Thermoanaerobacter uzonensis sp. nov., an anaerobic thermophilic bacterium isolated from a hot spring within the Uzon Caldera, Kamchatka, Far East Russia

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Several strains of heterotrophic, anaerobic thermophilic bacteria were isolated from hot springs of the Uzon Caldera, Kamchatka, Far East Russia. Strain JW/IW010^T was isolated from a hot spring within the West sector of the Eastern Thermal field, near Pulsating Spring in the Winding Creek area. Cells of strain JW/IW010^T were straight to slightly curved rods, 0.5 µm in width and variable in length from 2 to 5 µm and occasionally up to 15 µm, and formed oval subterminal spores. Cells stained Gram-negative, but were Gram-type positive. Growth was observed between 32.5 and 69 °C with an optimum around 61 °C (no growth occurred at or below 30 °C, or at or above 72 °C). The pH^{60 °C} range for growth was 4.2–8.9 with an optimum at 7.1 (no growth occurred at or below pH^{60 °C} 3.9, or at 9.2 or above). The shortest observed doubling-time at pH^{60 °C} 6.9 and 61 °C was 30 min. Strain JW/IW010^T was chemo-organotrophic; yeast extract, peptone, Casamino acids and tryptone supported growth. Yeast extract was necessary for the utilization of non-proteinaceous substrates, and growth was observed with inulin, cellobiose, maltose, sucrose, glucose, fructose, galactose, mannose, xylose, trehalose, mannitol, pyruvate and crotonate. The G + C content of the genomic DNA of strain JW/IW010^T was 33.6 mol% (HPLC method). The major phospholipid fatty acids were iso-15 : 0 (53.5 %), 15 : 0 (11.8 %), 16 : 0 (7.3 %), 10-methyl 16 : 0 (7.3 %) and anteiso-15 : 0 (5.3 %). 16S rRNA gene sequence analysis placed strain JW/IW010^T in the genus *Thermoanaerobacter* of the family 'Thermoanaerobacteriaceae' (Firmicutes), with *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T (97 % 16S rRNA gene sequence similarity) and *Thermoanaerobacter kivui* DSM 2030^T (94.5 %) as the closest phylogenetic relatives with validly published names. The level of DNA–DNA relatedness between strain JW/IW010^T and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T was 64 %. Based on the physiological, phylogenetic and genotypic data, strain JW/IW010^T represents a novel taxon, for which the name *Thermoanaerobacter uzonensis* sp. nov. is proposed. The type strain is JW/IW010^T (=ATCC BAA-1464^T=DSM 18761^T). The effectively published strain, 1501/60, of '*Clostridium uzonii*' [Krivenko, V. V., Vadachloriya, R. M., Chermikh, N. A., Mityushina, L. L. & Krasilnikova, E. N. (1990). *Microbiology* (English translation of *Mikrobiologiya*) **59**, 741–748] had approximately 88.0 % DNA–DNA relatedness with strain JW/IW010^T and was included in the novel taxon.

Abbreviations: AMC, 7-amino-4-methylcoumarin; 4MU, 4-methylumbelliferone; PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JW/IW010^T is EF530067.

The Uzon Caldera and Geyser Valley region of Kamchatka contain a wide variety of old and recently formed geothermal features in close proximity. Water and sediment samples were collected from hot springs of the Uzon Caldera and, concomitantly, temperature and pH

measurements were taken and geochemical properties were assessed. For the isolation of novel anaerobic thermophilic prokaryotes, enrichment cultures were prepared from Uzon Caldera hot spring water and sediment samples using various carbon substrates, including methanol, crotonate, glucose, fructose and xylose.

The enrichment cultures were prepared using an anaerobic, carbonate-buffered basal medium [2 mM KH_2PO_4 , 0.5 g NH_4Cl l^{-1} , 0.5 g $(\text{NH}_4)_2\text{SO}_4$ l^{-1} , 1.5 g NaHCO_3 l^{-1} , 0.1 mM MgCl_2 , 0.1 mM CaCl_2 , 1.0 ml vitamin solution l^{-1} and 1.0 ml Na_2SeO_3 - Na_2WO_3 trace-element solution l^{-1} (Widdel & Bak, 1992)] at a $\text{pH}^{25^\circ\text{C}}$ of 6, supplemented with 3 g carbon substrate l^{-1} and 1 g yeast extract l^{-1} . From enrichment cultures with visible growth, pure cultures were obtained using serial dilutions in anaerobic agar-shake-roll tubes with the carbonate-buffered basal medium supplemented with 2.15 % agar (Ljungdahl & Wiegel, 1986) and multiple rounds of colony isolation. Purity was checked microscopically and by means of 16S rRNA gene sequencing.

