***Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil**

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Four moderately thermophilic, carboxydotrophic streptomycetes were the subject of a comparative taxonomic investigation designed to establish their taxonomic relationships. Almost complete sequences of the 16S rRNA genes of the test strains were determined following the isolation and direct sequencing of the amplified genes. The resultant nucleotide sequences were aligned with the sequences of previously studied streptomycetes, and phylogenetic trees generated by using the neighbour-joining, Fitch–Margoliash, maximum-likelihood and maximum-parsimony methods. It was evident from the phylogenetic analyses that strains AT50, AT51 and AT52 were most closely related to *Streptomyces thermodiastaticus* DSM 40573^T and strain AT37 to *Streptomyces glaucescens* DSM 40716 and *Streptomyces pseudogriseolus* NRRL 3985. Random DNA amplification profiles clearly distinguished strains AT50, AT51 and AT52 from *Streptomyces thermodiastaticus* and from strain AT37. The molecular systematic evidence, together with phenotypic data derived from this and previous studies, indicate that the test strains merit species status within the genus *Streptomyces*. The name *Streptomyces thermocarboxydovorans* sp. nov. is proposed for strains AT50, AT51 and AT52 (type strain) and *Streptomyces thermocarboxydus* sp. nov. for strain AT37.

Keywords: *Streptomyces thermocarboxydovorans* sp. nov., *Streptomyces thermocarboxydus* sp. nov., thermophile, carboxydotrophy, soil

INTRODUCTION

Much of the attention in streptomycete systematics has been focused on chemo-organotrophic strains that are able to use a wide range of organic compounds as sole sources of carbon for energy and growth (50). These organisms are predominantly mesophilic though their thermophilic counterparts, which grow between 28 °C and 55 °C, form a taxonomically diverse group (13). Thermophilic streptomycetes have been shown to form two distinct clades (19), one of which encompasses *Streptomyces megasporus* and related species and the other *Streptomyces thermodiastaticus* and allied taxa. The thermophilic streptomycete group also includes moderately thermophilic, carboxydotrophic streptomycetes isolated from composts, soils and

sewage (32). Carboxydotrophic streptomycetes oxidize carbon monoxide via a soluble and inducible carbon monoxide dehydrogenase; they also oxidize hydrogen and can grow on complex media (1,2). In contrast, *Streptomyces thermoautotrophicus* is unable to grow on complex media or assimilate heterotrophic substrates but can grow by using carbon monoxide alone or a mixture of carbon dioxide and hydrogen (10). This organism is also able to fix dinitrogen (11).

The most comprehensive taxonomic study of carboxydotrophic actinomycetes was carried out by O'Donnell *et al.* (32) who examined 54 moderately thermophilic, carboxydotrophic strains and marker mesophilic and thermophilic streptomycetes for 119 unit characters. The resultant data were analysed by using three resemblance coefficients and a mean linkage algorithm. The carboxydotrophic actinomycetes were assigned to one major (9 strains), 11 minor (2–4 strains) and 22

Abbreviation: RAPD, random amplified polymorphic DNA.

single-membered clusters in the analysis based on the simple matching coefficient. Most of these organisms were recovered in two aggregate cluster-groups which were sharply separated from two corresponding aggregate taxa which contained the marker mesophilic and thermophilic streptomycetes, respectively. All but two of the carboxydophilic strains had a profile of chemical properties consistent with their assignment to the genus *Streptomyces*. Quantitative fatty acid analyses of the carboxydophilic strains revealed two statistically significant groups which corresponded to the two aggregate cluster-groups circumscribed in the numerical phenetic survey. Members of the two fatty acid groups were also distinguished by their phospholipid composition.

Molecular systematic methods, notably 16S rDNA sequencing, are having an ever increasing impact on streptomycete systematics (19, 42, 43, 45, 48, 52). Similarly, molecular fingerprinting techniques, such as random amplified polymorphic DNA (RAPD) PCR, are being used to establish relationships within and between closely related actinomycete species (30, 47). In the present investigation, the almost complete sequences of the 16S rRNA genes (rDNAs) of four carboxydophilic strains, i.e. representatives of minor cluster 4 (AT37) and major cluster 5 (AT50, AT51, AT52) of O'Donnell *et al.* (32), were determined and compared by using several tree-making algorithms (14, 17, 26) to establish their position within the evolutionary radiation encompassed by streptomycetes. The organisms were also compared with appropriate marker strains using a RAPD-PCR procedure (47).

