Designation of Neotype Strains for *Bacteroides amylophilus* Hamlin and Hungate 1956 and *Bacteroides succinogenes* Hungate 1950

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Bacteroides amylophilus ATCC 29744 and *B. succinogenes* ATCC 19169 are described and here designated the neotype strains of these two species.

Bacteroides amylophilus and *B. succinogenes* have been shown to be important in metabolic activities in rumens: *B. amylophilus* in the digestion of starch and *B. succinogenes* in the degradation of cellulose (2, 13, 15-17). Each of these organisms was well described when first isolated, but type strains were not formally designated, and the original isolates on which the descriptions were based have been lost.

In 1956, Hamlin and Hungate (13) described and named *B. amylophilus*, a new bacterial species that they had isolated from a bovine rumen. Although they sent cultures of the species to the American Type Culture Collection (ATCC), the strains were reported not to be viable when received by the ATCC. However, with the extensive description published by Hamlin and Hungate, strains later isolated by other investigators (1, 7, 10, 14) could be identified with confidence as members of this species.

In 1950, R. E. Hungate (16) described in great detail organisms that he had isolated earlier (15) from a bovine rumen, and he regarded them as belonging to a new species which he named *B. succinogenes*. Hungate did not designate a type strain for the species. However, since these bacteria were abundant in the rumen, fresh isolations were made, and many studies were made of their nutritional requirements and their activities (2-11). In 1953, a strain isolated by Bryant and Doetsch (5) from the rumen of a heifer was found to have the characteristics of *B. succinogenes* as reported by Hungate. This strain (S85) was deposited in the ATCC (ATCC 19169) as a representative strain of that species but was not formally designated as the neotype strain.

The purpose of this paper is to designate ATCC 29744 the neotype strain of *B. amylophilus* and ATCC 19169 the neotype strain of *B. succinogenes*, in accord with the rules of the *Bacteriological Code* (18).

MATERIALS AND METHODS

Bacterial strains. *B. amylophilus* ATCC 29744 (← VPI 2502B ← Bryant [Hobson H18] ← Hobson

H18) was isolated from a sheep rumen and described by Blackburn and Hobson (Rowett Research Institute, Aberdeen, Scotland) (1) in 1962. Characteristics reported for this strain were similar to those of *B. amylophilus* as listed by Hamlin and Hungate (13). *B. succinogenes* ATCC 19169 (← Bryant S85 → VPI 12249) was isolated from a bovine rumen by Bryant and Doetsch and described in 1954 (5).

Media and methods. The morphological characteristics and biochemical reactions of these strains were determined with media and by methods described previously (14) except where otherwise indicated. The basal broth medium used throughout (PY) contained (per 100 ml) 0.5 g of peptone, 0.5 g of Trypticase, 1.0 g of yeast extract, 0.5 mg of crystalline equine hemin, 0.1 µl of vitamin K₁ (Sigma Chemical Co.), salts solution, resazurin, and cysteine as previously described (14). All media used to determine the reactions of *B. succinogenes* were supplemented with 30% (vol/vol) sterile prereduced rumen fluid and 0.4% Na₂CO₃ (PYRC basal medium). An atmosphere of 10% CO₂ and 90% hydrogen was used for anaerobic incubation of plates.

RESULTS AND DISCUSSION

Bacteroides amylophilus Hamlin and Hungate 1956, 552 (later synonym: *Ruminobacter amylophilus* [Hamlin and Hungate] Prévot 1966, 121 [19]).

The cells of *B. amylophilus* ATCC 29744, after incubation for 2 days in peptone-yeast extract-maltose broth, were pleomorphic, gram-negative, oval to long rods with tapered or round ends and included some swollen forms and irregularly curved cells, 0.9 to 1.2 µm wide and 1.1 to 8.0 µm long.

The organism was strictly anaerobic and non-motile. It did not form spores or survive heating at 80°C for 10 min. Only bicarbonate or CO₂, a fermentable carbohydrate, and minerals were required for growth. NH₃ was essential as a nitrogen source; amino acids were not used. Of the sugars tested, only starch, maltose, glycogen, or dextrin would support growth. Glucose was not utilized and rumen fluid was not stimulatory. Growth was equally good at 37 and 45°C; there was no growth at 25 or 30°C.

