***Coprothermobacter platensis* sp. nov., a new anaerobic proteolytic thermophilic bacterium isolated from an anaerobic mesophilic sludge**

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A new anaerobic, proteolytic, moderately thermophilic bacterium, strain 3R^T, was isolated from a methanogenic mesophilic reactor treating protein-rich wastewater. The cells were Gram-negative, non-spore-forming, non-motile rods. The DNA base composition was 43 mol% G+C. The optimum pH and temperature for growth were 7.0 and 55 °C respectively. The bacterium fermented gelatin, casein, bovine albumin, peptone and yeast extract. Glucose, fructose, sucrose, maltose and starch were poorly fermented. The major fermentation products from glucose were acetate, CO₂ and H₂ and, from gelatin, propionate was also detected. Growth on glucose was stimulated by thiosulfate, which was reduced to sulfide. Sulfate and nitrate were not reduced. 16S rRNA gene analysis revealed that the isolated bacterial strain was phylogenetically related to *Coprothermobacter proteolyticus* (96.3% sequence similarity), the only known species within the genus. DNA–DNA hybridization analysis demonstrated a very low level of homology, indicating that the isolated strain and *C. proteolyticus* were not related at species level. Therefore, it is proposed to classify the described strain in the genus *Coprothermobacter* as a new species, *Coprothermobacter platensis*. The type strain of *C. platensis* is strain 3R^T (= DSM 11748^T).

Keywords: *Coprothermobacter platensis* sp. nov., proteolytic bacteria, thermophiles, anaerobe, thiosulfate reduction

INTRODUCTION

Anaerobic digestion is increasingly used for carbon decontamination of agroindustrial wastewaters (Speece, 1996). Proteins are frequently a major component of such wastes, and their degradation, initiated by extracellular proteases, is often incomplete (McInerney, 1988). The vast majority of full-scale digestors are mesophilic, however thermophilic treatment is also being explored as it may have advantages, especially for effluents produced at high temperature (Lettinga, 1995). To start thermophilic reactors, mesophilic anaerobic sludge may be adapted by gradually increasing the incubation temperature (Van Lier *et al.*, 1993).

Although proteolytic activity is a common characteristic among mesophilic bacteria, very few anaerobic, thermophilic proteolytic bacteria have been characterized. In the past 10 years four novel genera have been described, namely *Thermobrachium* (Engle *et al.*, 1996), *Anaerobranca* (Engle *et al.*, 1995), *Caloramator*

(Tarlera *et al.*, 1997) and *Coprothermobacter* (Ollivier *et al.*, 1985; Rainey & Stackebrandt, 1993; Kersters *et al.*, 1994). Among them, the genus *Coprothermobacter* (formerly classified as *Thermobacteroides*) represents a very deep-branching phylum within the domain *Bacteria*, with only one species, *Coprothermobacter proteolyticus*, reported so far (Rainey & Stackebrandt, 1993). The other genera, *Anaerobranca*, *Thermobrachium* and *Caloramator*, are related to the clostridia, belonging to the large low G+C content branch of the Gram-positive division (Rainey *et al.*, 1993; Collins *et al.*, 1994).

In this paper, we describe a novel species within the genus *Coprothermobacter*, *Coprothermobacter platensis*, isolated from a methanogenic mesophilic reactor treating a protein-rich wastewater. Furthermore, we present evidence of the ability of both species of *Coprothermobacter* to use thiosulfate as an electron acceptor. Many mesophilic facultative anaerobes and strict anaerobes share this physiological trait. Among the thermophiles and hyperthermophiles of the do-

main *Bacteria*, it has been reported for the genera *Thermoanaerobacter* and *Thermoanaerobacterium* (Lee *et al.*, 1993) and more recently for *Thermotogales* (Fardeau *et al.*, 1997; Ravot *et al.*, 1995, 1996).

