Parvularcula bermudensis gen. nov., sp. nov., a marine bacterium that forms a deep branch in the *α-Proteobacteria*

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Two bacterial strains, HTCC2503^T and HTCC2517, were isolated from the Bermuda Atlantic Time Series Station in the western Sargasso Sea, Atlantic Ocean, by new high-throughput culture methods that rely on dilution to extinction in very-low-nutrient media. Characterization of the two strains by polyphasic approaches revealed that they belonged to the same species. These isolates are Gram-negative, strictly aerobic, chemoheterotrophic, slightly motile short rods with a single flagellum. The temperature, pH and NaCl concentration ranges for growth were 10–37 °C, 6·0–9·0 and 0·75–20 % (w/v), respectively. Colonies on marine agar were very small (0·3–0·8 mm in diameter), yellowish-brown and very hard. Carotenoid pigments were synthesized but bacteriochlorophyll *a* was not. Several kinds of pentose, hexose, sugar alcohol, oligosaccharide and amino acid were utilized as sole carbon sources. Oxidase was produced, but catalase was not. All cellular fatty acids were even-numbered monounsaturated or saturated fatty acids and the major fatty acid was *cis*-7-octadecenoic acid (73·3 %). The DNA G+C content of strain HTCC2503^T was 60·8 mol%. Phylogenetic analyses of 16S rRNA gene sequences clearly indicated that the strains formed a distinct lineage, allied with activated sludge environmental clone H9, in the *α-Proteobacteria*. The clade containing strains HTCC2503^T and HTCC2517 and clone H9 could not be phylogenetically associated with any of the six known orders of the *α-Proteobacteria*. From this polyphasic evidence, it is proposed that the novel strains should be classified as *Parvularcula bermudensis* gen. nov., sp. nov. The type strain is HTCC2503^T (=ATCC BAA-594^T=KCTC 12087^T) and the reference strain is HTCC2517.

INTRODUCTION

It is generally accepted that standard plate-count methods on solid-surface media recover <1 % of all microbial cells from marine environments (Kogure *et al.*, 1979; Ferguson *et al.*, 1984). Molecular biological tools, such as 16S rRNA gene cloning and sequencing, have elucidated this issue for a decade (Giovannoni *et al.*, 1990; DeLong, 1992; Suzuki *et al.*, 1997; Béjà *et al.*, 2000), and have led to the conclusion that the most abundant marine microbial groups are as yet uncultivated and probably play a significant role in marine biogeochemical cycling (Giovannoni & Rappé, 2000). Acquisition of pure cultures for the study of their physiology, ecology and taxonomy is an important goal of research in this field. Recently, high-throughput culture (HTC) methods were developed, which allowed large numbers of microbial isolates to be recovered by dilution to extinction in natural sea-water media (Connon & Giovannoni, 2002). The first cultured representative of the SAR11 clade (Rappé *et al.*, 2002) and many novel strains in the *Proteobacteria* (Connon & Giovannoni, 2002) were isolated from the Oregon coast using this approach.

Here, we describe the application of similar HTC methods to the western Sargasso Sea. Several novel bacteria affiliated to the α- and γ-*Proteobacteria*, the families *Flavobacteriaceae* and *Nocardioidaceae* and the phylum *Cyanobacteria* were isolated. This study focuses on strains HTCC2503^T and HTCC2517, which form a very deeply branching lineage in the α-subclass of the *Proteobacteria*. The strains were characterized by polyphasic approaches (Vandamme *et al.*, 1996) and we propose their classification as *Parvularcula bermudensis* gen. nov., sp. nov.

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Abbreviation: HTC, high-throughput culture.

The GenBank accession numbers for the 16S rRNA gene sequences of strains HTCC2503^T and HTCC2517 are AF544015 and AF544016, respectively.

