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Streptomyces thermoautotrophicus sp. nov., a Thermophilic CO- and H_2-Oxidizing Obligate Chemolithoautotroph

DILIP GADKARI,[1] KARL SCHRICKER,[1] GEORG ACKER,[1] REINER M. KROPPENSTEDT,[2] AND ORTWIN MEYER[1]*

Lehrstuhl für Mikrobiologie, Universität Bayreuth, D-8580 Bayreuth,[1] and Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-3300 Braunschweig 2,[2] Federal Republic of Germany

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The novel thermophilic CO- and H_2-oxidizing bacterium UBT1 has been isolated from the covering soil of a burning charcoal pile. The isolate is gram positive and obligately chemolithoautotrophic and has been named *Streptomyces thermoautotrophicus* on the basis of G+C content (70.6 ± 0.19 mol%), a phospholipid pattern of type II, MK-9(H_4) as the major quinone, and other chemotaxonomic and morphological properties. *S. thermoautotrophicus* could grow with CO (t_d = 8 h), H_2 plus CO_2 (t_d = 6 h), car exhaust, or gas produced by the incomplete combustion of wood. Complex media or heterotrophic substrates such as sugars, organic acids, amino acids, and alcohols did not support growth. Molybdenum was required for CO-autotrophic growth. For growth with H_2, nickel was not necessary. The optimum growth temperature was 65°C; no growth was observed below 40°C. However, CO-grown cells were able to oxidize CO at temperatures of 10 to 70°C. Temperature profiles of burning charcoal piles revealed that, up to a depth of about 10 to 25 cm, the entire covering soil provides a suitable habitat for *S. thermoautotrophicus*. The K_m was 88 μl of CO liter^{-1} and V_{max} was 20.2 μl of CO h^{-1} mg of protein^{-1}. The threshold value of *S. thermoautotrophicus* of 0.2 μl of CO liter^{-1} was similar to those of various soils. The specific CO-oxidizing activity in extracts with phenazinemethosulfate plus 2,6-dichlorophenolindophenol as electron acceptors was 246 μmol min^{-1} mg of protein^{-1}. In exception to other carboxydotrophic bacteria, *S. thermoautotrophicus* CO dehydrogenase was able to reduce low potential electron acceptors such as methyl and benzyl viologens.

Carboxydotrophic bacteria are characterized by the chemolithoautotrophic utilization of carbon monoxide (CO) under aerobic conditions (23, 25). Some can denitrify (8a). Defined on physiological grounds, the bacterial grouping is taxonomically diverse (27). Most carboxydotrophic bacteria are also hydrogenotrophs, feeding on H_2 and CO_2 as well (27). Without exception, the carboxydotrophic bacteria known to date have a facultative metabolism and are capable of utilizing organic growth substrates. So far, obligate chemolithoautotrophic CO-oxidizing microorganisms are not known.

In the course of our search for new types of carboxydotrophic bacteria, especially those which might contribute to the sink of CO in nature, we became interested in the microflora of burning charcoal piles located in Bavaria. We report here on the isolation and characterization of a thermophilic CO- and H_2-oxidizing obligate chemolithoautotroph, *Streptomyces thermoautotrophicus* sp. nov., from these sites.

MATERIALS AND METHODS

Bacteria. *Pseudomonas carboxydovorans* OM5 (DSM 1227) (26) and the newly isolated strain UBT1 were used throughout this study.

Soils. Samples of covering soil were taken from burning charcoal piles in the upper Franconian Mountains (Plech valley) and Bavarian Forest (Zwieselau). Soil samples were also obtained from a pine-spruce forest (L/Oh horizon) and cornfield (upper layer) in the vicinity of Bayreuth (Bavaria).

Growth conditions. Bacteria were cultivated autotrophically in the mineral medium of Meyer and Schlegel (26) supplemented with 1 ml of trace element solution TS2 (27) liter^{-1}. If not otherwise indicated, the pH was adjusted to 7.5. Enrichments were at pH 7, and the phosphate content of the enrichment media was reduced to 20% of that of the above mineral medium. In addition, 1 ml of vitamin solution (41) was added per liter of enrichment medium. Media were solidified with 1.5% (wt/vol) Difco agar.

Enrichment cultures. Soil samples (0.5 g, wet weight) were incubated in 100-ml Erlenmeyer flasks containing 20 ml of mineral medium. Flasks were kept unshaken in desiccators under a gas atmosphere of 5% CO_2–45% CO–50% air (vol/vol/vol). After 1 month of incubation at 50°C, the first subcultures were made; later, they were done every 2 weeks. Growth occurred after 1 to 2 weeks in the form of a dry white pellicle covering the surface of liquid media. Mycelia were removed from pellicles, suspended in saline, streaked on mineral plates, and incubated under CO as above; emerging single colonies were restreaked. This procedure was repeated until a uniform shape of colonies was established.

