***Caldicellulosiruptor kristjanssonii* sp. nov., a cellulolytic, extremely thermophilic, anaerobic bacterium**

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A cellulolytic anaerobic bacterium, strain I77R1B^T, was isolated from a biomat sample of an Icelandic, slightly alkaline, hot spring (78 °C). Strain I77R1B^T was rod-shaped, non-spore-forming, non-motile and stained Gram-negative at all stages of growth. It grew at 45–82 °C, with an optimum growth temperature around 78 °C. At 70 °C, growth occurred at pH 5.8–8.0, with an optimum near pH 7.0. At the optimum temperature and pH, with 2 g cellobiose l⁻¹ as substrate, strain I77R1B^T had a generation time of 2 h. During growth on Avicel, strain I77R1B^T produced acetate, hydrogen and carbon dioxide as major fermentation products together with small amounts of lactic acid and ethanol. The strain fermented many substrates, including cellulose, xylan, starch and pectin, but did not grow with casein peptone, pyruvate, D-ribose or yeast extract and did not reduce thiosulfate to H₂S. The G+C ratio of the cellular DNA was 35 mol%. Comparative 16S rDNA analysis placed strain I77R1B^T among species of *Caldicellulosiruptor*. The closest relative was *Caldicellulosiruptor lactoaceticus*. Hybridization of total DNA showed 42% hybridization to *C. lactoaceticus* and 22% hybridization to *Caldicellulosiruptor saccharolyticus*. A new species, *Caldicellulosiruptor kristjanssonii* sp. nov. (I77R1B^T) is proposed.

Keywords: *Caldicellulosiruptor kristjanssonii* sp. nov. strain I77R1B^T, cellulolytic anaerobic bacterium, thermophile

INTRODUCTION

Anaerobic micro-organisms from extreme environments and, in particular, their fermentation products and thermostable enzymes, have been the subject of much research over the past 15–20 years. Much interest has been centred on the cellulose- and hemicellulose-degrading bacteria, as these polymers constitute a large renewable resource in nature.

Many of the validly described anaerobic, thermophilic, cellulose-degrading bacteria form spores and are placed in the genus *Clostridium* (Wiegel, 1992). In contrast, a number of anaerobic, cellulolytic, extremely thermophilic, non-spore-forming bacteria have been isolated (Huang *et al.*, 1998; Hudson *et al.*, 1990;

Mladenovska *et al.*, 1995; Rainey *et al.*, 1993a; Sissons *et al.*, 1987; Svetlichnii & Svetlichnaya, 1988; Svetlichnii *et al.*, 1990; Taya *et al.*, 1985; Wiegel, 1992). The cellulolytic strains have been mostly isolated from neutral to alkaline hot springs. Two strains were isolated from compost (Rainey *et al.*, 1993a) and *Caldicellulosiruptor owensensis* was isolated from sediment taken from Owens Lake, California, USA (Huang *et al.*, 1998). Common features of the isolates are their high temperature habitat, ability to ferment polysaccharides, high optimum growth temperature (68–75 °C), a maximum growth temperature lower than 85 °C, a pH range for growth between pH 4.0 and 9.0, and their apparent lack of ability to form spores. By comparative 16S rDNA sequence studies, Rainey and co-workers, and later Huang and co-workers, have shown that these organisms cluster together in one group (Huang *et al.*, 1998; Rainey *et al.*, 1993a, b; Rainey & Stackebrandt, 1993).

In the present study, we have characterized a new

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The EMBL accession number for the 16S rRNA gene sequence of strain I77R1B^T is AJ004811.

anaerobic, cellulolytic, extremely thermophilic bacterium, strain I77R1B^T. The physiological characteristics of this strain, phylogenetic analysis of rDNA and DNA–DNA hybridization suggest that this strain represents a new species of cellulolytic, extremely thermophilic, anaerobic bacteria, *Caldicellulosiruptor kristjanssonii* sp. nov., named in honour of Dr Jakob K. Kristjansson, a substantial contributor to the ecology and biotechnology of thermophilic bacteria.

METHODS

All diagnostic tests were performed at least twice, with triplicate determinations. Mean values are reported.

