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In vitro and in vivo sulfate reduction in the gut contents of the termite *Mastotermes darwiniensis* and the rose-chaffer *Pachnoda marginata*

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Sulfate-reducing bacteria (SRB) from termites have been assigned to the genus *Desulfovibrio*. *Desulfovibrio intestinalis* lives in the gut of the Australian termite *Mastotermes darwiniensis*. For the first time we were able to enrich and identify a sulfate-reducing bacterium from the gut of the rose-chaffer *Pachnoda marginata*, which showed the highest 16S rDNA sequence identity (93%) to *Desulfovibrio intestinalis* and *Desulfovibrio* strain STL1. Compared to *Mastotermes darwiniensis* (1×10^7 cells of SRB per ml gut contents), sulfate-reducing bacteria occurred in higher numbers in the gut contents of *Pachnoda marginata* reaching cell titers of up to 2×10^8 cells per ml gut contents. In vitro sulfate reduction rates were determined with SRB from the gut contents of the termite *Mastotermes darwiniensis* and the beetle *Pachnoda marginata*. Due to the higher cell titer, the sulfate reduction rate of *Pachnoda marginata* was $10^4 \text{ nmol} \times \text{h}^{-1} \times \text{ml}^{-1}$ and therefore, 21 times higher than that of *Mastotermes darwiniensis*. In addition, we detected in vivo sulfate reduction in *Mastotermes darwiniensis*, which indicates that sulfate reducers play an active role in the sulfur metabolism in the termite gut.

Key Words—*Desulfovibrio*; insect gut; *Mastotermes darwiniensis*; *Pachnoda marginata*; sulfate reduction; sulfur species

Introduction

Sulfate-reducing bacteria (SRB) are of great significance in the final step of carbon recycling and in the sulfur cycle in anaerobic ecosystems. SRB are found mainly in marine and freshwater sediments and sewage sludge, but were also isolated from the intes-

nal tract of humans, ruminants and pigs (Gebhart et al., 1993; Gibson et al., 1988a, b; Howard and Hungate, 1976; Sass et al., 1998). In anaerobic marine sediments these bacteria can catalyze more than 50% of the total carbon oxidation (Jørgensen, 1982). SRB were found beside methanogens and homoacetogens as a further H_2 -utilizing bacterial group in the gut of higher and lower termites and in the wood-eating cockroach *Cryptocercus punctulatus* (Brauman et al., 1990; Fröhlich et al., 1999; Kuhnigk et al., 1996; Ohkuma and Kudo, 1996; Trinkerl et al., 1990) and as ectosymbionts of anaerobic ciliates (Fenchel and Ramsing, 1992).

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Interestingly, nearly all SRB isolates from human and animal gut systems identified so far belong to the genus *Desulfovibrio*. Typical features of this genus are an incomplete oxidation of electron donors such as lactate to acetate and the capability of hydrogen consumption (Cord-Ruwisch et al., 1988; Widdel, 1988; Widdel and Hansen, 1992). In relatively sulfate-poor intestinal systems SRB are in direct competition with acetogenic and methanogenic bacteria for H_2 (Cord-Ruwisch et al., 1988), but it was shown that SRB are also able to use lactate without SO_4^{2-} in co-culture with methanogenic bacteria (Esnault et al., 1988). In order to examine the activity of SRB in intestinal systems of insects we determined their sulfate-reducing rates in the gut contents of the termite *Mastotermes darwiniensis* and the rose-chaffer *Pachnoda marginata*.

Materials and Methods

Organisms. Adults of *Pachnoda marginata* (Coleoptera; rose-chaffer) were obtained from the Zoological Garden in Frankfurt/Main, Germany. The beetles were fed with carrots. *Mastotermes darwiniensis* (Isoptera; termites) was obtained from the Bundesanstalt für Materialforschung und Materialprüfung (BAM, Berlin, Germany). The termites were fed with pine, beech or poplar wood and cultured at 28°C in metallic vessels which contained humid vermiculit. *Desulfovibrio intestinalis* strain KMS2 (DSM 11275^T; Fröhlich et al., 1999) was from the culture collection of our institute.

