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Thermoanaerobium brockii gen. nov. and sp. nov., A New Chemoorganotrophic, Caldoactive, Anaerobic Bacterium

J. G. Zeikus*, P. W. Hegge, and Mary Ann Anderson

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

Abstract. The isolation of a new anaerobic thermophilic bacterium, *Thermoanaerobium brockii*, from volcanic features is described. Successful enrichment required a complex medium containing glucose or other fermentable sugars and incubation temperatures of 55–80° C. Strains of *T. brockii* were gram positive, rods of uneven length that existed singly, in pairs, chains or filaments. Electron micrographs of thin sections of cell revealed a monolayered cell wall and a constrictive or "pinching off" cell division process. The organism was nonsporeforming, obligately anaerobic and chemoorganotrophic. The optimal temperature for growth was 65–70° C, the maxium was below 85° C and the minimum above 35° C. The doubling time at the optimal temperature for growth was about 1 h. The DNA base composition of three strains of *T. brockii* varied from 30.0–31.4 mol % guanosine plus cytosine. Fermentable carbohydrates included glucose, sucrose, maltose, lactose, cellobiose and insoluble starch. The fermentation products of cells grown on glucose were ethanol, lactic acid, acetic acid, hydrogen and carbon dioxide. Growth of all strains tested was inhibited by fairly low concentrations of cycloserine, penicillin, streptomycin, tetracycline and chloramphenicol. The possible ecological, evolutionary, and industrial significance, and taxonomic relationships of *Thermoanaerobium* are discussed.

Key words: *Thermoanaerobium brockii* – Genus, Species description – Obligate anaerobe – Sugar and starch fermentation – Ethanol production – Lactic acid – Thermophile ecology and physiology.

* To whom offprint requests should be sent

Abbreviations. TYEG = complex medium containing mineral salts, 0.3% yeast extract, 1.0% tryptone and 0.5% glucose
O.D. = optical density
G + C = guanosine plus cytosine

Understanding of the biology of the thermophilic microorganisms has been greatly advanced in the past ten years because of a most productive research effort. The past decade may well have been the golden era of thermophily. The discovery and description of *Thermus aquaticus* by Brock and Freeze (1969) initiated systematic studies on extreme (or caldoactive) bacteria that grow optimally in nature at temperatures near or greater than 70° C (even in boiling water). Present knowledge of species diversity, physiological ecology and molecular biology of thermophilic microorganisms has been more than adequately presented by recent books (Brock, 1978; Kushner, 1978) and reviews (Williams, 1975; Ljungdahl, 1979). By and large previous studies have been limited to aerobic thermophiles. Little is known about species diversity and the ecologically significant caldoactive anaerobic bacteria in nature. Notwithstanding many of the thermal features (e.g. hot springs, sediments and photosynthetic bacterial mats) associated with volcanic activity (see Brock, 1978) may be more conducive for growth of anaerobes than aerobes because of the low solubility of O_2 at high temperatures, the presence of H_2S, and high organic content associated with decomposing biomass.

This communication reports on the isolation and characterization of a new nonsporulating caldoactive anaerobe which is widespread in thermal features of Yellowstone National Park. The organism is a gram positive chemoorganotroph that ferments a variety of saccharides including starch to ethanol, lactic acid, acetic acid and H_2/CO_2. The name *Thermoanaerobium* is proposed for this new genus. The type species *Thermoanaerobium brockii* sp. nov. is named in honor of T. D. Brock, a pioneer in the golden era of thermophily.

Materials and Methods

Chemicals. All chemicals were reagent grade. The N_2/CO_2 gas mixture (95/5, V/V) was obtained from Matheson (Joliet, Ill.) and

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was passed over heated (370° C) copper filings to remove traces of O_2. Antibiotics were obtained from Sigma Chemical Co.

