gene sequence analysis indicated that the two strains formed an independent branch that was clearly separate from all the other Shewanella species with sequence similarities from 68.49 to 95.74 %. The DNA G+C content of strain 08MAS2251T was 52.68 mol%. Genomic relatedness of in silico DNA–DNA hybridization between strain 08MAS2251T and phylogenetic neighbours ranged from 50.5–51.8 %, below the cutoff of 70 %. In addition, corresponding average nucleotide identity values were between 93.01 to 93.49 %, which were lower than 95 % threshold. The major fatty acids of strain 08MAS2251T were C17:1ω8c (27.2 %), iso-C15:0 (22.5 %), summed feature 3 (C16:1ω6c and/or C16:1ω7c; 8.7 %), C16:0 (6.2 %), iso-C13:0 (5.6 %) and C17:0 (4.5 %). Based on phenotypic and genetic analysis, strains 08MAS2251T and LZ2016-166 are identified as a novel species of the genus Shewanella, for which the name Shewanella carassii sp. nov. is proposed. The type strain is 08MAS2251T (DSM 104682T=CGMCC 1.16033T).

The genus Shewanella, first described by MacDonell and Colwell [1], comprises a group of bacteria that are Gram-negative, oxidase-positive, facultative anaerobic and motile rods [2–4]. At the time of writing, the genus Shewanella contains 66 recognized species (www.bacterio.net/shewanella.html), with three novel species Shewanella invensitans [5], Shewanella gelidii [6] and Shewanella algicola [7] proposed recently. Shewanella species have been isolated from various sources such as marine habitats [8–11], invertebrates [12], fish [13, 14] and clinical samples [15–18]. In addition, several species are identified as potential agents of food contamination and opportunistic pathogens of humans [15, 17].

In this study, two strains designated 08MAS2251T and LZ2016-166 were isolated from freshwater fish and a diarrhoea patient, respectively. On the basis of polyphasic approaches including gene sequence analysis, genomic relatedness together with phenotypic and chemical characteristics, these two isolates were determined as a novel species of the genus Shewanella represented by the strain 08MAS2251T.

Strain 08MAS2251T was isolated from surface swabs of crucian carp collected in Ma’anshan city (31° 67’N 118° 53’E), Anhui province, China, on 10 September 2008. Strain LZ2016-166 was isolated from faecal specimens of a 1-month-old infant with diarrhoea collected in Laizhou city (37° 51’N 121° 27’E), Shandong province, China, on 28 July 2016. Samples were diluted with sterile water and incubated on Luria–Bertani (LB) agar at 35 °C for 24 h.
Strain purification was obtained by streaking of the colonies repeatedly on nutrient agar. Monomorphic pink-orange coloured colonies were isolated and designed strains 08MAS2251T and LZZ2016-166, respectively. Pure cultures of the two strains were stored routinely at −80 °C in LB broth supplemented with 25 % (v/v) glycerol.

Strains DNA was extracted for PCR amplification and sequencing of the 16S rRNA and gyrB genes as well as genome sequencing by means of the commercial genomic DNA purification kit (Promega) by following the manufacturer’s instructions. The 16S rRNA gene for strains 08MAS2251T and LZZ2016-166 was amplified with two universal primers (27F and 1492R), as described previously [19], and the fragments of the gyrB gene were obtained by primers UP-1 and UP-2r by referring to Yamamoto and Harayama [20]. For phylogenetic analysis, the sequences were aligned using MEGA version 6 [21] with reference sequences of Shewanella species available in the GenBank database and Escherichia coli ATCC 11775T served as an outgroup.

Phylogenetic trees were reconstructed by using the neighbour-joining and maximum-likelihood algorithms and evaluated by 1000 bootstrap replications. The model selected was Kimura’s two-parameter with the pairwise-deletion option. The evolutionary distance matrices and sequence similarities were also calculated by using MEGA 6 software.

The phylogenetic analysis of the 16S rRNA gene (1427 bp) was performed by the neighbour-joining method between strains 08MAS2251T, LZZ2016-166 and the 66 type strains of the Shewanella species (Fig. 1). In light of the monophyletic clade and high sequence similarity (99.93 %), the two novel strains represented the same species as members of the genus Shewanella. The neighbour-joining phylogenetic tree demonstrated that strains 08MAS2251T and LZZ2016-166 formed an independent cluster next to Shewanella chilikensis JC5T with 98.70 and 98.62 % similarities, respectively. In addition, the similar phylogenetic relationships was also observed in the maximum-likelihood tree (Fig. S1, available in the online version of this article).