METHODS

Sampling, isolation procedure, cultivation and maintenance. A sea-water sample was collected from a depth of 10 m at the Bermuda Atlantic Time Series (BATS) Station, an oligotrophic region in the western Sargasso Sea, Atlantic Ocean, in August 2001. The water sample was diluted to 10 cells ml^{-1} in low-nutrient

heterotrophic medium (0·2 μm-filtered and autoclaved sea water, modified with 1·0 μM NH_4Cl and 0·1 μM KH_2PO_4), supplemented with 0·001 % (w/v) D-glucose, D-ribose, succinic acid, pyruvic acid, glycerol and *N*-acetyl-D-glucosamine, 0·002 % (v/v) ethanol and Va vitamin solution at 10^{-4} dilution (Davis & Guillard, 1958). The initial liquid cultures of two bacterial strains (designated HTCC2503^T and HTCC2517) were obtained using the high-throughput approaches outlined by Connon & Giovannoni (2002). Microtitre dish wells were scored for growth by microscopic examination, using a procedure for creating cell microarrays. The positive cultures were then spread onto marine agar 2216 (Difco) and colonies of the two strains were isolated after 14 days incubation at 25 °C, purified by subsequent streaking onto marine agar and stored as 10 % (v/v) glycerol suspensions in liquid nitrogen. The strains were revived on agar plates and in broth from frozen stocks every 3 months to ensure their purity.

Microscopy. The strains were grown to late-exponential phase in marine R2A broth (Reasoner & Geldreich, 1985; Suzuki *et al.*, 1997) at 30 °C and 150 r.p.m. on a rotary shaker. Cell size and morphology were examined by DAPI (4′,6-diamidino-2-phenylindole) staining according to Porter & Feig (1980), using a Leica model DMRB epifluorescence microscope equipped with a Hamamatsu model ORCA-ER cooled CCD (charge-coupled device) camera and IPLab version 3.5 scientific imaging software (Scanalytics). Motility was examined from wet mounts of exponential-phase cells under dark field microscopy (DMRB; Leica). Exponential-phase cells were prepared for electron microscopy after they had been concentrated with Vivaspin 500 ultrafiltration concentrators (Vivascience), washed twice with PBS (pH 8·0), fixed with 1·5 % glutaraldehyde and negatively stained with 2 % aqueous ammonium molybdate (pH 6·3) on Formvar-filmed, carbon-coated and glow-discharged 300-mesh copper grids. Transmission electron microscopy was carried out using a Philips CM12 transmission electron microscope, operated at 60 kV in transmission mode.

Phenotypic characterization. Standard methods for phenotypic characterization were performed as described by Smibert & Krieg (1994), unless otherwise noted. Colony morphology, size and colour were examined from cultures grown aerobically on marine agar 2216 (Difco) at 30 °C for 14 days. Pigments were extracted using a methanol/acetone mixture (1 : 1) from cultures grown on marine agar 2216 for 14 days, and their absorption spectra were determined by using a scanning UV/visible spectrophotometer (Biospec-1601; Shimadzu). Gram reaction was confirmed by the non-staining KOH method (Buck, 1982).

The temperature and pH ranges for growth were determined in marine R2A broth by measuring OD_{600} during incubation for 20 days. Temperature range and optimum were tested in the range 4–44 °C. The pH range and optimum were examined at pH values 4·0–12·0 at 30 °C. The pH was adjusted with 0·1 M HCl and 0·1 M NaOH. The NaCl concentration range and optimum for growth were determined in a medium that contained (l^{-1}): 1·0 g $MgCl_2.6H_2O$, 5·0 g $MgSO_4.7H_2O$, 0·7 g KCl, 0·15 g $CaCl_2.2H_2O$, 0·5 g NH_4Cl, 0·1 g KBr, 0·27 g KH_2PO_4, 0·04 g $SrCl_2.6H_2O$, 0·025 g H_3BO_3, 5·0 g peptone and 1·0 g yeast extract (pH 8·0) with 0–20 % NaCl (w/v). Anaerobic growth was tested by using both the Oxoid Anaerobic and Merck Anaerocult C Mini systems.

The catalase test was performed by addition of 3·0 % hydrogen peroxide to fresh colonies; oxidase activity was determined using Kovacs' solution (Kovacs, 1956). Other biochemical tests were carried out on API 20NE strips (bioMérieux), following the manufacturer's instructions.

