Sporomusa aerivorans sp. nov., an oxygen-reducing homoacetogenic bacterium from the gut of a soil-feeding termite

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Previously undescribed, homoacetogenic bacteria were isolated from gut homogenates of the soil-feeding termite *Thoracotermes macrothorax*. The isolates were slightly curved, banana-shaped rods (0·6–0·7 × 1·3–7·0 μm) and were motile by one or more lateral flagella. In older cultures, cells formed club-like sporangia that developed into terminal, heat-resistant endospores. Cells stained Gram-positive but were Gram-negative in the KOH test. The isolates were mesophilic and grew homoacetogenically on H_2/CO_2 and L-lactate. Strain TmAO3^T, which was characterized further, also grew homoacetogenically on pyruvate, citrate, L-alanine, D-mannitol, ethanol, formate and methanol. Succinate was decarboxylated to propionate; fumarate, L-malate and oxaloacetate were fermented to propionate and acetate. Hexoses were not used as substrates. Resting cells had a large capacity for hydrogen-dependent oxygen reduction [826 nmol min^{-1} (mg protein)$^{-1}$], which enabled them to initiate growth in non-reduced basal medium that originally contained up to 1·5 kPa oxygen in the headspace, although growth commenced only after the medium had been rendered anoxic. Redox difference spectra of cell extracts indicated the presence of membrane-bound *b*-type cytochrome(s). Comparative 16S rRNA gene sequence analysis revealed that strain TmAO3^T belongs to a subgroup of the phylum of Gram-positive bacteria that is characterized by low DNA G+C content and a Gram-negative cell wall. It is related most closely to representatives of the genus *Sporomusa*. Based on morphological and physiological properties and on 16S rRNA gene sequence similarity of 94–97 % to other *Sporomusa* species, the isolates are assigned to *Sporomusa aerivorans* sp. nov. (type strain, TmAO3^T = DSM 13326^T = ATCC BAA-625^T).

Reductive acetogenesis from H_2 and CO_2 in gut homogenates of wood-feeding termites was first reported by Breznak & Switzer (1986). In the following years, the presence of homoacetogenic activity has been demonstrated for a large number of termite species from all major feeding guilds, including representatives of wood-feeding, fungus-cultivating and soil-feeding termites (Brauman *et al.*, 1992). Although reductive acetogenesis was always out-competed as a hydrogen sink by methanogenesis in gut homogenates of soil-feeding termites (Brauman *et al.*, 1992), microinjection of $H^{14}CO_3^-$ into intact hindguts of soil-feeding *Cubitermes* spp. has identified a high potential for reductive acetogenesis (Tholen & Brune, 1999), indicating that the contribution of reductive acetogenesis to overall electron flow in the guts of soil-feeding termites may be larger than expected.

In order to define the metabolic potential of termite gut homoacetogens and to identify specific adaptations to the gut environment, it is necessary to study these bacteria in pure culture (Brune *et al.*, 2000). However, only five strains of homoacetogenic bacterium have so far been isolated from termite guts, four of them from wood-feeding species. They comprise *Sporomusa termitida* and *Acetonema longum* from *Nasutitermes nigriceps* and *Pterotermes occidentis* (Breznak *et al.*, 1988; Kane & Breznak, 1991) and two spirochaetal isolates, strains ZAS-1 and ZAS-2, from *Zootermopsis angusticollis* (Leadbetter *et al.*, 1999). Only one homoacetogenic bacterium, *Clostridium mayombei* (Kane *et al.*, 1991), has been obtained from a soil-feeding termite (*Cubitermes*

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains TmAO3^T and TmAM3 are AJ506191 and AJ506192, respectively.

speciosus). Nevertheless, each of these isolates was a member of a novel genus or species affiliated with a different phylogenetic group and it is reasonable to assume that they represent only a negligible fraction of the diversity of homoacetogens that colonize the guts of more than 2600 known species of termite (Kambhampati & Eggleton, 2000).

Clearly, more isolates are needed to understand the physiological role and ecological significance of homoacetogenic bacteria in termite guts. In this study, we describe the isolation and characterization of a novel homoacetogenic bacterium from the gut of the soil-feeding termite *Thoracotermes macrothorax*. Further physiological characterization of this strain, which is the subject of a separate study (Boga & Brune, 2003), led to the surprising discovery of high oxygen-reducing capacity in this and other homoacetogenic bacteria isolated from termite guts.

