Caldanaerovirga acetigignens gen. nov., sp. nov., an anaerobic xylanolytic, alkalithermophilic bacterium isolated from Trego Hot Spring, Nevada, USA

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An anaerobic thermophilic bacterium, designated strain JW/SA-NV4^T, was isolated from a xylan-supplemented enrichment culture from Trego hot spring located within the Black Rock Desert (NV, USA). Cells were generally straight or slightly bent rod-shaped, 0.4–0.8 µm in width and 3–6 µm in length during exponential growth. Cells from stationary phase were variable in size and shape, showing curved or bent morphology. Motility was not seen and flagella were not observed in electron micrographs. Sporulation was not observed. Strain JW/SA-NV4^T stained Gram-negative but is phylogenetically Gram-type positive. Growth occurred at pH^{25 °C} 6.8–8.8, with optimum growth at pH 8.4; no growth occurred at pH 9.0 or above or at 6.5 or below. With glucose or xylose as the carbon source, strain JW/SA-NV4^T grew at 44–74 °C; no growth occurred at 76 °C or above or at 42 °C or below. However, the optimum temperature was 62 and 66 °C when grown on glucose and xylose, respectively. The shortest doubling time observed with glucose was approximately 4 h, and with xylose approximately 3.4 h. Strain JW/SA-NV4^T tolerated an atmosphere containing up to 0.1 % O₂; no growth occurred at a gas atmosphere of 0.2 % O₂. Chemo-organotrophic growth occurred with xylose, glucose, mannose, xylan, pyruvate, fructose, ribose, Casamino acids, mannitol, tryptone, peptone, cellobiose and yeast extract. When grown in mineral media containing 1 g yeast extract l⁻¹ as an electron donor, thiosulfate and sulfur were reduced to sulfide. The G + C content of the DNA was 38.6 mol% (HPLC). 16S rRNA gene sequence analysis placed strain JW/SA-NV4^T within the order *Thermoanaerobacterales* and within the *Thermoanaerobacterales* Incertae Sedis Family III, specifically between taxa classified within the genera *Thermosediminibacter* and *Thermovenabulum*. The closest phylogenetic neighbours were *Thermosediminibacter oceani* JW/IW-1228P^T (94.2 % 16S rRNA gene sequence similarity) and *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T (94.0 %) [Lee, Y.-J., Wagner, I. D., Brice, M. E., Kevbrin, V. V., Mills, G. L., Romanek, C. S. & Wiegel, J. (2005). *Extremophiles* **9**, 375–383]. Based on physiological and genotypic characteristics, strain JW/SA-NV4^T (=DSM 18802^T=ATCC BAA-1454^T) is proposed to represent the type strain of a novel species in a novel genus, *Caldanaerovirga acetigignens* gen. nov., sp. nov.

Abbreviation: PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JW/SA-NV4^T is EF530069.

The numerous hot springs distributed throughout northern Nevada, USA, are the result of the Pacific and North American tectonic plates colliding and the circulation of meteoric waters along Basin and Range faults (Hose & Taylor, 1974; Pearson *et al.*, 2004). In addition, several of the hot springs in northern Nevada are alkaline from the bleaching of carbonate or silica-bearing rocks by hot water. Alkaline hot springs with pH values above 8.5 are less common than slightly and strongly acidic hot springs and are studied less frequently. One consequence is that alkalithermophiles, *Bacteria* and *Archaea* that grow at elevated temperatures (optimum $>50^{\circ}\text{C}$) and high pH (optimum ≥ 8.5), have received less attention than the acidophilic or neutrophilic thermophiles (Kevbrin *et al.*, 2004; Wiegel, 1998). Here we report on the isolation of a moderately alkalithermophilic anaerobe growing on hemicellulosic material. Xylan is a component of plant hemicellulose and the second-most abundant renewable polysaccharide. Xylanolytic micro-organisms attract interest because the conversion of xylan to useful products could possibly be coupled to increasing the efficiency of processing lignocellulose (e.g. pulping processes) and to the production of energy from renewable resources (Biely, 1985; Himmel *et al.*, 2007). Xylan is widely used among thermophilic anaerobic *Bacteria*, especially among members of the *Firmicutes*, but is less studied in alkalithermophiles (Shao *et al.*, 1995; Wagner & Wiegel, 2008; Wiegel & Kevbrin, 2004).

