Thermobrachium celere gen. nov., sp. nov., a Rapidly Growing Thermophilic, Alkalitolerant, and Proteolytic Obligate Anaerobe

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More than 40 isolates of a novel, ubiquitous, proteolytic, moderately alkaliphilic, thermophilic obligate anaerobe were obtained from geothermally and anthropogenically heated environments and mesobiotic environments located on three continents. Whole-cell protein sodium dodecyl sulfate gel electrophoresis revealed that most of these organisms are very similar. Eight of the isolates were characterized in detail; this analysis included 16S ribosomal DNA sequence analysis. The cells of those organisms are (depending on the isolate) 0.5 to 0.8 μm in diameter and 1.5 to 13 μm long, exhibit tumbling motility, and have a positive Gram stain reaction. The temperature range for growth is 43° to 75°C (optimum temperature, 66°C), and the pH range for growth is 5.4 to 9.5 (optimum pH, 8.2); the shortest doubling time is around 10 min. Yeast extract is required for growth, and (depending on the strain) glucose, galactose, and ribose are utilized. The fermentation products from glucose in the presence of yeast extract are CO2, H2, acetate, formate, and ethanol. The G+C content is 30 to 31 mol%. On the basis of these characteristics, which differentiate these strains from all alkalitolerant thermophiles described previously, and the results of a comparison of the 16S ribosomal DNA sequences of these organisms with previously described sequences, we propose that our isolates be placed in a single species of the new genus Thermobrachium; strain JW/YL-NZ35 is the type strain of the type species, Thermobrachium celere.

There are relatively few alkaliphilic environments that occur naturally (13, 34). The most common and best-studied alkaliphilic environments are the soda lakes and deserts that have pH values of 10 to 11.5 (10). These environments usually result from the presence of large amounts of sodium carbonate and other salts (17). However, alkaline conditions can also be found in niches of other environments in which the pH of a microenvironment can be different from the pH of the environment as a whole (17).

Only a few thermophiles are able to grow at pH values of more than 9.0 (31). Aside from characterization of recently described anaerobic alkalithermophiles (5, 14, 15, 34), our knowledge of alkaliphilic bacteria is based mainly on the characteristics of Bacillus species (13, 20, 21). In this paper, we describe a novel, ubiquitous, moderately alkaliphilic, anaerobic thermophile, Thermobrachium celere, which was isolated from samples obtained from various mesobiotic and thermobiotic environments on three continents; the pH values of these samples ranged from 4.8 to 9.0.

MATERIALS AND METHODS

Sample collection. Separated and mixed sediment and water samples were obtained from various locations by completely filling 100- to 500-ml sterile glass jars which had been flushed with nitrogen and were sealed with butyl rubber stoppers. Although the samples were kept at temperatures below 10°C as much as possible during transport to the laboratory in Athens, Ga., they were temporarily exposed to ambient temperatures for various lengths of time. The pH values given below for the samples are usually the values determined at the site of collection; the values given in parentheses were determined later in a laboratory in Athens, Ga. The sampling sites from which the strains analyzed in detail were obtained are described in Table 1. Additional strains were isolated from geothermally heated sites in Iceland, from compost which had received manure from elephants and plant material in Bangkok, Thailand, and from algal mats at Laguna LaFigareda, Baja California, Mexico.

Isolation. Anaerobic enrichment medium M-5, which was used to isolate many of the strains, including strain JW/YL-NZ35T (T = type strain), was prepared by using the modified Hucker technique and contained 3.7 mM KH2PO4, 11.6 mM Na2HPO4, 17H2O, 3.8 mM (NH4)2SO4, 9.3 mM NH4Cl, 0.2 mM MgCl2·6H2O, 0.3 mM CaCl2·2H2O, 0.3% (wt/vol) yeast extract, 0.2% (wt/vol) skim milk (or casein), 5.0 ml of a trace element solution (8) per liter, 0.5 ml of a vitamin solution (6), per liter, and 10 ml of a solution, 1.25 g of Na2S per 100 ml and 1.25 g of cysteic acid per 100 ml) per liter. After the medium was autoclaved for 45 min at 121°C, the pH was adjusted to 9.5 (at 25°C) or other values (see below and Table 1) with aqueous 2% Na2CO3. After 30 to 125-ml portions of the medium in 50- to 150-ml serum bottles were inoculated with 0.5- to 2.5-g or 5- to 20-ml samples, the enrichment cultures were incubated for 2 to 3 days at 50 or 60°C. The variations of the isolation medium used included media which contained higher concentrations of yeast extract (up to 2.5% [wt/vol]), media which contained 0.5% (wt/vol) tryptone and peptone, and media which did not contain skim milk or casein. After one or two subcultures (in which a 5% [vol/vol] transfer inoculum was used) under the same conditions, the strains were purified by repeatedly isolating single colonies in agar shake roller tubes containing medium M-5 (pH 9.5) containing only 0.1% (wt/vol) skim milk and solidified with 2.2% (wt/vol) agar (Difco Laboratories, Detroit, Mich.). The roll tubes were incubated for 2 to 3 days at 60°C. Only colonies that exhibited clearing zones were selected.

