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TAXONOMIC DESCRIPTION
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Whole-genome sequencing reveals that *Shewanella haliotis* Kim *et al.* 2007 can be considered a later heterotypic synonym of *Shewanella algae* Simidu *et al.* 1990

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Abstract

Previously, experimental DNA–DNA hybridization (DDH) between *Shewanella haliotis* JCM 14758^T and *Shewanella algae* JCM 21037^T had suggested that the two strains could be considered different species, despite minimal phenotypic differences. The recent isolation of *Shewanella* sp. MN-01, with 99 % 16S rRNA gene identity to *S. algae* and *S. haliotis*, revealed a potential taxonomic problem between these two species. In this study, we reassessed the nomenclature of *S. haliotis* and *S. algae* using available whole-genome sequences. The whole-genome sequence of *S. haliotis* JCM 14758^T and ten *S. algae* strains showed ≥97.7 % average nucleotide identity and >78.9 % digital DDH, clearly above the recommended species thresholds. According to the rules of priority and in view of the results obtained, *S. haliotis* is to be considered a later heterotypic synonym of *S. algae*. Because the whole-genome sequence of *Shewanella* sp. strain MN-01 shares >99 % ANI with *S. algae* JCM 14758^T, it can be confidently identified as *S. algae*.

The genus *Shewanella* of the class *Gammaproteobacteria* is an extensively studied cosmopolitan group of species [1–3] with substantial genetic diversity [4–6] reflecting versatile respiratory and central metabolic pathways [2, 7–11]. *Shewanella algae* was described for the first time in 1985 [12] with the type strain designated OK-1^T, originally deposited as strain IAM 14159^T, and subsequently transferred to other collections (JCM 21037^T=ATCC 51192^T=CCUG 39064^T =CECT 5071^T=CIP 106454^T=DSM 9167^T=LMG 18393^T =NBRC 103173^T=NCIMB 13178^T).

S. algae is an emergent opportunistic human pathogen [11] that also carries great biotechnological potential as the only species in this genus that can carry out acetate-driven extracellular electron transfer [13]. However, perhaps due to lack of available sequences, *S. algae* taxonomy and phylogeny have not been investigated in previous studies [4–6]. Experimental DNA–DNA hybridization (DDH), a measure of the percentage relatedness of DNA genomes, is the current gold standard for species delineation, with a minimum DNA relatedness cut-off value of 70 % [14, 15]. Whole-genome sequence-based *in silico* analyses, such as average nucleotide identity (ANI) implementations [16–18] and digital DDH [19], are statistically valid alternatives to conventional experimental DDH. For ANI, the recommended species cut-off value is 95 % [4], representing 70 % DNA relatedness [16], while digital DDH values are equivalent to experimental DDH values. ANI and digital DDH yield more reproducible and absolute results than experimental DDH owing to the high accuracy of modern sequencing and assembly methods, and are therefore increasingly used for pragmatic microbial species delineation [19, 20].

To classify *Shewanella* sp. MN-01, we initially queried the nearly full-length 16S rRNA gene sequence to the nr/nt NCBI database. The *Shewanella* sp. MN-01 16S rRNA sequence matched *S. algae* OK-1^T and *Shewanella haliotis* DW01^T at 99 % sequence identity, revealing a potential taxonomic problem in the presumptive identification of similar strains based solely on the 16S rRNA gene. A 16S rRNA phylogenetic reconstruction with *S. algae* species, *S. haliotis* DW01^T and closely-related strains *Shewanella upenei* 20-23R^T [21], *S. indica* KJW27^T [22] and *S. chilikensis* JC5^T [23] (Fig. 1) revealed a close phylogenetic association of *S. haliotis* DW01^T, *Shewanella* sp. MN-01 and *S. upenei* 20-23R^T with *S. algae* strains (hereafter called the '*S. algae*' clade; Fig. 1). In contrast, *S. algae* BrY grouped with *S. indica* KJW27^T in a separate clade (hereafter called the 'BrY clade', Fig. 1).

