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Sporomusa silvacetica sp. nov., an Acetogenic Bacterium Isolated from Aggregated Forest Soil

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Sporomusa silvacetica **sp. nov. DG-1^T (= DSMZ 10669^T) (T = type strain) was isolated from well-drained, aggregated forest soil (pH 6.0) in east-central Germany. The cells were obligately anaerobic, slightly curved rods and were motile by means of laterally inserted flagella on the concave side of each cell. Typical cells were approximately 3.5 by 0.7 μm. Cells stained weakly gram positive, but thin sections revealed a complex multilayer cell wall. Spores were spherical and distended the sporangia. Growth and substrate utilization occurred with ferulate, vanillate, fructose, betaine, fumarate, 2,3-butanediol, pyruvate, lactate, glycerol, ethanol, methanol, formate, and H_2-CO_2. With most substrates, acetate was the primary reduced end product and was produced in stoichiometries indicative of an acetyl-coenzyme A pathway-dependent metabolism. Fumarate was dismutated to succinate and acetate. Methoxyl and acrylate groups of various aromatic compounds were O-demethylated and reduced, respectively. Yeast extract was not required for growth. Cells grew optimally at approximately 30°C and pH 6.8; under these conditions and with fructose as the substrate, the doubling time was approximately 14 h. The lowest temperature that supported growth was between 5 and 10°C. The carbon monoxide dehydrogenase and hydrogenase activities were approximately 9 and 102 μmol min^{-1} mg of protein^{-1}, respectively. A type *b* cytochrome was detected in the membrane. The G+C content was approximately 43 mol%. Phylogenetic analysis of the 16S ribosomal DNA indicated that DG-1^T was most closely related to members of the genus *Sporomusa* in the *Clostridium* subphylum of the gram-positive bacteria.**

Low-molecular-weight aliphatic organic acids are present in mineral forest soil solutions and are believed to play roles in soil formation, solubility of toxic metals, and plant growth (23, 24, 30, 49, 58, 59). In this regard, acetate is a dominate organic acid detected in mineral soils (59), and it has been proposed that the acetate in mineral soils is produced primarily through the collective action of facultatively and obligately anaerobic microorganisms (35, 63). Although well-drained soils are not considered typical acetogenic habitats, supplementation of forest (34, 35), prairie (63), and tundra (46) soils with H_2 or CO results in the utilization of substrates and the production of acetate in stoichiometries approximating those expected for H_2- or CO-dependent acetogenesis. In addition, acetogenic consortia are readily enriched from mineral forest soils (35, 48) and leaf litter (36). To further evaluate the occurrence of acetogens in well-drained, aggregated soils, an acetogen was isolated from a beech forest in east-central Germany. The collective characteristics of this isolate (strain DG-1^T [T = type strain]) are not consistent with the characteristics of any previously described acetogenic bacterium, and it is proposed that this organism should be placed in a new species, *Sporomusa silvacetica.*

MATERIALS AND METHODS

Soil collection. Forest soil (a silty loam) was obtained from the mineral (Ah) horizon of a slightly acidic beech site in the Geisberger Forest in east-central Germany. The soil had a pH of approximately 6, a dry weight of 60%, and an organic carbon content of 80 g kg^{-1} (dry weight) (see reference 35 for additional site and soil characteristics). The soil was collected in sterile glass containers and was utilized immediately after transportation to the laboratory.

Enrichment cultures. Soil samples were placed in an anaerobic chamber (100% N_2 gas phase; room temperature; Mecaplex, Grenchen, Switzerland) and added to anaerobic medium (approximately 1 g [wet weight] of soil per 10 ml of medium). The medium was then supplemented with either H_2-CO_2 (80:20) or a combination of vanillate (5 mM) and CO (100%) (the gas volumes were 20 ml per 120-ml vial or 10 ml per 30-ml vial). Enrichment cultures were incubated at 15°C, and stable enrichment cultures were obtained by repeated transfer in the same medium. After several transfers, enrichment cultures were streaked onto solid media (media supplemented with 1.5% agar). Isolated colonies were transferred to liquid media and assayed for substrate utilization and product formation.

