Shewanella woodyi sp. nov., an Exclusively Respiratory Luminous Bacterium Isolated from the Alboran Sea

JOHN C. MAKEMSON,^{1*} NADA R. FULAYFIL,¹ WARREN LANDRY,² LISA M. VAN ERT,³
CHARLES F. WIMPEE,³ EDITH A. WIDDER,⁴ AND JAMES F. CASE⁵

Department of Biological Sciences, Florida International University, University Park, Miami, Florida 33199¹;
Food and Drug Administration, Dallas, Texas 75204²; *Department of Biological Sciences, University of*
Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201³; *Harbor Branch Oceanographic Institution,*
Fort Pierce, Florida 34946⁴; *and Bioluminescence Group, Marine Science Institute,*
University of California, Santa Barbara, California 93106⁵

Thirty-four strains of nonfermentative, respiratory, luminous bacteria were isolated from samples of squid ink and seawater from depths of 200 to 300 m in the Alboran Sea. Although these strains had a few properties similar to properties of *Shewanella* (*Alteromonas*) *hanedai*, they did not cluster phenotypically with any previously described bacterium. The nucleotide sequence of a 740-bp segment of *luxA* was not homologous with other known *luxA* sequences but clustered with the *luxA* sequences of *Shewanella hanedai*, *Vibrio logei*, *Vibrio fischeri*, and *Photobacterium* species. The 16S RNA gene from two strains was sequenced and was found to be most closely related to the *S. hanedai* 16S RNA gene. Based on the differences observed, we describe the new isolates as members of a new species, *Shewanella woodyi* sp. nov. Strain ATCC 51908 (= MS32) is the type strain of this new species.

The first isolates of exclusively respiratory (nonfermentative) marine luminous bacteria were assigned to *Alteromonas hanedai* (2, 5a) and later were reassigned to the genus *Shewanella* (5b, 9). The genus *Shewanella* is composed of four species, and only one of these species, *Shewanella hanedai*, is bioluminescent. Compared to species of the genera *Alteromonas*, *Pseudomonas*, and *Deleya*, *S. hanedai* and *Shewanella putrefaciens* have unique quinone compositions, and these organisms possess menaquinones that are not present in *Alteromonas* species (1). Further confirmation that these *Shewanella* species form an independent clade that can be recognized as a genus came from 16S rRNA sequence analysis (4).

Water samples and squid ink samples from intermediate-depth dives of the submersible Johnson Sea Link contained luminous bacteria that were found to be unable to ferment sugars but could grow anaerobically with nitrate or nitrite as a terminal electron acceptor. Because these organisms did not seem to be similar to *S. hanedai*, we performed a more complete phenotypic characterization along with a hybridization and sequence analysis of a 740-bp section of the *luxA* gene, and we also sequenced the 16S RNA gene from two strains. We designated these new isolates *Shewanella woodyi* sp. nov. The species name is in honor of J. Woodland Hastings, known to most as Woody, an American biologist who, among other things, has made numerous and valuable contributions to our understanding of the biochemistry and function of bacterial bioluminescence.

MATERIALS AND METHODS

All of the strains analyzed in this study (Table 1) were picked as luminous bacterial colonies after squid ink, seawater, or detritus was plated from samples taken at depths of 200 to 430 m during three dives (35°29.05'N, 4°26.40'W for dives 2929 and 2930; 35°45.34'W, 2°45.42'W for dive 2942) of the submersible Johnson Sea Link in the Alboran Sea by two of us (E.A.W. and J.F.C.). The luminous isolates were restreaked and purified on glycerol marine agar (GMA) containing (per liter) 3 ml of glycerol, 5 g of Bacto Peptone (Difco), 1 g of Bacto Yeast Extract (Difco), 15 g of Bacto Agar (Difco), and 0.5× sea salts (pH 7.5). The sea salts preparation was made as a 5× stock solution (2.58 M NaCl, 0.125

