**Clostridium orbiscindens** sp. nov., a Human Intestinal Bacterium Capable of Cleaving the Flavonoid C-Ring

J. WINTER, M. R. POPOFF, P. GRIMONT, and V. D. BOKKENHEUSER

Department of Pathology, St. Luke's-Roosevelt Hospital Center, New York, New York 10025, and Unite des Anaerobies, and Unite des Enterobacteries, Institut National de la Sante et de la Recherche Medicale, Unite 199, Institute Pasteur, 75724 Paris Cedex 15, France

*Clostridium orbiscindens* sp. nov. is an obligate anaerobe that is capable of cleaving the C-3-C-4 bond of the natural anticarcinogen quercetin. The metabolic products, 3,4-dihydroxyphenylacetic acid and presumably phloroglucinol, are not known to possess anticarcinogen properties. This organism was isolated from human feces. On sheep blood agar plates *C. orbiscindens* forms minute, irregular, convex, gray or white, shiny, smooth, nonhemolytic colonies. It is beta-hemolytic on rabbit blood agar. The motile peritrichous rods are gram variable. Subspores are common. Cultures are resistant to 80°C for 10 min. Capsules are absent. This asaccharolytic organism does not metabolize esculin, urea, meat, gelatin, casein, or nitrate. The G+C content is 56 to 57 mol%. DNA hybridization experiments did not reveal relatedness to phenotypically similar *Clostridium* strains. Strain 265 (= ATCC 49531) is the type strain.

During investigations of flavonoid metabolism by the human intestinal flora, we isolated four bacterial strains that are capable of cleaving the C-ring of the aglycone quercetin. The metabolic products, 3,4-dihydroxyphenylacetic acid and presumably phloroglucinol, are not known to possess anticarcinogen properties. This organism was isolated from human feces. On sheep blood agar plates *C. orbiscindens* forms minute, irregular, convex, gray or white, shiny, smooth, nonhemolytic colonies. It is beta-hemolytic on rabbit blood agar. The motile peritrichous rods are gram variable. Subspores are common. Cultures are resistant to 80°C for 10 min. Capsules are absent. This asaccharolytic organism does not metabolize esculin, urea, meat, gelatin, casein, or nitrate. The G+C content is 56 to 57 mol%. DNA hybridization experiments did not reveal relatedness to phenotypically similar *Clostridium* strains. Strain 265 (= ATCC 49531) is the type strain.

**MATERIALS AND METHODS**

**Media.** Quercetin conversion was performed in prereduced brain heart infusion broth obtained from Scott Laboratories, Fiskeville, R.I., and in brain heart infusion broth obtained from BBL Microbiology Systems, Cockeysville, Md.; the latter was prepared in our laboratory according to the manufacturer's instructions and was biologically reduced with a noncleaving strain of *Escherichia coli* (16).

**Source of microorganisms.** Four bacterial strains (St. Luke's strains 257, 258, 264, and 265) that are capable of cleaving the C-ring of quercetin were isolated from normal human fecal flora. The characteristics of the cleaving organisms suggested that they might belong to a previously undescribed species (15). Accordingly, phenotypically related organisms were obtained from other collections for DNA hybridization experiments (Table 1).

**Substrates, reference compounds, and solvents.** Substrates and chromatographic reference compounds (Fig. 1) were obtained from Pfaltz and Bauer, Stamford, Conn., and from Sigma Chemical Co., St. Louis, Mo. Reagent grade solvents and high-pressure liquid chromatography grade solvents were used throughout the experiments.

**Isolation of bacteria that cleave the C-ring.** The methods used for isolating the active organisms from fecal flora have been described previously (3). The fecal samples were obtained from a healthy subject on a Western diet.

**Identification of C-ring-cleaving bacteria.** (i) **Microbiological characteristics.** Colonial morphology was determined from growth on sheep blood agar plates. Cell morphology, motility, and Gram-staining properties were determined by microscopically examining broth cultures that were incubated for 18 to 24 h.

(ii) **Phenotypic characteristics.** Biochemical characteristics and fermentation products were determined after 3 days of incubation (8). Antibiotic susceptibility was tested by using broth disk methods (8, 14).

(iii) **Electron microscopy.** Young bacterial cells of a pure culture of C-ring-cleaving bacteria were harvested from sheep blood agar plates, centrifuged at 7,000 × *g* 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 colloidal film grids and examined immediately with an electron microscope (model EM-200; Philips Electronic Instruments, Mahwah, N.J.) at 60 kV.

(iv) **Extraction and purification of DNA.** Bacterial DNA was isolated, purified, and tritiated as previously described (4, 5, 7). Hybridization was performed at 60°C by using the S1 nuclease-trichloroacetic acid method described by Grimont et al. (7). The denaturation temperature (*T*~*m*~) for 50% of the reassociated DNA became hydrolyzable by S1 nuclease was determined by using the method of Crosby et al. (5).

(v) **G+C contents.** The G+C contents were determined from the *T*~*m*~ values of DNA solutions (50 μg/ml) in buffer by using a spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio) (10). The DNA of *E. coli* K-12 was used as a standard; its G+C content was 51.2 mol%.

