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Carboxydobrachium pacificum gen. nov., sp. nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough

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A new anaerobic, thermophilic, CO-utilizing marine bacterium, strain JM^T, was isolated from a submarine hot vent in Okinawa Trough. Cells of strain JM^T were non-motile thin straight rods, sometimes branching, with a cell wall of the Gram-positive type, surrounded with an S-layer. Chains of three to five cells were often observed. The isolate grew chemolithotrophically on CO, producing equimolar quantities of H_2 and CO_2 (according to the equation $CO+H_2O \rightarrow CO_2+H_2$) and organotrophically on peptone, yeast extract, starch, cellobiose, glucose, galactose, fructose and pyruvate, producing H_2, acetate and CO_2. Growth was observed from 50 to 80 °C with an optimum at 70 °C. The optimum pH was 6·8–7·1. The optimum concentration of sea salts in the medium was 20·5–25·5 g l^{-1}. The generation time under optimal conditions was 7·1 h. The DNA G+C content was 33 mol%. Growth of isolate JM^T was not inhibited by penicillin, but ampicillin, streptomycin, kanamycin and neomycin completely inhibited growth. The results of 16S rDNA sequence analysis revealed that strain JM^T belongs to the *Thermoanaerobacter* phylogenetic group within the *Bacillus–Clostridium* subphylum of Gram-positive bacteria but represents a separate branch of this group. On the basis of morphological and physiological features and phylogenetic data, this isolate should be assigned to a new genus, for which the name *Carboxydobrachium* is proposed. The type species is *Carboxydobrachium pacificum*; the type strain is JM^T (= DSM 12653^T).

Keywords: submarine hot vents, carbon monoxide utilization, thermophilic anaerobe, *Carboxydobrachium pacificum*

INTRODUCTION

Deep-sea hydrothermal fields are inhabited by unique microbial communities that rely on the energy of reduced inorganic compounds of volcanic origin (Jannasch, 1995). Many thermophilic prokaryotes have been isolated from such habitats over the past decade (Prieur *et al.*, 1995). However, most of these organisms were hyperthermophilic archaea of the *Euryarchaeota* and *Crenarchaeota* kingdoms. Among them were methanogens of genera *Methanopyrus* (Kurr *et al.*, 1991) and *Methanococcus* (Jones *et al.*, 1983; Jeanthon *et al.*, 1998); sulfate and/or sulfite reducers of the genus *Archaeoglobus* (Burggraf *et al.*, 1990; Huber *et al.*, 1997); *Pyrolobus fumarii*, oxidizing molecular hydrogen either aerobically or via nitrate reduction (Blöchl *et al.*, 1997); and numerous anaerobic organotrophs belonging to the genera *Staphylothermus* (Fiala *et al.*, 1986), *Thermococcus* (Kobayshi *et al.*, 1994; González *et al.*, 1995, etc.), *Pyrococcus* (Erauso *et al.*, 1993; González *et al.*, 1998) and *Pyrodictium* (Pley *et al.*, 1991). The information on the presence of thermophilic bacteria in the microbial communities of deep-sea hot vents is up to now much more sparse. The presence of organotrophic anaerobes and aerobes belonging to the genera *Thermotoga*, *Thermus* and *Bacillus* in deep-sea hydrothermal habitats has been reported (Marteinsson *et al.*, 1995, 1996a, b), but only *Thermosipho melan-*

The GenBank accession number for the 16S rDNA sequence of strain JM^T is AF174484.

esiensis has been isolated in pure culture (Antoine *et al.*, 1997). The only lithotrophic thermophilic bacterium found in hydrothermal fields of East Pacific Rise is *Desulfurobacterium thermolithotrophum* (L'Haridon *et al.*, 1998). This extremely thermophilic organism obtains energy for growth from anaerobic oxidation of molecular hydrogen coupled with the reduction of elemental sulfur. In this publication, we describe a new anaerobic thermophilic bacterium isolated from a deep-sea hydrothermal system, which is capable of lithotrophic growth on CO with the production of molecular hydrogen.