Enrichment of SRB from the gut of *Pachnoda marginata*. The guts were prepared under aseptic conditions (Fröhlich et al., 1999; Trinkerl et al., 1990). For cell count determination and enrichment of SRB strains, Postgate medium B (Postgate, 1984) containing sodium lactate (2 g/L) and sodium sulfate (1 g/L) as electron donor and acceptor, respectively, and $FeSO_4 \cdot 7H_2O$ (500 mg/L) in addition was used. The titer of viable cells was determined with the MPN method (5 replicates) (Näveke and Tepper, 1979). The MPN series were incubated at 28°C for up to 3 weeks. An enrichment culture of the sulfate-reducing clone was obtained from the highest positive serial dilution step (FeS precipitation).

Nucleic acid preparation. For DNA preparation from bacteria 1 ml enrichment culture was centrifuged. The pellet was washed twice in 1 ml sterile water and then suspended in 200 µl InstaGeneTM DNA Purifica-

tion Matrix (BioRad, Munich, Germany). The suspension was incubated for 20 min at 56°C, and then for 10 min at 95°C, and finally, centrifuged for 2 min at 16,000×g. The supernatant was used for PCR.

DNA amplification. The 16S rDNA gene sequences were amplified by PCR (Medlin et al., 1988) with primer SRB687 specific for SRB from the δ -*Proteobacteria* (Devereux et al., 1992) and primer Eubak5F (AGAGTTTGATCMTGGCT; W. Ludwig, pers. communication; Berchtold et al., 1994) specific for Bacteria. The amplification step was performed in a mixture (100 µl) containing 2.5 units *Taq* polymerase (Applied Biosystems, Weiterstadt, Germany), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.4 µM of each primer in 1× PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 8.3). The reaction mixtures were heated at 94°C for 5 min, followed by 30 cycles (94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min) and a final extension step at 72°C for 10 min. The amplified DNA was purified from nucleotides, primers and *Taq* polymerase using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Sequencing of the amplicates with primer SRB687r (tacggatttcactcct) was performed by Genterprise (Mainz, Germany).

Phylogenetic analysis. The resulting 16S rDNA sequence was compared with RDP (Maidak et al., 2000) and EMBL (Baker et al., 2000) database sequences to find the most closely related species. For phylogenetic analysis we used 18 data sets of sulfate-reducing bacteria and *Escherichia coli* as the prokaryotic outgroup. Sequence data were aligned using the CLUSTAL X package (Thompson et al., 1997). Only those alignment positions were used which could be unambiguously aligned. To determine the evolutionary relationship of the organism, the programs DNADIST (Jukes and Cantor, 1969), NEIGHBOR (neighbor joining method, Saitou and Nei, 1987) and FITCH (least squares distance matrix analysis, Fitch and Margoliash, 1967) included in the PHYLIP program package (Felsenstein, 1993), were used. The reliability of the constructed trees was tested with the program SEQBOOT (Felsenstein, 1985).

Nucleotide sequence accession numbers. The nucleotide sequence data of the obtained 16S rDNA sequence (673 bp) from clone PM6 is available at the EMBL nucleotide sequence database under the accession No. AJ630406. The 16S rDNA sequence of the closest related clone Rs-K75 was obtained from gut contents of the termite *Reticulitermes speratus*

(Accession No. AB089105, Hongoh et al., 2003).

Determination of *in vivo* sulfate reduction. The *in vivo* reduction of sulfate was determined by comparing the concentration of reduced sulfur compounds in the liquid gut contents of the termite *Mastotermes darwiniensis* which were fed for 3 to 5 days with and without sulfate enriched sawdust. Subsequently the determination of the sulfur compounds was carried out by HPLC. Sawdust of pine-wood (0.25 g) was mixed with 1 ml 40 mM Na_2SO_4 (0.16 $\mu\text{mol}/\text{mg}$ sawdust) or 1 ml Milli-Q-water as a control. Twenty termites were fed for 3 or 5 days with and without (control) sulfate enriched sawdust.

