Collimonas fungivorans gen. nov., sp. nov., a chitinolytic soil bacterium with the ability to grow on living fungal hyphae

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A polyphasic approach was used to describe the phylogenetic position of 22 chitinolytic bacterial isolates that were able to grow at the expense of intact, living hyphae of several soil fungi. These isolates, which were found in slightly acidic dune soils in the Netherlands, were strictly aerobic, Gram-negative rods. Cells grown in liquid cultures were flagellated and possessed pili. A wide range of sugars, alcohols, organic acids and amino acids could be metabolized, whereas several di- and trisaccharides could not be used as substrates. The major cellular fatty acids were $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$. DNA G+C contents were 57–62 mol%. Analysis of nearly full-length 16S rDNA sequences showed that the isolates were related closely to each other (>98·6 % sequence similarity) and could be assigned to the *β-Proteobacteria*, family '*Oxalobacteraceae*', order '*Burkholderiales*'. The most closely related species belonged to the genera *Herbaspirillum* and *Janthinobacterium*, exhibiting 95·9–96·7 % (*Herbaspirillum* species) and 94·3–95·6 % (*Janthinobacterium* species) 16S rDNA sequence similarity to the isolates. Several physiological and biochemical properties indicated that the isolates could be distinguished clearly from both of these genera. Therefore, it is proposed that the isolates described in this study are representatives of a novel genus, *Collimonas* gen. nov. Genomic fingerprinting (BOX-PCR), detailed analysis of 16S rDNA patterns and physiological characterization (Biolog) of the isolates revealed the existence of four subclusters. The name *Collimonas fungivorans* gen. nov., sp. nov. has been given to one subcluster (four isolates) that appears to be in the centre of the novel genus; isolates in the other subclusters have been tentatively named *Collimonas* sp. The type strain of *Collimonas fungivorans* gen. nov., sp. nov. is Ter6^T (=NCCB 100033^T=LMG 21973^T).

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Abbreviation: FISH, fluorescent *in situ* hybridization.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains Ter6^T, Ter331, Ter118/Ter113, Ter227/Ter72, Ter14, Ter90, Ter91, Ter94, Ter165, Ter166, Ter266, Ter282, Ter291, Ter299, Ter300, Ter10, Ter146, Ter228, Ter252 and Ter330 are AJ310394, AJ310395, AJ496444, AJ496445 and AY281135–AY281150, respectively.

Physiological characteristics of the Ter isolates, respiratory responses to four Biolog substrates and chitosan, an unrooted phylogenetic tree based on 16S rDNA sequences and a UPGMA cluster analysis of BOX-PCR-generated genomic fingerprints are available as supplementary material in IJSEM Online.

INTRODUCTION

The ability of soil bacteria to produce chitinases is commonly indicated by the formation of clear zones (haloes) on agar plates that contain colloidal chitin (Gooday, 1990). This halo-forming ability is also the basis for enumeration of culturable chitinolytic bacteria. By using this method, it was observed that the dominant chitinolytic bacteria in slightly acidic dune soils in the Netherlands formed haloes with translucent colonies of little biomass on minimal chitin agar (De Boer *et al.*, 1998).

Twenty-two of these chitinolytic isolates were tested for their ability to degrade particulate chitin in sand; they were

poor degraders compared to filamentous fungi, actinomycetes and gliding bacteria (De Boer *et al.*, 1999). Further study revealed that these isolates could use their chitinase activity to facilitate mycoparasitic growth, i.e. growth at the expense of living fungal hyphae (De Boer *et al.*, 2001). Based on whole-cell fatty acid analysis, these isolates were identified provisionally as belonging to the genus *Pseudomonas* of the *γ-Proteobacteria* (De Boer *et al.*, 1998). However, preliminary 16S rDNA sequence analysis subsequently revealed that they were affiliated to the *β-Proteobacteria* (De Boer *et al.*, 2001). Here, results of polyphasic taxonomic characterization of these isolates are presented; these results support their classification in a novel genus, *Collimonas* gen. nov.

