Members of this monophyletic cluster were previously assigned to the family "*Thermoanaerobacteriaceae*" by Garrity et al. (2005). Because the current outline proposes that *Thermoanaerobacter* and not *Thermoanaerobacterium* is the type of the, now, *Thermoanaerobacteraceae* fam. nov., the taxonomic status of these genera is ambiguous. Although classified within a single family *incertae sedis*, the evidence for the relationship between *Thermoanaerobacterium* and the other members of this group is not strong. Thus, subsequent analyses may suggest reorganization.

Genus I. **Caldicellulosiruptor** Rainey, Donnison, Janssen, Saul, Rodrigo, Bergquisy, Daniel, Stackebrandt and Morgan 1995, 197^{VP} (Effective publication: Rainey, Donnison, Janssen, Saul, Rodrigo, Bergquist, Daniel, Stackebrandt and Morgan 1994, 264.) emend. Onyenwoke, Lee, Dabrowski, Ahring and Wiegel 2006, 1394

Fred A. Rainey

Cal.di.cel.lu.lo.si.rup'tor. L. adj. *caldus* hot; N.L. n. *cellulosum* cellulose; L. masc. n. *ruptor* breaker; N.L. masc. n. *Caldicellulosiruptor* cellulose-breaker living under hot conditions.

Cells are straight **rods. Gram-stain-negative.** Endospores not observed. Growth not obtained after heat treatment at 115 °C for 20 min. Growth **strictly anaerobic**, occurring over the temperature range of 45–82 °C with optima in the range of 65–75 °C. No growth detected below 45 °C or above 82 °C. Monosaccharides, disaccharides and polysaccharides serve as fermentable substrates. **Cellulose is hydrolyzed by most strains.**

DNA G+C content (mol %): 35–37.5 (T_m, HPLC).

Type species: **Caldicellulosiruptor saccharolyticus** Rainey, Donnison, Janssen, Saul, Rodrigo, Bergquisy, Daniel, Stackebrandt and Morgan 1995, 197^{VP} (Effective publication: Rainey, Donnison, Janssen, Saul, Rodrigo, Bergquist, Daniel, Stackebrandt and Morgan 1994, 265.)

Further descriptive information

The genus *Caldicellulosiruptor* contains five species: *Caldicellulosiruptor saccharolyticus* (Rainey et al., 1994) *Caldicellulosiruptor acetigenus* (Onyenwoke et al., 2006), *Caldicellulosiruptor kristjanssonii* (Bredholt et al., 1999), *Caldicellulosiruptor lactoaceticus* (Mladenovska et al., 1995) and *Caldicellulosiruptor owensensis* (Huang et al., 1998). All strains are rod-shaped, varying in length from 1.5 to 9.4 µm and in diameter from 0.4 to 1.0 µm. Cells have rounded ends and occur singly, in pairs or in short chains. In the case of *Caldicellulosiruptor acetigenus* chains of up to eight cells have been observed (Nielsen et al., 1993). During the exponential-phase, *Caldicellulosiruptor owensensis* cultures contain small coccoid cells (Huang et al., 1998). Although this genus falls within the radiation of the low G+C Gram-positive taxa (*Firmicutes*), all strains tested stain Gram-negative. A Gram-positive cell-wall structure based on electron microscopy studies has been shown in *Caldicellulosiruptor owensensis* (Huang et al., 1998) and *Caldicellulosiruptor acetigenus* (Nielsen et al., 1993; Onyenwoke et al., 2006). Flagella have been observed in two of the five species of this genus; *Caldicellulosiruptor kristjanssonii* is nonmotile, but peritrichous flagella are observed in exponential-phase cultures and two subterminal flagella were seen in old cultures (Bredholt et al., 1999). Although motility has not been observed, young cells of *Caldicellulosiruptor owensensis* have lophotrichous flagella (Huang et al., 1998). One of the main phenotypic characteristics differentiating the cellulose-degrading species of *Caldicellulosiruptor* from cellulolytic species of the genus *Clostridium* is the absence of endospores in species of *Caldicellulosiruptor*. Endospores or similar heat resistant structures have not been observed in any species of the genus *Caldicellulosiruptor* and in some cases this has been confirmed by heat treatments such as boiling for 5 min. *Caldicellulosiruptor kristjanssonii* (Bredholt et al., 1999) and *Caldicellulosiruptor acetigenus* (Nielsen et al., 1993), or heating culture to 115 °C for 20 min., *Caldicellulosiruptor saccharolyticus* (Rainey et al., 1994). Sphaeroplasts have been observed in *Caldicellulosiruptor acetigenus* when it was grown on minimal salts media and xylose (Nielsen et al., 1993). Colony descriptions have been provided for all species of the genus *Caldicellulosiruptor*, but growth on different media and under different conditions makes these characteristics of little comparative value. Colonies of *Caldicellulosiruptor saccharolyticus* grown on 2/1 cellobiose agar are 2–5 mm in diameter, cream-colored, and umbonate (Rainey et al., 1994). *Caldicellulosiruptor acetigenus* on xylan containing agar forms colonies that are off white, milky-colored and have clearing zones around them of up to 2 cm in diameter (Nielsen et al., 1993); after 7 d incubation in agar roll tubes surface colonies of *Caldicellulosiruptor lactoaceticus* are circular, white, opalescent, 1 mm in diameter with an entire edge (Mladenovska et al., 1995). *Caldicellulosiruptor owensensis* forms circular, convex, opaque, yellowish colonies with smooth edges that are up to 2 mm in diameter (Huang et al., 1998). Colonies of *Caldicellulosiruptor kristjanssonii* in roll tubes containing Avicel (microcrystalline cellulose) are 0.5–1.0 mm in diameter, flat with fringed edges and after 9–14 d incubation show 2–4 mm zones of clearing (Bredholt et al., 1999).