Composition of media and growth conditions. Media were prepared anaerobically (31). Medium A was an undefined, carbonate-buffered medium (16). Medium B was medium A without yeast extract or resazurin. These media were dispensed under CO_2 into 120-ml serum vials (50 ml of medium per vial) or 27-ml culture tubes (7 ml of medium per tube), which were then crimp sealed and autoclaved. Medium C was a defined, carbonate-buffered medium containing trace element solution SL10 (64, 65). This medium was adjusted to pH 6.5 prior to autoclaving, and an alternative vitamin solution (67) was utilized (1 ml liter^{-1}); after autoclaving, the medium was dispensed under N_2-CO_2 (90:10). Leaf leachate medium was prepared by incubating leaves (500 g) from the litter layer at Geisberg, Germany, in distilled water (750 ml) for 1 week at 20°C. The liquid was then decanted, centrifuged (10,000 × *g*, 20 min), filter sterilized, and made anoxic by gassing with 100% argon. The leaf leachate (pH 5.9) was diluted 1:1 with distilled water and was added to sterile 30-ml serum vials (10 ml per vial) under 100% argon. Soil extract medium was prepared by adding 20 g of soil to 75 ml of deionized, anoxic water. The resulting soil suspensions were placed on an end-over-end shaker (40 cycles min^{-1}) for 2 h at 4°C. The aqueous phase was then centrifuged (10,000 × *g*, 20 min), filter sterilized, and gassed with and dispensed under 100% argon.

The reduction of sulfate was determined by using a sulfate-enriched lactate medium (50) supplemented with 0.1 g of cysteine · HCl liter^{-1}; tubes were visually inspected for sulfide production (i.e., blackening of the precipitate). The reduction of nitrate was determined by using medium A supplemented with 5 mM KNO_3. The fixation of N_2 was determined by using a medium designed to assess N_2-fixing microorganisms (52); a trace element solution (1) (1 ml liter^{-1}) replaced yeast extract, and the medium was prepared with a 100% N_2 gas phase. Unless otherwise indicated, the temperature of incubation was 25°C.

Transmission electron microscopy. Cells were cultivated at 30°C in medium A supplemented with 10 mM fructose and harvested by centrifugation. The cells were negatively stained with uranyl acetate (62). For thin-section preparations, cells were fixed in glutaraldehyde-OsO_4 and prepared by a standard protocol (60). Thin sections were stained with uranyl acetate and lead acetate (53). Specimens were observed with a model CEM 902A microscope (Zeiss, Oberkochen, Germany).

Preparation of cell extract and enzyme assays. Cells were cultivated in medium A supplemented with 5 mM fructose. Cultures (total volume, 1.3 liters) were dispensed into centrifuge bottles in a Mecaplex anaerobic chamber (100% N_2 gas phase; room temperature) and centrifuged (10,000 × *g*, 20 min, 4°C). The

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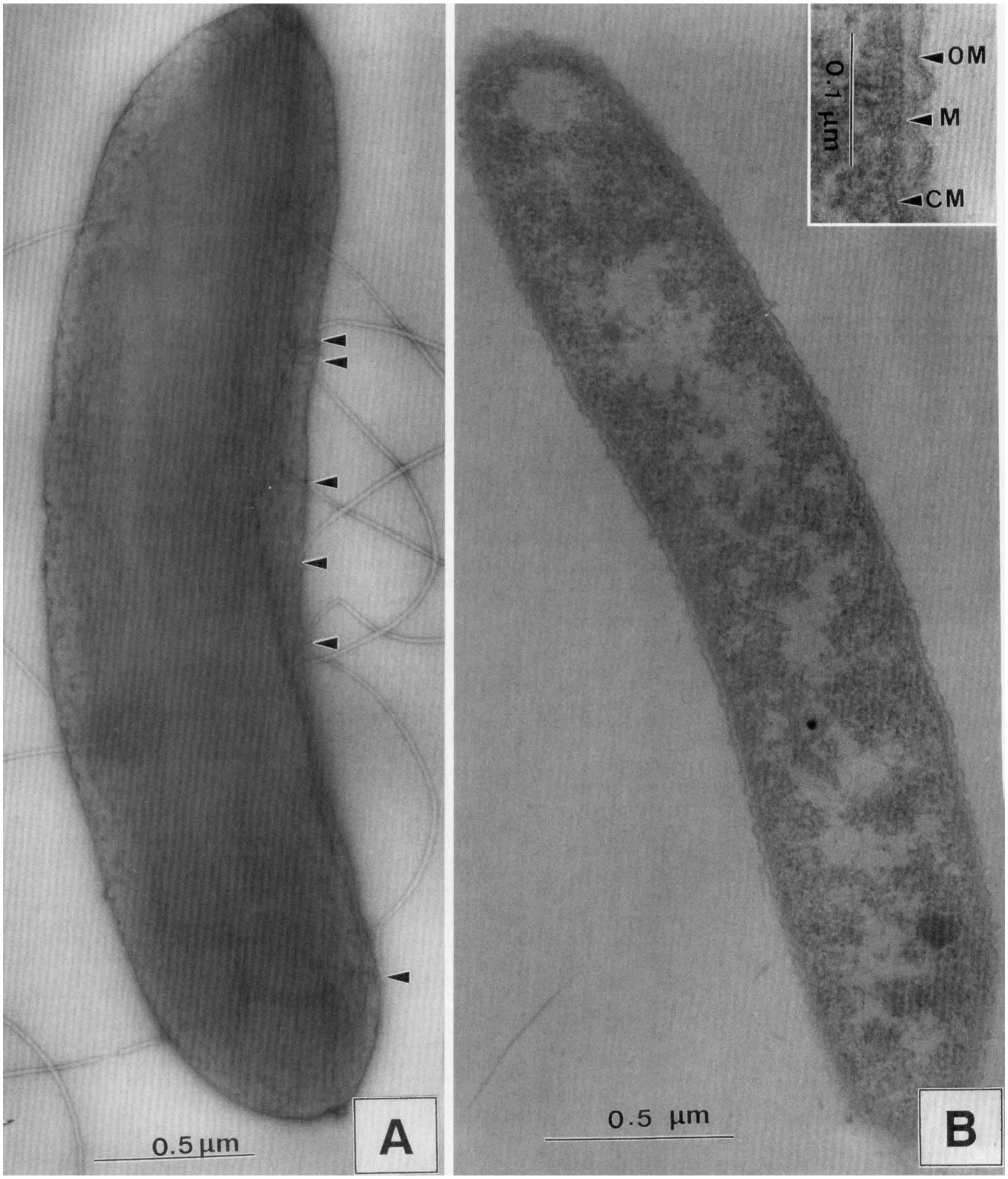