METHODS

Bacterial isolates. Isolates ($n=22$) were taken from chitin agar plates of samples of slightly acidic dune soils located on the Wadden Island Terschelling, as described previously (De Boer *et al.*, 1998). The isolates, referred to as Ter isolates, were stored at −80 °C and maintained on chitin/yeast agar and 0·1× tryptone soy broth (TSB; Oxoid) agar for routine culturing. Both media contained (l^{-1}): 1 g KH_2PO_4, 5 g NaCl and 20 g agar. In addition, 0·1× TSB agar contained 3 g TSB l^{-1} and chitin/yeast agar contained 2·0 g colloidal chitin l^{-1} and 0·05 g yeast extract (Difco) l^{-1}. Media were adjusted to pH 6·5 with 1 M NaOH before autoclaving. Colloidal chitin was prepared as described by Hsu & Lockwood (1975) from crab-shell chitin (Sigma).

As it appeared that the 16S rDNA sequences of the isolates were related most closely to those of members of the genus *Herbaspirillum*, three *Herbaspirillum* type strains (*Herbaspirillum seropedicae* DSM 6445^T, *Herbaspirillum rubrisubalbicans* ATCC 19308^T and *Herbaspirillum frisingense* DSM 13128^T) were included in some parts of the study (metabolic profiling and cellular fatty acid composition) for comparison.

Phenotypic characterization. Gram-reaction was tested indirectly by determining lysis in KOH. Motility was tested by measuring the spread of cells inoculated into 0·1× TSB that contained 0·2 % agar. Colony morphology of cells grown at 20 °C on 0·1× TSB agar (3 days) was assessed. Cellular morphology of five isolates (Ter6^T, Ter146, Ter227, Ter252 and Ter331), representing different colony forms, was studied by using a Philips CM10 transmission electron microscope.

Whole-cell fatty acid composition was determined after growth for 48 h at 28 °C on full-strength TSB agar by using the Microbial Identification system (Microbial ID). Extraction and preparation of fatty acid methyl esters, chromatography, reference library generation and principal component and cluster analyses were performed as described by Janse (1991).

Ability to oxidize various carbon sources was tested by using Biolog GN plates. Presence of oxidase was determined by using a test card (bioMérieux). Catalase activity was checked by holding a loop of bacterial cells in a drop of 3 % hydrogen peroxide solution on a microscopic slide. Direct appearance of gas bubbles indicated the presence of catalase. Chitosanase, β-glucanase, cellulase and protease activities were assayed visually, i.e. by production of clear zones after 2–3 weeks at 20 °C on plates that contained 2 % agar and (l^{-1}): 1 g KH_2PO_4, 5 g NaCl and 0·05 g yeast extract (pH 6·5). Colloidal chitosan was obtained by preparing water-soluble chitosan from crab chitosan (Sigma) and mixing it to a final concentration of 0·2 % with a hot agar/salt solution. Lichenan (Sigma) was used as the substrate (0·1 % final concentration) for indicating glucanase activity (Walsh *et al.*, 1995). Glucanolytic haloes were visualized after staining with Congo red. Cellulose agar contained 2 % cellulose powder (Sigma). Protease activity was demonstrated by using milk agar, which contained 3 % skimmed milk.

Ability to hydrolyse colloidal chitin was tested in both minimal agar and agar mixed with TSB (3 g l^{-1}). In addition, ability of the isolates to hydrolyse colloidal chitin in liquid minimal medium (pH 6·5) was tested by measuring the release of NH_4^+.

Effects of pH and temperature on growth were studied in four isolates: one representative of each of the three colony types that were observed (Ter146, Ter227 and Ter331; see Results and Discussion) and the proposed type strain (Ter6^T). Optimum pH for growth in minimal liquid medium was 5·0–8·0, using the ethanesulfonic acid buffers (10 mM; Sigma) MES (pH 5·0, 5·5, 6·0 and 6·5) or HEPES (pH 7·0, 7·5 and 8·0). For determination of temperature optimum and tolerances, minimal medium that contained 10 mM MES (pH 6·5) was used. The growth substrate in both experiments was *N*-acetylglucosamine (2 mM). Growth was recorded by measurement of OD_{660}.

Tolerance to antibiotics was tested by growing the isolates on TSB agar that contained one of the following compounds: ampicillin (20 and 60 μg ml^{-1}), streptomycin (25 and 50 μg ml^{-1}), oxytetracycline (10 μg ml^{-1}), chloramphenicol (25 and 100 μg ml^{-1}) and kanamycin (10 μg ml^{-1}).