Preparation of the guts and removal of the solid contents of the gut by centrifugation (2 times 10 min; 16,000 $\times g$) took place under an N_2 -atmosphere. Reagent (220 μl) consisting of 100 μl HEPES buffer (50 mmol/L HEPES, 5 mmol/L EDTA, pH 8.0), 100 μl acetonitrile and 20 μl monobromobimane (48 mmol/L in acetonitrile; Sigma) was added to 100 μl of the gut supernatant. The mixture was incubated for 30 min in the dark by shaking. The reaction was stopped by adding 200 μl methanesulfonic acid (65 mmol/L; Roth, Karlsruhe, Germany). The determination of the sulfur species S^0 , SO_4^{2-} , SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$ and S^{2-} were carried out as described by Rethmeier et al. (1997).

Determination of *in vitro* sulfate reduction rates. The sulfate concentration in gut contents was determined by HPLC (System S1021, Sykam, Gilching, Germany) with an electrochemical detector (ED 40; sensitivity: 5 μS ; Dionex, Idstein, Germany) equipped with a suppressor (ASRS II; Dionex). The gut contents were separated on a CarboPacAs11 column (Dionex) with a flow rate of 0.8 ml per min, using a 10 mM NaOH solution as eluent. For *Mastotermes darwiniensis* or *Pachnoda marginata*, the contents of 10 guts or 3 guts, respectively, were transferred into 1 ml H_2O . The insoluble material was removed by centrifugation.

The sulfate concentration and the sulfate reduction rates were determined in gut contents of the termite *Mastotermes darwiniensis* fed with sawdust (pine-wood) or with sulfate-enriched sawdust (2.5 g calcium sulfate per 10 g saw dust) and of the beetle *Pachnoda marginata*. *Desulfovibrio intestinalis* strain KMS2 was used as the reference strain. For each test, 94 termite guts of *Mastotermes darwiniensis* or 5 guts of *Pachnoda marginata* were used. From the gut contents of each insect three replicates were measured. The experiments were carried out as described by Rosser

and Hamilton (1983) and by Sass (1997) using $^{35}\text{SO}_4^{2-}$ (0.07 MBq; specific radioactivity: 0.9–1.5 TB/mg S). The collected gut contents were suspended in 3 ml basal medium (Widdel and Bak, 1992) containing 10 mM lactate and 0.3 mM sodium sulfate, but lacking FeSO_4 . The inoculum was cultured in the presence of 10 mM sulfate. Into the incubation vessels containing 10 ml medium, 500 μl of this suspension was injected. The cultures were incubated at 28°C for 5.5 h. The reaction was stopped with 1 ml 6 M HCl. The vessels were shaken for 20 h and the produced H_2^{35}S was measured as Zn^{35}S in 15 ml scintillation liquid (Rotiszint eco plus; Roth) with a scintillation counter (model LS 6000; Beckman Instruments, Munich, Germany). The titer of the control containing the reference species *Desulfovibrio intestinalis* was 10^6 cells per ml at the beginning of the incubation.

Results and Discussion

Enrichment and identification of sulfate-reducing bacteria from gut contents of the rose-chaffer Pachnoda marginata

SRB have been detected or isolated from the gut of higher and lower termites and the wood-eating cockroach *Cryptocercus punctulatus* (Brauman et al., 1990; Fröhlich et al., 1999; Kuhnigk et al., 1996; Ohkuma and Kudo, 1996; Trinkerl et al., 1990). Therefore, we were interested to know whether SRB are more widespread and occur also in gut contents of insects which branched off later in the phylogenetic tree such as Coleoptera. For the enrichment of SRB from *Pachnoda marginata* the gut contents were transferred into Postgate medium B and anaerobically incubated at 28°C for up to 3 weeks. The titer of SRB was in the range of up to 2×10^8 cells per ml gut content. The inoculated enrichment media turned black due to sulfide production after an incubation time of 12 h. A 16S rDNA sequence was obtained from serial dilution cultures by PCR with a SRB-specific primer. As shown by phylogenetic 16S rDNA analysis (Fig. 1) the identified sulfate reducer belonged to the genus *Desulfovibrio* and had the closest relationship to clone Rs-K75 (Hongoh et al., 2003) and *Desulfovibrio cuneatus* strain STL1 (Sass et al., 1998).