Utilization of organic compounds as sole carbon sources was tested using custom-made 48-well microplates that contained 47 different carbon compounds. Each compound was added to a final concentration of 0·2 % (w/v), following sterilization by either filtration or autoclaving. Strains were grown on marine agar plates and cell densities were adjusted to approximately $5{\cdot}0 \times 10^3$ cells ml^{-1} in artificial sea-water medium (ASW; 25·0 g NaCl, 1·0 g $MgCl_2.6H_2O$, 5·0 g $MgSO_4.7H_2O$, 0·7 g KCl, 0·15 g $CaCl_2.2H_2O$, 0·5 g NH_4Cl, 0·1 g KBr, 0·27 g KH_2PO_4, 0·04 g $SrCl_2.6H_2O$ and 0·025 g H_3BO_3 l^{-1}). Three microplates were inoculated with 1 ml cell suspension per well and incubated at 30 °C for 10 days. Cellular growth and purity were assessed by DAPI-stained epifluorescence microscopy. Cultures were scored for positive growth when a minimum of two cell doublings was detected. In addition to the sole carbon source test, the ability to oxidize organic carbon compounds was tested using the Biolog SF-N2 microplates (Rüger & Krambeck, 1994). The procedures for the carbon source oxidation tests were the same as those for the sole carbon source tests, except that 150 μl cell suspension was used for inoculation.

Susceptibility to antibiotics was determined by the diffusion plate method. Bacterial cultures (100 μl) were spread on marine agar 2216 plates, discs impregnated with antibiotics were placed onto the plate surfaces and the plates were incubated at 30 °C for 5 days. The following antibiotics were tested: chloramphenicol (25 μg), nalidixic acid (25 μg), kanamycin (30 μg), carbenicillin (25 μg), tetracycline (30 μg), streptomycin (50 μg), ampicillin (10 μg), puromycin (25 μg), erythromycin (15 μg), vancomycin (30 μg), rifampicin (50 μg), benzylpenicillin (100 U), gentamicin (10 μg) and cycloheximide (50 μg).

Cellular fatty acid analysis. Cells were grown on marine agar at 28 °C for 7 days. Cellular fatty acid methyl esters were prepared and analysed using GC according to the instructions of the Microbial Identification System (MIDI). The samples were analysed by Microbial ID.

Determination of DNA base composition. Genomic DNA was extracted and purified using the Qiagen DNeasy tissue kit. The G+C content was measured using HPLC according to Mesbah *et al.* (1989), with the Platinum EPS reverse-phase C18 column (150 mm, 4·6 mm, 5 μm pore size; Alltech).

16S rDNA sequencing and phylogenetic analyses. PCR amplification of bacterial 16S rDNA was performed using two bacterial universal primers, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Lane, 1991). PCR products were purified using a Qiagen QIAquick PCR purification column and sequenced by the chain-termination method on an ABI 377 automated sequencer. Initially, nearly complete sequences of the 16S rRNA gene were compared with sequences available in GenBank by BLAST network services (Altschul *et al.*, 1997), to determine their approximate phylogenetic affiliations. Sequences were aligned using the ARB software package (Ludwig *et al.*, 1998) and 1239 unambiguously aligned nucleotide positions were used for phylogenetic analyses with PAUP* version 4.0 beta 10 (Swofford, 2002). The similarity values between sequences were calculated from distance matrices by reversing the Jukes–Cantor distance formula (Jukes & Cantor, 1969). Phylogenetic trees were inferred by both neighbour-joining (Saitou & Nei, 1987) with the Kimura two-parameter model, and maximum-parsimony with a heuristic search. The resulting neighbour-joining and parsimony trees were evaluated by bootstrap analyses based on 1000 and 100 resamplings, respectively.

RESULTS AND DISCUSSION

Phenotypic characteristics

Strains HTCC2503^T and HTCC2517 had the same phenotypic and genotypic characteristics in all tests performed,

therefore only the characterization results for strain HTCC2503^T are described here. Strain HTCC2503^T was a Gram-negative (by Gram staining and KOH test), weakly motile short rod (sometimes coccoid), 0·4–1·3 (mean 0·8) μm in diameter and 0·6–1·8 (mean 1·0) μm in length, that divided by binary fission (Fig. 1a). The strain had a short flagellum with a mean length of 2·4 μm. A hook was clearly visible at one end of flagella detached from cells (Fig. 1b). Some cells had barely visible short fimbrae. Neither endospores nor poly-β-hydroxybutyrate granules were evident. When grown on marine agar 2216 at 30 °C for 8 days, colonies were 0·3–0·8 mm in diameter, yellowish-brown, uniformly circular, convex, dry and opaque with smooth surfaces and entire margins; they penetrated inside the agar and stuck to the agar surface. Strain HTCC2503^T is an obligately aerobic, NaCl-requiring chemoheterotroph. No growth was detected under anaerobic conditions, even with prolonged incubations of 40 days. The temperature range for growth was 10–37 °C, with optimum growth at 30 °C. No growth was observed at 4 or 44 °C. Extended incubation of up to 40 days was required at 10 °C before growth was observed. The pH range for growth was 6·0–9·0, with optimum growth at pH 8·0. No growth was detected at pH 5·5 or 9·5. Strain HTCC2503^T was moderately halophilic; it showed good growth at NaCl concentrations of 0·75–25 % (w/v) and optimal growth at 3·0 % (w/v).