METHODS

Sampling. Hydrothermal vent samples were collected during a scientific cruise on board the Natsushima research vessel. The manned Shinkai 2000 submersible was used for sample collection. In this study, mud samples were collected from an area with hydrothermal vent activity at the Okinawa Trough (27° 33′ N, 126° 56′ E) at 1395 m depth. This sampling site has been described by Sakai *et al.* (1990). Hot vent fluids at this location had a temperature of 110–130 °C as measured by a thermistor probe inserted into the vent opening. Densely populated colonies of invertebrates can be found around the vents and several thermophilic archaea have been previously isolated from this deep-sea location (Kobayashi *et al.*, 1994).

Enrichment and isolation. For enrichments and cultures, unless otherwise mentioned, Medium 1 was used, which contained (l^{-1}): NaCl, 18 g; KCl, 0·7 g; $MgSO_4.7H_2O$, 3·9 g; $CaCl_2.2H_2O$, 0·4 g; NH_4Cl, 0·3 g; Na_2HPO_4, 0·15 g; Na_2SiO_3, 0·03 g; trace element solution (Pfennig & Lippert, 1966), 1 ml; vitamin solution (Wolin *et al.*, 1963), 1 ml; resazurin, 1 mg; $NaHCO_3$, 0·5 g; $Na_2S.9H_2O$, 0·5 g; cysteine/HCl, 0·5 g. The medium was prepared anaerobically. It was supplemented with 0·05 g yeast extract l^{-1}. pH was adjusted to 7·0–7·2 by the addition of 6 M HCl. Portions of the medium (10 ml) were placed into 50 ml bottles under the flow of oxygen-free nitrogen. Then the gas phase was changed to CO at 100–110 kPa; the bottles were tightly stoppered and sterilized at 120 °C.

To obtain enrichments of CO-utilizing anaerobes, bottles inoculated with mud samples (2 cm^3) were incubated at 55, 60, 70 or 80 °C. Growth was determined using light microscopy and GLC detection of CO utilization and formation of gaseous growth products.

Colonies were obtained on Medium 1 solidified by 5 % agar in roll-tubes with CO as the gas phase. Large-scale cultures were grown at 70 °C in a Biostat fermenter equipped with a gas recirculation system.

Morphological and ultrastructural studies. Cell morphology was studied using an MBI-11 light microscope and a JEM-100 electron microscope. Electron microscopic methods have been previously described (Svetlichny *et al.*, 1991a).

Physiological tests. Potential substrates for growth were tested at a concentration of 0·5 % (w/v) in the same liquid medium under a N_2 atmosphere. Potential electron acceptors were added at 0·2 % (w/v), except for elemental sulfur, which was added at 1 % (w/v). Growth was detected using phase-contrast microscopy.

The influence of salts on growth was tested by varying the NaCl concentration from 0 to 5 % (w/v) in the Medium 1 supplemented with 0·2 g yeast extract l^{-1} under a CO gas phase. To determine whether NaCl can replace artificial seawater, various concentrations of NaCl, from 0·5 to 5 % (w/v), were added to Medium 2, which contained (l^{-1}): NH_4Cl, 0·066 g; $MgCl_2.6H_2O$, 0·016 g; $CaCl_2.2H_2O$, 0·010 g; KCl, 0·033 g; KH_2PO_4, 0·033 g; yeast extract, 0·2 g; trace element solution (Pfennig & Lippert, 1966), 1 ml; vitamin solution (Wolin *et al.*, 1963), 1 ml; resazurin, 1 mg; $NaHCO_3$, 0·5 g; $Na_2S.9H_2O$, 0·5 g; cysteine/HCl, 0·5 g. The medium was prepared anaerobically. pH was adjusted to 7·0–7·2.

The effect of pH on growth was studied under a CO atmosphere in Medium 1 except that the phosphate component of the medium was added in the form of 0·01 M phosphate buffer with a required pH.

Sensitivity of strain JM^T to penicillin (50 μg ml^{-1}), ampicillin (50 μg ml^{-1}), streptomycin (50 μg ml^{-1}), kanamycin (20 μg ml^{-1}) and neomycin (20 μg ml^{-1}) was tested in cultures grown on CO.

Temperature, pH and medium mineralization optima were determined from the growth rates. The cell density was measured by direct cell counting.