METHODS

Organisms and culture conditions. The carboxydophilic streptomycetes were compared with marker streptomycetes which included the type strains of validly described species which contain thermophilic organisms. Biomass of the four carboxydophilic organisms, namely AT37, AT50, AT51 and AT52, was prepared in shake flasks of tryptic soy broth at 45 °C for 5–7 d. At maximum growth, the broth cultures were checked for purity, harvested by centrifugation, washed twice in TE buffer (pH 8.0) (36) and kept at –20 °C. The strains were maintained on modified Bennett's agar plates (15) and as spore suspensions (20%, v/v, glycerol).

Morphology. Aerial spore mass colour was observed on inorganic salts-starch agar (ISP medium 4: Difco) (37) after incubating the four carboxydophilic strains at 45 °C for 7 d. The morphology of the spore chains and spore surface ornamentation were examined by scanning electron microscopy as described previously (32).

Phenotypic tests. The carboxydophilic strains were examined for a balanced set of phenotypic properties using procedures described in a previous study (32).

DNA extraction. Chromosomal DNA was isolated from the test strains using a procedure (5) slightly modified from that of Pitcher *et al.* (34).

Sequencing of 16S rDNA. PCR amplification of 16S rDNA was performed on the four carboxydophilic strains as

described by Kim *et al.* (19). The amplified fragments were directly sequenced by using a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and previously described oligonucleotide primers (5). Sequencing gel electrophoresis was carried out and nucleotide sequences were automatically obtained using an Applied Biosystems DNA sequencer (model 373A) and software provided by the manufacturer.

Phylogenetic analysis. The four 16S rDNA sequences were aligned manually with streptomycete nucleotide sequences (Table 1) derived from the Ribosomal Database Project (27) and EMBL/GenBank databases using the AL16S program (4). Two datasets were generated and analysed separately as some of the nucleotide sequences obtained from the databases were partial sequences (<1300 nt). The first dataset included nearly complete sequences and the second one partial sequences (Table 1). Dataset 1 contained information on 1463 nt positions for 36 streptomycetes and dataset 2 contained information on 1134 nt positions for 49 streptomycetes. Evolutionary trees for the two datasets were inferred by using four algorithms, namely the neighbour-joining (35), least squares (9), maximum-parsimony (20) and maximum-likelihood (8) methods.

Evolutionary distance matrices for the neighbour-joining and least-squares methods were generated as described by Jukes & Cantor (16). The PHYLIP package (8) was used for making trees with the four algorithms. The resultant unrooted tree topologies were evaluated by carrying out bootstrap analyses of the neighbour-joining method data based on 1000 re-samplings using the SEQBOOT and CONSENSE programs in the PHYLIP package (8). The root position of the unrooted tree based on the neighbour-joining method was estimated by using four outgroup organisms (*Arthrobacter globiformis* DSM 20214^T, accession no. M23411; *Bacillus subtilis*, K00637; *Escherichia coli*, J01695; *Nocardia asteroides* ATCC 19247^T, Z36934) as described by Swofford & Olsen (44).

DNA base composition. The base composition of the genomic DNA isolated from the four carboxydophilic strains was determined using the reverse phase HPLC method described by Tamaoka (46) and the HPLC conditions outlined by Gerke *et al.* (12). The analyses were performed on a Supelcosil LC-18S column (Supelco) with a 5 µm particle size and column dimensions of 15 cm × 4.6 mm i.d. Molar G + C ratios were calculated using the methods described by Mesbah *et al.* (31).

Random primer DNA amplification. The procedures described by Trujillo & Goodfellow (47) were followed with modifications to the reaction mixture and PCR conditions; DMSO was added to the PCR mixture (10% final concn) and the annealing temperature used for the PCR amplification was 37 °C. The amplified fragments were separated by agarose gel electrophoresis (2%), together with a *Pst*I digest of phage λ DNA (Sigma) in 0.5 × Tris borate/EDTA buffer (36). DNA bands were visualized by staining with ethidium bromide solution and the gels photographed under UV light.