An unexpected high cell count of sulfate reducers (2×10^8 cells per ml gut content) of the beetle *Pachnoda marginata* corresponded to 4.6% of the total bacterial population (9.2×10^9 cells per ml gut content)

Fig. 1. Phylogenetic relationship of clone PM6 from the gut of *Pachnoda marginata* to species of the genus *Desulfovibrio* and related genera with *Escherichia coli* as the outgroup.

The phylogenetic tree was constructed by the neighbor joining method based on 584 alignment positions. Bootstrap values are indicated at the respective nodes. Bootstrap values under 50% are not shown. Accession numbers are given in brackets. The bar represents 0.1 changes per nucleotide position.

grown in Postgate medium B. So far, SRB have also been found in the human large intestine (Gibson et al., 1988a, b) and in lower and higher animals (Brauman et al., 1990; Fröhlich et al., 1999; Gebhart et al., 1993; Howard and Hungate, 1976; Kuhnigk et al., 1996; Ohkuma and Kudo, 1996; Trinkerl et al., 1990). All isolates from termites examined so far could be assigned to the genus *Desulfovibrio*. All *Desulfovibrio* clones and isolates belonged to one of the four species *Desulfovibrio desulfuricans*, *Desulfovibrio giganteus*, *Desulfovibrio termitidis* and *Desulfovibrio intestinalis*. The 16S rDNA sequence identified in the gut contents of *Pachnoda marginata* must have originated from a new *Desulfovibrio* species.

Sulfate-reducing bacteria in intestinal systems may be involved in the removal of both oxygen and hydrogen, as well as acetate production and nitrogen fixation (Cypionka, 2000; Kuhnigk et al., 1996). Sulfate re-

ducers from the termite gut have the highest sulfate respiration rate among SRB determined so far (Cypionka, 2000; Kuhnigk et al., 1996). Meanwhile, it is well established that SRB are able to use hydrogen as an electron donor and oxygen as an electron acceptor (Cypionka, 2000; Dilling and Cypionka, 1990). Their activity leads to a depletion of oxygen and helps to reestablish reducing conditions (Kuhnigk et al., 1996; Sass et al., 1998) in their environment, which could also be important in intestinal systems.

In vitro sulfate reduction rates in gut contents

The concentration of sulfate in the gut of different termites was earlier determined to be in the range of 0.3–0.7 mM (Kuhnigk et al., 1996). In the present study a sulfate concentration of 0.14 mM was found in the gut of *Mastotermes darwiniensis*, which could increase up to about 23.9 mM when the termites were fed a sulfate-

Table 1. In vitro sulfate reduction rates in gut contents.

Species	Gut volume [μl]	SRB ^a [cells per ml gut content]	Sulfate concentration in gut content [mM]	Sulfate reduction rate [per ml gut content; nmol×h ⁻¹]	Sulfate reduction rate [per 10 ⁷ cells of SRB; nmol×h ⁻¹]
<i>Mastotermes darwiniensis</i>	10	1×10 ⁷	0.14	4.90	4.90
<i>Pachnoda marginata</i>	173	2×10 ⁸	1.10	104.30	5.20
<i>Desulfovibrio intestinalis</i> ^b strain KMS2 (reference strain)	n.d.	1×10 ⁶	0.30	1.40	0.20–0.70

n.d.=not determined, ^a cell number determined in this study, ^b titer of *Desulfovibrio intestinalis* is given as cells per ml culture and sulfate concentration at the beginning of the incubation.

The volume of the total gut was determined by weight (1 g≅1 ml; *M. darwiniensis*, 10 guts; *P. marginata*, 3 guts). When *Mastotermes darwiniensis* was fed with sulfate-enriched wood (2.5 g calcium sulfate per 10 g sawdust) for 22 days the sulfate concentration in the gut contents of *Mastotermes darwiniensis* increased to 18.3 mM and the sulfate reduction rate was 13.4 nmol×h⁻¹×ml⁻¹.

Table 2. Detection of sulfur species in gut contents of *Mastotermes darwiniensis* after feeding with and without sulfate-enriched sawdust.

