5

Characterization of two sulfate-reducing bacteria from the gut of the soil-feeding termite, *Cubitermes speciosus*

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Abstract

Two sulfate-reducing bacteria (SRB) were isolated from a mixed culture enriched with benzoate obtained from gut homogenate of the soil-feeding higher termite, *Cubitermes speciosus*. The organisms were vibrioid rods, staining Gram-negative, which performed incomplete substrate oxidation. They differed in several features. The smaller one, strain STp, was motile with a single polar flagellum. This strain differed from *Desulfovibrio desulfuricans* only by its inability to oxidize malate and pentanol. The bigger one, strain STg, differed from *Desulfovibrio giganteus* only by its nonmotility and a lower length. It is the first evidence of the presence of SRB in termite gut.

Termites, owing to their large biomass and the diversity of their diet, are among the main ligno-cellulosic degraders in tropical areas (Lee & Woods 1971; Lepage 1974). It has by now been clearly established that their considerable digestive capacity (65 to 99% of their food intake consists of cellulose and hemicelluloses) (Ezenther & Kirk 1974) is linked, in the case of the lower termites, to the presence of symbiotic microflora in their hind-gut (Hungate 1943).

The exact activity of this microflora in higher termites (70% of species) caracterized by the absence of cellulolytic symbiotic protozoa, has not yet been fully elucidated, specially for the soil-feeding termites which constitute a high proportion of he biomass in tropical soils (Lee & Woods 1971). These termites thrive on soil rich in polyaromatic compounds originating from lignin and tannin degradation (Sillam 1987).

In Soil-feeding termites, enrichment on several aromatic compounds was performed in order to understand the relationship between their symbiotic microflora and termite digestive capacity (Brauman et al. in preparation).

We report in this paper on the isolation and characterization of two species of sulfate-reducing bacteria belonging to the genus *Desulfovibrio*. These bacteria were isolated from a benzoate enrichment obtained from a gut homogenate of a soil-feeding termite. In this enrichment culture, the sulfate reducers were not the only hydrogenotrophic bacteria since methanogens belonging to the genera *Methanobrevibacter*, *Methanosphaera* and *Methanogenium* were also present (data not shown).

Strains STp and STg were isolated on lactate medium, from a mixed bacterial culture enriched on benzoate (5 mM) and inoculated with ground guts of the soil-feeding termite *Cubitermes speciosus,* from the Mayombe tropical rainforest, Congo (central Africa). Initial enrichment cultures from gut homogenate on benzoate were transferred be-

Fig. 1. Phase contrast photomicrograph of strain STp. Bar is 10 μm.

Fig. 2. Phase contrast photomicrograph of strain STg. Bar is 10 μm.

fore SRB isolation, during two months in an anaerobic bicarbonated pH 7.2 buffered sulfide-reduced medium containing benzoate (5 mM) and vitamins as sole organic substrate. The composition of this medium was described previously (Widdel & Pfennig 1984).

Two strains were used for comparison: *Desulfovibrio desulfuricans* (strain essex) was obtained from the Laboratoire de Chimie Bactérienne (CNRS, Marseille); *Desulfovibrio giganteus* (DSM 4123) was isolated in our laboratory.

The basal medium contained in g/l: KH_2PO_4, 0.2; NH_4Cl, 0.3; KCl, 0.5; $NaCl$, 1; $CaCl_2.2H_2O$, 0.15; $MgCl_2.6H_2O$, 0.4.1 ml/l trace element solution (Imhoff-Stuckle & Pfennig 1983) and 1 mg/l resazurin were added. The medium was sterilized for 40 min at 110° C. After autoclaving, the medium was immediately cooled under a continuous flow of N_2-CO_2 (80–20%); then 30 ml/l of $NaHCO_3$ 0.5 M and 3 ml/l of Na_2S 0.5 M were added from separately sterilized anoxic solutions. 1 ml/l of vitamin solution (Pfennig 1978) sterilized by filtration was also added. The medium was finally adjusted to pH 7.0–7.4 and distributed into Hungate tubes as described by Pfennig et al. (1981). The electron donnors and acceptors were added from separately sterilized anoxic solutions, as required, using disposable syringes.