FIG. 1. Transmission electron micrographs of strain DG-1^T. (A) Negatively stained preparation. The arrowheads indicate insertion points of flagella. (B) Thin section. Abbreviations: CM, cytoplasmic membrane; M, murein layer; OM, outer membrane.

cell pellet was washed three times with sodium phosphate buffer (50 mM, pH 7). The cell pellet was resuspended in 2 volumes of freshly prepared, anoxic lysozyme buffer (39), incubated for 1 h at room temperature, and centrifuged at 10,000 × *g* for 20 min at 4°C. The supernatant fluid was transferred to a serum vial (100% N_2 gas phase) and assayed immediately. Hydrogenase and carbon monoxide dehydrogenase activities were assayed at 30°C with Tris-hydrochloride (100 mM, pH 8.5)–benzyl viologen (1 mM)–dithiothreitol (1 mM) in the presence of H_2 (100%) or CO (100%) (19). Polyacrylamide gel electrophoresis (19) was preformed in a temperature-controlled Mecaplex anaerobic chamber (100% N_2 gas phase) at 10°C, and in situ staining of gels (19) for carbon monoxide

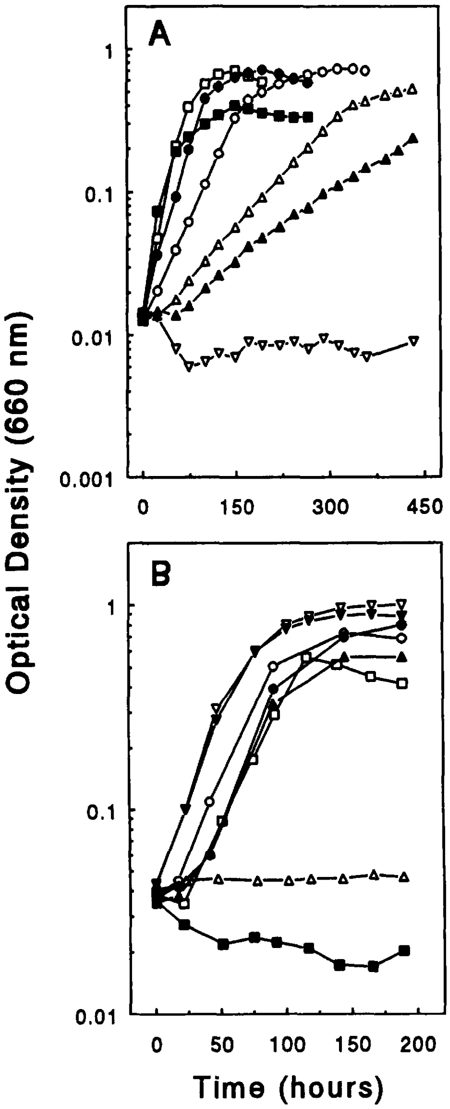

FIG. 2. Effect of temperature (A) and pH (B) on the growth of strain DG-1^T in medium A (unless otherwise indicated) supplemented with 10 mM fructose. (A) The temperatures tested were 10°C (▲), 15°C (△), 20°C (○), 25°C (●), 30°C (□), 35°C (■), and 5 or 42°C (▽). (B) The initial pHs were pH 4.6 (tryptic soy broth [3 g liter^{-1}]) (△), pH 5.5 (▲), pH 5.9 (●), pH 6.2 (○), pH 6.8 (▽), pH 7.2 (▼), pH 7.7 (□), and pH 8.0 (■).