Feeding conditions ^a	Sulfur [μM]	Sulfate [mM]	Sulfite [μM]	Thiosulfate [μM]	Sulfide [μM]	Total sulfur compounds [mM]
1 [3 days]	0.0	0.6	0.0	0.0	4.1	0.6
2 [3 days]	0.0	0.4	0.0	0.0	0.0	0.4
3 [5 days]	0.0	1.0	0.0	0.0	12.5	1.0
4 [3 days]	0.0	17.5	0.0	391.0	197.5	18.1
5 [3 days]	0.0	6.0	0.0	29.5	116.0	6.1
6 [5 days]	6.2	23.9	0.0	14.6	115.4	24.0

^a 1–3: sawdust without sulfate; 4–6: sawdust with sulfate; feeding time in brackets.

The experiments shown in Tables 1 and 2 were performed independently at different times. The same is true for feeding conditions 1 and 2 as well as 4 and 5.

rich diet. The sulfate concentration in the gut differed, when different feeding conditions were applied (Tables 1, 2). The sulfate concentration in the gut of *Pachnoda marginata* was determined to be 1.1 mM (Table 1).

The determined sulfate reduction rates in the gut contents of the termite *Mastotermes darwiniensis*, the gut contents of the beetle *Pachnoda marginata* and of a pure culture of *Desulfovibrio intestinalis* with lactate as electron donor are summarized in Table 1. Due to the higher number of sulfate reducers, the sulfate reduction rate per ml gut content of *Pachnoda marginata* is 21-fold higher than that of *Mastotermes darwiniensis*.

In vivo sulfate reduction

In addition to the in vitro determination of the sulfate

reduction rates in gut contents of the termite *Mastotermes darwiniensis*, the in vivo concentrations of sulfur species were determined after feeding with and without sulfate-enriched sawdust. In Table 2 the concentrations of the sulfur species in the gut contents are listed. After feeding with the sulfate-enriched diet the sulfide concentration increased significantly indicating that sulfate is reduced in the gut to a significant amount. In addition sulfur and thiosulfate were found under certain conditions, while sulfite was not present. These results indicate that sulfate reducers play a significant role in the participation of the sulfur metabolism in the termite gut as discussed previously by Kuhnigk et al. (1996).

General conclusions

So far, sulfate reduction rates have been described in sediments of oligo-, meso-, eu- and hypertrophic lakes (Bak and Pfennig, 1991; Fukui and Takii, 1990; Köhl and Jørgensen, 1992; Rothfuss et al., 1997; Sass et al., 1997). The highest rate ($20 \mu\text{mol}/\text{ml} \times \text{day}$) was found in biofilms corresponding to $4.2 \text{ nmol}/10^7 \text{ bacteria} \times \text{h}$ (Köhl and Jørgensen, 1992). Sass et al. (1997) reported that 2.1×10^5 SRB occurred per ml sediment sample proceeding with a sulfate reduction rate of $1.7 \text{ nmol}/\text{ml} \times \text{day}$ corresponding to $5.8 \text{ nmol}/10^7 \text{ bacteria} \times \text{h}$ in the oligotrophic Lake Stechlin. These reports showed that the sulfate-reducing activity per sulfate-reducing bacterium in gut homogenates of wood-fed termites and of the rose-chaffer *Pachnoda marginata* (in vitro activity; Table 1) is in a similar range to that in the Lake Stechlin and in biofilms.

The world's termite population is estimated to be approximately 2×10^{17} animals (Zimmermann et al., 1982). Based on the data obtained with *Mastotermes darwiniensis* and taking into account that the gut volume of smaller termites e.g. *Reticulitermes santonensis* is only one twentieth of that of *Mastotermes darwiniensis*, the annual hydrogen sulfide production by termites is supposed to be in the range of 0.4 to $8.6 \times 10^{10} \text{ mol}$ (≈ 0.15 to $2.90 \times 10^{12} \text{ g}$). According to this rough estimate, this amount represents 0.9% of the annual molar emanation of methane ($9.4 \times 10^{12} \text{ mol}$; $\approx 1.5 \times 10^{14} \text{ g}$) from termites, whereas the molar hydrogen production ($1 \times 10^{14} \text{ mol}$; $\approx 2 \times 10^{14} \text{ g}$) is about thousandfold higher and the molar carbon dioxide production ($1.1 \times 10^{15} \text{ mol}$; $\approx 5 \times 10^{16} \text{ g}$) is about ten thousand times higher (Zimmermann et al., 1982) than the hydrogen sulfide production. Since no data exists about the total population of the rose-chaffer *Pachnoda marginata* the total annual sulfide production of this species can not be calculated. The impact of termites on total biogenic sulfide production is difficult to estimate, since reliable data on global biogenic sulfide production are not available.

