Emendation of the Genus Thermobacteroides: Thermobacteroides proteolyticus sp. nov., a Proteolytic Acetogen from a Methanogenic Enrichment

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Thermobacteroides proteolyticus sp. nov. was isolated from a methanogenic enrichment culture inoculated from a thermophilic digestor (55°C) that was fermenting tannery wastes and cattle manure. This organism stains gram negative; it is a nonsporing bacterium which uses proteins and sugars as substrates. We propose the name Thermobacteroides proteolyticus sp. nov. for this organism.

MATERIALS AND METHODS

Inoculum and enrichment. Tannery wastes and cattle manure were digested under thermophilic conditions (55°C) at the Institut de Recherche de Chimie Appliquée, Vert le Petit, France. Digestor samples were inoculated into a 50% rumen fluid medium made up in the salt solution of Balch et al. (1) with the modifications of Balch et al. (1) were used throughout this work. Culture media and reagents were made anaerobically. Growth substrates used included yeast extract, peptone, casein, gelatin, and Trypsin case peptone. Fructose, glucose, maltose, sucrose, and mannose were weakly used as growth substrates; however, addition of yeast extract and either rumen fluid or Trypsin case peptone stimulated utilization of these carbohydrates. Acetate, H₂, and CO₂ were the major products of growth in medium containing gelatin or glucose. The cells were resistant to kanamycin. The type strain is strain BT (= ATCC 35242).

We report the isolation and characterization of a new thermophilic anaerobic species obtained from a methanogenic enrichment inoculated from a thermophilic digestor that was operated on tannery wastes and cattle manure. This organism stains gram negative; it is a nonmotile, pleomorphic, with some filamentous cells. The deoxyribonucleic acid base composition was 45 mol% guanine plus cytosine. The temperature optimum was 63°C (growth range 35, to 75°C); the pH optimum was 7.5 (growth range, pH 5.0 to 8.5). The growth substrates used included yeast extract, peptone, casein, gelatin, and Trypsin case peptone. Fructose, glucose, maltose, sucrose, and mannose were weakly used as growth substrates; however, addition of yeast extract and either rumen fluid or Trypsin case peptone stimulated utilization of these carbohydrates. Acetate, H₂, and CO₂ were the major products of growth in medium containing gelatin or glucose. The cells were resistant to kanamycin. The type strain is strain BT (= ATCC 35242).

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TABLE 1. Effect of yeast extract and rumen fluid on H₂ production from various substrates and growth of Thermobacillus proteolyticus

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Yeast extract*</th>
<th>Yeast extract + rumen fluid*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optical density</td>
<td>H₂ concn (μmol/10 ml of medium)</td>
</tr>
<tr>
<td>None</td>
<td>0.08</td>
<td>14.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.11</td>
<td>56.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.09</td>
<td>45.1</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.10</td>
<td>43.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.09</td>
<td>51.5</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.11</td>
<td>52.4</td>
</tr>
<tr>
<td>Trypticase</td>
<td>0.17</td>
<td>29.2</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.27</td>
<td>40.6</td>
</tr>
<tr>
<td>Peptone</td>
<td>0.38</td>
<td>24.9</td>
</tr>
<tr>
<td>Gelatin</td>
<td>0.32</td>
<td>24.7</td>
</tr>
<tr>
<td>Casein</td>
<td>0.21</td>
<td>23.2</td>
</tr>
</tbody>
</table>

* Sugar substrates were tested at concentrations of 10 mM; other compounds were tested at concentrations of 3 g/liter. We also tested the following compounds which did not support growth: xylose, b-glucose, maltose, lactose, trehalase, glycerol, mannool, and Casamino Acids.

* Yeast extract and rumen fluid were added at concentrations of 0.1% and 0.02% (v/v) respectively. Growth was measured by determining absorbance at 580 nm after 40 h at 65°C. The inoculum used was a 40-h culture grown on medium containing yeast extract and gelatin (1 g/liter).

RESULTS AND DISCUSSION

A non-methanogenic bacterium, strain BT (T = type strain), was isolated from a thermophilic, methanogenic enrichment culture. Table 1 shows that this organism grew well on peptides; fermentation of sugars in medium containing 0.1% yeast extract was poor unless rumen fluid was added. Trypticase peptone also stimulated the fermentation of glucose, but Casamino Acids did not (Table 2). Acetate was the major volatile acid produced (Table 3), and no significant quantities of ethanol, lactate, or succinate were formed.

Strain BT stained gram negative. Electron micrographs of thin sections revealed a thin inner wall layer and a heavy outer wall (Fig. 1).

Strain BT was resistant to kanamycin. In the presence of 300 mg/liter growth was reduced by 50%.

Strain BT differed from Thermoaerobium brockii (17) and Thermoaerobacter ethanolicus (16) by its Gram reaction, its substrate range, and its inability to use sugars readily in the presence of 0.1% yeast extract.