Growth medium. The anaerobic medium used for cultivation was as previously described (Angelidaki *et al.*, 1990), with the following adjustments. Avicel (2 g l⁻¹; microcrystalline cellulose, FMC International) was used as carbon and energy source. The medium was supplemented with 0.10 g yeast extract l⁻¹ and no cysteine was added. The medium was adjusted to pH 7.0 and autoclaved at 140 °C for 20 min. Before inoculation, the medium was reduced with 0.5 g Na₂S l⁻¹ and 10 ml vitamin solution l⁻¹ (DSMZ medium no. 141) was added. Carbohydrate and vitamin solutions were filter-sterilized and added just before inoculation. Cultures were maintained by transferring a 10% (v/v) inoculum to fresh medium and were incubated in the dark without shaking. Fully grown cultures were removed from the incubator and stored at room temperature. Cultures were transferred at least weekly.

Isolation. Isolation was carried out anaerobically at 70 °C and pH 7.0 using the roll-tube technique (Hungate, 1969). Dilutions of the enrichment cultures were transferred into medium containing 2 g Avicel l⁻¹ and solidified with 11 g Gelrite l⁻¹ and 1 g MgCl₂ l⁻¹. Colonies were picked anaerobically with sterile Pasteur pipettes, diluted and transferred to new roll-tubes. The procedure was repeated until only one colony type was present.

Analytical methods. GC was used for identification of fermentation products; flame-ionization detection was used for short chain fatty acids and alcohols (Sørensen *et al.*, 1991) and thermal conductivity detection was used for measurement of gases (Angelidaki *et al.*, 1990). Lactate and formate were quantified by HPLC (Hörber *et al.*, 1998).

Growth experiments. Growth rates were determined by measuring culture turbidity (OD₅₇₈) with cellobiose as substrate. Media with different pH values were obtained by varying the ratios of HCO₃⁻ and Na₂CO₃ in the medium (1 ml of appropriate 0.3 M buffer was added to 9 ml medium) and the concentration of CO₂ in the headspace gas. The actual pH of the media was measured at the experimental temperature (70 °C). Growth rate measurements at various pH values were made in the early growth phase when the pH of the media showed no significant changes. Lowering of pH occurred in late exponential phase due to accumulation of fermentation products. Cultures were adapted to each temperature and pH by transferring the culture to the new conditions three times before measurements were made. Growth on soluble carbon and energy substrates was determined turbidimetrically (OD₅₇₈). Growth on insoluble substrates, e.g. xylan, was determined by measurement of the increase in acetate concentration. Cultures were inoculated with 2.5% (v/v) from the same medium.

Microscopic examination. Agar-coated glass slides were used for phase-contrast photomicroscopy. Gram-staining was performed according to standard methods (Gerhardt, 1981). A *Streptococcus* species and *Escherichia coli* were used as positive and negative controls, respectively. Endospore formation was tested using the methods of Schink & Zeikus (1983) and Cook *et al.* (1991). In addition, cultures in the exponential growth phase were transferred to an incubator at 15–20 °C below the optimum growth temperature of the cultures to induce sporulation. Cultures with cells in the stationary growth phase were boiled for 60 min. At intervals of 5, 10, 20, 30 and 60 min, samples were transferred to roll-tubes with Avicel as substrate and incubated at 70 °C to determine survival. The Schaeffer–Fulton method (Gerhardt, 1981) was used for staining endospores. The Leifson method was used for staining flagella (Gerhardt, 1981). Positive controls were *Clostridium thermocellum* (strain DSM 4150 or ATCC 31449) for boiling experiments and *Clostridium thermohydrosulfuricum* strain A2 (Sonne-Hansen *et al.*, 1993) for flagella staining.

Tests for sulfide production. To test for sulfide production, cultures were grown in the anaerobic medium described above with 0.5 g cysteine–HCl l⁻¹ as reducing agent and 2 g cellobiose l⁻¹ as the carbon source. Na₂S was omitted from the medium. The medium was gassed and autoclaved for 30 min; 0.1 ml 2 M Na₂SO₄, 0.1 ml 2 M Na₂S₂O₃ or 0.1 ml 2 g peptone l⁻¹ was then added. Controls without additions were also performed. Sulfide in the culture medium was detected using a modification of the method described by Cord-Ruwisch (1985). Copper reagent (2 ml; 5 mM CuSO₄ in 50 mM H₂SO₄) was added to 0.5 ml culture fluid. A black precipitate of CuS confirmed the presence of sulfide. Positive controls were *Clostridium thermohydrosulfuricum* strain A2 (Sonne-Hansen *et al.*, 1993) and *Desulfovibrio gigas* strain DSM 1382^T (= NCIB 8403^T). The latter organism was grown on lactate.

