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Novel Psychropiezophilic *Oceanospirillales* Species *Profundimonas piezophila* gen. nov., sp. nov., Isolated from the Deep-Sea Environment of the Puerto Rico Trench

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The diversity of deep-sea high-pressure-adapted (piezophilic) microbes in isolated monoculture remains low. In this study, a novel obligately psychropiezophilic bacterium was isolated from seawater collected from the Puerto Rico Trench at a depth of ~6,000 m. This isolate, designated YC-1, grew best in a nutrient-rich marine medium, with an optimal growth hydrostatic pressure of 50 MPa (range, 20 to 70 MPa) at 8°C. Under these conditions, the maximum growth rate was extremely slow, 0.017 h^{-1}, and the maximum yield was 3.51×10^7 cells ml^{-1}. Cell size and shape changed with pressure, shifting from 4.0 to 5.0 μm in length and 0.5 to 0.8 μm in width at 60 MPa to 0.8- to 1.0-μm diameter coccoid cells under 20 MPa, the minimal pressure required for growth. YC-1 is a Gram-negative, facultatively anaerobic heterotroph. Its predominant cellular fatty acids are the monounsaturated fatty acids (MUFAs) C$_{16:1}$ and C$_{18:1}$. Unlike many other psychropiezophiles, YC-1 does not synthesize any polyunsaturated fatty acids (PUFAs). Phylogenetic analysis placed YC-1 within the family of *Oceanospirillaceae*, closely related to the uncultured symbiont of the deep-sea whale bone-eating worms of the genus *Osedax*. In common with some other members of the *Oceanospirillales*, including those enriched during the Deepwater Horizon oil spill, YC-1 is capable of hydrocarbon utilization. On the basis of its characteristics, YC-1 appears to represent both a new genus and a new species, which we name *Profundimonas piezophila* gen. nov., sp. nov.

Psychropiezophilic (low-temperature and high-pressure adapted) and obligately psychropiezophilic (requiring high pressure for growth) bacteria have been isolated from a variety of deep-sea environments below 2,000 m and 5,000 m, respectively, as reviewed by Eloe et al. (1). To date, all of these bacteria fall into the genera *Colwellia*, *Moritella*, *Psychromonas*, and *Shewanella* within the *Alteromonadaceae* family within the *Gammaproteobacteria*, the genus *Photobacterium* within the *Vibrionaceae* family within the *Gammaproteobacteria* (2, 3), and (for one recent isolate) within the *Roseobacter* clade of the *Rhodobacterales* order within the *Alphaproteobacteria* (1). A common characteristic shared by all psychropiezophiles examined is the production of large proportions of their membrane phospholipid-associated fatty acids as unsaturated fatty acids, present as monounsaturated fatty acids (MUFAs), long-chain omega-3 polyunsaturated fatty acids (PUFAs), or both (4). Unsaturated fatty acids are needed to counteract high-pressure increases in the packing density of fatty-acyl chains, which reduce membrane fluidity, permeability, and elasticity, as well as membrane protein function (5, 6). In the psychrotolerant piezophilic bacterium *Photobacterium profundum* strain SS9, the monounsaturated fatty acid *cis*-vaccenic acid (C$_{18:1}$) is required for high-pressure adaptation, but surprisingly, eicosapentaenoic acid (EPA; C$_{20:5}$), a PUFA produced in greater abundance at high pressure than at low pressure, is not required for high-pressure growth (7). In contrast, EPA is required for high-pressure growth, a late step in cell division, and the maintenance of proper membrane fluidity in the psychropiezophile *Shewanella violacea* strain DSS12 (8, 9). Thus, while unsaturated fatty acids appear to be a universal requirement for psychropiezophiles, the relative importance of MUFAs and PUFAs may depend on the species and physiological state. Additional adaptations for life at

low temperature and high pressure are also needed. For example, conditionally pressure sensitive mutants of *P. profundum* strain SS9 indicate the occurrence of high-pressure-specific modifications in chromosome structure and function and in ribosome assembly and function (10).