RESULTS AND DISCUSSION

Initial studies showed that the four carboxydophilic strains formed extensively branched substrate mycelia and aerial hyphae which carried long chains of spores,

Table 1. Strains used and their nucleotide sequence accession numbers

Species or subspecies	Strain*	Source†	Accession no.	Reference
Organisms whose complete sequences (>1463 nt) were used				
<i>S. acidiscabies</i>		ATCC 49003 ^T	D63865	45
<i>S. ambofaciens</i>	ISP 5053 ^T	ATCC 23877 ^T	M27245	33
<i>S. bikiniensis</i>	ISP 5581 ^T	DSM 40581 ^T	X79851	29
<i>S. bluensis</i>	ISP 5564 ^T		X79324	29
<i>S. bottropensis</i>	ISP 5262 ^T	ATCC 25435 ^T	D63868	45
<i>S. caelestis</i>	ISP 5084 ^T	NRRL 2418 ^T	X80824	29
<i>S. diastatochromogenes</i>	ISP 5449 ^T	ATCC 12309 ^T	D63867	45
' <i>S. espinosus</i> '		NRRL 5729	X80826	29
<i>S. eurythermus</i>	ISP 5014 ^T	ATCC 14975 ^T	D63870	45
<i>S. galbus</i>	ISP 5089 ^T	DSM 40089 ^T	X79852	29
<i>S. glaucescens</i>		DSM 40716	X79322	29
<i>S. griseocarneus</i>	ISP 5004 ^T	DSM 40004 ^T	X99943	29
<i>S. griseus</i> subsp. <i>griseus</i>	ISP 5236 ^T	KCTC 9080 ^T	X61478	18
' <i>S. hygroscopicus</i> subsp. <i>limoneus</i> '		ATCC 21431	X79853	29
<i>S. lincolnensis</i>	ISP 5355 ^T	NRRL 2936 ^T	X79854	29
<i>S. macrosporus</i>		DSM 41449 ^T	Z68099	19
<i>S. mashuensis</i>	ISP 5221 ^T	DSM 40221 ^T	X79323	29
<i>S. megasporus</i>		DSM 41476 ^T	Z68100	19
<i>S. neyagawaensis</i>	ISP 5588 ^T	ATCC 27449 ^T	D63869	45
<i>S. pseudogriseolus</i>		NRRL 3985	X80827	29
<i>S. sampsonii</i>	ISP 5394 ^T	ATCC 25495 ^T	D63871	45
<i>S. scabiei</i>		ATCC 49173 ^T	D63862	45
<i>S. subrutilus</i>	ISP 5445 ^T	DSM 40445 ^T	X80825	29
<i>S. tendae</i>	ISP 5101 ^T	ATCC 19812 ^T	D63873	45
<i>S. thermodiastaticus</i>	ISP 5573 ^T	DSM 40573 ^T	Z68101	19
<i>S. thermolineatus</i>		DSM 41451 ^T	Z68097	19
<i>S. thermotritificans</i>	ISP 5579 ^T	DSM 40579 ^T	Z68098	19
<i>S. thermoviolaceus</i> subsp. <i>apingens</i>		DSM 41392 ^T	Z68095	19
<i>S. thermoviolaceus</i> subsp. <i>thermoviolaceus</i>	ISP 5443 ^T	DSM 40443 ^T	Z68096	19
<i>S. thermovulgaris</i>	ISP 5444 ^T	DSM 40444 ^T	Z68094	19
<i>S. violaceoruber</i> (coelicolor)		A3(2)	X60514	48
<i>S. virginiae</i>	ISP 5094 ^T	IFO 3729 ^T	D85119	29
<i>Streptomyces</i> sp.	AT50	DSM 44294	U94487	This study
<i>Streptomyces</i> sp.	AT51	DSM 44295	U94488	This study
<i>Streptomyces</i> sp.	AT52 ^T	DSM 44296 ^T	U94489	This study
<i>Streptomyces</i> sp.	AT37 ^T	DSM 44293 ^T	U94490	This study
Organisms whose complete sequences (<1242 nt) were used				
<i>S. abikoensis</i>		DSM 40831 ^T	X53168	42
<i>S. albus</i> subsp. <i>albus</i>	ISP 5313 ^T	DSM 40313 ^T	X53163	42
<i>S. brasiliensis</i>		DSM 43159 ^T	X53162	42
' <i>S. cinnamoneus</i> subsp. <i>azacoluta</i> '		DPDU 0074 ^T	X53165	42
<i>S. cinnamoneus</i> subsp. <i>cinnamoneus</i>	ISP 5005 ^T	DPDU 0093 ^T	X53171	42
<i>S. diastaticus</i> subsp. <i>diastaticus</i>	ISP 5496 ^T	DSM 40496 ^T	X53161	42
<i>S. mobaraensis</i>	ISP 5587	DSM 40587	X53167	42
<i>S. lavendulae</i> subsp. <i>lavendulae</i>	ISP 5069 ^T	DSM 2014 ^T	X53173	42
' <i>S. luteoreticuli</i> '	ISP 5509	ATCC 27446	X53172	42
<i>S. olivoreticuli</i> subsp. <i>cellulophilus</i>		DPDU 0278 ^T	X53166	42
<i>S. purpureus</i>		DSM 43360	X53170	42
<i>S. roseovorticillatus</i>		DPDU 0819	X53164	42
<i>S. salmonis</i>		DPDU 00098 ^T	X53169	42