M MgCl₂, 0.125 M MgSO₄, 0.1 M KCl) and was diluted 1:10 in the medium. Our previous experience with luminous marine bacteria indicated that this medium was suitable for most luminous marine bacteria; however, *S. woodyi* grew but did not produce luminescence in GMA broth (this was later shown to be due to a salt requirement for luminescence). We also found that the strains whose designations begin with MS (MS strains) did not utilize glycerol. Consequently, the medium used for these strains was changed to *Shewanella* marine agar (SMA) containing (per liter) 5 g of Bacto Peptone (Difco), 1 g of Bacto Yeast Extract (Difco), 15 g of Bacto Agar (Difco), and 1× sea salts (pH 7.5). The designations used for the *S. woodyi* isolates all begin with MS, and strains of other luminous marine bacteria either were maintained as frozen glycerol stock preparations or were kept on slants of GMA or SMA.

Our phenotypic characterization included determining the growth temperature (by using 4, 25, 37, and 42°C) on either GMA or SMA and performing tests for hydrolases (amylase, gelatinase, lipase, chitinase) (by using the methods of Reichelt and Baumann [11]), oxidase, catalase, API 20E reactions (Analytab Products), luciferase kinetics (10), and the Biolog substrates (Biolog, Inc., Hayward, Calif.). For the API 20E test strip study, as well as the Biolog GN plate study, *S. woodyi* strains were grown on SMA and suspended in 1× sea salts, whereas the other strains were grown on GMA and suspended in 0.5× sea salts. For bacteria that grew equally well on both media, no difference between the reaction patterns with the Biolog substrates was noticed when inocula were prepared with the two procedures. The Biolog database could not be used for identification because it was derived from the reactions of cells grown on a different medium and suspended in standard saline rather than seawater salts. Furthermore, all of the previously described bacteria (Table 1) used in this study gave Biolog reactions that were different from the reactions in the Biolog database.

The phenotypic characterization data were codified to obtain 131 traits, which included Gram staining and morphology (5 traits). The following traits were used in a cluster analysis: Gram stain reaction (trait no. 1), morphology (trait no. 2 through 4), growth temperatures (4, 25, 37, and 42°C) (trait no. 5 through 8), hydrolase activities (amylase, gelatinase, lipase, and chitinase activities) (trait no. 9 through 12), oxidase and catalase activities (trait no. 13 and 14), API 20E reactions (trait no. 15 through 34), luciferase kinetics (trait no. 35 and 36), and Biolog substrate utilization (95 substrates) (trait no. 37 through 131). Clusters were analyzed by using cluster analysis (unweighted pair group method with arithmetic means [UPGMA]) to determine Euclidian distances and then multidimensional scaling with the distance matrix produced by Cluster Analysis in the STATISTICA 4.5 program (StatSoft, Tulsa, Okla.). Representative strains of clusters were tested with the MIDI (fatty acid analysis) system for relatedness to themselves and known strains in a marine bacterial library by one of us (W.L.).

luxA probes were amplified from either chromosomal DNA (*S. woodyi* MS32¹) or *Vibrio harveyi luxAB* on plasmid pLAV1 (a gift from T. O. Baldwin, Texas A and M University) in *Escherichia coli* LE392 by using standard PCR techniques (14) under stringent (55°C) and nonstringent (35°C) annealing conditions with the *luxA* primers of Wimpee et al. (14). Amplified DNA was random prime labeled with digoxigenin as described by the manufacturer (Boehringer Mannheim) and was used as a probe to hybridize chromosomal or plasmid DNA. The PCR product was sequenced with an Amplitaq cycle sequencing kit (Perkin-

* Corresponding author. E-mail: makemson@squ.edu.