G + C measurements. The G + C content (mol%) of the cellular DNA of strain I77R1B^T was measured by HPLC at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Phylogenetic analysis. DNA isolation, PCR amplification of the 16S rRNA gene and sequencing of the PCR product was performed at the DSMZ. The 16S rRNA nucleotide sequence of I77R1B^T was aligned against a ribosomal database comprising sequences of anaerobic bacteria of the Gram-positive phylum. The following 16S rRNA gene sequences were applied in the final phylogenetic analysis (EMBL no. is given in parentheses): *Caldicellulosiruptor saccharolyticus* strain Tp8T.6331^T (L09178); strain Comp.B1 (L09179); strain SP83 (X93020); *Caldicellulosiruptor owensensis* strain ATCC 700167^T (U80596); *Caldicellulosiruptor lactoaceticus* strain 6A^T (X82842); ‘*Anaerocellum thermophilum*’ strain Z-1203 (L09180); strain R12.B1 (L09181); strain Wai35B1 (L09182); ‘*Thermoanaerobacter cellulolyticus*’ strain IFO 14436 (L09183); *Fervidobacterium islandicum* strain DSM 5733^T (M59176); *Fervidobacterium gondwanense* strain AB39^T (Z49117); *Thermotoga maritima* strain DSM 3109^T (M21774); *Dictyoglomus thermophilum* strain DSM 3960 (X69194); *Thermoanaerobacter brockii* strain DSM 1457^T (L09165); *Thermoanaerobacter ethanolicus* strain ATCC 33223 (L09164). Corrected pair-wise evolutionary distances were computed with the correction of Jukes & Cantor (1969). A phylogenetic tree was constructed by neighbour-joining (Saitou & Nei, 1987). Bootstrap values were calculated for 500 phylogenetic trees. The phylogenetic analysis

was made using facilities collected in the ARB program package (Strunk & Ludwig, 1997).

DNA–DNA hybridization. Cells were harvested from cultures incubated overnight at 70 °C with lactose as substrate. Chromosomal DNA was isolated according to the following protocol. Cells were washed twice in TE buffer [10 mM Tris(hydroxymethyl) aminomethane, 1 mM EDTA, pH 8]. Egg-white lysozyme (12.5 mg ml⁻¹; Sigma) was added and the cells were incubated at 37 °C for 30 min. The lysed cells were incubated at 50 °C in 1% SDS and proteinase K (0.5 mg ml⁻¹; Boehringer Mannheim) for several hours or overnight. After two treatments with phenol:chloroform:isoamyl alcohol (25:24:1), DNA was extracted with chloroform, precipitated with 0.1 vol. sodium acetate and 2 vol. absolute ethanol and washed twice in 70% ethanol. The DNA pellets were dried, resuspended in TE buffer and incubated with RNase (0.25 µg 100 µl⁻¹; from bovine pancreas, Boehringer Mannheim) at 37 °C for 30 min. The DNA was re-precipitated, washed and resuspended in TE buffer. The quality of the DNA was measured in a 0.7% agarose gel and the concentration and purity of the DNA were determined by measuring the absorbance at 260 and 280 nm.

The DNA hybridization experiments were performed using a modification of a previously described method (Seldin & Dubnau, 1985) with DNA of strain I77R1B^T as a probe. The probe was prepared from 0.1 µg chromosomal DNA by nick translation (Boehringer Mannheim) and radiolabelled with α-³²P-labelled deoxycytosine (Amersham). The probe was denatured just before use by heating it up to 95 °C for 10 min followed by cooling in an ice-water bath.