All isolates were tested for their ability to grow in purified sand at the expense of intact, living hyphae of the fungi *Mucor hiemalis*, *Chaetomium globosum* and *Fusarium culmorum* (De Boer *et al.*, 2001). Growth response of bacteria was determined 3 weeks after introduction of the fungal inoculum. In zones where hyphae were present, bacterial numbers were determined by plate-counting and corrected for numbers that were present in the controls (i.e. without fungal invasion).

DNA G+C content. Genomic DNA isolation was performed by using Qiagen genomic-tips 500/G according to the manufacturer's recommendations. Determination of G+C content was carried out on four isolates (Ter6^T, Ter10, Ter252 and Ter331) by using thermal denaturation, as described by Marmur & Doty (1962).

BOX-PCR. BOX-PCR genomic fingerprinting was performed by using the method described by Rademaker & de Bruijn (1997). DNA was extracted from 3 ml liquid culture by a standard mini-chromosomal DNA extraction method (Sambrook *et al.*, 1989). Genomic fingerprints were compared by using the program IMAGE MASTER 1D ELITE (version 4.20; Amersham Pharmacia). Cluster analysis of pairwise similarity values was performed by using the UPGMA algorithm (Sneath & Sokal, 1973).

Cloning, sequencing and phylogenetic analysis. Almost-complete sequences of 16S rRNA genes were determined commercially by the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. A 5 μl aliquot of a colony resuspended in Tris/EDTA buffer was spotted onto CloneSaverCards (Whatman Bioscience) according to the manufacturer's recommendations. PCR was performed on 1·2 mm punches of the card with primers 16S1500F (5′-ccgaattcgtcgacaacagagtttgatcctggctcag-3′) and 16S1500R (5′-cccgggatccaagcttacggctaccttgttacgactt-3′). PCR products were cleaned by using GFX columns (Amersham Biosciences) and sequenced by using DYE-ET terminator cycle sequencing (Amersham Biosciences) with the following primers (sequences in 5′→3′ direction): 16S500F (tggagagtttgatcctggctcag), 16S500R (taccgcggctgctggcac), BSF1099/16 (gyaacgagcgcaaccc), BSF349/17 (aggcagcagtggggaat),

BSF748/15 (rggattagatacccc), BSF8/20 (agagtttgatcctggctcag), BSR1114/16 (gggttgcgctcgttrc), BSR357/15 (ctgctgcctyccgta) and BSR798/15 (ggggtatctaatccc). Prior to separation on an ABI 3700 system (Applied Biosystems), sequencing products were purified by using Sephadex G-50 Superfine. Contigs were built with the DNASTAR package and the Phrap/Phred/Consed package (http://www.phrap.org/).

Sequence alignments were performed by using CLUSTAL_W (Thompson *et al.*, 1994). Trees were constructed by using the neighbour-joining method.

Sequence similarity searches with previously determined database entries were performed with the BLAST program as supported by the NCBI website (Altschul *et al.*, 1997; http://www.ncbi.nlm.nih.gov/blast).

Design of a *Collimonas*-specific FISH (fluorescent *in situ* hybridization) probe. The 16S rDNA sequences of all Ter isolates were aligned and the consensus sequence was compared to 16S rDNA sequences of members of the family '*Oxalobacteraceae*'. One unique continuous stretch of 18 bp was shared between all Ter isolates, but was absent in all other strains. A probe complementary to this sequence was designed (CTE998–1015: 5′-CTCTTCGGGATT-CTGTAC-3′) and was found to be unique in a search of more than 72 000 sequences by using the PROBE_MATCH program of Ribosomal Database Project II (http://rdp.cme.msu.edu/cgis/probe_match.cgi?su=SSU). The probe was synthesized, labelled with Cy3 (Thermo Hybaid) and tested by FISH analysis on paraformaldehyde-fixed cells of all Ter isolates and three *Herbaspirillum* strains (Egli *et al.*, 2001).