Strains JW/IW007, JW/IW010^T and JW/IW015 were derived from water and sediment samples from a small pool 2 m east of Pulsating Spring (54.5007° N 160.0076° E), which had a temperature of about 77 °C and a pH of 5.6. These pools feed Winding Creek, which is located on the northern side of the central portion of the Uzon Eastern Thermal field and flows southwards into Chloride Lake. Strain JW/IW007 was isolated using methanol as the provided carbon substrate. Axenic cultures of strains JW/IW010^T and JW/IW015 were isolated using crotonate as the carbon substrate. Similarly, strain JW/HL-KA15 was isolated from a glucose-supplemented enrichment from the hot spring 'Blue Pool', located at the Northern Thermal field (78.8 °C and pH 6.3).

In order to check culture purity and for phylogenetic analyses, DNA was extracted using an UltraClean DNA isolation kit (Mo Bio) and 16S rRNA gene sequences were PCR-amplified and sequenced. Routine PCRs were prepared with the bacterial domain-specific primer set 27F and 1492R (Lane, 1991), using GoTaq (Promega) and the thermocycler conditions described previously by Lee *et al.* (2005). The 16S rRNA gene sequence of strain JW/IW010^T was amplified using high-fidelity, PrimeStar HS polymerase (Takara Mirus Bio). Amplification with PrimeStar HS polymerase proceeded with thermocycler conditions of 10 s at 98 °C, 5 s at 58 °C and 90 s at 72 °C, repeated for a total of 30 cycles. The amplified 16S rRNA gene sequences were purified using a QIAquick PCR purification kit (Qiagen) and sequenced by Macrogen. Sequencing results were aligned using Sequencher v4.1.4 (Gene Codes) and a multiple sequence alignment was created with CLUSTAL W (Thompson *et al.*, 1994). Editing of alignments was performed using GeneDoc v2.6.001 (Nicholas & Nicholas, 1997). The phylogenetic tree (Fig. 1) was inferred by using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes and Cantor model (Jukes & Cantor, 1969), using the PHYLIP v3.6a2.1 phylogenetic analysis package (Felsenstein, 2001).

From the 16S rRNA gene sequence analysis, strain JW/IW010^T and related strains were clearly placed in the genus *Thermoanaerobacter* of the family 'Thermoanaerobacteriaceae' (Firmicutes) (Fig. 1). Subsequent 16S rRNA gene sequence-based phylogenetic analyses revealed that strain JW/IW010^T had a high similarity (99 %) to strain 1501/60 with the effectively, but not validly, published name '*Clostridium uzonii*' (Krivenko *et al.*, 1990). '*C. uzonii*' has been described as a deeply branching member of the *Clostridium* cluster V, which contains the genus *Thermoanaerobacter* (Stackebrandt *et al.*, 1999). Based on 16S rRNA gene sequence analysis, the closest phylogenetic relatives with validly published names were *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T (97 % similarity) (Lee *et al.*, 2007a) and the acetogenic *Thermoanaerobacter kivui* DSM 2030^T (96 % similarity) (Onyenwoke & Wiegel, 2008). Strains 1501/60 (DSM 9752) and 1611 (DSM 9753), effectively described by Krivenko *et al.* (1990), were isolated from a mud cauldron and hydrothermal silt, respectively, within the Uzon Caldera and were obtained from the German Collection of Microorganisms and Cell Cultures for comparison with strain JW/IW010^T. *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T was obtained from laboratory stock.