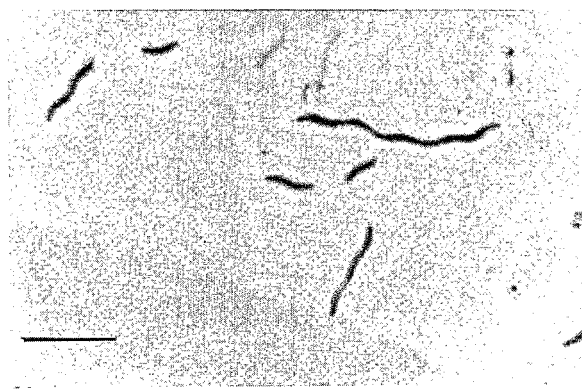Pure cultures were obtained by repeated application of the shake dilution method in anaerobic Hungate tubes as described by Pfennig et al. (1981). Purity was checked on a complex sulfate-free medium containing 0.25% glucose, 0.25% Biotrypcase (Biomérieux) and 0.25% yeast extract (Difco).

Growth was quantified by measuring the optical density at 580 nm. H_2S was determined spectrophotometrically as colloidal CuS (Cord-Ruwisch 1985). The presence of sulfate was revealed by addition of $BaCl_2$.NH_4^+ was determined by a potentiometric method using an Orion specific electrode, model 95-10 (Orion Research inc., Cambridge, Ma, USA). Disappearance and formation of metabolites were measured by HPLC (Cord-Ruwisch et al. 1986).

Whole cell DNA was extracted after disruption of the cells and purified using the Marmur method (1961) at the German Collection of Microorganisms (DSM), Braunschweig, FRG. The mole % G + C of the DNA was determined by buoyant density centrifugation in a $CsCl_2$ gradient.

Strain STp consisted of small Gram-negative curved rods, 0.5–1.0 by 1.0–3.0 μm, occuring either singly or in pairs (Fig. 1), motile with a single polar flagellum. Strain STg was a pleomorphic Gram-negative, nonmotile bacterium with both straight and curved rods, 1.0 by 2.0–6.0 μm. These occurred either singly, in pairs or in short chains (Fig. 2).

In the medium described by Pfennig et al. (1981), the optimum temperature and pH for growth were 30° C and 7.0 with strain STp and 37° C and 7.5 with

strain STg, respectively. The optimum NaCl concentration for growth were less than $1\,g.l^{-1}$ for STp and $10\,g.l^{-1}$ for STg.

The substrates tested as energy sources for strain STp are listed in Table 1. Growth occurred on lactate (20 mM), pyruvate (20), fumarate (20), formate (20), choline (20), ethanol (20), propanol (20), butanol (10) and H_2 (2 bars) + acetate (10); neither DL-malate (20) nor pentanol (10) were used. Pyruvate, fumarate and choline were fermented by this strain.

The substrates tested as energy sources for strain STg are listed in Table 1. This strain grew on lactate (20 mM), pyruvate (20), cysteine (20), glycerol (20), ethanol (20), propanol (20), isobutanol (10), H_2 (2 bars) + acetate (10). It was able to ferment pyruvate but not malate, fumarate and glycerol. It used formate (20), isopropanol (20), butanol (10) and pentanol (20) without growth.

Sulfate (20 mM), sulfite (5), thiosulfate (10), elemental sulfur (10), fumarate (20) and nitrate (5) were used as electron acceptors by strain STp. The

Table 1. Substrate utilization by strains STp and STg compared with that of *D. desulfuricans* and *D. giganteus*. Concentrations are expressed in (mM).