TABLE 1. Sources of *S. woodyi* strains and other bacteria^a

Taxon	Strain(s)	Source ^b
<i>Alteromonas haloplanktis</i>	ATCC 14393 ^T	ATCC
<i>Escherichia coli</i>	B	FIU Culture Collection
<i>Photobacterium angustum</i>	ATCC 25915 ^T	ATCC
<i>Photobacterium leiognathi</i>	ATCC 25521 ^T	ATCC
	721	Kenneth Neelson
<i>Photobacterium phosphoreum</i>	ATCC 11040 ^T	ATCC
<i>Shewanella hanedai</i>	ATCC 33224 ^T	ATCC
<i>Shewanella putrefaciens</i>	ATCC 8071 ^T	ATCC
<i>Shewanella woodyi</i>	MS1, MS7 through MS11	Dive 2929, 435 m, squid ink
	MS15 through MS18	Dive 2930, 198 m, seawater
	MS19 through MS30	Dive 2942, 220 m, seawater
	MS31 through MS34	Dive 2942, 370 m, detritus
	MS35 through MS38	Dive 2942, 384 m, seawater
	MS39 through MS41	Dive 2942, 370 m, seawater
<i>Vibrio alginolyticus</i>	ATCC 17749 ^T	ATCC
<i>Vibrio anguillarum</i>		Richard Morita
<i>Vibrio carchariae</i>	ATCC 35084 ^T	ATCC
<i>Vibrio cholerae</i> O1	ATCC 14035 ^T	ATCC
<i>Vibrio cholerae</i> El Tor	ATCC 14033	ATCC
<i>Vibrio fischeri</i>	ATCC 7744 ^T	ATCC
<i>Vibrio harveyi</i>	B-392, MAV	J. W. Hastings
<i>Vibrio logei</i>	MS12	Dive 2929, 435 m, squid ink
<i>Vibrio natriegens</i>	ATCC 14048 ^T	ATCC
<i>Vibrio parahaemolyticus</i>	B22	Michael Silverman
<i>Vibrio vulnificus</i>	ATCC 27562 ^T	ATCC

^a Other strains that were used included *Vibrio harveyi*-like strains from Bahrain, the Gulf of Oman, and Biscayne Bay and strains WH69 and WH70, which are undescribed *Photobacterium* strains isolated from the Gulf of California by Paul Dunlap.

^b ATCC, American Type Culture Collection; FIU, Florida International University.

Elmer Cetus) by using the methods recommended by the manufacturer. A phylogenetic analysis in which heuristic methods were used was performed by using the PAUP program (12).

The DNA G+C content was estimated by a modification (9a) of a fluorescence method (8) by using two DNA-binding fluorescent dyes (bis-benzimide, chromomycin A3) and ethanol-fixed whole cells in Tris-saline-Mg buffer.

The 16S RNA genes of strains MS1 and MS32^T were sequenced by using the PCR method of Lane et al. (7) and the following forward and reverse primers (kind gifts from Reinhardt Rosson): primers 519r and 907r (7); primers 805f, 342r, 8f ("fD2"), and 1492rL ("rP2") of Weisberg et al. (13); and primer 530f (GTGCCAGCMGCCGCGG), as suggested by D. Distel. The final PCR mixtures contained 2 U of *Taq* polymerase (Promega, Madison, Wis.), 2.75 mM MgCl₂, 7% glycerol, 3% formamide, each deoxynucleoside triphosphate at a concentration of 0.2 mM, 20 pmol of each primer, and 50 µg of template. The Perkin-Elmer model 480 thermal cycler conditions used were 3 min at 94°C, followed by 30 cycles consisting of 1 min at 50°C, 1 min at 74°C and 1 min at 94°C. The amplified fragments were purified on 1% agarose, and the slices were cleaned with Wizard PCR Preps (Promega) and sequenced with an Applied Biosystems model ABI 373a automated sequencer. The sequences obtained were aligned by using Sequencer 3.0 (Gene Codes Corp., Ann Arbor, Mich.) and were used to construct trees. The trees were constructed by performing a parsimony analysis with the PAUP program (10).