RESULTS AND DISCUSSION

Morphological characteristics

On chitin/yeast agar, halo formation as a result of chitin degradation by the Ter isolates was detected after 5–7 days incubation at 20 °C. Haloes reached a final size of 4–10 mm after about 14 days. As the isolates produced translucent biomass in small amounts, the colonies and surrounding haloes appeared as clear spots. On 0·1× TSB agar, three different colony types were observed. Nine isolates, including the designated type strain, Ter6^T, produced type I colonies, which appeared as flat, glossy, turbid, whitish colonies with a diameter of 3–7 mm and a layered structure. Type II colonies (five isolates) were flat with a diameter of 3–7 mm, a yellowish central part and a translucent, granular-structured periphery. The remaining eight isolates grew as small, glossy, whitish colonies with a diameter of 1–3 mm (type III).

Light and electron microscopic observations showed that the cell morphology of all colony types of Ter isolates was very similar. They were straight or slightly curved rods, 0·3–0·5 μm in diameter and 1·0–2·0 μm long (Fig. 1). Transmission electron microscopic inspection demonstrated the presence of one to three polar flagella and pili (Fig. 1a). In some instances, lateral flagella were also seen. Presence of flagella was, however, only observed when the isolates were grown in liquid media. Most cells produced polyphosphate granules (about 0·1 μm diameter) in the media used. Transmission electron micrographs further indicated that the cell-wall ultrastructure was Gram-negative, which was confirmed by KOH tests.

Most Ter isolates were highly motile on low-percentage agar plates, with a rate of >40 mm in 48 h. Isolates that belonged to colony type III were less motile (spreading <20 mm in 48 h).

Growth characteristics

A summary of growth characteristics (Supplementary Table A) is available as in IJSEM Online. None of the nine Ter isolates tested was able to grow under anaerobic conditions. These isolates were positive for oxidase and negative or weakly positive for catalase.

Four isolates (Ter6^T, Ter146, Ter227 and Ter331) were selected for testing the effects of environmental conditions on growth. These isolates had a broad temperature optimum; highest growth rates were observed at 20–30 °C. Growth rates decreased gradually at lower temperatures (minimum temperature, <4 °C), but dropped dramatically

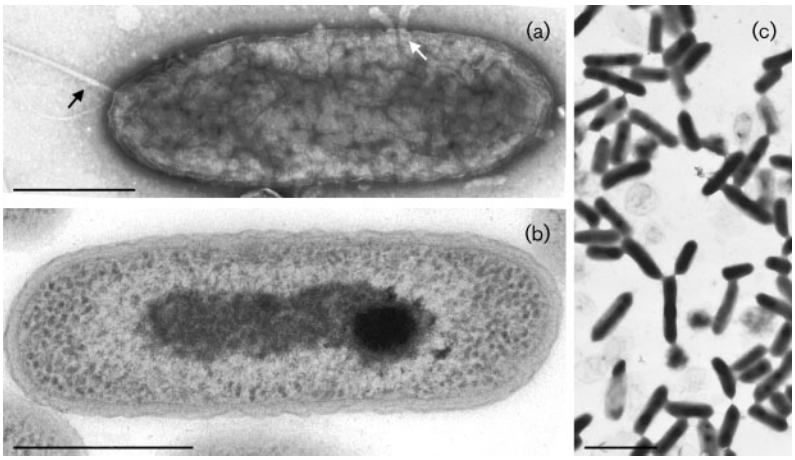

Fig. 1. Transmission electron micrographs of Ter isolates (*Collimonas*) grown on chitin/yeast agar, showing: (a) a polar flagellum (black arrow) and pili (white arrow) of cells (Ter331) grown in liquid culture (bar, 0·5 μm); (b) Gram-negative cell-wall structure (Ter227) and polyphosphate granules (bar, 0·5 μm); and (c) general cell morphology (Ter6^T, negatively stained) (bar, 1 μm).

at temperatures above 35 °C. Optimal pH for growth was 6·5, but the effect of pH on growth was minor within the range studied (5·0–8·0).