Isolation and morphological characterization

Gut homogenates were prepared from different gut sections of the termite *Thoracotermes macrothorax* Sjöstedt (Tholen & Brune, 1999) and diluted serially in anoxic, bicarbonate-buffered mineral medium (AM-5) supplemented with yeast extract and Casamino acids (each 0·1 %, w/v) (Boga & Brune, 2003). Enrichment cultures with lactate (8 mM) as an additional substrate and DTT (1 mM) as a reducing agent were incubated in an H_2/CO_2 atmosphere (80 : 20, v/v; 150 kPa). The highest dilutions where net acetate production indicated the presence of reductive acetogenesis were transferred into fresh medium. Subsequent agar dilution series (Pfennig & Trüper, 1981), conducted in the same medium but incubated in an N_2/CO_2 atmosphere, yielded mostly light-brown, lentil-shaped colonies from which several pure cultures were obtained.

Phase-contrast microscopy revealed morphologically indistinguishable, curved rods with slightly tapered ends, which resembled the shape of a banana. Strain TmAM3, which was derived from a homogenate of midgut and mixed segment, and strain TmAO3^T, which stemmed from a homogenate that comprised the third and fourth proctodeal segment, were selected for further characterization. In each case, the dilution steps indicated an original population of approximately 10^3–10^4 cells per gut section.

Both strains stained Gram-positive but reacted Gram-negative in the KOH test (Gregersen, 1978); *Bacillus megaterium* (DSM 32^T) and *Escherichia coli* (DSM 498) were used as controls. Cells of strain TmAO3^T were 3–7 μm long and 0·6–0·7 μm wide and occurred singly or in pairs (Fig. 1a). In the stationary growth-phase, they formed terminal endospores in club-shaped sporangia that tended to aggregate in a characteristic manner (Fig. 1b). Older cultures sporulated completely and remained viable when pasteurized (80 °C, 10 min). Cells were motile by means of one or more lateral flagella (Figs 1c and d). Identical results were obtained with strain TmAM3.

Growth and nutrition

Cultures were grown routinely at 30 °C and pH 7. Growth was determined photometrically by following increase in OD_{578}. Growth yields were estimated by using an OD-to-cell-mass conversion factor that was determined with cultures (1 l) grown on lactate in N_2/CO_2. Substrate utilization and product formation were assayed by HPLC with an ion-exclusion column and a refractive index detector (Tholen *et al.*, 1997). Aromatic acids were quantified by reversed-phase HPLC as described previously (Brune & Schink, 1990).

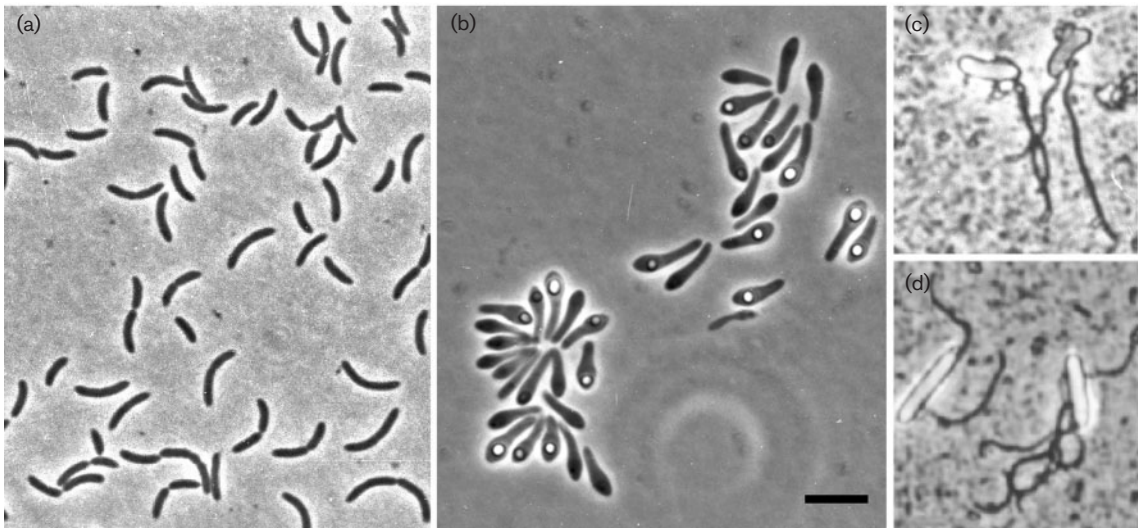

Fig. 1. Phase-contrast photomicrographs of cells from a growing culture of strain TmAO3^T (a), during sporulation (b) and after a flagella stain (c, d). Wet mounts for microphotography (Pfennig & Wagener, 1986) and flagella stains (Blenden & Goldberg, 1965) were prepared as described. Bar, 5 μm.