Inocula. Source materials for initiation of enrichment cultures were obtained from Yellowstone National Park. Octopus spring is located in the Lower Geyser Basin. The chemical, thermal and microbial characteristics of the primary production portion of the bacterial mat ecosystem associated with this spring have been well characterized (Doemel and Brock 1977, Brock 1978). Washburn pool A and B were located several meters NW from Devils ink pot in the Washburn springs area southwest of Mount Washburn. The chemical and thermal properties of Washburn springs have been characterized (Gunter and Musgrave, 1966). Firehole pool A is located next to the Firehole river in the Midway Geyser Basin halfway between Ojo Caliente and the Fountain Freight road bridge. Collection of thermal spring water, edge sediment or bottom portions of bacterial mats and inoculation of enrichment cultures were performed anaerobically by using techniques described (Zeikus and Winfrey, 1976, Winfrey and Zeikus, 1979) for analysis of methanogenic bacteria. Initiation of enrichment cultures was performed in the field and thermal features were used as "in site" incubators. Successful enrichment of *Thermoanaerobium* strains from all the thermal features reported was accomplished in July, 1977 and repeated in October, 1978.

Media and Cultivation Conditions. The anaerobic culture technique described by Bryant (1972) was used. Basal medium contained per liter of distilled water: NH_4Cl, 0.9 g; NaCl, 0.9 g; $MgCl_2$, 0.2 g; KH_2PO_4, 0.75 g; K_2HPO_4, 1.5 g; trace mineral solution, 9 ml; 10% $FeSO_4$, 0.03 ml; 0.2% resazurin, 1 ml; vitamin solution (Wolin et al., 1963), 5 ml; and 10 ml of 10% Na_2S was added before autoclaving. Trace mineral solution contained per liter distilled H_2O: 12.8 g nitrilotriacetic acid neutralized to pH 6.5 with KOH, $FeCl_3 \times 4H_2O$, 0.2 g; $MnCl_2 \cdot 4H_2O$, 0.1 g; $CoCl_2 \cdot 6H_2O$, 0.17 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnCl_2$, 0.1 g; $CuCl_2$, 0.02 g; H_3BO_3, 0.01 g; $NaMoO_4 \cdot 2H_2O$, 0.01 g; NaCl, 1.0; and Na_2SeO_3, 0.02 g. The pH of the medium was 7.2–7.4 and 95% N_2/5% CO_2 was used in the culture medium gas phase. Where indicated the basal medium was supplemented with yeast extract and various additions. Additions (e.g. energy sources) were sterilized seperately and then added to yeast extract supplemented basal medium. Growth inhibitors and antibiotics were not autoclaved befor addition. TYEG medium consisted of basal medium supplemented with 0.3% yeast extract, 1.0% tryptone and 0.5% glucose (autoclaved seperately). Cells used for growth, nutritional and morphological studies were cultured in 23 ml anaerobic culture tubes (size 18 × 142 mm) from Bellco (Vineland, New Jersey) which were sealed with Nr. 1 neoprene stoppers (Scientific products, McGaw Park, Ill.). Cells used for DNA Base composition determination and analysis of cytochromes were cultured at 65° C in a 14L New Brunswick Microferm Fermentor that contained 12L of TYEG medium. Cells were used immediately or stored at −20° C.

Growth Characterization. Growth was determined by measuring the increase in turbidity at 540 nm. Optical density was quantified directly by insertion of the anaerobic culture tubes into a Spectronic 20 (Bausch and Lamb, Rochester, N. Y.). All growth experiments employed duplicate tubes and results represent mean values. All individual experiments were duplicated or triplicated.

Fermentation products formed during growth were routinely determined by gas chromatography. H_2 and CO_2 were analyzed using the procedures described by Nelson and Zeikus (1974). Ethanol, acetic acid and lactic acid were determined by modification of the procedures described by Weimer and Zeikus (1977). The following parameters were modified for product analysis: glass column (1.83 m × 2 mm) contained Chromosorb 101 (Supelco, Bellefonte, Pa.), 100/120 mesh; column temperature, 160° C for fatty acids and alcohols; 200° C for lactic acid, injection temperature, 210° C detector temperature, 230° C carrier gas, nitrogen 35 cm^3 per min; H_2 flow rate, 30 cm^3 per min; air flow rate, 300 cm^3 per min. Formic and succinic acids were analyzed by isoionic exchange chromatography (Thauer et al., 1970). The production of sulfide from sulfite was determined as described by Hollaus and Sleytr (1972).

