Reclassification of *Desulfovibrio desulfuricans* Norway 4 as *Desulfomicrobium norvegicum* comb. nov. and Confirmation of *Desulfomicrobium escambiense* (corrig., Formerly “*escambium*”) as a New Species in the Genus *Desulfomicrobium*

BARBARA R. SHARAK GENTHNER,^{1,2*} STEPHANIE D. FRIEDMAN,^{1,2†} AND RICHARD DEVEREUX³

Biogeochemical Systems Center, University of New Hampshire, Durham, New Hampshire 03824¹; Center for Environmental Diagnostics and Bioremediation, University of West Florida, Pensacola, Florida 32514²; and Environmental Research Laboratory, Microbial Ecology and Biotechnology Branch, U.S. Environmental Protection Agency, Gulf Breeze, Florida 32561³

***Desulfomicrobium escambiense*, *Desulfomicrobium baculatum*, *Desulfomicrobium apsheronum*, and *Desulfovibrio desulfuricans* Norway 4 are closely related as determined by a 16S rRNA comparison (levels of relatedness, 0.976 to 0.997) and are distinct on the basis of levels of DNA-DNA similarity (11.1 to 27.4%), genomic restriction fragment length polymorphism patterns, and certain phenotypic characteristics. We proposed that *Desulfovibrio desulfuricans* Norway 4 be renamed *Desulfomicrobium norvegicum* comb. nov.**

The genus *Desulfomicrobium* contains dissimilatory sulfate-reducing species that are physiologically similar to *Desulfovibrio* species and are straight rods which lack desulfovibridin (25), a pigment considered diagnostic for the genus *Desulfovibrio* (21). Recently, we described the isolation and classification of the new species *Desulfomicrobium escambiense* (type strain, ATCC 51164) (27). In the current study we compared the genotypic and phenotypic characteristics of *Desulfomicrobium escambiense*, *Desulfomicrobium baculatum* DSM 4028, *Desulfomicrobium apsheronum* DSM 5918, and *Desulfovibrio desulfuricans* Norway 4 (= NCIMB 8310). Our results showed that these four organisms are members of separate *Desulfomicrobium* species.

Growth was measured by determining increases in optical density at 600 nm (OD₆₀₀) in anaerobic medium supplemented with 0.02% yeast extract (26, 28, 29). Volatile and nonvolatile fatty acids were quantified after derivitization (9, 10) by comparison to derivatized authentic standards and by using heptanoate as an internal derivitization control. Sulfate concentrations were determined by ion chromatography (27).

The 16S ribosomal DNA sequence of *Desulfomicrobium apsheronum* (GenBank accession no. U64865) was determined as described by Weisberg et al. (27, 32). Levels of similarity among *Desulfomicrobium* 16S rRNAs (26) were calculated as described previously (11). DNA-DNA hybridization was performed on Immobilon-N transfer membranes (23) as previously described (1), with the size variations of purified genomic DNA minimized by passage through a 29-gauge needle and sonication. Genomic DNA was labeled with [³²P]dCTP by using a random primed DNA labeling kit (Boehringer Mannheim). Levels of heterologous DNA-DNA binding were calculated by determining percentages of similarity (8). *Xba*I-digested genomic DNA was analyzed by pulse-field gel electrophoresis (2, 14).

Utilization of lactate, pyruvate, formate, and hydrogen as

electron donors for sulfate reduction and the failure to utilize acetate, propionate, or butyrate as electron donors for sulfate reduction have been described previously for *Desulfomicrobium* species (4, 16, 19, 20, 24, 25, 27). However, previous authors have disagreed on the utilization of ethanol, malate, fumarate, or choline as electron donors. Our growth and chromatographic data indicated that the four strains used in our study utilized ethanol, but not choline (data not shown), for sulfate reduction, in contrast to previous reports that *Desulfomicrobium baculatum* and strain Norway 4 did not use ethanol as an electron donor (4, 24) and that strain Norway 4 used choline (16), like other *Desulfovibrio desulfuricans* (22) strains.