Both strains required an oxygen-free, reduced medium for growth. They grew best in medium reduced with DTT (1 mM) or cysteine (2 mM). Good growth was also obtained when a palladium catalyst was added to reduce medium incubated in an H_2/CO_2 atmosphere (Tholen *et al.*, 1997). The strains grew homoacetogenically on H_2/CO_2 or L-lactate but required small amounts (0·1 %, w/v) of yeast extract or Casamino acids, which were fermented to acetate and traces of propionate. As the results of all initial growth tests were identical for both isolates, only strain TmAO3^T was characterized in more detail.

On basal medium with lactate, cells grew at pH 6·2–8·2 [pH adjusted by adding sterile Na_2CO_3 (1 M) or HCl (1 M)] and at 19–35 °C, but not at 4 or 40 °C. Highest growth yields were obtained at 30 °C and pH 7. Under these conditions, cultures reached similar densities on H_2/CO_2 (80 : 20; 150 kPa) or lactate (8·1 mM) (Fig. 2), but the respective growth yields differed considerably [2·5 and 6·0 g dry wt (mol acetate)$^{-1}$, corrected for background growth and acetate production on basal medium]. Doubling times of cultures growing on H_2/CO_2 or lactate were 8·9 and 4·4 h, respectively. In cultures growing on H_2/CO_2, growth was exponential only at lower cell densities. Most likely, mass transfer of H_2 from the gas phase into the liquid medium became limiting as cell density increased.

Strain TmAO3^T also grew on alanine, citrate, mannitol, formate, pyruvate, ethanol and methanol. In all cases, acetate was the only product detected by HPLC analysis.

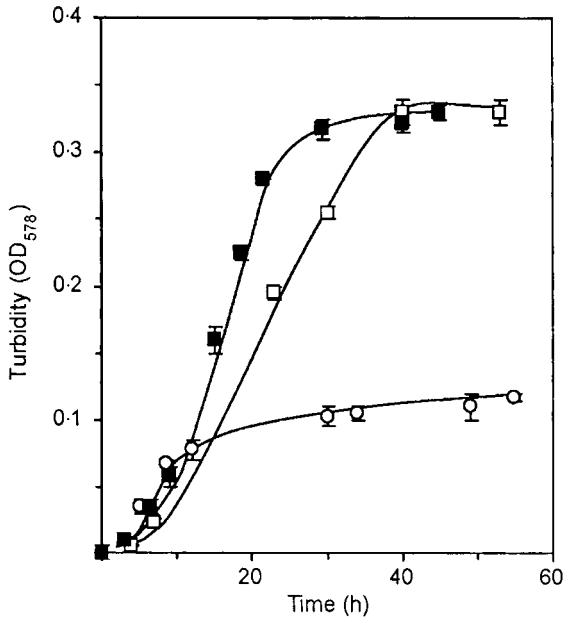

Fig. 2. Growth of strain TmAO3^T on lactate (8·1 mM) (■) or H_2/CO_2 (80 : 20; 150 kPa) (□) in basal medium that contained yeast extract and Casamino acids (each 0·1 %, w/v). Background growth on basal medium alone (○). Values are means of two cultures.

Substrate-free controls showed that growth and acetate production on basal medium were substantial. Nevertheless, the product pattern and electron recovery obtained with L-lactate, L-alanine and D-mannitol were close to the theoretical values expected for a purely homoacetogenic metabolism (Table 1), i.e. all reducing equivalents from substrate oxidation to acetate were apparently used for further (reductive) acetogenesis from CO_2.

In contrast, dicarboxylic acids such as L-malate, fumarate and oxaloacetate were fermented to varying amounts of propionate and acetate; consistently incomplete utilization of the racemic mixture of DL-malate indicates that D-malate was not used. Propionate was virtually the only product formed from succinate and the growth-yield increase in the presence of succinate indicates that strain TmAO3^T derives energy from succinate decarboxylation, most likely via the methylmalonyl–CoA pathway. However, no increased growth yield was associated with the decarboxylation of malonate to acetate (Table 1).