Light microscope and electron microscope. A model PM-10AD phase-contrast microscope (Olympus Optica Co., Ltd., Tokyo, Japan) was used for routine examination and for taking photomicrographs of cells placed on dried agarose-coated slides. Electron microscopy and preparation of cells for ultrathin sectioning were carried out as described previously (7, 12, 26) by using uranyl acetate and lead citrate for poststaining. Cells were negatively stained as described by Valentine et al. (27) and Beucher et al. (2) by using 2% uranyl acetate.

Gram reaction and Gram type. The Gram stain reaction was determined by using the modified Hucker method and an Enhanced Gram Stain kit obtained from Carr Scarborough Microbiologics, Inc., Decatur, Ga. Cells from both the exponential and stationary growth phases were used. The Gram type was unequivocally determined by the polymyxin B-lipopolysaccharide assay as described previously (33).

Determination of G+C content. DNA was isolated from cells in the exponen-
Hot spring environments: Anthropogenic environment: Mesobiotic environments (sediment and water samples):

<table>
<thead>
<tr>
<th>Location</th>
<th>Enrichment conditions</th>
<th>Strain(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment and water near bathhouse in Ohinomutu, New Zealand, pH 7.7, 56–75°C</td>
<td>pH 10.0, 50°C</td>
<td>JW/YL-NZ25⁷</td>
</tr>
<tr>
<td>Sediment and water, Larderello district, Toscani, Italy, pH 4.8, 60–65°C</td>
<td>pH 9.0, 60°C</td>
<td>JW/YL-T148</td>
</tr>
<tr>
<td>Anthropogenic environment: Horse manure compost, Athens, Ga., pH 7.8, 50–55°C</td>
<td>pH 10.0, 60°C</td>
<td>JW/YL-M105</td>
</tr>
<tr>
<td>Mesobiotic environments (sediment and water samples): Platte River, Buenos Aires, Argentina, pH 5.0, ambient temperature</td>
<td>pH 9.0, 60°C</td>
<td>JW/YL-PR6, JW/YL-PR7</td>
</tr>
<tr>
<td>Platte River (flood plain), Buenos Aires, Argentina, pH 6.8, ambient temperature</td>
<td>pH 9.0, 60°C</td>
<td>JW/YL-PR32</td>
</tr>
<tr>
<td>Elbe River, Brunssbuttel, Germany, pH 7.0, 18°C</td>
<td>pH 9.0, 60°C</td>
<td>JW/YL-ER21</td>
</tr>
<tr>
<td>Mono Lake, Calif., pH 9⁰C, 9.0⁰, ambient temperature</td>
<td>pH 9.9, 60°C</td>
<td>JW/YL-L115</td>
</tr>
</tbody>
</table>

*The pH is the initial pH.

16S rDNA sequence determination and analysis. Genomic DNA was extracted and PCR-mediated amplification of the 16S rDNA was performed as described previously (24). Purified PCR products were sequenced with a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) as recommended by the manufacturer. Sequence reaction mixtures were electrophoresed by using an Applied Biosystems model 373A DNA sequencer. The 16S rDNA sequences were aligned manually with the sequences of representatives of the genus *Clostridium* and related taxa. Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (11). Both the least-squares distance method of De Soete (4) and the neighbor-joining method of Saitou and Nei (25) were used to construct phylogenetic dendrograms from distance matrices. A maximum-likelihood analysis was performed by using the PHYLYP package (6). Bootstrap values were calculated from 1,000 trees by using the programs MFFT and NIBOOT. The reference sequences included in the analysis were obtained from the Ribosomal Database Project (18).