Water and sediment samples were collected from hot springs of northern Nevada at various sites, including Trego hot spring ($40^{\circ}46'12''\text{ N } 119^{\circ}6'46''\text{ W}$; 46.8°C and pH 8.9). The samples were transferred to sterile anaerobic serum bottles, and sealed with butyl rubber stoppers. Enrichment cultures were prepared from these Nevada Hot Spring samples using an anaerobic carbonate- and TAPS-buffered medium containing (l^{-1}): 0.272 g KH_2PO_4 , 0.5 g

NH_4Cl , 0.5 g $(\text{NH}_4)_2\text{SO}_4$, 1.5 g NaHCO_3 , 2.43 g TAPS buffer, 0.02 g MgCl_2 , 0.015 g CaCl_2 , 1.0 ml vitamin solution and 1.0 ml $\text{Na}_2\text{SeO}_3\text{-Na}_2\text{WO}_3$ trace-element solution (Widdel & Bak, 1992) adjusted to either pH 8.5 or 9.5; 0.125 g cysteine HCl l^{-1} was added as a reducing agent. Xylose or xylan was added after the medium had been autoclaved to a final concentration of 0.3 or 3.0 g l^{-1} , respectively. Initial incubations were performed at 62°C . After 5–7 days of incubation, growth was observed within the pH 8.5 xylan- or xylose-supplemented enrichment cultures from Trego hot spring. Agar (2.15 % w/v) shake-roll tubes were prepared, and single colonies from dilution series were sequentially isolated to obtain pure cultures (Ljungdahl & Wiegel, 1986). Culture purity was checked by using phase-contrast microscopy and analysis of the 16S rRNA gene sequence. Strain JW/SA-NV4^T was obtained and chosen for further study. For subsequent comparisons (see 16S rRNA gene sequence analysis below), *Thermosediminibacter oceani* JW/IW-1228P^T and *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T were obtained from laboratory stocks.

Within agar shake-roll tubes, colonies of JW/SA-NV4^T were 0.1–1.5 mm in diameter and irregularly shaped. During exponential growth in a liquid medium, cells of strain JW/SA-NV4^T were uniformly straight or slightly bent rod-shaped, 0.4–0.8 μm in width and 3–6 μm in length. Short chains of two or three cells were frequently observed (Fig. 1a), but occasionally cells with lengths of 20–30 μm were observed during exponential growth. Cells from stationary phase cultures were variable in size and shape; most noticeably, cells were often curved or bent (Fig. 1b). Strain JW/SA-NV4^T stained Gram-negative at all growth phases but is phylogenetically Gram-type positive (Wiegel, 1981). The formation of spores was not observed under various conditions including during growth on glucose,

Fig. 1. Phase-contrast and electron micrographs of strain JW/SA-NV4^T. (a) Cells from exponential phase cultures; bar, 10 μm . (b) Cells from stationary phase cultures; bar, 10 μm . Cells from stationary phase cultures are more variable in size and shape compared with cells from exponential phase cultures, which are generally straight or slightly bent rod-shaped, 0.4–0.8 μm in width and 3–6 μm in length. (c) Electron micrograph of strain JW/SA-NV4^T; bar, 2 μm .

beef extract, yeast extract-containing agar slants. No viability was observed when cultures were heated to 100 °C for 2 min as usual for thermophilic spore-forming anaerobic *Firmicutes*. Motility was not observed in cells from liquid culture and electron micrographs did not reveal flagella (Fig. 1c).

Temperature profile, pH profile, NaCl tolerance and substrate range 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 JW/SA-NV4^T using the carbonate- and TAPS-buffered basal medium complemented with 1.0 g yeast extract l⁻¹ and either 3.0 g glucose l⁻¹ or 3.0 g xylose l⁻¹. With glucose as a provided carbon source (pH^{25 °C} 8.1), strain JW/SA-NV4^T grew between 44 and 74 °C, and optimally at about 62 °C; after more than 200 h of incubation, no growth was observed at or below 42 °C, or at 77 °C or above. With xylose as the provided carbon substrate (pH^{25 °C} 8.2), strain JW/SA-NV4^T grew between 46 and 76 °C, and optimally around 66 °C; after more than 300 h of incubation, no growth was observed at or below 44 °C, or at 78 °C or above. In medium containing 1.0 g yeast extract l⁻¹ and 3.0 g glucose l⁻¹ (pH^{25 °C} 8.1) or xylose (pH^{25 °C} 8.2), the shortest doubling time was approximately 4 h (glucose) and 3.4 h (xylose). To determine the pH profile of strain JW/SA-NV4^T, 3 g l⁻¹ glucose-containing media were buffered with 10 mM each of TES, TAPS and CAPS. Growth occurred between pH^{25 °C} 6.8 and 8.8, with optimum growth at pH 8.4; no growth occurred at pH 9.0 or above or at 6.5 or below. Strain JW/SA-NV4^T grew in medium containing up to 4.0 % (w/v) NaCl by successive transfer of the culture to media with increased NaCl concentrations; no growth was recorded at 4.5 % (w/v) NaCl or higher.