Strain TmAO3^T also grew by demethylation of vanillate, syringate and 3,4,5-trimethoxybenzoate (each 2 mM), forming acetate and the corresponding phenolates (protocatechuate or gallate) as demethylation products. No growth occurred with glucose, fructose, lactose, cellobiose, trehalose or ethylene glycol (5 or 10 mM each) or with glycerol, oxalate, glyoxylate, aspartate or glutamate (10 or 20 mM each). Sulfate and nitrate (10 mM each) were not reduced (this was tested with lactate as electron donor).

Cytochrome content

Cultures were centrifuged at 10 000 ***g*** for 30 min and cells were washed and then resuspended in anoxic buffer. Cell extracts were prepared by repeatedly passing the cell suspension through a French pressure cell at 138 MPa. Crude extract was centrifuged again (30 000 ***g***, 20 min) and the supernatant (cell-free extract) was fractionated into a membrane fraction and a soluble fraction by ultracentrifugation (126 000 ***g***, 1 h). Fractions were assayed for the presence of cytochromes by recording difference spectra of $N_2S_2O_4$-reduced minus air-oxidized samples, as described previously (Breznak *et al.*, 1988). All procedures were carried out in potassium phosphate buffer (0·1 M, pH 7).

Redox difference spectra of cell extracts from lactate-grown (Fig. 3) and hydrogen-grown (not shown) cells of strain TmAO3^T showed absorption maxima at 562, 545 and 432 nm, which are characteristic of *b*-type cytochrome(s) (Dickerson & Timkovich, 1975). Absence of other absorption maxima and the fact that maxima were found only in the spectra of the membrane fraction after ultracentrifugation indicated that *a*- or *c*-type cytochromes were not present.

Phylogenetic analysis

16S rRNA genes were amplified *in vitro* by PCR and the sequences were analysed as described previously (Springer *et al.*, 1992). The 16S rRNA gene sequences (homologous to

Table 1. Growth yields and fermentation products of strain TmAO3^T grown on selected substrates in an N_2/CO_2 atmosphere

Background growth and product formation on basal medium were subtracted for the calculation of electron recoveries and substrate-specific growth yields. As cells were grown in bicarbonate-buffered medium, CO_2 formation could not be analysed. Values are means of two replicates (<5 % variance). ND, Not determined.

Substrate	(mM)	Cell mass formed (mg l^{-1})*	Substrate assimilated (mM)†	Product formed (mM)‡			Electron recovery (%)§	Growth yield [g (mol substrate)$^{-1}$]
				Acetate	Propionate	Succinate		
D-Mannitol	(4·5)	145	0·6	20·6	0·2	0·0	95·0	20·8
L-Alanine	(8·9)	ND	ND	19·5	0·2	0·0	93·6‖	ND
L-Lactate	(8·9)	123	1·0	20·0	0·2	0·0	96·5	8·1
L-Malate	(8·9)	115	0·9	8·7	6·3	0·1	90·5	7·1
Fumarate	(8·9)	155	1·4	9·0	5·2	0·7	93·7	11·6
Succinate	(8·9)	78	0·4	8·2	7·9	0·0	87·5	3·0
Succinate	(17·8)	116	0·8	7·7	16·8	0·0	94·8	3·7
Malonate	(8·9)	50	0·0	16·6	0·4	0·0	94·7	0·0
Control¶	(−)	51	−	8·2	0·4	0·0	−	−

*Calculated from increase in optical density by using the OD-to-dry-mass ratio of 343 mg l^{-1} at $OD_{578}=1$, experimentally determined for lactate-grown cells of strain TmAO3^T.

†Assimilated substrate was calculated as the amount of substrate necessary for formation of the indicated amount of cell mass, observing a closed oxidation–reduction balance and assuming a cell composition of '$C_4H_7O_3$'.

‡Values are not corrected for background growth and product formation on basal medium.

§Calculated after subtracting the amount of substrate assimilated and the electrons contained in the products also formed in the control.

‖Electron recovery calculated by using assimilated substrate of culture growing on lactate.

¶Substrate-free controls contained yeast extract and Casamino acids (0·1 % each, w/v).