Oceanospirillales are heterotrophic bacteria both associated with oil spills, including the Deepwater Horizon deep-sea oil plume (11, 12), and involved in symbiotic interactions with various marine invertebrates, including sea urchins, corals, mussels, and bone-eating worms (13). The connection between members of the *Oceanospirillales* and bone-eating worms is particularly relevant to this study. Bone-eating worms of the genus *Osedax* have been discovered in whale carcasses from both shallow-water and deep-sea benthoses (14). Female worms possess a posterior root-like structure that infiltrates whale bones, apparently as a result of acid secretion (15). Although *Osedax* females lack a mouth and a gut, they harbor heterotrophic symbiotic bacteria in bacteriocytes within their roots. It is believed that the root epithelium absorbs bone collagen and/or lipids, which are utilized by the bacterial symbionts, and that the symbionts, in turn, serve as a food source for their hosts (16, 17). Phylogenetic analyses together with fluorescent *in situ* hybridization (FISH) experiments have shown that *Osedax* species harbor intracellular microbes belonging to the or-

der *Oceanospirillales* (13, 16). The free-living hydrocarbon-degrading bacterium *Neptunomonas naphthovorans*, isolated from Puget Sound sediment at a depth of 15.5 m, and *Neptunomonas japonica* strains isolated from sediment adjacent to a sperm whale carcass off Kagoshima, Japan, at a depth of 226 to 246 m are the cultured relatives most closely related to the shallow-water *Osedax* symbionts (13, 18, 19). However, no close relatives of the clades of *Osedax* symbionts that include those present at a deep-sea whale fall have yet been cultivated under laboratory conditions (13).

In this study, we report on the isolation of the first psychropiezophilic member of the order *Oceanospirillales* of the *Gammaproteobacteria*. In order to understand more about *Oceanospirillales* bacteria in the deep ocean, we characterized the general growth, morphology, and phylogenetic and physiological properties of strain YC-1. This piezophilic bacterium is identified as the first cultured deep-sea bacterium closely related to the clade *Osedax* symbionts containing deep-sea whale fall representatives. The purpose of this study was to describe the isolation of strain YC-1 and to place it in a meaningful taxonomic framework. Strain YC-1 represents a new genus and species that we have designated *Profundimonas piezophila* gen. nov., sp. nov.

MATERIALS AND METHODS

Sample collection and high-pressure cultivation conditions. Strain YC-1 was isolated from deep-sea seawater collected from the Puerto Rico Trench at a depth of 6,000 m (19.667°N, 65.966°W), by the R/V *Atlantic Explorer* (Bermuda Atlantic Time Series [BATS]) with a conductivity-temperature-depth (CTD) rosette (with 24 12-liter bottles) in October 2008. The water sample was carried to the sea surface and was then placed in sterilized bags (Kapak, USA). The plastic bags were sealed and were subsequently pressurized to 60 MPa in pressure vessels that were maintained at 2°C. High-pressure cultivation utilized a liquid hydraulic system. For enrichment, the water sample was cultivated in marine broth 2216 (Difco, USA) at 2°C and 60 MPa for about 6 weeks. Strain YC-1 was isolated using the silica gel method by following the general procedures described by Dietz and Yayanos (20). The piezophilic strain was maintained in pressurizable polyethylene transfer pipette bulbs containing marine broth 2216, and it grew at 8°C and 60 MPa. Optimal growth pressure and temperature were determined by total-cell counts with epifluorescence microscopy. The culture samples were fixed with formaldehyde and were stained with 4′,6-diamidino-2-phenylindole (DAPI).

Morphological analysis. Cell morphologies at low pressure (20 MPa) and high pressure (60 MPa) were determined by scanning electron microscopy as described previously (21). Strain YC-1 was cultivated in marine broth 2216 at 8°C for each pressure. Mid-exponential-phase cultures were fixed with glutaraldehyde (final concentration, 2%) and were filtered through 0.2-μm polycarbonate filters (Nuclepore, USA). Cells were dehydrated by transferring the filters through a series of increasing concentrations of ethanol (10, 30, 50, 70, and 95%, and three times at 100%, for 10 min each time), followed by critical-point drying with CO_2 and sputter-coating with gold. Samples were examined with an environmental scanning electron microscope (Quanta 600; FEI, USA).

