Eubacterium cylindroides (Rocchi) Holdeman and Moore: Emended Description and Designation of Neotype Strain

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Twenty-five strains that conform closely to the original and subsequent descriptions of the organism now known as *Eubacterium cylindroides* (Rocchi) Holdeman and Moore have been studied, and previously undescribed characteristics of this species are presented. This organism is a numerically significant member of the normal intestinal flora of man and animals, and it has also been found in clinical specimens. ATCC 27803 (=Virginia Polytechnic Institute 3654) is designated the neotype strain of *Eubacterium cylindroides*.

In 1908, G. Rocchi isolated from the feces of a normal human adult an organism which he named *Bacterium cilindroide* (sic) (8). He described it as a large filamentous rod, 6 to 8 μ m in length, with swellings and granules, sometimes having the appearance of tortuous noodles. It did not stain uniformly and was gram-negative. It was a strict anaerobe and grew slowly and only in the presence of a fermentable carbohydrate. The temperature for optimum growth was 38 C. It did not grow on the surface of agar, but in deep agar the colonies were small and roundish with no distinctive characteristics. Milk was not changed, albumins were not attacked, and proteoses were slightly attacked. Glucose and sucrose were weakly acidified; there was no action on lactose, galactose, mannitol, or dulcitol. It was non-pathogenic.

This group of organisms was renamed *Ristella cylindroides* by Prévot in 1938 (7), who added to the description the information that gelatin was not liquefied and gas and spores were not produced. In Krasil'nikov's classification (4), this organism was named *Pseudobacterium cylindroides*. Another synonym for this organism is *Bacteroides cylindroides* (Rocchi) Kelly (3).

The strain on which the original description of this organism was based is not extant, and there are no strains of this organism in Prévot's collection at the Pasteur Institute (Paris). Possibly no isolates have been reported because of a typographical error which has persisted from at least as far back as 1937 (9), when it was stated that the organism only grew at 18 C. As originally described, best growth occurred at 38 C (8).

Many strains that possess the morphological

characters given in the original description of this organism (8) have been isolated in our laboratory. Organisms in this group do not stain uniformly with the Gram stain. They are often gram-negative in 24-h-old cultures. However, young (2- to 4-h-old) cultures of the same strains are actually gram-positive but decolorize readily. The major products of fermentation by these strains are lactic and butyric acids, sometimes with acetic, formic, and succinic acids. Because they are anaerobic, gram-positive, non-sporeforming rods that produce butyric and other acids, they were reclassified as *Eubacterium cylindroides* (2, 6).

On the basis of a study of 25 strains whose characters conform closely with those given in the original description of *B. cilindroide* Rocchi (8), the description has been emended and a neotype strain selected.

MATERIALS AND METHODS

Bacterial strains. The 25 strains included in this study were isolated in our laboratory: 19 were from feces of clinically normal humans, two were from chicken intestinal contents, one was from the intestine of a hog, one was a contaminant in a Pasteur Institute (Paris) culture labeled *Staphylococcus anaerobius* from pus from an inguinal cyst, and two were isolated as the only organisms detected from the blood of a patient with congestive heart failure and acute myocardial infarction. Both strains from this patient were included in this study, since only one fermented sucrose and the other fermented inulin; there were also slight differences in their colony types.

Methods. The methods for the isolation and characterization of these organisms have been described previously (2). The thermal melting point method of Marmur and Doty (5) was used for determination of the guanine + cytosine (G+C)

content of the deoxyribonucleic acid (DNA) preparations.

RESULTS AND DISCUSSION

Morphologically (Fig. 1), the cells of *Eubacterium cylindroides* are as originally described (8): they are rather pleomorphic rods with granules and swellings; they occur singly, in pairs, or in short chains; they may be straight or curved with tapered or rounded ends; they do not stain uniformly, and, although gram-positive in young cultures, they rapidly become gram-negative; they are nonmotile and measure 0.3 to 1.2 by 0.8 to 50.0 μm . Filamentous forms are often seen.

Surface colonies on blood agar plates (2 days) are pinpoint to 2 mm in diameter, circular to slightly irregular, entire to diffuse, convex, translucent to opaque, and often have a mottled or granular appearance when viewed by obliquely transmitted light. Some strains are slightly hemolytic on sheep blood agar.