Cellular Characterization. A Carl Zeiss photomicroscope was used for phase contrast observations including determination of cell size. The methods used for preparing cells for thin sectioning and electron microscopic examination were described by Zeikus and Bowen (1975). Thin sections were stained by 1% uranyl acetate and then by 1% lead citrate. Sections were examined with a Hitachi Hu 11E electron microscope by R. Heinzen.

DNA was isolated and purified from lysozyme treated cells by the method of Marmur (1961). DNA base compositions were calculated according to the method of DeLey (1970) from thermal denaturation in 0.015M NaCl and 0.0015M trisodium citrate as determined in a Gilford Model 250 spectrophotometer equipped with a Model 2527 thermoprogrammer. *Escherichia coli* DNA VIII, lot No. 57C-6830 from Sigma Chemical Co. served as standard. Data reported represent the mean of 4 seperate determinations. The %G + C of *E. coli* standard was 52.8. (± 2%). Cytochromes were examined in cells by methods previously described (Badziong et al., 1978).

Results

Enrichment, Isolation and Cultivation. Water, sediment and decomposing photosynthetic bacterial biomass from diverse thermal features in Yellowstone National Park were added into anaerobic culture tubes that contained 10 ml of TYEG Medium. The tubes were gassed with N_2, sealed with neoprene rubber stoppers and incubated in the field at 55–80° C. Within 12–24 h enrichments had developed a positive pressure and visible turbidity. Microscopic observation in the laboratory revealed a bacterial population comprised of rods with spores visible only in enrichments that used decaying bacterial mats as inocula (see Table 1). Enrichments were maintained by repeated transfer (1% inoculum) of the culture fluid in anaerobic culture tubes that contained TYEG medium. Repeated transfer of all enrichments into fresh medium selected for non-sporeforming rods.

Direct isolation of the organism from enrichment cultures of the thermal features indicated in Table 1 was obtained by streaking plates of TYEG medium that contained 3.5% agar in an anaerobic chamber (Coy Lab Products, Ann Arbor, Mich.) followed by in-

Table 1. Thermal features yielding *Thermoanaerobium* isolates

Location	Inoculum source temperature (°C)	strain designation
Firehole pool A edge sediment	65	HTA1
Octopus Spring bacterial mat	65	HTB1
Washburn pool A water	55	HTD1
Washburn pool B bacterial mat	65–80	HTD2, HTD4, HTD6

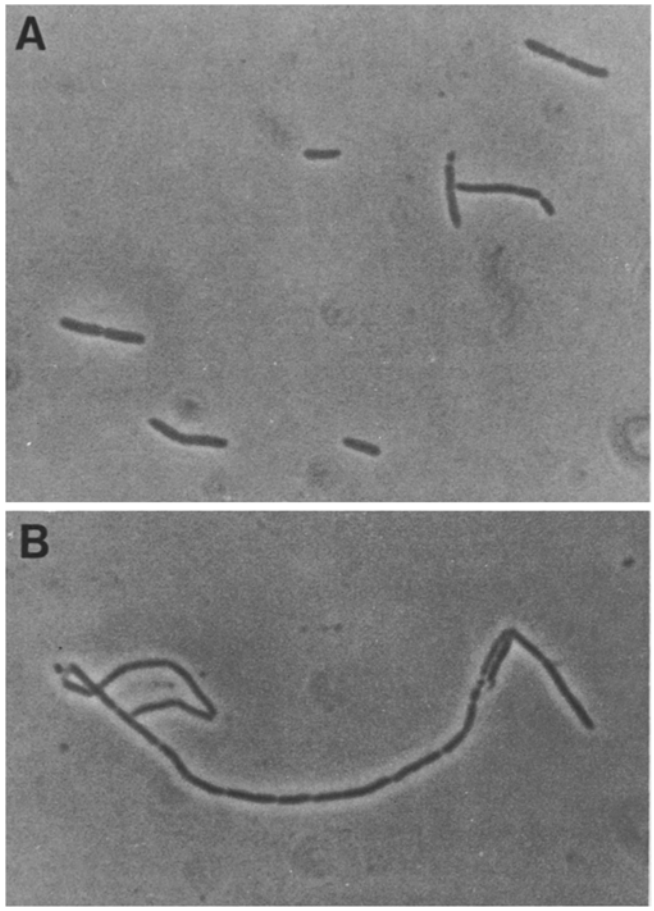

Fig. 1A and B. Phase contrast photomicrographs of *Thermoanaerobium brockii* strain HTD4 grown in TYEG Medium at 65° C. **A** Single and paired cell morphologies. **B** Chain cell morphology. Note the presence of small cells (mini-cells) associated with paired cells and filaments. Magnification: ×1778