Hybridization. To samples containing 3 µg DNA in 200 µl TE buffer, 200 µl 20 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was added. The samples were denatured by heating them up to 95 °C for 15 min followed by cooling on ice. Nitrocellulose membranes (Highbond-N; Amersham) were soaked in 20 × SSC for 60 min before assembling of the slotblotter. Prior to loading the denatured DNA (in triplicate), 400 µl 10 × SSC was added to the wells. Immediately after loading, the membranes were UV-fixed (2000 × 100 µJ cm⁻²) for 2 min in a UV Cross-linker (Hoefer). The membranes were placed in hybridization tubes containing 5 ml hybridization solution [2 × SSC, 1 mM EDTA, 0.1% blocking reagent (Boehringer Mannheim)] and were pre-hybridized for 2 h at 65 °C. The denatured probe was added and the hybridizations were carried out overnight in a hybridization oven (Hybridizer HB-1D; Techne) at 65 °C. The membranes were then washed twice in 2 × SSC and 0.1% SDS at 25 °C followed by two washes in 0.1 × SSC and 0.1% SDS at 37 °C. The membranes were wrapped in plastic foil and exposed to X-ray film (Bio-maxMS; Kodak) for 24 h. After exposure, the individual slots were cut out of the membrane and their radioactivity measured with a scintillation counter. The percentage homology was calculated using the ratio: (c.p.m. heterologous DNA/c.p.m. homologous DNA) × 100. Homologous DNA was used as the probe in the hybridization experiments (Seldin & Dubnau, 1985).

RESULTS

Strain I77R1B^T was isolated from an enrichment culture grown at 78 °C with Avicel as substrate and inoculated with a biomat sample from a slightly

Fig. 1. Phase-contrast photomicrographs showing the isolated strain I77R1B^T. Cells were grown on 5.0 g xylose l⁻¹ at 70 °C. Bar, 10 µm.

alkaline (pH 8.7) Icelandic hot spring (Sonne-Hansen & Ahring, 1997).

Colony and cell morphology of strain I77R1B^T

Clearing zones around colonies in roll-tube cultures with 2 g Avicel l⁻¹ were 2–4 mm in diameter after 9–14 d incubation, indicating solubilization of the Avicel by the actions of extracellular cellulases. Colonies were 0.5–1.0 mm, flat, cream in colour and had a fringed edge. Liquid cultures with 2.0 g Avicel l⁻¹ were fully grown after 3–4 d at 78 °C. Xylose-grown cultures in exponential growth phase comprised rod-shaped cells with rounded ends, 2.8–9.4 µm long and 0.7–1.0 µm wide. Cells occurred singly, in pairs and in short chains (Fig. 1). Longer cells and chains of cells were observed under non-optimal conditions of temperature and pH. Cells were generally shorter when Avicel was used as substrate. Cells were observed both free in the liquid medium and attached to the cellulose fibres. Motility was never observed. Strain I77R1B^T had peritrichous flagella in exponential phase cultures whereas older cultures had two subterminal flagella.

Formation of endospores or spore-like structures was never observed on any of the different substrates or media or at any temperatures used. Accordingly, the strain did not survive boiling for 5 min.

Fermentation end-products

The major fermentation end-products on 2.0 g Avicel l⁻¹ at 78 °C were up to 16 mM acetic acid, 3.1 mM lactic acid, 1.1 mM ethanol and 0.6 mM formic acid.

Fig. 2. Unrooted phylogenetic tree comparing the 16S rRNA gene sequence of strain I77R1B^T and sequences of three validly described species of the genus *Caldicellulosiruptor* and six genetically related species. Sequences from other thermophilic non-spore-forming bacteria were used as outgroups. Bootstrap values indicate the percentage probability for the appropriate branches of the tree. Bar, 10% corrected sequence variation.

At 70 °C and 2.0 g substrate l⁻¹, strain I77R1B^T produced up to 19 mM acetic acid from xylan, 9 mM from cellobiose and 9 mM from glucose. In addition to the organic acids, the strain produced hydrogen and carbon dioxide on all the different substrates tested.

Optimum pH and temperature for growth

With 2.0 g cellobiose l⁻¹ as substrate, strain I77R1B^T grew at 50–82 °C and had an optimum growth temperature of 78 °C. At 70 °C, growth occurred at pH 5.8–8.0 with an optimum pH at approximately 7.0. At the optimum temperature and pH with 2 g cellobiose l⁻¹ as substrate, strain I77R1B^T had a generation time of 2.0 ± 0.08 h.