Growth measurements. Since strain UBT1 did not grow dispersed but developed in the form of a pellicle on the surface of liquid media, biomass was measured as dry weight. To reduce hydrophobic interactions, Tween 80 (0.5 ml) was added to an entire culture (15 ml) prior to filtration on cellulose acetate membrane filters of 0.2-μm pore size (Sartorius, Göttingen, Federal Republic of Germany). Filters were dried overnight at 80°C. The growth curves of Fig. 2 were obtained as follows. Bacteria were cultured in a series of 50-ml Erlenmeyer flasks containing 15 ml of mineral medium. All flasks were inoculated and treated in the same way. They were kept unshaken at 65°C in desiccators

* Corresponding author.

supplied with the indicated gas mixtures. With time, entire cultures were removed for dry-weight determinations. All experiments were done in triplicate, and the data presented are average values.

The utilization of organic compounds (0.05 to 0.5% by weight or volume) for aerobic growth was tested in liquid culture. Volatile organic compounds were applied as vapor. Methane and home heating gas were applied at 30 and 50% (vol/vol) in air.

Determination of pH and temperature of a burning charcoal pile. Samples of 1 g (wet weight) were taken from the covering soil of a pile at Zwieselau (Bavaria), suspended in 2.5 ml of 0.01 M aqueous $CaCl_2$, mixed, and kept for 30 min at ambient temperature. Subsequently, pH values were determined by using a combined glass electrode.

Temperatures were measured in situ by means of a battery-powered instant action thermometer (model Technoterm 9300; Testoterm, Lenzkirch, Federal Republic of Germany) equipped with an NiCr-NiAl thermocouple probe of 1-m length. Isotherms were calculated from plots of temperature versus height.

Electron microscopy. For scanning electron microscopy, bacteria were grown on mineral plates with CO for 3 to 6 days. Colonies were removed and fixed for 4 h at room temperature in glutaraldehyde (2%, vol/vol, in 50 mM phosphate buffer, pH 6.9) and subsequently overnight at 4°C in OsO_4 (2%, wt/vol, in phosphate buffer) following published procedures (35). The fixed samples were dehydrated in a graded aqueous acetone series (percent, vol/vol), 25, 50 (containing 1%, [wt/vol] uranyl acetate), 70, 90, and absolute acetone, and critical-point dried in CO_2 (1). Uranyl acetate treatment was for 2 h. The samples were mounted on specimen stubs, immediately sputter coated with gold, and observed in a scanning electron microscope (model Novascan 30; Zeiss, Oberkochen, Federal Republic of Germany).

Transmission electron microscopy was with CO-grown bacteria. Cells were harvested from liquid cultures as described above, transferred to distilled water, and negatively stained with 2% (wt/vol) aqueous uranyl acetate solution, pH 4.5 (45). For thin-section preparation, 3-day-old CO-grown bacterial colonies were fixed in glutaraldehyde-OsO_4 and prepared as described previously (44). Embedment was in Spurr resin (Serva, Heidelberg, Federal Republic of Germany). Thin sections were stained with uranyl acetate (2%, wt/vol) and lead citrate (36). Samples were viewed in a transmission electron microscope (model EM 109; Zeiss).

Cell chemistry. Cell wall amino acids and whole-cell sugars were analyzed by thin-layer chromatography (42). The phospholipids and menaquinones were extracted by the integrated method of Minnikin et al. (31). Menaquinones were separated by high-pressure liquid chromatography by the method of Kroppenstedt (14). The fatty acids were saponified and methylated by the method of Miller (28) as modified by Lambert and Moss (16). The fatty acid methyl esters were analyzed gas chromatographically (14a). For determination of mycolic acids, the transmethylation method of Minnikin et al. (29) was used.

Measurement of CO oxidation. CO oxidation by intact bacteria was determined as follows. Wheaton vials (37-ml total volume) were filled with 10 ml of mineral medium, inoculated with 0.3 ml of a 4- to 6-day-old homogenized CO-grown preculture, provided with loosely fitting metal caps, and incubated at 65°C under a gas atmosphere of 45% CO–5% CO_2–50% air (vol/vol/vol). In the exponential growth phase (3 to 6 days), the gas atmosphere was replaced by air, the vials were serum stoppered, and up to 300 ppmv of CO was injected. The vials were kept at 65°C. Gas samples of 50 to 100 μl were removed with time and analyzed in a CO analyzer (RG D2; Trace Analytical, Düsseldorf, Federal Republic of Germany) based on HgO to Hg vapor conversion as detailed before (5, 39, 40). Prior to detection, CO was gas chromatographically separated from H_2 and other compounds reactive under these conditions. The gas chromatograph was equipped with a 0.5-nm molecular sieve steel column (2 m by 4 mm) operated at 110°C. Helium was used as the carrier gas at a flow rate of 25 ml min^{-1}.

Plasmids. Conventional methods were used for detection of plasmids (3, 12, 21).

Characterization of DNA. DNA was isolated (21), and the G+C content of the DNA was determined by the thermal denaturation method (6); values were corrected by the method of Mandel and Marmur (20). A DNA sample of *Escherichia coli* B served as a reference (9). The spectrophotometer (model Response II; Gilford, Giessen, Federal Republic of Germany) was equipped with a thermoprogrammer and an automatic cuvette changer.

