Vol. 41, No. 3

Isolation of Sulfate-Reducing Bacteria from Human Thoracoabdominal Pus

Julien Loubinoux,[1,2]* Benoit Jaulhac,[3] Yves Piemont,[3] Henri Monteil,[3] and Alain E. Le Faou[1]

Laboratoire de Bactériologie-Virologie UMR CNRS 7565, Faculté de Médecine, 54505 Vandoeuvre-lès-Nancy Cedex,[1] and Laboratoire de Bactériologie, Faculté de Médecine et Hôpitaux Universitaires de Strasbourg, 67000 Strasbourg,[3] France, and Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, 2780-156 Oeiras, Portugal[2]

To evaluate the prevalence of sulfate-reducing bacteria in septic processes, we searched for these bacteria by culture in 100 consecutive abdominal and pleural pus specimens. Twelve isolates were obtained from abdominal samples and were identified by a multiplex PCR as *Desulfovibrio piger* (formerly *Desulfomonas pigra*) (seven strains), *Desulfovibrio fairfieldensis* (four strains), and *Desulfovibrio desulfuricans* (one strain).

Sulfate-reducing bacteria (SRB) represent a class of anaerobic microorganisms that conduct dissimilatory sulfate reduction to obtain energy. In this process, sulfate reduction permits the dissimilatory oxidation of organic matter with the release of hydrogen sulfide, a corrosive and cytotoxic compound. SRB are present in the digestive tract (mouth and gut) of animals and humans (3, 17, 18). Human isolates consist of *Desulfovibrio* species, as *Desulfomonas pigra* has been recently reclassified as *Desulfovibrio piger* (2, 9, 19). So far, three species of *Desulfovibrio* have been isolated from human specimens: *Desulfovibrio desulfuricans*, *Desulfovibrio fairfieldensis*, and *D. piger* (7, 8, 10). These bacteria may play a role in the onset or the perpetuation of inflammatory chronic diseases such as inflammatory bowel disease (4, 8, 14) and periodontitis (10). *Desulfovibrio* spp. have also been isolated from abdominal and brain abscesses, blood, and urine (1, 5, 6, 7, 11, 12, 16). Most of these infectious processes were consecutive to digestive surgery. Whether SRB are etiologic agents of surgical abdominal infections is not known. Their prevalence in clinical samples is underestimated, as most medical laboratories do not specifically search for SRB. Additionally, these bacteria are seldom isolated because of their slow growth. Colonies appear after more than 3 days of incubation and are generally not noticed, being overgrown by the accompanying flora. Thus, their isolation requires a specific or selective growth medium. Once isolated, identification at the species level may be difficult. For example, it is not possible to differentiate *D. desulfuricans* and *D. fairfieldensis* by phenotypic tests, necessitating the use of genotypic tests to differentiate the two species. The aim of this study was to determine the prevalence of SRB in abdominal and pleural pus by using a specific liquid growth medium (Test-kit Labège; Compagnie Française de Géothermie, Orléans, France). A multiplex PCR was devised for the identification of the isolates at the species level.

Over a 6-month period, the consecutive purulent collections of 100 patients (55 women and 45 men) from different surgery units of the University Hospital of Strasbourg, Strasbourg, France, were studied. They consisted of abdominal ($n = 88$) and pleural ($n = 12$) samples. The mean age of the patients was 57 years (range, 2 to 95 years). Aerobic and anaerobic bacterial cultures were performed on all samples at the Laboratory of Bacteriology of the University Hospital of Strasbourg. For the specific culture of SRB, 200 µl of each sample was inoculated through the rubber cap of a ready-to-use specific liquid growth medium (Test-kit Labège). This medium was chosen, as it is more sensitive than the commonly used Postgate medium for isolating SRB from clinical samples (8, 10, 15). The inoculated media were incubated at 37°C for 1 month in an anaerobic chamber. SRB were detected by the formation of a black precipitate (ferrous sulfide) and were subsequently identified to species level by a multiplex PCR (8).