dehydrogenase activity was performed at room temperature. Reduction of acetylene was used to evaluate nitrogenase activity (37).

Membrane preparation and redox difference spectra. Cells were cultivated in medium A supplemented with 10 mM fructose. Membranes were prepared from cell extracts by ultracentrifugation under aerobic conditions (26). Washed membranes were reduced with sodium dithionite, and reduced-minus-oxidized spectra were obtained with a model Uvikon 930 double-beam recording spectrophotometer (Kontron Instruments, Milan, Italy) at room temperature (26).

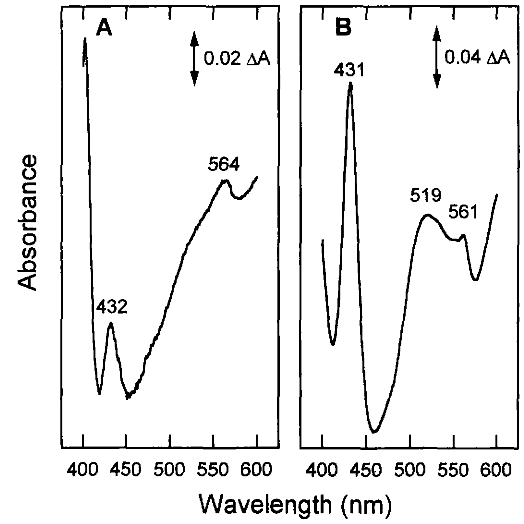

FIG. 3. Redox difference spectra for strain DG-1^T membranes (A) and cytoplasm (B). The protein concentrations used for the spectra shown in panels A and B were 1.4 and 11.2 mg ml^{-1}, respectively.

G+C content. Cells were washed with phosphate buffer (50 mM, pH 7.0), and DNA was extracted by the NaOH method (3). The G+C content was determined by high-performance liquid chromatography (42).

Analysis of 16S rDNA. Approximately 95% of the 16S rRNA gene (rDNA) sequence of strain DG-1^T was determined by directly sequencing PCR-amplified 16S rDNA. The analysis was performed by workers at the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA, and purification of the PCR products were performed by using previously described protocols (51). Purified PCR products were sequenced by using an ABI PRISM Ready Reaction dye terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.). Sequence reaction mixtures were electrophoresed with an Applied Biosystems model 373A DNA sequencer. The sequence data were put into alignment editor ae2 (40), aligned manually, and compared with available 16S rDNA sequences of representative organisms belonging to the *Clostridium* subphylum of the gram-positive bacteria. A dendrogram was constructed by using standard phylogenetic methods and the PHYLIP package (22, 32, 54).

Additional analytical methods. Growth and cell dry weight were determined as previously described (16). For fructose-containing cultures, a culture optical density at 660 nm of 1 corresponded to 133 mg (dry weight) of cells liter^{-1}. Protein content was determined by the Bradford method (6). The amounts of substrates and products present in culture fluids and gas phases were determined by high-performance liquid chromatography and gas chromatography (16, 27, 35, 41). Soil pH was determined by using 1:2.5 suspensions of soil in 0.02 N $CaCl_2$, and soil dry weight was obtained by weighing samples before and after drying at 105°C for 16 h. The amount of total carbon in oven-dried (65°C), homogenized organic matter was determined with an element analyzer (CHN-O-Rapid instrument; Foss-Heraeus, Hanau, Germany). The nitrate content was determined colorimetrically (11). The results and values given below are representative of the results and values obtained in duplicate experiments.

TABLE 1. Substrate-product stoichiometries for strain DG-1^T grown in medium A at 25°C

Substrate	Maximum optical density at 660 nm	Amt of substrate consumed (mM)	Amt of product (mM)		Acetate/substrate ratio[b]
			Acetate[a]	Protocatechuate	
Fructose	0.46	4.8	13.8	NA[c]	2.88 (3.0)
Vanillate	0.11	6.5	4.5	6.2	0.69 (0.75)
H_2	0.08	23.3	6.6	NA	0.28 (0.25)

[a] Values were corrected for the amount of acetate formed in controls lacking additional substrates.