The genus *Caldicellulosiruptor* can be differentiated from the thermophilic, cellulolytic *Clostridium* species (*Clostridium stercorarium, Clostridium thermocellum* and *Clostridium thermocopriae*) and related strains based on maximum growth temperature. In the numerical taxonomy study of Rainey et al. (1993a) growth at 75 °C and at 80 °C was determined for 51 strains, 43 of which did not form endospores and clustered separately from the eight spore-forming strains (including the type strains of the species *Clostridium stercorarium, Clostridium thermocellum* and *Clostridium thermocopriae*). The strains falling in clusters A–D are considered to be *Caldicellulosiruptor* strains (including the strains Tp8T 6331^T, COMP. B1, Wai35. B1, RI2. B1, Z-1203, Rt8. B7, Rt8. B15, and Ok9. B1) and all grow at 75 °C and ~70% of them at 80 °C, in contrast to the endospore forming strains, 60% of which grow at 75 °C and none at 80 °C (Rainey et al., 1993a). Growth of the described species of the genus *Caldicellulosiruptor* is in the temperature range of 45–82 °C with optima

in the range of 65–78 °C (see species descriptions below). *Caldicellulosiruptor kristjanssonii* has both the highest temperature maximum for growth at 82 °C and optimum for growth at 78 °C (Bredholt et al., 1999). *Caldicellulosiruptor* species are neutrophiles growing optimally at pH ~7.0. The pH range for growth of the species of this genus is 5.2–9.0 (see species descriptions below). *Caldicellulosiruptor owenensis* is described as an alkalitolerant species growing up to pH 9.0 (Huang et al., 1998).