* ISP, International *Streptomyces* Project.

† ATCC, American Type Culture Collection, Rockville, MD, USA; DPDU, Instituto di difesa delle Plante, Università degli Studi di Udine, Udine, Italy; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; KCTC, Korean Collection of Type Cultures, Korean Research Institute of Bioscience and Biotechnology, Taejeon, Republic of Korea; NRRL, Northern Regional Research Laboratory, Agricultural Research Service, US Department of Agriculture, Peoria, IL, USA.

Fig. 1. Scanning electron micrographs showing spore chain morphology. (a) *Streptomyces* strain AT52^T. (b) *Streptomyces* strain AT37^T. Bars, 5 µm.

contained LL-diaminopimelic acid and were rich in iso- and anteiso-fatty acids and hexahydrogenated menaquinones with nine isoprene units (32). The results of the present morphological studies confirm and extend those carried out in the previous investigation. Strains AT50, AT51 and AT52 formed smooth-surfaced spores in long straight to flexuous spore chains whereas the warty-surfaced spores of strain AT37 were borne in open-spiral or looped chains (Fig. 1). The molar G + C ratio of the DNA of strain AT37 was $70.9 \pm 0.4\%$; the corresponding G + C ratios for strains AT50, AT51 and AT52 were $74.7 \pm 1.0\%$, $74.8 \pm 0.6\%$ and $74.7 \pm 0.4\%$, respectively. All of the properties outlined above are consistent with the classification of the four carboxydutrophic strains in the genus *Streptomyces* (28, 32, 50).

The almost complete 16S rDNA sequences [1463 nt between *E. coli* positions 28 and 1524 (3)] of the four carboxydutrophic streptomycetes were compared with the corresponding nucleotide sequences of 24 mesophilic and 8 thermophilic streptomycetes by using four tree-making algorithms (i.e. the neighbour-joining, least squares, maximum-parsimony and maximum-likelihood methods). It is evident from the phylogenetic tree that strains AT50, AT51 and AT52 belong

to the *Streptomyces thermodiastaticus* clade recognized by Kim *et al.* (19). Strains AT50 and AT51 had identical 16S rDNA sequences, the corresponding sequence of strain AT52 differs from this common sequence by a single nucleotide.

Strains AT50, AT51 and AT52 share high percentage 16S rDNA nucleotide similarity values with other members of the clade, namely *Streptomyces thermodiastaticus* (99.0), *Streptomyces thermonitrificans* (97.6–97.7), *Streptomyces thermovulgaris* (97.7), *Streptomyces thermoviolaceus* subsp. *apingens* (98.5–98.6) and *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* (98.7–98.8). Nucleotide similarity values in this range have been recorded for several validly described thermophilic *Streptomyces* species, for instance between *Streptomyces macrosporus* and *Streptomyces megasporus* (98.0%) and between *Streptomyces thermoviolaceus* and *Streptomyces thermovulgaris* (98.5%), which are clearly distinct on the basis of phenotypic properties (13, 19). Similarly, the type strains of *Streptomyces* (*Kitasatosporia*) *griseosporus*, *Streptomyces* (*Kitasatosporia*) *phosalacineus* and *Streptomyces* (*Kitasatosporia*) *setae* share high 16S rDNA sequence similarity values with one another (98.8–99.0%; 19) but can be separated on the basis of several genotypic and phenotypic properties, notably on DNA:DNA pairing data (21).