Nucleotide sequence accession number. The 16S rDNA nucleotide sequence of strain JW/YL-NZ25⁷ has been deposited in the EMBL database under accession number X92938.

RESULTS AND DISCUSSION

Isolation and habitats. More than 40 strains of alkali-tolerant thermophiles were isolated from samples collected from mesobiotic habitats (e.g., river and lake sediments in South America, North America, and Europe), geothermally heated habitats (e.g., hot springs in the United States, Italy, Iceland, and New Zealand, pH 7.7, 56–75°C) by using a 0.008 N H₂SO₄ mobile phase at a flow rate of 0.6 mm/min and a model 156 refractive index detector (ALTEN, San Ramon, Calif.). Whole-cell protein pattern (sodium dodecyl sulfate [SDS]-polyacrylamide gel electrophoresis [PAGE]). The strains were grown in Hungate tubes containing 10 ml of prerduced medium M-5 (see above) containing 0.5% yeast extract, 0.25% tryptone, and 0.5% (wt/vol) filter-sterilized glucose. Duplicate cultures were incubated at 60°C until the OD₆₀₀ values were 0.2 and 0.4 (early and late exponential growth phases) for each strain. The cultures were immediately centrifuged at 8,000 × g for 20 min to pellet the cells. The pellets from cultures at each OD₆₀₀ were combined, resuspended in 4.0 ml of 0.125 M Tris HCl buffer (pH 7.0), and frozen at −20°C until they were lyzed. Cells were lysed by passing them through a chilled French pressure cell (catalog no. 4-3359; Aminco) at 17,000 lb/in². The resulting lysates were centrifuged at 8,000 × g for 20 min to remove whole cells and debris and were frozen at −20°C until they were electrophoresed.

For electrophoresis, SDS-polyacrylamide separating gels (thickness, 1 mm) containing 10% acrylamide and 4% acrylamide stacking gels were cast and electrophoresed in a Bio-Rad Mini Protein II system at 150 V for approximately 1.5 h. High-molecular-weight standards for SDS were obtained from Pharmacia. Protein samples in 0.7-microcentrifuge tubes were diluted into denaturing loading buffer and heated in a boiling water bath for 20 min. Approximately 10 μg of protein was loaded in each lane. The gels were stained with 0.1% (wt/vol) Coomassie brilliant blue R-250, dissolved in methanol-deionized water-acetic acid (9:9:2, vol/vol/vol) for at least 2 h and were destained in a solution containing 40% methanol and 10% acetic acid. 16S rDNA sequence determination and analysis. Genomic DNA was extracted and PCR-mediated amplification of the 16S rDNA was performed as described previously (24). Purified PCR products were sequenced with a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) as recommended by the manufacturer. Sequence reaction mixtures were electrophoresed by using an Applied Biosystems model 373A DNA sequencer. The 16S rDNA sequences were aligned manually with the sequences of representatives of the genus *Clostridium* and related taxa. Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (11). Both the least-squares distance method of De Soete (4) and the neighbor-joining method of Saitou and Nei (25) were used to construct phylogenetic dendrograms from distance matrices. A maximum-likelihood analysis was performed by using the PHYLYP package (6). Bootstrap values were calculated from 1,000 trees by using the programs MFFT and NIBOOT. The reference sequences included in the analysis were obtained from the Ribosomal Database Project (18).

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Isolation and habitats. More than 40 strains of alkali-tolerant thermophiles were isolated from samples collected from mesobiotic habitats (e.g., river and lake sediments in South America, North America, and Europe), geothermally heated habitats (e.g., hot springs in the United States, Italy, Iceland, and

FIG. 1. SDS gel electrophoresis of whole-cell proteins of *Thermobacterium celer* strains and similar thermophile anaerobios. For details see Materials and Methods. Lane 1, *Caloramator fervidus*; lane 2, JW/YL-PR6; lane 3, JW/YL-T148; lane 4, JW/YL-YS199 (unidentified strain); lane 5, JW/YL-NZ25⁷; lane 6, JW/YL-PR7; lane 7, JW/YL-ER21; lane 8, JW/SD-EPPFY (unidentified strain).
FIG. 2. Phylogenetic dendrogram based on the results of a 16S rDNA sequence comparison, showing the position of five Thermobrachium celere strains within the radiation of the clostridia and related taxa. The dendrogram was reconstructed by using the neighbor-joining method and the bootstrap values calculated from 1,000 trees. Scale bar = 5 nucleotide changes per 100 nucleotides.