Characterization of growth under high-pressure conditions. All high-pressure physiological tests were repeated three times with uninoculated blank controls. Acid production from sugars (L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, D-mannose, maltose, D-raffinose, L-rhamnose, D-ribose, sucrose, starch, D-trehalose, and D-xylose) was assessed in modified O/F medium (22) containing 0.5× artificial seawater (1.5% NaCl, 0.27% $MgSO_4\cdot7H_2O$, 0.035% KCl, 0.05% $CaCl_2\cdot2H_2O$, 0.54% $MgCl_2\cdot6H_2O$), 0.05% $NH_4H_2PO_4$, 0.005% yeast extract, 0.1% Na_2CO_3, 1% sugar, and 0.003% bromothymol blue (the pH was adjusted to 7.1 on ice). The cultures were incubated at 60 MPa and 8°C for 4 weeks. Tests for the production of hydrogen sulfide from thio-

sulfate, indole production, and cell motility were performed using SIM (sulfide indole motility) medium (23) prepared with 0.5× artificial seawater instead of water. Enzymatic activities were tested as described previously (24). Cells were cultivated in marine broth 2216. After centrifugation at 14,000 rpm for 20 min, the cell pellet was spread on oxidase test paper (25) for the oxidase test. Catalase activity was tested by putting 3% H_2O_2 on the cell pellet. The DNase test was performed by inoculating stab cultures in DNase medium (Difco, USA) containing 0.5× artificial seawater and 0.01% methyl green. To test for gelatinase activity, cells were inoculated by stab cultures in marine broth 2216 medium containing 2% gelatin. After incubation, the stab culture was decompressed and was placed in a 30% trichloroacetic acid solution for clear-zone examination. Tests for growth with sole carbon sources and sole electron acceptors were performed according to the methods described by DeLong et al. (26). For instance, tests for lactate as the sole carbon source and trimethylamine oxide (TMAO) as the sole electron acceptor were performed as follows: four parts of inoculated mineral medium (27) were mixed with no addition, 15 mM lactate, 25 mM TMAO, and 15 mM lactate plus 25 mM TMAO, respectively, and after incubation, the cultures were decompressed and checked for growth.

The cellular fatty acid content of strain YC-1 were analyzed at Microbial Identification, Inc. (MIDI), using the MIDI system (28). Chromosomal DNA was extracted and was purified according to the standard method (29). The DNA G+C content was analyzed using reversed-phase high-performance liquid chromatography (HPLC) (30).

16S rRNA sequencing and phylogenetic analysis. For phylogenetic analysis, the 16S rRNA gene was PCR amplified with primers 27F and 1492R (31). The PCR conditions were as follows: initial denaturation at 94°C for 10 min; 35 cycles of denaturation (94°C for 30 s), annealing (52°C for 2 min), and extension (72°C for 2 min); and a final extension of 72°C for 7 min. The purified PCR product was sequenced by SeqXcel Inc. using BigDye Terminator chemistry (Applied Biosystems, USA) with the 3730 DNA analyzer (Applied Biosystems, USA). The 16S rRNA gene sequence of strain YC-1 was first compared to other gene sequences in the NCBI GenBank database using a BLAST search (32). The sequence was later aligned using the ARB software package with the ARB_EDIT4 tool (33). Aligned sequences were exported to PHYLIP (34) and FastTree (35) for neighbor-joining (NJ) and maximum-likelihood (ML) analysis with bootstrap sampling, respectively. *Profundimonas piezophilia* strain YC-1 is available through the American Type Culture Collection (catalog no. BP-BAA-2591).

Nucleotide sequence accession number. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YC-1 is HQ230045.