When *S. haliotis* strain DW01^T (=KCTC 12896^T=JCM 14758^T) was originally described [24], it was proposed as a novel species based on 98.3 % 16S rRNA gene sequence similarity and 35.8 % digital DDH with *S. algae* ATCC 51192^T

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Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization.

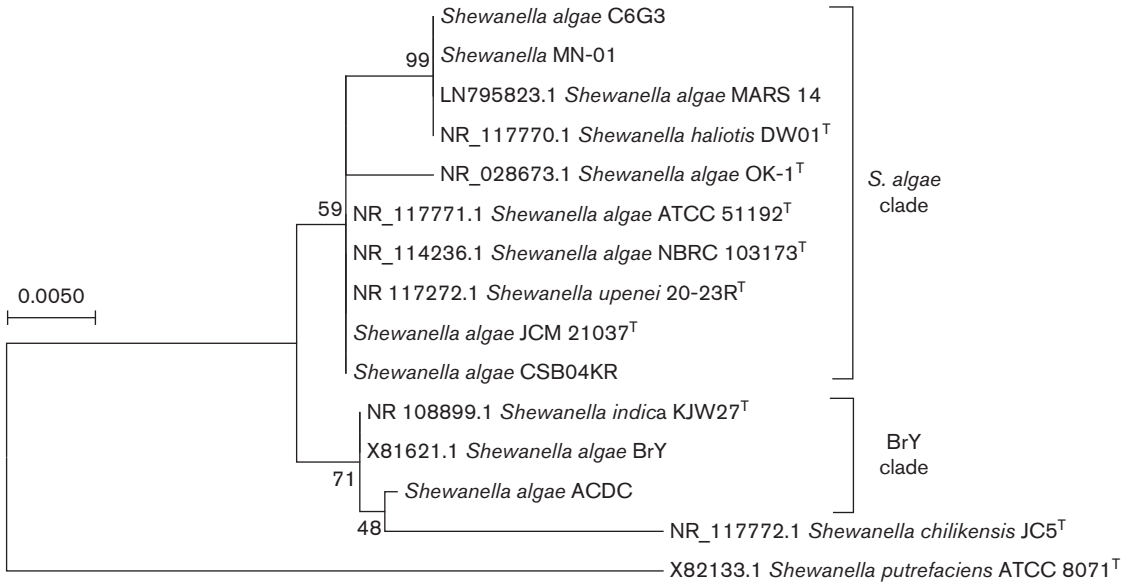

Fig. 1. Molecular phylogenetic analysis of 16S rRNA genes of *S. algae* and closely related strains. The evolutionary history was inferred by using the maximum-likelihood method based on the Tamura–Nei model [32]. The tree with the highest log likelihood (−2483.15) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths proportional to the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 1139 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [33].

[24]. However, phenotypic differences between the two strains were limited to carbohydrate assimilation (inability of *S. haliotis* to assimilate mannose and fructose and inability of *S. algae* to assimilate malate), and fatty acid composition (undetectable $C_{12:0}$ 3-OH in *S. algae* and undetectable $C_{13:0}$ 3-OH in *S. haliotis*). Because strains from the same species may display phenotypic differences reflecting distinct ecological niches [25], genomic identity typically prevails as the deciding factor for nomenclature. We used ANI and digital DDH to determine the taxonomic position of available whole-genome sequences of eight *S. algae* genomes from purified isolates, *S. haliotis*, and three closely related strains (Table 1). For the type strain of *S. algae*, we used the first publicly available genome, JCM

Table 1. *S. algae* genomes and their neighbours sequenced to date in the NCBI database (accessed March 2017)