New Zealand), and anthropogenically heated habitats with elevated temperatures (e.g., manure and bark compost in the United States and Thailand). The environmental conditions of these habitats ranged from slightly acidic (pH 4.0) to alkaline (pH 9.5) and from freshwater (e.g., Elbe River in Germany) to mineral salt-containing water (hot springs in New Zealand) to algal mats containing high salt concentrations (e.g., Laguna La Figuera, Baja California, Mexico). The wide distribution of these organisms, which included environments in which the macroscopic conditions were outside the growth conditions determined for the organisms, may be due to the fact that we used composite water and sediment samples for enrichment and to the short doubling time obtained under optimal conditions for most of the strains (see below), which allowed the organisms to grow in temporary microniches. Microniches, such as more alkaline conditions, can be formed through microbial processes, such as sulfate reduction, photosynthesis, and ammonification by acid-tolerant thermophiles (9). Riverbanks and similar environments can be heated by the sun and reach temperatures of 45°C or more or can contain microniches where the temperatures are elevated by rapid biodegradative processes.

Differentiation of the isolates on the basis of total cell protein profiles. The total cell proteins of the isolates were separated on SDS-PAGE gels. The protein profiles obtained were compared with the profiles of Caloramator fervidus (used because of the 16S rDNA sequence data [see below]), three other alkalophilic and alkalitolerant anaerobic thermophiles, Clostridium paradoxum (15), Clostridium thermoalcalophilum (14), and Anaerobranca horikoshii (5), and other thermophiles, such as Thermoanaerobacter and Thermoanaerobacterium strains (Fig. 1 and data not shown). The band patterns of most of the novel isolates were very similar, indicating that these organisms are members of the same species; two exceptions were strains JW/SD-EPPFY and JW/YL-Y5199. It should be pointed out that the three strains isolated from Plate River samples that we investigated, which came from two locations that were within 50 m of each other and exhibited practically identical protein
Anaerobranca horikoshii, Lachnospira pectinoschiza, Roseburia cecicola were determined by all of the phylogenetic analysis methods used. When a larger number of sequences of thermophilic Clostridium oceanicum, Clostridium argentinense, Clostridium limosum, and Clostridium cadaveris were identical in this region. The phylogenetic position of Thermobrachium celere JW/YL-NZ35T, JW/YL-PR6, JW/YL-PR32, and JW/YL-M105, Partial 16S rDNA sequences at the 5' end of the gene (approximately 500 to 800 nucleotides) were determined for other strains. Because of ambiguous nucleotides or missing bases in the reference sequences, only positions 98 to 1520 were used in the phylogenetic analyses. The 16S rDNA sequences of the four strains indicated above were identical in this region. The phylogenetic position of strain JW/YL-NZ35T within the radiation of the clostridia and related taxa is shown in Fig. 2. The distinctness of the cluster containing the four strains of Thermobrachium celere and the relationship between this cluster and Caloramator fervidus were determined by all of the phylogenetic analysis methods used. When a larger number of sequences of thermophilic anaerobes were included in the analysis, there was a tendency for the strains included in this study and Caloramator fervidus to group with members of the genera Thermoanaerobacterium, Thermoanaerobacter, and Moorella. A similar artifact in phylogenetic analysis was determined for strains JW/YL-NZ35T, JW/YL-PR6, JW/YL-PR32, and JW/YL-M105.
FIG. 3. (A and B) Light micrographs of *Thermobrachium celere* JW/YL-NZ35\(^T\); cells in the early exponential growth phase (A) and the stationary growth phase (B). (C and D) Cells of strain JW/YL-YS199 in the early exponential growth phase (C) and the late exponential growth phase (D). (E and F) Cells of strains JW/YL-T148 (E) and JW/YL-PR32 (F) in the early stationary growth phase. (G and H) Cells of strain JW/YL-PR6 in the early stationary growth phase (G) and the late stationary growth phase (H) with a high tendency to produce L-shaped cells. The arrowheads indicate branch formation, which was more common in the freshly isolated cultures than in those cultured in the laboratory for several years. Bar = 5 μm.
FIG. 4. Electron micrographs of strain JW/YL-NZ35T. (a) Negatively stained cell, showing peritrichously inserted flagella (F). Because of drying, the cell shrank, which resulted in clearly visible outer layers of the cell envelope (W). (b and c) Ultrathin cross sections through a cell at a low magnification (b) and a high magnification (c). CM, cytoplasmic membrane; P, peptidoglycan; S, surface layer composed of regularly arranged units. (d) Ultrathin section through cells, showing extracellular material (C) on top of the surface layer. S, S-layer; P, peptidoglycan layer. (e) Negatively stained sample containing cells in a state of partial disintegration. Patches of the hexagonal surface layer (circles labeled S), the peptidoglycan (P), and the cytoplasmic membrane (CM) are visible. The Arrow PS indicates a cell inclusion that looks like a polysheet (defective bacteriophage). (f) Cell in an advanced state of disintegration. The peptidoglycan and surface layer are no longer visible, but the cytoplasmic membrane (CM) contains defective bacteriophage particles (PS) in two forms; one form (PS$_1$) resembles a hollow tube, whereas the other form (PS$_2$) contains additional material.
genetic analyses involving 16S rDNA sequence data for ther-
mophilic bacteria has been observed previously with Caloramato-
ria fervidus (3, 23). The dendrogram in Fig. 2 is based on a
reconstruction in which the neighbor-joining method was used.
Although there is obviously a relationship between the Ther-
mobrachium celere strains and Caloramator fervidus, a boot-
strap analysis of 1,000 trees revealed that the common branching
position was recovered in only 73% of the trees sampled. A
bootstrap value of 73% is not significant and indicates that the
Thermobrachium celere strains are distinct from Caloramator
fervidus. The lack of phylogenetic relatedness to previously
described taxa was also demonstrated by the low levels of
similarity between the 16S rDNA sequences determined in this
study and the 16S rDNA sequences of the reference organisms
(Table 2). The highest level of 16S rDNA sequence similarity
was the level of similarity with Caloramator fervidus (93.3%)
(Table 2). The levels of similarity between strain JW/YL-
NZ35T and the reference organisms that were analyzed, in-
cluding the thermophilic alkaliphiles Clostridium thermoacido-
philum, Clostridium paradoxum, and Anaerobranca horkoshii,
were less than 90% (Table 2).