All species of the genus are obligately anaerobic chemoorganotrophs with fermentative metabolism. Monosaccharides, disaccharides, sugar alcohols, and polysaccharides serve as fermentable substrates. The species of the genus can be differentiated based on substrate utilization patterns (Table 252). *Caldicellulosiruptor lactoaceticus* uses a limited number of substrates including Avicel, starch and xylan (Table 252, Mladenovska et al., 1995). Growth of the species *Caldicellulosiruptor saccharolyticus, Caldicellulosiruptor lactoaceticus* and *Caldicellulosiruptor kristjanssonii* is supported by Avicel, starch and xylan (Bredholt et al., 1999; Mladenovska et al., 1995; Rainey et al., 1994). *Caldicellulosiruptor owensensis* also grows on starch, xylan and cellulose but the type of cellulose tested is not provided, therefore the ability of this species to degrade crystalline cellulose is undetermined (Huang et al., 1998). *Caldicellulosiruptor acetigenus* is the only species of the genus *Caldicellulosiruptor* that does not degrade cellulose either filter paper or Avicel, but does display cellulase enzyme activity when grown on carboxymethylcellulose (Nielsen et al., 1993; Onyenwoke et al., 2006). The end products of fermentation of sugars for the species of the genus include acetate, ethanol, CO_2 and H_2. Lactate is produced as the main product of fermentation by *Caldicellulosiruptor lactoaceticus* and in smaller amounts by *Caldicellulosiruptor saccharolyticus, Caldicellulosiruptor owenensis* and *Caldicellulosiruptor kristjanssonii*. *Caldicellulosiruptor acetigenus* does not produce lactate as an end product of fermentation, but does produce trace amounts of iso-butyrate (Nielsen et al., 1993; Onyenwoke et al., 2006).

The G+C content is in the range of 35.0–37.5 mol% for the type strains of the species of the genus *Caldicellulosiruptor*. The values for the species *Caldicellulosiruptor acetigenus, Caldicellulosiruptor lactoaceticus* and *Caldicellulosiruptor kristjanssonii* were determined by the HPLC method while those of *Caldicellulosiruptor saccharolyticus* and *Caldicellulosiruptor owenensis* were determined by the thermal denaturation and buoyant gradient density methods respectively.

Enrichment, isolation and growth conditions

Four of the five species of the genus *Caldicellulosiruptor* have been isolated from thermal springs or material associated with thermal springs, the exception being *Caldicellulosiruptor owensensis* which was isolated from a freshwater pond with a temperature of 32 °C in the dry lake bed of Owens Lake, California, USA. Species of this genus can be isolated using anaerobic techniques with xylan or cellulose as substrates in enrichment cultures. The roll tube method (Hungate, 1969) can be used for the isolation of single colonies that give zones of clearing in the xylan- or cellulose-containing agar. *Caldicellulosiruptor saccharolyticus* strain Tp8T 6331^T was isolated from the decomposed end of a *Pinus radiata* plank in the downstream flow of a thermal spring TP10, Taupo, New Zealand (Sissons et al., 1987). Thermal spring TP10 had a temperature of 78 °C and the downstream flow at which the pine wood plank was located (TP8) had a temperature of 48 °C (Sissons et al., 1987). Strain Tp8T 6331^T was isolated from the homogenized wood sample inoculated into basal salts medium containing 0.5% MN 300 cellulose and subsequent serial dilution with cellobiose (Sissons et al., 1987). A cellulose-clearing colony was selected and further purified on cellobiose agar and liquid cultures containing cellobiose and Sigmacell 50 (Sissons et al., 1987). *Caldicellulosiruptor acetigenus* was originally isolated as a xylanolytic, non-spore-forming bacterium, and described as *Thermoanaerobium acetigenum* (Nielsen et al., 1993) before being transferred to the genus *Caldicellulosiruptor* (Onyenwoke et al., 2006). The type strain XB6^T of *Caldicellulosiruptor acetigenus* was isolated from sediment and biomat collected from a thermal spring with

TABLE 252. Substrate utilizations differentiating species of the genus *Caldicellulosiruptor*[a]

Substrate	*C. saccharolyticus*[b]	*C. acetigenus*[c]	*C. kristjanssonii*[d]	*C. lactoaceticus*[e]	*C. owensensis*[f]
Arabinose	+	+	–	–	+
Fructose	+	+	+	–	+
Glucose	+	+	+	–	+
Galactose	+	+	+	–	+
Ribose	–	ND	–	–	+
Sucrose	+	+	+	–	+
Raffinose	–	+	–	–	+
Trehalose	+	+	+	–	–
Inositol	–	ND	ND	ND	+
Mannitol	–	ND	–	–	+
Cellulose	+	–	+	+	+
Xylan	+	+	+	+	+

[a]ND, Not determined.
[b]Data from Rainey et al. (1994).
[c]Data from Nielsen et al. (1993) and Onyenwoke et al. (2006).
[d]Data from Bredholt et al. (1999).
[e]Data from Mladenovska et al. (1995).
[f]Data from Huang et al. (1998).