Although, due to the high interspecies sequence similarity among the closely phylogenetic neighbours as well as the existence of sequence variation in copies, the 16S rRNA gene sequences lacked the resolving power to distinguish some closely related Shewanella species [3]. Therefore, the more rapidly evolving housekeeping gene of gyrB was employed for the definition of Shewanella species [22]. The gyrB gene sequences of strains 08MAS2251T and LZZ2016-166, consisting of 1156 bases, were aligned with those of the Shewanella species available from GenBank. In the neighbour-joining phylogenetic tree based on the gyrB gene (Fig. S2), strains 08MAS2251T and LZZ2016-166 formed an aggregated cluster with 99.83 % similarity clearly separated from the other Shewanella species. In addition, the novel strains shared gyrB gene sequence similarities of 68.49–95.74 % to the type strains of genus Shewanella. In addition, phylogenetic trees were reconstructed by the maximum-likelihood method (Fig. S3), showing that strains 08MAS2251T and LZZ2016-166 formed a separate branch outside the cluster that comprised five close species, S. chilikensis, Shewanella indica, Shewanella algae, Shewanella putenei and Shewanella halotis. On the basis of the phylogenetic analysis and sequence similarity data, it was clear that strains 08MAS2251T and LZZ2016-166 served as a novel species in the genus Shewanella.

The draft genomes of novel type strain 08MAS2251T and three type strains, S. chilikensis KCTC 22540T, S. indica KCTC 23171T and S. putenei KCTC 22806T, were sequenced by means of Illumina HiSeq 2000 sequencing. Two paired-end libraries with average insert lengths of 500 and 2000 bp were established, as well as 100× libraries obtained with clean paired-end read data. The filtered Illumina reads were assembled with SOAPdenovo version 2.04. Strain 08MAS2251T generated a 4375287 bp genome, and the DNA G+C content was 52.68 mol % within the bound of the genus Shewanella.

The taxonomic relationship between the strain 08MAS2251T and phylogenetically related Shewanella species was further evaluated by genomic relatedness with the in silico DNA-DNA hybridization (isDDH) and average nucleotide identity (ANI) methods, which are regarded as a replacement for wet-lab DDH [23]. IsDDH values were determined using the genome-to-genome distance calculator (GGDC) (http://ggdc.dsmz.de/) and ANI values were measured by using the EZ Biocloud platform (www.ezbiocloud.net/tools/ani). The isDDH values between strain 08MAS2251T and five closely related Shewanella species, namely S. chilikensis KCTC 22540T, S. indica KCTC 23171T, S. algae JCM 21037T, S. halotis JCM 14758T and S. putenei KCTC 22806T, were 50.5, 51.2, 51.5, 51.7 and 51.8 %, respectively, and the corresponding ANI values ranged from 93.01–93.49 % (Table 1). The isDDH and ANI values were clearly below the species-delimiting threshold, i.e. 70 and 95 %, respectively [24–26], supporting the conclusion that the type strain 08MAS2251T represented a distinct species in the Shewanella genus.

The following phenotypic characteristics were further tested for strains 08MAS2251T and LZZ2016-166, as described previously [27]. The type strains of the most related species based on phylogenetic analysis, S. chilikensis KCTC 22540T, S. indica KCTC 23171T, S. putenei KCTC 22806T and S. algae JCM 21037T, were examined in parallel under the same conditions. All tested strains were Gram-negative, oxidase-positive, facultative anaerobic and motile, in agreement with the description of the genus Shewanella. The temperature range (4, 10, 25, 30, 35, 42, 46 °C) and salt tolerance (0–12 % [w/v] at intervals of 1 % NaCl) for growth were measured in LB broth incubated for up to 7 days. Meanwhile, the optimal growth conditions were assessed by monitoring the optical density of strains cultured in LB broth after 2–18 h at intervals of 2 h. The results indicated that strains 08MAS2251T and LZZ2016-166 were able to grow at 10–42 °C and with 0–9 % (w/v) NaCl. The optimum temperature and salt concentration for growth were 35 °C and 1 % (w/v) NaCl,
Fig. 1. Phylogenetic tree reconstructed by the neighbour-joining method based on the nucleotide sequences of the 16S rRNA gene. The phylogenetic tree was reconstructed from an alignment of 1427 nt and all known type strains of the Shewanella species were included. *E. coli* ATCC 11775\textsuperscript{T} was used as an outgroup. Numbers at nodes indicate bootstrap values (percentage of 1000 replicates).
respectively. The ability to grow on different media was determined with LB agar, sheep blood agar, thiosulfate-citrate-bile-sucrose (TCBS) agar and MacConkey agar (Oxoid) at 35 °C for 48 h. Colonies of strains 08MAS2251 T and LZ2016-166 on LB agar were circular, convex, pink-orange coloured and 2–3 mm in diameter. Haemolysis was observed on blood agar. Besides, two novel strains grew well on TCBS agar with green colonies but weakly on MacConkey agar.