METHODS

Strains. *Coprothermobacter proteolyticus* BT^T (T = type strain) (DSM 5265^T) was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

Enrichment and isolation. A sample (5 ml) taken from the sludge of a mesophilic anaerobic digester treating wastewater from baker's yeast production in Montevideo, Uruguay, was anaerobically transferred to 50 ml of BCYT medium – a basal medium containing yeast extract (Difco; 1 g l⁻¹) and triptone (Difco; 1 g l⁻¹) (Touzel & Albagnac, 1983) – supplemented with glucose (5 g l⁻¹) and gelatin (Sigma; 5 g l⁻¹). Gelatin and glucose were used to enrich the medium because many thermophilic glycolytic anaerobes exhibit protease activity (Wiegel, 1992). This medium was prepared as previously described (Muxí *et al.*, 1992), sparged with N₂/CO₂ (80:20, v/v), autoclaved for 15 min at 121 °C and reduced with filter-sterilized sulfide-cysteine solution (Touzel & Albagnac, 1983). Enrichments were incubated at 55 °C and examined for growth with a phase-contrast microscope (Axioplan; Zeiss). Positive cultures were transferred periodically to fresh medium by using 10% (v/v) inocula. Isolation was performed in plates with the same medium solidified with agar (1.8%, w/v) and incubated at 55 °C in an anaerobic chamber (Coy Laboratory Products) with a gas atmosphere containing N₂/H₂/CO₂ (80:10:10, by vol.). After 10 d, a colony was picked and reisolated twice in the same medium. Purity was checked microscopically and by growth in the same medium incubated anaerobically and aerobically at 55 °C. A purified strain (3R^T) was grown under N₂ atmosphere, in pre-reduced PY broth medium – a basal medium containing peptone (Difco; 10 g l⁻¹) and yeast extract (Difco; 10 g l⁻¹) (Smibert & Krieg, 1994) – and stored at room temperature.

Substrate utilization and end product formation. Substrate utilization was determined in pre-reduced BC medium (BCYT medium without tryptone and with yeast extract 0.2 g l⁻¹). Stock solutions of soluble substrates (100 g l⁻¹) were anaerobically prepared, filter sterilized and anaerobically dispensed into tubes containing pre-reduced BC medium (10 ml) with a N₂ atmosphere. Insoluble substrates were weighed and transferred to culture tubes. Then, BC medium was dispensed (10 ml) into the tubes under an N₂ atmosphere and autoclaved. The complex substrates, bovine albumin (Sigma), xylan (Sigma), cellulose (Sigma), gelatin (Sigma), peptone (Difco), yeast extract (Difco), casein (Merck) and starch (Fluka), were added to a final concentration of 5 g l⁻¹. For the other substrates, a final concentration of 2 g l⁻¹ was used. Growth was measured spectrophotometrically (Genesys 5; Spectronic, Milton Roy) at 660 nm. In cultures containing insoluble substrates, growth was monitored by microscopic examination and by analysis of end products (volatile fatty acids). The effect of sulfate, nitrate and thiosulfate on growth was tested using BCYT medium, supplemented with equimolar concentrations of glucose (20 mM) and electron acceptor (20 mM).

Analytical procedures. Fermentation products, volatile fatty acids and alcohols were measured by HPLC using a refractive index detector (Waters Millipore) with an OA1000

(Alltech Associates) column. The mobile phase was H₂SO₄ (0.005 M), the flow rate was 0.8 ml min⁻¹ and the temperature was 35 °C. Hydrogen was measured by gas chromatography using a 14A gas chromatograph with a thermal conductivity detector (Shimadzu), equipped with a Carbo-sieve S II column and argon as the gas carrier. The flow rate was 30 ml min⁻¹ and the initial temperature was 35 °C, with an increase of 32 °C min⁻¹ up to 225 °C. Anions (nitrate, nitrite, sulfate) were measured by HPLC using a UV detector (Shimadzu) and an ICPAK ANION (Waters Millipore) column, with a flow rate of 1.2 ml min⁻¹ and a temperature of 42 °C. The mobile phase was phosphate buffer (0.01 M, pH 6.8). Sulfide was detected by the methylene blue method (Rand *et al.*, 1975). Gelatin was measured as described by Bradford (1976).