Analytical methods. Gaseous and liquid fermentation products were detected by GLC (Svetlichny *et al.*, 1991a). Lactate was detected by GLC as acetaldehyde after periodic acid treatment of culture liquid samples (Teunissen *et al.*, 1989).

DNA analyses. DNA was prepared as described by Marmur (1961). The DNA G+C content was determined by the melting point analysis (Marmur & Doty, 1962) using *Escherichia coli* K-12 DNA as a reference.

The 16S rRNA gene from strain JM^T was sequenced by the dideoxynucleotide chain termination method on an ABI 373A sequencer (Applied Biosystems). The JM^T 16S rDNA sequence was manually aligned with 16S rDNA sequences from closely related species obtained from the GenBank database. The initial sets of prealigned eubacterial sequences were obtained from the Ribosomal Databases Project (Larsen *et al.*, 1993). Regions that either were not sequenced in one or more reference organisms or exhibited alignment uncertainty, i.e. positions 1–101, 201–218, 453–479, 839–847, 999–1035, 1134–1140, 1449–1455 and 1483–1486–3′ terminus (*E. coli* numbering), were omitted from the analysis. Pairwise evolutionary distances (expressed as estimated changes per 100 nt) were computed as suggested by Jukes & Cantor (1969) and Swofford & Olsen (1990). A rooted phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with the bootstrap analysis of 100 trees performed by using the TREECON program (Van de Peer & De Wachter, 1994) using *Streptomyces griseus* as an outgroup.

Reference accession numbers of sequences used in this study are as follows: *Thermoanaerobacter thermohydrosulfuricus* DSM 567, L09161; *Thermoanaerobacter sulfurophilus* L-64^T, Y16940; *Thermoanaerobacter wiegelii* Rt8.$B1^T$, X92513; *Thermoanaerobacter acetoethylicus* ATCC 33265^T, L09163; *Thermoanaerobacter kivui* DSM 2030^T, L09160; *Thermoanaerobacter brockii* DSM 1457^T, L09165; *Thermoanaerobacter mathranii* $A3^T$, Y11279; *Thermoanaerobacter thermocopriae* JT-3^T, L09167; *Thermoanaerobacter ethanolicus* JW-200^T, L09162; '*Thermoanaerobacter lacticus*' SEBR 5268^T, U14330; *Thermoanaerobacter siderophilus* $SR4^T$, AF120479; *Thermoanaerobacterium thermosulfurigenes* E100-69^T, L09161; *Thermoanaerobacterium aotearoense* JW/SL-