Sulfate-reducing *Desulfovibrio* species are thus able to catalyze all reactions of a complete sulfur cycle. Members of the genus *Desulfovibrio* are anaerobes, although some exhibit strong oxygen tolerance (Cypionka, 2000). The sulfate reducers of the termite gut have been assigned to the genus *Desulfovibrio* (König et al., 2002). The sulfate-reducing bacterium detected in the gut of the rose-chaffer *Pachnoda marginata* belonged also to this genus. Termites and the rose-

chafers benefit from the *Desulfovibrio* species, which produce acetate, and thus provide nutrition for their hosts. In co-culture the intestinal species *Desulfovibrio desulfuricans* from the termite gut used the fermentation products of other fermentative gut bacteria and produced mainly acetate. They also contribute to the anoxic milieu of the gut by producing hydrogen sulfide and removing oxygen with hydrogen or low molecular weight organic or reduced sulfur compounds. The isolates were able to oxidize sulfur compounds such as sulfide, thiosulfate and sulfite. Sulfide was oxidized to sulfate as indicated by the amount of sulfide to oxygen consumed (molar ratio: 1 : 2). Performing a complete sulfur cycle the desulfovibrios can balance the redox status of the gut contents. Some of the isolates were able to oxidize sulfide or other sulfur compounds to sulfate. This activity would allow sulfate recovery and keep a complete sulfur cycle running. Sulfide reoxidation could prevent toxic effects of H_2S . Only substrates that are of no direct use for the termites (H_2 , formate) were oxidized completely, while others were transformed to acetate. Rates of oxygen consumption were as high as $1,570 \text{ nmol O}_2 \text{ min}^{-1} \text{ mg protein}^{-1}$ with electron donors in excess, which is also the case in the termite gut. These are the highest respiration rates of sulfate-reducing bacteria ever measured. These rates exceed by far the respiration rates of typical aerobes or facultative anaerobes such as *Escherichia coli* ($300 \text{ nmol O}_2 \text{ min}^{-1} \text{ mg protein}^{-1}$) (Kuhnigk et al., 1996).

In the final stage of the degradation of organic material the methanogens, acetogens and sulfate reducers may principally coexist, although differences in the number of methanogens and acetogens have been found in wood-feeding and soil-feeding termites (Braumann et al., 1992). The nutritional versatility of acetogens may be one of the reasons why they outcompete methanogens in xylophagous termites (Breznak and Blum, 1991). Under hydrogen limitation sulfate reducers may outcompete acetogenic and methanogenic bacteria.