Growth conditions. Optimum pH, temperature and NaCl concentration ranges for growth were determined in PY medium, using a 5% (v/v) inoculum. The optimum pH was determined by incubating cultures at 55 °C at initial pH values from 4.3 to 9.1 adjusted by adding NaOH or HCl. The optimum concentration of NaCl was determined at pH 7 and 55 °C.

Susceptibility tests. Antibiotic resistance was determined in liquid PY medium at pH 7 and 55 °C. Antibiotics were filter sterilized and the following concentrations were tested: vancomycin (2.5 and 5.0 mg l⁻¹), neomycin (0.15 g l⁻¹), polymyxin B (20 and 40 mg l⁻¹), penicillin G (20 U ml⁻¹), kanamycin (300 and 600 ng ml⁻¹), sodium azide (0.5 and 1.0 g l⁻¹).

Protease assays. Protease activity was assayed using the azocasein method under anaerobic conditions (N₂ atmosphere) as described by Brock *et al.* (1982). The specific activity (in units) was expressed as µg azocasein hydrolysed h⁻¹ (mg bacterial protein)⁻¹. Protein was determined as described by Bradford (1976).

Electron microscopy. For electron microscopic studies, the culture was centrifuged for 5 min at 3000 r.p.m. The supernatant was discarded and the pellet fixed in glutaraldehyde (2%) in sodium cacodylate buffer (0.1 M) at pH 7.4 for 30 min. The process was repeated, the pellet was then washed in phosphate buffer (pH 7.4) and post-fixed with osmium tetroxide (1%). Specimens were dehydrated in an ascending gradient of ethanol (50, 70, 80, 90 and 95%) and then impregnated in propylene oxide. Finally, they were embedded in Poly/Bed 812 resin (Polysciences 18976-2590). Ultrathin sections were cut with an ultratome Super Nova (Reichert-Jung) and mounted in a copper grid, stained with uranyl acetate and lead citrate and examined in a JEM-1200 Ex II transmission electron microscope at 80 kV.

DNA base composition. DNA was isolated (Sambrook *et al.*, 1989) and the G + C content was determined by HPLC at the DSMZ (Mesbah *et al.*, 1989).

DNA–DNA hybridization. The genetic relatedness of strains was determined by DNA–DNA hybridization on nylon membranes (Johnson, 1991). Serial dilutions of DNA in denaturation solution (0.5 M NaOH, 1.5 M NaCl) were applied to a Pall Biodyne nylon membrane. The membrane was neutralized with 1.5 M NaCl, 0.5 M Tris/HCl pH 8 and baked for 2 h at 80 °C. Prehybridization (2 h) and hybridization (18 h) were performed at 65 °C in hybridization buffer containing 0.15 M NaCl, 1% (w/v) SDS, 0.3% skim milk, using as a probe chromosomal DNA from strain 3R^T, digested with the *AluI* endonuclease and ³²P-labelled using the Random Primers DNA Labelling System (Gibco BRL).

Fig. 1. (a) Phase-contrast micrograph of strain 3R^T grown on PY medium, showing straight rods occurring as single cells, in pairs or in chains. Bar, 10 µm. (b) Ultrathin section electron micrograph of strain 3R^T showing the cell wall structure. An intensely stained inner layer (i) and a less densely stained outer layer (o) were observed. Bar, 0.2 µm.

The results were scored by autoradiography. Similarity values were calculated as described by Johnson (1991).