a temperature of 70 °C and a pH of ~8.5 in the Hverðagerdi-Hengill geothermal area, Iceland (Nielsen et al., 1993). The enrichment cultures were established at 68 °C in mineral medium at pH 7.0 containing 4 g/l of beech wood xylan and 10% (v/v) of sediment as an inoculum (Nielsen et al., 1993). Enrichments showing solublization of xylan, and over pressure, were transferred to fresh medium several times before pure cultures were isolated on xylan containing media solidified with Gelrite using the roll tube technique (Nielsen et al., 1993). The isolation of *Caldicellulosiruptor kristjanssonii* followed the same techniques used in the isolation of *Caldicellulosiruptor acetigenus*, except that xylan was replaced with Avicel. The inoculum was a biomat from a hot spring at pH 8.7 and the enrichment was established at 78 °C (Bredholt et al., 1999). Avicel was also used as the substrate in the enrichment culture from which *Caldicellulosiruptor lactoaceticus* was isolated (Mladenovska et al., 1995). The isolation source of *Caldicellulosiruptor owensensis* indicated that species of this genus can be isolated from non-thermal sources. *Caldicellulosiruptor owensensis* was isolated from sediment samples (pH 9.0 and 32 °C) collected from a small freshwater pond in the dry bed of Owens Lake (Huang et al., 1998). The enrichment temperature of 75 °C selected for thermophilic organisms and xylan added to the carbonate buffered culture medium selected for species that were xylanolytic (Huang et al., 1998).

Taxonomic comments

It is the lack of spore formation and ability to grow at >75 °C that differentiated these thermophilic cellulolytic bacteria from the spore-forming, thermophilic, cellulolytic species of the genus *Clostridium*. The numerical taxonomy study of Rainey et al. (1993a) involved the determination of 92 characteristics for 51 strains, 43 that did not form endospores and eight spore-forming strains (including the type strains of the species *Clostridium stercorarium, Clostridium thermocellum* and *Clostridium thermocopriae*). At the 72% similarity level in a S_{SM} unweighted pair-group method with averages (UPGMA) analysis, five clusters (designated A–E) were formed (Rainey et al., 1993a). The thermophilic, cellulolytic *Clostridium* species all fell into cluster E, while the non-spore-forming strains that grew at 75 °C or above fell into clusters A–D. This study demonstrated that at the phenotypic level the non-spore-forming, cellulolytic, thermophiles could be differentiated from *Clostridium* species. Phylogenetic analysis based on partial 16S rRNA gene sequences of 16 of the strains from clusters A through D grouped them separately from the cellulolytic thermophilic species of the genus *Clostridium* (Rainey et al., 1993a). Comparison of seven full 16S rRNA gene sequences of strains from clusters A through D with other thermophilic anaerobic bacteria demonstrated that they warranted novel genus status (Rainey et al., 1993b). The genus *Caldicellulosiruptor* was described for the strain Tp8T 6331 with the species *Caldicellulosiruptor saccharolyticus* designated the type species (Rainey et al., 1994). Since then four additional species have been added to the genus based on 16S rRNA gene sequence comparisons, phenotypic differences and DNA–DNA hybridization studies (Bredholt et al., 1999; Huang et al., 1998; Mladenovska et al., 1995; Nielsen et al., 1993; Onyenwoke et al., 2006). Figure 256 shows the phylogenetic relationships of the type strains of the species of *Caldicellulosiruptor*, as well as a number of undescribed strains for which full 16S rRNA gene sequences are available. The 16S rRNA gene sequence similarities between the described species of *Caldicellulosiruptor* are in the range of 95.5–99.6, with the most closely related species being *Caldicellulosiruptor kristjanssonii* and *Caldicellulosiruptor acetigenus* (clone 1). These two species share 99.6% 16S rRNA gene sequence similarity, but it should be noted that the type strain of the species *Caldicellulosiruptor acetigenus* contains two copies of the 16S rRNA gene which share only 98.87% similarity (Onyenwoke et al., 2006). This considerably large difference between the two gene copies results in the16S rRNA gene copy designated clone 2 (AY772477) being more similar (99.43%) to the type strain of *Caldicellulosiruptor lactoaceticus* than to the other 16S rRNA gene copy designated clone 1 (AY772476) (98.78% similarity). DNA–DNA hybridization studies demonstrated the true species status of *Caldicellulosiruptor acetigenus* when compared to *Caldicellulosiruptor kristjanssonii, Caldicellulosiruptor lactoaceticus* and *Caldicellulosiruptor saccharolyticus*, with reassociation values of 53.1, 50.9 and 34.3 respectively (Onyenwoke et al., 2006). The inclusion of the additional strains of the genus for which full 16S rRNA gene sequences are available (Figure 256) clearly demonstrates the species status of some of these strains including those not validly named, "*Anerocellum thermophilum*" Z-1203 (Svetlichnyi et al., 1990) and "*Thermoanaerobacter cellulolyticus*" NA10 (Taya et al., 1988, 1985).