Substrates	strain STp	*D. desulfuricans*	strain STg	*D. giganteus*
Acetate (20) + sulfate	–	–	–	–
Lactate (20) + sulfate	+	+	+	+
Citrate (10) + sulfate	–	nd	–	–
Choline (20) + sulfate	+	+	–	–
Choline (20)	+	+	–	–
Pyruvate (20) + sulfate	+	+	+	+
Pyruvate	+	+	+	+
Fumarate (20) + sulfate	+	+	–	–
Fumarate	+	+	–	–
Formate (20) + sulfate	+	+	(+)	(+)
Malate (20) + sulfate	–	+	–	–
Malate	nd	nd	–	–
Succinate (20) + sulfate	nd	nd	–	–
Butyrate (10) + sulfate	nd	nd	–	–
isoButyrate (20) + sulfate	nd	nd	–	–
2-methylButyrate (20) + sulf.	nd	nd	–	–
Stearate (2) + sulfate	–	–	–	–
Palmitate (2) + sulfate	–	–	–	–
Methanol (20) + sulfate	–	nd	–	–
Ethanol (20) + sulfate	+	+	+	+
Propanol (20) + sulfate	+	+	+	+
isoPropanol (20) + sulfate	–	nd	(+)	(+)
Butanol (10) + sulfate	+	+	(+)	(+)
isoButanol (10) + sulfate	–	nd	+	+
Pentanol (10) + sulfate	–	+	(+)	(+)
Glycerol (20) + sulfate	–	nd	+	+
Glycerol	nd	nd	–	–
Fructose (20) + sulfate	–	–	–	–
Fructose (20)	–	–	–	–
Glutamate (10) + sulfate	–	nd	–	–
Alanine (10) + sulfate	–	nd	–	–
Cysteine (20) + sulfate	nd	nd	+	+
Benzoate (10) + sulfate	–	–	–	–
H2 (2 bars) + acetate (10) + sulf.	+	+	+	+

nd: not determined
(+): degradation without growth

doubling times of this strain grown on lactate with sulfate, sulfite, fumarate or nitrate were 10.2, 8.3, 8.1 and 9.5 h, respectively.

Strain STg used only sulfate, sulfite, thiosulfate and elemental sulfur as electron acceptors. Doubling times of strain STg grown on lactate with sulfate, sulfite or thiosulfate were 7.7, 6.5 and 7.4 h, respectively.

The mole % G + C content of DNA of strains STp (DSM 4369) and STg (DSM 4370) were respectively 55.2 and 56.0 (mean value of three determinations).

Strains STp and STg consist of non-sporeforming curved rods, and reduce sulfate. The isolates incompletely oxidize pyruvate and lactate to acetate + CO_2. Neither acetate, propionate nor butyrate are oxidized. Based on these characteristics, the isolates can be assigned to the genus *Desulfovibrio* (Pfennig et al. 1981) but they do not belong to the *sapovorans* group since saturated fatty acids were not oxidized.

The morphological and physiological characteristics of strain STp are similar to those of *D. desulfuricans* (Widdel & Pfennig 1984); STp differs from this species only in that it does not oxidize malate and pentanol. Both strains ferment pyruvate, fumarate and choline.

The morphological and physiological characteristics of strain STg can be related to the newly described species *D. giganteus* (Esnault et al. 1988). STg differs only in that it is nonmotile and has a lower length. *D. giganteus* requires an upper NaCl concentration for optimum growth. Both strains ferment pyruvate and use formate, isopropanol, butanol and pentanol without growth.

Sulfate reducing bacteria (SRB) are known to be widespread in anoxic areas containing sulfates such as marine environments and salt marsh. They are also to be found in sulfate-free habitats, rich in organic substances such as animals and human feces (Beerens & Romond 1977), plant wastes and rumen contents (Coleman 1960; Huising et al. 1974). Here we described the first sulfate-reducing bacteria isolated from termite gut. Their exact role in the metabolism of the insect has not yet been determined, however. In such ecosystem they could act as hydrogen scavengers through interspecies hydrogen transfer with methanogens, during the degradation of highly reduced compound such as benzoate and relatives. In the absence of sulfate, strains STp and STg can also oxidize organic substrates as pyruvate, fumarate or choline.

D. desulfuricans is a bacterium currently isolated from various biotopes through out the world and it's not surprising to found this organism in the gut of soil-feeding termites. On contrary *D. giganteus* is a newly described strain isolated from a brackish coastal lagoon, having an optimal salt requirement (2.5% NaCl) and growing up to 5%. Its presence in the gut of soil-feeding termites is unexplained.

Acknowledgement

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