Sequence analysis of 16S rDNA and phylogenetic analysis. DNA from strain 3R^T was purified (Sambrook *et al.*, 1989) and the ribosomal 16S genes were amplified by PCR (Johnson, 1994), using the following universal primers: 1492R (5'-GGTTACCTTGTTACGACTT-3'), corresponding to positions 1510–1492 in reverse *Escherichia coli* numbering, and 27F (5'-AGAGTTTGATCMTGGCTC-AG-3'), corresponding to positions 8–27 in forward *E. coli* numbering. The following temperature cycles were performed: 94 °C for 5 min, 30 cycles of 94 °C for 60 s, 60 °C for 60 s and 72 °C for 60 s, followed by a final 7 min incubation at 72 °C. The PCR products were purified using WIZARD PCR Preps columns (Promega). The PCR product was manually sequenced using the fmol DNA Sequencing kit (Invitrogen). The 16S rDNA sequence was aligned, using the CLUSTAL V program, with similar sequences belonging to various members of the domain *Bacteria* retrieved from the EMBL database and the Ribosomal Database Project (Maidak *et al.*, 1994). Only unambiguously aligned positions were used for phylogenetic analysis (1235 positions, from 227 to 1416 by *E. coli* numbering, were selected). An unrooted tree was constructed using the DNADIST (Jukes & Cantor option) and the NEIGHBOR-JOINING programs contained in the PHYLIP Phylogeny Inference Package, version 3.5 (Felsenstein, 1993). A bootstrap analysis (1000 replicates) was also performed using a program included in the same package.

RESULTS

Enrichment and isolation of strain 3R^T

The bacterial strain used in this study was isolated from a mesophilic anaerobic wastewater digester at a baker's yeast factory located in Montevideo, Uruguay. Bacterial growth was evident after incubation in glucose-gelatin medium at 55 °C within 5–7 d after inoculation. Microscopic examination of this primary enrichment culture revealed micro-organisms with diverse morphologies, but after eight subcultures small, rod-shaped cells were dominant. Purification was performed on agar plates incubated at 55 °C under anaerobic conditions. After 10 d incubation, colonies were about 1 mm in diameter, circular with smooth

margins, transparent to whitish. A single colony was picked and purified. This purified strain, designated 3R^T (T = type strain), was characterized.

Morphology and cell structure

Cells of strain 3R^T grown in PY medium were non-motile, straight rods, occurring singly or in pairs (Fig. 1a). They were generally 1.5–2 µm long and 0.5 µm wide. Long chains were also observed in old cultures. Spores were never observed, and the cultures did not survive a heat treatment of 2 h at 90 °C. Lysis was observed in late stationary phase. Young cultures of strain 3R^T stained Gram-negative but had a negative KOH test (Gregersen, 1978). Electron micrography of thin sections revealed a cell wall with an intensely stained inner layer and a less densely stained outer layer (Fig. 1b).

Physiological characteristics

(i) Growth requirements. Strain 3R^T required strictly anaerobic conditions for growth. The addition of 0.02% yeast extract was necessary for growth in medium with glucose as sole carbon source. The temperature range for growth at pH 7 was 35–65 °C, with an optimum at 55 °C. The pH range for growth at 55 °C was 4.3–8.3, with an optimum at 7.0. During growth at pH values close to neutrality, the pH decreased by not more than 0.4 units. The doubling time under optimal conditions in PY medium was 16.1 h. Growth was inhibited in PY medium supplemented with NaCl to a concentration of 0.4 M or higher.

(ii) Substrate utilization and fermentation products. Cells of strain 3R^T were proteolytic. Growth was observed using gelatin, casein, bovine albumin and other proteinaceous substrates, such as yeast extract and peptones, as energy sources. An extracellular protease activity was demonstrated for cells grown on gelatin. Protease activity increased during growth, showing a maximum of 116 ± 20 U at the end of the exponential growth phase.

Table 1. Effect of thiosulfate on growth of *Coprothermobacter proteolyticus* BT^T and strain 3R^T

Optical density, sulfide and glucose were measured after 7 d incubation at 55 °C for *C. proteolyticus* BT^T and after 9 d for strain 3R^T.

