Clostridium celatum sp.nov., Isolated from Normal Human Feces

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Clostridium celatum sp.nov. was isolated from a number of fecal specimens at concentrations of 10^3 to 10^6 per gram (wet weight). The isolates are nonmotile, saccharolytic, nontoxic, nonhemolytic, and non-proteolytic. They reduce sulfite and nitrite and produce urease but no lecithinase or lipase; the major organic acids produced in peptone-glucose cultures are acetic and formic. American Type Culture Collection no. 27791, GD-1, is designated the type strain.

During enumerations of fecal *Clostridium perfringens* spores (6) we encountered, in 14 out of 60 stool specimens from healthy adults, a nonmotile sulfide- and nitrite-producing clostridium which might be falsely identified as *C. perfringens* by the confirmatory nitrite motility test of Angelotti et al. (1). The spore concentrations per gram (wet weight) of feces varied from log 2.9 to log 5.8 and often exceeded those of *C. perfringens* by 1 to 2 logs.

The present work was undertaken to characterize our recent clostridial isolates and, if possible, to relate them to a known species.

MATERIALS AND METHODS

The methods used for determining some of the characteristics of the clostridial isolates are listed in Table 1. Some additional comments are warranted. (i) To ascertain production of sulfide from sulfite, the isolates were also incubated in SFP (13) agar without metabisulfite; no black colonies were formed. (ii) Nitrite formation in supplementary NM (4) agar was determined after 40 h of incubation. (iii) For toxicity tests, 24-h cultures in CP-2V (7) and 24- and 72-h cultures in chopped meat glucose (8) were centrifuged at $10,000 \times g$ for 10 min; 0.5-ml volumes of supernatant fluid were injected intravenously and in triplicate into 20- to 22-g white mice; these were observed for 4 days. (iv) For determining lecithinase in SFP agar, the plates were incubated without overpour (6). (v) Hemolysis was determined on human, bovine, sheep, and rabbit blood agar (6). (vi) The incubation temperature was 37 C.

The concentration of carbohydrates and polyalcohols in peptone yeast (PY) medium (8) were from 0.5 to 1.0%. The pH decreases were measured after 3 days of incubation. Free and methylated organic acids were determined chromatographically from 3-day cultures (8). Hydrogen production was determined by

chromatographic analysis of head gas from PY-glucose broth cultures (10).

Only a few nonmotile sulfide- and nitrite-producing clostridial species have been reported (2, 8, 9, 12). Strains of other species described as nonmotile and nitrate- or sulfite-reducing clostridia, or as closely related to *C. perfringens*, were also tested for the characteristics listed in Table 1. Cultures tested included several strains of *C. perfringens* and strains of *C. perenne* (Prévot 1116D), *C. absonum* (Nakamura HA-7103, -7107, -9103), *C. paraperfringens* (Nakamura 3-3, G), and two strains (1236 and 5223) of *C. barati* (*Inflabilis barati*) received from the Pasteur Institute (Paris). [*C. paraperfringens* has been proposed (11) as the legitimate name for strains formerly designated *I. barati* (Prévot) and *C. barati* (Prévot) Holdeman and Moore.] We also examined strains labeled *I. indolicus* (Pasteur Institute PeI) and *I. lacustris* (Pasteur Institute 12/10, 1022D, 06/43D, 6/33B, 9/43C, 12/16C) and isolates of Mead and Chamberlain (9) from pheasant intestine.

RESULTS AND DISCUSSION

Our isolates from the 14 stool specimens were nonmotile, nitrite- and sulfide-producing, obligately anaerobic gram-positive rods which formed long filaments (up to 0.2 mm in CP-2V) and grew as flat colonies with irregular edges on blood agar. Large central, subterminal, or terminal spores were produced. The strains were nontoxic for mice, nonhemolytic, produced no opaque zones on SFP agar, and did not liquefy gelatin. Four strains were examined in greater detail, and some of their characteristics are summarized in Table 1.

The isolates were distinctly different from all the strains of related clostridia that were examined. Strains of *C. perfringens*, *C. perenne*, *C. paraperfringens* (*C. barati*), and *C. absonum*

TABLE 1. *Characteristics of four isolates of C. celatum*^a

Characteristic	Method	Isolate			
		GD-1 ^b	OD	AD	HD-1
Motility	(i) Supplemented NM agar (4) (ii) Slide method (8)	—	—	—	—
Flagella	Leifson's flagella strain (8)	—	—	—	—
Reduction of: sulfite to sulfide	Black colonies in SFP agar (11)	+	+	+	+
nitrate to nitrite	(i) Indole nitrite medium (8)	+	—	+	—
	(ii) Supplemented NM agar (4)	+	+	+	+
Spore formation	Blood agar (5), E broth, CMC broth (8)	+	+	+	+
Toxicity	24-h culture in CP-2V (7)	—	—	—	—
Hemolysis	(6)	—	—	—	—
Liquefaction of gelatin	Lactose gel. (5), gel. medium (8)	—	—	—	—
Digestion of casein	Casein agar (8)	—	—	—	—
Production of: lecithinase	Opaque zone on SFP (11), EYA (8)	—	—	—	—
lipase	Iridescent sheen on EYA (8)	—	—	—	—
catalase	Gas from H ₂ O ₂ on EYA (8)	—	—	—	—
urease	Growth from EYA in PY-urea broth (8)	+	+	+	+
indole	Ehrlich reagent (8)	—	—	—	—

^a Abbreviations: CMC, chopped meat carbohydrate; gel., gelatin; EYA, egg yolk agar.