Strain BT might belong in the genus Bacteroides because it ferments proteins more strongly than sugars and does not use Casamino Acids. This latter physiological property is shared by two mesophilic Bacteroides species, Bacteroides asaccharolyticus (15) and Bacteroides melaninogenicus (13). B. asaccharolyticus grows on peptides but has a limited ability to ferment free amino acids (15); however, free amino acids...
Acids stimulate growth on peptides (8). Rumen fluid or Trypsin-case peptone stimulated the growth of strain BT\(^T\) on sugars, but Casein or 2-methylbutyrate did not. Trypsin-case peptone also stimulates glucose utilization in *Bacteroides ruminicola* (12), but synthesis of cell material is not always proportional to the amount of carbon and energy source utilized. A similar lack of proportionality was also observed when strain BT\(^T\) was grown on maltose and mannose. Regardless of these physiological similarities, strain BT\(^T\) cannot be placed within a recognized species of the genus *Bacteroides* because of its high growth temperature.

Recently, another thermophilic, nonsporeforming, gram-negative rod-shaped organism was described (3, 6). This organism was placed in a newly described genus, *Thermobacteroides*, as the type species, *Thermobacteroides proteolyticus*. *Thermobacteroides acetoneoehylicus* would have been placed in the genus *Bacteroides* except for its thermophilic nature. The high growth temperature was the primary reason for creating a new genus for this isolate. Thus, any new thermophilic isolates with the generic characteristics of *Bacteroides* should also be placed in the genus *Thermobacteroides*. For these reasons, we propose placing strain BT\(^T\) in the recently created genus *Thermobacteroides* as a separate species. No formal genus description has been given for *Thermobacteroides*. Therefore, we propose the emended genus description given below.

**Thermobacteroides** Ben-Bassat and Zeikus 1983, 673. (Ther.mo.bac.ter.o'i.des. Gr.n. thermus heat; M.L.n. bacte'rium a staff or rod; Gr.n. idus form, shape; Thermobacteroides rodlike thermophile) cells are gram-negative, nonsporeforming rods. Nonmotile or motile with peritrichous flagella.

Obligately anaerobic. Thermophilic: optimum growth temperature, 55 to 70°C; no growth occurs below 35°C.

*FIG. 2.* Phase-contrast photomicrograph of young cells grown on gelatin at 65°C. Bar = 10 μm.

**Thermobacteroides proteolyticus** sp. nov. *Thermobacteroides proteolyticus* (pro.te.o.ly'ti.cus. Gr. adj. protos first; Gr. adj. lyticus loosening, dissolving; N.L. masc.adj. proteolyticus proteolytic) cells are rod shaped and 0.5 by 1 to 6 μm and occur singly or in pairs in young cultures (Fig. 2); pleomorphic in old cultures. No lysis is observed in the stationary phase. Colonies in roll tubes are 1

**FIG. 3.** Effect of temperature on growth, as determined by measuring absorbancy at 580 nm after 40 h on 0.2% yeast extract medium.

The deoxyribonucleic acid guanine-plus-cytosine content ranges from 30 to 46 mol%.

Found in thermophilic anaerobic digestors and natural thermophilic environments where organic matter is being vigorously decomposed.

Type species: *Thermobacteroides acetoneoehylicus* Ben-Bassat and Zeikus 1983, 673.

We propose the species description below for *Thermobacteroides proteolyticus*.

**Thermobacteroides proteolyticus** sp. nov. (Ther.mo.bac.ter.o'i.des pro.te.o.ly'ti.cus. Gr. neut.n. bacterium a staff or rod; Gr.n. idus form, shape; Thermobacteroides rodlike thermophile) cells are gram-negative, nonsporeforming rods. Nonmotile or motile with peritrichous flagella.

Obligately anaerobic. Thermophilic: optimum growth temperature, 55 to 70°C; no growth occurs below 35°C.

**FIG. 4.** Effect of pH on growth of cells on 0.2% yeast extract. Absorbancy at 580 nm was determined after 24 h.
to 2 mm in diameter after 3 to 4 days; they are white, entire, circular, smooth, and convex. Gram negative. Nonmotile. Nonsporeforming.

Obligately anaerobic. Ferments peptone, gelatin, casein, and Trypticase peptone in the presence of 0.1% yeast extract. Grows on the following sugars when yeast extract and either rumen fluid or Trypticase peptone are added: glucose, fructose, maltose, sucrose, and mannose. The fermentation products from gelatin or glucose in the presence of yeast extract are acetic acid, \( \text{H}_2 \), and \( \text{CO}_2 \), along with smaller quantities of isobutyric, isovaleric, and propionic acids.

Optimum temperature, 63°C (range, 35 to 70°C) (Fig. 3). Optimum pH, 7.5 (range, pH 5.0 to 8.5) (Fig. 4).

Yeast extract is required for growth on protein substrates. Sugars are used poorly unless yeast extract and either rumen fluid or Trypticase peptone are added.

The guanine-plus-cytosine content of deoxyribonucleic acid is 45 mol%, as determined by buoyant density.

Isolated from a thermophilic (55°C) digester that was fermenting tannery wastes and cattle manure.

The type strain is strain BT (= ATCC 35242).

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FIA-MCS 2171 (from the Gas Research Institute and by research R.

fermenting tannery wastes and cattle manure.

Fluid or Trypticase peptone are added. Optimum pH, 7.5 (range, 35 to 70°C) (Fig. 3). Optimum pH, 7.5 (range, pH 5.0 to 8.5) (Fig. 4).

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LITERATURE CITED