JOURNAL OF CLINICAL MICROBIOLOGY, July 1997, p. 1805–1808 Vol. 35, No. 7
0095-1137/97/$04.00+0

Bacteremia Caused by a Recently Described Novel *Desulfovibrio* Species

RODNEY McDOUGALL,[1]* JENNIFER ROBSON,[1] DAVID PATERSON,[2] AND WEE TEE[3]

Drs. J. J. Sullivan, N. J. Nicolaides & Partners Consulting Pathologists, Taringa, Queensland,[1] and Victorian Infectious Diseases Reference Laboratory, Fairfield Hospital, Fairfield, Victoria,[3] Australia, and Infectious Disease Section, Veterans Affairs Medical Center, Pittsburgh, Pennsylvania[2]

Received 22 November 1996/Returned for modification 10 March 1997/Accepted 9 April 1997

An obligately anaerobic, fastidious, slowly growing, spiral, gram-negative bacterium was isolated from the blood of a 75-year-old man with acute onset of pyrexia. The patient responded rapidly to appropriate antibiotic therapy. Extensive investigation failed to detect a focus for the infection. Phenotypically, the organism was consistent with *Desulfovibrio* species. Microscopic investigation revealed an organism with a vibrioid or spirillioid morphology with rapidly progressive motility by means of a single polar flagellum. Biochemically, the organism produced large amounts of H_2S and contained desulfovirdin. The 16S rRNA gene sequence of the organism was found to be most similar to those of members of the genus *Desulfovibrio*, with identical sequence homology to the newly proposed species described by Tee et al. (W. Tee, M. Dyall-Smith, W. Woods, and D. Eisen, J. Clin. Microbiol. 34:1760–1764, 1996). This is a second unrelated isolation of this novel species from two widely different locations in Australia. The two isolates show some phenotypic differences, indicating that they are different strains of the same species.

Large numbers of sulfate-reducing, anaerobic, gram-negative bacteria are found in the human bowel and mouth. A variety of nutritionally and morphologically distinct genera can be found, including *Desulfovibrio*, *Desulfomonas*, *Desulfobacter*, *Desulfotomaculum*, and *Desulfobulbus* (3, 11, 12, 19). *Desulfovibrio* spp. are ubiquitous in nature and can be isolated from most water and soil types, yet only five cases of human infection have been described previously (2, 13, 17, 18). It is an organism of considerable industrial and economic importance (8, 18). A recent report has documented the isolation of a new species of *Desulfovibrio* from a liver abscess of an elderly Australian man (18). This organism has been designated strain FH26001/95 (ATCC 700045) and has been provisionally named *Desulfovibrio fairfieldensis*. In this report, we describe a second case of bacteremia caused by this novel species and present the phenotypic and genotypic characteristics of the isolate.

CASE REPORT

A 75-year-old man presented with a 3-day history of fevers, chills, sweats, lethargy, and anorexia. At presentation he was moderately unwell but still ambulatory. There was no associated diarrhea. He was treated with oral cephalexin for 5 days without improvement. His past medical history included nonspecific sclerosing glomerulonephritis, chronic obstructive airway disease, hypertension, and deep venous thrombosis. The medications that he was taking included prednisolone, ranitidine, warfarin, metoprolol, enalapril, and hydralazine.

Due to continued pyrexia he was assessed by an infectious diseases physician. Physical examination revealed a soft systolic cardiac murmur with no clinical evidence of bacterial endocarditis. Examination of other organ systems was normal.

Laboratory investigations revealed a microcytic anemia and neutrophilia (leukocyte count, 18.5×10^9/liter; neutrophils, 15.9×10^9/liter). Electrolyte and liver function test results were within the normal range. Urine and sputum cultures were negative. Chest radiography demonstrated changes consistent with chronic obstructive airway disease, without evidence of consolidation. Ultrasonographic examination of the liver and biliary tract demonstrated the presence of a nonobstructing 0.8-cm stone in the gallbladder.