cubation of plates in Gas Pak Jars (BBL, Cockeysville, Md.) at 60–65° C. All colonies observed were smooth uniformly round, mucoid, non-pigmented and flat. The maximum colony diameter after 48 h incubation was 0.3 cm for strain HTD6 and 0.2 cm for HTD4 and HTA1.

Stock cultures were prepared by inoculating anaerobic culture tubes containing TYEG medium with single, well-isolated colonies from agar plates followed by incubation at 60–80° C. Stocks were incubated overnight and then stored at 4° C for up to 2 weeks before transfer. Stock cultures can be stored anaerobically at 4, −20 or −80° C and remain viable for many weeks. Cultures were not incubated for more than 16 h at optimal growth temperatures because this normally resulted in complete lysis of the culture. All strains indicated in Table 1 appeared morphologically similar and further studies were limited to strains HTA1, HTD4, and HTD6. All three strains belong to the type species *Thermoanaerobium brockii* and strain HTD4 is the type strain.

Cellular Features. The morphology of *T. brockii* varied somewhat with cultural conditions. Cells grown in TYEG medium at 65° C appeared as rods (Fig. 1). The rods can exist singly or in pairs, in chains and often in filaments. The average size of mid exponential grown cells of HTD6 was 0.8–1.0 μm in width and varied considerably in length from 2 μm to greater than 20 μm. Paired cells were often not of equal length and mini-cells are associated with paired cells and filaments. Cell granulation, elongation-swelling, and lysis was observed in stationary phase cultures. *T. brockii* was gram positive, non-sporeforming and motility was not observed in wet mounts. All *Thermoanaerobium* strains were grown under many different conditions to attempt to induce sporulation. Included in these conditions were growth on starch agar, growth in *Clostridium perfringens* sporulation medium (Duncan and Strong, 1968), GC medium and xylose soil extract medium (Hollaus and Sleytr, 1972), and growth in TYEG medium with varying extremes in temperature, pH, specific ion concentration, etc. Spores were never observed under any of the growth conditions employed.

Electron microscopic observations of thin sections revealed that cells had a mono-layered cell wall envelope (Fig. 2B) similar in profile to that of certain other Gram positive bacteria. Thin sections of cells grown in TYEG medium displayed a constrictive or "pinching off" type cell division process (Fig. 2). Intracellular membranes were commonplace, appeared to form by invagination of the plasma membrane, and were associated with cell division.

DNA isolated from *T. brockii* had a base composition of 30.0, 31.0 and 31.4 (±1) mol percent guanosine + cytosine for strains HTD6, HTD4 and HTA1, respectively. Air-oxidized spectra of all strains lacked absorbance bands corresponding to cytochromes. Catalase was not detectable.

Growth and Nutritional Properties. The relationship between growth of *T. brockii* strain HTD4 and temperature is shown in Fig. 3. The optimum temperature for growth was 70° C, the maximum was less than 85° C and the minimum was greater than 35° C. The optimum temperature for growth of strains HTD6 and HTA1 was 65° C, and temperature ranges for growth of these strains was similar to HTD4. A typical growth curve for *T. brockii* is shown in Fig. 4. The generation time at the optimum temperature for growth was about 1 h. The cessation of growth observed at 8 h was not due to substrate limitation (i.e. glucose). The pH of the medium at the end of growth was 5.5. The fermentation products of all three strains when grown on TYEG medium were lactic acid, ethanol, acetic acid, hydrogen