Within agar-shake-roll tubes, colonies of strain JW/IW010^T were visible following overnight incubation at 60 °C; the colonies were circular, 1–2 mm in diameter, slightly convex and creamy white in colour. When grown on plates of carbonate-buffered basal medium with 2.3 % agar within anaerobic jars at 60 °C, strain JW/IW010^T formed colourless, nearly transparent circular colonies up to 1 mm in diameter, whereas *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T formed circular colonies of up to 1 mm in diameter that were opaque and bluish white in colour. Cells of strain JW/IW010^T were rod-shaped, straight to slightly curved and approximately 0.5 µm in width and 2–5 µm in length (Figs 2 and 3). Occasionally, cells up to 15 µm in length were observed. Shorter or longer chains of cells frequently contained intermittent coccoid cells (Fig. 3), typical of *Thermoanaerobacter* (Wiegel & Ljungdahl, 1981). Gram stain reactions were performed on both early exponential and stationary growth phase cultures of strain JW/IW010^T using Fisherbrand Gram/Control/Test Slides (Thermo Fisher Scientific). Strain JW/IW010^T stained Gram-negative under both growth conditions although phylogenetically it is Gram-type positive (Wiegel, 1981).

Sporulation of strain JW/IW010^T was observed, though only rarely. Spores were oval, located subterminal to terminal and caused the mother cell to swell (Fig. 2a). Viable subcultures were obtained from liquid cultures heated to 100 °C for 5 min. Although sporulation was rarely seen in strain JW/IW010^T, other *Thermoanaerobacter* species also produce spores infrequently, whereas sporulation has never been observed in other taxa of this genus, e.g. *Thermoanaerobacter ethanolicus* JW 200^T (Onyenwoke & Wiegel, 2008; Wiegel & Ljungdahl, 1981). Cells of strain JW/IW010^T had peritrichously inserted

Fig. 1. 16S rRNA gene sequence-based phylogenetic dendrogram of strains JW/IW007, JW/IW010^T, JW/IW015 and JW/MH015 amongst members of the genus *Thermoanaerobacter* and selected members of the family 'Thermoanaerobacteriaceae'. *Clostridium butyricum* was used as an outgroup; numbers at nodes are bootstrap percentages (100 replicates). The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the Jukes and Cantor distance corrections (Jukes & Cantor, 1969). Bar, 2 nucleotide substitutions per 100 nucleotides.

flagella (Fig. 3) and tumbling motility was observed when liquid cultures were viewed using phase-contrast microscopy. When grown in media containing 50 or 100 mM thiosulphate, cells of strain JW/IW010^T were more motile compared with cells grown in medium lacking thiosulphate.

The temperature and pH profiles, substrate range and tolerance of thiosulphate and sulphite were determined through measurement of ΔOD_{600} using a Spectronic 21 spectrophotometer (Bausch and Lomb). A temperature-gradient incubator (Scientific Industries) was used to determine the temperature range and optimum for growth of strain JW/IW010^T using the aforementioned basal carbonate-buffered medium complemented with 4 g glucose l⁻¹ and 2 g yeast extract l⁻¹. Up to 96 h, growth was observed between 32.5–69 °C, with optimum growth at 61 °C; growth was not observed at or below 30 °C, or at or

above 72 °C (Fig. 4a). Media used for the determination of the pH range of strain JW/IW010^T was buffered with 10 mM of each trisodium citrate, MES, Tris and Na₂CO₃, rather than NaHCO₃, and also contained 4 g glucose l⁻¹ and 2 g yeast extract l⁻¹. Up to 75 h, growth was observed between an initial pH^{60 °C} of 4.2–8.9, with an optimum at 7.1; no growth was observed at or below pH^{60 °C} 3.9, or at 9.2 or above (Fig. 4b). The shortest doubling-time for strain JW/IW010^T was approximately 30 min and occurred at pH^{60 °C} 6.9 and 61 °C in medium containing 2 g yeast extract l⁻¹ and 4 g glucose l⁻¹. No growth was observed in the absence of yeast extract while using glucose as a carbon source. Using 0.5 g yeast extract l⁻¹ as the sole substrate led only to limited growth. Proteinaceous carbon sources added to the carbonate-buffered basal medium to a concentration of 5 g l⁻¹ were tested for their ability to support the growth of strain JW/IW010^T. Growth was assumed when ΔOD_{600} increased or when cell numbers

Fig. 2. Morphology of cells of strain JW/IW010^T, studied using an Olympus VANOX phase-contrast microscope (Tokyo, Japan). (a) Spore formation within a cell of strain JW/IW010^T. The arrow indicates the position of oval spores within the swollen mother cell. Bar, 5 µm. (b) Sulphur granules within cells of strain JW/IW010^T grown for approximately 24 h in medium containing 50 mM thiosulphate. Bar, 10 µm.