Blood samples for culture were collected and a gram-negative, spiral, rapidly motile organism was observed by wet preparation and Gram and acridine orange staining of a sample from the anaerobic bottle after 6 days of incubation. A provisional diagnosis of *Campylobacter* bacteremia was made, and intravenous ciprofloxacin (200 mg daily) was commenced. This was continued for 4 days, resulting in prompt defervescence and clinical improvement. Oral ciprofloxacin therapy was continued for a further 5 days. Subsequent blood cultures remained negative, and there was no recurrence of fevers. Endoscopy was normal, but at colonoscopy to investigate continued anemia, bleeding colonic polyps were detected. Histological examination confirmed the presence of benign adenomatous polyps.

MATERIALS AND METHODS

The organism was detected in an anaerobic blood culture bottle (BacT/Alert; Oreganon Technika Corporation, Durham, N.C.) after 6 days of incubation. Wet preparations of the blood culture were examined by light and phase-contrast microscopy, and fixed smears stained by standard Gram and acridine orange staining methods were examined. The blood culture was subcultured onto 4% horse blood and MacConkey agars incubated in air, chocolate agar incubated in 5% CO_2, and Wilkins-Chalgren anaerobic blood agar incubated anaerobically; all cultures were incubated at 37°C. Skirrow's campylobacter agar was inoculated with a sample from the blood culture and was incubated in a microaerophilic atmosphere at 37 and 42°C. All plates were examined daily for evidence of growth. Identification was initially attempted by using the Vitek ANI card (bioMérieux Vitek-Aust. Pty. Ltd.) and the RapID ANA II system (Innovative Diagnostic Systems, Inc., Norcross, Ga.). Temperature tolerance, atmospheric growth conditions, and other phenotypic aspects including Gram stain morphology, motility, and biochemical reactions were determined by standard methods (6). The desulfovirdin test was performed by adding two drops of 2.0 N NaOH to a turbid (equivalent to the turbidity of a no. 3 McFarland standard) cell suspension in distilled water and immediately exposing the cell suspension to UV light at 365 nm in a darkened room (6). The organism was inoculated into Postgate media, which are used to cultivate and study the growth requirements

* Corresponding author. Mailing address: Microbiology Department, Drs. J. J. Sullivan, N. J. Nicolaides & Partners Consulting Pathologists, 134 Whitmore St., Taringa 4068, Queensland, Australia. Phone: 61 7 3377 8543. Fax: 61 7 3371 9277.

of sulfate-reducing bacteria (1a, 21). Transmission electron microscopy was performed on glutaraldehyde-fixed bacteria harvested from blood agar after 7 days of anaerobic incubation and negatively stained with 1% aqueous uranyl acetate.

Antibiotic susceptibility testing. Antibiotic susceptibility was determined by both the disc diffusion and the Epsilometer test (Etest; AB Biodisk, Solna, Sweden) methodologies. Etests were performed and interpreted as detailed elsewhere (1). Due to slow organism growth, MIC results were read after 96 h of incubation. Quality control of Etest materials and the Etest method was performed by using *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Eubacterium lentum* ATCC 43055, as recommended by the National Committee for Clinical Laboratory Standards (16). MIC results were interpreted with reference to the guidelines of the National Committee for Clinical Laboratory Standards mentioned above (16). The disc diffusion test was performed on Wilkens-Chalgren agar plus 5% defibrinated horse blood by using an inoculum with a turbidity equivalent to that of a no. 1 McFarland standard, and zone sizes were read after 96 h. To allow for a comparison of antibiotic susceptibility test results, both the Etest and the disc diffusion test were repeated with an increased inoculum of 10^9 organisms and 5 days of incubation under anaerobic conditions, as detailed by Tee et al. (18).

Chromosomal DNA extraction, PCR, and sequencing of rRNA genes. The genomic DNA was extracted and purified from plate-grown bacteria by the cetyltrimethylammonium bromide method as described previously (22). The 16S rRNA gene sequence was determined by a previously published method (18). Briefly, the entire 16S rRNA gene (~1,500 nucleotides) of the test strain was amplified by PCR with consensus terminal primers, and the purified full-length product was sequenced directly by using terminal and internal primers specific for 16S rRNA genes. Sequencing was performed in an automated DNA sequencer (model 373A DNA sequencer; Applied Biosystems Inc., Foster City, Calif.) by a dye-labelled dideoxy termination method (Taq Dye-Deoxy Terminator Cycle Sequencing kit; Applied Biosystems Inc.). Analysis of the 16S rRNA sequence was mainly performed by using the programs and database of small-subunit rRNA sequences available in the Ribosomal Database Project database (University of Illinois, Urbana) (15).