[b] The values in parentheses are the theoretical ratios expected for acetogenic consumption of substrates (20).

[c] NA, not applicable.

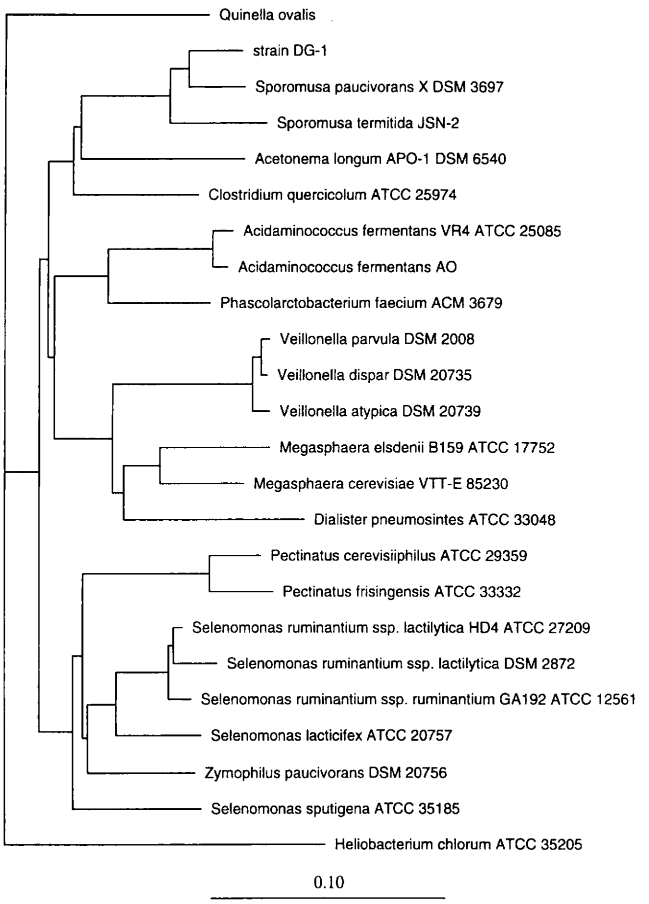

FIG. 4. Dendrogram showing the phylogenetic position of strain DG-1^T (based on an analysis of 16S rDNA). Scale bar = 10 nucleotide substitutions per 100 nucleotides.

Nucleotide sequence accession number. The 16S rDNA sequence data have been deposited in the EMBL nucleotide sequence database (Cambridge, United Kingdom) under accession number Y09976.

RESULTS

Enrichment cultures. Aggregated soil was added to defined (medium B or C), soil extract, and leaf leachate media supplemented with H_2-CO_2 or vanillate-CO. H_2 and CO_2 were utilized, vanillate was O-demethylated, and acetate was produced by all soil suspensions within 3 or 4 weeks; repeated transfers yielded stable acetate-producing enrichment cultures. These observations indicated that enrichment of acetogenic consortia occurred. In the leaf leachate and soil extract media, exogenous substrate utilization was retarded; however, acetate production occurred at rates similar to those observed in other media, suggesting that endogenous carbon sources were also utilized.

Isolation. Isolate DG-1^T was obtained from a stable medium B enrichment culture containing H_2-CO_2. DG-1^T produced acetate as the sole soluble end product from utilization of H_2. The purity of isolate DG-1^T was ensured by restreaking an isolated colony three times. Cultures of DG-1^T were considered to be pure based on uniform colony and cellular morphologies.

Morphology. Isolate DG-1^T was a strictly anaerobic, motile, rod-shaped organism. Its cells were approximately 3.5 by 0.7 μm and slightly curved (Fig. 1). Flagellar staining (4) and electron microscopy revealed laterally inserted flagella on the concave side of each cell (Fig. 1A). Cells exhibited both tumbling motility and short, directional motility in wet mounts. Although Gram staining (13) and the KOH test (28) indicated that DG-1^T was weakly gram positive, thin sections revealed a more complex, multilayer cell wall (Fig. 1B, inset). Small inclusion bodies were observed in thin sections. With fructose as the substrate, cells occasionally swelled and became teardrop shaped after growth began; terminal, spherical spores were observed in wet mounts prepared from old cultures. Free spores were rarely observed, and the capacity to sporulate appeared to decrease with prolonged laboratory cultivation. Colonies on solidified medium A supplemented with fructose were shiny, beige to slightly yellow, and 2 to 3 mm in diameter. The colony form was irregular with entire or slightly undulate margins.