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References

- Bak, F. and Pfennig, N. (1991) Microbial sulfate reduction in littoral sediment of Lake Constance. *FEMS Microbiol. Ecol.*, **85**, 31–42.
- Baker, W., van den Broek, A., Camon, E., Hingamp, P., Sterk, P., Stoesser, G., and Tuli, M. A. (2000) The EMBL Nucleotide Sequence Database. *Nucleic Acids Res.*, **28**, 19–23.
- Berchtold, M., Ludwig, W., and König, H. (1994) 16S rDNA sequence and phylogenetic position of an uncultivated spirochete from the hindgut of the termite *Mastotermes darwiniensis* Froggatt. *FEMS Microbiol. Lett.*, **123**, 269–274.
- Brauman, A., Koenig, J. F., and Dutreix, J. L. (1990) Characterization of two sulfate-reducing bacteria from the gut of the soil-feeding termite, *Cubitermes speciosus*. *Antonie Van Leeuwenhoek*, **58**, 271–275.
- Braumann, A., Kane, M. D., Labat, M., and Breznak, J. A. (1992) Genesis of acetate and methane by gut bacteria of nutritionally diverse termites. *Science*, **257**, 1384–1386.
- Breznak, J. A. and Blum, J. S. (1991) Mixotrophy of the termite gut acetogen, *Sporomusa termitida*. *Arch. Microbiol.*, **156**, 105–110.
- Cord-Ruwisch, R., Seitz, H.-J., and Conrad, R. (1988) The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. *Arch. Microbiol.*, **149**, 350–357.
- Cypionka, H. (2000) Oxygen respiration by *Desulfovibrio* species. *Annu. Rev. Microbiol.*, **54**, 827–848.
- Devereux, R., Kane, M. D., Winfrey, J., and Stahl, D. A. (1992) Genus- and group-specific hybridization probes for determinative and environmental studies of sulfate-reducing bacteria. *Syst. Appl. Microbiol.*, **15**, 601–609.
- Dilling, W. and Cypionka, H. (1990) Aerobic respiration in sulfate-reducing bacteria. *Arch. Microbiol.*, **71**, 123–128.
- Esnault, G., Caumette, P., and Garcia, J. L. (1988) Characterization of *Desulfovibrio giganteus* sp. nov., a sulfate-reducing bacterium isolated from a brackish coastal lagoon. *Syst. Appl. Microbiol.*, **10**, 147–151.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.
- Felsenstein, J. (1993) Phylip 3.5. Department of genetics SK-50, University of Washington, Seattle, Wash. 98195.
- Fenchel, T. and Ramsing, N. B. (1992) Identification of sulfate-reducing ectosymbiotic bacteria from anaerobic ciliates using 16S rDNA-binding oligonucleotide probes. *Arch. Microbiol.*, **158**, 394–397.
- Fitch, W. M. and Margoliash, E. (1967) Construction of phylogenetic trees. *Science*, **155**, 279–284.
- Fröhlich, J., Sass, H., Babenzien, H. D., Kuhnigk, T., Varma, A., Saxena, S., Nalepa, C., Pfeiffer, P., and König, H. (1999) Isolation of *Desulfovibrio intestinalis* sp. nov., from the hindgut of the lower termite *Mastotermes darwiniensis*. *Can. J. Microbiol.*, **45**, 145–152.
- Fukui, M. and Takii, S. (1990) Seasonal variations of population density and activity of sulfate-reducing bacteria in offshore and reed sediments of a hypertrophic freshwater lake. *Jpn. J. Limnol.*, **51**, 63–71.
- Gebhart, C. J., Barn, S. M., McOrist, S., Lin, G.-F., and Lawons, H. K. (1993) Ileal symbiont intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. *Int. J. Syst. Bacteriol.*, **43**, 533–538.
- Gibson, G. R., Cummings, J. H., and MacFarlane, G. T. (1988a) Competition for hydrogen between sulfate-reducing bacteria and methanogenic bacteria from the human large intestine. *J. Appl. Bacteriol.*, **65**, 241–247.
- Gibson, G. R., Cummings, J. H., and MacFarlane, G. T. (1988b) Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl. Environ. Microbiol.*, **54**, 2750–2755.
- Hongoh, Y., Ohkuma, M., and Kudo, T. (2003) Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). *FEMS Microbiol. Ecol.*, **44**, 231–242.
- Howard, B. H. and Hungate, R. E. (1976) *Desulfovibrio* of the sheep rumen. *Appl. Environ. Microbiol.*, **32**, 598–602.
- Jørgensen, B. B. (1982) Mineralization of organic matter in the sea bed: The role of sulfate reduction. *Nature*, **296**, 643–645.
- Jukes, T. H. and Cantor, C. R. (1969) Evolution of protein molecules. In *Mammalian Protein Metabolism*, ed. by Munro, H. N., Academic Press, New York, pp. 21–132.
- König, H., Fröhlich, J., Berchtold, M., and Wenzel, M. (2002) Diversity and microhabitats of the hindgut flora of termites. *Recent Res. Devel. Microbiol.*, **6**, 125–156.
- Kühl, M. and Jørgensen, B. B. (1992) Microsensor measurements of sulfate reduction and sulfide oxidation in compact microbial communities of aerobic biofilms. *Appl. Environ. Microbiol.*, **58**, 1164–1174.
- Kuhnigk, T., Branke, J., Krekeler, D., Cypionka, H., and König, H. (1996) A feasible role of sulfate-reducing bacteria in the termite gut. *Syst. Appl. Microbiol.*, **19**, 139–149.
- Maidak, B. L., Cole, J. R., Lilburn, T. G., Parker, C. T., Jr., Saxman, P. R., Stredwick, J. M., Garrity, G. M., Li, B., Olsen, G. J., Pramanik, S., Schmidt, T. M., and Tiedje, J. M. (2000) The RDP (Ribosomal Database Project) continues. *Nucleic Acids Res.*, **28**, 173–174.
- Medlin, L., Elwood, H. J., Stickel, S., and Sogin, M. L. (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, **71**, 491–499.
- Näveke, R. and Tepper, K.-P. (1979) Einführung in die mikrobiologischen Arbeitsmethoden, Gustav Fischer Verlag, Stuttgart pp. 91–92.