RESULTS AND DISCUSSION

All of the strains were motile rods. Each cell possessed a single polar flagellum (Fig. 1), which was not surrounded by a sheath like that found in some other luminous bacteria (2). An unsheathed flagellum is characteristic of the genera *Shewanella*, *Alteromonas*, and *Pseudoalteromonas* (4, 6). None of the strains could ferment sugars, which led us initially to suspect that these organisms were strains of *S. hanedai*. *S. hanedai* is psychrophilic with an optimum growth temperature of 15 to 20°C. The MS strains grew at 4°C, grew best at 25°C, and did not grow at 30°C. Thus, to determine if these organisms were related, all of the phenotypic characteristics (see above) were tested and coded for a cluster analysis. This analysis emphasized substrate utilization in the Biolog GN plates (95 of 131 traits). The UPGMA cluster analysis in which Euclidean distances were used showed that the MS strains had relatively high levels of similarity (>92%). The UPGMA distance matrix was analyzed by multidimensional scaling in two dimensions (Fig. 2), which showed that the MS strains were clearly sepa-

FIG. 1. Transmission electron micrograph of *S. woodyi* MS32^T stained with 1% uranyl acetate on a Formvar-coated grid. Bar = 0.5 µm.

FIG. 2. Multidimensional scaling analysis of MS strains (*S. woodyi* cluster) and previously described luminous bacteria, performed by using the Euclidean distance matrix and all of the traits listed in the text. All known strains are listed in Table 1. Abbreviations: A.HALO, *Alteromonas haloplanktis*; E.COLI, *Escherichia coli*; P.ANG, *Photobacterium angustum*; P.LEIO, *Photobacterium leiognathi*; P.L-721, *Photobacterium leiognathi* 721; P.PHOS, *Photobacterium phosphoreum*; S.HAN, *Shewanella hanedai*; S.PUT, *Shewanella putrefaciens*; V.ALGIN, *Vibrio alginolyticus*; V.ANG, *Vibrio anguillarum*; V.CARCHA, *Vibrio carchariae*; V.CHO-01, *Vibrio cholerae* O1; EL TOR, *Vibrio cholerae* El Tor; V.H-B392, *Vibrio harveyi* B-392; V.LOGEI, *Vibrio logei*; V.NAT, *Vibrio natriegens*; V.PARA, *Vibrio parahaemolyticus*; V.VULN, *Vibrio vulnificus*.

TABLE 2. Fatty acid profiles, as determined by the MIDI method

Fatty acid	% in:					
	<i>S. woodyi</i> MS1	<i>S. woodyi</i> MS32 ^T	<i>S. putrefaciens</i> ATCC 8071	<i>S. hanedai</i> ATCC 33224	<i>P. phosphoreum</i> ATCC 11040	<i>P. leiognathi</i> ATCC 25521
12:0	4.64	4.13	2.76	6.04	5.55	4.75
11:0 3OH			0.48			
13:0 iso	10.5	10.04	3.74	14.07		
13:0	1.09	0.87	1.15	0.94		
12:0 3OH	2.06	1.93	1.49	3.94	5.13	3.38
14:0 iso	0.54					
14:0	6.42	4.65	1.26	10.66	4.66	4.81
13:0 iso 3OH	5.35	6.54	2.97	6.14		
15:0 iso	14.32	19.53	22.56	7.19		
15:0 anteiso	0.40	0.65	0.37			
15:0	4.80	4.05	4.85	3.35		
16:0 iso			0.88			
16:1 B	1.09	1.18	0.051	1.55		
16:1 <i>cis</i> 9	16.19	17.26	18.25	23.40	55.05	38.31
16:0	26.07	20.81	12.91	17.85	20.36	25.05
15:0 iso 3OH			0.53			
17:1 iso F			0.80			
17:0 iso	0.74	1.66	2.19	0.77		
17:1 B	1.55	2.15	9.07	1.37		
17:0 cyclo						0.63
17:0	1.41	1.30	2.85			
16:0 3OH			0.41			
18:1 <i>cis</i> 9	1.03	0.98	2.12	0.77		
18:0			0.76			0.61
20:0 iso	0.74	0.68				
Unknown 12.486					1.29	0.42

rated from *S. hanedai*, as well as other known luminous bacteria. The G+C content of MS32^T was determined to be 39 mol% by using the modified fluorescence method. The G+C content of *S. hanedai* DNA is 45.2 mol% (6). Thus, the new MS strains could be considered members of a separate taxon on phenetic grounds.