Glucose broth cultures are turbid with a smooth or ropy sediment. The average pH after 5 days of incubation is 5.2 compared to 6.1 in a basal noncarbohydrate medium. The organisms are strict anaerobes, and growth is stimulated in the presence of a fermentable carbohydrate as stated in the original description. The temperature for optimal growth is 37 C, although most strains grow equally well at 45 C.

Biochemical reactions of the strains studied are listed in Table 1. In addition, neither lecithinase nor lipase was formed on McClung-Toabe egg yolk agar; catalase and deoxyribonuclease were not present; hippurate was not hydrolyzed; indole was not formed, and nitrate was not reduced; acetylmethylcarbinol was formed by 8 of 21 strains tested; little or no gas was produced in glucose agar deeps; neither H_2 nor increased CO_2 was present in the head space above fructose broth cultures inoculated under N_2 ; H_2S was not formed, but ammonia was produced by some strains; and all strains

TABLE 1. Biochemical reactions of strains of *E. cylindroides*^a

Substrate or reaction	Rocchi	No. strains tested	Test result	% +	Neo-type strain
Cellobiose		25	—w	32	—
Dulcitol	—	25	—	0	—
Fructose		25	A	100	A
Galactose	—	19	—	0	—
Glucose	w	25	A	100	A
Inulin		18	A—	61	A
Lactose	—	25	—	0	—
Maltose		25	—	0	—
Mannitol	—	25	—	0	—
Mannose		25	Aw	100	A
Raffinose		25	—w	44	—
Salicin		25	A—	72	—
Sucrose	w	25	A—	64	A
Trehalose		25	—A	12	—
Starch hydrolyzed		25	—+	28	—
Esculin hydrolyzed		25	+	100	+
Gelatin liquefied	—	22	—	0	—
Milk changed	—	24	—	0	—

^a No strain fermented arabinose, ribose, xylose, erythritol, glycerol, inositol, sorbitol, amygdalin, dextrin, esculin, glycogen, melezitose, melibiose, rhamnose, or sorbose. Two weakly fermented starch. Where a single result is listed, the reaction is produced by >90% of the cultures. Where two reactions are listed, the first is the result observed with most cultures, the second is less frequently observed. Legend: + = reaction positive, — = reaction negative or pH >6.0, w = pH 5.5 to 6.0, A = pH <5.5.

reduced neutral red, but only 12 of the 25 reduced resazurin.

Products of fermentation of peptone-yeast extract-glucose broth include DL-lactic acid (1.0 to 8.9 meq/100 ml), butyric acid (0.4 to 2.6 meq/100 ml), and occasionally traces of formic acid (0.0 to 0.7 meq/100 ml) and succinic acid (0.0 to 0.5 meq/100 ml). Neither threonine, lactate, gluconate, glucuronate, nor galacturonate was utilized, but pyruvate was converted to acetate, butyrate, and formate.

Reactions of individual representative strains of this species are listed in Table 2. The G+C content of the DNA of three of these representative strains was 26, 31, and 35 mol%, indicating that there may be heterogeneity within this group of organisms. However, strain ATCC 27805, giving a low G+C value of only 26%, also yielded little DNA. Thus, this result may be questionable. Other reactions did not differ and are as described above. Strain

FIG. 1. *E. cylindroides* ATCC 27803. Cells from a 24-h-old peptone-yeast extract-glucose agar slant (left); cells from a 24-h-old sweet E (2) agar slant (right). Bars represent 10 μm .

(Virginia Polytechnic Institute) 0689B was isolated from the anterior small intestine of a hog, VPI 1278 from the cecum of a chicken, strains ATCC 27803, 27804, and 27805 were from feces of clinically normal humans, and strain ATCC 27528 was from the blood of a patient with congestive heart failure. ATCC 27803 (= VPI 3654) is here designated the neotype strain of *E. cylindroides* (Rocchi) Holdeman and Moore.

Characteristics which qualify *E. cylindroides* as a member of the genus *Eubacterium* Prévot: *E. cylindroides* is a member of the genus *Eubacterium* because it is an obligately anaerobic, gram-variable, non-sporeforming rod whose major fermentation products are lactic and butyric acids (6).