Fig. 3. Scanning electron micrographs of cells of strain JW/IW010^T. Arrows indicate characteristic coccoid cells observed within the chain. Bar, 5 µm.

Fig. 4. Temperature and pH^{60 °C} profiles of strain JW/IW010^T and *Thermoanaerobacter sulfurifigens* JW/SL-NZ826^T. Growth rates over a range of temperatures (a) and pH^{60 °C} values (b) are shown. □, Strain JW/IW010^T; ○, *Thermoanaerobacter sulfurifigens* JW/SL-NZ826^T (data from Lee *et al.*, 2007a).

increased as determined through the use of phase-contrast microscopy. At 5 g l⁻¹, yeast extract (ΔOD₆₀₀=0.04), peptone (ΔOD₆₀₀=0.03), Casamino acids (ΔOD₆₀₀=0.03) and tryptone (ΔOD₆₀₀=0.03) served as sole carbon substrates, whereas beef extract did not support growth. The ability of strain JW/IW010^T to utilize other carbon substrates was determined with substrates at a concentration of 3 g l⁻¹, supplemented with 1 g yeast extract l⁻¹. Utilization was recorded as positive when ΔOD₆₀₀ was twice that of cultures containing only the basal (1 g l⁻¹) concentration of yeast extract. Growth of strain JW/IW010^T was observed with inulin, cellobiose, maltose, sucrose, glucose, fructose, galactose, mannose, xylose, trehalose, mannitol, pyruvate and crotonate. Growth was not observed with casein, cellulose, xylan, starch, olive oil, pectin, lactose, arabinose, rhamnose, ribose, xylitol, sorbitol, methanol, glucuronic acid or glycerate.

Enhanced growth of strain JW/IW010^T was not observed in media containing a headspace of H₂/CO₂, in comparison with cultures grown in the same basal media containing 1 g yeast extract l⁻¹, with an N₂ gas headspace. When grown with 2 g glucose l⁻¹ and 1 g yeast extract l⁻¹, strains JW/IW010^T and 1501/60 produced lactic acid, acetic acid, ethanol, CO₂ and H₂ as fermentation end products, though in different proportions. Strain JW/IW010^T primarily produced ethanol and acetate, and only a trace amount of lactic acid, yielding a simplified fermentation equation of: 1 glucose + 0.85 H₂O → 0.85 acetic acid + 1.15 ethanol + 2 CO₂ + 1.7 H₂, whereas strain 1501/60 produced lactic acid, acetic acid and ethanol, yielding the simplified fermentation equation of: 1 glucose + 0.6 H₂O → 0.84 lactic acid + 0.6 acetic acid + 0.56 ethanol + 1.16 CO₂ + 1.76 H₂.

The phylogenetically close neighbour, *Thermoanaerobacter sulfurigignens* (two isolates), exhibits resistance to 1 M thiosulphate and 90 mM sulphite (Lee *et al.*, 2007a). In contrast, strain JW/IW010^T tolerated only up to 200 mM thiosulphate (growth did not occur at or above 250 mM) and up to 40 mM sulphite (no growth at or above 50 mM) using mineral media supplemented with 1 g yeast extract l⁻¹ and 1 g glucose l⁻¹ as carbon and energy sources. The reduction of thiosulphate to sulphide was assessed by the use of lead acetate-impregnated paper. A culture that was supplemented only with yeast extract served as a control. In media containing 50 or 100 mM thiosulphate, and 1 g l⁻¹ yeast extract and glucose, strain JW/IW010^T formed H₂S and elemental sulphur, observed as intracellular sulphur deposits, from thiosulphate. Sulphur granula could be differentiated and from spores by the little to no swelling of the mother cell, occasional multiple inclusions per cell, and the late appearance of sulphur granula in the stationary phase after spore formation (Fig. 2a and b). The formation of elemental sulphur deposits in addition to H₂S formation is similar to what has been observed for *Thermoanaerobacter italicus* (Kozianowski *et al.*, 1997), whereas *Thermoanaerobacter sulfurigignens* (Lee *et al.*, 2007a, b) only produces elemental sulphur, similar to most species of *Thermoanaerobacterium* (Lee *et al.*, 1993; Onyenwoke & Wiegel, 2008; Schink & Zeikus, 1983).

