Barophilic bacteria grow optimally or exclusively at hydrostatic pressures greater than 1 atm (1 atm = 1.013 MPa = 101.3 bar). Barophilic isolates have been obtained from a variety of different deep-sea habitats by a number of different laboratories (5, 8, 9, 15, 16, 20, 34–36, 38, 39). The phylogenetic affiliations of several barophilic isolates have been inferred by 5S rRNA (8, 22) or 16S-like rRNA sequence comparisons (9, 13, 15, 16, 20). Studies based on 5S rRNA sequences showed that two facultatively barophilic bacterial isolates, *Colwellia psychroerythrus* (9). Additionally, small-subunit rRNA sequence analysis indicated that two strains of Antarctic obligate barophiles, initially grown in mixed culture, are members of the gamma subdivision of the class *Proteobacteria* (*γ*-Proteobacteria); *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and a new group containing strain CNPT3. This data indicate that the barophilic phenotype has evolved independently in different *γ*-Proteobacteria genera.

**Bacterial strains and growth media.** The pressure physiology of most barophilic bacterial strains in this study have been previously described (Table 1) (7, 35, 37–39). *Isolate SS9 originated from a depth of 2,551 m in the Sulu Sea and was obtained from an amphipod homogenate enrichment (5). Barophilic strain F1A was kindly provided by Carl O. Wirsen (34). All other reference strains were obtained from the culture collection of A. A. Yayanos or the American Type Culture Collection (ATCC). Nonbarophilic strains were grown at 3°C in marine broth 2216 (Difco). Barophilic strains were grown at 3°C in marine broth 2216 at the appropriate hydrostatic pressure (Table 1), as previously described (Table 1) (6, 7, 39), decompressed, harvested by centrifugation, and stored frozen at −80°C. All barophilic strains examined in this study (Table 1), with the exception of strain MT41, which was not tested, were successfully stored as frozen stock cultures at 1.1 × 10⁵ Pa and −80°C. Strains were grown at the appropriate growth pressure at 3°C to approximately 1 × 10⁶ cells/ml, decompressed, immediately diluted 1:1 in ice-cold 2× freeze buffer (1% yeast extract, 10% glycerol, 10% dimethyl sulfoxide, 0.1 M K₂HPO₄ [pH 7.0]), quickly frozen in a dry ice-ethanol bath, and stored at −80°C. Frozen cultures were revived by inoculation of ice-cold marine broth with scrapings from the frozen stock and incubation under the appropriate hydrostatic pressure at 3°C.

**Phenotypic characterization.** DNA base composition was determined from thermal melting profiles performed under standard conditions as previously described (24). Other physiological tests were performed by slight modification of the general procedures described by Baumann and Baumann (1). All high-pressure physiological tests were performed in tandem with uninoculated blank controls. Acid production from glucose was assessed in a modified OF medium (1) containing 0.5× ASW (1× ASW is 400 mM NaCl, 100 mM MgSO₄·H₂O, 20 mM KCl, 20 mM CaCl₂·2H₂O, 10 mM morpholinepropanesulfonic acid (MOPS; pH 7.5) at 22°C, 1% glucose, 0.2% agar, 0.5% yeast extract, and 0.003% bromcresol purple. Cultures were inoculated as stabs and sealed with molten media containing 4% gelatin. After topping the tube with 4% gelatin, the tubes were chilled to 4°C, sealed with Parafilm, and incubated at the appropriate pressure at 3°C (Table 1). After decompression, the stabs were examined for growth and acid production. Hydrogen sulfide production from thiocarbonate was assessed in triple sugar iron agar (TSI; Difco) prepared with 0.5× ASW instead of water. DNAse activity was tested by inoculation of stab cultures in DNase medium (Difco) containing 0.5× ASW and 0.01% methyl green. Oxidase tests were performed as previously described (1). Tests for anaerobic growth with lactate as the sole carbon source and trimethylamine oxide (TMAO) as the sole electron acceptor were performed as follows. Two parts of a defined mineral medium (SM medium [10]) was mixed with 12% gelatin plus the following combinations of electron donors and acceptors: (i) no addition, (ii) 25 mM TMAO, (iii) 15 mM lactate, and (iv) 25 mM TMAO plus 15 mM lactate. The tubes were chilled to solidify the gelatin, inoculated as a stab, overlaid with 1 ml of 4% gelatin, sealed with Parafilm, and incubated at 2°C and the pressure appropriate for the specific strain. Strains which showed growth in the presence of TMAO and lactate were scored as positive. Motility determinations were performed on cultures grown at their optimal growth pressure in marine broth. Exponentially growing cultures were decompressed to 1.1 × 10⁵ Pa, placed on a chilled microscope slide, and immediately examined by phase microscopy for motility at 1.1 × 10⁵ Pa.

**Phylogenetic characterization.** Nucleic acids were purified by standard methods (30). In some cases (SC2A, F1A, *Shewanella hanedai* ATCC 33224, MT41, PT48, and PT99), rRNA templates
were directly sequenced with reverse transcriptase (Seikagaku) as previously described (17, 18). All other small subunit rRNA sequences (PE36, Mortiella marinus MP1, CNPT3, SS9, S. hanedai ATCC 35256, S. benthica ATCC 43991, and Shewanella putrefaciens ATCC 8072) were obtained by direct sequencing of PCR-amplified ribosomal DNA genes.