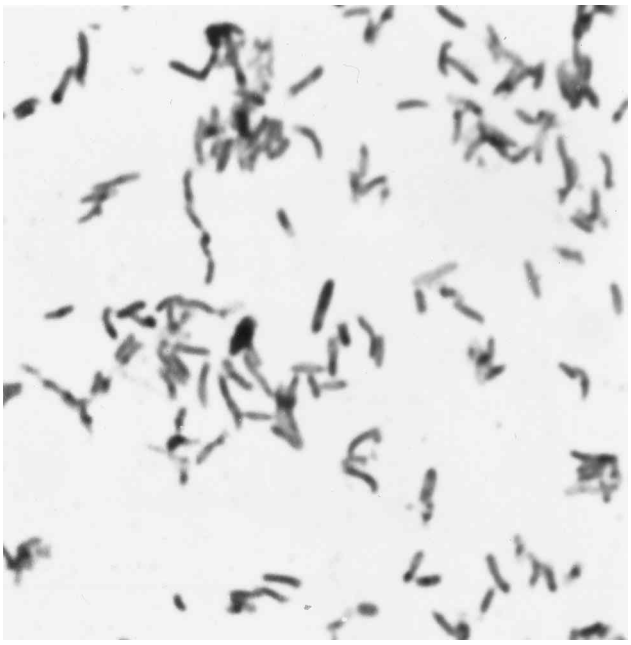

FIG. 1. Photomicrograph of a Gram-stained smear of the *Desulfovibrio* isolate showing by light microscopy, wavy spiral and vibrioid forms. Magnification, ×1,000.

RESULTS

Direct microscopic examination of the anaerobic blood culture revealed gram-negative spiral bacteria with rapidly progressive motility. Colonies on Wilkins-Chalgren anaerobic blood agar at 5 days of incubation were small (1 mm), nonhemolytic, pinpoint, grey, round, smooth, and butyrous. No growth was evident on the remaining primary culture media after 10 days of incubation. A strong sulfur smell was noted on opening the anaerobic chamber. Gram staining of the colony showed gram-negative, spiral, wavy, and vibrioid bacteria of various lengths (Fig. 1). Rapidly progressive, nondarting motility with a consistent rapid "wobble" was present by the hanging drop test.

The desulfovirdin test was positive, with an intense red fluorescence indicating the presence of the serohydrochlorin chromatophore of desulfovirdin. The organism was unreactive in tests with the Vitek ANI card and the RapID ANA II system. The organism failed to utilize carbohydrates. Other negative reactions included those of oxidase, urease, indole, nitrate, lecithinase, esculin, and gelatin hydrolysis. The slide catalase test produced a moderately positive result. A notable feature of the organism was the extremely large amount of H_2S produced, demonstrated with the use of triple sugar iron agar, Kligler iron agar, and lead acetate strips in thioglycolate broth. Production of H_2S was more pronounced at 42 and 45°C than at 37°C. Like most members of the genus *Desulfovibrio*, Postgate medium B supported the organism's growth, and a characteristic black precipitate was present, indicating sulfate reduction of ferrous salts. The organism also grew readily in Postgate basal medium D with pyruvate in the absence of sulfate. This supports the growth of only a few species of the genus *Desulfovibrio* including *Desulfovibrio desulfuricans* (21). The organism grew on bile esculin agar incubated under anaerobic conditions. It was moderately salt tolerant, growing in the presence of 2% NaCl, but this was not essential for growth. Growth was detected on subcultures incubated anaerobically at 21, 25, 37, 42, and 45°C but not at 60°C.