Growth and the end products formed with pyruvate, fumarate, and malate during sulfate reduction or fermentation were compared (Table 1). Acetate was a major product of all strains in both media used. All strains formed succinate in fumarate-sulfate medium, although *Desulfomicrobium baculatum* and strain Norway 4 formed much more succinate (25 mM) than *Desulfomicrobium apsheronum* or *Desulfomicrobium escambiense* formed (1.0 to 8 mM). Only *Desulfomicrobium baculatum* and strain Norway 4 formed succinate (7.4 to 9.3 mM) in malate-sulfate medium. Lactate was formed by *Desulfomicrobium escambiense* and strain Norway 4 in fumarate-sulfate and malate-sulfate media, but only by *Desulfomicrobium escambiense* in pyruvate-sulfate medium. Interestingly, *Desulfomicrobium apsheronum* and *Desulfomicrobium escambiense* grew better (OD₆₀₀, 0.3 to 0.4) with fumarate or malate in sulfate medium than *Desulfomicrobium baculatum* or strain Norway 4 grew (OD₆₀₀, 0.2). In these tests, *Desulfomicrobium apsheronum* and *Desulfomicrobium escambiense* reduced the sulfate, while *Desulfomicrobium baculatum* and strain Norway 4 preferentially reduced fumarate to succinate despite the less favorable thermodynamics (30) of the latter reaction. We found that *Desulfomicrobium escambiense* used fumarate and malate as electron donors in media containing yeast extract, in contrast to the negative results obtained with defined anaerobic medium (27).

Previous reports indicated that strain Norway 4 grew poorly (20) and *Desulfomicrobium baculatum* failed to grow (19, 24) by pyruvate fermentation, while *Desulfomicrobium baculatum* failed to ferment fumarate (24) or malate (25) and strain Norway 4 fermented both of these compounds (15, 17). In the

* Corresponding author. Mailing address: Center for Environmental Diagnostics and Bioremediation, University of West Florida, 11000 University Parkway, Pensacola, FL 32514. Phone: (904) 857-6097. Fax: (904) 474-3130. E-mail: bgenthne@uwf.edu.

† Present address: U.S. Environmental Protection Agency, Gulf Breeze, FL 32561.

TABLE 1. Comparison of *Desulfomicrobium* species and strain Norway 4 for growth by sulfate reduction or fermentation with fumarate, malate, or pyruvate

Growth conditions	Species or strain	Growth ^a	Major organic product(s)	SO ₄ reduction ^a
Fumarate and SO ₄	<i>Desulfomicrobium apsheronum</i>	+++	Acetate, succinate	++
	<i>Desulfomicrobium baculatum</i>	+++	Acetate, succinate	—
	<i>Desulfomicrobium escambiense</i>	+++	Acetate, lactate, succinate	++
	Norway 4	++	Acetate, lactate, succinate	—
Malate and SO ₄	<i>Desulfomicrobium apsheronum</i>	+++	Acetate	++
	<i>Desulfomicrobium baculatum</i>	++	Acetate, succinate	—
	<i>Desulfomicrobium escambiense</i>	+++	Acetate, lactate	++
	Norway 4	++	Acetate, lactate, succinate	—
Pyruvate and SO ₄	<i>Desulfomicrobium apsheronum</i>	++	Acetate	+
	<i>Desulfomicrobium baculatum</i>	++	Acetate	+
	<i>Desulfomicrobium escambiense</i>	++	Acetate, lactate	+
	Norway 4	++	Acetate	+
Fumarate alone	<i>Desulfomicrobium apsheronum</i>	+++	Acetate, succinate	NA
	<i>Desulfomicrobium baculatum</i>	+++	Acetate, succinate	NA
	<i>Desulfomicrobium escambiense</i>	+	Acetate, lactate, succinate	NA
	Norway 4	+	Acetate, lactate, succinate	NA
Malate alone	<i>Desulfomicrobium apsheronum</i>	+	Acetate, lactate, succinate	NA
	<i>Desulfomicrobium baculatum</i>	+	Acetate, succinate	NA
	<i>Desulfomicrobium escambiense</i>	+	Acetate, lactate, succinate	NA
	Norway 4	+	Acetate, lactate, succinate	NA
Pyruvate alone	<i>Desulfomicrobium apsheronum</i>	+	Acetate, lactate, succinate	NA
	<i>Desulfomicrobium baculatum</i>	±	Acetate, succinate	NA
	<i>Desulfomicrobium escambiense</i>	+	Acetate, lactate, succinate	NA
	Norway 4	±	Acetate, lactate, succinate	NA

^a +++ , very good; ++ , good; + , fair; ± , poor; — , none; NA , not applicable.

current study, pyruvate fermentation and malate fermentation were indicated by the formation of acetate and succinate by *Desulfomicrobium baculatum* and by the formation of acetate, lactate, and succinate by *Desulfomicrobium escambiense*, *Desulfomicrobium apsheronum*, and strain Norway 4. *Desulfomicrobium apsheronum* and *Desulfomicrobium baculatum* grew better (OD₆₀₀, 0.24 to 0.36) by fumarate fermentation than *Desulfomicrobium escambiense* or strain Norway 4 grew (OD₆₀₀, 0.10) and formed acetate and succinate, in contrast to *Desulfomicrobium escambiense* and strain Norway 4, which formed acetate, lactate, and succinate. Thus, lactate was formed by *Desulfomicrobium escambiense* and strain Norway 4 during fermentation of all three substrates and by *Desulfomicrobium apsheronum* during fermentation of pyruvate or malate, but not by *Desulfomicrobium baculatum* during fermentation of any of the three substrates. Choline was not fermented by any of the strains tested.