The remaining carboxydutrophic organism, strain AT37, was most clearly related to *Streptomyces glaucescens* DSM 40716 (Fig. 2). However, the nucleotide sequence of strain AT37 differed from the corresponding sequences of its nearest neighbours, *Streptomyces glaucescens* and *Streptomyces pseudogriseolus*, by 23 nt. A similar number of nucleotide differences have been recorded in the 16S rDNA sequences of the type strains of *Streptomyces thermoviolaceus* and *Streptomyces thermovulgaris* (19).

The positions of the test and marker strains in the phylogenetic tree were not markedly affected by either the tree-making algorithms or by the outgroup strains used. However, the close relationship between strains AT50, AT51, AT52 and *Streptomyces thermodiastaticus* was not supported when the data were analysed using the maximum-likelihood (8) and maximum-parsimony (20) methods. Comparable results were obtained in the analyses based on the partial 16S rDNA sequences (Fig. 3); once again the close relationship between strains AT50, AT51, AT52 and *Streptomyces thermodiastaticus* was not supported by the results of the maximum-likelihood (8) and maximum-parsimony methods (20). Similarly, the relationships between these organisms and *Streptomyces bluenensis* were not supported by the results of the Fitch–Margoliash (9), maximum-likelihood (8), and maximum-parsimony (20) methods. In contrast, the close relationship between strain AT37 and *Streptomyces glaucescens* DSM 40716 was supported in all of the phylogenetic analyses, though a relatively low bootstrap value (63%) was recorded for the neighbour-

Fig. 2. Neighbour-joining tree (34) based on nearly complete 16S rDNA sequences of 36 streptomycetes. f, l and p indicate branches that were also found when we used the Fitch-Margoliash (9), maximum-likelihood (8) and maximum-parsimony (20) methods, respectively; the asterisks indicate branches recovered with all four methods. The numbers at the nodes indicate the level of bootstrap support based on a neighbour-joining analysis of 1000 re-sampled datasets; only values that were >50% are given. The arrow indicates the estimated position of the four root organisms. The test strains were type strains unless otherwise indicated. The scale bar indicates 0.01 substitutions per nucleotide position.

joining tree based on the partial nucleotide sequence dataset.

The present 16S rDNA sequence data confirm and extend the results of an earlier study (19) which showed that thermophilic streptomycetes can be assigned to several clades and hence cannot be classified as a subgenus within the genus *Streptomyces* as suggested by Craveri & Pagani (6). It is also interesting that numerical phenotypic data support the assignment of mesophilic, thermophilic and thermophilic carboxydophilic streptomycetes to distinct aggregate taxa (32).

The number of DNA amplification products obtained for the test and marker strains varied from 5 to 12 with the lengths of the products lying between 0.2 and

2.2 kbp (Fig. 4). Several strongly amplified fragments were detected in all of the preparations. Amplified products were not observed in the control assays in which either the cells or primers were omitted. Strains AT50, AT51 and AT52 gave almost identical RAPD patterns which were sharply distinguished from those derived from strain AT37 and the marker organisms. Similarly, *Streptomyces thermonitrificans* DSM 40579^T and *Streptomyces thermovulgaris* DSM 40444^T had similar profiles, albeit ones that were markedly different from those of *Streptomyces thermodiastaticus* DSM 40573^T and *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T. The RAPD profiles of the latter two organisms were also readily distinguished.

3985 and from members of the other thermophilic taxa. It is also interesting in this context that *Streptomyces* species defined on the basis of a combination of genotypic and phenotypic features are homogeneous with respect to certain traditionally weighted morphological and pigmentation properties (24, 25).

The species concept remains a controversial subject in streptomycete systematics, partly due to the extreme taxonomic variation encompassed by the genus and to the absence of recognized minimal standards for the

Fig. 4. DNA amplification fingerprints of the test strains analysed using M13f as the primer. Lanes: M, *Pst*I-digested phage λ DNA; 1–8, RAPD products of AT37, AT50, AT51, AT52, *S. thermodiastaticus* DSM 40573^T, *S. thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T, *S. thermovulgaris* DSM 40444^T and *S. thermotrophicus* DSM 40579^T, respectively.