Strain HTCC2503^T was catalase-negative and oxidase-positive. The bacterium reduced nitrate to nitrite, but not nitrite to N_2. It did not produce indole, nor did it deaminate arginine. Urea and gelatin were hydrolysed, but no hydrolysis of aesculin was detected. Acid was not produced from glucose. The strain produced carotenoid pigments with spectral absorbance peaks at 321 and 465 nm. The peak at 465 nm was much higher than that at 321 nm. There was no difference in spectral peaks between light-grown and dark-grown cultures. No bacteriochlorophyll peaks were detected.

The strain utilized some pentoses, hexoses, sugar alcohols, oligosaccharides and amino acids as sole carbon sources. C1–C4 compounds and organic acids were not utilized as sole carbon sources. The following compounds were utilized: D-arabinose, D-glucose, L-rhamnose, sucrose, D-cellobiose, D-maltose, D-mannose, D-melezitose, D-mannitol, D-sorbitol, myo-inositol, L-glutamic acid, L-lysine, L-serine, L-leucine and L-isoleucine. However, DL-glyceraldehyde, D-ribose, D-xylose, D-galactose, D-fructose, L-sorbose, β-lactose, D-trehalose, D-melibiose, D-raffinose, adonitol, methanol, ethanol, glycerol, *N*-acetyl-D-glucosamine, succinic acid, itaconic acid, citric acid, gluconic acid, D-malic acid, malonic acid, formic acid, pyruvic acid, propionic acid, lactic acid, L-ornithine, L-proline, D-glucosamine, L-alanine, glycine and L-arginine were not utilized as sole carbon sources. In the test using Biolog SF-N2 microplates, the following carbon compounds were oxidatively utilized: dextrin, glycogen, adonitol, L-arabinose, D-arabitol, D-cellobiose, L-fucose, gentiobiose, α-D-glucose, *m*-inositol, maltose, D-mannitol, D-mannose, β-methyl-D-glucoside, L-rhamnose, sucrose, turanose, xylitol, D-galacturonic acid, α-ketobutyric acid, α-ketoglutaric acid, propionic acid, L-alaninamide, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic