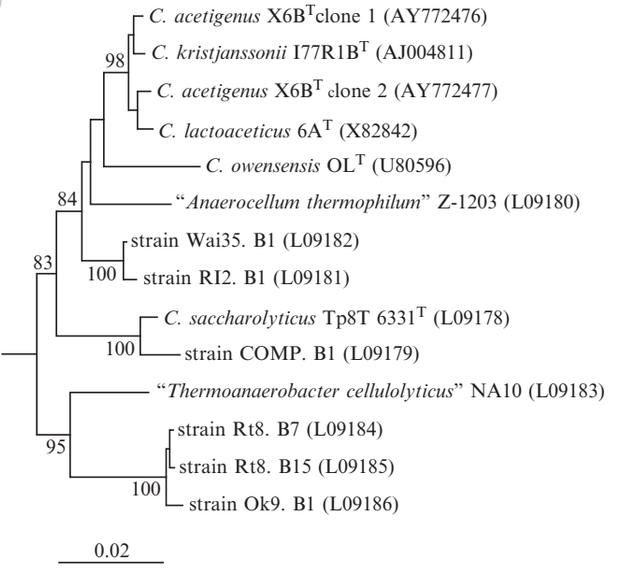

FIGURE 256. 16S rRNA based phylogeny of the species and strains of the genus *Caldicellulosiruptor*. The tree was constructed from distance matrices using the neighbor-joining method. The scale bar represents 2 nucleotide substitutions per 100 nucleotides.

1. **Caldicellulosiruptor saccharolyticus** Rainey, Donnison, Janssen, Saul, Rodrigo, Bergquisy, Daniel, Stackebrandt and Morgan 1995, 197^{VP} (Effective publication: Rainey, Donnison, Janssen, Saul, Rodrigo, Bergquist, Daniel, Stackebrandt and Morgan 1994, 265.)
sac.cha.ro.ly′ti.cum Gr. n. *sakkharon* sugar; Gr. adj. *lytikos* able to loosen; N. L. masc. adj. *saccharolyticus* breaking up polysaccharides.

   Cells are straight rods 0.4–0.6 µm in diameter and 3.0–4.0 µm in length, occurring both singly and in pairs. Endospores are not formed. On 2/1 cellobiose agar colonies are 2–5 mm in diameter, cream-colored and umbonate. The optimum temperature for growth is 70 °C in the range of 45–80 °C. The pH range is 5.5–8.0, with an optimum at pH 7.0. The main fermentation products on 2/1 cellobiose medium are acetate and lactate with trace amounts of ethanol. Hydrogen sulfide is not produced. Acid is produced from arabinose, amorphous cellulose, Avicel, cellobiose, fructose, galactose, glucose, glycogen, gum guar, gum locust bean, lactose, laminarin, lichenin, maltose, mannose, pullulan, pectin, rhamnose, Sigmacell 20, Sigmacell 50, Sigmacell 100, starch, sucrose, xylan, and xylose, Acid is not produced from amygdalin, erythritol, glycerol, inositol, inulin, mannitol, melibiose, melezitose, raffinose, ribose, sorbitol, or sorbose. Culture supernatants hydrolyse carboxymethylcelluose, gum guar, gum locust bean, laminarin, lichenin, pullulan, starch, and xylan, but not arabinoglactan, chitin, glycogen, gum karaya, inulin, or mannan. Growth is inhibited by penicillin, neomycin and polymyxin B at 10ug/ml and by novobiocin at 100 µg/ml. Rifampin is not inhibitory at 1000 µg/ml. *Caldicellulosiruptor saccharolyticus was* isolated from pinewood in the flow of a geothermal spring, Taupo, New Zealand.
DNA G+C content (mol%): 37.5 (T_m).
Type strain: Tp8T 6331, ATCC 43494, DSM 8903.
GenBank accession number (16S rRNA gene): L09178.

