Unusual *Sphaerophorus* Species from the Large Intestine of Man

TED A. PEARSON and EDWARD BALISH

Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

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An obligately anaerobic, gram-negative microorganism identified as a *Sphaerophorus* species was recovered from the fecal material of two cancer (chronic myelogenous leukemia and idiopathic thrombocytopenia) patients receiving cobalt radiation therapy. The organism, isolated on sheep blood-agar, exhibited extreme pleomorphism (rods, filaments, and spheroids) and was a major component of the anaerobic fecal microflora. In one patient the numbers of *Sphaerophorus* species (designated as isolate 6-13-68), *Bacteroides* species, and *Clostridium perfringens* declined after irradiation; however, they were stable in this same patient after a second therapeutic dose of radiation. The numbers of anaerobes in the other patient remained fairly consistent after radiation. The biochemical and morphological characteristics and carbohydrate fermentation reactions of isolate 6-13-68 most closely resembled those of *Sphaerophorus ridiculosis*.

The predominant components of the fecal microflora of normal humans are the anaerobic, nonsporeforming gram-negative bacilli of the family *Bacteroidaceae* (1, 2, 4, 9). This heterogeneous group is composed almost entirely of members of the genus *Bacteroides* and members of *Sphaerophorus* and *Fusobacterium* to a lesser degree. The gram-negative anaerobes may sometimes outnumber the coliforms by 10 to 1000. In this study, we determined the levels of *Sphaerophorus*, designated as isolate 6-13-68, and other anaerobic microorganisms in the feces of two patients before, and for several weeks after, cobalt radiation therapy.

**MATERIALS AND METHODS**

Culture methods for fecal material. A weighed sample of feces (0.5 to 1.5 g) was placed in 100 ml of sterile NaCl (1%, w/v) and agitated until uniformly suspended. This initial dilution of feces, used as a 1:100 dilution, was further diluted with sterile NaCl to 10⁻⁴. A portion (0.1 ml) was spread on dry agar plates with a bent glass rod, immediately placed in anaerobe jars, and incubated for 3 to 7 days at 37 C.

*Bacteria.* *Sphaerophorusfreundii* (9817) and *S. varius* (8501) were obtained from the American Type Culture Collection. We isolated strain 6-13-68 from both patients. The isolates from both patients possessed identical morphological and biochemical properties. Cultures were maintained in thioglycollate broth (BBL) and transferred every 7 days.

*Anaerobiosis.* We used Gaspak anaerobe jars (BBL) with an atmosphere of 90% hydrogen and 10% carbon dioxide. Methylene blue indicators (BBL) were always used to insure removal of oxygen from the jars.

**Media for characterization.** Blood-agar plates with 10% defibrinated sheep blood (BBL) were used to demonstrate hemolysis. We studied carbohydrate fermentation in basal thioglycollate broth containing (grams per liter): Trypticase, 15; Phytone, 3; sodium thioglycollate, 0.5; cysteine, 0.125; agar, 0.70; and fermentable substrate, 10. To measure acid production, we compared the change in pH after 72 hr of incubation of thioglycollate broth cultures with and without the test substrate. Indole production and nitrate reduction were determined in Indole-Nitrite medium (BBL), hydrogen sulfide production in S IM medium (BBL), and gas and odor production in thioglycollate broth. For more reduced conditions, all liquid media contained sodium thioglycollate (BBL) at a concentration of 0.05% (w/v), and were boiled and cooled just before use.

To detect hemagglutinins in fresh isolates, we mixed equal amounts of microorganisms and sheep erythrocytes (2% in normal saline) on glass slides. The organisms were grown for 24 hr on sheep blood-agar plates, removed with sterile cotton swabs, and suspended in NaCl (1%, w/v) before testing.

**RESULTS**

We isolated 6-13-68 from patient A (Table 1) in concentrations of 10⁷ to 10¹⁰ viable cells per gram of feces (dry weight). Throughout the 4-week study, the numbers of isolate 6-13-68 and *Bacteroides* were fairly consistent; three samples (days -4, +4, and +10) of feces did not contain isolate 6-13-68 at a 10⁻⁴ dilution (the lowest dilution plated). Isolate 6-13-68 and *Bacteroides*...
TABLE 1. Anaerobic fecal microflora of patient A treated with a single dose of 242 rads of splenic irradiation

| Time (days) | Bacteroides sp. | Isolate 6-13-68 | C. perfringens |
|-------------|-----------------|-----------------|---------------|
| -4          | <4<sup>a</sup>  | <4              | 6             |
| 0           | 10              | 9               | 7             |
| +4          | <4              | <4              | <4            |
| +6          | 10              | 10              | 9             |
| +10         | 10              | <4              | 5             |
| +12         | 7               | 6               | 6             |
| +14         | 10              | 10              | <4            |
| +23         | 9               | 9               | 4             |

<sup>a</sup> Log<sub>10</sub> of viable microorganisms per gram of stool (dry weight).

TABLE 2. Anaerobic fecal microflora of patient B before and after 150-r total-body irradiation<sup>a</sup>

| Time (days) | Bacteroides sp. | Isolate 6-13-68 | C. perfringens |
|-------------|-----------------|-----------------|---------------|
| -3          | 9<sup>b</sup>   | 9               | 7             |
| 0           | 8               | <4              | 8             |
| +3          | 8               | 8               | 9             |
| +8          | 9               | 9               | <4            |
| +10         | 9               | 5               | <4            |
| +18         | <4              | <4              | 4             |
| +24         | <4              | <4              | <4            |
| +31         | <4              | <4              | <4            |
| +45         | <4              | <4              | <4            |

<sup>a</sup> Patient was irradiated in June 1968 with <sup>60</sup>Co at 1.5 r/hr in a special low dose rate facility.
<sup>b</sup> Log<sub>10</sub> of viable microorganisms per gram of stool (dry weight).

were isolated more consistently (only two samples were negative) during the second study than during the first. Although not isolated in consistently high numbers, C. perfringens was also present in the feces of patient B. Colonies of isolate 6-13-68 on sheep blood-agar have a fried-egg shape (Fig. 1). They are circular, undulate, brownish-gray, possess a slight metallic gray sheen in the raised central portion of the colony, and are 3 to 6 mm in diameter after 96 hr of incubation at 37°C.