Colony and cell morphology. Colonies, either embedded in
the agar or growing on the surface, were white and circular.
The cells were rod shaped, 0.5 to 1.2 μm wide, and 1.5 to 14
μm long, and there were some noticeable variations among
the isolates (Table 3 and Fig. 3). The cells of several of the strains,
including YW/YL-NZ35T, occurred mainly in chains during
the early exponential growth phase and then separated during
the mid-exponential phase into individual cells (Fig. 3A
through D). During the stationary growth phase, L-form cells
occurred frequently (Fig. 3H) and the average cell length was
slightly greater than the average cell length in the exponential
phase (Table 3). All of the isolates formed primary branches at
a frequency of about 1 to 5% (Fig. 3B, F, and G). However,
this property was less pronounced in the type strain after it was
cultivated in the medium described above for more than 2
years. Endospore formation was not observed in liquid or solid
medium containing 0.1 to 0.5% (wt/vol) yeast extract in the
presence of low concentrations of the carbohydrates utilized by
the strains or in the absence of these carbohydrates. The cells
were peritrichously flagellated (Fig. 4a). The cells stained gram
positive in both the exponential and stationary phases of
growth, which is consistent with the Gram type-positive cell
wall structure of these organisms (Fig. 4c and e). However,
cells were easily destained with ethanol in the Gram staining
procedure. The cells had an S-layer with a weak hexagonal
lattice (Fig. 4b, c, and e); the molecular weight of the subunit
was 145,000, as estimated from SDS gels, and the estimated
diameter of the subunit was 8.5 nm, as determined by exam-
ining electron micrographs. In addition, strain JW/YL-NZ35T
(other strains were not tested) contained capsular material
(Fig. 4a and d). The cells contained a granular material, pos-
sibly a carbohydrate storage material, that reacted purple-blue
with iodine solutions. Polysheath-like inclusions were observed
frequently in negatively stained cells (Fig. 4f), and these inclu-
sions were assumed to be defective bacteriophage particles on
the basis of data obtained previously for other bacteria (16, 28,
29).