All Ter isolates were able to hydrolyse colloidal chitin in solid and liquid minimal media. The rate of chitin hydrolysis in liquid media was relatively slow for isolates that belonged to colony type III (not shown). None of the isolates hydrolysed colloidal chitin in agar that contained TSB, possibly due to catabolic repression. Catabolic repression of chitinase production has been reported for several bacteria, e.g. *Cytophaga*, *Arthrobacter* and *Streptomyces* (Morrissey *et al.*, 1976; De Boer *et al.*, 1998; Saito *et al.*, 1998). However, in contrast to most other soil and rhizosphere bacteria, repression of chitinase activity of the Ter isolates on mixed chitin/TSB agar was complete and long-lasting (De Boer *et al.*, 1998; W. de Boer & P. J. A. Klein Gunnewiek, unpublished results).

Chitosanase activity was observed in about half of the isolates. All isolates were proteolytic, but did not show glucanolytic or cellulolytic activity under the conditions tested.

All Ter isolates proliferated in sand when hyphae of the fungi *C. globosum*, *F. culmorum* or *M. hiemalis* invaded. The strongest increase in cells was induced by hyphae of *M. hiemalis*. All isolates adhered rapidly to heat-fixed hyphae of the three fungi, as observed by microscopic inspection (not shown).

Growth of the Ter isolates on TSB agar was not inhibited by ampicillin (60 μg ml^{-1}), streptomycin (25 μg ml^{-1}), chloramphenicol (25 μg ml^{-1}) or kanamycin (10 μg ml^{-1}). Oxytetracycline (10 μg ml^{-1}) inhibited all but two isolates (Ter146 and Ter300).

Phylogenetic position based on 16S rDNA nucleotide sequences

Comparison of nearly full-length 16S rDNA sequences (corresponding to *Escherichia coli* positions 68–1451) of all 22 Ter isolates revealed between 98·6 and 99·9 % similarity. Taking into account only those nucleotides that were phylogenetically informative, 12 unique 16S rDNA sequences could be distinguished that were related most closely to representatives of the genera *Herbaspirillum* (95·9–96·7 % similarity) and *Janthinobacterium* (94·3–95·6 % similarity) (Fig. 2). These genera belong to the family '*Oxalobacteraceae*', order '*Burkholderiales*' (Garrity *et al.*, 2001). Another close relative is a hydrogen-oxidizing isolate designated *Aquaspirillum autotrophicum* (Aragno & Schlegel, 1978). This isolate, however, does not show a close affiliation with other *Aquaspirillum* species, including the genus-defining species *Aquaspirillum serpens*. Ding & Yokota (2002) demonstrated that the genus *Aquaspirillum* is polyphyletic, with at least four proposed phylogenetic groupings, including placement of *A. autotrophicum* in the family '*Oxalobacteraceae*'. Therefore, re-evaluation of the phylogenetic position of *A. autotrophicum* is needed.

The Cy3-labelled probe CTE998–1015 hybridized to all fixed cells of Ter isolates at 45 °C, 10 % formamide, but not to cells of *H. frisingense*, *H. rubrisubalbicans* or *H. seropedicae* (not shown).

Metabolic profile

The Ter isolates had a similar respiratory response to 80 of the 95 substrates in the Biolog GN plates. Substrates that induced respiratory activity were: Tween 40, Tween 80, acetyl-D-glucosamine, L-arabinose, D-arabitol, D-fructose,