delineation of new species (28). However, it is apparent that *Streptomyces* species need to be circumscribed using a combination of complementary genotypic and phenotypic data (22–25). The present molecular sys-

tematic data when taken together with extensive phenotypic information (32) show that strains AT50, AT51 and AT52 form a homogeneous taxon which can be distinguished from corresponding validly described *Streptomyces* species which encompass mesophilic or moderately thermophilic streptomycetes. Similarly, strain AT37 can be distinguished from representatives of mesophilic and moderately thermophilic streptomycetes, including *Streptomyces glaucescens* DSM 40716 and *Streptomyces pseudogriseolus* NRRL 3985. The four carboxydotrophic strains can also be readily distinguished from *Streptomyces thermoaerophilus* which is an obligate chemolithotroph (10). Accordingly, the new species *Streptomyces thermocarboxydovorans* and *Streptomyces thermocarboxydus* are proposed for strains AT50, AT51 and AT52, and AT37, respectively.

Description of *Streptomyces thermocarboxydovorans* sp. nov.

Streptomyces thermocarboxydovorans (ther.mo.car.-box.y.do'vor.ans. Gr. n. *therme* heat; L. n. *carbo* charcoal, carbon; Gr. adj. *oxys* sharp, acid; L. v. *voro* to consume; M.L. part. adj. *thermocarboxydovorans* thermophilic, carbon monoxide consuming).

The description is based on data taken from this and earlier studies (7, 32). Aerobic, Gram-positive, moderately thermophilic, facultatively chemolithotrophic actinomycete with extensively branched substrate and aerial mycelia. *Rectiflexibles* chains of smooth-surfaced spores are borne on aerial hyphae. The aerial spore mass is grey; distinctive substrate mycelium colours are not produced. Diffusible pigments are not formed. Melanin pigments are not produced on pep-

Table 2. Phenotypic properties separating test strains from related *Streptomyces* species

Abbreviations: RF, *rectiflexibles*; SP, *spirales*; RA, *retinaculiaperti*. +, Positive; –, negative.

Characteristic	<i>Streptomyces</i> strains AT50–AT52	<i>S. thermodiastaticus</i> *	<i>S. thermoviolaceus</i> subsp. <i>thermoviolaceus</i> *	<i>S. thermoviolaceus</i> subsp. <i>apingens</i> *	<i>S. thermovulgaris</i> *	<i>S. thermotrophicus</i> *	<i>Streptomyces</i> strain AT27	<i>S. glaucescens</i> *	<i>S. pseudogriseolus</i> *
Aerial spore mass colour	Grey	Brownish grey	Grey-yellowish pink	Violet-grey	Greyish brown	Brownish grey	Grey	Greyish green	Greyish buff
Spore chains	RF	SP	SP	RA	SP	RF/SP	RA	SP	SP/RA
Spore surface	Smooth	Warty or spiny	Warty	Warty	Smooth	Smooth	Warty	Hairy	Smooth
Melanin production	–	–	–	–	–	–	–	+	–
Nitrate reduction	+	–	–	–	+	–	+	–	–
Degradation of:									
Elastin	+	+	+	+	–	+	+	–	+
Starch	+	+	+	+	+	+	+	–	+
L-Tyrosine	+	+	+	+	+	+	–	+	+
Growth in the presence of:									
Phenol (0.1%, w/v)	–	+	+	+	+	+	–	–	+
Phenyl ethanol (0.3%, w/v)	+	–	–	–	+	–	+	–	–
Sodium azide (0.02%, w/v)	–	–	+	+	+	+	–	–	+
Growth on sole carbon sources:									
<i>meso</i> -Inositol	–	+	–	+	+	+	+	+	+
Mannitol	–	+	+	+	+	+	+	+	+
Raffinose	–	+	–	–	+	–	–	–	–
Sucrose	–	–	–	–	+	+	–	–	–
Sodium pyruvate	+	+	+	+	+	+	–	–	+

* Data taken from previous studies (13, 38–41, 49).