Phylogenetic analyses were conducted with reference sequences and software (GDE 2.2 [32]) obtained via anonymous file transfer program from the Ribosomal RNA Database sequences and software (GDE 2.2 [32]) obtained via anonymous file transfer program from the Ribosomal RNA Database.

**Nucleotide sequence accession number.** The sequences of the ribosomal DNA genes used in this study have been deposited in GenBank under accession no. U91586 to U91600.

**Results and discussion.** Strain designations, sites and depths of origin, and pressure optima for barophilic strains characterized in this study are shown in Table 1. Phenotypic characteristics of barophilic isolates and reference strains are shown in Table 2. Physiological tests conducted at 3°C and in situ growth pressures (Tables 1 and 2) were consistent with the placement of F1A, PT99, PT48, and SC2A within the genus Shewanella. Characteristics common to Shewanella species (19) and found in S. benthica strains PT99, PT48, and F1A included H₂S production on TSI agar, lactate oxidation coupled to TMAO reduction, being oxidase and DNase positive, and showing no fermentation of glucose. At 1.1 × 10⁵ Pa and 8°C, S. benthica ATCC 43991 tested positive for anaerobic growth on lactate with Fe³⁺ but not sulfite as a terminal electron acceptor. Strain SC2A, phylogenetically affiliated with S. putrefaciens, grew anaerobically on lactate with Fe³⁺, TMAO, or sulfite as the terminal electron acceptors. Unlike most other barophilic strains examined, strain CNPT3 fermented glucose vigorously as indicated by both acid production and gas production on glucose. Strain CNPT3 was the only strain examined which tested positive for arginine dihydrolase.

**TABLE 1. Deep-sea bacterial strains used in this study**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Physiology</th>
<th>Geographic origin</th>
<th>Depth (m)</th>
<th>Approx growth pressure optimum (10⁵ Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT41</td>
<td>Yananos (38)</td>
<td>Obligate barophile</td>
<td>Mariana Trench</td>
<td>10,476</td>
<td>1,034</td>
</tr>
<tr>
<td>C. psychroerythrus</td>
<td>ATCC 27364 (9)</td>
<td>Psychrophile</td>
<td>Norwegian fjord</td>
<td>Surface</td>
<td>1.1</td>
</tr>
<tr>
<td>CNPT3</td>
<td>Yananos (37)</td>
<td>Facultative barophile</td>
<td>Central North Pacific</td>
<td>5,800</td>
<td>517</td>
</tr>
<tr>
<td>SC2A</td>
<td>Yananos (39)</td>
<td>Psychrophile</td>
<td>California coast</td>
<td>1,957</td>
<td>1.1</td>
</tr>
<tr>
<td>F1A</td>
<td>Jannasch and Wirschen (34)</td>
<td>Facultative barophile</td>
<td>North Atlantic</td>
<td>4,800</td>
<td>414</td>
</tr>
<tr>
<td>PT99</td>
<td>Yananos (7)</td>
<td>Obligate barophile</td>
<td>Philippine Trench</td>
<td>8,600</td>
<td>621</td>
</tr>
<tr>
<td>S. benthica</td>
<td>ATCC 43991 (8)</td>
<td>Facultative barophile</td>
<td>Puerto Rico Trench</td>
<td>5,920</td>
<td>414</td>
</tr>
<tr>
<td>PT48</td>
<td>Yananos (7)</td>
<td>Obligate barophile</td>
<td>Philippine Trench</td>
<td>6,163</td>
<td>621</td>
</tr>
<tr>
<td>Photobacterium sp. strain S99</td>
<td>DeLong (5)</td>
<td>Facultative barophile</td>
<td>Sulu Sea</td>
<td>2,551</td>
<td>276</td>
</tr>
<tr>
<td>M. marinus MP1</td>
<td>ATCC 15361 (4)</td>
<td>Psychrophile</td>
<td>Oregon coast</td>
<td>1,200</td>
<td>1.1</td>
</tr>
<tr>
<td>PE36</td>
<td>Yananos (35)</td>
<td>Facultative barophile</td>
<td>California coast</td>
<td>3,584</td>
<td>414</td>
</tr>
</tbody>
</table>

The genus *Shewanella* is composed of an ecologically diverse group of obligately respiratory, gram-negative *Proteobacteria* isolates of both marine and terrestrial origin (9, 13, 14, 21, 22, 27, 29, 31, 33, 37). A large number of barophilic isolates, obtained by five different laboratories working in disparate locales (7, 8, 15, 16, 20, 34), are *S. benthica* strains or extremely close relatives (Fig. 1). The data indicate that *S. benthica* is the most commonly isolated barophilic species, recovered from a variety of abyssal to hadal environments in Pacific, Atlantic, and Antarctic seas. It is difficult to judge from available data how numerically abundant or ecologically significant this species actually is, since its representation in deep-sea bacterial
culture collections may simply reflect its selective enrichment in culture and not its numerical abundance.

In total, our analyses indicate that the evolution of barophilic phenotype is not limited to one or a few genera, but may be fairly widely distributed among the \textit{g}-Proteobacteria and possibly other eubacterial or archaeal phyla. Considering that many naturally occurring microorganisms have proven difficult or impossible to recover by standard cultivation techniques, it is likely that many new lineages of deep-sea barophilic bacteria remain to be discovered and described.

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