E. coli positions 8–1542) of the isolates were fitted into an alignment of about 40 000 homologous full or partial primary structures that are available in public databases (Ludwig, 1995) by using the automated tools of the ARB software package (Ludwig & Strunk, 1996). Distance-matrix, maximum-parsimony and maximum-likelihood methods were applied for tree construction, as implemented in the ARB software package. Different datasets, which varied with respect to the selection of outgroup reference organisms as well as alignment positions, were analysed.

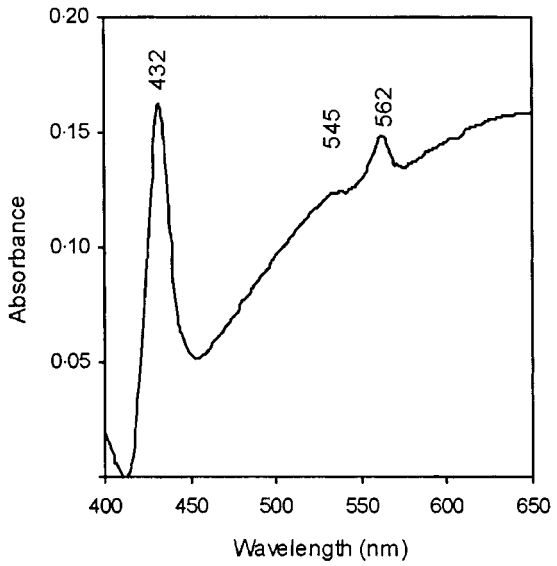

Fig. 3. Redox difference spectrum of the membrane fraction of strain TmAO3^T grown on lactate, showing absorption maxima that indicate the presence of a *b*-type cytochrome. No absorption maxima were detected in the soluble fraction. The assay contained 5 mg protein ml^{-1}.

The almost-complete 16S rRNA gene sequences obtained for strains TmAO3^T and TmAM3 were nearly identical (>99·5 % sequence similarity). Comparative sequence analysis revealed that both strains were phylogenetically most closely related to members of the genus *Sporomusa* (Fig. 4), which belongs to a subgroup of Gram-positive bacteria with a low DNA G+C content that is characterized by organisms with a Gram-negative cell wall (Willems & Collins, 1995). All phylogenetic analyses placed the sequences of strains TmAO3^T and TmAM3 at the base of the *Sporomusa* species cluster; sequence similarity to other members of the genus *Sporomusa* ranged from 94 to 97 %. Highest values were obtained with *Sporomusa sphaeroides* (96·8 %) and *Sporomusa* sp. strain DR5 (97·0 %), which was isolated from anoxic bulk soil of flooded rice microcosms (Rosencrantz *et al.*, 1999). Sequence similarity to representatives of other genera in the subgroup was <91·6 %.

Taxonomy

The physiological characteristics of strain TmAO3^T clearly identify it as a homoacetogenic bacterium. Like most other