2. **Caldicellulosiruptor acetigenus** (Nielsen, Mathrani and Ahring 1993) Onyenwoke, Lee, Dabrowski, Ahring and Wiegel 2006, 1394^{VP} (*Thermoanaerobium acetigenum* Nielsen, Mathrani and Ahring 1993, 464)
a.ce.ti.ge′nus. L. n. *acetum* vinegar; L. v. *genere gignere* to produce; N.L. masc. adj. *acetigenus* vinegar- or acetic acid-producing.

   Cells are rod-shaped, 0.7–1.0 µm in diameter and 3.6–5.9 µm in length, occurring singly, in pairs or in chains of up to eight cells. *Caldicellulosiruptor acetigenus* strains are Gram-stain-negative, but have a Gram-positive cell-wall structure. On solidified xylan-containing medium, off-white, milky-colored colonies are observed. It is a strictly anaerobic chemoorganoheterotroph.

   The optimum temperature for growth is 65–68 °C in the range of 50–78 °C. The pH range is 5.2–8.6, with an optimum at pH 7.0. Doubling time under optimal conditions is approximately 4 h. Growth is supported by arabinose, cellobiose, fructose, D-galactose, D-glucose, lactose, maltose, mannose, raffinose, soluble starch, sucrose, trehalose, D-xylose, and xylan. Growth and CMC-cellulase activity is observed when grown on carboxymethylcellulose (Hercules CMC, 7LT, or 7M) in the presence of traces of yeast extract, but not with filter paper or crystalline cellulose (Avicel). The end products of growth on glucose or D-xylose include acetate, CO_2, H_2, ethanol and traces of isobutyric acid (but not lactate). Isolated from a combined biomat and sediment sample from a slightly alkaline hot spring at Hverðagerdi, Iceland.
DNA G+C content (mol%): 35.7 (HPLC).
Type strain: X6B, ATCC BAA-1149, DSM 7040.
GenBank accession number (16S rRNA gene): AY772476, AY772477.

3. **Caldicellulosiruptor kristjanssonii** Bredholt, Sonne-Hansen, Nielsen, Mathrani and Ahring. 1999, 995^{VP}
kris.tjans.son′ii. N.L. gen. n. *kristjanssonii* named after Jakob K. Kristjansson.

   Cells are rod-shaped with rounded ends, 0.7–1.0 µm in diameter and 2.8–9.4 µm in length, occurring singly, in pairs or in short chains, and are Gram-stain-negative. Endospores are not found. Motility is not observed, but two subterminal flagella are present. When grown in roll-tubes on mineral medium plus Avicel, colonies are flat, cream in color, with a fringed edge. After 9–14 d, clearing zones around colonies are 2–4 mm. The optimum temperature for growth is 78 °C with a range of 45–82 °C. The pH range is 5.8–8.0, with an optimum at pH 7.0. Obligately anaerobic chemoorganotroph. Growth is supported by Avicel, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, lactose, maltose, mannose, pectin, salicin, soluble starch, sucrose, trehalose, xylan, and xylose. It does not utilize D-ribose, L-arabinose, esculin, glycerol, inulin, lactic acid, mannitol, pyruvate, raffinose, L-rhamnose, D-ribose, sorbitol, casein peptone, or yeast extract. Growth is inhibited by air, chloramphenicol, neomycin, penicillin, streptomycin, tetracycline, and vancomycin. The major end products of growth are acetic acid, H_2 and CO_2, with smaller amounts of lactic acid and ethanol and trace amounts of formic acid. Hydrogen sulfide is not produced from sulfate, thiosulfate, or casein peptone. *Caldicellulosiruptor kristjanssonii* was isolated from microbial mats and cellulosic materials, e.g., wood or straw, in Icelandic thermal and slightly alkaline springs.
DNA G+C content (mol%): 35.0 (HPLC).
Type strain: I77R1B, ATCC 700853, DSM 12137.
GenBank accession number (16S rRNA gene): AJ004811.