16S rRNA sequence comparisons (Table 2) revealed a close relationship among the four strains examined. *Desulfomicrobium baculatum*, *Desulfomicrobium apsheronum*, and strain Norway 4 were more closely related to each other (levels of sequence similarity, 99.3 to 99.7%) than to *Desulfomicrobium escambiense* (97.6 to 98.1%). Since 16S rRNA analysis alone is not sufficient to distinguish closely related species (6), DNA-DNA hybridization experiments were performed with the four organisms. The levels of DNA-DNA similarity (Table 2) among the four strains used ranged from 11.1 to 27.4%, which is consistent with each taxon representing a unique species. The similarity value of 24.7% reported previously (18, 25) for *Desulfomicrobium baculatum* and *Desulfomicrobium apsheronum* is close to our value (19.8%). Wayne et al. (31) stated that a species should include strains that exhibit ≥70% DNA-DNA relatedness. Strain Norway 4 exhibited only 2.1% similarity with *Desulfovibrio desulfuricans* ATCC 27774.

TABLE 2. Levels of DNA-DNA similarity and 16S rRNA similarity for *Desulfomicrobium* species

Species or strain	% Homology or % similarity ^a				
	<i>Desulfomicrobium escambiense</i> ESC1	<i>Desulfomicrobium apsheronum</i>	<i>Desulfomicrobium baculatum</i>	Norway 4	<i>Desulfovibrio desulfuricans</i> ^b
<i>Desulfomicrobium escambiense</i> ESC1		0.981	0.981	0.976	0.871
<i>Desulfomicrobium apsheronum</i>	11.1 ± 13.2		0.997	0.992	0.870
<i>Desulfomicrobium baculatum</i>	12.3 ± 5.4	19.8 ± 22.7		0.993	0.877
Norway 4	13.0 ± 5.4	13.1 ± 9.7	27.4 ± 7.1		0.877
<i>Desulfovibrio desulfuricans</i> ^b	1.5 ± 1.7	0.14 ^c	1.8 ± 1.3	2.1 ± 2.0	

^a The values on the upper right are levels of 16S rRNA similarity, and the values on the lower left are levels of DNA-DNA similarity.

^b Negative control.

^c There was no sample deviation, and the analysis was not repeated.

FIG. 1. Macrorestriction fragment analysis of *Desulfomicrobium apsheronum* (lane A), *Desulfomicrobium escambiense* ATCC 51164 (lane B), *Desulfomicrobium baculatum* (lane C), *Desulfomicrobium escambiense* ESC1 (lane D), strain Norway 4 (lane E), and a λ ladder size standard (lane F) by pulse-field gel electrophoresis.

Genotypic divergence was also indicated by the results of a macrorestriction analysis (2, 3, 7) which revealed unique fragmentation patterns for the strains examined (Fig. 1). We calculated that the G+C content of strain Norway 4, which has not been published previously, is 54.2 mol% on the basis of a DNA thermal melting point of 92.0°C (13). The G+C contents of *Desulfomicrobium baculatum* (24), *Desulfomicrobium escambiense* (27), and *Desulfomicrobium apsheronum* (25) are 56.8, 59.9, and 52 mol%, respectively.

Desulfovibrio desulfuricans Norway 4 was first isolated from Oslo Harbor in 1949 (16). It was classified as a strain of *Desulfovibrio desulfuricans* on the basis of physiological similarity, despite the fact that it was a straight rod that lacked desulfoviridin. More recently, bacterial strains having these characteristics were placed in a separate genus, the genus *Desulfomicrobium* (25). On this basis, Fauque et al. (5) considered strain Norway 4 a strain of *Desulfomicrobium baculatum* (12, 16). Placement of a strain in a species should be based on both phylogenetic and phenotypic characteristics (31). We therefore reexamined the members of the genus *Desulfomicrobium* and *Desulfovibrio desulfuricans* Norway 4. Based on DNA-DNA hybridization values and the organic products formed from growth on malate, fumarate, and pyruvate, strain Norway 4 appears to be sufficiently different from *Desulfomicrobium baculatum* to be considered a member of a separate species. We propose that *Desulfovibrio desulfuricans* Norway 4 be renamed *Desulfomicrobium norvegicum* comb. nov. In addition, our phenotypic and phylogenetic information confirmed that *Desulfomicrobium escambiense* (es.cam.bi.en'se. M. L. adj. *escambiense*, pertaining to the Escambia River) is a distinct species.