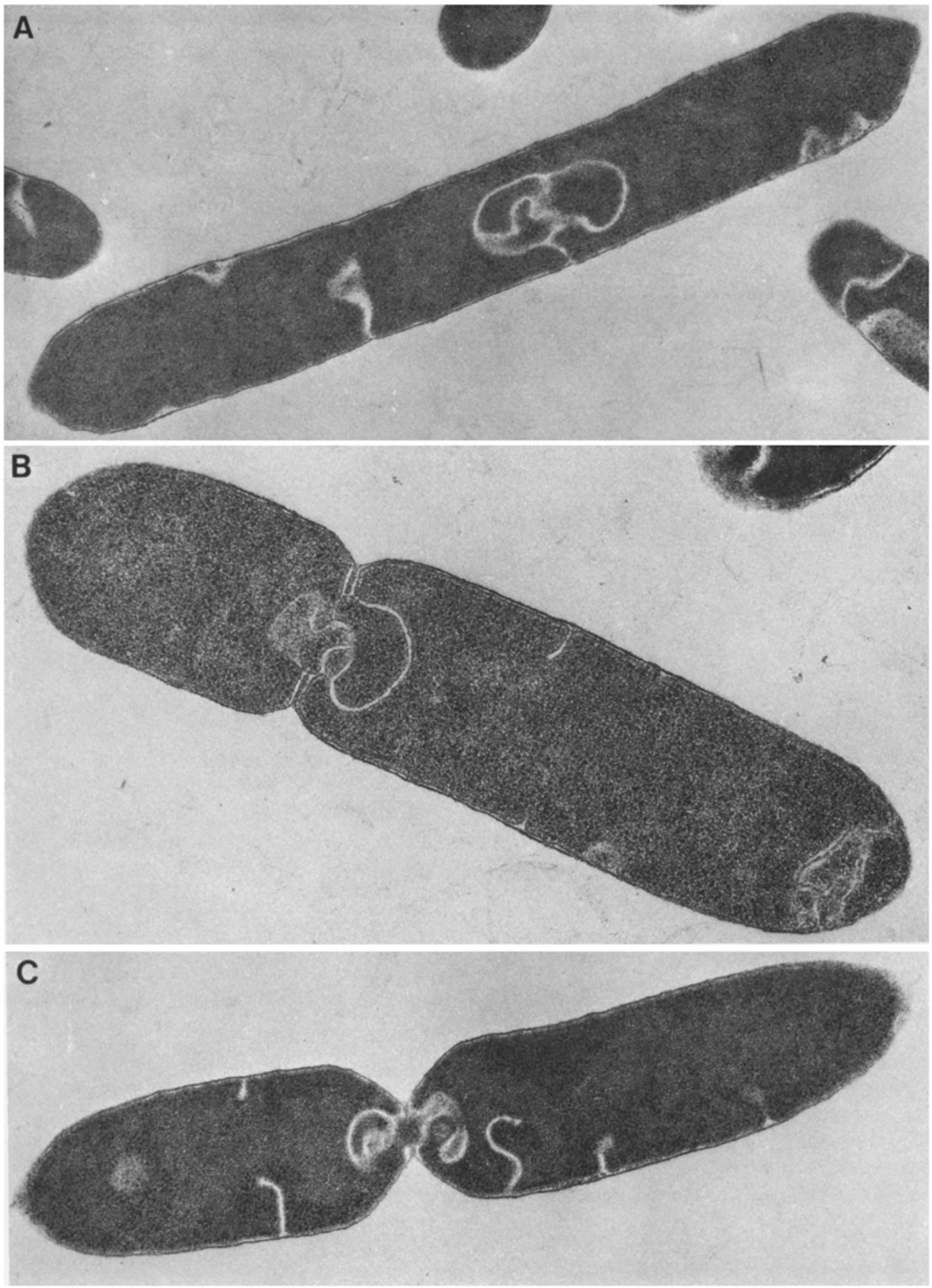

Fig. 2A—C. Electron photomicrographs of thin sections of *T. brockii* strain HTA1 grown in TYEG medium at 65° C. **A** Initiation of cell division. Magnification: ×44,202. **B** During cell division; magnification: ×58,830. **C** Nearing end of cell division. Magnification: ×44,202

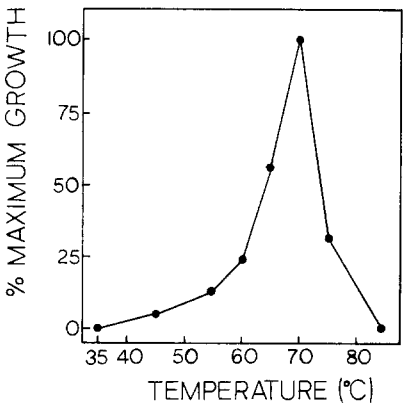

Fig. 3. Optimum temperature for growth of *T. brockii* strain HTD4 in TYEG medium