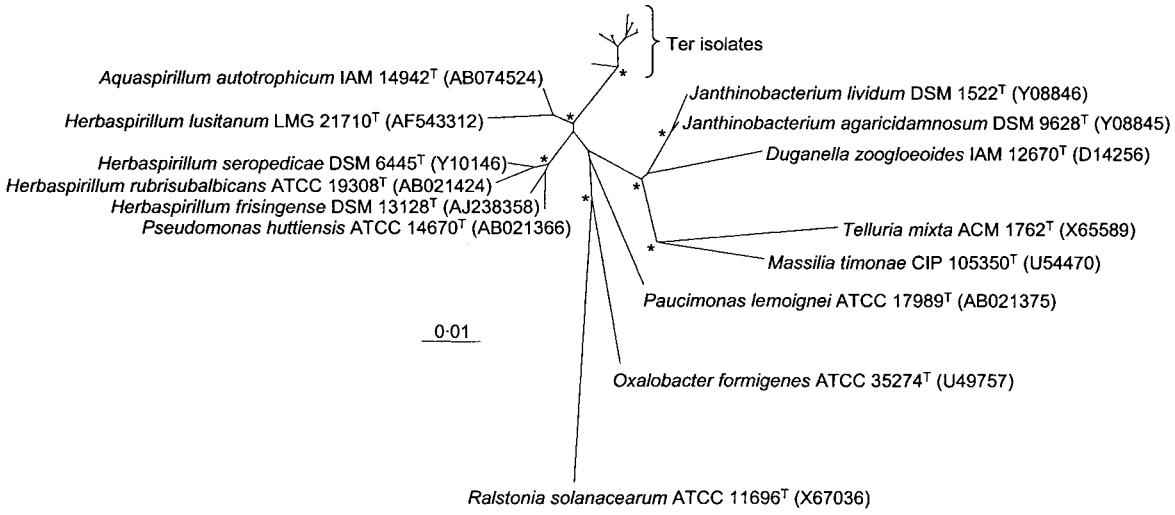

Fig. 2. Unrooted tree showing the phylogenetic relationship between the 12 unique 16S rDNA sequences of 22 Ter isolates (*Collimonas*) and other members of the family '*Oxalobacteraceae*'. The 16S rDNA sequence of *Ralstonia solanacearum* ATCC 11696^T (GenBank no. X67036) was used as the outgroup. Tree construction was by neighbour-joining. Asterisks indicate nodes with bootstrap values of 950 or higher (1000 replicates). Bar, 0·01 nucleotide substitutions.

L-fucose, D-galactose, D-glucose, *m*-inositol, D-mannitol, D-mannose, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-acetonic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, β-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, propionic acid, D-saccharic acid, succinic acid, bromosuccinic acid, alaninamide, L-alanine, L-alanylglycine, L-aspargine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-leucine, L-proline, L-pyroglutamic acid, L-threonine, γ-aminobutyric acid, inosine, uridine and glycerol. Substrates that were negative or gave very weak respiratory activity were: α-cyclodextrin, dextrin, glycogen, acetyl-D-galactosamine, cellobiose, *m*-erythritol, gentiobiose, α-D-lactose, maltose, D-melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, sucrose, turanose, α-hydroxybutyric acid, γ-hydroxybutyric acid, hydroxyphenylacetate, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, sebacic acid, D-alanine, glycyl L-aspartic acid, hydroxy-L-proline, L-ornithine, L-phenylalanine, D-serine, DL-carnitine, thymidine, phenylethylamine, putrescine and glucose 1-phosphate. The isolates varied for (responses for strain Ter6^T are given in parentheses): adonitol (−), lactulose (+), D-sorbitol (+), D-trehalose (+), xylitol (+), D-galacturonic acid (−), D-glucosamic acid (−), quinic acid (−), succinamic acid (−), glucuronamide (−), L-histidine (−), L-serine (−), 2-aminoethanol (+), DL-glycerol phosphate (−), glucose 6-phosphate (−) and urocanic acid (+).

The metabolic profiles of the *Herbaspirillum* type strains were very similar to each other (not shown). Most notable differences between the Ter isolates and the *Herbaspirillum* strains were found in the metabolism of itaconic acid, glycyl L-glutamic acid, inosine and uridine (Table 1). Furthermore, none of the *Herbaspirillum* strains was able to degrade colloidal chitin.

From Table 1, it can be seen that the Ter isolates share some metabolic properties with *Herbaspirillum* strains and others with *Janthinobacterium* strains. However, the ability of the Ter isolates to metabolize uridine and L-histidine distinguishes them from both of these genera.

Table 1. Differentiating respiratory activities (Biolog GN) of Ter isolates (*Collimonas*) and *Herbaspirillum* and *Janthinobacterium* strains

Taxa: 1, *Herbaspirillum*; 2, *Janthinobacterium*; 3, Ter isolates. +, Activity present in all isolates; M, present in most isolates (>90 %); L, present in <10 % of isolates; −, not present; V, variable among isolates.

Substrate	1*	2†	3
Adonitol	+	−	L
D-Trehalose	−	V	M
Monomethyl succinate	+	−	+
cis-Aconitic acid	+	−	+
D-Galacturonic acid	+	−	L
Itaconic acid	+	−	−
D-Saccharic acid	+	−	+
Glycyl L-glutamic acid	−	+	+
L-Histidine	−	−	M
L-Leucine	+	−	+
Inosine	−	V	+
Uridine	−	−	+
Glucose 1-phosphate	−	+	−

*Tests carried out with three type strains (*H. seropedicae* DSM 6445^T, *H. rubrisubalbicans* ATCC 19308^T and *H. frisingense* DSM 13128^T) and complemented with data given by Baldani *et al.* (1996) and Lincoln *et al.* (1999).