Cell harvesting and preparation of extracts. Since cells of strain UBT1 did not sediment upon centrifugation, mycelia were harvested by filtration through a narrow mesh (0.2 to 0.5 mm) household sieve. Recoveries were better than 80%. Mycelia (about 1 g, wet weight) were suspended in 1 ml of 50 mM phosphate buffer, pH 7. Extracts were prepared by passing suspensions three times through a precooled French pressure cell operated at 1,500 lbs/in^2. Cell debris was removed by low-spin centrifugation. Supernatants were designated the crude extract.

Electron acceptor specificities. Reduction of artificial electron acceptors by crude extracts of strain UBT1 in the presence of CO was examined spectrophotometrically (Uvikon 720 LC micro; Kontron, Eching, Federal Republic of Germany). Assays were in serum-stoppered cuvettes containing 50 μM electron acceptor in 1 ml of the above phosphate buffer, flushed for 10 min with pure CO. Reactions were started by injecting crude extract.

Chemicals. Benzyl viologen, phenazine methosulfate, iodonitrotetrazolium chloride, 1-methoxyphenazine methosulfate, methylene blue, and thionine (Lauth's violet) were from Serva. Methyl viologen and 2,6-dichlorophenolindophenol were from Fluka, Buchs, Switzerland. Neutral red was from Merck, Darmstadt, Federal Republic of Germany. All other chemicals were from commercial sources.

RESULTS

Isolation and properties of isolate UBT1. Bacterium UBT1 was isolated from the covering soil of a burning charcoal pile as detailed under Materials and Methods. Our attention was attracted by cultures showing growth of bacteria in the form of a dry pellicle entirely covering the surface of liquid cultures.

Growth of strain UBT1 was obligately dependent on O_2. The cells of strain UBT1 were gram positive and catalase and tetramethyl *p*-phenylenediamine oxidase negative. The G+C content of the DNA was 70.6 ± 0.19 mol%.

Strain UBT1 formed rough colonies of 4 to 6 mm in diameter. Their color was grey to white, and they sat loosely on solid surfaces; i.e., with the help of an inoculation loop, they could easily be moved around.

The isolate formed a substrate mycelium and a scanty aerial mycelium. In liquid media UBT1 covered the surface as a white, dry, and hydrophobic pellicle of 40 to 50 μm in

thickness (Fig. 1A), composed of an irregular web of hyphae (Fig. 1B). Strain UBT1 formed branching mycelia. Individual cells had a width of 0.2 to 0.5 μm and a length of up to 5 μm. Branching always occurred close to the septa (Fig. 1D).

UBT1 formed spores of 1 by 2 μm (Fig. 1C, E, and F). Two to eight oval spores were arranged in a short chain (Fig. 1C). The pellicles turned grey after sporulation. Thin-sectioned spores revealed vacuoles and granulated bodies and were surrounded by a sheath (Fig. 1E and F). Spores of UBT1 which were allowed to dry and kept exposed to the environment at ambient temperature retained their viability for at least 4 months. Spore suspensions in saline were resistant to boiling for 15 min.

Plasmids were not detected in UBT1.

Chemotaxonomy. The cells of UBT1 contained LL-diaminopimelic acid. In addition, the following phospholipids were resolved: phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and two to three phosphatidylinositol mannosides, i.e., phospholipid type II (Lechevalier et al. [18]). Menaquinone MK-9(H_4) was the main quinone (90% of the total quinone fraction). Small amounts of MK-9(H_6) (10%) were also present. Iso and anteiso branched fatty acids were predominant (93.5% of the total fatty acid fraction). The total fatty acid fraction contained 67% 15-methyl- and 14-methyl-hexadecanoic acids (iso- and anteiso-C_{17}) and 16% 14-methyl-heptadecanoic acid (iso-C_{16}). In addition, 0.6% hydroxy fatty acid (iso-$C_{17:0}$-OH_2, 2-hydroxy-15-methyl-hexadecanoic acid) and 2% cyclopropane fatty acid (*cis*-9,10-methyl-enoctadecanoic acid) were present. Small amounts of unsaturated branched (3%) and saturated unbranched (3.5%) fatty acids were also resolved. Ribose was the only sugar found in bacterial hydrolysates. Sugars of diagnostic value, e.g., arabinose, galactose, xylose, madurose, or rhamnose, were absent, as were mycolic acids.

Chemolithoautotrophic growth. Strain UBT1 could grow chemolithoautotrophically in a mineral medium with CO or H_2 plus CO_2 as sole sources of carbon and energy under aerobic conditions (Fig. 2). Growth with CO showed no special requirement for CO_2.

Growth was observed between pH 6.5 and 8.5 and was best at pH 7.5. The doubling time with CO or H_2 plus CO_2 was 8 or 6 h, respectively. Growth also occurred with the constituents of gas collected from the exhaustion pipe of a car without a catalytic converter and the smoke derived from the incomplete burning of wood. Since in liquid media growth of UBT1 was not dispersed, the yields obtained depended on the available surface and not on the culture volume.