DNA extracts were obtained from 500 µl of the SRB-positive culture media (Test-kit Labège). After centrifugation and resuspension in 500 µl of Tris-EDTA buffer (10 mM Tris HCl, 1 mM EDTA, pH 8), bacterial cells were lysed by successively using lysozyme (3 mg ml^{-1}), sodium dodecyl sulfate (1% [wt/vol]), and proteinase K (0.25 mg ml^{-1}). After an overnight incubation at 37°C, DNA was then extracted by the standard phenol-chloroform-isoamyl alcohol method. Each 50-µl PCR mixture contained 5 µl of DNA extract (approximately 50 ng of DNA) and final 0.4 µM concentrations of each of the six primers listed below, as well as a 0.8 mM concentration of each deoxynucleoside triphosphate (Boehringer Mannheim Biochemicals, Mannheim, Germany), 20 mM Tris HCl buffer (pH

TABLE 1. Primers for the identification of *Desulfovibrio* strains

Strain	Primers	Target site on 16S ribosomal DNA[a]	Length of PCR product (bp)
D. piger[b]	Pig-F, P687-R	453–708	255
D. desulfuricans Essex 6	Essex-F, P687-R	453–708	255
D. desulfuricans MB	27K-F, 27K-R	630–1026	396
D. fairfieldensis	Fair-F, P687-R	174–708	534

[a] *Escherichia coli* numbering on 16S ribosomal RNA gene.
[b] Formerly *D. pigra*.

* Corresponding author. Mailing address: Service de Microbiologie, Hôtel Dieu, 1 place du Parvis Notre-Dame, 75181 Paris Cedex 04, France. Phone: (33) 1 42 34 82 73. Fax: (33) 1 42 34 87 19. E-mail: j.loubinoux@voila.fr.

TABLE 2. Characteristics of patients from whom SRB were isolated

Patient no.	Sex	Age (yr)	Sample	Clinical findings	SRB species[a]
1	Male	64	Peritoneal fluid	Peritonitis	*D. piger*[b]
2	Female	83	Peritoneal fluid	Peritonitis	*D. piger*
3	Female	81	Peritoneal fluid	Rectal cancer	*D. piger*
4	Male	32	Peritoneal fluid	Appendicitis, peritonitis	*D. fairfieldensis*
5	Female	88	Peritoneal fluid	Peritonitis	*D. piger*
6	Male	14	Intra-abdominal collection	Appendicitis	*D. piger*
7	Male	18	Peritoneal fluid	Peritonitis	*D. piger*
8	Female	29	Peritoneal fluid	Appendicitis, peritonitis	*D. fairfieldensis*
9	Male	9	Peritoneal fluid	Appendicitis, peritonitis	*D. piger*
10	Female	53	Peritoneal fluid	Peritonitis	*D. fairfieldensis*
11	Male	80	Peritoneal fluid	Peritonitis	*D. desulfuricans*
12	Male	21	Intra-abdominal collection	Appendicitis	*D. fairfieldensis*

[a] SRB were identified by PCR.
[b] Formerly *D. pigra*.

8.4), 1.5 mM MgCl$_2$, and 1.5 U of *Taq* DNA polymerase (Gibco-BRL Life Technologies, Paisley, United Kingdom). The primers, designed previously from 16S rRNA gene sequences (8), were Pig-F (5′-CTA GGG TGT TCT AAT CAT CAT CCT AC-3′), P687-R (5′-GAT ATC TAC GGA TTT CAC TCC TAC ACC-3′), Essex-F (5′-CTA CGT TGT GCT AAT CAG CAG CGT AC-3′), 27K-F (5′-CTG CCT TTG ATA CTG CTT AG-3′), 27K-R (5′-GGG CAC CCT CTC GTT TCG GAG A-3′), and Fair-F (5′-TGA ATG AAC TTT TAG GGG AAA GAC-3′) (Table 1). All reactions were carried out by using the GeneAmp PCR System 2400 (Applied Biosystems, Norwalk, Conn.). An initial denaturation step of 94°C for 4 min was followed by 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min), and extension (72°C, 2 min), with a final extension (72°C, 5 min). Amplified products were resolved by electrophoresis in 1.5% (wt/vol) agarose gels containing ethidium bromide (1.6 mg ml^{-1}). A 100-bp DNA ladder was used as a size marker (Gibco-BRL Life Technologies). *D. piger*, *D. desulfuricans* strain Essex 6, *D. desulfuricans* strain MB, and *D. fairfieldensis* were identified by 255-, 255-, 396-, and 534-bp bands, respectively (Table 1). *D. piger* and *D. desulfuricans* Essex 6 were further differentiated by separate PCR assays with their respective specific primers.