Temperature and pH optima. Isolate DG-1^T grew at temperatures ranging from 10 to 35°C (Fig. 2A); no growth was observed at 5 or 42°C during incubation for 1 month. The optimal temperature was 25 to 30°C. Growth was observed after a sporulated culture was heated for 10 min at 80°C. Growth was relatively rapid when the initial pH was between 5.5 and 7.7 (Fig. 2B).

Doubling time. In fructose-supplemented medium A (pH 6.8), the doubling times at 30 and 10°C were approximately 14 and 80 h, respectively.

Fermentation stoichiometries. Strain DG-1^T produced acetate concomitant with growth on fructose, vanillate, or H_2-CO_2 (data not shown). The substrate-product stoichiometries obtained from such cultures indicated that acetogenic consumption of substrates occurred (Table 1). The growth yield for cells grown in medium A supplemented with 5 mM fructose was approximately 61 mg (dry weight) of cells liter^{-1}, and 13.8 mM acetate was formed, yielding an acetate/biomass ratio of 226 mmol of acetate g (dry weight) of cells^{-1}. Protocatechuate was produced in near stoichiometry with the amount of vanillate consumed, indicating that the aromatic ring was not subject to breakage or to further transformation.

Additional physiological characteristics. In medium A, growth and substrate utilization were observed with the following substrates: ferulate, vanillate, fructose, betaine, fumarate, 2,3-butanediol, pyruvate, lactate, glycerol, ethanol, methanol, formate, and H_2-CO_2. In all cases, the substrate was converted to acetate. Protocatechuate and hydroferulate were additional products observed with vanillate and ferulate, respectively. H_2 and the *O*-methyl group of vanillate were utilized as cosubstrates. Fumarate was dismutated to succinate and acetate. Fumarate is also dismutated by *Clostridium aceticum* and *Clostridium formicoaceticum* (18, 41). H_2 was not produced. Traces of methane were produced in addition to acetate in cultures grown with H_2-CO_2; production of trace levels of methane has also been observed with the acetogens *Clostridium thermoautotrophicum* (55), *Acetobacterium woodii* (10), and *Acetobacterium carbinolicum* (10).

No growth or substrate utilization was observed with cellobiose, glucose, citrate, 4-hydroxybenzaldehyde, xylose, succinate, oxalate, acetate, or CO. According to the results of an API analysis (BioMérieux Deutschland GmbH, Nürtingen, Germany), mannitol, lactose, saccharose, maltose, salicin, arabinose, mannose, melezitose, raffinose, sorbitol, rhamnose, and trehalose were not utilized. Additional results of the API analysis indicated that esculin was hydrolyzed, gelatin was not hydrolyzed, and tryptophanase, urease, catalase, and oxidase activities were absent. Yeast extract (1 g liter^{-1}) stimulated growth, but was not required. The growth rates and final cell yields were not appreciably affected when cells were cultivated repeatedly in sodium-deficient medium B (prepared by using potassium salts rather than sodium salts) supplemented with either fructose or H_2-vanillate (data not shown).

Nitrate, rather than CO_2, is the preferred terminal electron acceptor for *Clostridium thermoaceticum* and *C. thermoautotrophicum* and inhibits the ability of these acetogens to form acetate by repressing the electron transport system normally engaged in the acetyl coenzyme A (acetyl-CoA) pathway (21, 26, 57). When strain DG-1^T was grown in the presence of nitrate, nitrate was not appreciably utilized and acetate production was not appreciably affected, indicating that nitrate was not used as an alternative electron acceptor by DG-1^T. Sulfate was also not used as an alternative electron acceptor. Strain DG-1^T did not fix N_2.

Enzyme activities and membrane spectrum. The carbon monoxide dehydrogenase and hydrogenase activities in cell extracts were approximately 9 and 102 μmol min^{-1} mg of $protein^{-1}$, respectively. Two electrophoretically distinct carbon monoxide dehydrogenase bands were observed on in situ-stained polyacrylamide gels. A type *b* cytochrome was detected in membranes; absorption maxima were observed at 432 and 564 nm (Fig. 3A). The cytoplasmic fraction had absorption maxima at 431 and 561 nm (Fig. 3B), indicating that the cytoplasm also contained a type *b* cytochrome or that a portion of the membranous type *b* cytochrome was lost to the cytoplasmic fraction during membrane preparation. The chromophoric origin of the absorption maximum at 519 nm in the cytoplasmic fraction (Fig. 3B) was not resolved, but this absorption maximum was characteristic of the β-absorption peak of a type *c* cytochrome.