RESULTS AND DISCUSSION

YC-1 is an obligate psychropiezophile with morphology varying as a function of pressure. Strain YC-1 grew well in pressure vessels incubated at temperatures of 4 to 14°C at 60 MPa, with a temperature optimum of 8°C. No growth was observed at temperatures higher than 16°C. Growth occurred under hydrostatic pressures of 20 to 70 MPa at 8°C. The optimal pressure for growth was determined to be 50 MPa at 8°C, and no growth occurred at pressures either below 10 MPa or above 80 MPa (Fig. 1). The minimal doubling time was about 41 h, and the optimal growth yield was 3.5×10^7 cells ml^{-1}. The maximal growth rate of YC-1 (0.017 h^{-1}) was much lower than those of other cultured psychropiezophilic isolates within the *Gammaproteobacteria* (0.07 to 0.5 h^{-1}). The growth rate of YC-1 is comparable to that of the recently isolated psychropiezophilic *Alphaproteobacterium* strain PRT1, which was isolated from hadal seawater (collected from 8,350 m) in the Puerto Rico Trench and has a minimal doubling time of 36 h (1). This suggests that the low growth rates of YC-1 and PRT1 could reflect adaptation to their oligotrophic environments.
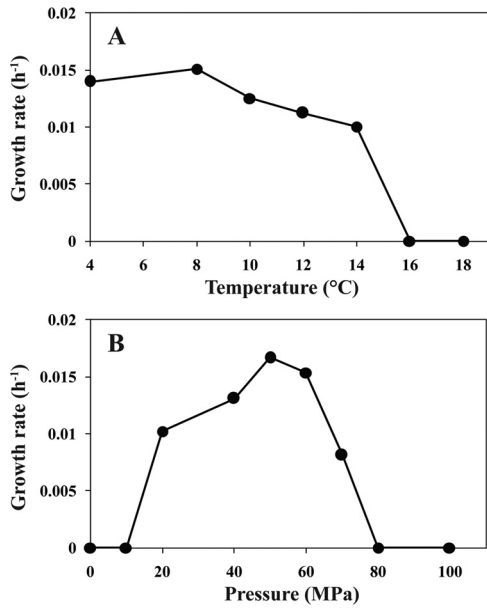
FIG 1 Growth of strain YC-1. (A) Effect of temperature on the growth rate of strain YC-1 under 60 MPa, equivalent to the pressure at a depth of 6,000 m. (B) Growth rate of strain YC-1 under different pressures at 8°C.

Cells of strain YC-1 were Gram-negative rods, 4.0 to 5.0 μm long and 0.5 to 0.8 μm wide under 60 MPa, which is equivalent to the hydrostatic pressure at its depth of isolation, 6,000 m. Coccoid bodies (diameter, 0.8 to 1.0 μm) were observed at a pressure of 20 MPa, which is the minimal pressure permitting growth of strain YC-1 (Fig. 2). These results indicate that strain YC-1 changes morphology under different pressures, shifting toward more filamentous forms at higher pressures, a result similar to those obtained previously for pressure-sensitive bacteria (36).

MUFAs are the predominant cellular fatty acids of YC-1. The whole-cell fatty acid compositions of the novel strain YC-1 and related species are shown in Table 1. The fatty acid profile is similar to those of members of the genus *Neptunomonas* (37). The predominant nonpolar cellular fatty acids of YC-1 were $C_{16:1}$ (palmitoleic acid) and $C_{18:1}$ (oleic acid and *cis*-vaccenic acid). YC-1 produced more $C_{16:1}$ (44.9%) than $C_{18:1}$ (30.7%) at low pressure (20 MPa). At high pressure (60 MPa), the proportions of $C_{18:1}$ fatty acids (45.6%), especially *cis*-vaccenic acid, of YC-1 increased dramatically, while that of the $C_{16:1}$ fatty acid (35.8%) was re-
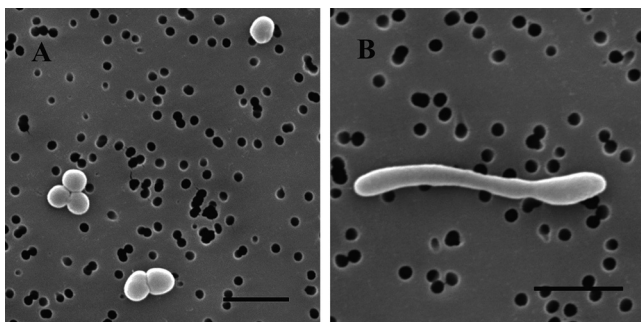
FIG 2 Scanning electron micrographs of cells of strain YC-1 cultivated in marine broth 2216 at 8°C under pressures of 20 MPa (A) and 60 MPa (B). Bars, 2 μm.