Motility was rapid at lower temperatures and absent at temperatures above 37°C. Organism growth in the absence of sulfates was poor. Thioglycolate broth with increasing sulfate concentrations was inoculated with the test organism and incubated at 37°C. The result was abundant growth, with the amount of black iron salt precipitation being proportional to the broth sulfate content. At high sulfate concentrations the motility of the organism was decreased. This has been noted by other investigators (21).

Transmission electron microscopy revealed a curved, sometimes sigmoid-shaped bacterium 0.75 to 1 μm by 2 to 4 μm in size with a single, long, polar, unsheathed flagellum (Fig. 2 and 3). Many organisms lacked flagella, but this was most likely due to detachment during manipulation. Small globular forms 0.5 to 0.75 μm in diameter were observed in small numbers in 37°C cultures (Fig. 3). In cultures incubated for longer periods and at temperatures above 37°C, large numbers of bacteria with internal bulbous swellings were noted (Fig. 4). Spore stains on cultures incubated at various temperatures and containing different sulfate concentrations failed to detect the presence of endospores.

The complete 16S rRNA gene sequence of the isolate was determined and compared with more than 3,000 rRNA sequences found in the sequence databases (Ribosomal Database Project and GenBank). The sequence was found to be most similar to those of organisms of the genus *Desulfovibrio*, and specifically with the new species proposed by Tee et al. (18) (GenBank accession no. U42221), to which it had an identical sequence across the entire length of the 16S rRNA gene. The type strain of *Desulfovibrio*, *D. desulfuricans* ATCC 27774, had only 97% sequence identity (45 nucleotide substitutions) with the new isolate, as reported before with the previous isolate of Tee et al. (18).

Antibiotic susceptibility testing indicated that the isolate was resistant to penicillin, ampicillin, cephalothin, cefaclor, ticarcillin, trimethoprim, sulfamethoxazole-trimethoprim, gentami-

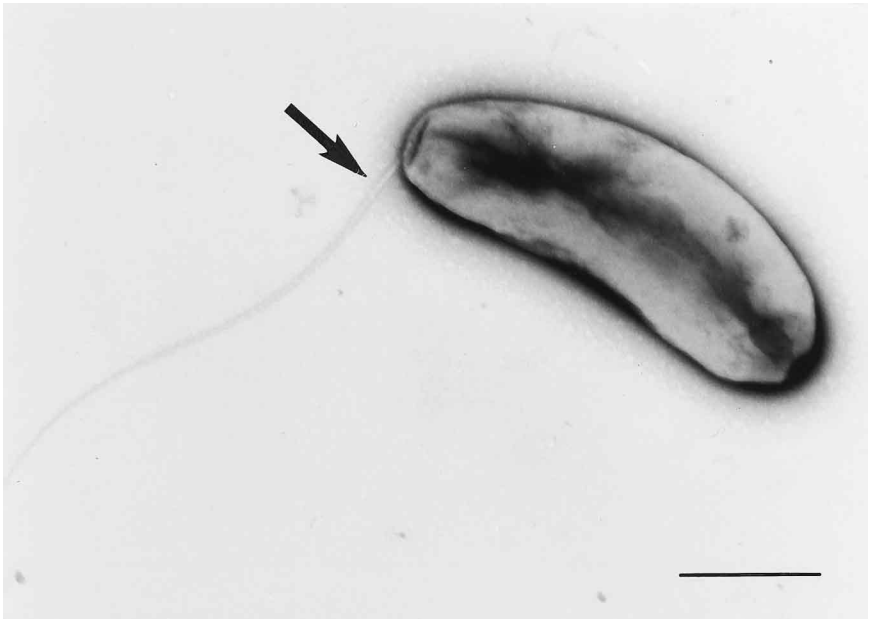

FIG. 2. Electron micrograph of the *Desulfovibrio* isolate showing the vibrioid morphology and single polar unsheathed flagellum (arrow). Bar, 1 μm.

cin, cefotaxime, and vancomycin. It was sensitive to metronidazole (MIC, 0.006 μg/ml), chloramphenicol (MIC, 0.125 μg/ml), ciprofloxacin (MIC, 0.5 μg/ml), imipenem (MIC, 0.125 μg/ml), amoxicillin-clavulanate (MIC, 1.0 μg/ml), ticarcillin-clavulanate (MIC, 1.0 μg/ml), azithromycin (MIC, 1.0 μg/ml), and clindamycin (MIC, 0.125 μg/ml).