Organism name	Strain	Source	GenBank accession no.	G+C (mol%)	Original publication
Shewanella sp.	MN-01	Sediment	LIRM00000000	53.0	[13]
S. algae	MARS 14	*Homo sapiens*	CDQH00000000	52.9	[34]
S. algae	YHL	*Homo sapiens*	LVDU00000000	53.0	NA
S. algae	JCM 21037^T	Alga (*Jania* sp.)	BALO00000000	53.0	[12, 26]
S. algae	NBRC 103173^T	Alga (*Jania* sp.)	BCZT00000000	53.1	[12, 26]
S. algae	C6G3	Sediment	JPMA00000000	53.1	[35, 36]
S. algae	CSB04KR	Sea cucumber (*Apostichopus japonicus*)	MBFW00000000	53.1	[37]
Shewanella sp.	38A_GOM_205m	Seawater	2546825529 (JGI-IMG)	53.1	NA
S. haliotis	JCM 14758^T	Abalone (*Haliotis discus)*	BALL00000000	52.9	[24]
S. algae	BrY (ATCC 51181)	Sediment	MDKA00000000	52.4	[27]
S. algae	ACDC	Soil	2510461018 (JGI-IMG)	52.4	[38]
Shewanella sp.	ECSMB14102*	East China Sea	JWGX00000000	52.2	[39]

*The 16S rRNA gene was missing from the genome assembly and therefore not included in phylogenetic analysis.
NA, Not available (unpublished).