SRB were isolated from 12 samples, all of abdominal origin (10 peritoneal fluids and 2 intra-abdominal collections). The SRB-positive samples were from patients presenting with peritonitis, appendicitis, or abscess after surgery for rectal cancer (Table 2). SRB were identified by PCR as *D. piger* (seven strains), *D. fairfieldensis* (four strains), and *D. desulfuricans* MB (one strain) (Fig. 1). In all cases, SRB isolates were part of mixed aerobic and anaerobic flora. No SRB were detected in the 12 purulent pleural fluids studied.

SRB have been described in the digestive tract of animals and humans for many years. In humans, neither prevalence nor identification as to predominant species has been determined. Recently, the study of feces from healthy individuals and patients has shown that *D. piger* is the predominant species in humans (8). Until that study, *D. piger* had been reported only once in human feces (13). Our results also suggest that it is the most common species of SRB in human abdominal pus, which is a logical finding since the flora of abdominal pus is of enteric origin. In contrast, *D. fairfieldensis* occurs less commonly in the gut and in purulent abdominal specimens but has been isolated from blood (7, 12) and periodontal pockets (10). *D. fairfieldensis* is the only species of SRB found in pure culture in septic processes outside the abdomen.

The type species of the genus *Desulfovibrio*, *D. desulfuricans*, is commonly isolated from the environment. It has also been considered the most prevalent species of *Desulfovibrio* in humans (4, 19). However, this species appears to be an uncommon organism in the human intestinal tract, as evidenced by the fact that there was only one isolate obtained from 88 abdominal specimens in this work and that only one isolate was obtained from 151 human feces samples in a previous study
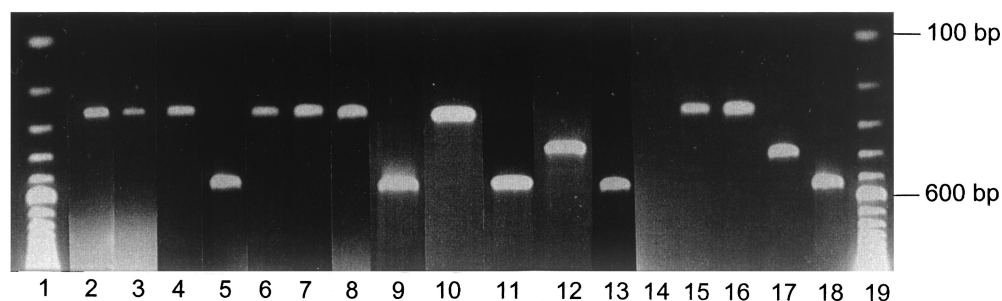

FIG. 1. Multiplex PCR products obtained with 12 clinical samples and 4 collection strains of *Desulfovibrio*. Lanes: 1 and 19, 100-bp DNA ladder; 2 to 13, patients 1 to 12; 14, negative control (water); 15, *D. piger* (formerly *D. pigra*) ATCC 29098[T]; 16, *D. desulfuricans* strain Essex 6 (ATCC 29577[T]); 17, *D. desulfuricans* strain MB (ATCC 27774); and 18, *D. fairfieldensis* ATCC 700045.

(8). These results suggest that *D. desulfuricans* may occasionally be present in humans as a result of a contamination from the environment but that it is not a common commensal or infectious agent.

The involvement of SRB in pathological processes has recently been proposed. *D. fairfieldensis* may present with invasive properties that explain its presence in the bloodstream and abscesses (7, 12, 16). Its association with periodontitis deserves further investigation. While *D. piger* has never been isolated from extra-abdominal samples, recent findings suggest a relationship between this bacterium and inflammatory bowel diseases (8). However, the role of SRB in the digestive tract is still not understood. *D. piger* and *D. fairfieldensis* have never been isolated outside human samples, indicating that they may be natural residents of the human digestive tract. The search for these bacteria in various ecological niches should be undertaken in an attempt to further elucidate their role in the human host.