tone-iron agar. The organism oxidizes hydrogen and can use carbon monoxide as a sole source of carbon for energy and growth. Positive activity is seen for chemotrypsin, cystine arylamidase, α -galactosidase and lipase but not for α -glucosidase (API-ZYM). Similarly, *N*-acetyl- β -D-glucosamidase is produced but not β -D-cellobiosidase or β -D-xylosidase. Arbutin, DNA, elastin, guanine, hypoxanthine, RNA, starch, L-tyrosine and xanthine are degraded. Nitrate is reduced and hydrogen sulphide produced. Neither allantoin nor urea are hydrolysed. D-Fructose, D-lactose, sodium fumarate, sodium pyruvate, but not adonitol or xylitol, are used as sole carbon sources and L-arginine, D-aminobutyric acid, L-cysteine, L-histidine, L-methionine, potassium nitrate, L-serine, L-threonine, L-valine, but not L-hydroxyproline, as sole nitrogen sources. Growth occurs between 20 °C and 55 °C and in the presence of adenine (0.5%, w/v), crystal violet (0.001%, w/v), phenyl ethanol (0.3%, v/v), potassium tellurite (0.001%, w/v), sodium azide (0.01%, w/v), chloramphenicol (2 µg ml⁻¹), chlor-tetracycline (4 µg ml⁻¹), gentamicin sulphate (1 µg ml⁻¹), novobiocin (0.5 µg ml⁻¹), oleandomycin phosphate (1 µg ml⁻¹), penicillin G (16 µg ml⁻¹), polymyxin B (32 µg ml⁻¹) and tobramycin sulphate (1 µg ml⁻¹). In contrast, growth was inhibited in the presence of phenol (1%, w/v), sodium azide (0.02%, w/v), rifampicin (0.5 µg ml⁻¹) and streptomycin sulphate (2 µg ml⁻¹). The DNA base composition is 74.7 ± 0.05 mol% G + C. Isolated from soil. The type strain is AT52 (= DSM 44296).

Description of *Streptomyces thermocarboxydus* sp. nov.

Streptomyces thermocarboxydus (*ther.mo.car.box'y.dus*). Gr. n. *therme* heat; L. n. *carbo* charcoal, carbon; Gr. adj. *oxys* sharp, acid; L. n. *carboxydus* pertaining to carbon monoxide).

The description is based on data taken from this and earlier studies (7, 32). Aerobic, Gram-positive, moderately thermophilic, facultatively chemolithotrophic actinomycete with extensively branched substrate and aerial mycelia. *Retinaculiaperti* chains of warty-surfaced spores are borne on aerial hyphae. The aerial spore mass is grey; distinctive substrate mycelium colours are not produced. Diffusile pigments are not formed. Melanin pigments are not produced on peptone-iron agar. The organism oxidizes hydrogen and can use carbon monoxide as a sole source of carbon for energy and growth. Positive activity is seen for chemotrypsin, cystine arylamidase, α -glucosidase, lipase and trypsin but not for α -galactosidase (API-ZYM). *N*-Acetyl- β -D-galactosamidase, *N*-acetyl- β -D-glucosamidase and α -L-arabinosidase are produced but not β -D-cellobiosidase, β -D-fucopyranosidase, α -D-mannosidase or β -D-xylosidase. Aesculin, arbutin, DNA, elastin, guanine, hypoxanthine, starch, testosterone and xanthine are degraded. Nitrate is reduced and hydrogen sulphide produced. Neither allantoin nor urea are hydrolysed. D-Fructose, D-glucose, *meso*-

inositol, D-lactose, mannitol, trehalose and sodium propionate, but not adonitol, raffinose, sucrose, xylitol, sodium acetate, sodium fumarate or sodium pyruvate, are used as sole carbon sources and L-arginine, D-aminobutyric acid, L-cysteine, L-histidine, potassium nitrate, L-serine, L-threonine and L-valine, but not L-hydroxyproline or L-methionine, as sole nitrogen sources. Growth occurs between 20 °C and 55 °C and in the presence of adenine (0.5%, w/v), crystal violet (0.001%, w/v), phenyl ethanol (0.3%, v/v), potassium tellurite (0.01%, w/v), sodium azide (0.01%, w/v), chloramphenicol (32 µg ml⁻¹), chlor-tetracycline (4 µg ml⁻¹), gentamicin sulphate (8 µg ml⁻¹), neomycin sulphate (2 µg ml⁻¹), novobiocin (2 µg ml⁻¹), oleandomycin phosphate (32 µg ml⁻¹), penicillin G (16 µg ml⁻¹), polymyxin B (32 µg ml⁻¹), rifampicin (4 µg ml⁻¹), streptomycin sulphate (2 µg ml⁻¹), tobramycin sulphate (8 µg ml⁻¹) and vancomycin (4 µg ml⁻¹). The DNA base composition is 70.9 ± 0.4 mol% G + C. Isolated from soil. The type strain is AT37 (= DSM 44293).

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