Colonies in laked-blood roll-streak tubes after incubation for 4 days were pinpoint to 2 mm in diameter, circular, entire to slightly erose, convex to umbonate, translucent, colorless, shiny, and smooth. No internal structure was visible. Growth was very sparse on freshly prepared blood agar plates incubated anaerobically, and no colonies appeared on egg yolk agar plates.

Maltose broth cultures were turbid with a stringy sediment; the final pH after 5 days of incubation was 5.45. Products of the fermentation of maltose were (in milliequivalents per 100 ml of culture): succinic acid, 3.0; formic acid, 0.9; acetic acid, 0.7; and lactic acid, 0.3.

The reactions of this strain after 5 days of incubation and the reactions of this species as described by Hamlin and Hungate (13) are listed in Table 1. They reported that gelatin, when added in a 5% concentration to peptone-yeast extract-starch broth, was hydrolyzed by some strains. ATCC 29744 did not hydrolyze 5% gelatin plus 0.1% glucose but completely liquefied 5% gelatin plus 0.1% maltose after 4 days of incubation.

In addition to the reactions in Table 1, ATCC 29744 produced acid from dextrin and glycogen, as was reported by Blackburn and Hobson (1). There was no growth in the basal broth medium (14) or in the basal medium with adonitol, amygdalin, dulcitol, erythritol, inositol, melezitose, melibiose, pectin, ribose, sorbitol, sorbose, or trehalose; milk was not curdled or digested; lactate, pyruvate, gluconate, and threonine were not utilized; catalase was not formed; cellulose was not digested.

The normal habitat of *B. amylophilus* is the bovine or ovine rumen, where it may be one of the principal starch digesters (1, 17).

The guanine-plus-cytosine content of the deoxyribonucleic acid of ATCC 29744 is 42.1 mol% (20).

The characteristics of ATCC 29744 agree entirely with those given in the original description of *B. amylophilus* by Hamlin and Hungate (13). This strain is therefore designated the neotype strain of *B. amylophilus* Hamlin and Hungate.

Bacteroides succinogenes Hungate 1950, 13 (later synonym: *Ruminobacter succinogenes* [Hungate] Prévot 1966, 122 [19]).

The cells of *B. succinogenes* ATCC 19169, after 24 h in PY-glucose-30% rumen fluid-Na₂CO₃ broth, were coccoid to oval in shape, gram negative, 0.8 to 1.6 µm wide and 0.9 to 1.6 µm long, and occurred mostly singly or in pairs but occasionally in short chains.

The organism was strictly anaerobic and non-motile. It did not form spores or survive heating at 80°C for 10 min. In earlier studies (5) of the

exact nutritional requirements supplied by the rumen fluid and yeast extract, a branched-chain fatty acid (either isovaleric or isobutyric) and a straight-chain fatty acid (either butyric or valeric) as well as biotin, CO₂, NH₃, sulfide or cysteine, and some minerals including sodium were found to be essential for growth (3, 6-8). The organism did not use amino acid nitrogen. Growth was best at 37°C and moderate at 30°C. There was no growth in 24 h at 25 or 45°C.

Colonies in rumen fluid-glucose-cellobiose-agar roll tubes (14) after 3 days of incubation were punctiform, circular, entire, convex, transparent, colorless, shiny, and smooth. No internal structure was visible. No individual colonies appeared on freshly prepared blood agar plates incubated anaerobically, although a film appeared on the surface and a few cells were seen in a Gram stain. There was no growth on blood agar plates incubated aerobically or under 5% CO₂ or on egg yolk agar plates incubated anaerobically.