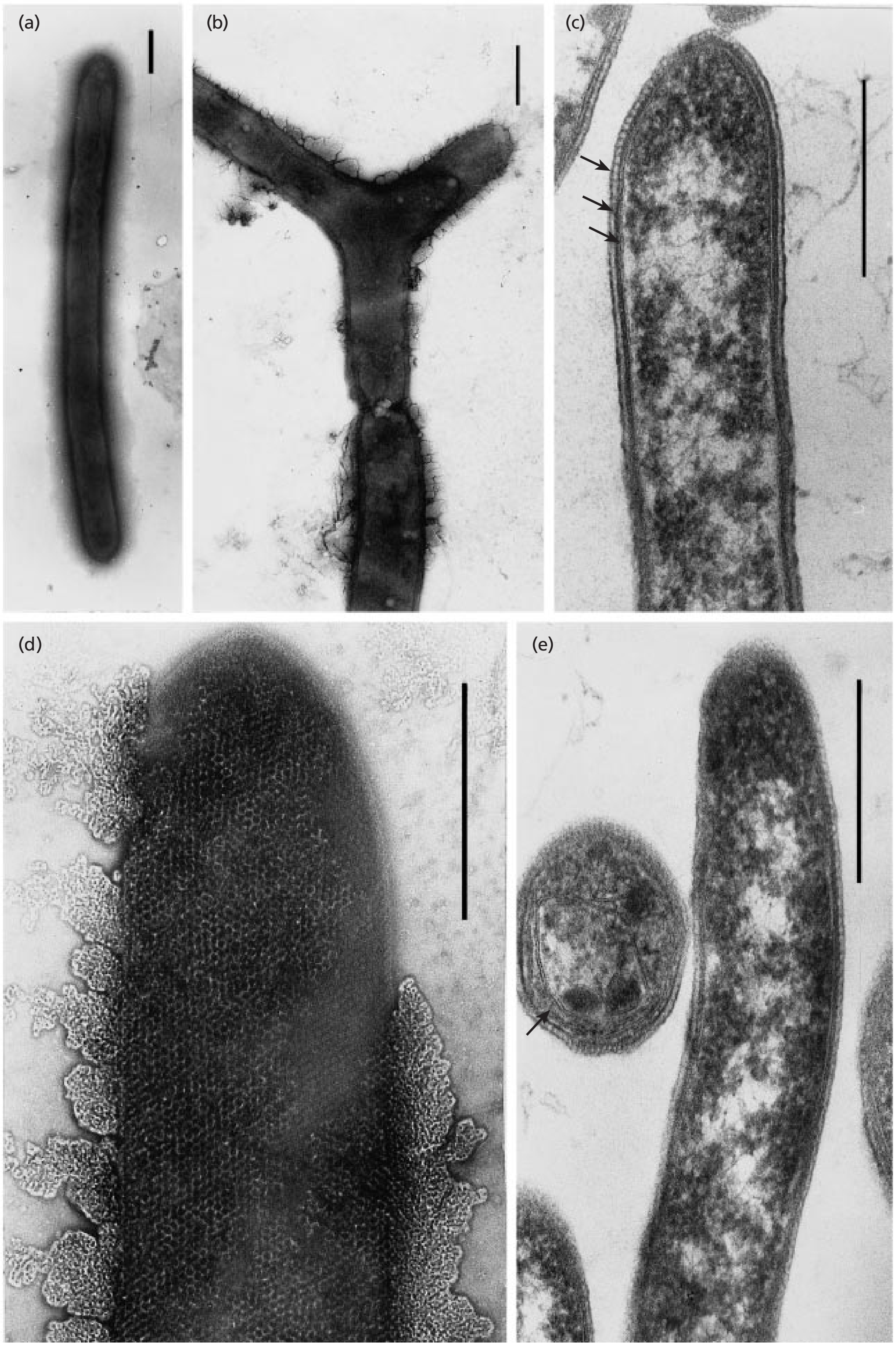

Fig. 1. Electron micrographs of cells of strain JM^T. (a, b, d) Negative staining; (c, e) thin sections. Bars 0·5 μm. Arrows in (c) indicate (from inside to outside) a cytoplasmic membrane, an electron-dense layer and an S-layer formed by global subunits. The arrow in (e) indicates an invaginated cytoplasmic membrane.

NZ613^T; X93359; *Thermoanaerobacterium saccharolyticum* DSM 7060^T, L09169; *Thermoanaerobacterium xylanolyticum* DSM 7097^T, L09172; *Thermoanaerobacterium thermosaccharolyticum* ATCC 7956^T, M59119; *Dictyoglomus thermophilum* H-6-12^T, X69194; *Anaerobranca horikoshii* JW/YL-138^T, U21809; *Moorella thermoautotrophica* JW 701/5^T, X58354; *Moorella thermoacetica* LJD^T, X58352; *Moorella glycerini* YS6^T, U82327; *Thermoterrabacterium ferrireducens* JW/AS-Y7^T, U76363; *Ammonifex degensii* DSM 10501^T, U34975; *Syntrophospora bryantii* DSM 3014B, M26491; *Carboxydothermus hydrogenoformans* DSM 6008^T, AF244579.

RESULTS

Enrichment and isolation

Bottles inoculated with mud samples were incubated at 55, 60, 70 and 80 °C. Incubation at 55 and 60 °C did not result in significant microbial growth or CO consumption. In the bottles incubated at 80 °C for 5 d, CO consumption and hydrogen production were detected, but stable growth was not obtained. At 70 °C, growth of thin rods was observed and the gas pressure in these bottles increased from 100 to 150–160 kPa after 4–5 d incubation. During growth, CO concentration dropped in the gas phase, whereas H_2 and CO_2 appeared. After a number of serial dilution transfers, homogeneous culture of long thin rods was obtained. Branching cells were observed. On solid medium under CO, round, white, semitransparent colonies (up to 1 mm diam.) developed after 3 d of incubation at 70 °C. After several consequent isolations of a single colony, the pure culture of strain JM^T was obtained.