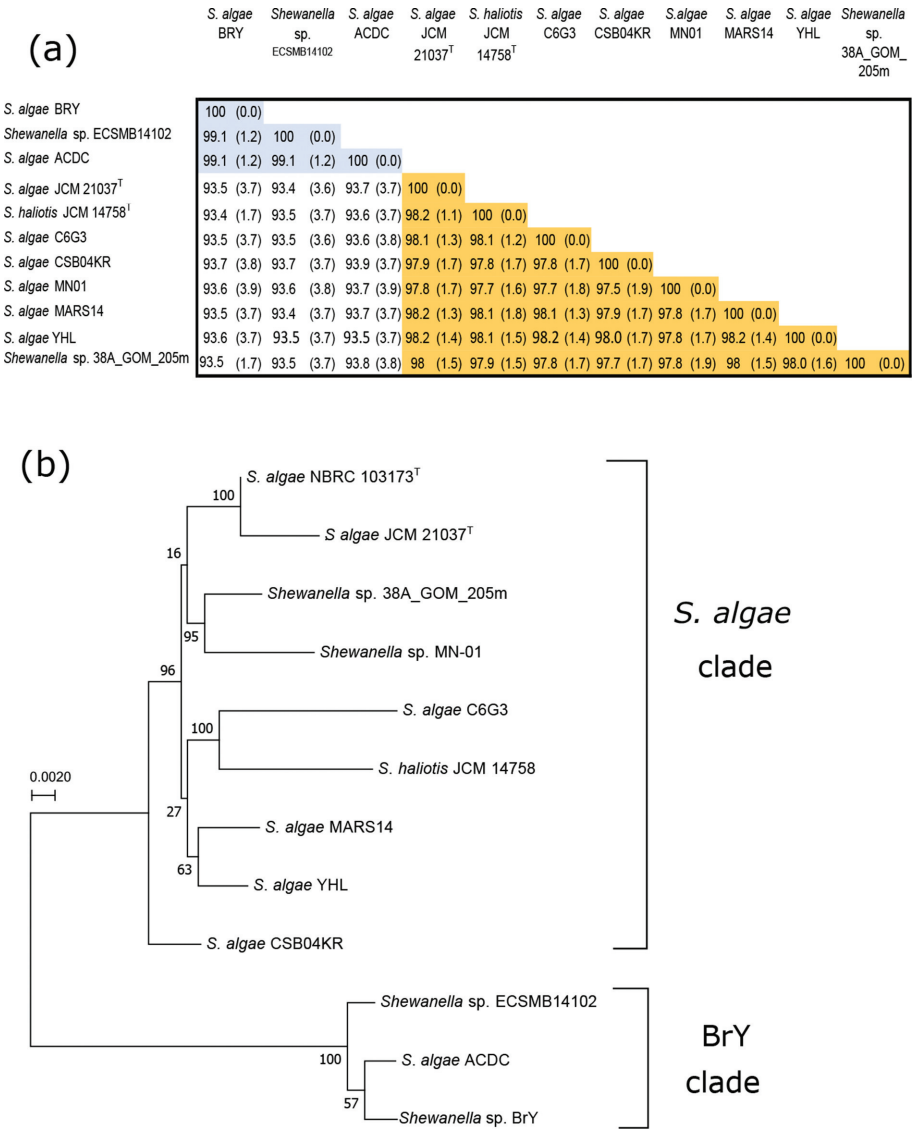

Fig. 2. Taxonomic relationship of *S. algae* strains. (a) Pairwise comparison of ANI values among *S. algae* and *S. haliotis* public genomes (Table 1) was obtained using a Web-based ANI calculator (http://enve-omics.ce.gatech.edu/g [40]). Standard deviation values are shown in parentheses. The ANI values in this table suggest the distinction of two *Shewanella* species: shaded in orange are ANI values >95 % corresponding to the same species of *S. algae* and shaded in blue are ANI values of >95 % between each other, but <95 % with *S. algae* OK-1^T. *S. algae* NBRC 103173^T has an ANI of 99.6% with JCM 21037^T and is therefore not shown in this matrix. (b) The evolutionary history of *S. algae* and *S. haliotis* strains was inferred using the maximum-likelihood method by RAxML with refinement in PATRIC [41].

21037^T [26]. Unfortunately, no genome sequences from *S. indica*, *S. upenei* and *S. chilikensis* were available as of November 2017.

S. algae strains JCM 21037^T, NBRC 103173^T, CSB04KR, C3G6 and MARS14, *Shewanella* sp. MN-01, *Shewanella* sp. 38A_GOM_205m and *S. haliotis* JCM 14758^T shared ≥97.7 % ANI (Fig. 2a), ≥78.9 % DDH (Fig. 3a) and <0.2mol % G+C content variation (52.9–53.1 %; Table 1, Fig. 3b), suggesting that they all belong to the same species. In agreement with 16S rRNA gene phylogeny (Fig. 1), a whole-genome phylogeny grouped the strains into a monophyletic clade (Fig. 2b). Based on these data, *Shewanella* sp. MN-01 and *Shewanella* sp. 38A_GOM_205m can confidently be classified as members of the species *S. algae*. In contrast to previous results, our present *in silico* analyses show that *S. haliotis* appears to fall within the species *S. algae*.

When originally isolated, *S. algae* strain BrY was not classified as an *S. algae* species [27], but in subsequent studies it became known as *S. algae* BrY based solely on its 16S rRNA

(a)

	S. algae BrY	*Shewanella* sp. ECSMB14102	S. algae ACDC	*S. algae* JCM 21037^T	*S. algae* NBRC 103173^T	*S. haliotis* JCM 14758^T	*S. algae* C6G3	*S.algae* MN01	*S. algae* MARS14	*Shewanella sp.* CBRK04R	*S. algae* YHL	*Shewanella* sp. 38A_GOM_205m
S. algae BrY	**100.0**											
Shewanella sp. ECSMB14102	92.4	100.0										
S. algae ACDC	94.3	94.0	**100.0**									
S. algae JCM 21037^T	54.4	54.1	57.7	100.0								
S. algae NBRC 103173^T	53.9	53.5	53.7	99.8	100.0							
S. haliotis JCM 14758^T	54.5	54.4	57.5	84.1	84.8	100.0						
S. algae C6G3	54.4	57.4	57.4	84.8	84.4	91.1	100.0					
S.algae MN01	54.7	54.4	58.3	81.2	81.3	80.6	80.4	100.0				
S. algae MARS14	54.4	30.2	57.7	85.2	85.3	92.5	84.2	81.30	100.0			
Shewanella sp. CBRK04R	55.6	54.6	58.6	82.6	82.5	81.6	81.9	79.1	82.4	100.0		
S. algae YHL	53.4	53.3	53.4	84.7	85.1	84.4	84.5	81.1	53.5	82.5	100.0	
Shewanella sp. 38A_GOM_205m	55.6	53.5	58.0	82.8	83.2	88.7	81.9	78.9	82.4	80.4	98.8	100.0

