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Isolation of a Cellulolytic *Bacteroides* sp. from Human Feces

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An anaerobic cellulolytic bacterium, identified as a *Bacteroides* sp., was present in 10^{-8} g of feces from only one of five human subjects.

With the current emphasis on the importance of fiber in the human diet (10) and the paucity of information on cellulolytic bacteria in the human bowel (7), a study of cellulose-hydrolyzing bacteria in human feces seemed timely.

By the techniques of Eller et al. (2), five samples, one from each of five young adults on unrestricted diets, were processed in the following manner: 1 g of freshly voided feces was diluted with 9 ml of anaerobic dilution solution (1) to make a 10^{-1} dilution; fractions of this suspension were then diluted, in triplicate, in cellulose broth in serial 10-fold steps through a 10^{10} dilution of the original sample. The cellulose broth contained (per liter): clarified rumen fluid, 400 ml; cellulose (3-day ball-milled 2% slurry of Whatman no. 1 filter paper), 1.5 g; mineral solutions 1 and 2 (1), 40 ml each; Na_2CO_3, 4.0 g; cysteine hydrochloride, 0.5 g; and resazurin, 0.002 g. For cellulose roll tubes, cellulose was increased to 4 g, and 15 g of agar (Difco Laboratories) was added. All incubations were at 37°C.

Only one of the five samples showed cellulolytic activity as indicated by a visible disappearance of most of the insoluble cellulose, whereas a control of cellulolytic strain 7, *Ruminococcus albus,* always showed rapid digestion. The most probable number of cellulolytic organisms present in the one active fecal sample was 2.4×10^8/g of feces (all three tubes of 10^8 dilution were positive after about 2 weeks, whereas none of the 10^9 tubes was positive after 3 months), or about 0.3% of the total viable count of bacteria present in feces of the same individual, determined by using the rumen fluid roll tube medium with glucose, cellobiose, and starch in place of cellulose as an energy source.

Isolation of a pure culture of the cellulolytic bacterium was accomplished by a multistep procedure as described by Hungate (7). A cellulose broth culture representing the 10^7 dilution of the fecal sample showing active cellulose digestion was transferred (5% inoculum) every 2 days through several transfers. A portion was then diluted in serial 10-fold steps, and 0.2- and 0.5-ml volumes of the 10^4 through 10^8 dilutions were used to inoculate cellulose agar roll tubes (18 by 150 mm), each containing 5 ml of medium. Although non-cellulolytic colonies could be seen after 3 days of incubation, clearing of the cellulose around cellulolytic colonies was not clearly visible until 4 weeks after inoculation. At this time, a zone of clearing 2 to 3 mm in diameter appeared around celluloyltic colonies in roll tubes representing a 10^6 dilution of the serially transferred culture. These colonies were picked, with a sterile Pasteur pipette (7), into 2 ml of anaerobic dilution solution and serially diluted to 10^5. A second series of cellulose agar roll tubes was then inoculated by using 0.2 and 0.5 ml of each dilution. After 18 days of incubation, clearing of cellulose around the colonies was observed. These colonies were picked and diluted as described above, and 0.2- and 0.5-ml volumes of each dilution were used to inoculate roll tubes containing cellobiose instead of cellulose as the energy source. After 3 days of incubation, eight colonies on cellobiose agar were picked into cellulose broth. Cellulose digestion was observed in all eight tubes 5 days after inoculation, confirming that the organisms that grew on cellobiose were indeed cellulolytic. Gram stains of the colonies on the second series of cellulose agar, of the colonies on cellobiose agar, and of the final cellulose broth cultures all revealed only a gram-negative, coccus-to-rod-shaped organism (0.8 to 1.0 μm by 1 to 3 μm) with blunt, round ends and with a few short chains. The organism was nonmotile and sometimes appeared weakly gram positive in young cultures. Morphologically identical cells of cellulolytic cultures were also isolated from cellulose broth subcultures of the original cellulose broth enrichment by dilution and direct inoculation into cellobiose agar roll tubes. In this case, only a few of the colonies picked were the cellulose digestor.

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The cellulolytic strain JLB was identified at the Virginia Polytechnic Institute as an unknown species of the genus *Bacteroides* (5, 6). The organism fermented lactose, pectin, and cellobiose in addition to cellulose and hydrolyzed esculin in rumen fluid-supplemented media but was inactive on other energy sources tested (6). Fermentation products from lactose included mainly succinate and H_2 and a small amount of acetate and formate. In lactose medium, it was stimulated by the addition of rumen fluid or Tween 80 but was inhibited by bile. It was not active in milk and did not liquefy gelatin, produce indole, or reduce nitrate.

It is somewhat similar to *Bacteroides capillosus* and *B. succinogenes* (5, 6) but differs from the former in that it fails to ferment glucose, very actively ferments lactose, is inhibited by bile, and is morphologically different from H_2-producing strains of *B. capillosus*. It differs from *B. succinogenes* in features such as its production of H_2, lack of glucose fermentation, lack of pointed ends and fragile cells, slow fermentation of cellulose, and formation of discrete colonies in the center of the zone of cellulose digestion in agar roll tubes.

Unpublished data of Allen Scott (1973, in the laboratory of M. P. Bryant) also indicated a count of about 10^8 cellulolytic bacteria per g in one sample of human feces; the organism was a gram-negative, nonmotile, nonsporing anaerobic rod but was lost before further studies could be done.

Our finding of significant numbers of cellulose-digesting bacteria in only one of the five individuals samples was of interest since some isolates of normal fecal flora are currently being identified as *Ruminococcus albus* (4, 9). Rumen strains of *R. albus* are usually cellulolytic (11), but all three recently isolated human fecal strains were non-cellulolytic (M. Wozny, M. P. Bryant, L. V. Holdeman, and W. E. C. Moore, manuscript in preparation). Studies such as deoxyribonucleic acid homologies may indicate that they actually represent a species different from the rumen strains.

Whether significant amounts of any form of cellulose are catabolized in persons harboring cellulolytic strains of *Bacteroides* sp. is not known. It seems certain that, since they are relatively slow in attacking easily degraded cellulose, i.e., Whatman no. 1 filter paper, they would be unable to degrade more-resistant cellulose such as native cotton fibers or chromatographic powder (3).

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