In the carboxydotrophic bacteria examined to date, CO dehydrogenase was found to be a molybdo iron-sulfur flavoprotein (22). Consequently, the utilization of CO by these bacteria depends on the availability of molybdate in the culture medium (22). It was thus of interest to examine whether growth of UBT1 with CO also would depend on molybdenum. Since growth was only obtained when molybdate was present (Fig. 2), CO dehydrogenase in UBT1 very likely also is a molybdoenzyme. That growth with H_2 occurred in the absence of molybdate (Fig. 2) indicates that the molybdenum requirement is not a general metabolic requirement, but is specific for CO cultures. Growth of the bacterium with H_2 plus CO_2 did not require nickel in the medium, referring to an Ni-free hydrogenase.

There was no specific vitamin requirement.

Growth with CO was inhibited by antibiotics, as follows (concentrations in milligrams per liter): penicillin, 25; ampicillin, 16; streptomycin, 5; rifamycin, 8; chloramphenicol, 8; kanamycin, 8. The MICs of metal salts for UBT1 growing with CO were as follows (in milligrams of metal per liter): $Pb(NO_3)_2$, 250; Na_2HAsO_4, 1,200; Na_2SeO_3, 7; $CuSO_4$, 7.

Heterotrophic growth. Since the carboxydotrophic bacteria studied so far are capable of feeding on a wide variety of organic substrates (27), we were interested in the utilization of organic compounds by strain UBT1. None of the following compounds supported growth under aerobic conditions at 65°C: methane, home heating gas, glucose, fructose, sucrose, lactose, nutrient broth, peptone, yeast extract, malt extract, meat extract, casein hydrolysate, Casamino Acids, starch, methanol, ethanol, propanol, isoamyl alcohol, glycerol, pyruvate, succinate, acetate, formate, dichloromethane, diethyl ether, di-isopropyl ether, acetone, benzol, formamide, terpenaline, carbon tetrachloride, acetaldehyde. Growth of UBT1 with CO in liquid media was not affected by the above compounds.

Effect of temperature on growth and CO oxidation. Strain UBT1 could grow in the temperature range of 40 to 65°C (Fig. 3). No growth occurred below 40°C, indicating that UBT1 is a true stenothermophile. The optimal growth temperature was 65°C. However, bacteria grown at 65°C were capable of oxidizing CO at temperatures ranging from 10 to 70°C with an optimum similar to that of growth (Fig. 3).

Kinetics. CO oxidation by intact cells of strain UBT1 and *P. carboxydovorans* did not follow Michaelis-Menten kinetics. Hill coefficients were 1.1 and 1.4 for *P. carboxydovorans* and UBT1, respectively. The K_ms were 88 and 112 μl of CO $liter^{-1}$ for UBT1 and *P. carboxydovorans*. V_{max}s for UBT1 and *P. carboxydovorans* were 20.2 and 1.6 μl of CO h^{-1} mg of $protein^{-1}$. The kinetics of agricultural soil and pine-spruce forest soil also did not follow Michaelis-Menten kinetics. The Hill coefficients were 1.2 and 1.5 for agricultural soil and pine-spruce forest soil. The K_m for pine-spruce forest was 8 μl of CO $liter^{-1}$ and that for a cornfield was 38 μl of CO $liter^{-1}$. V_{max}s for cornfield soil and pine-spruce forest soil were 0.6 and 3.6 μl of CO h^{-1} mg of $protein^{-1}$.

Electron acceptor utilization. The utilization of natural and artificial electron acceptors by CO dehydrogenase with CO as electron donor was examined in crude extracts of bacterium UBT1. The following electron acceptors were not utilized: NAD^+, $NADP^+$, flavin adenine dinucleotide, flavin mononucleotide, neutral red, and thionine. Those utilized are listed in Table 1. The range of electron acceptors used by UBT1 was unique, since it differed considerably from that found with all other carboxydotrophic bacteria in ability to act on viologen dyes and inability to reduce thionine. Electron acceptor utilization by UBT1 refers to structural differences of its CO dehydrogenase compared with the enzymes from other carboxydotrophic bacteria, which are known to be very similar in their N-terminal amino acid sequences of subunits (13) and biochemical (27) and immunological (37) properties.

CO oxidation at ambient concentrations. At CO concentrations exceeding that of natural air very slightly, strain UBT1 oxidized CO very rapidly (15.7 μl of CO h^{-1} mg of dry $weight^{-1}$) at rates similar to those observed with pine-spruce forest soil (Fig. 4). On the other hand, CO oxidation by cells of *P. carboxydovorans* or agricultural soil was much slower (Fig. 4). Similar to other carboxydotrophic bacteria (37), CO oxidation by UBT1 was about fourfold faster with exponential versus stationary cells. The threshold values obtained with strain UBT1, *P. carboxydovorans*, and different types of soil (Fig. 4) were very similar (about 0.2 μl of CO $liter^{-1}$) and favorably compared with the mixing ratio of CO in