(b)

	G+C difference vs. *S. algae* JCM21037^T (mol%)
S. sp 38A_GOM_205m	0.01
S. algae C6G3	0.02
S. algae NBRC 103173^T	0.02
Shewanella sp. CSB04KR	0.03
S.algae MN01	0.04
S. algae YHL	0.05
S. haliotis JCM 14758^T	0.16
S. algae MARS14	0.18
S. algae ACDC	0.47
S. algae BrY	0.71
Shewanella sp. ECSMB141	0.87

Fig. 3. Pairwise comparison of digital DDH values among *S. algae* and *S. haliotis* genomes sequenced to date. (a) The digital DDH values were calculated using the DSMZ digital DDH calculation tool with the option for BLAST+ (http://ggdc.dsmz.de/). Reported are values from Formula 2, which computes identities/high scoring *p* length. All bootstrap confidence intervals were <0.5 % DDH. Shaded in orange are DDH values >70 % corresponding to the same species of *S. algae* and shaded in blue are DDH values of >70 % with each other, but <70 % with *S. algae* JCM 21037^T. Strains that tested positive for urease are marked in bold. (b) G+C differences between *S. algae* and *S. haliotis* strains compared with JCM 21037^T.

gene sequence similarity to other *S. algae* strains [28]. Using whole-genome sequences, however, we found that strains in the BrY clade [*S. algae* BrY (ATCC 51181), *S. algae* ACDC and *Shewanella* sp. ESCMB14102] shared ≥99 % ANI and >92 % DDH with one another (Figs 2a, 3a), but ≤94 % ANI, ≤58 % DDH, and significantly lower G+C contents (52.2–52.4 %, ≤0.2 % difference) than strains in the *S. algae* clade (Table 1, Fig. 3b). In addition, *Shewanella* sp. BrY produce a distinct O-lipopolysaccharide containing malic acid, fucosamine and rhamnose as repetitive monosaccharide residues, which has not been found in other *S. algae* strains [29]. Collectively, this evidence suggests that the BrY clade may represent a new species. *S. indica* genomes are needed to determine if the BrY clade and *S. indica* are the same species. Until further studies are conducted, strains BrY, ACDC and ESCMB1102 should be regarded as *Shewanella* spp. A formal proposal for the reclassification of *Shewanella* spp. BrY requires a thorough characterization, and we are currently in the process of performing a polyphasic analysis of both the strains with the aim of producing a formal description of a new *Shewanella* species at a later date.

Further genomic inspection revealed that urease genes are encoded in strains of the BrY clade, and are absent from strains of the *S. algae* clade. A urease test confirmed that *Shewanella* spp. BrY and ACDC are both urease-positive, unlike urease-negative *S. algae* strains [ATCC 51192^T [30], JCM 14758^T [24] and MN-01 (this study)]. Based on this phenotypic difference, we propose that the urease test could be a simple method to quickly distinguish *S. algae* from BrY strains when conducting phenotype-based identifications.

Based on the data discussed above, and in accordance with the rules of priority, we hereby propose *S. haliotis* as a later heterotypic synonym of *S. algae* (Rule for Prokaryotic Nomenclature 24b [31]), and emend the description of *S. algae*.

EMENDED DESCRIPTION OF *SHEWANELLA ALGAE* SIMIDU *ET AL.* 1990, EMEND. NOZUE *ET AL.* 1992

The properties are as given in the previous species description [26, 30], with the following amendments. *S. algae* cannot grow without NaCl. The genomic G+C content ranges from 52.9 to 53.1 %. *S. algae* shows positive reduction of nitrate to nitrite [24], and reduction of solid iron (Fe(III)) oxides with lactate as electron donor, as well as positive anaerobic oxidation of acetate with soluble manganese or iron as electron acceptor [13]. Type strain is OK-1^T (=JCM 21037^T=ATCC 51192^T=CCUG 39064^T=CECT 5071^T=CIP 106454^T=DSM 9167^T=LMG 18393^T=NBRC 103173^T =NCIMB 13178^T).

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

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