The most significant attribute of these organisms, both taxonomically and economically, is their ability to degrade cellulose. In our first

Table 1. Biochemical characteristics of *Bacteroides amylophilus*^a

Reaction	Hamlin ^b and Hungate (13)	ATCC ^b 29744
Hydrolysis of:		
Gelatin	variable	+
Starch	+	+
Fermentable carbohydrate required	+	+
Products of carbohydrate fermentation	ASF(L) ^c	SFAI
Final pH in maltose broth	5.5-5.7	5.5
Acid from:		
Maltose	+	+
Starch	+	+

^aSymbols: -, negative reaction; +, positive reaction (carbohydrate cultures, pH < 5.5); A, acetic acid; S, succinic acid; F, formic acid; L, lactic acid.

^bNo acid produced from arabinose, cellobiose, esculin, fructose, galactose, glucose, glycerol, inulin, lactose, mannitol, raffinose, rhamnose, salicin, sucrose, or xylose; indole, hydrogen sulfide and acetylmethylcarbinol not produced; nitrate not reduced; no hydrogen detected from fermentation of maltose.

^cProducts are listed in order of relative amounts detected. Small letter indicates trace amount. (), Product sometimes detected.

attempts to demonstrate cellulolytic ability of ATCC 19169, we used pure cellulose fiber, ground and supplied to us by Celanese Corp., as a substrate in the basal medium. There was no visible growth in this medium and no apparent disappearance of the cellulose over a period of 3 weeks.

We were equally unsuccessful in demonstrating cellulose digestion with either Whatman no. 1 filter paper strips or Whatman chemically prepared cellulose powder.

Forsberg (12) has recently shown that *B. succinogenes*, in pure culture, is one of the rumen organisms most sensitive to trace elements such as mercury, copper, chromium, selenium, nickel, cadmium, and arsenic. Since any or all of these could be present in the water in our area, or since there might be some volatile inhibitory agent present, we took special care in distilling the water used in making cellulose medium, collected it at 90°C, and prepared the medium as soon as possible after collection. With 0.2% (wt/vol) Whatman ashless cellulose powder (standard grade) and 0.01% cellobiose as a growth booster, the cellulose was visibly and microscopically about 80% digested in 7 days.

During these studies, it was suggested by P. J. Van Soest that native cellulose such as that in carrots, radishes, or turnips should be tested as a substrate. Accordingly, we added small, thin strips (ca. 2 mm by 0.5 cm by 3 cm) of each of these raw vegetables to the basal medium before sterilization and repeated the test. Although growth was good and digestion of the substrate was evident in all of the vegetable broths, the results were most dramatic in the broth with the turnip strip. Disintegration of the strip was evident within 24 h, and only small bits were apparent after incubation for 5 days. By 11 days, no gross structures were visible in the broth.

According to Van Soest, turnips have about 10% dry matter. The approximate composition on a dry matter basis is 20% cellulose, 20% pectin (calcium-pectate), 2 to 4% lipid, 20 to 30% sugar (principally sucrose), 20% protein, and ash. The plant cell walls contain the native cellulose and the intracellular layer contains primarily pectin.

To establish that the degradation of the turnip strip was a result of cellulose digestion and not of pectin fermentation, *B. vulgatus* (VPI B8-11), a pectin fermenter, was used as a control culture. With *B. vulgatus*, the turnip slice broke in two within 5 days and by 11 days was reduced to large flakes. The absence of cellulose digestion was confirmed by microscopic examination of the culture under phase contrast (low-power magnification: $\times 100$). Many whole, undigested cell walls were readily apparent, and none

showed any evidence of destruction (Fig. 1a). However, in the *B. succinogenes* culture, careful microscopic examination revealed no intact cell walls and only a few partially digested plant cell wall fragments (Fig. 1b).

Determination of cellulolytic activity with turnip slices appears to be a simple and sensitive procedure. Turnip cellulose may more nearly represent the natural substrates of cellulolytic bacteria than highly crystalline or ball-milled cellulose. However, careful attention should be given to possible limitations presented by a possible sparing effect of the other carbohydrates in the structure. Gross disintegration of the plant tissue may be caused by destruction of pectin, and therefore microscopic examination is required to confirm cellulose digestion of the plant cell walls.

