Eubacterium desmolans sp. nov., a Steroid Desmolase-Producing Species from Cat Fecal Flora

G. N. MORRIS,1 J. WINTER,1 E. P. CATO,2 A. E. RITCHIE,3 AND V. D. BOKKENHEUSER1*

Department of Pathology, St. Luke's-Roosevelt Hospital Center, New York, New York 10025; Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061; and National Animal Disease Center, Ames, Iowa 50010

Eubacterium desmolans sp. nov., an obligate anaerobe with desmolytic activity, was isolated from cat feces. Desmolase, which was previously observed only in a Clostridium species isolated from human fecal flora, cleaves the carbon-carbon bond of 17-hydroxylated corticoids between C-17 and C-20, thereby converting these compounds to androstenes (C19 steroids). In primary cultures on sheep blood agar plates, E. desmolans forms nonhemolytic, circular to irregular, convex, shiny, entire, white to colorless colonies that are barely visible to the unaided eye. The cells are plump, short rods that are 0.8 to 1.1 μm wide by 1.7 to 2.3 μm long and have capsules and flagella. They occur singly or in short chains and are gram positive. Inositol is the sole carbohydrate fermented. Indole is produced. The major fermentation products in inositol-enriched media are acetic and butyric acids. The type strain is strain ATCC 43058.

For almost 30 years it has been suspected that human feces contain bacteria which are capable of metabolizing 17α-hydroxysteroids to androstenes (4, 5, 13, 15). This conversion involves a cleavage of the side chain (Fig. 1). Clostridium scindens, which synthesizes the specific enzyme desmolase, was recently isolated from human fecal flora (18). Cortisol, which is an ideal substrate for this enzyme, is metabolized rapidly and completely to 11β-hydroxyandrostenedione (18). However, cortisol is not a biliary steroid in humans (12) and, therefore, is not the natural substrate for the enzyme. The only 17α-hydroxysteroid present in significant amounts in the bile of humans is ring A-reduced 17α-hydroxyprogesterone, but this compound is a poor substrate for the C. scindens desmolase (18). Since cortisol undergoes enterohepatic circulation in cats (14), it seemed probable that this animal could be the natural host for C. scindens. The investigations described in this paper showed that not only was this hypothesis incorrect, but the demolase-producing organism isolated from cat feces is a hitherto undescribed species of Eubacterium.

MATERIALS AND METHODS

Media. Supplemented peptone broth (SPB) was purchased from Becton Dickinson and Co., Rutherford, N.J.; chopped meat broth was obtained from Carr-Scarborough Microbiological, Decatur, Ga.; and prereduced brain heart infusion broth was purchased from Scott Laboratories, Fiskeville, R.I. Columbia agar plates supplemented with 5% sheep blood (BAP) and the GasPak systems used to establish anaerobic conditions were obtained from BBL Microbiology Systems, Cockeysville, Md.

Substrates. The steroids used as substrates and reference compounds were purchased from Sigma Chemical Co., St. Louis, Mo.

Detection of desmolase activity. SPB supplemented with 20 μg of cortisol per ml (1) was seeded either with 0.1-ml portions of a bacterial suspension from feces at dilutions of 10^2 to 10^10 or with 0.1-ml portions of the growth harvested from BAP seeded with the same suspensions. These conversion cultures were incubated at 37°C for 7 days. The steroids were extracted and identified by thin-layer chromatography, gas-liquid chromatography, and mass spectrometry as previously described (1, 3).

Isolation. The desmolase-synthesizing strain was isolated by using a modified version of the dilution technique described previously (1, 3). Briefly, within 1 h of defecation, 1 g of cat feces was decimally diluted to 10^6 in SPB; 0.1-ml samples were spread onto BAP and incubated anaerobically for 2 days. Repeated subcultures of desmolase-active growth from BAP onto SPB resulted in a relative increase in the population of desmolase-synthesizing organisms from 1 per 1,000 to 1 per 35 live bacteria. A total of 40 colonies were transferred to chopped meat broth (1), and as expected, one colony consisted of organisms with desmolase activity.

Electron microscopy. Young bacterial cells of a pure culture of the desmolase-producing organism were harvested from BAP, centrifuged at 7,000 × g for 15 min, dispersed in distilled water, and suspended in 0.2 to 0.4% potassium phosphotungstate (pH 6.7) containing 0.05% bovine serum albumin (1). The resulting suspension was applied to carbon colloidin film grids and examined immediately with an electron microscope (model EM-200; Philips Electronic Instrument, Mahwah, N.J.) at 60 kV.

Microbiological characteristics. Colonial morphology was determined from growth on BAP. Cell morphology, motility, and Gram staining properties were determined by microscopic examination of 16- to 24-h-old SPB cultures.

Phenotypic characterization. Biochemical characteristics and fermentation products were determined as described previously (6). Biochemical tests were read after 3 days of incubation. Antibiotic susceptibility was tested by the broth disk method (6, 17).