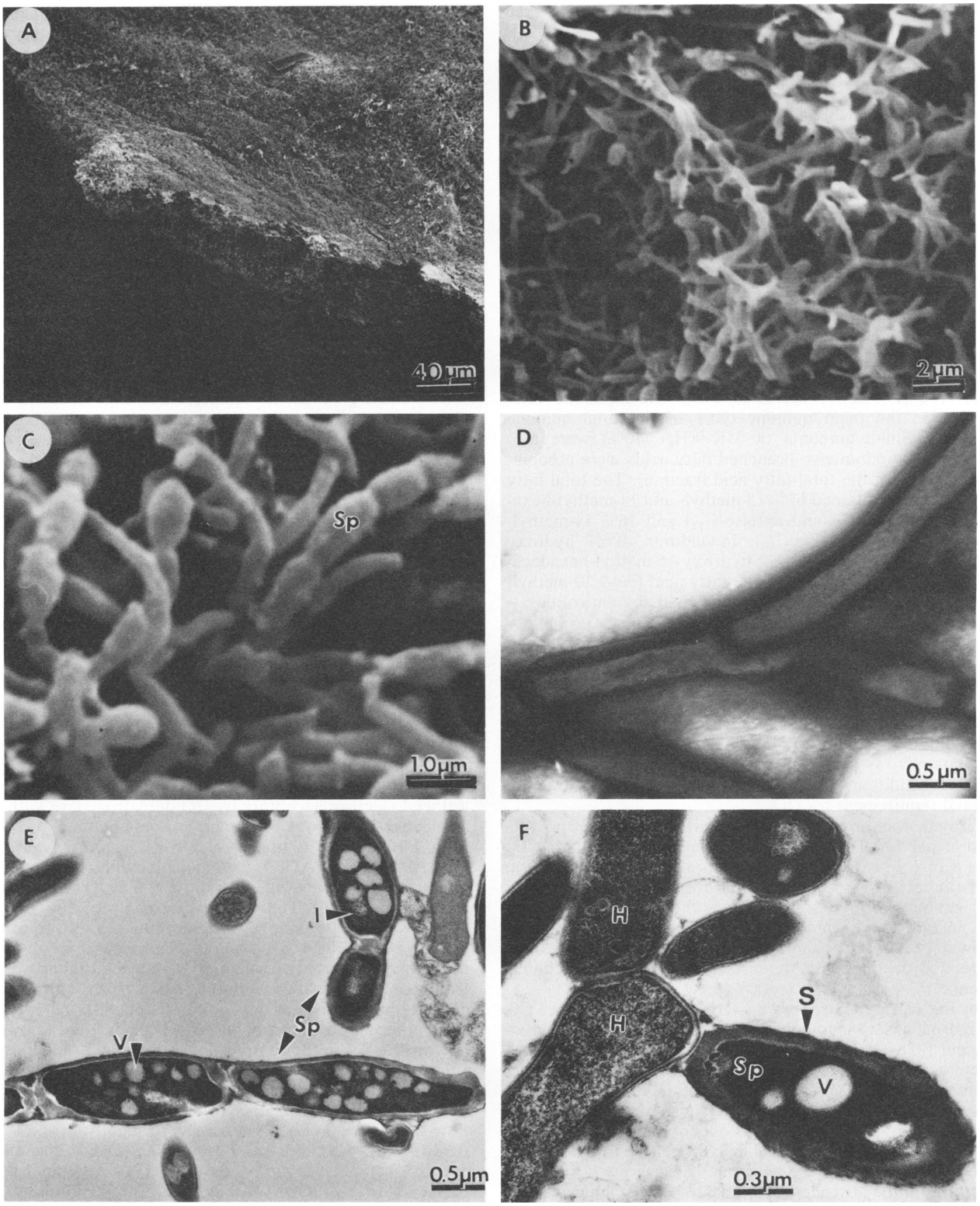

FIG. 1. Electron micrographs of strain UBT1. Scanning (A to C) and transmission (D to F) electron micrographs of vegetative mycelia and spore chains of CO-grown cells. (A) Pellicle at low-power magnification with a fracture edge demonstrating the thickness. Preparation: glutaraldehyde-OsO_4 fixation before critical-point drying. (B) Interwoven arrangement of hyphae within the pellicle shown in panel A. (C) Vegetative and sporogenous hyphae showing shape and arrangement of spores. (D) Branching and septation of negatively stained vegetative hyphae. (E) Thin-sectioned sporogenous hyphae (Sp) revealing vacuoles (V) and inclusion bodies (I). (F) Thin section showing spore (Sp) formation at the edge of vegetative hyphae (H), outer sheath (S), and vacuole (V).