Substrate and nutritional requirements and G + C content

Strain I77R1B^T grew on Avicel, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, lactose, maltose, mannose, pectin, salicin, soluble starch, sucrose, trehalose, xylan and xylose. The strain did not grow on casein peptone, pyruvate, D-ribose, yeast extract, L-arabinose, aesculin, glycerol, inulin, lactic acid, mannitol, raffinose, L-rhamnose or sorbitol. Strain I77R1B^T did not produce H₂S from any sulfuroxy anions or from peptone.

The G + C content of strain I77R1B^T is 35 ± 0.8 mol %.

Antibiotic, hydrogen and NaCl sensitivity

At 70 °C, the strain was sensitive to chloramphenicol, neomycin, penicillin-S, streptomycin, tetracycline and vancomycin, all added at 100 mg l⁻¹. At 70 °C and an antibiotic concentration of 10 mg l⁻¹, the strain was

still sensitive to neomycin, penicillin-S, streptomycin, tetracycline and vancomycin. NaCl (0.2%) or 0.5 atm overpressure of hydrogen did not inhibit growth of strain I77R1B^T.

Phylogenetic characterization

The 16S rDNA was amplified by PCR and 1508 bp of the sequence were obtained. The sequence was manually aligned to sequences of clostridia and related taxa. Preliminary analysis revealed that this 16S rRNA gene sequence grouped with those of other anaerobic thermophilic non-spore-forming organisms. The tree demonstrated that strain I77R1B^T was related to species of the genus *Caldicellulosiruptor* (Fig. 2). The closest relative was *Caldicellulosiruptor lactoaceticus* with 98.9% sequence similarity. The distance to the type species, *Caldicellulosiruptor saccharolyticus*, was 95.6% and the distance to the most recently described species, *Caldicellulosiruptor owensensis*, was 96.5%. The phylogenetic tree includes seven organisms that are not validly described. The sequence similarities between these strains and the validly described *Caldicellulosiruptor* species are 99.9–94.4%.

DNA–DNA hybridization

Genomic DNA from the closest relative, *Caldicellulosiruptor lactoaceticus*, and from the type species of the genus, *Caldicellulosiruptor saccharolyticus*, were hybridized to genomic DNA from strain I77R1B^T. DNA of strain I77R1B^T shared 42 ± 3% hybridization homology with *Caldicellulosiruptor lactoaceticus* and 22 ± 2% with *Caldicellulosiruptor saccharolyticus*. The reliability of the method was tested using different strains of *Thermoanaerobacter mathranii* with nearly identical 16S rDNA. This resulted in high (86–93%)

hybridization homology. Likewise, very low hybridization homology (5%) was found when hybridizing *Thermoanaerobacter mathranii* and *Caldicellulosiruptor lactoaceticus*.

DISCUSSION

Strain I77R1B^T is anaerobic, extremely thermophilic, Gram-negative and highly saccharolytic. The strain hydrolyses crystalline cellulose and is, therefore, a 'true' cellulose degrader (Wiegel, 1992). Xylan, pectin and starch are also degraded. Based on 16S rRNA sequence analysis, strain I77R1B^T is a member of the domain *Bacteria* and belongs to the subdivision containing Gram-positive bacteria with a DNA G + C content of less than 55 mol % and within the radiation of members of the genus *Clostridium* and related taxa (Rainey *et al.*, 1993b). Strain I77R1B^T is non-spore-forming and therefore may not be included in the spore-forming genera *Clostridium* or *Thermoanaerobacter* (Lee *et al.*, 1993). The strain does not reduce thiosulfate and, therefore, may not be included in the genus *Thermoanaerobacterium* (Lee *et al.*, 1993). These characteristics are shared with the three validly described species of *Caldicellulosiruptor*, *Caldicellulosiruptor saccharolyticus*, *Caldicellulosiruptor lactoaceticus* and *Caldicellulosiruptor owensensis*, as well as other non-spore-forming, anaerobic, extremely thermophilic, cellulolytic bacteria (Huang *et al.*, 1998; Hudson *et al.*, 1990; Mladenovska *et al.*, 1995; Rainey *et al.*, 1994; Sissons *et al.*, 1987; Svetlichnii & Svetlichnaya, 1988; Svetlichnii *et al.*, 1990; Taya *et al.*, 1985; Wiegel, 1992).