Morphology of the new isolate

During growth on CO, cells of isolate JM^T were straight rods, about 0·4 µm wide and from 4 to 10 µm long (Fig. 1a). They appeared as single cells or short chains of three to five cells. Branching (Fig. 1b) was usually observed at the end of the exponential phase during growth on CO. In the stationary phase, round bodies, probably spheroplasts, appeared at the ends of lysed cells or even separately. In media with organic substrates, cell length and branching frequency were substrate-dependent. Branching was not observed on glucose or cellobiose, but was frequent on galactose. Flagella were never observed. Electron microscopy of ultrathin sections revealed that the cell envelope was composed of the cytoplasmic membrane, an electron-dense layer (Fig. 1c) and a hexagonal S-layer formed by global subunits (Fig. 1c, d, e). The cytoplasmic membrane was often invaginated and formed lamella-like structures (Fig. 1d)

Growth characteristics

Growth of isolate JM^T occurred within a temperature range of 50–80 °C with an optimum at 70 °C; no growth was observed at 40 or 85 °C. Strain JM^T grew in a pH range of 5·8–7·6, with an optimum at 6·8–7·2. The optimum concentration of sea salts for strain JM^T was between 20·5 and 25·5 g^{-1}. Artificial seawater could not be replaced by corresponding concentrations of NaCl. In Medium 2 supplemented with 0·5–5 % NaCl, no growth of strain JM^T was observed.

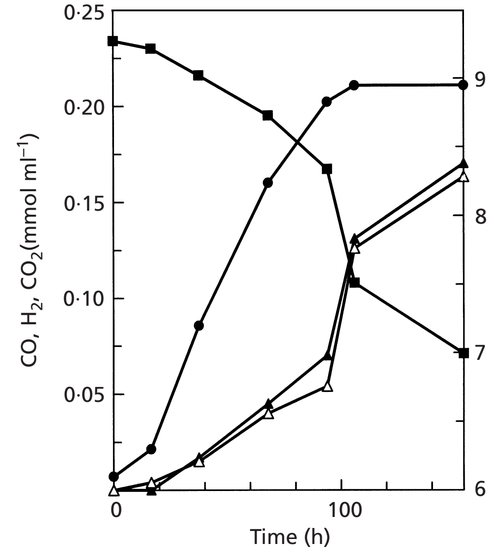

Fig. 2. Growth of strain JM^T at 70 °C in Medium 1 supplemented with 0·2 g yeast extract l^{-1} under a CO atmosphere (●), CO consumption (■), H_2 production (▲) and CO_2 production (△).

Strain JM^T grew only under strictly anaerobic conditions. It did not grow in Medium 1 without reducing agents, nor under the mixture of CO and air (4:1, v/v). It grew in an atmosphere of 100 % CO in Medium 1 supplemented with 0·5 g yeast extract l^{-1} with a final cell yield of 3×10^8 cells ml^{-1}. When the concentration of yeast extract was increased up to 0·2 g l^{-1} the cell yield was 1×10^9 cells ml^{-1}. CO oxidation was coupled with H_2 and CO_2 formation (Fig. 2). Methane, lactate, acetate or other volatile fatty acids were not formed during growth on CO. The amount of hydrogen formed in control bottles with the same medium under 100 % N_2 did not exceed 1 % of that formed in the presence of CO. This suggests that H_2 production is connected mainly with CO utilization. The stoichiometry of the reaction corresponded to an equimolar ratio of CO consumption and H_2 and CO_2 production (Fig. 2), according to the equation: $CO + H_2O \rightarrow H_2 + CO_2$. The generation time of strain JM^T under optimal conditions with 100 % CO in the gas phase and 0·2 g yeast extract l^{-1} was 7·1 h. Yeast extract could be replaced by 0·2 g peptone or acetate l^{-1} with the same effect. Peptone or acetate supported stable growth of isolate JM^T under a CO atmosphere over at least five transfers.

Apart from CO, strain JM^T was able to grow on peptone, yeast extract, starch, cellobiose, glucose, galactose, fructose and pyruvate, but not on sucrose.