REFERENCES

1. **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.
2. **Gibson, G. R., G. T. McFarlane, and J. H. Cummings.** 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:**103–111.
3. **Gibson, G. R.** 1990. Physiology and ecology of sulphate-reducing bacteria. J. Appl. Bacteriol. **69:**769–797.
4. **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.
5. **Johnson, C. C., and S. M. Finegold.** 1987. Uncommonly encountered, motile, anaerobic gram-negative bacilli associated with infection. Rev. Infect. Dis. **9:**1150–1162.
6. **La Scola, B., and D. Raoult.** 1999. Third human isolate of a *Desulfovibrio* sp. identical to the provisionally named *Desulfovibrio fairfieldensis*. J. Clin. Microbiol. **37:**3076–3077.
7. **Loubinoux, J., F. Mory, I. A. C. Pereira, and A. E. Le Faou.** 2000. Bacteremia caused by a strain of *Desulfovibrio* related to the provisionally named *Desulfovibrio fairfieldensis*. J. Clin. Microbiol. **38:**931–934.
8. **Loubinoux, J., J.-P. Bronowicki, I. A. C. Pereira, J.-L. Mougenel, and A. E. Le Faou.** 2002. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiol. Ecol. **40:**107–112.
9. **Loubinoux, J., F. M. A. Valente, I. A. C. Pereira, A. Costa, P. A. D. Grimont, and A. E. Le Faou.** 2002. Reclassification of the only species of the genus *Desulfomonas*, *Desulfomonas pigra*, as *Desulfovibrio piger* comb. nov. Int. J. Syst. Evol. Microbiol. **52:**1305–1308.
10. **Loubinoux, J., C. Bisson-Boutelliez, N. Miller, and A. E. Le Faou.** 2002. Isolation of the provisionally named *Desulfovibrio fairfieldensis* from human periodontal pockets. Oral Microbiol. Immunol. **17:**321–323.
11. **Lozniewski, A., P. Maurer, H. Schuhmacher, J. P. Carlier, and F. Mory.** 1999. First isolation of *Desulfovibrio* sp. as part of a polymicrobial infection from a brain abscess. Eur. J. Clin. Microbiol. Infect. Dis. **18:**602–603.
12. **MacDougall, R., J. Robson, D. Paterson, and W. Tee.** 1997. Bacteremia caused by a recently described novel *Desulfovibrio* species. J. Clin. Microbiol. **35:**1805–1808.
13. **Moore, W. E. C., J. L. Johnson, and L. V. Holdeman.** 1976. Emendation of *Bacteroidaceae* and *Butyrivibrio* and descriptions of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas*, *Butyrivibrio*, *Eubacterium*, *Clostridium*, and *Ruminococcus*. Int. J. Syst. Bacteriol. **26:**238–252.
14. **Pitcher, M. C. L., and J. H. Cummings.** 1996. Hydrogen sulphide: a bacterial toxin in ulcerative colitis? Gut **39:**1–4.
15. **Postgate, J. R.** 1984. The sulphate-reducing bacteria, 2nd ed. Cambridge University Press, Cambridge, United Kingdom.
16. **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.
17. **Van der Hoeven, J. S., C. W. A. Van den Kieboom, and M. J. M. Schaeken.** 1995. Sulfate-reducing bacteria in the periodontal pocket. Oral Microbiol. Immunol. **10:**288–290.
18. **Willis, C. L., G. R. Gibson, C. Allison, S. MacFarlane, and J. S. Holt.** 1995. Growth, incidence and activities of dissimilatory sulfate-reducing bacteria in the human oral cavity. FEMS Microbiol. Lett. **129:**267–272.
19. **Willis, C. L., J. H. Cummings, G. Neale, and G. R. Gibson.** 1997. Nutritional aspects of dissimilatory sulfate reduction in the human large intestine. Curr. Microbiol. **35:**294–298.