TABLE 1 Whole-cell fatty acid compositions of piezophilic strain YC-1, related *Neptunomonas* strains, and piezophilic species of related genera

| Fatty acid | Whole-cell fatty acid composition (%)[a] of: | | | | | | | | | | |
| | Strain YC-1 grown at: | | *N. concharum* LHW37[T] | *N. japonica* JAMM 0745[T] | *N. antarctica* S3-22[T] | *N. naphthovorans* NAG-2N-126[T] | *Colwellia piezophila* Y223G[T] | *Moritella japonica* JCM 10249[T] | *Photobacterium profundum* JCM 10084[T] | *Psychromonas kaikoae* JCM 11054[T] | *Shewanella benthica* ATCC 43992[T] |
	20 MPa	60 MPa									
$C_{10:0}$	Tr	Tr	2.9	2.9	TR	—	1–2	—	2	1	—
$C_{12:0}$	Tr	Tr	1.3	1.7	TR	3.7	9	18	3	6	2
$C_{14:0}$	Tr	Tr	—	—	—	—	3	1	1	1	14
$C_{15:0}$	Tr	Tr	—	—	—	—	—	—	—	—	—
$C_{16:0}$	7.7	7.0	8.7	7.0	14.7	17.1	31–33	21	9	15	14
$C_{13:0}$ iso	—	—	—	—	—	—	—	—	2	—	5
$C_{14:0}$ iso	—	—	—	—	—	—	—	—	4	—	—
$C_{15:0}$ iso	—	—	—	—	—	—	—	—	2	—	11
$C_{16:0}$ iso	Tr	Tr	—	—	—	—	—	—	15	10	—
$C_{14:1}$	Tr	—	—	—	—	—	2	2	3	—	—
$C_{15:1}$	Tr	Tr	—	—	—	—	3	—	—	—	—
$C_{16:1}$	44.9	35.8	38.0	45.2	64.0	37.9	48–50	50	31	54	31
$C_{18:1}$	30.7	45.6	39.9	32.5	9.9	34.3	—	2	9	2	2
$C_{20:5}$ (EPA)	—	—	—	—	—	—	—	—	—	2	16
$C_{22:6}$ (DHA)	11.0	7.3	5.1	4.8	4.5	6.1	—	6	13	—	—
$C_{10:0}$ 3-OH	—	—	—	—	—	—	1	—	—	2	1
$C_{12:0}$ 3-OH	—	—	—	—	—	—	—	—	5	—	—
$C_{14:0}$ 3-OH	—	—	—	—	—	—	—	—	—	4	—
$C_{13:0}$ iso 3-OH	—	—	—	—	—	—	—	—	—	—	5