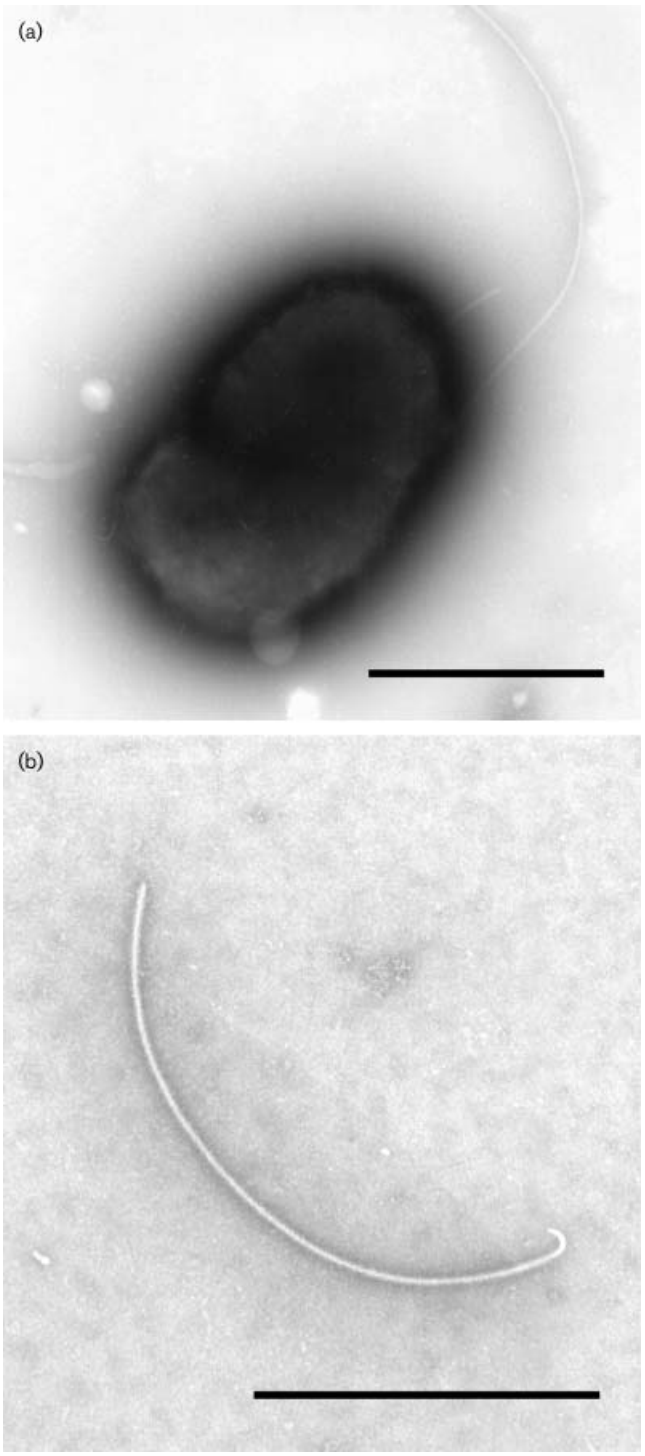

Fig. 1. Electron micrographs of negatively stained cells of strain HTCC2503^T. (a) Cell with single flagellum and fimbrae; (b) detached flagellum with hook at one end. Bars, 1 μm.

Table 1. Cellular fatty acid composition of strain HTCC2503^T grown on marine agar

Fatty acid	% of total
Dodecanoic acid ($C_{12:0}$)	5·2
Tetradecanoic acid ($C_{14:0}$)	2·9
Hexadecanoic acid ($C_{16:0}$)	8·6
cis-9-Octadecenoic acid ($C_{18:1}\omega 9c$)	6·0
cis-7-Octadecenoic acid ($C_{18:1}\omega 7c$)	73·3
Octadecanoic acid ($C_{18:0}$)	4·0

acid, L-leucine, L-serine, urocanic acid, inosine and DL-α-glycerolphosphate.

Strain HTCC2503^T was susceptible to chloramphenicol, carbenicillin, tetracycline, streptomycin, puromycin, erythromycin and rifampicin. However, it was resistant to nalidixic acid, kanamycin, vancomycin, ampicillin, benzylpenicillin, gentamicin and cycloheximide.

Fatty acid composition and DNA base composition

Only six kinds of fatty acid, containing 12–18 carbon atoms, were detected (Table 1). All fatty acids were even-numbered and either monounsaturated or saturated. The predominant fatty acid was *cis*-7-octadecenoic acid (73·3 %) and the total percentage of saturated fatty acids ($C_{12:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$) was 20·7 %. The DNA G+C content of strain HTCC2503^T was $60{\cdot}8 \pm 0{\cdot}3$ mol% (mean $\pm$ SD; $n=3$), determined by HPLC.

Phylogenetic analyses for 16S rDNA sequences

A total of 1455 and 1440 nt of the 16S rRNA gene sequences were determined for strains HTCC2503^T and HTCC2517, respectively. Their sequences were identical, so they were considered to belong to the same species by this criterion, as well as by comprehensive phenotypic classification. Preliminary sequence analyses using the BLAST network service and subsequent phylogenetic analyses showed that the strains belong to the α-*Proteobacteria*, and were most closely related to the sludge environmental clone H9 (GenBank no. AF234706; Juretschko *et al.*, 2002), albeit with a low similarity value (90·4 %). The sequences were also aligned with representative 16S rRNA gene sequences of organisms that belong to the six different orders of the α-*Proteobacteria* from the ARB database. Sequence comparisons to validly published bacteria indicated that the strains are most closely related to *Aminobacter aminovorans* (89·6 % similarity), *Aminobacter aganoensis* (89·9 %) and *Mesorhizobium loti* (89·5 %) in the order '*Rhizobiales*', and *Silicibacter lacuscaerulensis* (88·6 %) and *Rhodovulum sulfidophilum* (87·9 %) in the order '*Rhodobacterales*' [the ordinal names in quotation marks represent proposed, but not yet approved, names that appear in the second edition of *Bergey's Manual of Systematic Bacteriology*; Garrity & Holt (2001)]. As shown in the phylogenetic tree (Fig. 2), strains HTCC2503^T and HTCC2517 and environmental clone H9 formed a unique clade that did not associate significantly with any of the known six orders of the α-*Proteobacteria* (Fig. 2). This clade appeared to be monophyletic in both neighbour-joining and maximum-parsimony trees, with strong bootstrap support (99 and 98 %, respectively). The strains formed a supraordinal monophyletic clade with the orders '*Rhodobacterales*' and '*Rhizobiales*' in the neighbour-joining tree, but this relationship was not strongly supported by bootstrap analysis (69 %) and the clade broke down in maximum-parsimony analysis. These phylogenetic analyses indicate the uniqueness of the 16S rDNA sequences of strains