Positive utilization of a carbon substrate was recorded for strain JW/SA-NV4^T when ΔOD_{600} was twice that of cultures containing only the basal 0.5 g l⁻¹ concentration of yeast extract ($\Delta\text{OD}_{600}=0.015$). Strain JW/SA-NV4^T utilized xylose, glucose, mannose, xylan, pyruvate, fructose, ribose, cellobiose, Casamino acids, mannitol, tryptone, peptone and yeast extract. Strain JW/SA-NV4^T did not utilize sucrose, glycine betaine, galactose, lactose, xylitol, sorbitol, inositol, arabinose, starch or cellulose. Yeast extract was required for growth, with about 10 g l⁻¹ saturation in media containing 5 g xylose l⁻¹. Acetate was the major fermentation product of strain JW/SA-NV4^T when grown with glucose as the carbon substrate with 2.2–2.4 mol acetate produced per mol glucose consumed, suggesting a homoacetogenic fermentation, although a trace amount of lactate was occasionally detected (<0.1 mol lactate produced per mol glucose consumed; H₂ and CO₂ were not quantified) (Drake *et al.*, 2008). Fermentation products were similar when strain JW/SA-NV4^T was grown with either xylan or xylose.

Biochemical features of strain JW/SA-NV4^T, and phylogenetically related taxa (see 16S rRNA gene sequence

analysis below) *Thermosediminibacter oceani* JW/IW-1228P^T and *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T were tested using the API ZYM 25200 (bioMérieux) system (*Thermovenabulum ferriorganovororum* strain Z-9801^T is not available from DSMZ). For all three strains, strong positive reactions were observed for acid phosphatase and naphthol-AS-BI-phosphohydrolase. A strong α -glucosidase reaction was observed for *Thermosediminibacter oceani* JW/IW-1228P^T, but not for strain JW/SA-NV4^T or *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T (Table 1).

The potential for strain JW/SA-NV4^T to utilize various electron acceptors was studied using mineral media containing 1 g yeast extract l⁻¹ as an electron donor. Thiosulfate (15 mM) and sulfur (0.96 g l⁻¹) were reduced to sulfide, as determined by the formation of CuS upon the addition of CuSO₄. Sulfate (15 mM) and sulfite (5 mM) were not reduced to sulfide. To determine the tolerance of oxygen by strain JW/SA-NV4^T, 140 ml of the inoculated mineral media (containing 0.5 g yeast extract l⁻¹ and 1 g glucose l⁻¹) were incubated in 1 l Media Storage Bottles model 1395 (Pyrex) in the horizontal position. Media were prepared to be anoxic, by gassing with N₂ without reducing agents, and volumes of air were injected via syringes into the headspace. Liquid cultures of strain JW/SA-NV4^T tolerated an atmosphere containing up to 0.1 % O₂; no growth was observed with an atmosphere of 0.2 % O₂ or higher.

Lyophilized cell material was used to analyse the phospholipid fatty acid (PLFA) content of strain JW/SA-NV4^T. Lipid extraction was performed as described by Wagner *et al.* (2008) based on the protocols of Zhang *et al.* (2004) and White *et al.* (1979). GC-MS analyses of the fatty acid methyl esters were performed using an HP5890 GC equipped with a 30 m, HP5-MSI column and programmable temperature vaporizing inlet and coupled to a HP5972 mass spectrometer. The major PLFAs of strain JW/SA-NV4^T were i15:0 (46.8 %), a15:0 (26.7 %), 15:0 (6.3 %) and 16:0 (5.5 %) (Table 2).