To compare the biochemical and enzymic features of strain JW/IW010^T with those of its closest recognized relative, *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T, the BBL Crystal RGP (Becton Dickinson) system was used. The procedures used were according to the manufacturer's instructions with the modification that cells were obtained via centrifugation of 250 ml cultures that were grown overnight (basal medium described previously, containing 2 g yeast extract l⁻¹ and 4 g glucose l⁻¹). The cells were then washed twice with a solution of the basal medium that did not contain yeast extract or other carbon substrate. Under these conditions, both strain JW/IW010^T and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T tested positive for the following: hydrolysis of the glycosidic bond of 4-methylumbelliferone (4MU) β-D-glucoside,

hydrolysis of the amide bond of L-methionine-7-amino-4-methylcoumarin (AMC) and L-valine-AMC; hydrolysis of the aryl substituted glycoside of *o*-nitrophenyl β-D-glucoside and *p*-nitrophenyl β-D-glucoside, *p*-nitrophenyl β-D-glucoside, *p*-nitrophenyl phosphate, and *p*-nitrophenyl β-D-galactoside and *p*-nitrophenyl α-D-galactoside. Both taxa tested negative for the following: hydrolysis of the amide bond of L-proline-AMC, L-arginine-AMC, L-tryptophan-AMC and L-phenylalanine-AMC; hydrolysis of the glycosidic bond of 4MU β-cellobiose, 4MU-phosphate, 4MU α-D-glucoside; utilization of arabinose, maltose, dextrin, mannitol, galactose, *N*-acetyl-D-glucosamine, trehalose, mannose, maltotriose, ornithine; the enzymic hydrolysis of the aryl substituted glycoside of *p*-nitrophenyl α-D-glucoside; and hydrolysis of urea and aesculin. However, the two taxa differed in that *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T hydrolysed the amide bond of L-pyrroglutamic acid-AMC, whereas strain JW/IW010^T did not, and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T hydrolysed the aryl substituted glycoside of *p*-nitrophenyl α-cellobiose, whereas JW/IW010^T did not.

Genomic DNA for the determination of the G + C content was extracted using a large-scale phenol/chloroform-based procedure essentially as described by Wilson (1997), without the final caesium chloride gradient purification step. The DNA G + C content was measured by using HPLC (Mesbah *et al.*, 1989) with the modification of Lee *et al.* (2005), using S1 nuclease (Invitrogen) and 0.3 M sodium acetate (pH 5.0). The DNA G + C content of strain JW/IW010^T was 33.6 mol% (SD=0.4; *n*=9). Krivenko *et al.* (1990) reported the DNA G + C content of strain 1501/60 as being 34.4 mol%, determined using the thermal denaturation technique (Marmur & Doty, 1962). Using the HPLC-based method of Mesbah *et al.* (1989), the DNA G + C content of strain 1501/60 was 33.3 mol% (SD=0.08; *n*=4).

To assess the genotypic similarity between strain JW/IW010^T and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T, and also strains JW/IW010^T and 1501/60, whole genome DNA–DNA relatedness and Δ*T*_m values were determined. DNA–DNA hybridizations performed at DSMZ revealed that strain JW/IW010^T and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T had 64 % DNA–DNA relatedness, whereas strains JW/IW010^T and 1501/60 had 87–88.0 % DNA–DNA relatedness. Whole-genome Δ*T*_m values between strains JW/IW010^T, 1501/60 and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T were determined using the fluorimetric, SYBR Green I (Invitrogen)-based technique described by Gonzalez & Saiz-Jimenez (2005). The mean Δ*T*_m between hybridized JW/IW010^T and JW/SL-NZ826^T DNA and homologous JW/IW010^T DNA was 5.85 °C (*n*=4). The mean Δ*T*_m between hybridized JW/IW010^T and 1501/60 DNA and homologous JW/IW010^T DNA was 4.95 °C (*n*=4). At present, Δ*T*_m values of >5 °C and DNA–DNA relatedness values of less than 70 %, considered along with