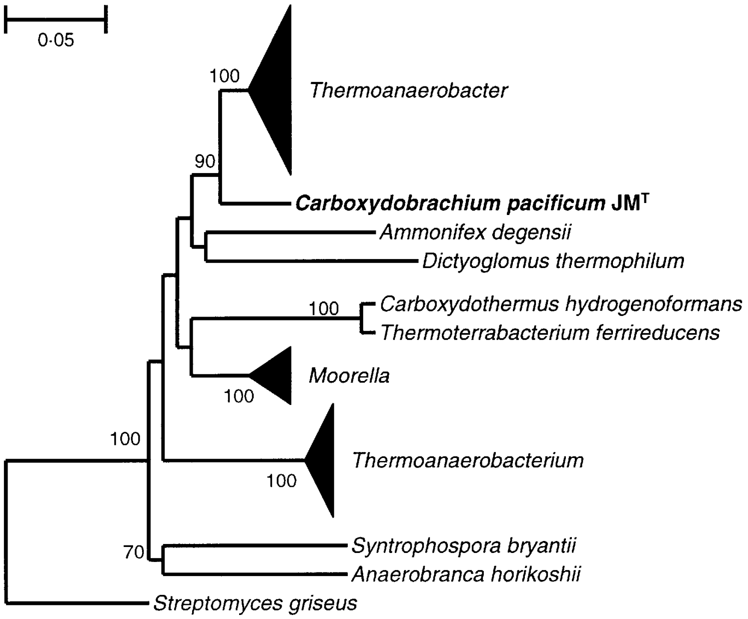

Fig. 3. Dendrogram showing the phylogenetic position of strain JM^T. The tree was derived by Jukes & Cantor (1969) distance analysis. Bar represents 5 nt substitutions per 100 nt. Numbers refer to bootstrap values.

The products of organotrophic growth were H_2, acetate and CO_2. Among organic substrates, galactose supported the best growth of strain JM^T, with a doubling time of about 10 h. Acetate, CO_2 and hydrogen were produced from galactose in a 2:1:1 ratio. An H_2/CO_2 gas mixture (4:1, v/v) did not support growth. No growth was observed on acetate, formate, methanol, ethanol or lactate in the presence or absence of elemental sulfur or sulfate.

Penicillin did not inhibit growth, CO consumption and H_2/CO_2 production; ampicillin, streptomycin, kanamycin and neomycin completely inhibited both growth and CO utilization.

DNA base composition

The DNA G+C content of strain JM^T was 33 ± 1 mol%.

Phylogenetic analysis

A total of 1490 nt of the 16S rRNA gene of strain JM^T, corresponding to positions 8–1512 (*E. coli* numbering) were sequenced. A total of 78 sequences of representative species from various phyla of the *Bacteria* domain were compared with the sequence of strain JM^T. This analysis revealed that strain JM^T is a member of the low-G+C, Gram-positive phylum and falls into the '*Thermoanaerobacter* and relatives' group (RDP database). Additional sequence alignments and phylogenetic analysis performed with members of this group revealed that strain JM^T was related to species of the genus *Thermoanaerobacter* (sequence similarity of 92·3–93·5%). However, this value was lower than the interspecies level of similarity within the phylogenetic cluster of the genus *Thermoanaerobacter* (94·7–99·4%). The level of sequence similarity with other members of this group was no more than 89·1%. A phylogenetic dendrogram showing these results is presented in Fig. 3. Bootstrap values indicate that the relationship of strain JM^T to *Thermoanaerobacter* species is not robust (90%). The thermophilic strain JM^T shows a relatively high G+C level in the 16S rDNA gene, i.e. 58·8 mol%, which falls into the characteristic range for *Thermoanaerobacter* species. The transversion distance method (Woese *et al.*, 1991; Swofford & Olsen, 1990) for the generation of phylogenetic trees revealed that the high G+C content of rDNA has introduced bias, resulting in the clustering of the members of the *Thermoanaerobacter* group. In this tree, strain JM^T is not clustered together with *Thermoanaerobacter* species or other reference species (data not shown).