[a] Data for strain YC-1 are from this study; data for *Neptunomonas* strains are from the work of Lee et al. (37); and data for the *C. piezophila*, *M. japonica*, *P. profundum*, *P. kaikoae*, and *S. benthica* strains are from the work of Nogi et al. (38). Tr, trace (<1.0%); —, not detected/not reported.
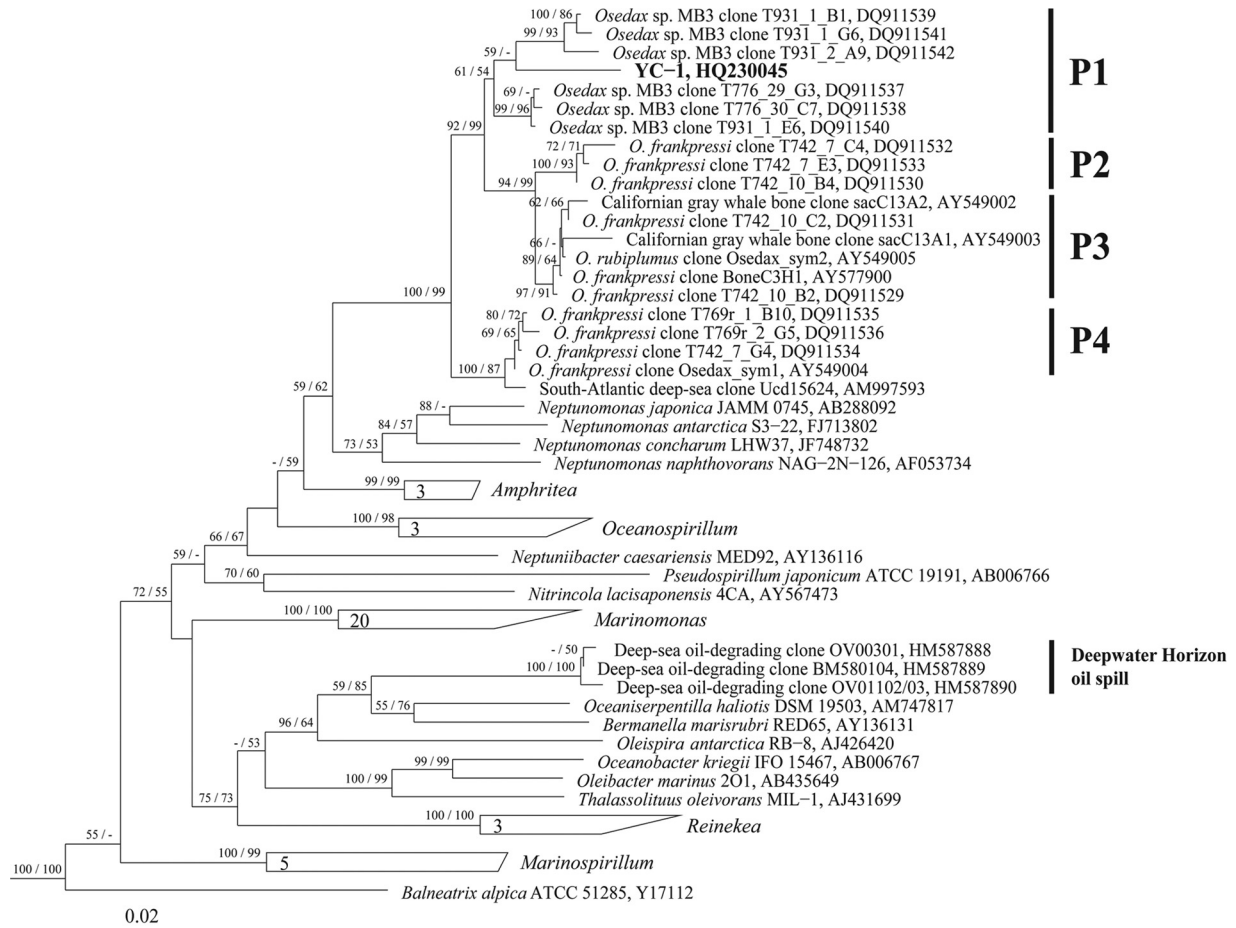
FIG 3 Phylogenetic tree depicting the relationship, based on 16S rRNA gene sequences, of strain YC-1 (shown in boldface) from the Puerto Rico Trench and related strains within the family *Oceanospirillaceae*. Bootstrap support for nodes is indicated (PHYLIP NJ method/FastTree ML method; only values above 50% are shown). The outgroup used to calculate phylogeny was *Escherichia coli* ATCC 11775[T] (GenBank accession number X80725). The bar represents 0.02 substitution per nucleotide position. Clusters P1 to P4, previously identified by Goffredi et al. (14), and Deepwater Horizon oil spill-associated clones identified by Hazen et al. (11) are indicated.

duced. The major hydroxy fatty acid was $C_{10:0}$ 3-OH at both low and high pressures. Unlike most other piezophilic bacteria within the *Gammaproteobacteria*, strain YC-1, like *Colwellia piezophila* Y223G, did not produce either EPA or DHA (38). The ratio of unsaturated fatty acids to saturated fatty acids in YC-1 changed from 6.8 to 8.8 when the pressure was increased from 20 MPa to 60 MPa.

YC-1 is a novel member of the *Oceanospirillales*. Phylogenetic analyses based on the 16S rRNA gene sequence place YC-1 within the *Oceanospirillales* order of the *Gammaproteobacteria* (Fig. 3). More specifically, YC-1 is localized within the P1 clade, a grouping that, until now, was associated exclusively with uncultured symbionts of bone-eating worms of the genus *Osedax* (13, 14). All of the sequences assigned to this clade are from the first and the deepest whale fall yet characterized for *Osedax* worms, a gray whale carcass present at 2,891 m within Monterey Canyon, CA (16). Strain YC-1 is closely related to the symbiont bacterial clone Osedax_sym1 (97.2% 16S rRNA sequence identity). Curiously, the worm tissues from which Osedax_sym1 nucleic acid were derived were also found to be rich in vaccenic acid ($C_{18:1}$) and the long-chain polyunsaturated fatty acid EPA, features shared