Growth conditions	<i>C. proteolyticus</i> BT ^T			Strain 3R ^T		
	OD ₆₆₀	H ₂ S (mM)	Glucose consumed (%)	OD ₆₆₀	H ₂ S (mM)	Glucose consumed (%)
Basal medium*	0.145	3.2	—	0.109	3.5	—
Basal medium + glucose†	0.387	3.2	58	0.210	3.8	19
Basal medium + glucose + thiosulfate†	0.756	20.6	100	1.132	23.5	100

* Basal medium BC supplemented with yeast extract (1 g l⁻¹) and tryptone (1 g l⁻¹).

† Glucose (20 mM) was used as substrate and thiosulfate (20 mM) as electron acceptor.

Table 2. Main characteristics of strain 3R^T, *Coprothermobacter proteolyticus* and *Thermobacteroides leptospartum*

Character	Strain 3R ^T	<i>C. proteolyticus</i> *	<i>T. leptospartum</i> †
Morphology	Short rods, pleomorph	Short rods, pleomorph	Long, thin rods
Optimum pH	7.0	7.5	7.5
Optimum temperature (°C)	55	63	60
Maximum temperature (°C)	65	70	71
G + C content (mol %)	43	45	43
Fermentation end products from glucose	Acetate, H ₂ , CO ₂	Acetate, H ₂ , CO ₂	Ethanol, acetate
Thiosulfate reduction to sulfide	+	+	NR
Proteolytic	+	+	+
Utilization of:			
Glucose	+	+	+
Sucrose	+	+	—
Fructose	+	+	—
Maltose	+	+	+
Xylose	—	+	+
Starch	+	+	NR
Growth in the presence of antibiotics			
Vancomycin (2.5 mg l ⁻¹)	—	+	+
Neomycin (0.15 g l ⁻¹)	—	—	—
Polymixin B (20 mg l ⁻¹)	—	+	—
Sodium azide (0.5 g l ⁻¹)	+	+	+
Kanamycin (600 ng l ⁻¹)	+	+	NR
Penicillin G (20 U ml ⁻¹)	+	—	+
Isolated from	Mesophilic anaerobic reactor	Thermophilic anaerobic reactor	Cattle manure

NR, Not reported.

* Data from this study, Ollivier *et al.* (1985) and Kersters *et al.* (1994).

† Data from Toda *et al.* (1987).

Cells of strain 3R^T were also able to grow on glucose, fructose, sucrose, maltose and starch. However, xylose, lactose, sorbitol, glycerol, inositol, xylans and cellulose were not fermented. After growth in BC medium supplemented with glucose (2 g l⁻¹), 10 % of the glucose was consumed and the major fermentation products were acetate (2.1 mM), hydrogen (2.3 mM) and CO₂ (not determined). After growth in BC medium supplemented with gelatin (5 g l⁻¹), 80 % of the gelatin

was consumed and the major fermentation products were acetate (5.6 mM), hydrogen (1.1 mM) and propionate (1.9 mM). Butyrate (4.6 mM), isobutyrate (traces) and isovalerate (traces) were detected in similar amounts as in control cultures in BC medium. Growth on glucose was not affected by the addition of nitrate or sulfate. However, thiosulfate had a pronounced effect on growth and glucose utilization by strain 3R^T and *C. proteolyticus* (BT^T) (Table 1).