The relatedness of strain JM^T and the genus *Thermoanaerobacter* was supported by the presence of specific oligonucleotide motifs at positions 841–846 and 998–1025 (*E. coli* numbering). To determine the affiliation of strain JM^T with genera of the *Thermoanaerobacter* group, its 16S rDNA was analysed for the presence of genus-specific signature nucleotides. The genera *Thermoanaerobacter*, *Thermoanaerobacterium* and *Moorella* can be defined by unique 16S rDNA signature nucleotides at 50 sites in the gene (Rainey *et al.*, 1993). Strain JM^T has seven differences at signature positions with *Thermoanaerobacter* species and no less than 16 differences with other genera of this group.

Comparison of secondary structures of 16S rRNAs of thermophilic members of the genus *Thermoanaerobacter* with those reported for other prokaryotes revealed the presence of unique helixes in four sequence regions (Rainey *et al.*, 1993; Slobodkin *et al.*, 1999). The secondary structure that is specific for the genus *Thermoanaerobacter* in these regions was not found in strain JM^T. Hence, on the basis of phylogenetic analysis, strain JM^T does not belong to the *Thermoanaerobacter* cluster and should be considered as a separate phylogenetic branch.

DISCUSSION

CO can be used as a growth substrate by many metabolically and phylogenetically diverse prokaryotic micro-organisms. O_2-dependent CO oxidation is a well known process carried out by a voluminous group of aerobic carboxydobacteria (Zavarzin, 1978; Meyer *et al.*, 1993).

Among anaerobes, utilization of CO occurs via the acetyl-CoA pathway found in homoacetogenic bacteria (Ljungdahl, 1994), methanogenic (Deppenmeier *et al.*, 1996; Ferry, 1999) and non-methanogenic archaea (Möller-Zinkhan & Thauer, 1990; Vorholt *et al.*, 1995, 1997), sulfate-reducing bacteria (Schauder *et al.*, 1987; Janssen & Schink, 1995; Elferink *et al.*, 1999; Fukui *et al.*, 1999) and photosynthetic bacteria (Uffen, 1983; Ensign, 1995). Some of the organisms possessing the acetyl-CoA pathway are able to utilize CO as an energy substrate during growth. During growth on CO, homoacetogenic and methanogenic bacteria produce acetate and methane, respectively. Several acetogens produce, apart from acetate, other volatile fatty acids or alcohols during growth on CO. Anaerobic oxidation of CO to CO_2 with concurrent formation of H_2 from water is another catabolic reaction which has been found so far only in two groups of micro-organisms: mesophilic purple bacteria (Uffen, 1976; Dashekvicz & Uffen, 1979; Kerby *et al.*, 1995) and extremely thermophilic bacteria of the genus *Carboxydothermus* (Svetlichny *et al.*, 1991a, 1994). Strain JM^T carried out the same process of anaerobic CO oxidation coupled with only CO_2 and H_2 production as the representatives of the genus *Carboxydothermus* and was found to be a thermophile growing in the temperature range from 50 to 80 °C. *Carboxydothermus hydrogenoformans* (Svetlichny *et al.*, 1991a) was isolated from freshwater hot springs and grows only chemolithotrophically with CO or chemoorganotrophically with pyruvate. Another thermophilic obligately carboxydotrophic bacterium, *Carboxydothermus restrictus*, was isolated from a freshwater hydrothermal vent at Raoul Island (Archipelago Kermadek, New Zealand) and grows only chemolithotrophically with CO (Svetlichny *et al.*, 1994). Unidentified thermophilic anaerobic bacteria performing the reaction of CO oxidation to CO_2 with concurrent H_2 production from water are widely distributed in terrestrial hydrothermal springs of the Kuril Islands (Svetlichny *et al.*, 1991b) and the Kamchatka Peninsula (Bonch-Osmolovskaya *et al.*, 1999). There is also the evidence of the presence of such organisms in shallow-water submarine hot vents of the Kuril Islands (Svetlichny *et al.*, 1991b). Strain JM^T is the first anaerobic marine organism growing on CO and producing hydrogen from water which has been isolated in pure culture. It was obtained from a deep-sea hot vent and its growth was dependent on salt concentrations similar to those found in seawater. Strain JM^T differs from previously known anaerobic CO-utilizing hydrogen-producing thermophiles both in morphological and physiological characteristics. Whereas cells of *Carboxydothermus* spp. are short motile rods, the new isolate has long non-motile branching cells. Another important differentiating feature of strain JM^T is the wide range of growth substrates. In addition to lithoheterotrophic growth with CO, it can grow organotrophically on different fermentable substrates, though with a lower growth rate than on CO.