For cell-wall analysis of strain JW/SA-NV4^T, peptidoglycan was isolated after disintegration of cells with glass beads in a Vibrogen cell mill (Edmund Bühler) and then subjected to a trypsin digestion according to the method of Schleifer & Seidl (1985). Strain JW/SA-NV4^T had a peptidoglycan type A1 γ (Schleifer & Kandler, 1972; P. Schumann, personal communication).

Genomic DNA for the analysis of the G+C content of strain JW/SA-NV4^T was obtained through a large-scale phenol/chloroform genomic DNA extraction, essentially as described by Wilson (1997), but without performing the final caesium chloride gradient purification step described. The DNA G+C content was measured by using the HPLC method of Mesbah *et al.* (1989), with S1 nuclease (Invitrogen) and 0.3 M sodium acetate (pH 5.0) modifications described by Lee *et al.* (2005). The DNA G+C content of strain JW/SA-NV4^T was 38.6 mol%; standard deviation, 0.4 ($n=4$).

Table 1. Morphological and physiological characteristics of strain JW/SA-NV4^T and close relatives

Strains: 1, JW/SA-NV4^T (*C. acetigignens* sp. nov.; data from this study); 2, *Thermosediminibacter oceani* JW/IW-1228P^T (Lee *et al.*, 2005; this study); 3, *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T (Lee *et al.*, 2005; this study); 4, *Thermovenabulum ferriorganovororum* Z-9801^T (Zavarzina *et al.*, 2002). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Isolation source	Terrestrial hot spring, Nevada, USA	Subseafloor, Peru Margin	Subseafloor, Peru Margin	Terrestrial hot spring, Uzon Caldera, Russian Far East
Cell size (μm)	0.4–0.8 × 3–6	0.2–0.7 × 1.5–16	0.3–0.5 × 2–10	0.5–0.6 × 1.5–7.0
Flagella	No flagella observed	Flagella observed	Retarded peritrichous flagella	Peritrichous flagella
Temperature range for growth (°C)	44–74	52–76	43–76	45–76
Optimum temperature (°C)	61	68	64	63–65
pH range for growth	6.8–8.8	6.3–9.3	5–9.5	4.8–8.2
Optimum pH	8.4	7.5	7.9–8.4	6.7–6.9
Salinity (% NaCl, w/v)	0–4.0	0–6.0	0–4.5	0–3.5
DNA G + C content (mol%)	38.6	46.3	45.2	36
Gram stain	–	–	–	+
Spores observed	–	–	–	+
α-Glucosidase	–	+	–	ND
Utilization of:				
Galactose	–	+	+	–
Glucose	+	+	+	–
Inositol	–	+	+	ND
Sorbitol	–	+	–	ND
Sucrose	–	+	+	+
Trehalose	ND	+	ND	ND
Xylitol	–	–	+	ND
16:1 PLFA	+	–	–	ND
18:0 PLFA	–	+	+	ND

Table 2. Phospholipid fatty acid contents (%) of strains JW/SA-NV4^T, JW/IW-1228P^T and JW/YJL-1230-7/2^T

Strains: 1, JW/SA-NV4^T (*C. acetigignens* sp. nov.; data from this study); 2, *Thermosediminibacter oceani* JW/IW-1228P^T (Lee *et al.*, 2005); 3, *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T (Lee *et al.*, 2005). ND, Not detected.

Fatty acid methyl ester	1	2	3
i13:0	2.7	ND	ND
14:0	3.0	1.8	1.7
i15:0	46.8	56.2	16.7
a15:0	26.7	6.7	3.8
15:0	6.3	5.0	2.6
i16:0	ND	1.9	1.6
16:1ω9c	ND	5.6	19.9
16:1	2.8	ND	ND
16:0	5.5	7.5	15.5
i17:0	2.4	9.6	4.5
17:0	ND	1.0	ND
18:2ω6c	ND	ND	1.4
18:1ω9c	3.9	3.3	20.3
18:1ω9t/18:1ω7c*	ND	ND	6.3
18:0	ND	1.5	5.7

*18:1ω9t/18:1ω7c were unresolved in this analysis.