Fig. 2. Phylogenetic tree derived from 16S rDNA sequence data analysis, showing the position of strain 3R^T. The bar corresponds to evolutionary distance of 0.1. The numbers shown next to the nodes indicate percentage bootstrap values from 1000 data sets. The EMBL accession numbers for the sequences used in the phylogenetic analysis were as follows: *Aquifex pyrophilus* strain Ko15a^T, M83548; *Chloroflexus aurantiacus* strain J-10-fl^T, M34116; *Coprothermobacter proteolyticus* strain ATCC 35245^T, X69335; *Deinococcus radiodurans* strain ATCC 35073, M21413; *Fervidobacterium islandicum* strain H21^T, M59176; *Thermotoga maritima* strain MSB8^T, M21774; *Thermomicrobium roseum* strain ATCC 27502^T, M34115; *Thermodesulfobacterium commune* strain YSRA-1^T, L10662; *Thermus aquaticus* strain X-1, X58340; *Hydrogenobacter thermophilus* strain TK-6^T, Z30214; *Methanococcus jannaschii* strain JAL-1^T, M59126.

(iii) **Antibiotic susceptibility.** Growth of strain 3R^T was inhibited by vancomycin (2.5 mg l⁻¹), neomycin (0.15 g l⁻¹) and polymyxin B (20 mg l⁻¹). Inhibition was not detected in the presence of penicillin G (20 U ml⁻¹), kanamycin (600 ng ml⁻¹) or sodium azide (0.5 g l⁻¹) (Table 2).

DNA base composition

The G + C content of strain 3R^T was 43 mol%.

Phylogenetic analysis

A total of 1446 nucleotides of the 16S rDNA gene were sequenced from positions 30 to 1481 according to *E. coli* numbering. Comparison with rDNA sequences available in databases revealed that strain 3R^T is related to *Coprothermobacter proteolyticus* and peripherally related to species of the genera *Thermotoga* and *Fervidobacterium*. The percentage sequence similarities, corrected for multiple changes by the method

of Jukes & Cantor, obtained from the phylogenetic distances matrix, were 96.3% for *Coprothermobacter proteolyticus*, 76.1% for *Thermotoga maritima* and 75.2% for *Fervidobacterium islandicum*, followed by *Thermodesulfobacterium commune* (73.4%) and *Hydrogenobacter thermophilus* (73.3%). According to the phylogenetic analysis (Fig. 2) strain 3R^T and *Coprothermobacter proteolyticus* are in the same clade. However DNA–DNA hybridization analysis demonstrated less than 12% similarity between the chromosomal DNAs of these bacteria.

DISCUSSION

The new isolate, strain 3R^T, is an anaerobic, moderately thermophilic, proteolytic bacterium. To our knowledge, six anaerobic, thermophilic, proteolytic micro-organisms of the domain *Bacteria* have been described in the past 10 years: *Coprothermobacter proteolyticus* (Rainey & Stackebrandt, 1993), formerly

Thermobacteroides proteolyticus (Ollivier *et al.*, 1985), *Thermobacteroides leptospartum* (Toda *et al.*, 1988), *Clostridium* P2 (Örlygsson, 1994), *Anaerobranca horikoshii* (Engle *et al.*, 1995), *Thermobrachium celere* (Engle *et al.*, 1996) and *Caloramator proteoclasticus* (Tarlera *et al.*, 1997). The last four belong to the large low G+C content branch of the Gram-positive division of the domain *Bacteria* (Rainey *et al.*, 1993; Collins *et al.*, 1994). The phylogenetic position of *Thermobacteroides leptospartum* remains unknown, while *Coprothermobacter proteolyticus* belongs to one of the deepest divisions of the domain *Bacteria* (Rainey & Stackebrandt, 1993).

The phylogenetic analysis indicated that strain 3R^T clusters with *Coprothermobacter proteolyticus*. This result was supported by the high bootstrap value (100% of 1000 replicates). The closest relatives are *Thermotoga maritima* and *Fervidobacterium islandicum*, both members of the order *Thermotogales*. However, the phylogenetic relationship between the genus *Coprothermobacter* and the *Thermotogales* is still uncertain, as suggested by the low bootstrap value (69.5%) and the short evolutionary distance of the common branch.