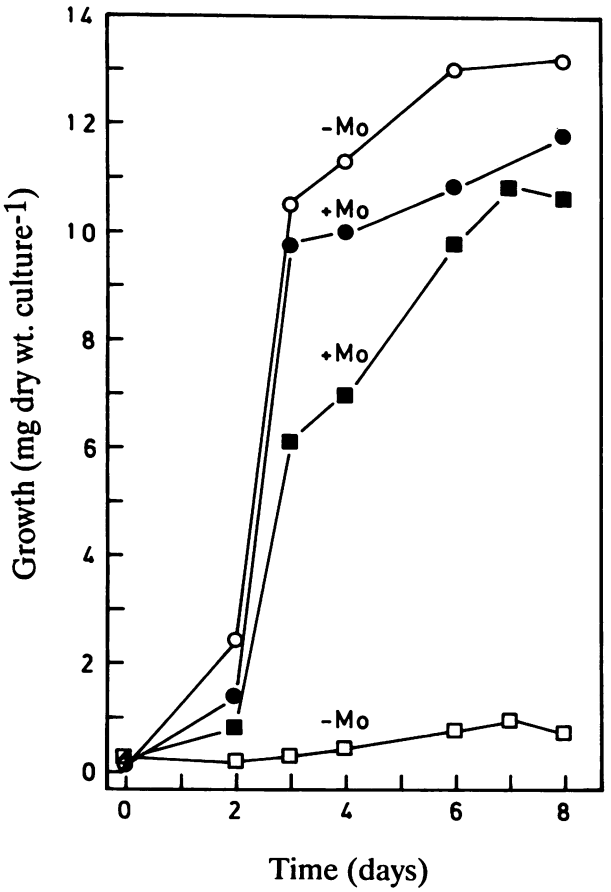

FIG. 2. Requirement of molybdenum for growth of strain UBT1. Growth was in 15 ml of mineral medium at 65°C under a gas atmosphere of 5% CO_2–45% CO–50% air (vol/vol/vol) (□, ■) or 10% CO_2–10% O_2–80% H_2 (vol/vol/vol) (○, ●). Molybdate was omitted from the trace element solution as indicated. For experimental details, refer to Materials and Methods.

unpolluted air (32, 38). In the range examined (7 to 270 μl of CO liter^{-1}), threshold values were not influenced by the initial CO concentration.

Temperature profile in burning charcoal piles. Strain UBT1 is characterized by the aerobic utilization of CO and H_2 in the temperature range of 40 to 68°C and at neutral pH. Since we were interested to know in which part of a burning charcoal pile these conditions would be established, the temperature profile was determined in the covering soil at different depths. The 40 and 68°C isotherms show that the temperature range for growth of strain UBT1 was found in the entire covering layer (Fig. 5). At a height of 0.5 m, it extends to a depth of 75 cm, and up to the highest point of the pile (3 m) it gradually decreases to 10 cm. The pH of the covering soil ranged from 7.5 to 8. The water content of the covering soil depends on the freshness of the woods used and the weather conditions. They ranged from 79 to 107% after a period of heavy rainfall and from 34 to 56% on a sunny day.

DISCUSSION

Taxonomic considerations. Strain UBT1 is to be assigned to the order *Actinomycetales* because it was gram positive, formed branching filaments with chains of nonmotile spores

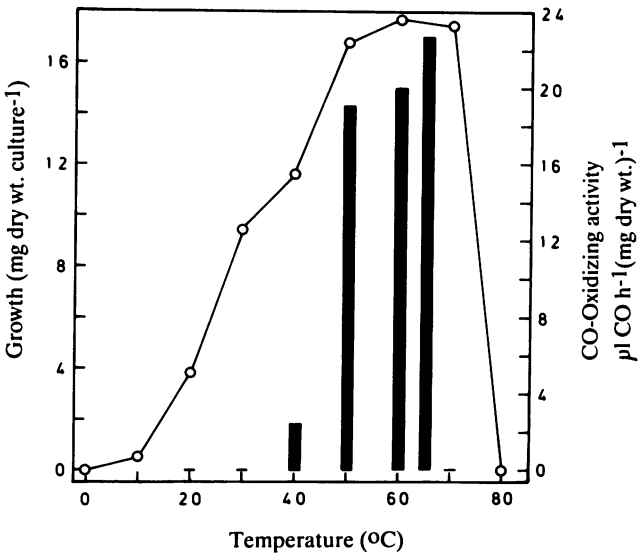

FIG. 3. Effect of temperature on growth and CO oxidation of strain UBT1. Measurements of CO oxidation were done on bacterial cultures (volume, 15 ml) grown for 6 days at 65°C. Symbols: bars, growth yields; ○, CO-oxidizing activity.

(conidia), and grew with a fungal appearance as a dry pellicle on the surface of solid and liquid media.

The actinomycetes are separated into groups on the basis of physiology (fermentative versus oxidative metabolism), morphology (type and stability of mycelium; types, number, and disposition of spores; formation of sclerotia, sporangia, or synnemata; formation of flagellate elements), physical qualities (heat resistance), and chemistry (cell wall and whole-cell composition, types of lipids, isoprenoid quinones) (15, 17). That strain UBT1 should be assigned to the family *Streptomycetaceae* is supported by the following considerations. Its metabolism was respiratory and strictly aerobic. The presence of LL-diaminopimelic acid in the peptidoglycan of UBT1 indicates a cell wall of type I. The fatty acid spectrum of strain UBT1 was characterized by the presence of saturated straight, iso- and anteiso, branched fatty acids typical of members of the family *Streptomycetaceae*. That UBT1 belongs to *Streptomycetaceae* is also supported by the results of sugar analysis in whole-cell hydrolysates.