Genomic DNA for amplification of the 16S rRNA gene sequence was extracted using an UltraClean DNA isolation kit (Mo Bio). PCRs were prepared with the bacterial domain-specific primer set 27F and 1492R (Lane, 1991), using GoTaq (Promega), and thermocycler conditions described previously by Lee *et al.* (2005). Amplification products were purified using a QIAquick PCR purification kit (Qiagen) and sequenced by Macrogen. Due to unexplained difficulties with the 27F sequencing reaction, an additional reverse sequencing reaction was performed using a modified Univ907 R primer (5'-CGTCAA-TTCCTTTGAGTTT-3') (Amann *et al.*, 1992; Loy *et al.*, 2007). Sequencing results were aligned using Sequencher v4.1.4 (Gene Codes). A multiple sequence alignment was created with CLUSTAL W (Thompson *et al.*, 1994) and subsequent phylogenetic analyses were performed using MEGA4 software (Tamura *et al.*, 2007). Phylogenetic trees (Fig. 2) were inferred by using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes and Cantor model (Jukes & Cantor, 1969). The 16S rRNA gene sequence of JW/SA-NV4^T was deposited at GenBank. Phylogenetic analyses placed strain JW/SA-NV4^T within the *Thermoanaerobacterales* Incertae Sedis Family III (Ludwig *et al.*, 2009) of the phylum *Firmicutes* (Fig. 2). The closest phylogenetic neighbours were the marine

Fig. 2. 16S rRNA gene sequence-based phylogenetic dendrogram of strain JW/SA-NV4^T and related taxa. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the Jukes and Cantor distance corrections (Jukes & Cantor, 1969). Numbers at nodes are bootstrap percentages based on 1000 replicates. Bar, 2 nucleotide substitutions per 100 nucleotides. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There was a total of 1836 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007).

Thermosediminibacter oceanii JW/IW-1228P^T (94.2 % gene sequence similarity) and *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T (94.0 %), both isolated from eastern equatorial Pacific sediment of the Peru Margin (Lee *et al.*, 2005), and *Thermovenabulum ferriorganovorum* Z-9801^T (90.3 %), isolated from a terrestrial hot spring in the Uzon Caldera, Kamchatka, Far East Russia (Zavarzina *et al.*, 2002).

Nearly all of the recognized anaerobic alkalithermophilic *Bacteria* belong to the *Firmicutes* (Kevbrin *et al.*, 2004; Wiegel, 1998); indeed, strain JW/SA-NV4^T belongs within this clade, specifically within the *Thermoanaerobacterales* Incertae Sedis Family III (Ludwig *et al.*, 2009). Within the *Thermoanaerobacterales* Incertae Sedis Family III, the genera *Thermoanaerobacterium*, *Caldicellulosiruptor* and *Caldanaerobius* form well-defined phylogenetic clades (Fig. 2). The other recognized genera of the *Thermoanaerobacterales* Incertae Sedis Family III include *Thermosediminibacter*, *Thermovenabulum* and *Tepidanaerobacter*. A hindrance to assigning a taxonomic affiliation for strain JW/SA-NV4^T is that at present there are only four recognized species classified within the three genera set of *Thermosediminibacter*, *Thermovenabulum* and *Tepidanaerobacter*. Naturally, as additional species are described within these genera, a more complete view of their phylogenetic relationships will emerge. Strain JW/SA-NV4^T had 94.2 % 16S rRNA gene sequence similarity to *Thermosediminibacter oceanii* JW/IW-1228P^T and 94.0 % similarity to *Thermosediminibacter litoriperuensis* JW/YJL-

1230-7/2^T. Although it is a topic of continued discussion, some microbial systematists regard distance values of 5 % as differentiation at the genus level (Schloss & Handelsman, 2004; references therein). The notion that strain JW/SA-NV4^T should be classified as a novel species within a novel genus is also supported by biochemical and physiological differences (Table 1). Thus, we propose that strain JW/SA-NV4^T belongs to a novel genus. The differences include the following properties. The *Thermosediminibacter* species have flagella (Lee *et al.*, 2005), whereas no flagella were observed for strain JW/SA-NV4^T. A small percentage of the cells in cultures of *Thermosediminibacter oceanii*, *Thermosediminibacter litoriperuensis* and *Thermovenabulum ferriorganovorum* exhibit a characteristic branched cell morphology (Lee *et al.*, 2005; Zavarzina *et al.*, 2002), which was not observed with cells of strain JW/SA-NV4^T. There are differences in the PLFA profiles of strain JW/SA-NV4^T and the *Thermosediminibacter* species. The 16:1 PLFA was detected for JW/SA-NV4^T, but not for either *Thermosediminibacter oceanii* JW/IW-1228P^T or *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T; the 18:0 PLFA was found within *Thermosediminibacter oceanii* JW/IW-1228P^T and *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T, but was not found in strain JW/SA-NV4^T; the a15:0 amount of 26.7 % was found for strain JW/SA-NV4^T, with much lower values of 6.7 and 3.8 % for *Thermosediminibacter oceanii* JW/IW-1228P^T and *Thermosediminibacter litoriperuensis* JW/YJL-1230-7/2^T, respectively (Table 2). There are also differences in the utilization of carbon substrates, e.g., both *Thermosediminibacter* species