Additional physiological and biochemical properties of two novel strains and four reference type strains were determined using API 20E strips, API 20NE strips and the GN VITEK-2 compact system (bioMérieux) in accordance with the manufacturer’s instructions. Detailed phenotypic results of the tested strains are presented in Table 2. It was demonstrated that the two novel strains utilized malic acid weakly as well as being unable to grow in the presence of 10 % (w/v) NaCl. Several distinctive characteristics could be further evidence for differentiating strains 08MAS2251 T and LZ2016-166 from reference species: S. chilikensis KCTC 22540 T by the absence of urease; from S. indica KCTC 23171 T by a negative result in l-lactate alkalinization; from S. upenei KCTC 22806 T and S. algae JCM 21037 T by the presence of l-proline arylamidase and the absence of ornithine decarboxylase; additionally from S. upenei KCTC 22806 T by the inability of l-lactate alkalinization and γ-glutamyl transferase production (Table 2).

Antimicrobial susceptibilities of strains 08MAS2251 T, LZ2016-166 and related type strains of genus Shewanella to 23 antibiotics, including ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefuroxime, ceftroxime axetil, cefotetan, ceftazidime, ceftriaxone, cepfime, aztreonam, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, cefazolin, imipenem, penicillin and clindamycin, were determined in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [28]. After 24 h incubation at 35 °C, the strains were classified as susceptible, intermediate or resistant according to standards of the CLSI. Antibiotic susceptibility results showed that two novel strains were resistant to cefazolin, intermediate to clindamycin and susceptible to the other antibiotics tested apart from the resistance of type strain 08MAS2251 T to penicillin. The drug resistance profiles that differentiated the novel strains from four related type strains are shown in Table 2.

The determination of chemical characteristics comprising isoprenoid quinones, polar lipids and fatty acids were performed for type strain 08MAS2251 T. Phylogenetically related species were examined as control strains in some cases. Cells of strains cultivated on the LB agar for 24 h at 35 °C were tested following the standard procedure described previously [29–31]. Both menaquinones and ubiquinones as respiratory quinones were discovered in strain 08MAS2251 T. The predominant menaquinones were MK-7 (33.0 %) and MK-8 (65.5 %). The major ubiquinones were Q-7 and Q-8 with area ratios of 44.5 and 55.5 %, respectively. These quinones were commonly contained in the genus Shewanella [32, 33]. Polar lipid analysis conducted by two-dimensional thin-layer chromatography indicated that diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol were the main components in strain 08MAS2251 T, which was similar to that for other Shewanella species (Fig. S4) [32, 33]. The detailed fatty acid profiles of strains 08MAS2251 T and LZ2016-166 as well as five phylogenetic type neighbours are shown in Table S1. In all strains, C17:ω8c, iso-C15:0 summed feature 3 (C16:1ω6c and/or C16:1ω7c), C16:0, iso-C15:0 3-OH and C17:0 3-OH were the predominant fatty acids, which was consistent with previous reports [34]. The two strains, 08MAS2251 T and LZ2016-166, shared identical fatty acid profiles apart from C16:0 and C17:1ω8c. The novel species could be distinguished from all related type species by C15:1ω8c, from S. chilikensis by summed feature 3 (C16:1ω6c and/or C16:1ω7c), from S. indica by summed feature 1 (iso-C15:1 H and/or C13:0 3-OH), from S. upenei by summed feature 8 (C18:1ω6c and/or C18:1ω7c), from S. alga by iso-C15:0 and from S. haliotis by iso-C13:0 3-OH.