Like all other sporoactinomycetes, *Streptomyces* show a

TABLE 1. Electron acceptor specificity of CO dehydrogenase in crude extracts of strain UBT1

Electron acceptor[a]	E'_0 (mV)	Activity (%)[b]	ε (λ), cm^{-1} mmol^{-1} (nm)
Methyl viologen	−440	7	9.7 (578)
Benzyl viologen	−359	83	8.74 (560)
MPMS + INT	−83	80	17.98 (496)
Methylene blue	+11	49	37.11 (615)
PMS	+80	43	25 (387)
DCPIP	+217	34	16.1 (600)
PMS + DCPIP		100	16.1 (600)

[a] Electron acceptor specificities were examined in extracts of strain UBT1; for experimental details, see Materials and Methods. MPMS, 1-Methoxyphenazine methosulfate; INT, iodonitrotetrazolium; PMS, phenazine methosulfate; DCPIP, 2,6-dichlorophenolindophenol.

[b] Percentages of the CO-oxidizing activity with PMS plus DCPIP, which was 246 μmol of CO oxidized min^{-1} mg of protein^{-1}.

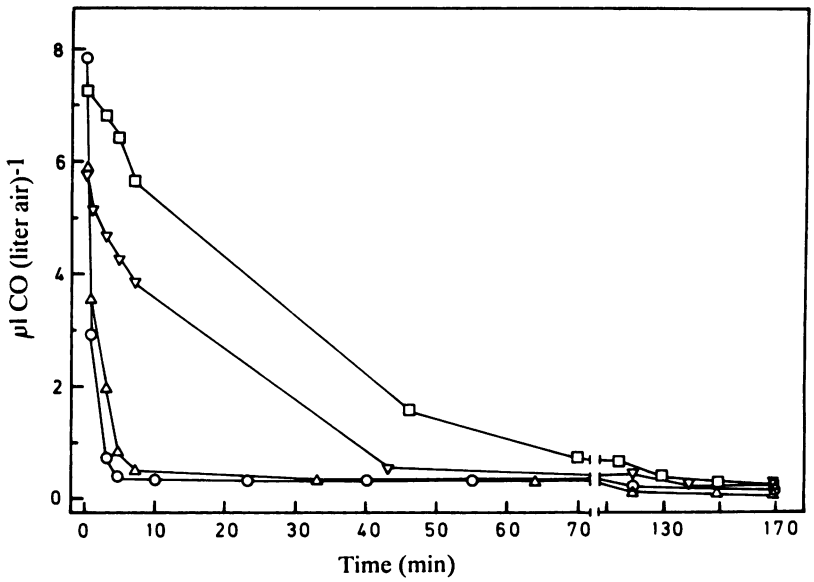

FIG. 4. Threshold for removal of CO from air by strain UBT1 (○); *P. carboxydovorans* (□); agricultural soil (cornfield) (▽); soil from a mixed forest of pine and spruce (△). Experiments with strain UBT1, *P. carboxydovorans*, and soils were performed at 65, 30, and 25°C, respectively.

high G+C content of DNA (69 to 78 mol%) (47). Their morphology is characterized by the formation of chains of nonmotile spores enclosed within a sheath (10). Fragmentation of the substrate mycelium is rare (10). Their major isoprenologs are MK-9(H_4), MK-9(H_6), and MK-9(H_8) (30), and they contain type II phospholipids (19). The morphology of strain UBT1 was typical of a streptomycete. In addition, the G+C content was high (70.6 mol%), falling into the range known for streptomycetes. The phospholipid pattern was of type PII, and MK-9(H_4) and MK-9(H_6) were the only quinones found, also typical of streptomycetes. Fatty acids typical of the other genera in this family (15) were absent, and isolate UBT1 is clearly separated from the other sporoactinomycetes on the basis of chemotaxonomic markers. Therefore, with respect to the properties discussed, isolate UBT1 should be assigned to the genus *Streptomyces*.

Chemolithoautotrophic streptomycetes are not known. *Streptomyces autotrophicus* was reported for growth with H_2 plus CO_2 (43). However, the bacterium was transferred to the genus *Nocardia* (11) and subsequently to the newly established genus *Amycolata* as *Amycolata autotrophica* (19). *Streptomyces* strain G26 can grow on CO but not on H_2 plus CO_2 (46). Its taxonomic characterization is marginal, and the authors recently tended not to use the genus name (46). Isolate UBT1 is a new thermophilic streptomycete characterized by an obligate chemolithoautotrophic metabolism. Because of these properties, we propose the species name *Streptomyces thermoautotrophicus*.