The MIC results obtained by disc diffusion susceptibility testing correlated well with the MIC results obtained by the Etest method. Antibiotic susceptibility results by the modified method with an enhanced inoculum and prolonged incubation time did not alter the susceptibility test results significantly. A test for beta-lactamase activity with nitrocefin discs was positive.

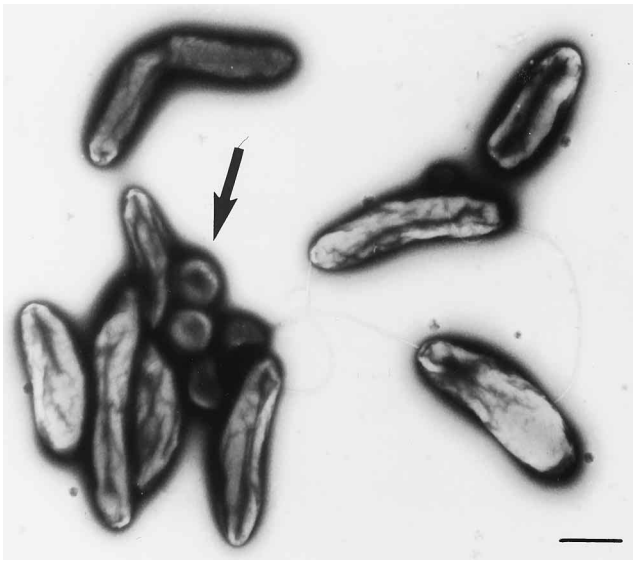

FIG. 3. Electron micrograph of the *Desulfovibrio* isolate showing globular forms (arrow) noted in 37°C cultures Bar, 1 μm.

DISCUSSION

The sulfate-reducing bacteria are found in the gastrointestinal tracts of humans and animals, but they also found in environmental habitats such as mud and fresh, brackish, and polluted waters (5). These bacteria, and in particular, *Desulfovibrio* spp., have a detrimental effect on industry and the environment. Problems include metal corrosion by cathodic depolarization and reduction of anaerobic digestion efficiency in the stabilization of wastewater sludges and sewage treatment. The

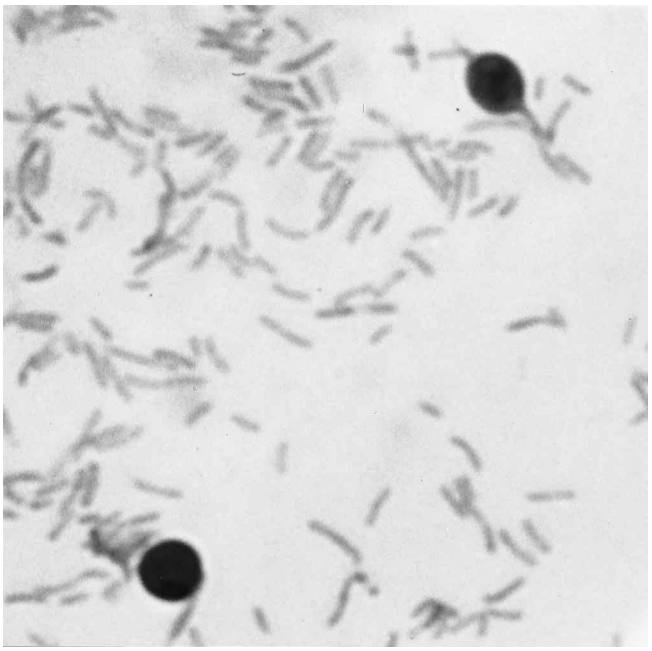

FIG. 4. Photomicrograph of a Gram-stained smear of the *Desulfovibrio* species showing by light microscopy globular forms (arrow) present in cultures in the stationary or decline phase. Magnification, ×1,000.