Neutral-to-alkaline hot springs, naturally solar-heated environments and compost are thermal environments which select for populations of cellulolytic and xylanolytic bacteria with many similar phenotypic characteristics, independent of geographic location. There are many similarities in the phenotypes of strain I77R1B^T, *Caldicellulosiruptor saccharolyticus*, *Caldicellulosiruptor lactoaceticus* and *Caldicellulosiruptor owensensis*. A comparison reveals differences in cell morphology (cell length), growth, range of substrates utilized and in proportions of fermentation end-products. Cells of *Caldicellulosiruptor saccharolyticus*, *Caldicellulosiruptor lactoaceticus* and *Caldicellulosiruptor owensensis* are shorter (3–4, 1.5–2.8 and 2–5 µm, respectively) than those of strain I77R1B^T (2.8–9.4 µm). *Caldicellulosiruptor owensensis* differs from the other strains by its production of small coccoid cells in the exponential growth phase (Huang *et al.*, 1998). At optimum temperature and pH with 2.0 g cellobiose l⁻¹ as substrate, strain I77R1B^T has a maximum generation time of 2.0 ± 0.08 h. Under the same conditions, but with glucose as substrate, *Caldicellulosiruptor owensensis* has a generation time of 7.3 h (Huang *et al.*, 1998). *Caldicellulosiruptor lactoaceticus* differs from strain I77R1B^T in producing mainly lactate rather than acetate as the main end-product and by its growth on a more limited range of

carbohydrates. Strain I77R1B^T differs from *Caldicellulosiruptor saccharolyticus* and *Caldicellulosiruptor owensensis* in the spectrum of carbohydrates used for growth. *Caldicellulosiruptor saccharolyticus* ferments L-arabinose and L-rhamnose, whereas strain I77R1B^T does not grow on these substrates. *Caldicellulosiruptor owensensis* differs from I77R1B^T, *Caldicellulosiruptor saccharolyticus* and *Caldicellulosiruptor lactoaceticus* by its ability to ferment mannitol, raffinose and D-ribose. Thus, *Caldicellulosiruptor lactoaceticus* is more exclusively polysaccharolytic, whereas strain I77R1B^T, *Caldicellulosiruptor saccharolyticus* and *Caldicellulosiruptor owensensis* are adapted to the utilize a wider range of soluble carbohydrates.

Comparative 16S rDNA sequence analysis places strain I77R1B^T among species of *Caldicellulosiruptor*. Strain I77R1B^T is most closely related to *Caldicellulosiruptor lactoaceticus*, with a sequence similarity of 98.9%. DNA–DNA hybridization homology clearly separates strain I77R1B^T and its nearest relative *Caldicellulosiruptor lactoaceticus* and strongly supports assignment of I77R1B^T to the genus *Caldicellulosiruptor* as a new species; the name *Caldicellulosiruptor kristjanssonii* sp. nov. is proposed.

Description of *Caldicellulosiruptor kristjanssonii* sp. nov.

Caldicellulosiruptor kristjanssonii (kris.tjans.son'ii. M.L. gen. n. *kristjanssonii* named after Dr Jakob K. Kristjansson).

Cells are rods with rounded ends, 2.8–9.4 µm by 0.7–1.0 µm. Cells occur singly, in pairs or in short chains. Gram-negative. Endospores not found. Motility not observed. Two subterminal flagella. The DNA G + C composition is 35 mol %. When grown in roll-tubes on mineral medium plus Avicel, colonies are flat, cream in colour with a fringed edge. After 9–14 d, clearing zones around colonies are 2–4 mm. Obligately anaerobic. The optimum, maximum and minimum temperatures for growth are 78, 82 and 45 °C, respectively. The optimum pH for growth is approximately 7.0; no growth above pH 8.0 or below pH 5.8. Chemo-organotroph. Grows on Avicel, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, lactose, maltose, mannose, pectin, salicin, soluble starch, sucrose, trehalose, xylan and xylose. Does not utilize D-ribose, L-arabinose, aesculin, 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 fermentation end-products are acetic acid, H₂ and CO₂, with smaller amounts of lactic acid and ethanol and trace amounts of formic acid. No H₂S from sulfate, thiosulfate or casein peptone. Habitat: microbial mats and cellulosic materials, e.g. wood or straw, in Icelandic thermal and slightly alkaline springs. Type strain is I77R1B^T (= DSM 12137^T) and has been deposited in the DSMZ.

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