can utilize galactose and sucrose, whereas strain JW/SA-NV4^T cannot use either. The isolation source of strain JW/SA-NV4^T was terrestrial hot spring whereas all the *Thermosediminibacter* strains were derived from marine sediment (Lee *et al.*, 2005). The difference in the G+C content of the genomic DNA of strain JW/SA-NV4^T and the *Thermosediminibacter* species is approximately 7 mol%.

Description of *Caldanaerovirga* gen. nov.

Caldanaerovirga (Cal.da.na.e.ro.vir'ga. L. adj. *caldus* hot; Gr. pref. *an* not; Gr. n. *aer* air; L. fem. n. *virga* rod; N.L. fem. n. *Caldanaerovirga* rod that grows in the absence of air at elevated temperatures).

Anaerobic thermophilic, Gram-type positive. Cell wall has a peptidoglycan type A1 γ . Belongs to the class *Clostridia*. The DNA G+C content is around 38 mol%. The type species is *Caldanaerovirga acetigignens*.

Description of *Caldanaerovirga acetigignens* sp. nov.

Caldanaerovirga acetigignens (a.ce.ti.gig'nens. L. n. *acetum* vinegar; L. v. *gignere* to produce; N.L. part. adj. *acetigignens* vinegar- or acetic acid-producing).

Exhibits the following properties in addition to those given in the genus description. During exponential growth, cells are straight or slightly bent rods of 0.4–0.8 μ m in width and 3–6 μ m in length. Cells from stationary phase are more variable in size and shape, showing a more expressed curved or bent morphology. Neither flagella or motility nor sporulation are observed. Cells stain Gram-negative but are phylogenetically Gram-type positive. Growth with glucose as carbon and energy source occurs between pH^{25 °C} 6.8 and 8.8 (optimum, pH 8.4); no growth occurs after 200 h of incubation at pH 9.0 or above, or at 6.5 or below. Grows at 44–74 °C (glucose); no growth occurs after 300 h of incubation at 76 °C or above, or at 42 °C or below. Optimum temperature for growth is around 62 and 66 °C when grown on glucose and xylose, respectively. The shortest doubling time observed was approximately 4 h (glucose) and 3.4 h (xylose). Chemo-organotroph. Positive for utilization of xylose, glucose, mannose, xylan, pyruvate, fructose, ribose, Casamino acids, mannitol, tryptone, peptone, cellobiose and yeast extract. Acetate (2.2–2.4 mol/glucose) is the major fermentation product when grown with glucose suggesting a homoacetogenic fermentation. Yeast extract is required for growth. Does not utilize sucrose, glycine betaine, galactose, lactose, xylitol, sorbitol, inositol, arabinose, starch or cellulose. Using mineral media containing 1 g yeast extract l⁻¹ as an electron donor, thiosulfate and sulfur, but not sulfate or sulfite, are reduced to sulfide. Growth occurs in the presence of an atmosphere containing 0.1 % O₂ but not 0.2 %. Positive for acid phosphatase and naphthol-AS-BI-phosphohydrolase. Major PLFAs of strain JW/SA-NV4^T are i15:0, a15:0,

15:0 and 16:0. The G+C content of the DNA of the type strain is 38.6 mol%; standard deviation 0.4 (HPLC).

The type strain, JW/SA-NV4^T (=DSM 18802^T=ATCC BAA-1454^T), was isolated from Trego hot spring (40°46'12" N 119°6'46" W), Black Rock Desert, north-western Nevada, USA.

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