Growth properties. All of the isolates were proteolytic (most
of the strains were isolated by using skim milk as a major
carbon source) and required at least 0.1% yeast extract for
growth. Yeast extract could serve as a sole carbon and energy
source. The strains were obligate anaerobes and did not grow
in medium in which the redox indicator resazurin had turned
slightly pink. Strain JW/YL-NZ35T, however, remained viable
in aerated medium for at least 24 h at room temperature and
60°C. Growth was not inhibited by the presence of sodium
azide.

For most strains the temperature range for growth at pH 8.0
(determined at 66°C) was >37 to <75°C (Table 3), and the
optimum temperature was between 62 and 67°C (Fig. 5A). The
pH80°C range for growth at 60 or 66°C was 5.0 to 9.3 (Table 3),
and the optimum pH80°C was around 8.5 (Fig. 5B). When strain
JW/YL-NZ35T was transferred frequently into modified me-
dium M-S (pH at 60°C, 8.5) containing 0.5% yeast extract and
0.5% glucose and incubated at 65°C, the doubling time was as
little as 10 min; hence, the species name Thermobrachium
celere was chosen. The doubling times for other strains tested
were between 10 and 25 min under similar conditions.

Maintenance. All strains of Thermobrachium celere were vi-
able after they had been stored for up to 3.5 years (the longest
time tested) in an anaerobic 50% (vol/vol) glycerol solution at
-70°C. Grown cultures could be stored at room temperature,
depending on the strain, for up to 48 h (strain JW/YL-PR6) or
up to about 2 months (strain JW/YL-NZ35T), after which they
lost viability.

Substrate utilization and fermentation products. Depen-
ding on the strain, the carbohydrates utilized included glucose,
sucrose, fructose, galactose, and maltose (Table 3). Strain JW/
YL-NZ35T was tested with other compounds, and in the pres-
ence of 0.5% yeast extract, no increase in optical density was
obtained with ribose, lactose, cellulbiose, rhamnose, arabinose,
raffinose, mannose, xylose, xylitol, glycerol, methanol, salicin,
lactate, pyruvate, succinate, glucuronic acids, Casamino Acids,
N-acetylglucosamine, and xylan. In contrast to all of the strains
tested, the organism that is most closely related phylogenet-
cally, Caloramator fervidus, utilizes xylan (Table 3). However,
not all of the more than 40 isolates which appeared to be
Thermobrachium celere were tested with this substrate. Dissim-
ilatory reduction of sulfate or thiosulfate in which glucose, acetate, or lactate was used as an electron donor was not observed. Formation of elemental sulfur granules was not observed when the organisms were grown in the presence of thiosulfate. Cultures grown in modified medium M-5 containing 0.5% yeast extract and 0.5% (wt/vol) glucose produced acetate, formate, CO₂, and H₂ as major fermentation products and lower amounts of ethanol.

**Differentiation of Thermobrachium celere from Caloramator fervidus and other thermophilic anaerobes.** On the basis of similarities in physiological properties and 16S rDNA sequences, all of the new strains were considered members of the new genus Thermobrachium and the new species Thermobrachium celere. None of the strains studied produced elemental sulfur granules from thiosulfate, which distinguishes Thermobrachium strains from members of the genus Thermoanaerobacter. The formation of nearly 1 mol of formate per mol of glucose fermented distinguishes these strains from members of the genus Thermobrachium. None of the strains hydrolyzed crystalline cellulose, which distinguishes them from the group of cellulosytic organisms that cluster on the basis of their 16S rDNA sequences around Caldocellulinsiruptor saccharolyticum (synonym, Caldocellum saccharolyticum) and Clostridium thermocellum.