The level of 16S rDNA sequence similarity between strain 3R^T and *C. proteolyticus*, the only species in the genus so far, was 96.3%. It has been proposed that strains sharing less than 97% similarity usually do not belong to the same species (Stackebrandt & Goebel, 1994). Furthermore, the level of chromosomal DNA–DNA homology between the strains, as measured by hybridization techniques, was less than 12%, far below the threshold value of 70% recommended for species delineation (Wayne *et al.*, 1987). According to these results, strain 3R^T can be considered as a new species within the genus *Coprothermobacter*.

Table 2 shows some characteristics of the two species of the genus *Coprothermobacter* and of *Thermobacteroides leptospartum*, which originally belonged to the same genus as *Coprothermobacter proteolyticus*. There are no significant differences in optimum pH and G+C content of the chromosomal DNAs. Strain 3R^T and *Coprothermobacter proteolyticus* show similar morphology and fermentation products, differing in both aspects from *Thermobacteroides leptospartum*. Strain 3R^T differs from *C. proteolyticus* and *Thermobacteroides leptospartum* in optimum temperature for growth, antibiotic susceptibility and sugar utilization.

We also tested the ability of strain 3R^T and *Coprothermobacter proteolyticus* strain BT^T to reduce thiosulfate with glucose as substrate. Thiosulfate was reduced to sulfide by both strains, and its addition clearly stimulated glucose utilization and growth, as was reported for, among others, *Thermotoga*, *Fervidobacterium* (Fardeau *et al.*, 1997; Ravot *et al.*, 1995) and *Thermosipho* species (Ravot *et al.*, 1996). This property has not been previously reported for members of the genus *Coprothermobacter*. Further studies are necessary to elucidate the mechanism of thiosulfate

reduction by bacteria of the *Coprothermobacter* genus as well as its ecological relevance.

According to the physiological and phylogenetic characteristics of strain 3R^T, we propose the creation of a new species within the genus *Coprothermobacter* to be named *Coprothermobacter platensis* sp. nov.

Description of *Coprothermobacter platensis* sp. nov.

Coprothermobacter platensis (pla.ten'sis. L. masc. adj. pertaining to Rio de la Plata, a river between Uruguay and Argentina, the region from where the strain was isolated).

Cells are straight rods, 0.5 × 1.5–2.0 µm, that occur singly or in pairs in young cultures. Long chains are present in old cultures. Lysis is observed in the stationary phase. Colonies in PY agar plates are circular, 1 mm in diameter, with an entire border, transparent to whitish. Stains Gram-negative but the cell wall under the electron microscope is atypical with a dense inner layer and a less dense outer layer. Non-motile, non-spore-forming. Obligately anaerobic. Proteolytic. Ferments gelatin, casein, bovine albumin, peptone and yeast extract. Glucose, fructose, sucrose, maltose and starch are poorly fermented. Fermentation products from glucose are acetate, H₂ and CO₂. The major fermentation products from gelatin are acetate, propionate, H₂ and CO₂. Growth on glucose is stimulated by thiosulfate, which is reduced to sulfide. Sulfate and nitrate are not reduced. Moderately thermophilic, optimum temperature 55 °C (range 35–65 °C). Optimum pH 7.0 (range 4.3–8.3). Yeast extract is required. Growth is inhibited by vancomycin (2.5 mg l⁻¹), neomycin (0.15 g l⁻¹) and polymyxin B (20 mg l⁻¹). Resistant to penicillin G (20 U ml⁻¹), kanamycin (600 ng ml⁻¹) and sodium azide (0.5 g l⁻¹). NaCl, 0.4 M or higher, is inhibitory. The G+C content of DNA is 43 mol% as determined by HPLC. Phylogenetically closely related to *Coprothermobacter proteolyticus* according to the 16S rDNA sequence analysis. Both are included in one of the earlier branches of the domain *Bacteria*. Isolated from a mesophilic upflow anaerobic sludge blanket reactor of a baker's yeast factory. The type strain is strain 3R^T (= DSM 11748^T).

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