As a test of taxonomic validity, several strains (MS1, MS32^T, MS36, MS38) were analyzed with the MIDI fatty acid analysis system and compared to other marine bacteria (Table 2). Like *S. putrefaciens* and *S. hanedai*, the MS strains had relatively high concentrations of 13:0 iso and 15:0 iso fatty acids, which were not present in vibrios (data not shown) or *Photobacterium* species. Furthermore, the ratio of 16:1 *cis* 9 to 16:0 was skewed

TABLE 3. Reactions of *luxA* probes prepared from *V. harveyi* and *S. woodyi* with chromosomal DNAs of luminous bacteria

Organism(s)	Reaction with <i>luxA</i> probe from:			
	<i>V. harveyi</i> B-392		<i>S. woodyi</i> MS32 ^T	
	High-stringency conditions	Low-stringency conditions	High-stringency conditions	Low-stringency conditions
<i>V. harveyi</i> MAV	+	+	+	+
<i>S. woodyi</i> MS32 ^T	—	+	+	+
<i>S. woodyi</i> MS1	—	—	+	+
<i>V. fischeri</i> ATCC 7744	—	—	—	—
<i>V. logei</i> MS12	—	+	—	+
Six nonluminous vibrios	—	3+, 3 ^a	—	—

^a The American Type Culture Collection type cultures that hybridized with the *V. harveyi* probe were the *V. damsela*, *V. natriegens*, and *V. parahaemolyticus* cultures, whereas those that did not hybridize were the *V. anguillarum*, *V. alginolyticus*, and *V. cholerae* cultures.

FIG. 3. Unrooted phylogenetic trees based on *luxA* sequences. (A) Tree based on direct *luxA* sequences. (B) Tree based on translated *luxA* sequences analyzed by PAUP. Abbreviations: Krya, *Kryptophaneron alfredi* symbiont; Vharv, *Vibrio harveyi*; Xla, *Xenorhabdus (Photorhabdus) luminescens*; MS1 and MS32, *S. woodyi* MS1 and MS32^T, respectively; Shan, *Shewanella hanedai*; Vlogei, *Vibrio logei*; Pla2 and Pla, two strains of *Photobacterium leiognathi*; Pphos, *Photobacterium phosphoreum*; Pfisc, *Vibrio (Photobacterium) fischeri*.

toward the unsaturated fatty acid (1.4 to 2.7) in the vibrios, *Photobacterium* species, *S. putrefaciens*, and *S. hanedai* but skewed toward the saturated fatty acid (0.62 to 0.83) in *S. woodyi*. Thus, the fatty acid analysis showed that *S. woodyi* is unique but is related to other shewanellae.

luxA hybridization data also confirmed the unique taxonomic nature of the MS strains. The primers of Wimpee et al. produced a PCR product with DNAs from MS strains only if

FIG. 5. Growth (closed symbols) and luminescence (open symbols) of MS25 in SMA broth at 25°C. Conditioned medium (□ and ■) was prepared by growing MS25 in unconditioned SMA broth (○ and ●) to an optical density at 660 nm of 1.0 and then centrifuging the culture (12,000 rpm [Eppendorf Microfuge], 5 min, 20°C) and sterilizing the supernatant by filtration through a 0.2-μm-pore-size membrane filter (Millipore). Both media were inoculated from the same culture to an initial optical density at 660 nm of 0.01 and incubated with shaking (200 rpm) at 25°C. OD, optical density.

FIG. 4. Unrooted phylogenetic tree based on 16S rRNA sequences analyzed by PAUP. Strains from GenBank are designated with species names; MS1 and MS32 are *S. woodyi* strains; and MS12 is a *V. logei* strain (unpublished sequence). The GenBank accession numbers are as follows: *E. coli*, J10695; *P. leiognathi*, 74686; *P. phosphoreum*, 74687; *Photobacterium luminescens*, 3368; *S. hanedai*, 103207T; *V. cholerae*, X74697; *V. vulnificus*, X74726; *Vibrio splendidus*, X74724; *Photobacterium fischeri* X74702; *V. harveyi*, X74706; and *V. logei*, X74708.