4. **Caldicellulosiruptor lactoaceticus** Mladenovska, Mathrani and Ahring 1997, 1274^{VP} (Effective publication: Mladenovska, Mathrani and Ahring 1995, 229.)
lac.to.acet.i cus. N.L. adj. *lacticum* lactic; N.L. adj. *aceticum* acetic. N.L. *lactoaceticum*, neut adj. referring to production of lactic acids as major fermentation products.

   Cells are Gram-stain-negative, rod-shaped with rounded ends, 0.7 µm in diameter and 1.5–3.5 µm in length, occurring singly, in pairs or in short chains. Endospores are not formed. Cells are non–motile and flagella are not observed. Colonies are 1 mm in diameter, round, white, opalescent and circular with an entire edge after incubation in agar roll tubes for approximately 1 week. The optimum temperature for growth is 68 °C with a range of 50–78 °C. The pH range is >5.6–<9.0, with an optimum at pH 7.0. Cells are obligately anaerobic and chemoorganoheterotrophic. Growth occurs with Avicel, cellobiose, lactose, maltose, pectin, soluble starch, xylan,

and xylose. No growth occurs on acetate, arabinose, arbutin, casein peptone, dextran, ethanol, fructose, galactose, glucose, lactate, mannitol, mannose, methanol, pyruvate, D-ribose, raffinose, rhamnose, sorbitol, sucrose, trehalose, yeast extract, or $H_2 + CO_2$. Fermentation products are lactate and acetate with small amounts of ethanol, CO_2, and H_2 are produced. Sulfate, thiosulfate, and sulfite are not reduced. Nitrate does not stimulate growth. No growth occurs in the presence of ampicillin, chloramphenicol, kanamycin, neomycin, streptomycin sulfate, penicillin V, tetracycline, or vancomycin (at 10 µg/ml). Cells are resistant to D-cycloserine (100 µg/ml). *Caldicellulosiruptor lactoaceticus* was isolated from microbial mats and cellulosic materials, e.g., wood or straw, in slightly alkaline Icelandic thermal springs.

DNA G+C content (mol%): 35.2 (HPLC).

Type strain: 6A, DSM 9545.

GenBank accession number (16S rRNA gene): X82842.

5. **Caldicellulosiruptor owensensis** Huang, Patel, Mah and Baresi 1998, 95[VP]

o.wen.sen′sis. N. L. adj. *owensensis* from Owens Lake, CA, USA.

Cells are rod-shaped, 0.5–0.8 µm in diameter and 2.0–5.0 µm in length, occurring singly, in pairs or in chains and arenonmotile, but have lophotrichous flagella. Gram-stain-negative. Endospores are not formed. Colonies are <2 mm in diameter, circular with smooth edges, convex, opaque and yellowish.

The optimum temperature for growth is 75 °C with a range of 50–80 °C. The pH range is 5.5–9.0, with an optimum at pH 7.5. Cells are alkali tolerant. Yeast extract or vitamin solutions are not required for growth. Growth is inhibited by penicillin G, streptomycin, chloramphenicol, and ampicillin at 100 µg/ml. Cells are resistant to D-cycloserine (100 µg/ml), erythromycin (200 µg ml/l) and tetracycline (100 µg/ml). Growth is strictly anaerobic. Chemoorganotrophic. Growth occurs with arabinose, cellobiose, cellulose, dextrin, fructose, galactose, glucose, glycogen, inositol, lactose, mannitol, maltose, mannose, pectin, raffinose, rhamnose, ribose, starch, sucrose, tagatose, xylan, xylose, and yeast extract. It does not grow on acetate, amygdalin, arbutin, erythritol, glycerol, lactate, melezitose, melibiose, methanol, pyruvate, sorbitol, trehalose, trypticase peptone, or $H_2 + CO_2$. The end products from glucose fermentation are acetate, lactate, ethanol, H_2, and CO_2. Cells do not reduce nitrate, sulfate, sulfite or thiosulfate. *Caldicellulosiruptor owenensis* was isolated from the Owens Lake in California, USA.