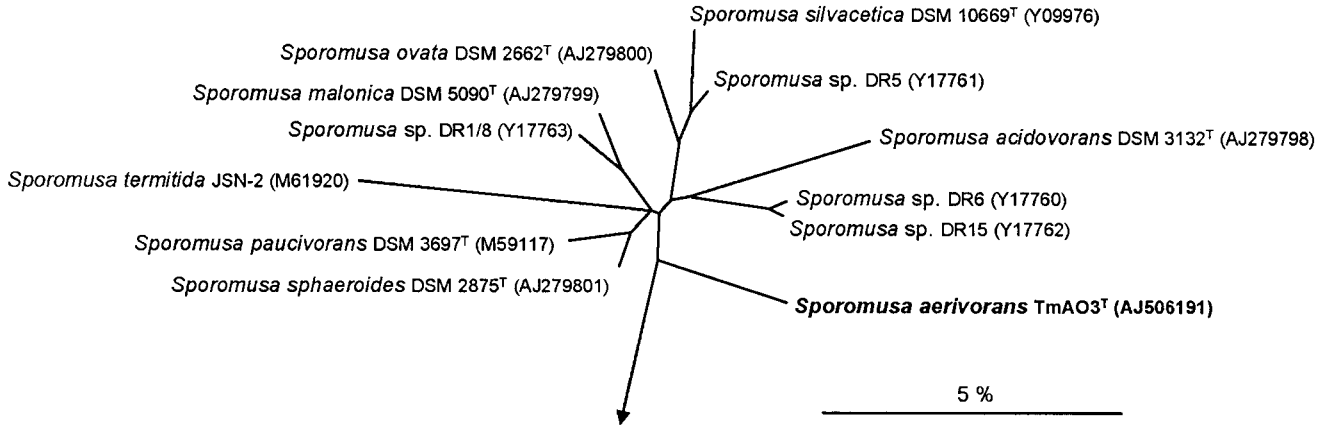

Fig. 4. Phylogenetic relationship of *Sporomusa aerivorans* (strain TmAO3^T) and other species in the genus *Sporomusa*. The tree is based on maximum-likelihood analysis of the 16S rRNA gene sequences of these strains and a selection of 100 reference organisms, including the closely related genera *Acidaminococcus*, *Anaeromusa*, *Dendrosporobacter*, *Desulfotomaculum*, *Dialister*, *Megasphaera*, *Pectinatus*, *Phascolarctobacterium*, *Quinella*, *Schwartzia*, *Selenomonas*, *Succiniclasticum*, *Succinispira*, *Veillonella* and *Zymophilus* and other Gram-positive bacteria with a low DNA G+C content, which also served to define the root of the tree. Only alignment positions that were invariant among at least 50 % of these sequences were included in the calculations. The tree topology was evaluated and corrected according to the results of distance matrix and maximum-parsimony analyses. Multifurcations indicate that a significant relative branching order could not be determined unambiguously or was not supported by the results of the alternative treeing procedures. GenBank/EMBL accession numbers of the sequences are given in parentheses. Bar, 5 % estimated sequence divergence.

bacteria in this physiological group, it catalyses the H_2-dependent reduction of CO_2 to acetate and ferments lactate, ethanol and other substrates homoacetogenically, i.e. all reducing equivalents from substrate oxidation to acetate are used for further (reductive) acetogenesis from CO_2. The presence of CO dehydrogenase [11·5 μmol CO oxidized min^{-1} (mg $protein^{-1}$)] has been demonstrated (Boga & Brune, 2003). Also, the demethylation of *O*-methyl ethers and subsequent fermentation of the methyl groups to acetate and the utilization of other C_1 compounds such as formate and methanol are typical of many homoacetogens (see reviews by Diekert, 1992; Drake *et al.*, 1994; Schink, 1994).

16S rRNA gene sequence analysis revealed that strains TmAO3^T and TmAM3 are most closely related to members of the genus S*poromusa*. Their phenotypic characteristics are also typical of members of this genus (Möller *et al.*, 1984): cells are curved, banana-shaped rods, form heat-resistant endospores, are motile by one or more lateral flagella, possess *b*-type cytochromes and form acetate as the major fermentation product.

In its substrate utilization spectrum, strain TmAO3^T most closely resembles *S. termitida*, a homoacetogenic isolate from a wood-feeding termite (Breznak *et al.*, 1988), which was included in this study as a reference strain. Both strains can be clearly differentiated from other members of the genus *Sporomusa* by their inability to grow on fructose and glycerol and their ability to grow on mannitol, citrate and succinate (Table 2). However, the strains differ significantly in their 16S rRNA gene sequence (94 % similarity) and the ability to ferment dicarboxylic acids. Strain TmAO3^T grows on L-malate and fumarate, whereas cells of *S. termitida* do not grow on these substrates (Table 2). Cells of strain TmAO3^T are also curved more strongly and, in contrast to *S. termitida*, are not sensitive to reducing agents such as DTT and cysteine (Breznak *et al.*, 1988; this study).

Physiology and ecology

Like most homoacetogens (Schink, 1994; Drake *et al.*, 1997), strain TmAO3^T is metabolically quite versatile. It ferments a large number of organic substrates homoacetogenically, utilizing reductive acetogenesis from CO_2 as an acceptor for the electrons released during substrate oxidation to acetate. The inability to ferment glucose or other sugars is unusual but encountered frequently among *Sporomusa* species (Table 2). Also, fermentation of dicarboxylic acids, e.g. fumarate and L-malate, is not a common trait among homoacetogens, but again, it is not uncommon in the genus *Sporomusa* (Table 2). It has been suggested that *b*-type cytochromes, which are present in many *Sporomusa* species (including strain TmAO3^T), are involved in fumarate reduction (Diekert, 1992; Diekert & Wohlfarth, 1994). However, *b*-type cytochromes are also present in species that do not grow on dicarboxylic acids (*S. termitida*, *Sporomusa ovata* and *S. sphaeroides*) and there is evidence for *S. sphaeroides* that *b*-type cytochromes are also involved in the oxidation of methyl groups (Kamlage & Blaut, 1993).