We do not know whether the *B. succinogenes* cells digested the turnip pectin or even whether pectin digestion is necessary for destruction of the tissue integrity or for exposing the plant cell wall cellulose for digestion. Although *B. succinogenes* showed no activity on citrus pectin (ICN Pharmaceuticals, Inc., Cleveland, Ohio) as a substrate (Table 2), bacterial pectinolytic activity differs with apple, potato, and presumably other pectins such as turnip pectin.

Cellobiose broth cultures of ATCC 19169 were turbid with a smooth sediment. The final pH after 4 days of incubation was 5.4. The products of the fermentation of cellobiose were (in milliequivalents per 100 ml): succinic acid, 6.4; and acetic acid, 1.2.

The reactions of ATCC 19169 after 13 days of incubation in PYRC-based media and the reactions of this species as described by Hungate (16) are listed in Table 3. Although the original isolates were reported to ferment starch, mal-

FIG. 1. Turnip cells after 11 days of incubation with: (a) *B. vulgatus*; (b) *B. succinogenes*, photographed under phase-contrast microscope (low power: $\times 10$ objective). Bar = 0.3 mm.

Table 2. *Substrate utilization by B. succinogenes and B. vulgatus*

Medium	Amount of growth ^a	Final pH	Digestion of turnip cell walls
Uninoculated			
PYRC ^b	—	6.4	
PYRC-turnip	—	6.2	—
PYRC-citrus pectin	—	5.8	
ATCC 19169			
PYRC	+	6.1	
PYRC-turnip	+++	5.6 ^c	++++
PYRC-citrus pectin	+	5.9	
<i>B. vulgatus</i>			
PYR	+++	6.2	
PYR-turnip	++++	5.1	—
PYR-citrus pectin	++++	5.2	

^aGrowth estimated on a + to ++++ basis.^bPYRC = PY-30% rumen fluid—Na₂CO₃.^cProducts of the fermentation of turnip were (in meq/100 ml): succinic acid, 8.14; acetic acid, 0.97; a trace of formic acid.Table 3. *Biochemical characteristics of Bacteroides succinogenes*^a

Reaction	Hungate ^b (16)	ATCC ^b 19169
Acid from:		
Cellulose	+	+
Cellulose	+	+
Dextrin	+	—
Glucose	+	+
Lactose	—	w
Maltose	+	—
Starch	+	—
Trehalose	—	—
Products of cellulose fermentation:		
Acids	SAf ^c	SAf

^aSymbols: —, negative reaction; +, positive reaction (culture pH below 5.5); w, pH between 5.5 and 6.0; S, succinic acid; A, acetic acid; F, formic acid.^bNo acid was produced from arabinose, dulcitol, fructose, galactose, glycerol, inositol, inulin, mannitol, mannose, melezitose, melibiose, pectin, raffinose, rhamnose, salicin, sorbitol, sucrose, or xylose; no hydrogen or carbon dioxide was detected from cellulose fermentation.^cProducts are listed in order of relative amounts. Small letters indicate trace amounts.

tose, trehalose, and dextrin, Hungate later stated (17) that fermentation of these, as well as of lactose or pectin, may be variable. We found, as was previously reported by Bryant and Doetsch (5), that ATCC 19169 did not ferment xylan, gum arabic, or esculin, did not produce indole or acetylmethylcarbinol, and did not reduce nitrate or hydrolyze gelatin.

In addition to these reactions, ATCC 19169 did not ferment adonitol, amygdalin, erythritol, glycogen, ribose, or sorbose. There was no change in milk and no growth in lactate, pyruvate, threonine, or gluconate broth. H₂S was not formed in SIM medium (BBL Microbiology Systems).

The normal habitat of *B. succinogenes* is the rumen.

The guanine-plus-cytosine content of the deoxyribonucleic acid of ATCC 19169 is 42.8 mol% (20).

Since the characteristics of ATCC 19169 agree essentially with Hungate's original and later descriptions of *B. succinogenes* (15–17), this strain is here designated the neotype strain of *Bacteroides succinogenes* Hungate.

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REPRINT REQUESTS

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