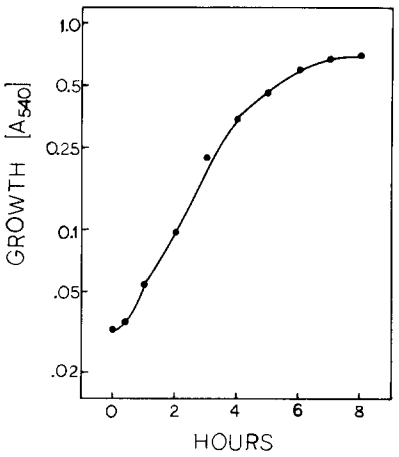

Fig. 4. Growth of *T. brockii* strain HTA1 on TYEG medium at 65° C

and carbon dioxide. End products not detectable in spent culture supernatants included: formic acid, succinic acid, butanediol, fatty acids larger than acetic, and alcohols other than ethanol. The pH optimum for growth of *T. brockii* strain HTD4 was about 7.5. No growth occurred when the initial culture medium pH was below 5.5 or above 9.5.

Nutritional studies were performed at 65° C using basal salts medium. Growth was not detected on basal salts medium that contained yeast extract (0.5%) and/or tryptone (0.5%). Growth required the presence of yeast extract (not replaced by tryptone) and a fermentable carbohydrate (see Table 2). Growth was proportional to the yeast extract concentration from 0.01 – 0.05% in basal salts medium that contained 0.5% glucose. The energy sources utilized by *T. brockii* are shown in Table 2. In addition to fermentation of various sugars, *T. brockii* also grew on insoluble starch as evidenced by H_2 production. This species did not hydrolyze or grow on proteins and did not grow via reduction of oxygen, sulfate, nitrate or fumarate.

Table 2. Organic substrates examined as energy sources for growth of *T. brockii*

	Growth (ΔA_{540})[a]	
Substrate[b]	HTD4	HTD6
Glucose	0.6	0.6
Sodium Pyruvate	0.2	0.3
Cellobiose	0.5	0.5
Lactose	0.5	0.2
Sucrose	0.4	0.4
Maltose	0.5	0.4
others tested[c]	0.0	0.0

[a] Growth determined after 16 h incubation at 65° C.
[b] Substrate was added (to 0.5%) to basal salts medium containing 0.1% yeast extract.
[c] Additions tested that did not serve as energy sources for growth: xylose, cellulose, arabinose, sodium tartrate, sodium lactate, ethanol, tryptone, casamino acids, pectin and mannose.

Table 3. Effect of antibiotics and inhibitors on growth of *T. brockii*

	% Inhibition of Growth[a]		
Agent[b]	HTA1	HTD4	*Clostridium pasteurianum*
Cycloserine	–	100	100
Penicillin	100	100	100
Streptomycin	94	96	100
Tetracycline	92	100	100
Chloramphenicol	100	100	100
Sodium Azide			
500 µg/ml	29	39	100
250 µg/ml	6	–	93
Sodium Chloride (2%)	82	84	73
Oxygen[c]	100	100	–

[a] Growth in TYEG medium ± agent was determined after 10 h incubation; 0% inhibition of growth represents a final A_{540} increase of 0.8 – 0.95 for the cultures examined. *T. brockii* was incubated at 65° C and *C. pasteurianum* at 37° C
[b] Final concentration; unless indicated, was 100 µg/ml
[c] Culture was incubated aerobically in a shake flask, sealed with a cotton stopper.

Strains HTD4 and HTD6, but not HTA1, produced sulfide from sulfite.