low-stringency annealing temperature (35°C) was used. This indicated that there may be sequence differences in the primer regions (a 23-mer and a 26-mer) between *S. woodyi* and other luminous bacteria. When an MS strain DNA PCR product was used as the PCR template, a good yield was obtained under high-stringency conditions (55°C) because the PCR product had the Wimpee et al. primer sequences at each end. Probes from MS32^T DNA and *V. harveyi* B392 DNA that were random prime labeled with digoxigenin were prepared and reacted with chromosomal DNAs prepared from two MS strains and known American Type Culture Collection strains under low- and high-stringency conditions (14). Our results (Table 3) show that the MS strain probe reacted under high-stringency conditions only with other MS strain DNAs but under low-stringency conditions reacted with *V. harveyi* and *Vibrio logei* and not with *Vibrio fischeri* or other nonluminous vibrios, whereas the *V. harveyi* probe reacted with five species (*S. woodyi*, *V. logei*, *Vibrio damsela*, *Vibrio natriegens*, *Vibrio parahaemolyticus*) under low-stringency conditions.

The sequences in the 740-bp sequence of *luxA* subtended by the primers of Wimpee et al. (11) from MS1 (639 bp) and from forward (206-bp) and reverse (119-bp) sections of the MS32^T sequence were compared to other known *luxA* sequences obtained from GenBank and analyzed with the PAUP program. Figure 3A shows the most-parsimonious unrooted consensus tree at the nucleotide level which demonstrated that for this *luxA* region, the two MS strains clustered together and were more closely related to *V. logei* and *V. (Photobacterium) fischeri* than the other luminous bacteria, including *S. hanedai*. At the translated amino acid level, the MS1 and MS32^T *luxA* sequences clustered closer to *S. hanedai* and still remained close to *V. fischeri* and *V. logei* (Fig. 3B).

TABLE 4. Sodium requirement for luciferase synthesis by *S. woodyi* MS32^T growing in SMA broth at 25°C with shaking (200 rpm)

% of NaCl concn in seawater ^a	Maximum growth (optical density at 660 nm)	Luminescence (light units) ^b
50	0.99	0.15
75	1.23	12,640
100	1.25	608,000
125	1.01	2,702
150	0.94	0.15
175	0.148	0

^a A value of 100% was equivalent to 0.517 M; the concentrations of all other sea salts were the same as the concentrations in SMA.

^b One light unit was equivalent to 3.9×10^6 photons $s^{-1} ml^{-1}$.

Figure 4 shows that the 16S RNA sequence data for MS1 and MS32^T (GenBank accession numbers AF003548 and AF003549) indicate a closer relationship between MS1 and MS32^T sequences and the *S. hanedai* sequence (GenBank accession no. 103207T) than is evident from the *luxA* sequence. Furthermore, the luminous shewanella 16S RNA sequences form a group that is separate from the 16S RNA sequences of all of the other luminous bacteria.

S. woodyi has a kinetically distinct luciferase (the apparent first-order decay constants at 25°C were $0.241 s^{-1}$ with dodecanal and $0.240 s^{-1}$ with decanal in the presence of 2 mg of bovine serum albumin per ml). These kinetics are intermediate between the kinetics of the kinetically slow luciferases of *V. harveyi* and the kinetics of the fast luciferases of the *Photobac-*

FIG. 6. Dithionite-reduced-minus-hydrogen-peroxide-oxidized spectra of MS32^T whole cells in 0.05 M Tris (pH 8.0) containing 0.01 M MgCl₂. (A) Difference spectrum for aerobically grown cells. Bar = optical density of 0.1. (B) Difference spectrum for nitrate-anaerobic cells. The following wavelengths are indicated on the spectrum: a, 522 nm; b, 530 nm; c, 551 nm. Bar = optical density of 0.01.