The morphology of isolate 6-13-68 is shown in Fig. 2. Short bacilli, long bent bacilli, long bacilli with blebs or swellings, and large spheroids were all present in the same thioglycollate broth (Fig. 1). Colonial morphology of isolate 6-13-68 after 96 hr of anaerobic incubation on sheep blood-agar.
culture grown for 24 hr at 37 C. After the organism had been subcultured repeatedly, it grew as regular bacilli (3 to 6 μm long).

The physiological characteristics of isolate 6-13-68 were compared with those of S. varius (98501) and S. freundii (9981), which were obtained from the American Type Culture Collection (Table 4). The data for S. ridiculosi were taken from Prevot (6). All four are found in the intestinal tract of man, produce gas, foul odor, and hydrogen sulfide, and are extremely pleomorphic. Only S. varius produced indole and only S. freundii reduced nitrate to nitrite. Colonies of isolate 6-13-68 were beta hemolytic on sheep blood-agar only after they were exposed to the atmosphere for 72 hr at room temperature. Fresh isolates of 6-13-68 also possess a hemagglutinin for sheep erythrocytes.

The capacity of some Sphaerophorus species to ferment sugars, as determined by acid production from various carbohydrates, is shown in Table 5. The data for S. ridiculosi were taken from Prevot (7). Sphaerophorus varius fermented only glucose and fructose; isolate 6-13-68 and S. freundii produced acid from several carbohydrates (Table 5).

Isolate 6-13-68 did not produce acid from sucrose but S. freundii did; isolate 6-13-68 fermented maltose and S. freundii did not. Sphaerophorus ridiculosi and isolate 6-13-68 differ only with acid production from sucrose, salicin, melibiose, and raffinose. The latter three carbohydrates were either negative or not tested by Prevot (6) when he characterized S. ridiculosi.

**DISCUSSION**

The predominant microorganisms found in the large bowel of normal humans are the gram-negative, anaerobic, nonsporeforming bacilli of the family Bacteroidaceae. Zubrzycki and Spaulding (9) showed that Bacteroides may outnumber coliforms by 100- or 1,000-fold in the feces of normal adults.

In two patients reported here, Bacteroides and Sphaerophorus were consistently present in feces. However, the Sphaerophorus population in the feces of patient B declined drastically after his first exposure to therapeutic TBI (150 r at 1.5 r/hr). The irradiation may have had some effect on the anaerobic flora, as the patient received no antibiotics or diet change during the study. This type of drastic change in the anaerobic population is the only one we have noticed in our microfloral studies of irradiated cancer patients. One year later the numbers of Bacteroides and Sphaerophorus in the same patient, after a similar exposure to TBI, were consistently high for 7 weeks.

The morphological and biochemical properties of the two Sphaerophorus isolates did not change during this 1-year period.

Although others have isolated Sphaerophorus
TABLE 4. Physiological characteristics of Sphaerophorus species

| Characteristic  | Isolate 6-13-68 | S. varius (ATCC 8501) | S. freundii (ATCC 9817) | S. ridiculosisb |
|-----------------|-----------------|-----------------------|------------------------|---------------|
| Habitat         | Intestinal tract—man | Intestinal tract—man | Intestinal tract—man | Intestinal tract—man |
| Rods, ovoids, spheroids, filaments | Rods, ovoids, spheroids, filaments | Rods, ovoids, spheroids, filaments | Rods, ovoids, spheroids, filaments |
| Gas             | +               | +                     | +                      | +             |
| Fetid odor      | —               | +                     | +                      | +             |
| Indole          | —               | —                     | —                      | —             |
| H2S             | +               | +                     | +                      | +             |
| Hemolysis       | β               | —                     | —                      | —             |
| Nitrate reduction| +             | —                     | —                      | —             |
| Hemagglutinin   | +               | +                     | +                      | +             |

a Designated as *Fusobacterium moriferum* by W. E. C. Moore (personal communication).
b Data taken from Prevot (7).

TABLE 5. Acid production by different Sphaerophorus species

| Substrate     | Isolate 6-13-68 | S. varius (ATCC 8501) | S. freundii (ATCC 9817) | S. ridiculosisb |
|---------------|-----------------|-----------------------|------------------------|---------------|
| Glucose       | A              | A                     | A                      | A             |
| Fructose      | A              | A                     | A                      | A             |
| Maltose       | A              | —                     | —                      | A             |
| Lactose       | A              | —                     | —                      | A             |
| Sucrose       | —d             | A                     | A                      | A             |
| Galactose     | A              | —                     | —                      | A             |
| Salicin       | A              | —                     | —                      | A             |
| Melibiose     | A              | —                     | —                      | A             |
| Raffinose     | A              | —                     | —                      | A             |
| Controlc      | —d             | —                     | —                      | —             |

a Designated as *Fusobacterium moriferum* by W. E. C. Moore (personal communication).
b Data taken from Prevot (7).
c Acid production.
d No acid.
c Control consisted of thioglycollate base without carbohydrate.

The data in Table 5 indicate that S. varius and S. freundii produce acid from all substrates listed, while S. ridiculosis does not produce acid from glucose, fructose, maltose, and lactose.

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