Combined with phylogenetic analysis, genomic relatedness data as well as phenotypic and chemical characteristics,

<table>
<thead>
<tr>
<th>Query genome</th>
<th>Reference genome</th>
<th>DDH (%)</th>
<th>Model CI</th>
<th>Distance</th>
<th>G+C difference</th>
<th>ANI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08MAS2251 T</td>
<td>S. chilikensis KCTC 22540 T</td>
<td>50.5</td>
<td>[47.9–53.1 %]</td>
<td>0.0705</td>
<td>0.31</td>
<td>93.01</td>
</tr>
<tr>
<td>08MAS2251 T</td>
<td>S. indica KCTC 23171 T</td>
<td>51.2</td>
<td>[48.6–53.9 %]</td>
<td>0.0688</td>
<td>0.31</td>
<td>93.34</td>
</tr>
<tr>
<td>08MAS2251 T</td>
<td>S. algae JCM 21037 T</td>
<td>51.5</td>
<td>[48.8–54.1 %]</td>
<td>0.0683</td>
<td>0.37</td>
<td>93.42</td>
</tr>
<tr>
<td>08MAS2251 T</td>
<td>S. haliotis JCM 14758 T</td>
<td>51.7</td>
<td>[49.0–54.3 %]</td>
<td>0.0678</td>
<td>0.22</td>
<td>93.46</td>
</tr>
<tr>
<td>08MAS2251 T</td>
<td>S. upenei KCTC 22806 T</td>
<td>51.8</td>
<td>[49.1–54.4 %]</td>
<td>0.0677</td>
<td>0.40</td>
<td>93.49</td>
</tr>
</tbody>
</table>
DESCRIPTION OF SHEWANELLA CARASSII
SP. NOV.

Shewanella carassii (ca.ras’si. i. N.L. gen. n. carassii of Carassius carassius, the scientific name of the crucian carp).

Strains are Gram-negative, oxidase- and catalase-positive, facultative anaerobic, haemolytic and motile rods. Grows well on LB agar, blood agar and TCBS agar, but weakly on MacConkey agar. Colonies on LB agar are pink/orange coloured, circular, convex and opaque with diameter of 2–3 mm after 48 h at 35 °C. Growth occurs with 0–9% (w/v) NaCl (optimum, 1%) and at 10–42 °C (optimum, 35 °C). Positive for H₂S production, nitrate reduction and gelatin hydrolysis. Negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, Voges–Proskauer reaction, indole production and aesculin hydrolysis. Able to utilize N-acetyl-glucosamine and capric acid but unable to assimilate D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose. In the GN VITEK-2 system analysis, strains are positive for Ala-Phe-Pro-arylamidase, β-N-acetyl-glucosaminidase, glutamyl arylamidase pNA, L-proline arylamidase, tyrosine arylamidase, phosphatase, Glu-Gly-Arg-arylamidase and H₂S production, variable for L-phenylalanin arylamidase and succinate alkalinization and negative for the remaining tests. Both strains are sensitive to ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefuroxime, cefuroxime axetil, cefotetan, ceftazidime, ceftriaxone, cefepime, aztreonam, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprime/sulfamethoxazole and imipenem, intermediate to clindamycin and resistant to cefazolin, whereas inconsistent in their sensitivity of penicillin. The predominant fatty acids are C₁₇:₀-ω₈c, iso-C₁₅:₀, summed feature 3 (C₁₆:₁-ω₆c and/or C₁₆:₁-ω₇c), C₁₆:₀, iso-C₁₅:₀ and C₁₇:₀.

The type strain, 08MAS2251T (=DSM 104682T=CGMCC 1.16033T), was isolated from surface swabs of crucian carp collected in Ma’anshan city, Anhui province, China, on 29 July 2008. An additional strain, LZ2016-166 was isolated from faecal samples of a 1-month-old diarrhoea patient collected in Laihou city, Shandong province, China, on 28 July 2016. The DNA G+C content of the type strain is 52.68 mol%.

Funding information
This work was supported by the NSFC of China (31570134) and the Priority Project on Infectious Disease Control and Prevention (2012ZX10004215) from the Ministry of Health, China.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study was approved by the Ethics Committee of National Institute for Communicable Disease Control and Prevention, Chinese Centre for Disease Control and Prevention. The study was performed according to the ethical standards.

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.