Molecular hydrogen is a typical product that is formed by

Table 2. Selected morphological and physiological properties that differentiate strain TmAO3^T from other members of the genus *Sporomusa*

Species: 1, strain TmAO3^T; 2, *S. termitida*; 3, *S. ovata*; 4, *S. sphaeroides*; 5, *Sporomusa acidovorans*; 6, *Sporomusa paucivorans*; 7, *Sporomusa malonica*; 8, *S. silvacetica*. +, Positive; −, negative; W, weakly positive; ±, most strains positive; ND, not determined.

Property	1	2	3	4	5	6	7	8
Width (μm)	0·6–0·7	0·5–0·8	0·9–1	0·5–0·9	0·7–1	0·4–0·7	0·7	0·7
Length (μm)	1·3–7	2–8	1·3–1·4	2–5	2–8	2–3	2·6–4·8	3
Formation of endospores	+	+	+	+	+	−	+	+
Catalase activity	+	+	W	+	ND	−	−	−
Utilization of:								
Fructose	−	−	±	−	+	−	+	+
Mannitol	+	+	−	−	ND	−	ND	−
Glycerol	−	−	−	+	+	+	−	+
Citrate	+	+	−	−	−	−	+	−
L-Malate	+	−	−	−	+	−	+	ND
Fumarate	+	−	−	−	+	−	+	+
Succinate	+	+	−	−	+*	−	+	−
Source of type strain	Termite gut	Termite gut	Silage	River sediment	Distillery waste water	Lake sediment	Freshwater sediment	Forest soil
Reference	This study	Breznak *et al.*, 1988	Möller *et al.*, 1984	Möller *et al.*, 1984	Ollivier *et al.*, 1985	Hermann *et al.*, 1987	Dehning *et al.*, 1989	Kuhner *et al.*, 1997; Karnholz *et al.*, 2002

*Produces acetate from succinate (Ollivier *et al.*, 1985), whereas the other species form propionate as the major product.

fermentative degradation of organic compounds (Schink, 1997) and hydrogen-consuming processes, such as reductive acetogenesis and methanogenesis, seem to be important electron sinks in the hindguts of all termites studied so far (Brauman *et al.*, 1992; Schmitt-Wagner & Brune, 1999; Tholen & Brune, 1999). In soil-feeding *Termitinae*, methanogenesis seems to dominate over reductive acetogenesis as a hydrogen sink, possibly due to the lower affinity of homoacetogens for H_2 (Breznak, 1994). Nevertheless, the presence of high potential rates of reductive acetogenesis in soil-feeding termites indicates that they are able to coexist with other hydrogenotrophic populations (Tholen & Brune, 1999).

It is possible that the metabolic versatility of homoacetogenic bacteria allows them to maintain an active metabolism during phases of low H_2 partial pressure in the gut (Tholen & Brune, 1999). Hindgut fluid of soil-feeding termites contains considerable concentrations of potential substrates for homoacetogens, e.g. lactate and ethanol (E. Miambi, H. I. Boga, A. Tholen & A. Brune, unpublished data), and probably also methoxylated aromatic compounds that are derived from lignins or humic acids. Recent findings indicate that peptides and amino acids may also be important substrates for the gut microbiota of soil-feeding termites (Ji *et al.*, 2000). Mixotrophy, i.e. the ability of homoacetogens to use H_2 and organic substrates simultaneously, as observed for *S. termitida* (Breznak & Blum, 1991), would add to their competitiveness (Breznak, 1994).

Strain TmAO3^T was isolated from a dilution step that indicated a population of approximately 10^3–10^4 cells per gut section, which is in good agreement with the estimated total number of homoacetogens growing in serial dilutions of gut homogenates of *Thoracotermes macrothorax* (E. Miambi, H. I. Boga, A. Tholen & A. Brune, unpublished data). Nevertheless, the large discrepancy between viable counts and total cell counts, together with the high potential rates of reductive acetogenesis in the guts of soil-feeding termites, indicate a strong cultivation bias against homoacetogens (Tholen & Brune, 1999).