Those characteristics most useful in distinguishing *E. cylindroides* from other members of the genus *Eubacterium* are shown in Table 3. *E.*

TABLE 2. Reactions of representative strains of *E. cylindroides*

Substrates and products	Strain					
	VPI 0689B	VPI 1278	ATCC 27804, VPI 3651 (35% G+C)	ATCC 27803, VPI 3654 (31% G+C)	ATCC 27805, VPI 3696 (26% G+C)	ATCC 27528, VPI 6849A
PY ^a	6.03 ^b	6.0	6.1	6.1	6.15	6.1
Cellobiose	5.8	6.1	6.0	6.1	6.1	5.5
Fructose	5.3	4.85	5.2	5.1	5.0	5.3
Glucose	5.45	5.1	5.1	5.1	5.35	5.3
Inulin	5.8	5.7	5.1	6.0	5.85	5.6
Mannose	5.7	5.0	5.15	5.3	5.1	5.55
Raffinose	5.5	6.0	6.0	6.05	5.6	5.65
Salicin	5.5	6.1	6.0	6.1	6.1	5.5
Sucrose	5.5	5.6	6.1	5.05	5.45	5.45
Trehalose	5.45	6.2	6.3	6.1	6.2	5.3
Products-PYG ^a	LBfs ^c	LB	LB	LB	LB	LBf

^a PY, Peptone-yeast extract; PYG, peptone-yeast extract-glucose.

^b pH after 5-day incubation.

^c L = at least 1 meq lactic acid/100 ml; B = at least 1 meq butyric acid/100 ml; f = <1 meq formic acid/100 ml; s = <1 meq succinic acid/100 ml.

TABLE 3. Characteristics that differentiate *E. cylindroides* from other species in the genus *Eubacterium*^a

<i>Eubacterium</i> species	Butyric produced	Fructose acid	Mannose acid	Starch acid	Esculin hydrolyzed	Maltose acid	Lactose acid	Products (PYG) ^b
<i>E. cylindroides</i>	+	+	+	—	+	—	—	LB(sf)
<i>E. multiforme</i>	+	+	+	—	+	—	+	LAB(fsp)
<i>E. nitritogenes</i>	+	+	+	—+	+	+	—+	LBAs(fsp)
<i>E. moniliforme</i>	+	+	+	—+	—	v	—	LBa(fsp2)
<i>E. budayi</i>	+	+	+	+	+	+	+	Lba
<i>E. rectale</i>	+	+	v	+	+	+	+	LB(sfa)
<i>E. ruminantium</i>	+	+	—	+	+	v	v	LFb(ap)
<i>E. tortuosum</i>	+	+	—	—	+	+	v	Lbs(af)
<i>E. cellulosolvens</i>	+	—	+	—	+	+	—	Lfbsa(p)
<i>E. combesii</i>	+	—	—	—	+	—+	—	ABibivp
<i>E. tenue</i>	—	—+	—	—+	—	—+	—	Af(lspibiv)
<i>E. contortum</i>	—	+	v	v	+	+	v	Af(sl2)
<i>E. aerofaciens</i>	—	+	+	—	+	+	+	LAF(s2)
<i>E. lentum</i>	—	—	—	—	—	—	—	(afsl)

^a Adapted from Holdeman and Moore (2); Legend: + = reaction positive for >90% of strains, — = reaction negative for >90% of strains, v = reaction positive for 40-60% of strains. Where two reactions are listed, the first is the result observed with most cultures, the second reaction less frequently observed.

^b Products: L = lactic acid; B = butyric acid; S = succinic acid; F = formic acid; P = propionic acid; A = acetic acid; 2 = ethyl alcohol; ib = isobutyric acid; iv = isovaleric acid; upper case = >1 meq/100 ml; lower case = <1 meq/100 ml; () = produced by some strains. PYG, peptone-yeast extract-glucose.

cylindroides produces butyric acid, hydrolyzes esculin, and acidifies glucose, fructose, and mannose, but not starch, maltose, or lactose.

Because *E. cylindroides* is easily decolorized, it may be confused with *Fusobacterium prausnitzii* (mol% G+C = 50 to 57); however, it is more fermentative than strains of *F. prausnitzii*.

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REPRINT REQUESTS

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