DNA G+C content (mol%): 36.6 (B_d).

Type strain: OL, ATCC 700167, DSM 13100.

GenBank accession number (16S rRNA gene): U80596.

Genus II. **Thermoanaerobacterium** Lee, Jain, Lee, Lowe and Zeikus 1993a, 48[VP]

ROB U. ONYENWOKE AND JUERGEN WIEGEL

Ther.mo.an.ae.ro.bac′te.ri.um. Gr. n. *thermos* hot, Gr. pref. *an* not, Gr. n. *aer* air, Gr. n. *bakterion* a small rod, N.L. neut. n. *Thermoanaerobacterium* a rod which grows in the absence of air at high temperatures.

Extreme thermophiles with growth temperature optima between 55 and 70 °C. Cells have **Gram-positive cell-wall structure**, but many strains are **Gram-stain-negative.** Cells are rod-shaped and motile by peritrichously inserted flagella, except for *Thermoanaerobacterium aciditolerans*, which has a single, polar flagellum. **Endospores are present in some species.** Hexagonal S-layer typically present. However, it may be difficult to visualize because of extracellular hydrolytic enzymes attached to the cells. **Obligate anaerobes** and catalase-negative.

The temperature growth range for the members of the genus is extremely broad (35–75 °C; for discussion of this topic, see Wiegel (1990, 1998). The pH range for growth is equally broad, 3.2–8.5. The lowest pH optimum is 5.2 for *Thermoanaerobacterium aotearoense*, which is the lowest pH optimum for a validly published, anaerobic, thermophilic bacterium. Similarly, *Thermoanaerobacterium aciditolerans* has the lowest minimum pH for growth, 3.2. The highest pH optimum is 7.8–8.0 for *Thermoanaerobacterium thermosaccharolyticum*. No true alkalithermophilic species have been isolated so far (Table 253). **Chemo-organotrophs** and yeast extract stimulates growth for most species. Fermentation end products from hexoses (unless noted otherwise below) are: acetic acid, ethanol, lactic acid, H_2, and CO_2 in various stoichiometries. In some instances, butyrate and butanol are formed (e.g., *Thermoanaerobacterium thermosaccharolyticum*). All strains with the exception of *Thermoanaerobacterium thermosaccharolyticum* contain menaquinone-7 as the major component of their isoprenoid quinone system. Members of the genus include some well-characterized species, such as *Thermoanaerobacterium thermosaccharolyticum (basonym Clostridium)* or the "swelling can food spoilers" (Collins et al., 1994; Dotzauer et al., 2002; McClung, 1935; Prevot, 1938). A comparison of the properties of the various *Thermoanaerobacterium* species is given in Table 253.

DNA G+C content (mol%): 29–46.

Type species: **Thermoanaerobacterium thermosulfurigenes** (Schink and Zeikus 1983a) Lee, Jain, Lee, Lowe and Zeikus 1993a, 48[VP] (*Clostridium thermosulfurogenes* Schink and Zeikus 1983a, 1156)

Further descriptive information

Sporulation Sporulation has only been observed in some of the species, and it is not regarded as a reliable taxonomic marker because a mutation in one of the many sequentially operating gene products can prevent a species from sporulating. Microorganisms that possess all or most of the sporulation genes but fail to sporulate are termed "asporulating" (Onyenwoke et al., 2004). Common sporulation genes are present in some of the asporulating *Thermoanaerobacterium* species (Brill and Wiegel, 1997; Onyenwoke et al., 2004; unpublished results). The inactivation kinetics of *Thermoanaerobacterium thermosaccharolyticum* endospores during pressure-assisted thermal processing have been studied to better understand the endospore inactivation (Ahn et al., 2007).

Thiosulfate metabolism The reduction of thiosulfate to elemental sulfur instead of H_2S was once considered a feature