excessive production of hydrogen sulfide by sulfate-reducing bacteria in anoxic waters and sediments may have adverse environmental impacts, resulting in plant and animal poisoning (4). *Desulfovibrio* spp. are the prominent species of sulfate-reducing bacteria in the large bowel (10), and as such, it is proposed that this was the portal of entry in this patient via bleeding colonic polyps. The patient was immunocompromised due to preexisting sclerosing glomerulonephritis and steroid therapy; this may have predisposed this patient to systemic infection. To date, five cases of infection with *Desulfovibrio* spp. have been documented. In most patients, the gastrointestinal tract was postulated to be the source of the infection (2, 13, 17, 18). *Desulfovibrio* spp. have been implicated as possible causes of ulcerative colitis in humans and proliferative bowel disease in other mammals (7–10, 14).

Desulfovibrio spp. use sulfate as a terminal electron acceptor during respiration, generating large amounts of H_2S directly, in contrast to other organisms that generate H_2S during metabolism (17). This isolate exhibited the classical phenotypic traits of the *Desulfovibrio* species, being a strictly obligate, anaerobic, gram-negative curved bacillus that exhibited rapid progressive motility and that contained the pigment desulfovirdin. Other confirmatory tests included the visualization of a single polar, unsheathed flagellum, copious amounts of H_2S production, and utilization of pyruvate as a carbon source in the absence of sulfate (21). The optimum temperature for growth was approximately 25 to 35°C, and the temperature range for growth was 21 to 45°C. As with other members of the genus, this strain did not have heat-resistant forms and was sensitive to heating for 5 min at 100°C.

The aberrant globular morphology of the organism readily noted in cultures incubated for longer periods or at increased temperatures appears similar to that of the sporoplasts seen in the stationary or decline phase of the curved anaerobic gram-negative bacilli, *Pectinatus* and *Selenomonas* (20).

Genetic analysis by 16S rRNA gene sequence homology showed that this isolate and the new species proposed by Tee et al. (18) are identical, indicating that the two organisms recovered from unrelated Australian subjects are of the same species. There are phenotypic differences between these two strains. Unlike the previous isolate, this isolate grew at temperatures below 25°C and produced larger (copious) amounts of H_2S, and it also exhibited a different antibiotic susceptibility profile. Both Australian isolates were resistant to penicillin and ampicillin, differing from other reported human isolates of *Desulfovibrio* spp. (14, 17). The new isolate was susceptible to amoxicillin-clavulanate, imipenen, chloramphenicol, and ciprofloxacin, whereas the previous isolate reported by Tee et al. (18) was resistant to these antibiotics. The two isolates were sensitive to clindamycin and metronidazole, two drugs commonly used for the treatment of anaerobic infections. The phenotypic differences observed between these two isolates show that they represent different strains within this novel species. Laboratories need to be aware of the anaerobic, motile, vibrioid-like organisms, in particular, *Desulfovibrio* species, as rare causes of human disease. Obligate anaerobic conditions and increased incubation times are essential for successful isolation. After primary isolation the organisms appear to be aerotolerant, and less stringent anaerobic conditions are required for further manipulation. The identification of *Desulfovibrio* species to the genus level is within the scope of most laboratories by the performance of simple tests. *Desulfovibrio* species and closely related organisms exhibit great phenotypic diversity, and the use of phenotypic characteristics in the taxonomy of these organisms is not consistent with the phylogenetic relationships within the group. Molecular techniques have and will continue to allow for the more accurate taxonomic classification of these and many other bacteria.

ACKNOWLEDGMENTS

We thank the staff of Drs. J.J. Sullivan, N.J. Nicolaides and Partners for their cooperation and Deborah Stenzel from the Queensland University of Technology for performing the electron microscopy. Michael Whitby is to be thanked for allowing us to report clinical aspects of this case, and Michael Dyall Smith of Microbiology Department, University of Melbourne, is thanked for technical advice.