phenotypic and chemotaxonomic differences, are acknowledged standards for the differentiation of species (Stackebrandt *et al.*, 2002). In comparing strain JW/IW010^T with *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T, the ΔT_m value of 5.85 °C and DNA–DNA relatedness of 64 % indicated that strain JW/IW010^T and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T represent separate species, which was also corroborated by phenotypic and chemotaxonomic differences. The ΔT_m value of 4.95 °C and DNA–DNA relatedness of 87–88 % demonstrate that strains JW/IW010^T and 1501/60 belong to the same species.

The phospholipid fatty acid (PLFA) contents of strain JW/IW010^T and the phylogenetically closely related taxon *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T were measured and compared. Cell material for lipid extraction was obtained according to previously described procedures (Zhang *et al.*, 2004), based on the protocol of White *et al.* (1979). In brief, an overnight extraction commenced with a single-phase organic solvent system comprising chloroform, methanol and aqueous 50 mM phosphate buffer (pH 7.4) in a ratio of 1:2:0.8 (by vol.). Chloroform and nanopure water were then added to the extract to obtain a final ratio of 1:1:0.9 (by vol.), resulting in a two-phase system. Neutral lipids, glycolipids and polar lipids were fractionated using a silicic acid column (Guckert *et al.*, 1985). The polar lipids were treated with mild alkaline methanolysis to produce fatty acid methyl esters. Analyses of the fatty acid methyl esters were performed using a 6890 gas chromatograph (Agilent) with a flame-ionization detector, equipped with a 30 m DB-5 column (5 % phenyl) and programmable temperature vaporizing inlet. The temperature programme for samples in hexane was: starting at 60 °C, 10 °C min⁻¹ to 180 °C, and 4 °C min⁻¹ to 320 °C and hold for 20 min. GC-MS analyses of fatty acid methyl esters were performed using an HP5890 gas chromatograph (Hewlett Packard) equipped with a 30 m HP5-MSI column and programmable temperature vaporizing inlet and coupled to an HP5972 mass spectrometer. The major PLFAs for strain JW/IW010^T were iso-15:0 (53.5 %), 15:0 (11.8 %), 16:0 (7.3 %), 10-methyl 16:0 (7.3 %), and anteiso-15:0 (5.3 %). The major PLFAs for *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T were iso-15:0 (57.7 %), 15:0 (18.3 %), anteiso-15:0 (6.1 %), iso-17:0 (5.7 %), and 10-methyl 16:0 (5.5 %). PLFA 14:0 (1.7 %) was found within strain JW/IW010^T but was not detected for *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T, analysed at the same time. Whereas both strain JW/IW010^T and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T contained 18:1 ω 9c (3.9 and 1.8 %, respectively), the saturated fatty acid 18:0 was only found within strain JW/IW010^T and 18:1 ω 7t was only detected in *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T (Table 1). The PLFA content of strain 1501/60 was also analysed; as for strain JW/IW010^T, iso-15:0 was the major lipid (56.9 %); however, strain 1501/60 was found to have more of anteiso-15:0 (9.4 %) than 15:0

Table 1. PLFA contents (%) of strain JW/IW010^T (*Thermoanaerobacter uzonensis* sp. nov.) and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T

ND, Not detected.

PLFA	Strain JW/IW010 ^T	Strain JW/SL-NZ826 ^T
iso-13:0	1.4	0.9
14:0	1.7	ND
iso-15:0	53.5	57.7
anteiso-15:0	5.3	6.1
15:0	11.8	18.3
16:0	7.3	2.2
10-methyl 16:0	7.3	5.5
iso-17:0	2.8	5.7
18:1 ω 9c	3.9	1.8
18:1 ω 7t	ND	1.8
18:0	5.0	ND
Total	100	100

(1.1 %), in addition to having iso-16:0 (7.0 %) and anteiso-17:0 (6.3 %), which were not detected in strain JW/IW010^T or *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T (data not shown).