with many psychropiezophilic bacteria. The cultivated organisms most closely related to strain YC-1 belong to the genus *Neptunomonas* and include *Neptunomonas antarctica* S3-22[T], *Neptunomonas japonica* JAMM 0745[T], *Neptunomonas naphthovorans* NAG-2N-126[T], and *Neptunomonas concharum* LHW37[T], which share 93.2, 93.1, 92.7, and 92.6% 16S rRNA sequence identity with YC-1, respectively. *Neptunomonas japonica* also is connected to whale falls. The one member of this species was isolated from sediment adjacent to a sperm whale carcass off Kagoshima, Japan (19), and is closely related to symbionts of *Osedax* present at shallow-water minke, pilot, and sperm whale falls in the North Atlantic (13). Phylogenetically, strain YC-1 falls next to the genus *Neptunomonas* and within clades P1 to P4 of uncultured California *Osedax* symbionts and uncultured sea ice and seawater microbes (14). The G+C content of the DNA of strain YC-1 is 44.6 mol%.

Previous FISH investigations of *Osedax* symbionts revealed that the symbionts are present in the ovisac and proliferative "root" tissues of mature females (13, 39) and are absent from *Osedax* eggs, sperm, and presettlement larvae (13, 40). It is believed that *Osedax* worms acquire the symbionts as free-living bacteria from the environment before settlement. Given that the

TABLE 2 Phenotypic characteristics of strain YC-1 and related species of the genus *Neptunomonas*[a]

Characteristic	Strain YC-1	*N. japonica* JAMM 0745[T]	*N. naphthovorans* NAG-2N-126[T]	*N. antarctica* S3-22[T]	*N. concharum* LHW37[T]
Cell shape	Rod/cocci	Rod	Rod	Rod	Rod
Motility	−	+	+	+	+
Growth temp (°C)					
Range	2–14	5–25	4–30	4–25	10–45
Optimum	8	20	ND	15	37
Optimum pressure (MPa)	50	0.1	0.1	0.1	0.1
Growth at atmospheric pressure	−	+	+	+	+
Oxidase	+	+	+	+	+
Catalase	+	+	+	+	+
DNase	−	+	−	−	+
Gelatinase	−	+	−	−	+
Indole production	−	−	+	−	−
Thiosulfate reduction	−	−	−	−	−
Nitrate reduction	+	+	−	+	+
Acid from sugars	−	−	+	+	+
DNA G+C content (mol%)	44.6	43.6	46.3	45.6	48.2

[a] Data for strain YC-1 are from this study; data for the *Neptunomonas* species are from the work of Zhang et al. (43) and Lee et al. (37). +, positive; −, negative; ND, no data.

symbionts of deep-sea *Osedax* hosts are distinct from those of their shallow-water counterparts, horizontal transfer of symbionts could be controlled by both host and nonhost environmental factors, including those associated with increasing water depth (13, 14). Indeed, a recent study of temporal variation with *Osedax* symbiont diversity provided evidence that the *Oceanospirillales* symbionts associated with *Osedax* are dynamic and differ according to environmental conditions. The acquisition of symbionts by *Osedax* was affected by the composition of free-living microbes in the local environment (41). Species related to the psychropiezophilic strain YC-1 could be selectively favored by *Osedax* larvae during symbiont acquisition in deeper waters. However, the relationship between free-living strain YC-1 and bone-eating worm symbionts still needs further study.

YC-1 can degrade hydrocarbons and is metabolically versatile. The characteristics of the isolated piezophilic strain YC-1 and reference *Neptunomonas* strains are shown in Table 2. YC-1 is a facultatively anaerobic heterotroph. The results of oxidase and catalase tests were positive, and the tests of DNase and gelatinase activities were negative. Hydrogen sulfide and indole were not produced, and motility was not detected. Acid was not produced from sugar. Strain YC-1 oxidized Tween 80, Triton X-100, Triton X-114, hexadecane, citrate, formate, lactate, L-alanine, L-arginine, L-aspartic acid, L-lysine, L-methionine, L-phenylalanine, L-serine, and L-threonine as sole carbon sources. An electron acceptor was required for anaerobic growth, and YC-1 was able to use nitrate, sulfate, and TMAO as terminal electron acceptors.