The sensitivity of various antibiotics and inhibitors on growth of *T. brockii* and *Clostridium pasteurianum* was compared (Table 3). *T. brockii* was sensitive to antibiotics that effectively inhibit cell wall synthesis and ribosome function in typical prokaryotes. *T. brockii* was noticeably more tolerant of sodium azide than *C. pasteurianum*. The possible detoxification of this substrate via decomposition at 65° C was not eliminated although growth of *T. aquaticus* at 70° C (Brock and Freeze, 1969) is completely inhibited by the concentrations of azide used here. Oxygen was an effective inhibitor of growth. However, cells posessed some

degree of O_2-tolerance because cultures exposed to air for 2 h retained their viability when incubated into fresh anaerobic culture tubes that contained TYEG medium.

Discussion

The discovery of *Thermoanaerobium* extends further the known diversity of caldoactive bacteria in nature. Previous studies (see Brock, 1978) on extreme thermophiles were limited to a few aerobic or facultative genera. *Thermoanaerobium* species should provide interesting material for evolutionary investigations (e.g., origin of thermophily, or origin of typical bacteria versus "Archaebacteria") because the organism is an obligately anaerobic, asporogenous chemoorganotroph. *Thermoanaerobium* is quite easily enriched and isolated from a variety of volcanic thermal features in Yellowstone National Park. Further ecological studies are required to establish that this organism is widespread in nature and an important agent of anaerobic metabolism in thermal features. Several trends that have been demonstrated in ecological studies of caldoactive aerobic microorganisms (Zeikus and Brock, 1972; Brock, 1978) appear applicable to anaerobic extreme thermophiles. Namely, species diversity within a particular metabolic group of thermophilic microorganisms (e.g., chemoorganotrophs) is limited and non-sporeforming rod-shaped bacteria predominate in caldoactive environments. Although not as widespread or predominant several *Clostridium* species, including strains similar to *C. thermohydrosulfuricum*, have been isolated (unpublished results) from Yellowstone National Park.

T. brockii appears to be a typical bacterium and not a member of the „Archaebacteria group" (Woese et al., 1978). Growth of this organism was inhibited by antibiotics that are effective against typical prokaryotes which contain peptidoglycan and 70S ribosomes. The only thermophiles known to belong to the "Archaebacteria group" are *Sulfolobus* and *Thermoplasma* (Woese et al., 1978), both of which are also obligate acidophiles, and *Methanobacterium thermoautotrophicum*. Nevertheless, the cell division process of *T. brockii* was not typical of other Gram positive bacteria. Standard fixation procedures used to demonstrate cell division in other anaerobic bacteria revealed a constrictive or "pinching off" type of division process in *T. brockii*. Septation has been the only form of division demonstrated in most Gram positive rods. Artifacts of fixation cannot be eliminated; and, Gilleland and Murray (1975) even suggest that in Gram negative rods (pseudomonads and enterics) constriction is an artifact.

The proper taxonomic disposition of *Thermoanaerobium* awaits further macromolecular characterization and then its evolutionary relatedness to other genera will be more definitive. Until then, it is suggested that *Thermoanaerobium* be placed in part 16 of Bergey's Manual (Buchanan and Gibbons, 1974) as a genus of uncertain affiliation. At first glance *Thermoanaerobium* may seen related to *Eubacterium*. Like its aerobic chemoheterotrophic counterpart in nature *Thermus*, which bears no defined relation to *Flavobacterium; Thermoanaerobium* may not be closely related to *Eubacterium*. In fact, the taxonomic relationships of *Eubacterium* to actinomycetes and other members of part 17 of Bergey's Manual appear to require revision. C. Woese (personal communication) suggests on the basis of 16S rRNA oligonucleotide homologies that *Eubacterium* is more closely related to *Clostridium* than actinomycetes. Unlike many saccharolytic eubacteria, *T. brockii* does not produce butyric or formic acids or large amounts of acetic acid. It is likely that other species of *Thermoanaerobium* exist. At first glance *T. brockii* may appear to be an asporogenous strain of *C. thermohydrosulfuricum* or *C. thermosaccharolyticum*. The DNA G+C content of both of these chemoheterotrophic anaerobic species (Matteuzzi et al., 1978) is similar to that of *T. brockii*, and the temperature range for growth of *C. thermohydrosulfuricum* (Hollaus and Sleytr, 1972) is nearly the same as *T. brockii*. *C. thermohydrosulfuricum* is recognized (Hollaus and Sleytr, 1972) as a species distinct from *C. thermosaccharolyticum* on the basis of possessing an hexagonal as opposed to a rectangular outer cell wall layer, producing H_2S from sulfite, and growing at higher temperatures. The lack of spore formation by *T. brockii* on similar media that results in sporulating cultures of *C. thermohydrosulfuricum* can not be used alone to designate a new species. It should be noted that spore formation is difficult to document in some clostridia (e.g. *C. perfringens*). Thus, the data presented do not rule out the possibility that the *Thermoanaerobium* species described here is an asporogenous *Clostridium* species. However, it is not now appropriate to name this new organism a *Clostridium*, since this logic would also necessitate taxonomic revision of *Eubacterium* and other Gram positive anaerobic rods that have not been demonstrated to possess endospores.