G+C content and phylogenetic analysis of 16S rDNA. The DNA base composition of DG-1^T was 42.7 ± 0.4 mol% G+C ($n = 6$). Phylogenetic analysis of the 16S rDNA indicated that strain DG-1^T was most closely related to the genus *Sporomusa* (Fig. 4). A similarity value of 97.2% was obtained when the 16S rDNA sequences of strain DG-1^T and *Sporomusa paucivorans* DSM 3697^T were compared. A similarity value of 95.9% was obtained when the 16S rDNA sequences of strain DG-1^T and *Sporomusa termitida* JSN-2 were compared.

DISCUSSION

Its cell shape, Gram reaction, formation of spores, and lack of sulfate reduction indicated that strain DG-1^T classically belongs to the genus *Clostridium*. However, thin sections revealed a multilayer cell wall characteristic of gram-negative bacteria, and the phylogenetic analysis of the 16S rDNA sequence indicated that DG-1^T is most closely related to members of the gram-negative, spore-forming genus *Sporomusa* in the *Sporomusa* subbranch of the *Clostridium* subphylum of the gram-positive bacteria (40, 66). As outlined below, the characteristics of DG-1^T are not consistent with the characteristics of previously described acetogenic strains of the genus *Clostridium* or the genus *Sporomusa*, and we propose that strain DG-1^T is a member of a new acetogenic species, *S. silvacetica*; strain DG-1 is the type strain of this new species.

The production of acetate as the primary reduced end product, the stoichiometries of acetate formation observed with fructose, vanillate, and H_2 (approximately 3:1, 3:4, and 1:4, respectively), and carbon monoxide dehydrogenase activity indicated that strain DG-1^T is an acetogen. Strain DG-1^T differs from other acetogenic clostridia (12, 20, 56) as follows: it differs from *C. aceticum*, *Clostridium fervidus*, *C. formicoaceticum*, *Clostridium magnum*, and *Clostridium* sp. strain CV-AA1 in its Gram reaction; it differs from *C. fervidus*, *C. formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium mayombei*, *Clostridium* (*Oxobacter* [15]) *pfennigii*, *C. thermoaceticum*, and *C. thermoautotrophicum* in its optimum temperature and temperature range; it differs from *C. magnum* in size; it differs from all other acetogenic clostridia except *C. magnum* in its cell shape during spore formation; it differs from all other acetogenic clostridia in its substrate range (i.e., it differs in specific substrates utilized on a case-by-case basis); it differs from *C. formicoaceticum* in N_2 fixation (5); and it differs from all other acetogenic clostridia in its G+C content.

The cell and spore morphologies of strain DG-1^T were typical of the genus *Sporomusa* (45). The following six *Sporomusa* species have been characterized previously: *Sporomusa acidovorans* (47), *Sporomusa malonica* (17), *Sporomusa ovata* (45), *S. paucivorans* (29), *Sporomusa sphaeroides* (45), and *S. termitida* (8). To date, only the 16S rDNAs of *S. paucivorans* and *S. termitida* have been sequenced. In contrast to strain DG-1^T, *S. paucivorans* does not utilize sugars, requires yeast extract for growth, has a G+C content of 47 mol%, and has not been observed to form spores (29). In contrast to DG-1^T, *S. termitida* does not utilize fructose, fumarate, or glycerol and has a G+C content of 49 mol%. On a case-by-case basis, DG-1^T differs from the other *Sporomusa* species in spore morphology, substrate range, yeast extract requirement, and G+C content. A type *b* cytochrome was detected in the membranes of DG-1^T, a trait characteristic of other *Sporomusa* species (8, 17, 45). Betaine stimulates the production of membranous type *b* cytochromes in *S. ovata* (33).

Strain DG-1^T is the first *Sporomusa* strain to be isolated from well-drained terrestrial soils, a habitat that is subject to fluctuations in aeration and redox potential. With the exception of *S. termitida*, all other *Sporomusa* species have been isolated from habitats that are classically considered to be anaerobic (e.g., freshwater sediments) (20). *S. termitida* was isolated from a termite gut (8), a microhabitat also subject to fluctuations in redox potential and anaerobiosis (9). It thus appears that certain *Sporomusa* species can adapt to habitats that are not strictly anaerobic. The origin of DG-1^T is also in marked contrast to the origins of numerous other mesophilic acetogens isolated from sewage, gastrointestinal tracts of animals, and water-logged habitats, such as marine sediment and ditch mud (20, 56).