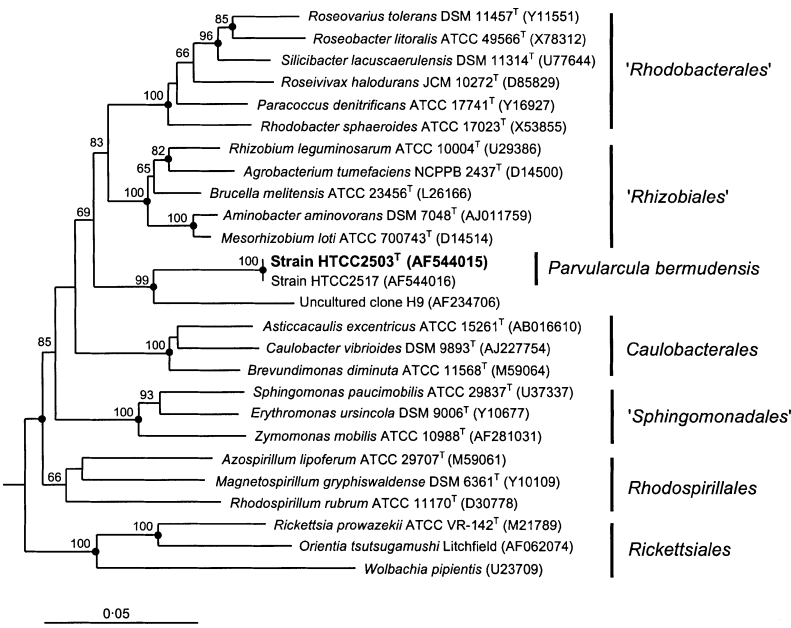

Fig. 2. Neighbour-joining tree showing relationships between strain HTCC2503^T and representatives of the α-*Proteobacteria*, inferred from 16S rRNA gene sequence analyses. Bootstrap values >65 % are shown. Closed circles at nodes indicate recovered nodes with >65 % bootstrap value in a maximum-parsimony tree. *Escherichia coli* (M87049) was used as an outgroup to define the root of the tree. Bar, 0·05 substitutions per nucleotide position.

HTCC2503^T and HTCC2517, compared to those of previously described α-*Proteobacteria*.

Polyphasic taxonomy of the novel strains

The α-*Proteobacteria* have been divided into four groups (α1, α2, α3 and α4) that have been historically used in the taxonomy of α-*Proteobacteria* (Woese *et al.*, 1984; Bhat *et al.*, 1991; Kosako *et al.*, 2000; Ruiz *et al.*, 2000). Recently, the second edition of *Bergey's Manual of Systematic Bacteriology* proposed that the class α-*Proteobacteria* should be divided into six orders: *Rhodospirillales*, *Rickettsiales*, *Caulobacterales*, '*Rhodobacterales*', '*Sphingomonadales*' and '*Rhizobiales*', on the basis of 16S rRNA gene sequences (Garrity & Holt, 2001). The phylogenetic analysis of strains HTCC2503^T and HTCC2517 was based on this classification, and employed some representative sequences that belonged to each of these six orders. Even though our strains were distantly related to the orders '*Rhodobacterales*' and '*Rhizobiales*' in the neighbour-joining phylogenetic tree, the sequence similarities of the strains to the other members of these orders were only 86·3–89·4 and 84·5–88·7 %, respectively. Furthermore, a specific relationship of the strains to these orders was not seen in the maximum-parsimony analysis. Thus, from the phylogenetic analyses, the strains could not be associated with any of the six known orders of the α-*Proteobacteria*; these novel strains therefore appear to constitute a new seventh order of the α-*Proteobacteria*.