RESULTS AND DISCUSSION

Cat feces at a maximum dilution of 10^6 produced growth in SPB. However, desmolase activity was present only in cultures diluted up to 10^2. The organism which synthesized...
the specific enzyme was isolated by the enrichment procedure described previously (1) (see above). This organism is an obligately anaerobic, chemoorganotrophic, gram-positive, nonsporeforming, rod-shaped bacterium. The main fermentation products in cultures in peptone-yeast extract supplemented with inositol are acetate, butyrate, and trace amounts of succinate and lactate; no ethanol is produced. These findings indicate that the organism belongs to the genus *Eubacterium* as described by Holdeman and Moore (7). This organism has characteristics which are unlike those of previously described species in the genus. For this new species we propose the name *Eubacterium desmolans*.

**Eubacterium desmolans** sp. nov. *Eubacterium desmolans* (des'mo.lans. Gr. n. desmos a bond; mod. chem. term desmolase, an enzyme splitting a carbon-carbon bond; N.L. part. adj. desmolans making desmolase) cells are gram-positive plump rods that are 0.8 to 1.1 μm wide by 1.7 to 2.3 μm long (Fig. 2) and occur singly or in short chains. Some cells are capsulated. The tumbling motility of the peritrichous cells is due to four to six long flagella. Internal mesosomes are present, especially in dividing cells. Spores are not formed, and the organism does not survive exposure to 80°C for 10 min or treatment with absolute alcohol for 30 min.

**Growth characteristics.** Primary colonies on BAP incubated at 37°C are invisible to the unaided eye. Subcultures on the same medium yield circular to slightly irregular, convex, shiny, entire, semiopaque, white to colorless colonies with a diameter of 0.6 to 0.8 mm. Neither sheep nor rabbit erythrocytes are hemolyzed. Growth in SPB is light and slightly turbid, with a fine precipitate after 3 to 4 days. Addition of 1% inositol enhances growth; 20% bile (2% oxgall) is tolerated but has no effect on multiplication.

**Aerotolerance.** *E. desmolans* does not form colonies on BAP incubated in atmospheric air or in air supplemented with 10 or 30% CO₂. However, organisms seeded on BAP
tolerate 4 but not 8 h of exposure to atmospheric air at 22°C, as evidenced by colony formation on plates incubated anaerobically after exposure. Subcultures of such organisms synthesize desmolase.

**Phenotypic reactions.** Inositol is fermented to a final pH of 5.1. Indole is produced. H2S is not produced on sulfide-indole motility medium. Gelatin, milk, and meat are not digested. As noted above, inositol is utilized, resulting in the formation of acetate and butyrate and trace amounts of succinate and lactate. Hydrogen gas is not produced. Amygdalin, arabinose, cellobiose, erythritol, esculin, glycerogen, fructose, glucose, lactose, maltose, mannotri, mannose, melezitose, melibiose, pectin, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, and xylose are not fermented. Starch is not hydrolyzed. Lipase, lecinthinase, oxidase, and catalase are not produced. Nitrate and resazurin are not reduced.

**Steroid metabolism.** *E. desmolans* synthesizes at least two enzymes with activity on corticoids, a desmolase which cleaves the side chain of 17α-hydroxysteroids between C-17 and C-20 and a 20β-hydroxysteroid dehydrogenase which reduces the 20-keto group of both 17α-hydroxy- and 17β-deoxysteroids.

Desmolase requires a C-21 hydroxy group and a keto or β-hydroxy group at C-20, but functions independently of the structure of the rest of the molecule. The metabolite formed is invariably a C-17 ketosteroid.

The organism also reduces many C20 keto steroids. The need for hydroxy groups at C-21 or C-17 or both is not clear. For example, 17α-hydroxyprogesterone, deoxycorticosterone, and cortisol are all reduced to the 20β-hydroxy compounds. In contrast, progesterone, aldosterone, and corticosterone are not reduced at C-20. Gas-liquid chromatography of the metabolites isolated compared with authentic compounds. In contrast, progesterone, aldosterone, and corticosterone are all reduced to the 20β-hydroxy compound, but functions independently of the structure of the rest of the molecule.

Kinetic experiments revealed that 20β-hydroxysteroid dehydrogenase is measurable in the late log phase and the early log phase (7 to 12 h of incubation). Desmolase is demonstrable from the beginning of the log phase (9 to 10 h of incubation) and reaches a maximum within 2 to 3 h, approximately 3 h before the beginning of the stationary phase.

**Natural habitat.** Although *E. desmolans* was isolated from the feces of an apparently normal cat, its host range is uncertain. Inositol is fermented to a final pH of 5.1. Indole is produced. H2S is not produced on sulfide-indole motility medium. Gelatin, milk, and meat are not digested. As noted above, inositol is utilized, resulting in the formation of acetate and butyrate and trace amounts of succinate and lactate. Hydrogen gas is not produced. Amygdalin, arabinose, cellobiose, erythritol, esculin, glycerogen, fructose, glucose, lactose, maltose, mannotri, mannose, melezitose, melibiose, pectin, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, and xylose are not fermented. Starch is not hydrolyzed. Lipase, lecinthinase, oxidase, and catalase are not produced. Nitrate and resazurin are not reduced.

**LITERATURE CITED**