16S rDNA sequence data showed that the strains which we studied represent one species and that the most closely related organism is Caloramator fervidus. The level of 16S rDNA sequence similarity between the Thermobrachium celere strains and Caloramator fervidus is 93.3%, a value considered low for the clostridia and related organisms. As the phylogenetic dendrogram shows, the level of similarity between Thermobrachium celere and Caloramator fervidus is low compared with the levels of similarity between other taxa which represent different genera. Some examples include the levels of 16S rDNA sequence similarity among Roseburia cecicola, Lachnospira pectininoschiza, and Coprococcus eutactus, which range from 93.9 to 94.9% (data not shown). The phylogenetic distinctness of Thermobrachium celere is also supported by the low bootstrap value for the branch point with Caloramator fervidus, a value which could become lower if additional sequences of newly isolated taxa are used in future analyses. The distinctness of the genus is also supported by the special branched-cell morphology observed for Thermobrachium celere (although this is less obvious after several years of cultivating compared with immediately after isolation) and the 8 mol% difference in the G+C contents of Thermobrachium celere and Caloramator fervidus. Thermobrachium celere consistently stains positive with modified Gram stain kits, whereas Caloramator fervidus stains negative (22). Furthermore, Thermobrachium celere is able to grow at more alkaline pH values than Caloramator fervidus (Table 3), which has a relatively restricted growth range (22); no spore formation has been observed for any of the new isolates, whereas Caloramator fervidus does form spores. Also, unlike Caloramator fervidus, Thermobrachium celere is not able to utilize xylan and is not able to utilize serine as a sole carbon source (Table 3) (22). Whereas Caloramator fervidus forms acetate as its major fermentation end product along with small quantities of valerate, butyrate, ethanol, lactate, CO₂, and H₂, Thermobrachium celere produces almost equal amounts of acetate, formate, CO₂, and H₂ does not produce valerate or butyrate, and produces only trace amounts of lactate. Thermobrachium celere also has a noticeably more rapid growth rate (doubling times, as little as 10 min) than Caloramator fervidus (doubling times, around 1 h). These physiological differences between Caloramator fervidus and Thermobrachium celere, along with the 16S rDNA sequence analysis data, support the placement of the novel isolates in a new species of the new genus Thermobrachium, Thermobrachium celere.

**Description of Thermobrachium gen. nov. Thermobrachium** (Ther.mo.bra'chi.um.) G. adj. thermos, hot; L. brachium, arm, branch; L. n. Thermobrachium, referring to the branched cells observed frequently with this thermophilic bacterium. The genus Thermobrachium belongs to the low-G+C-content, Gram type-positive Bacillus-Clostridium subphylum. The cells are usually rod shaped and frequently exhibit true branching (i.e., the cells are branched with no septa between the branches). Gram positive, although cells of some strains are easily decolorized. An S-layer with a hexagonal lattice is present. Cells are flagellated, but only sluggishly motile. The G+C content is around 30 mol%. The habitats include anthropogenically heated environments (composts) and geothermally heated environments (hot springs and sediments), as well as mesobiotic freshwater sediments in locations on several continents. Chemoorganoheterotrophs. The type species is Thermobrachium celere, which so far is the only species in the genus.

**Description of Thermobrachium celere sp. nov. Thermobrachium celere** (ce'le're). L. adj. celere, fast, referring to the rapid growth of all isolates). Colonies growing in agar or on agar surfaces are white and circular. The cells stain gram positive and are obligately anaerobic and rod shaped, ranging from 0.5 to 1.2 μm wide and from 1.5 to 14 μm long. The cells form primary branches at a low frequency (1 to 5% of the cells in a culture), and L-form cells are produced in the stationary growth phase. Spores have not been observed, Chemoorganotrophic and proteolytic but not cellulolytic. Yeast extract can serve as a sole carbon and energy source. In the presence of yeast extract, glucose, sucrose, fructose, galactose, and maltose are utilized. Substrates that are not utilized include cellobiose, rhamnose, mannose, arabinose, xylose, glucuronic acid, xylose, Casamino Acids, and pyruvate. The fermentation products from glucose in the presence of yeast extract are CO₂, H₂, acetate, formate, and ethanol. Thermophilic and alkali tolerant. The temperatures that permit growth range from >37 to <75°C, and the optimum temperature is 62 to 66°C at pH 6.0 to 8.0. The pH range for growth is 5.0 to 9.7 at 60 or 66°C, and the optimum pH 6.0 to 8.5. The DNA G+C content is 30 to 31 mol%.

Strain JW/YL-NZ35T is the type strain of Thermobrachium celere and has been deposited in the German Collection of Microorganisms as strain DSM 8682. The DNA G+C content of JW/YL-NZ35T is 31 mol%. The optimum pH is 8.2, and the optimum temperature is 67°C. The type strain utilizes glucose, sucrose, fructose, and galactose, but not ribose or Casamino Acids.

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