In addition, strain JW/IW010^T and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T differed morphologically. Cells of strain JW/IW010^T were approximately 0.5 µm in width and 2–5 µm in length; occasionally cells up to 15 µm in length were observed. Cells of *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T were much more variable, between 0.3–0.8 µm in diameter and 1.2–4.0 µm in length during exponential growth and, during stationary growth phase, the length of the cells increased up to 35 µm (Lee *et al.*, 2007a). Certain characteristics between strain JW/IW010^T and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T were similar; e.g. the measured DNA G + C content of 33.6 and 34.5 mol% (HPLC), respectively; cells of strains JW/IW010^T and JW/SL-NZ826^T stained Gram-negative; and the ability to use cellobiose, maltose, sucrose, xylose, galactose, mannitol and pyruvate for growth. However, these are characteristics held by many *Thermoanaerobacter* species. However, significant differences between strain JW/IW010^T and *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T include the optimum pH^{60 °C} for strain JW/IW010^T of 7.1 (Fig. 4b), whereas *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T had a broad optimum pH^{60 °C} of 5.0–6.5 (Lee *et al.*, 2007a). Under optimal conditions, the doubling times for strain JW/IW010^T and related strains were approximately 0.5 h to less than 1 h, and thus are notably faster than the doubling times reported for *Thermoanaerobacter sulfurigenens* JW/SL-NZ826^T as well as for strain JW/SL-NZ824 (2.4 h) (Lee *et al.*, 2007a). Metabolic differences between strains JW/IW010^T and *Thermoanaerobacter sulfurigenens*

Table 2. Differential characteristics of strain JW/IW010^T (*Thermoanaerobacter uzonensis* sp. nov.) and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T

+, Positive; –, negative.

Characteristic	Strain JW/IW010 ^T	Strain JW/SL-NZ826 ^T
Temperature optimum	61	63–67
pH ^{60 °C} optimum	7.1	5.0–6.5
Doubling time under optimal conditions (h)	0.5	2.4
Cell size (µm)	0.5 × 2–5; up to 15 in length	0.3–0.8 × 1.2–4.0; up to 35 in length
Growth with:		
Casamino acids	+	–
Tryptone	+	–
Starch	–	+
Pectin	–	+
Lactose	–	+
Methanol	–	+
Growth in:		
250 mM thiosulphate*	–	+
50 mM sulphite†	–	+
Production of H ₂ S from thiosulphate	+	–
Hydrolysis of:		
Amide bond of L-pyrrogluamic acid	–	+
Aryl-substituted glycoside of <i>p</i> -nitrophenyl	–	+
α-cellobiose		
Presence of:		
18:0	+	–
18:1ω7t	–	+
Production of lactic acid as a fermentation end product	Trace amounts	0.9 mol per mol glucose consumed

*Strain JW/IW010^T grew at thiosulphate concentrations up to 200 mM whereas *Thermoanaerobacter sulfurigignens* strain JW/SL-NZ826^T grew at thiosulphate concentrations up to 1 M.

†Strain JW/IW010^T grew at sulphite concentrations up to 40 mM whereas *Thermoanaerobacter sulfurigignens* grew at sulphite concentrations up to 90 mM sulphite (Lee *et al.*, 2007a).

JW/SL-NZ826^T included that strain JW/IW010^T utilized Casamino acids as well as tryptone whereas *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T utilized neither (Lee *et al.*, 2007a). *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T was also able to utilize both starch and lactose as growth substrates whereas strain JW/IW010^T could not (see Table 2). In addition, the thiosulphate and sulphite tolerance between strain JW/IW010^T and *Thermoanaerobacter sulfurigignens* strains JW/SL-NZ826^T and JW/SL-NZ824 is substantially different. Strain JW/IW010^T tolerated only up to 200 mM thiosulphate and 40 mM sulphite, whereas *Thermoanaerobacter sulfurigignens* exhibited a remarkable resistance to 1 M thiosulphate and 90 mM sulphite. Furthermore, strain JW/IW010^T reduced thiosulphate to hydrogen sulphide and also converted thiosulphate to sulphur, whereas *Thermoanaerobacter sulfurigignens* could convert thiosulphate to elemental sulphur without any detectable sulphide production (Lee *et al.*, 2007a). Differential characteristics of strain JW/IW010^T and *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T are summarized in Table 2.