REFERENCES

1. **AB Biodisk.** 1996. Etest technical guide 1B. AB Biodisk, Solna, Sweden.
1a. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 556–558. CRC Press, London, United Kingdom.
2. **Baron, E. J., R. Bennion, J. Thompson, C. Strong, P. Summanen, M. McTeague, and S. M. Finegold.** 1992. A microbiological comparison between acute and complicated appendicitis. Clin. Infect. Dis. **14:**227–231.
3. **Beerens, H., and H. Romond.** 1977. Sulfate-reducing anaerobic bacteria in human faeces. Am. J. Clin. Nutr. **30:**1770–1776.
4. **Bitton, G.** 1994. Wastewater microbiology, p. 69–71. John Wiley & Sons, Inc., New York, N.Y.
5. **Butlin, K. R., M. E. Adams, and M. Thomas.** 1949. The isolation and cultivation of sulfate-reducing bacteria. J. Gen. Microbiol. **3:**46–59.
6. **Engelkirk, P. G., J. Duben-Engelkirk, and V. R. Dowell, Jr.** 1992. Clinical anaerobic bacteriology. Star Publishing Company, Belmont, Calif.
7. **Florin, T. H. J., G. R. Gibson, G. Neale, and J. H. Cummings.** 1990. A role for sulfate reducing bacteria in ulcerative colitis? Gastroenterology **98:**A170.
8. **Fox, J. G., F. E. Dewhirst, G. J. Fraser, P. J. Paster, B. Shames, and J. C. Murphy.** 1994. Intracellular *Campylobacter*-like organism from ferrets and hamsters with proliferative bowel disease is a *Desulfovibrio* sp. J. Clin. Microbiol. **32:**1229–1237.
9. **Gebhart, C. J., S. M. Barns, S. McOrist, G. F. Lin, and G. H. K. Lawson.** 1993. Ileal symbiont intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. Int. J. Syst. Bacteriol. **43:**533–538.
10. **Gibson, G. R., J. H. Cummings, and G. T. McFarlane.** 1988. Competition of hydrogen between sulfate-reducing bacteria and methanogenic bacteria from the human large intestine. J. Appl. Bacteriol. **65:**241–247.
11. **Gibson, G. R., J. H. Cummings, and G. T. McFarlane.** 1991. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol. Ecol. **86:**103–112.
12. **Gibson, G. R., J. H. Cummings, and G. T. McFarlane.** 1988. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J. Appl. Bacteriol. **65:**103–110.
13. **Johnson, C. C., and S. M. Finegold.** 1987. Uncommonly encountered, motile, anaerobic gram-negative bacilli associated with infection. Rev. Infect. Dis. **9:**1150–1162.
14. **Lawson, G. H. K., S. McOrist, S. Jasni, and R. A. Mackie.** 1993. Intracellular bacteria of porcine proliferative enteropathy: cultivation and maintenance in vitro. J. Clin. Microbiol. **31:**1136–1142.
15. **Maidak, B. L., N. Larson, M. J. McCaughey, R. Overbeek, G. J. Olsen, K. Fogel, J. Blandy, and C. R. Woese.** 1994. The Ribosomal Database Project. Nucleic Acids Res. **22:**3485–3487.
16. **National Committee for Clinical Laboratory Standards.** 1990. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard, 2nd ed. NCCLS document M100-56 (11-A3 anaerobic dilution). National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **Porschen, R. K., and P. Chan.** 1977. Anaerobic vibrio-like organisms cultured from blood: *Desulfovibrio desulfuricans* and *Succinivibrio* species. J. Clin. Microbiol. **5:**444–447.
18. **Tee, W., M. Dyall-Smith, W. Woods, and D. Eisen.** 1996. Probable new species of *Desulfovibrio* isolated from a pyogenic liver abscess. J. Clin. Microbiol. **34:**1760–1764.
19. **van der Hoeven, J. S., C. W. van der Kieboom, and M. J. Schaeken.** 1995. Sulfate-reducing bacteria in the periodontal pocket. Oral Microbiol. Immunol. **10:**288–290.
20. **Widdell, F., and N. Pfenning.** 1984. Anaerobic gram-negative straight, curved and helical rods, p. 655–656. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore, Md.
21. **Widdell, F., and N. Pfenning.** 1984. Dissimilatory sulfate or sulfur reducing bacteria, p. 663–679. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore, Md.
22. **Wilson, K.** Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley & Sons, Inc., New York, N.Y.