TABLE 5. Comparison of *S. hanedai* and *S. woodyi*

Trait	<i>S. woodyi</i>	<i>S. hanedai</i> ^a
Respiratory	+	+
Fermentative	—	—
Oxidase	+	+
Growth at 4°C	+	+
Growth at 30°C	—	—
Amylase ^b	+ ^c	—
Gelatinase	+	+
Lipase ^b	—	+
Chitinase ^b	—	+
Biolog substrates		
D-Mannose	—	—
D-Galactose	+	+
Sucrose	—	—
Cellobiose ^b	+	—
D-Glucuronate ^b	+	—
N-Acetylglucosamine ^b	—	+
Glycerol	—	—
Acetate	+	+
α -Ketoglutarate ^b	+	—
Propionic acid	+	+
Succinic acid ^b	+	—
Citrate	—	—
L-Alanine	+	+
D-Alanine	—	—
L-Threonine	+	+
L-Leucine	+	+
L-Serine	+	+
Putrescine	+	+

^a Data from reference 3 were confirmed in this study by using the American Type Culture Collection type strain.

^b Differentiating characteristic.

^c Of the 34 strains tested, 27 were positive.

terium group. The *S. woodyi* luciferase kinetics are nearly the same as the *V. harveyi* luciferase kinetics with decanal, yet unlike the kinetics of *V. harveyi* luciferase, which exhibits very slow decay kinetics with dodecanal. The *S. woodyi* luciferase decay kinetics with dodecanal are almost identical to the decanal decay kinetics, a situation which is similar to the situation with the fast luciferases of the *Photobacterium* group.

S. woodyi synthesized luciferase late in batch growth and apparently could have autoinductive control of luciferase synthesis because bacteria freshly inoculated into "conditioned" media showed no lag in luciferase synthesis (Fig. 5). Even though the bacteria could grow over a wide range of salinities (Table 4), luciferase synthesis showed a pronounced requirement for sodium (at 1× seawater strength), like strains of *Photobacterium phosphoreum* (3, 5).

S. woodyi is a prototroph that grows well on single-carbon-source media with ammonium as the nitrogen source. Anaerobically, the MS strains grew with nitrate or nitrite as the terminal electron acceptor and grew slowly with fumarate as the terminal electron acceptor. Whether the organisms are grown aerobically or anaerobically, the reduced-minus-oxidized cytochrome spectra of whole cells have a single α peak at 552 nm, indicating a high cytochrome *c* content (Fig. 6). There were no cytochrome *a*, *d*, or *o* peaks. The very high cytochrome *c* content was reflected in colony coloration; MS strains growing on SMA all had a pinkish orange tint identical to that of *S. putrefaciens* growing on brain heart infusion agar.

Traits that separate *S. woodyi* from *S. hanedai* are indicated in Table 5; *S. woodyi* possesses amylase, lipase, and chitinase activities and is positive in Biolog reactions with cellobiose, D-glucuronate, N-acetylglucosamine, α -ketoglutarate, and suc-

cinic acid. Both *Shewanella* species were isolated from intermediate depths in the ocean (*S. hanedai* from Arctic sediments, Antarctic and Sannich Inlet, and British Columbia seawater at depths of 100 to 220 m; *S. woodyi* from the Alboran Sea), but the two species appear to be taxonomically separate.

Description of *Shewanella woodyi* sp. nov. *Shewanella woodyi* (wood'y.i. M.L. gen. n. *woodyi*, of Woody, in honor of the American biologist J. Woodland Hastings) cells are gram-negative, obligately respiratory, luminescent, marine, nonsporulating rods (0.4 to 1.0 by 1.4 to 2.0 μ m), and each cell possess a single, unsheathed, polar flagellum. The cells use oxygen, nitrate, and nitrite as terminal electron acceptors and possess catalase and oxidase activities. They grow at 4 to 25°C but not at 30°C. All strains produce gelatinase, and most strains (27 of 34 strains) produce amylase, but they do not produce lipase, chitinase, or agarase. They metabolize D-galactose, cellobiose, D-glucuronic acid, acetate, α -ketoglutarate, propionate, succinate, L-alanine, L-threonine, L-leucine, L-serine, and putrescine. The G+C content of the DNA is 39 mol%. The type strain is ATCC 51908 (= MS32).

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