Description of *Desulfomicrobium norvegicum* comb. nov. *Desulfomicrobium norvegicum* (nor.ve'gi.cum. M. L. adj. *norvegicum*, Norwegian). Isolated from Oslo Harbor in 1949. The type strain was originally designated *Desulfovibrio desulfuricans* Norway 4 and was deposited in the National Collection of Industrial Bacteria as strain NCIB 8310.

Non-spore-forming, gram-negative, sulfate-reducing, obli-

gately anaerobic rods (0.5 to 1.0 by 3.0 to 5.0 μ m). Uses lactate, pyruvate, ethanol, hydrogen, and formate but not acetate, propionate, butyrate, methanol, propanol, butanol, isobutanol, lactose, glucose, oxalate, or choline as electron donors for sulfate reduction. Ferments pyruvate poorly, malate well, and fumarate very well, forming acetate, lactate, and succinate. Does not ferment choline. Preferentially reduces fumarate and malate to succinate over reduction of sulfate to sulfide. Contains desulfoferritin, but not desulfoferritin. The DNA G+C content is 56.8 mol%. The levels of DNA-DNA homology with *Desulfomicrobium apsheronum*, *Desulfomicrobium baculatum*, and *Desulfomicrobium escambiense* are 13.1, 27.4, and 13.0%, respectively, and the level of DNA-DNA homology with *Desulfovibrio desulfuricans* ATCC 27774 is 2.1%. Exhibits 99.2, 99.3, and 97.6% 16S rRNA similarity with *Desulfomicrobium apsheronum*, *Desulfomicrobium baculatum*, and *Desulfomicrobium escambiense*, respectively, and 87.7% 16S rRNA similarity with *Desulfovibrio desulfuricans* ATCC 27774. The type strain is NCIMB 8310.

We thank Brendan Manning for assistance in pulse-field gel electrophoresis, Michael O'Keefe and Ling Wan for technical support, and the University of Florida Interdisciplinary Center for Biotechnology Research (Gainesville) for the *Desulfomicrobium apsheronum* 16S rRNA sequence determination.

This research was supported in part by U.S. Environmental Protection Agency Cooperative Agreement CR 820062 with Mark E. Hines (University of New Hampshire).

REFERENCES

- Amann, R. L., C. Lin, R. Key, L. Montgomery, and D. A. Stahl. 1992. Diversity among *Fibrobacter* isolates: towards a phylogenetic classification. *Syst. Appl. Microbiol.* 15:23-31.
- Cheng, H. P., and T. G. Lessie. 1994. Multiple replicons constituting the genome of *Pseudomonas cepacia* 17616. *J. Bacteriol.* 176:4034-4042.
- Chu, G. 1990. Pulsed-field gel electrophoresis: theory and practice. *Methods* 1:129-142.
- Devereux, R., S. H. He, C. L. Doyle, S. Orkland, D. A. Stahl, J. LeGall, and W. B. Whitman. 1990. Diversity and origin of *Desulfovibrio* species: phylogenetic definition of a family. *J. Bacteriol.* 172:3609-3619.
- Fauque, G., J. LeGall, and L. L. Barton. 1991. Sulfate-reducing and sulfur-reducing bacteria, p. 271-337. In J. M. Shively and L. L. Barton (ed.), *Variations in autotrophic life*. Academic Press, New York, N.Y.
- Fox, G. E., J. D. Wisotzkey, and P. Jurtshuk, Jr. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int. J. Syst. Bacteriol.* 42:166-170.
- Grothues, D., and B. Tummier. 1991. New approaches in genome analysis by pulsed-field gel electrophoresis: application to the analysis of *Pseudomonas* species. *Mol. Microbiol.* 5:2763-2776.
- Johnson, J. L. 1991. DNA reassociation experiments, p. 21-44. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons, New York, N.Y.
- Kawahara, F. K. 1971. Gas chromatographic analysis of mercaptans, phenols and organic acids in surface waters with use of pentafluorobenzyl derivatives. *Environ. Sci. Technol.* 5:235-239.
- Kuo, C.-W., and B. R. Sharak-Genthner. 1996. Effect of added heavy metal ions on biotransformation and biodegradation of 2-chlorophenol and 3-chlorobenzoate in anaerobic bacterial consortia. *Appl. Environ. Microbiol.* 62:2317-2323.
- Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Macke, T. L. Marsh, and C. R. Woese. 1993. The Ribosomal RNA Database Project. *Nucleic Acids Res.* 21:3021-3023.
- Lee, J. P., C. S. Yi, J. LeGall, and H. D. Peck. 1973. Isolation of a new pigment, desulfoferritin, from *Desulfovibrio desulfuricans* (Norway strain) and its role in sulfite reduction. *J. Bacteriol.* 115:453-455.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* 5:109-118.
- McClelland, M., R. Jones, Y. Patel, and M. Nelson. 1987. Restriction endonucleases for pulsed field mapping of bacterial genomes. *Nucleic Acids Res.* 15:5985-6005.
- Miller, J. D. A., P. M. Neumann, L. Elford, and D. S. Wakerley. 1970. Malate dismutation by *Desulfovibrio*. *Arch. Microbiol.* 71:214-219.
- Miller, J. D. A., and A. M. Saleh. 1964. A sulphate-reducing bacterium containing cytochrome c_3 but lacking desulfoferritin. *J. Gen. Microbiol.* 37:419-423.