^b Type strain (ATCC 27791 = NCTC 10947).

were hemolytic and produced lecithinase and large amounts of butyric acid; the pheasant isolates were hemolytic and produced lecithinase. The strain labeled *I. indolicus* was hemolytic, produced indole, and digested casein. The six strains labeled *I. lacustris* produced lecithinase and indole, digested milk and meat, and did not ferment lactose or sucrose; these strains appear to be nonmotile variants of *C. sordellii* or *C. bifermentans*. In addition, our isolates differed from the description of *I. lacustris* (12) in that they did not coagulate milk rapidly, ferment glycerol, or produce NH₃.

As discussed previously (6), it seems paradoxical that a clostridium which is as numerous as *C. perfringens* in many normal stools could not be identified. Similarly, Debono (3) described as "*Bacillus regularis filiformis*" a "common" clostridial isolate from normal feces that has apparently not been isolated from feces since 1912. Debono's isolates and ours are both nonmotile, form long filaments, and do not liquefy gelatin. However, according to Prévot (12), a strain that he isolated from tomato preserves and identified as *Inflabilis filiformis* ["*Bacillus regulares filiformis*" (Debono), *C. filiforme* (Debono) Bergey et al.] did not reduce nitrate to nitrite or sulfite to

sulfide. Apparently, no strains of this species are extant.

Therefore, our isolates appear to represent a new species, for which we propose the name *Clostridium celatum*.

Clostridium celatum sp.nov. [ce.la'tum. L. adj. *celatum* hidden].

The cells are gram-positive, nonmotile rods, 0.9 to 3.0 by 6.3 to >200 µm in diameter (filaments), with large central, subterminal, or terminal spores (Fig. 1 and 2). Spore formation was confirmed by growth in PY-starch medium which had been inoculated with spore cultures and placed in boiling water for 10 min (8).

After anaerobic incubation for 2 days, surface colonies are 2 to 4 mm in diameter, circular, lobate to erose, low convex to flat, and opaque with granular or mottled appearance. There is no growth on the surface of blood agar plates incubated in a candle jar or aerobic atmosphere. PY-glucose cultures are turbid with smooth sediment and a pH value of 5.2 to 5.5 in 24 h. There is poor growth in medium without a fermentable carbohydrate. The optimal temperature for growth is 37 C. There is usually good growth at 30 C, poorer growth at 45 C, and no growth at 25 C. Growth is inhibited by 6.5% NaCl and 20% bile.

FIG. 1. Vegetative cells of *C. celatum* GD-1; 24-h culture in CP-2V (7). $\times 850$.

FIG. 2. Sporulating cell of *C. celatum* GD-1; 2-day culture from blood agar (Difco blood agar base with 5% sheep blood). $\times 4,800$.

In addition to the characteristics given in Table 1, the type strain and the three other strains tested ferment (pH 5.2 to 5.8) amygdalin, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, ribose, salicin, sucrose, and trehalose. Esculin is weakly fer-

mented (pH 5.80 to 5.95). There is no fermentation and poor growth in PY with adonitol, arabinose, erythritol, glycerol, glycogen, inositol, inulin, mannitol, melezitose, melibiose, raffinose, rhamnose, sorbitol, or xylose. The type strain lowers the pH in PY-dextrin to

5.6 to 5.9, but does not ferment sorbose or starch. One other strain weakly ferments sorbose; another weakly ferments starch.

The type strain and the other strains tested reduce neutral red and produce a small amount of gas in glucose-agar deep cultures and abundant hydrogen from PY-glucose broth cultures. They do not produce acetylmethylcarbinol or NH_3 (in PY or chopped meat cultures), hydrolyze hippurate, or completely hydrolyze starch. They do not digest milk or meat. A soft curd is irregularly observed in milk cultures after incubation for 3 weeks.

Each of the four strains tested produced urease when growth from egg-yolk agar was incubated in urea broth (8). The type strain and two other strains produced urease in PY-glucose broth cultures. Strains GD-1 and AD produced the strongest urease reaction; these two strains also reduced nitrate to nitrite in indole-nitrite (BBL) medium (Table 1). None of the four cultures produced urease in PY-urea broth, possibly because these strains do not grow well without a fermentable carbohydrate.

Fermentation products (average meq/100 ml of culture):

From PY-glucose: Acetic (1) and formic (1) acids and ethanol, usually with a small amount of butyric acid (0.1) and occasionally with small amounts of pyruvic, lactic, succinic, fumaric, and/or propionic acids.

From PY: Acetic acid (0.2), sometimes with small amounts of lactic and butyric acids.

From PY-pyruvate: Acetate (4), usually with formate (0.8) and a small amount of butyrate.

Lactate and gluconate are not utilized.

Type strain: American Type Culture Collection no. 27791, strain GD-1, isolated from human feces.

Lack of lecithinase or hemolytic activity and production of acetic and formic acids and ethanol from fermentation of glucose differentiate this species from other nonmotile, nitrite- and sulfide-producing species of clostridia (8, 11).

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REPRINT REQUESTS

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