In addition to strains JW/IW007, JW/IW015 and JW/HL-KA15, other isolates with a high similarity to strain JW/

IW010^T (≥98 % 16S rRNA gene sequence similarity) have been found in hot springs throughout the Uzon Caldera. When the 16S rRNA gene sequences of strain JW/IW010^T and similar strains (data not shown) were compared with the sequence of the phylogenetically closely related recognized taxon *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T, noteworthy insertions/deletions were observed. A 7 bp region (nucleotides 183–189 of strain JW/SL-NZ826^T 16S rRNA gene sequence) is present within the *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T 16S rRNA gene sequence, but is absent from the 16S rRNA gene sequence of strain JW/IW010^T and 14 similar isolates from the Uzon Caldera. An additional 4 bp region (nucleotides 1428–1431 of the 16S rRNA gene sequence of strain JW/SL-NZ826^T) is also present within the *Thermoanaerobacter sulfurigignens* JW/SL-NZ826^T 16S rRNA gene sequence, but is absent from the 16S rRNA gene sequence of strain JW/IW010^T and 14 similar isolates from the Uzon Caldera.

Thus, strain JW/IW010^T is phenotypically, chemotaxonomically and phylogenetically different from the most closely related recognized species, *Thermoanaerobacter sulfurigignens*. For these reasons, strain JW/IW010^T is

proposed to represent a novel species with the name *Thermoanaerobacter uzonensis* sp. nov. Strain 1501/60 was described by Krivenko *et al.* (1990) as the type strain of the effectively published, but not recognized, taxon '*Clostridium uzonii*', which herewith is included in the novel taxon *Thermoanaerobacter uzonensis* sp. nov.

Description of *Thermoanaerobacter uzonensis* sp. nov.

Thermoanaerobacter uzonensis (u.zo.nen'sis. N.L. masc. adj. *uzonensis* pertaining to the Uzon Caldera, Kamchatka, Far East Russia).

Cells are straight to slightly curved rods, approximately 0.5 µm in width and 2–5 µm, and occasionally up to 15 µm, in length. Oval, subterminal to terminal, mother cell-distending spores are observed, though rarely. Cells have peritrichously inserted flagella and are motile. Cells stain Gram-negative and are Gram-type positive (Wiegel, 1981). Within agar-shake-roll tubes, colonies are circular, 1–2 mm in diameter, slightly convex and creamy white in colour. When grown on plates of carbonate-buffered basal medium with 2.3 % agar within anaerobic jars at 60 °C, forms colourless, nearly transparent circular colonies up to 1 mm in diameter. Temperature range for growth is 32.5–69 °C with an optimum at 61 °C (no growth is observed at 30 °C or below, or at 72 °C or above). pH^{60 °C} range for growth is 4.2–8.9 with an optimum at pH^{60 °C} 7.1 (no growth occurs at or below pH^{60 °C} 3.9, or at 9.2 or above). Chemo-organotrophic. Yeast extract, peptone, Casamino acids and tryptone support growth. In the presence of 0.1 % (w/v) yeast extract, growth occurs in the presence of inulin, cellobiose, maltose, sucrose, glucose, fructose, galactose, mannose, xylose, trehalose, mannitol, pyruvate and crotonate. Growth is not observed with beef extract, casein, cellulose, xylan, starch, olive oil, pectin, lactose, arabinose, rhamnose, ribose, xylitol, sorbitol, methanol, glucuronic acid or glycerate. Thiosulphate is converted to sulphide and also elemental sulphur. The G+C content of the genomic DNA of the type strain is 33.6 mol% (SD=0.4; HPLC method). Major PLFAs are iso-15:0, 15:0, 16:0, 10-methyl 16:0 and anteiso-15:0.

The type strain, JW/IW010^T (=ATCC BAA-1464^T=DSM 18761^T), was isolated from a hot spring 2 m east of Pulsating Spring (54.5007° N 160.0076° E) in the Winding Creek area, on the northern side of the central portion of the Uzon East Thermal field, Kamchatka, Far East Russia. This species is apparently widespread throughout the Uzon Caldera in various hot springs. Additional strains are 1501/60 (DSM 9752) and JW/IW007, JW/IW015 and JW/HL-KA15 (available from the authors).

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