Acetate is a major organic acid of mineral forest soils (59). Although acetate does not accumulate in situ, forest soils produce acetate and CO_2 as major carbonaceous products during the turnover of endogenous organic matter in anaerobic microcosms (35). The stoichiometry of acetate production from exogenously added H_2 by anaerobically incubated mineral soils (35, 63) suggests that acetate can be formed via the acetyl-CoA Wood-Ljungdahl pathway (20). The isolation of an acetogen from soil in Geisberg, Germany, confirms that acetogens are present and potentially active in anaerobic microsites in aggregated forest soils.

The degradation of lignocellulose yields lignin monomers, such as vanillate, syringate, and ferulate (14, 25). Strain DG-1^T exhibited a broad substrate range and utilized methoxylated lignin derivatives in addition to sugars, alcohols, organic acids, betaine, and H_2-CO_2. In contrast to *C. aceticum* and *C. formicoaceticum* (27), strain DG-1^T did not grow at the expense of 4-hydroxybenzaldehyde. A number of acetogens can utilize multiple substrates simultaneously (7, 38, 41). In addition, most acetogens can utilize electron acceptors other than CO_2 for the conservation of energy (20, 21, 56). Strain DG-1^T used H_2 and the methoxyl groups of vanillate as cosubstrates, a metabolic capacity that may enhance the in situ activities of certain acetogens (38). Strain DG-1^T did not engage in lactate fermentation concomitant with acetogenesis, as *Peptostrepto-*

coccus productus U-1 can (44), but did exhibit the ability to utilize aromatic acrylate groups as electron acceptors, as certain other acetogens, such as *A. woodii* (2, 61) and *P. productus* U-1 (43), do. In addition, fumarate was dismutated to succinate and acetate, suggesting that reduction of fumarate to succinate may be an energy-conserving process, as is the case in *C. aceticum* and *C. formicoaceticum* (18, 41). The capacity of other *Sporomusa* species to utilize fumarate and aromatic acrylate groups as terminal electron acceptors has not been reported previously (8, 17, 29, 45, 47). The broad substrate, pH, and temperature ranges of strain DG-1^{T} may contribute to its competitiveness in a soil-based ecosystem subject to large environmental changes and limited substrate availability.

It has been proposed that acetate forms a trophic link between anaerobic and aerobic processes in certain soils (21, 35, 63). This hypothesis is based on the observation that acetate is a stable end product under experimentally imposed anaerobic conditions and is converted to methane only after extensive lag periods (approximately 1 to 3 months) at in situ temperatures. In the presence of oxygen, however, anaerobically produced acetate is readily oxidized to CO_2 (21, 35, 63). The anaerobic and acetogenic capacities of aggregated soils are relatively stable during periods of O_2 enrichment and aerobic drying (63); oxic leaf litter also has acetogenic capacities (36). Spore formation may allow strain DG-1^{T} and other soil-dwelling anaerobes involved in acetate production to compete in an environment where redox conditions are constantly changing.

Description of *Sporomusa silvacetica* sp. nov. *Sporomusa silvacetica* (sil.va.ce'ti.cum. L. n. *silva*, forest; L. fem. adj. *acetica*, pertaining to vinegar, acetic acid; N. L. fem. adj. *silvacetica*, a forest organism producing acetic acid). Based on 16S rDNA analysis data, strain DG-1^{T} is most closely related to the genus *Sporomusa* in the *Clostridium* subphylum of the gram-positive bacteria.

Cells are weakly gram-positive, motile rods that are slightly curved (approximately 3.5 by 0.7 μm), occur singly or in pairs, and form round, terminal spores. The cell wall is multilayered, and flagella are laterally inserted on the concave side of each cell. Colonies growing on fructose are shiny, beige to slightly yellow, and 2 to 3 mm in diameter. Obligate anaerobe. The optimum temperature and optimum pH for growth are 25 to 30°C and 6.8, respectively; under these conditions and with fructose as the substrate, the doubling time is 14 h.

Uses the acetyl-CoA pathway for acetate synthesis. Grows with ferulate, vanillate, fructose, fumarate, betaine, 2,3-butanediol, pyruvate, lactate, glycerol, ethanol, methanol, formate, and H_2-CO_2. Reduces fumarate and the aromatic acrylate group of ferulate. Does not require yeast extract for growth. Does not grow by dissimilation of nitrate or sulfate. Nitrogen is not fixed. Catalase and oxidase negative. Contains a membranous type *b* cytochrome. The G+C content of the DNA is approximately 43 mol%.

The type strain, strain DG-1, was isolated from the mineral horizon of a beech forest soil in Bavaria, Germany, and has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSMZ 10669.

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