17. Miller, J. D. A., and D. S. Wakerley. 1966. Growth of sulphate-reducing bacteria by fumarate dismutation. *J. Gen. Microbiol.* **43**:101–107.
18. Nazina, T. N., A. B. Poltarau, and E. P. Rozanova. 1987. Estimation of genetic relationship of rod-shaped asporogenic sulfate-reducing bacteria. *Microbiology (Engl. Transl. Mikrobiologiya)* **56**:669–672.
19. Pfennig, N., F. Widdel, and H. G. Truper. 1981. The dissimilatory sulfate-reducing bacteria, p. 926–940. In M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes*. Springer-Verlag, Berlin, Germany.
20. Postgate, J. R. 1952. Growth of sulphate-reducing bacteria in sulphate-free media. *Research (London)* **5**:189–190.
21. Postgate, J. R. 1959. A diagnostic reaction of *Desulphovibrio desulphuricans*. *Nature (London)* **183**:481–482.
22. Postgate, J. R., and L. L. Campbell. 1966. Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. *Bacteriol. Rev.* **30**:732–738.
23. Raskin, L., W. C. Capman, M. D. Kane, B. R. Rittmann, and D. A. Stahl. 1996. Critical evaluation of membrane supports for use in quantitative hybridizations. *Appl. Environ. Microbiol.* **62**:300–303.
24. Rozanova, E. P., and T. N. Nazina. 1976. A mesophilic, sulfate-reducing, rod-shaped, nonsporeforming bacterium. *Microbiology (Engl. Transl. Mikrobiologiya)* **45**:711–716.
25. Rozanova, E. P., T. N. Nazina, and A. S. Galushko. 1988. Isolation of a new genus of sulfate-reducing bacteria and description of a new species of this genus, *Desulfomicrobium apsheronum* gen. nov., sp. nov. *Microbiology (Engl. Transl. Mikrobiologiya)* **57**:514–520.
26. Sharak Genthner, B. R., C. L. Davis, and M. P. Bryant. 1981. Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol- and H₂-CO₂-utilizing species. *Appl. Environ. Microbiol.* **42**:12–19.
27. Sharak Genthner, B. R., G. Mundfrom, and R. Devereux. 1994. Characterization of *Desulfomicrobium escambium* sp. nov. and proposal to assign *Desulfovibrio desulfuricans* strain Norway 4 to the genus *Desulfomicrobium*. *Arch. Microbiol.* **161**:215–219.
28. Sharak Genthner, B. R., W. A. Price, and P. H. Pritchard. 1989. Anaerobic degradation of chloroaromatic compounds in aquatic sediments under a variety of enrichment conditions. *Appl. Environ. Microbiol.* **55**:1466–1471.
29. Sharak Genthner, B. R., W. A. Price, and P. H. Pritchard. 1989. Characterization of anaerobic dechlorinating consortia derived from aquatic sediments. *Appl. Environ. Microbiol.* **55**:1472–1476.
30. Thauer, R. K., K. Jungermann, and K. Decker. 1977. Energy conservation in chemoautotrophic anaerobic bacteria. *Bacteriol. Rev.* **41**:100–180.
31. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Truper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* **37**:463–464.
32. Weisburg, W. G., S. M. Barnes, D. A. Pelleteir, and D. J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**:697–703.