(vi) **Conversion experiments.** Bacterial C-ring cleavage was examined in prereduced brain heart infusion broth and in brain heart infusion broth that was biologically reduced with a noncleaving strain of *E. coli* and was supplemented with quercetin (20 μg/ml). Conversion of quercetin was determined partly by following its concentration in the culture medium and partly by observing the formation of 3,4-dihydroxyphenylacetic acid (Fig. 1). Phloroglucinol, the other expected quercetin metabolite, was not detected by the methods which we used. The methods used for extrac-
TABLE 1. Levels of relatedness between DNAs of C. orbiscindens 265T and strains of other Clostridium species

<table>
<thead>
<tr>
<th>Source of unlabeled DNA</th>
<th>Origin of strain</th>
<th>% Relatedness</th>
<th>ATm (°C)</th>
</tr>
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<tbody>
<tr>
<td>C. orbiscindens 265T</td>
<td>SLRHC</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C. orbiscindens 257</td>
<td>SLRHC</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
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<td>95</td>
<td>1</td>
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<tr>
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<td>SLRHC</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
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<td>ATCC</td>
<td>0</td>
<td></td>
</tr>
<tr>
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<td>C. beijerinckii VPI 2983</td>
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</table>

*SLRHC, St. Luke’s-Roosevelt Hospital Center, New York, NY; ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; VPI, Virginia Polytechnic Institute and State University, Blacksburg.

† Relative values.

‡ ATm is the difference between the Tm of the heterologous DNA pair and the Tm of the homologous DNA.

RESULTS AND DISCUSSION

Conversion. Metabolic experiments revealed that all of the fecal specimens obtained from a group of five normal individuals cleaved quercetin. Four bacterial strains isolated from one of these individuals, St. Luke’s strains 257, 258, 264, and 265T, synthesized the enzymes that cleave the C-ring of quercetin. Polyacrylamide gel electrophoresis of the four strains (15) produced slightly different patterns (data not shown). DNAs from strains 257 and 265T contained 56 and 57 mol% G+C, respectively, values that are remarkably high for Clostridium spp., which usually have lower G+C contents (23 to 43 mol%) (9).

DNA-DNA hybridization. Labeled DNA from strain 265T hybridized 100% (relative value) with homologous DNA and 73 to 95% with DNAs from strains 257, 258, and 264 (Table 1). The Tm of strain 265T was 96.5°C, and the Tm values in experiments with the other three strains ranged from 9 to 1°C. Thus, the results of our DNA-DNA hybridization experiments indicated that the four isolates represent a single species. No relatedness was detected between this group and 10 Clostridium spp. with which strain 265T could be confused (Table 1).

DNA base composition. The results of neither the DNA hybridization experiments nor the G+C content determinations supported the inclusion of the C-ring-cleaving organism in the genus Clostridium. However, there is no genetic definition of a genus and phenotypic characteristics clearly placed strain 265T in the genus Clostridium. For this previously undescribed species (13) we propose the name Clostridium orbiscindens.

Description of Clostridium orbiscindens sp. nov. Clostridium orbiscindens (or. bi. scin’ dens. N. L. adj. orbiscindens, ring cutting). Cells in broth cultures are gram-variable, motile, peritrichous, sporeforming, straight rods that are 0.9 to 1.0 by 2 to 7 μm and occur singly or in pairs (Fig. 2). Spores are round to oval, subterminal, 0.7 by 0.5 μm, and thermoresistant (80°C for 10 min). Capsules are absent. The organisms are strictly anaerobic but survive exposure to atmospheric air on sheep blood agar plates at 20°C for at least 24 h. Good growth of all four strains occurs when they are incubated in brain heart infusion broth under anaerobic conditions (5% CO2, 95% N2) at 37°C for 2 days. One of the four strains grows on bile agar. Colonies on rabbit blood agar incubated anaerobically are 1 mm in diameter, circular, convex, slightly irregular, gray or white, shiny, smooth, and beta-hemolytic. Colonies on sheep blood agar are slightly larger and are not hemolytic. In brain heart infusion broth cultures produce distinct turbidity and sediment. The optimum temperature for growth is 37°C. The main specific enzymatic activity is C-ring cleavage of flavonoid molecules.

Amygdalin, arabinose, cellobiose, erythritol, glucose, glycogen, gum arabic, inositol, lactose, larch arabinogalactan, maltose, mannitol, melizitose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, xylan, and xylose are not fermented.

Production of indole (one of four strains) and H2S (three of four strains) is variable. Utilization of fumarate (two of four strains) and pyruvate (three of four strains) is variable. Gelatin, meat, and casein are not digested. Lecithinase, lipase, and urease are not produced. Nitrate is not reduced. The strains are susceptible to cephalothin (30 U), kanamycin (1,000 U), and metronidazole (80 U); they are resistant to nalidixic acid (30 U), rifampin (15 U), and vancomycin (5 U). Susceptibility to penicillin is variable (two of four strains are susceptible).

**FIG. 1.** C-ring cleavage of quercetin.

**FIG. 2.** Electron micrograph of C. orbiscindens.
The products of metabolism from peptone-yeast-glucose broth include large amounts of acetic acid and butyric acid and a small amount of propionic acid. The G+C content of the DNA is 56 to 57 mol% (as determined by the $T_{m}$ method). All four strains administered intramuscularly to mice are apathogenic. The outstanding characteristics of this species are the high G+C content, the lack of glycolytic activity, and the synthesis of a C-ring-cleaving enzyme.

Description of the type strain. Type strain 265 (= ATCC 49531) has all of the characteristics described above for the species except that pyruvate (but not fumarate) is utilized, indole is not produced, no growth occurs on bile agar, and it is not susceptible to penicillin and kanamycin.

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REFERENCES