- Ohkuma, M. and Kudo, T. (1996) Phylogenetic diversity of the intestinal bacterial community in the termite *Reticulitermes speratus*. *Appl. Environ. Microbiol.*, **62**, 461–468.
- Postgate, J. R. (1984) Genus *Desulfovibrio*. In *Bergey's Manual of Systematic Bacteriology*, ed. by Krieg, N. R. and Holt, J. G., Vol. I, Williams & Wilkins, Baltimore, pp. 666–672.
- Rethmeier, J., Rabenstein, A., Langer, M., and Fischer, U. (1997) Detection of traces of oxidized and reduced sulfur compounds in small samples by combination of different high-performance liquid chromatography methods. *J. Chromatogr. A*, **760**, 295–302.
- Rosser, H. R. and Hamilton, W. A. (1983) Simple assay for accurate determination of [³⁵S]sulfate reduction activity. *Appl. Environ. Microbiol.*, **45**, 1956–1959.
- Rothfuss, F., Bender, M., and Conrad, R. (1997) Survival and activity of bacteria in a deep, aged lake sediment (Lake Constance). *Microbiol. Ecol.*, **33**, 69–77.
- Saitou, N. and Nei, M. (1987) The neighbour-joining method: A new method for constructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.
- Sass, H. (1997) Vorkommen und Aktivität sulfatreduzierender Bakterien in der Oxykline limnischer Sedimente, Hartung-Gorre Verlag, Konstanz.
- Sass, H., Berchtold, M., Branke, J., König, H., Cypionka, H., and Babenzien, H. D. (1998) Psychrotolerant sulfate-reducing bacteria from an oxic freshwater sediment, description of *Desulfovibrio cuneatus* sp. nov. and *Desulfovibrio litoralis* sp. nov. *Syst. Appl. Microbiol.*, **21**, 212–219.
- Sass, H., Cypionka, H., and Babenzien, H.-D. (1997) Vertical distribution of sulfate-reducing bacteria at the oxic-anoxic interface in sediments of the oligotrophic Lake Stechlin. *FEMS Microbiol. Ecol.*, **22**, 245–255.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **24**, 4876–4882.
- Trinkerl, M., Breunig, A., Schauder, R., and König, H. (1990) *Desulfovibrio termitidis*, sp. nov., a carbohydrate-degrading sulfate-reducing bacterium from the hindgut of a termite. *Syst. Appl. Microbiol.*, **13**, 372–377.
- Widdel, F. (1988) Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In *Biology of Anaerobic Microorganisms*, ed. by Zehnder, A. J. B., John Wiley & Sons, New York, pp. 469–585.
- Widdel, F. and Bak, F. (1992) Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, ed. by Balows, A., Trüper, H. G., Dworkin, M., Harder, W., and Schleifer, K. H., Vol. 4, Springer-Verlag, New York, pp. 3352–3372.
- Widdel, F. and Hansen, T. A. (1992) The dissimilatory sulfate- and sulfur-reducing bacteria. In *The Prokaryotes*, ed. by Balows, A., Trüper, H. G., Dworkin, M., Harder, W., and Schleifer, K.-H., Vol. 1, Springer-Verlag, Heidelberg, pp. 583–627.
- Zimmermann, P. R., Greenberg, J. P., Wandiga, S. O., and Crutzen, P. J. (1982) Termites: A potentially large source of atmospheric methane, carbon dioxide, and molecular hydrogen. *Science*, **218**, 563–565.