Examination of the data presented her indicate that *T. brockii* is a distinct species and different from *C. thermohydrosulfuricum*. First, the cell wall ultrastructure and the mechanism of cell division in these species differs. The cell wall achitecture of both *C. thermohydrosulfuricum* and *C. thermosaccharolyticum* (Sleytr and Glauert, 1976) consists of a double-layered wall with an outer wall layer composed of distinct subunits. *T. brockii* has a monolayered cell wall and cells appear to divide by a constrictive mechanism. Secondly, the catabolic machinery of *T. brockii* differs from *C.*

thermosaccharolyticum and *C. thermohydrosulfuricum* (Hollaus and Sleytr, 1972; Ljungdahl, 1979) in that xylose, mannose and pectin are not fermented, and butyric acid is not a detectable end product. In addition, several minor differences between this new species and *C. hydrosulfuricum* (Hollaus and Sleytr, 1972) are worth noting. *T. brockii* does not hydrolyze proteins, cells are nonmotile when observed by phase microscopy, and stationary grown cells may appear swollen but are not thinner than exponentially grown cells.

The catabolic activity of *Thermoanaerobium* strains may be of industrial interest. For example, *T. brockii* is able to grow on a variety of sugars, including insoluble starch, and produces largely ethanol and lactic acid as end products. Thus, this organism may be of use in chemical feedstock production via thermophilic fermentation of biomass. *Thermoanaerobium* strains may also be useful model organisms for understanding molecular mechanisms of thermophily. Under non-optimized conditions in fermentor cultures, doubling times of less than 30 min and growth yields of greater than 40 g wet weight/10l at 65° C are observed without significant loss of media due to evaporation.

Thermoanaerobium brockii gen. nov. and sp. nov. Ther.mo.an.ae.ro.bium Gr. n. *thermus* heat; *anaerobium*, M.D.n. life without air. brock-ii; M. L. gen. n. named for Thomas Dale Brock, who pioneered studies on physiological ecology of extreme thermophiles.

Morphology

Rods measuring 1.0 by 2–20 μm. Cells frequently uneven in length (mini-cells) and occurring in chains, pairs and filaments. Gram positive, and endospores not formed. *Colony Characteristics.* Colonies are uniformly round, mucoid, non-pigmented flat, and grow to a diameter of 0.2–0.3 cm in 48 h.

Cellular Characteristics. DNA base composition of 30.0–31.4 (± 1) mol % G + C. Cytochrome pigments and catalase absent. Mono-layered cell wall architecture without an outer wall membrane.

Temperature and pH Relationships.

Optimum temperature for growth 65–70° C, maximum < 85° C and minimum > 35° C. pH optimum for growth ~ 7.5, no growth above 9.5 or below 5.5.

Physiology. Chemoorganotroph. Utilizes a variety of saccharides as energy sources including starch, maltose, glucose, lactose, sucrose and cellobiose. Obligate anaerobe. Growth inhibited by air, penicillin, cycloserine, streptomycin, tetracycline and chloramphenicol. Fermentation end products are ethanol, lactic acid, acetic acid, hydrogen and carbon dioxide.

Habitat. Anaerobic thermal features associated with volcanic activity including springs, decomposing photosynthetic biomass and sediments.

Type Strain HTD4. This strain was isolated from a Washburn thermal springs edge sediment located in Yellowstone National Park, U.S.A. and has been deposited in the Deutsche Sammlung von Mikroorganismen, (DSM 1457), Göttingen (FRG), and the American Type Culture Collection (ATCC 33075), Rockville, MD.

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