The 16S rDNA sequence analysis places strain JM^T in the Gram-positive *Bacillus–Clostridium* subphylum. Strain JM^T appears to be related to the genus *Thermoanaerobacter* but does not belong to it. Morphologically, strain JM^T differs from the representatives of genus *Thermoanaerobacter* by the ability to form branching cells. *Anaerobranca horikoshii* (Engle *et al.*, 1995) also forms branching cells but is quite distant phylogenetically (Fig. 3). The main physiological feature that separates strain JM^T from its closest relatives is that it grows rapidly on CO, producing only CO_2 and H_2. Among its relatives only *Thermoanaerobacter kivuii*, *Moorella thermoautotrophica* and *Moorella thermoacetica* can grow on CO with acetate as the end product. The insufficient level of sequence similarity ($< 93{\cdot}5\%$) and the peculiarities of signature positions and secondary structure of 16S rDNA sequence confirm the phenotypic evidence supporting that strain JM^T should be considered as separate from the members of the *Thermoanaerobacter* group at a generic level.

Based on morphological, physiological and phylogenetic features of the isolate JM^T, we propose the description of a new genus, *Carboxydobrachium*, with the type species *Carboxydobrachium pacificum* gen. nov., sp. nov.

Description of *Carboxydobrachium* gen. nov.

Carboxydobrachium (Car.bo.xy.do.bra′chi.um. N.L. neut. n. *Carboxydum* carbon monoxide; N.L. neut. n. *brachium* arm, branch; N.L. neut. n. *Carboxydobrachium* CO branch, i.e. CO-utilizing, branching bacterium).

Cells are long, thin, non-motile branching rods. The Gram-positive cell wall is covered with an S-layer. Obligate anaerobe. Thermophile. Obligately requires seawater-based medium. Grows chemolithotrophically on CO. Utilizes CO as the sole energy source with equimolar formation of H_2 and CO_2 according to the equation $CO + H_2O \rightarrow CO_2 + H_2$. Grows organotrophically on a number of fermentable substrates, producing H_2, CO_2 and acetate. The type species is *Carboxydobrachium pacificum*.

Description of *Carboxydobrachium pacificum* sp. nov.

Carboxydobrachium pacificum (pa.ci′fi.cum. L. neut. adj. *pacificum* peaceful, pertaining to the Pacific Ocean, from the western part of which the type strain was isolated).

Cells are long thin straight non-motile rods, of about 0·3 μm width and 4–10 μm length. Cells are single or form short chains of three to five cells. Cells sometimes branch. Cells have a Gram-positive cell wall covered with an S-layer. On solid medium, round, white, translucent colonies are produced. Obligate anaerobe. Extreme thermophile. Grows within the temperature range of 50–80 °C; optimum growth at 70 °C. pH for growth ranges from 5·8 to 7·6; optimum pH is 6·8–7·2. Growth and CO consumption are not inhibited by penicillin, but ampicillin, streptomycin, kanamycin and neomycin completely inhibit growth and CO utilization. Obligately requires seawater-based medium. The optimum concentration of sea salts is 20·5–25·5 g l^{-1}. Grows chemolithotrophically on CO. Utilizes CO as the sole energy source with equimolar formation of H_2 and CO_2 according to the equation $CO + H_2O \rightarrow CO_2 + H_2$. Requires yeast extract or peptone or acetate as carbon source. Grows organotrophically on peptone, yeast extract, starch, cellobiose, glucose, galactose, fructose and pyruvate, but not on sucrose. The products of organotrophic growth are acetate, CO_2 and H_2. An H_2/CO_2 gas mixture does not support growth. No growth occurs on acetate, formate, methanol, ethanol or lactate either in the presence or in the absence of elemental sulfur or sulfate. DNA G + C content is 33 ± 1 mol %. The type strain is *Carboxydobrachium pacificum* JM^T (= DSM 12653^T), isolated from a submarine hydrothermal vent in Okinawa Trough.

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