Oxygen reduction

Drake and coworkers were the first to document tolerance and metabolic response to the presence of oxygen for a number of homoacetogens, including *Sporomusa silvacetica* (Küsel *et al.*, 2001; Karnholz *et al.*, 2002). In an independent study, we have shown that strain TmAO3^T and other strains of homoacetogen isolated from termite guts consume oxygen at high rates (Boga & Brune, 2003).

Strain TmAO3^T has by far the highest capacity for hydrogen-dependent oxygen reduction [826 nmol min^{-1} $(mg\ protein)^{-1}$] of all homoacetogens tested (Boga & Brune, 2003); it is surpassed only by that reported for several *Desulfovibrio* species isolated from termite guts (Kuhnigk *et al.*, 1996; Cypionka, 2000). The activity is cyanide-sensitive, which indicates that cytochromes might participate in electron transport to oxygen. Strain TmAO3^T also possesses high catalase activity, whereas it is superoxide dismutase-negative in both the xanthine/xanthine oxidase

assay and the nitro blue tetrazolium salt reduction assay (Boga & Brune, 2003).

Owing to its large capacity for hydrogen-dependent oxygen reduction and its exceptional tolerance to a temporary exposure to oxygen, strain TmAO3^T is able to initiate growth in non-reduced basal medium that contains up to 1·5 kPa of oxygen in the headspace (Boga & Brune, 2003). However, closer investigation revealed that growth commences only after the cells have rendered the medium anoxic and that reductive acetogenesis from CO_2 is severely compromised if even traces of oxygen are present in the medium (Boga & Brune, 2003).

It has been proposed that the ability of obligate anaerobes to scavenge oxygen, together with their apparent tolerance of toxic oxygen reduction products, would not only enable them to survive a temporary exposure to oxygen but would also allow them to actively re-establish favourable conditions for growth (Boga & Brune, 2003). As the termite gut habitat is characterized by a large influx of oxygen via the epithelium (Brune, 1998; Brune *et al.*, 2000), it is possible that strain TmAO3^T contributes to oxygen consumption within the micro-oxic periphery of the gut.

Based on phylogenetic, morphological and physiological differences, strain TmAO3^T is proposed as a novel member of the genus *Sporomusa*, with the name *Sporomusa aerivorans* sp. nov.

Description of *Sporomusa aerivorans* sp. nov.

Sporomusa aerivorans [ae.ri.vo′rans. L. n. *aer* air; L. pres. part. *vorans* digesting, devouring; N.L. pres. part. *aerivorans* devouring air (oxygen), referring to the high capacity of the organism to reduce oxygen].

Curved rods, 1·3–7·0 μm long and 0·6–0·7 μm wide. Motile by one or more lateral flagella. Stain Gram-positive but react Gram-negative in the KOH test. Catalase-positive but superoxide dismutase-negative. Terminal, heat-resistant endospores in club-shaped sporangia are formed. Oxygen-sensitive; does not grow in air. Resting cells reduce oxygen in the presence of hydrogen or by endogenous reductant; therefore, they can initiate growth in non-reduced medium under micro-oxic conditions. Chemo-organotrophic fermentative metabolism. Nitrate and sulfate are not used as external electron acceptors. Grows lithotrophically by reductive acetogenesis on H_2 and CO_2 in the presence of yeast extract or Casamino acids. Homoacetogenic; ferments L-lactate, pyruvate, citrate, L-alanine, D-mannitol, ethanol, formate and methanol to acetate as sole product. Fumarate, L-malate and oxaloacetate are fermented to propionate and acetate. Decarboxylates succinate and malonate to propionate or acetate, respectively. The *O*-methyl groups of syringate, vanillate and 3,4,5-trimethoxybenzoate are fermented to acetate. Does not grow on hexoses. Yeast extract or Casamino acids are required for growth and are fermented mainly to acetate. Possesses membrane-bound *b*-type cytochrome(s). Temperature range for growth is 19–35 °C, optimum 30 °C. No growth at 4 or 45 °C. pH range for growth is 6·2–8·2, optimum pH 7.

Type strain: TmAO3^T (= DSM 13326^T = ATCC BAA-625^T). Habitat: intestinal tract of the termite *Thoracotermes macrothorax*.

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