Strain HTCC2503^T was also clearly different, both phenotypically and ecologically, from its closest neighbours as determined by comparative analyses of 16S rRNA gene sequences. The species *A. aminovorans* and *M. loti* in the order '*Rhizobiales*' and *S. lacuscaerulensis* in the order '*Rhodobacterales*' were all isolated from either soils or a geothermal lake, and exhibited low or moderate tolerance to elevated salt concentrations; *A. aminovorans* and *M. loti* can only grow in $<3·0$ % salt, and *S. lacuscaerulensis* in $<7·0$ % salt (Urakami *et al.*, 1992; Jarvis *et al.*, 1997; Petursdottir & Kristjansson, 1997). Strain HTCC2503^T grew at salt concentrations of up to 25·0 %. *A. aminovorans* utilizes methylamine, divides by budding, contains poly-β-hydroxybutyric acid granules and does not contain pigments (Urakami *et al.*, 1992). *M. loti* forms nitrogen-fixing nodules on the roots of leguminous plants (Jarvis *et al.*, 1997). *S. lacuscaerulensis* is a long (9–18 μm), non-motile, catalase-positive, gas vacuole-containing rod that grows optimally at 45 °C (Petursdottir & Kristjansson, 1997). *R. sulfidophilum* is a facultative anaerobic phototrophic organism that contains bacteriochlorophyll *a* (Hiraishi & Ueda, 1994). Therefore, strain HTCC2503^T cannot be identified as a member of any of the genera described above.

In conclusion, this polyphasic approach demonstrates that strain HTCC2503^T represents a novel genus within the α-*Proteobacteria*, for which the name *Parvularcula bermudensis* gen. nov., sp. nov. is proposed.

Description of *Parvularcula* gen. nov.

Parvularcula (Par.vu.lar′cu.la. L. adj. *parvulus* very small; L. fem. n. *arcula* a jewel-casket; N.L. fem. n. *Parvularcula* a very small jewel-casket).

Cells are Gram-negative, strictly aerobic short rods that occur singly, are sometimes coccoid, multiply by binary fission and are slightly motile with a single flagellum. Endospores and poly-β-hydroxybutyrate granules are not formed. Colonies on marine agar are very small (0·3–0·8 mm in diameter), yellowish-brown, circular, dry and very hard. Produces carotenoid pigments, but not bacteriochlorophyll *a*. Catalase-negative and oxidase-positive. Nitrate is reduced to nitrite. Urea and gelatin are hydrolysed, but aesculin is not. Chemoheterotrophic and moderately halophilic, requires NaCl for growth. Cellular fatty acids are even-numbered monounsaturated or saturated fatty acids. The major fatty acid is *cis*-7-octadecenoic acid (73·3 %). Based on the 16S rRNA sequence, the genus belongs to the α-*Proteobacteria*. Phylogenetically, the genus forms a novel seventh order of the α-*Proteobacteria*. The type species of the genus is *Parvularcula bermudensis*.

Description of *Parvularcula bermudensis* sp. nov.

Parvularcula bermudensis (ber.mu.den′sis. N.L. fem. adj. *bermudensis* from the Bermuda Islands, the geographical origin of the type strain of the species).

In addition to the characteristics reported for the genus, cells are 0·4–1·3 μm wide and 0·6–1·8 μm long. Able to grow at 10–37 °C and optimally at 30 °C, but not at 4 or 44 °C. Growth occurs at pH 6·0–9·0 and 0·75–25 % NaCl, and optimally at pH 8·0 and 3·0 % NaCl. Pentoses, hexoses, sugar alcohols, oligosaccharides and amino acids are utilized as sole carbon sources. Carbon source utilization patterns, including the sole carbon sources test and Biolog plate test, are given in the text. Susceptible to chloramphenicol, carbenicillin, tetracycline, streptomycin, puromycin, erythromycin and rifampicin. The DNA G+C content is 60·8 mol% (HPLC method).

The type strain, HTCC2503^T (=ATCC BAA-594^T=KCTC 12087^T) and reference strain HTCC2517 were isolated from the Bermuda Atlantic Time Series Station in the western Sargasso Sea, Atlantic Ocean.

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