***Streptomyces thermoautotrophicus* is a new thermophilic, CO- and H_2-oxidizing chemolithoautotroph.** *S. thermoautotrophicus* UBT1 is a new carboxydotrophic bacterium and a novel streptomycete. It harbors two ways of chemolithoautotrophic life: the utilization of CO and that of H_2 plus CO_2. *S. thermoautotrophicus* is unique in its exclusive use of lithotrophic substrates. In contrast, all other carboxydotrophic bacteria have a facultative metabolism (23). As with the other carboxydotrophic bacteria, molybdenum is specifically involved in the metabolism of CO of *S. ther-*

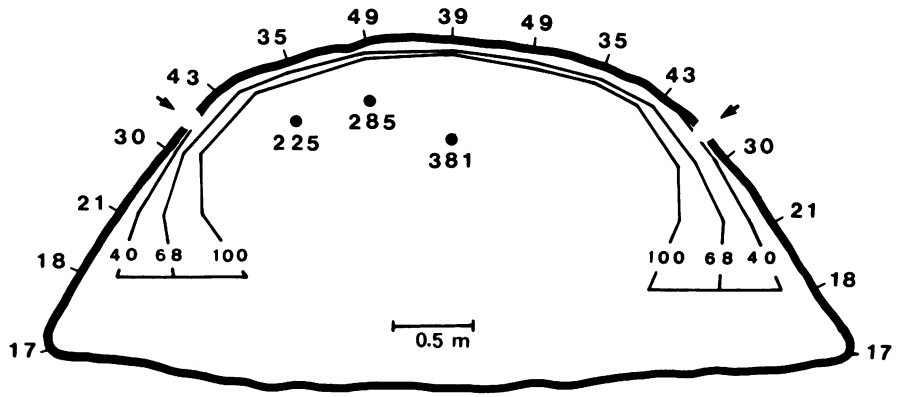

FIG. 5. Temperature profiles of a 7-day-old burning charcoal pile. If not otherwise indicated, values refer to temperatures in degrees Celsius. Isotherms at 40, 68, and 100°C have been calculated as detailed under Materials and Methods. Arrows point to holes for ventilation.

moautotrophicus. The reduction of low potential electron acceptors by *S. thermoautotrophicus* CO dehydrogenase (Table 1) is a specific feature of the isolate and refers to a new type of CO dehydrogenase. Viologen dye reduction has been observed before with *Streptomyces* strain G26 (2) and has so far been considered typical of the Ni-containing CO dehydrogenases occurring in anaerobes (7, 8, 24, 33, 34).

Habitat of *S. thermoautotrophicus*. Charcoal piles are composed of burning wood covered by a layer of soil mixed with small pieces of charcoal. Usually such layers are around 25 cm thick. Smoke is produced by the carbonization of wood. It consists of (percent, vol/vol) 49 CO_2, 34 CO, 13 CH_4, 2 ethylene, and 2 H_2 (48), and we analyzed similar CO concentrations. In addition, substantial amounts of acetic acid, methanol, acetone, methylacetate, acetaldehyde, and allylalcohol are also released (4). Since charcoal fumes and air continuously pass through the covering soil, it is obvious that a burning charcoal pile perfectly fulfills the substrate requirements of *S. thermoautotrophicus*. The moisture content of the covering soil is constantly kept in a range suitable for the proliferation of microorganisms by water vapor condensing from the charcoal fumes. Minerals are provided by the ashes. The temperature profile of *S. thermoautotrophicus* (Fig. 3) matches that of the covering soil (Fig. 5). Temperatures detrimental to *S. thermoautotrophicus* occurred only in the burning core of charcoal piles (Fig. 5). Charcoal piles are operated discontinuously. However, *S. thermoautotrophicus* can survive such periods of time since it is capable of co-oxidizing CO at ambient temperatures for maintenance. It also produces spores conferring resistance to desiccation for several months as well as to the temperature of boiling water. In addition, *S. thermoautotrophicus* oxidizes CO very rapidly and its affinity for CO is high, enabling the bacterium to consume CO even at concentrations approaching the threshold value of soil (Fig. 4).

Formal description of *Streptomyces thermoautotrophicus* sp. nov. *Streptomyces thermoautotrophicus* sp. nov. (ther.mo.auto.tro'phi.cus. G. adj. thermus hot; G.n. autos self; G. part trophikos nursing; M.L. masc. thermoautotrophicus, heat-loving self-nourishing, referring to the ability to grow at high temperature at the expense of CO or H_2 plus CO_2).

Streptomyces thermoautotrophicus forms relatively stable branching vegetative hyphae with a diameter of 0.2 to 0.5 μm. Formation of chains of two to eight oval spores produced in a sheath residing on substrate and scanty whitish aerial mycelium. No endospores, synnemata, sporangia, or sclerotia are found. Aerobic, non-acid fast, thermophilic, autotrophic, nonmotile, gram and catalase positive. Presence of LL-diaminopimelic acid and ribose (cell wall type I). Absence of mycolic acids. Predominant menaquinone is MK-9(H_4); small amounts of MK-9(H_6) also occur. Phospholipid pattern is composed of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides (PL-type 2). Iso and anteiso branched fatty acid pattern is synthesized, with iso-C_{17}, anteiso-C_{17}, and iso-C_{16} being the predominant fatty acids. Small amounts of cyclopropane fatty acids and traces of 2-hydroxy fatty acids are also present.

The G+C content of DNA is 70.6 ± 0.19 mol%.

A culture of *Streptomyces thermoautotrophicus* UBT1 has been deposited with the German Collection of Microorganisms (accession DSM 41605).

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