The utilization of hexadecane by YC-1 as its sole carbon source indicated an ability to degrade aliphatic hydrocarbon. Studies of the 2010 Deepwater Horizon oil spill in the Gulf of Mexico revealed that microbial processes were the key factor accounting for the degradation of the 1,100- to 1,200-m-deep plume of light crude oil and chemical dispersants (11, 12, 42). Microbial community analyses revealed that a single operational taxonomic unit (OTU) within the *Oceanospirillales* accounted for more than 90% of the 16S rRNA gene sequences recovered from plume samples (11). This OTU shares 90.0% similarity with YC-1. Metagenomics, metatranscriptomics, and single-cell sequencing of the Deep-

water Horizon plume-associated microbes has identified the entire pathways for degradation of alkanes in these uncultured *Oceanospirillales* species (12). However, the lack of a cultivated isolate within the *Oceanospirillales* from the deep sea has prevented in-depth physiological studies of hydrocarbon degradation within this order. The isolation of the psychropiezophilic *Oceanospirillales* isolate YC-1 provides an opportunity for such studies.

Consistent with the phylogenetic analysis, strain YC-1 shares a number of physiological and biochemical properties with members of the genus *Neptunomonas*, including positive oxidase and catalase activities, $C_{16:1}$ and $C_{18:1}$ fatty acids as dominant components of MUFAs, and DNA G+C contents of 43.6 to 46.3 mol%. However, there were also some major differences between strain YC-1 and members of the genus *Neptunomonas*. YC-1 is a deep-sea psychropiezophilic bacterium that cannot survive at atmospheric pressure. It also exhibits no motility. The low 16S rRNA gene sequence similarity and the significant phenotypic differences between strain YC-1 and the representatives of related genera within the family *Oceanospirillaceae* suggest that strain YC-1 represents a novel deep-sea species within a new genus, which we designate *Profundimonas piezophila* gen. nov., sp. nov.

Description of *Profundimonas* gen. nov. *Profundimonas* (Pro.fun.di.mo′nas. L. neut. n. *profundum*, the depths of the sea; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Profundimonas*, a unit [bacterium] from the depths of the sea). Cells are nonmotile, facultatively anaerobic, Gram-negative rods at optimal high hydrostatic pressures. Coccoid bodies formed under low pressures. Oxidase and catalase positive, and predominant fatty acids are $C_{16:1}$ and $C_{18:1}$. Psychropiezophilic growth, with pressure range from 20 to 70 MPa, temperature range from <4 to 14°C. 16S rRNA gene sequence analysis places the genus close to the genus *Neptunomonas* within the family *Oceanospirillaceae*. The type species is *Profundimonas piezophila*.

Description of *Profundimonas piezophila* sp. nov. *Profundimonas piezophila* (pi.e.zo.phi′la. Gr. v. *piezo*, to press; N.L. adj. *philus*, -a, -um [from Gr. adj. *philos*, -ê, -on], friend, loving; N.L. fem. adj. *piezophila*, loving pressure). The main characteristics of

the species are the same as those reported for the genus. The cell size is 4.0 to 5.0 by 0.5 to 0.8 μm as a rod under 60 MPa, 0.8 to 1.0 μm in diameter as a coccoid under 20 MPa. Optimal growth occurs at 50 MPa and 8°C when cells are cultivated in marine broth 2216. The main nonpolar cellular fatty acids are $C_{16:1}$ and $C_{18:1}$. The major hydroxy fatty acid is $C_{10:0}$ 3-OH. Tests for oxidase and catalase activities are positive, and negative reactions are obtained for DNase and gelatinase. Negative for indole production, and H_2S is not produced from thiosulfate. Acid is not produced from sugars. The following substrates are utilized as sole carbon sources for respiration: Tween 80, Triton X-100, Triton X-114, hexadecane, citrate, formate, lactate, L-alanine, L-arginine, L-aspartic acid, L-lysine, L-methionine, L-phenylalanine, L-serine, and L-threonine. The following compounds are used as terminal electron acceptors for anaerobic growth: nitrate, sulfate, and TMAO. The G+C content of the DNA is 44.6 mol%. The type strain, YC-1, was isolated from a deep-sea seawater sample collected in the Puerto Rico Trench. *Profundimonas piezophilia* strain YC-1 is available through the American Type Culture Collection (catalog no. BP-BAA-2591).

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