†Data from Lincoln *et al.* (1999) for three strains each of *Janthinobacterium lividum* and *Janthinobacterium agaricidamnosum*.

Cellular fatty acid composition

The cellular fatty acid composition of all Ter isolates is given in Table 2. In total, 13 fatty acids were found, of which eight were present in all isolates. The major fatty acids were $C_{16:0}$ and $C_{16:1}\omega7c$. The major cellular fatty acids of the three *Herbaspirillum* type strains were $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$ (Table 2). The absence of the fatty acid $C_{14:0}$ 2-OH in the Ter isolates is a characteristic that clearly distinguishes these strains from *Herbaspirillum* species (Table 2; Lincoln *et al.*, 1999). Furthermore, the Ter isolates

Table 2. Cellular fatty acid composition (%) of Ter isolates (*Collimonas*) and strains of *Herbaspirillum* and *Janthinobacterium*

Taxa: 1, Ter isolates ($n=22$); 2, *Herbaspirillum* ($n=3$); 3, *Janthinobacterium* ($n=6$).

Fatty acid*	1	2†	3‡
$C_{10:0}$	0·0–0·4	0·1–0·2	−
$C_{10:0}$ 3-OH	0·8–1·2	1·5–1·6	3·1–4·0
$C_{12:0}$	3·1–4·0	0·5–3·1	3·6–5·2
$C_{12:0}$ 2-OH	2·2–2·8	0·5–1·4	0·0–0·9
$C_{12:0}$ 3-OH	3·1–3·9	3·1–3·5	−
$C_{14:0}$	0·0–0·6	0·6–3·8	0·6–3·8
$C_{14:0}$ 2-OH	−	1·1–2·2	−
$C_{16:0}$	30·8–41·2	21·3–26·5	35·3–40·9
$C_{16:1}\omega7c$	31·8–42·5	29·7–36·0	22·4–40·7
$C_{16:1}$ 2-OH	0·0–4·7	0·4–0·5	−
$C_{17:0}$	0·0–0·7	0·2–0·4	−
$C_{17:0}$ cyclo	0·7–5·2	4·6–10·0	9·8–26·7
$C_{18:0}$	0·0–1·5	1·0–1·2	−
$C_{18:1}\omega7c$	7·1–14·9	21·2–23·6	1·4–3·5

*Fatty acids given in bold are distinctive between the genera.

†Data for *H. seropedicae* DSM 6445^T, *H. rubrisubalbicans* ATCC 19308^T and *H. frisingense* DSM 13128^T.

‡Data from Lincoln *et al.* (1999) for three strains each of *Janthinobacterium lividum* and *Janthinobacterium agaricidamnosum*.

had a much lower content of $C_{18:1}\omega7c$ than *Herbaspirillum* species.

Comparison with data given by Lincoln *et al.* (1999) indicated that the Ter isolates contain more $C_{18:1}\omega7c$ than *Janthinobacterium* species, whereas the $C_{17:0}$ cyclo content of the Ter isolates was considerably lower than that of *Janthinobacterium* species (Table 2). The content of $C_{12:0}$ 2-OH of the Ter isolates was relatively high, compared to both *Herbaspirillum* and *Janthinobacterium* species, whereas their $C_{10:0}$ 3-OH content was relatively low.

DNA G+C content

The DNA G+C content (T_m method) of four isolates, including strain Ter6^T and representatives of each of the three colony types, varied from 57 to 62 mol% (Ter6^T G+C content was 57·8 mol%). This is notably lower than the G+C contents of recognized species of *Herbaspirillum* and *Janthinobacterium*, with the exception of *Herbaspirillum lusitanum* (Valverde *et al.*, 2003).

Genomic and metabolic variation among the Ter isolates

The 12 unique 16S rDNA sequences among the 22 Ter isolates clustered in four groups (see Supplementary Fig. A in IJSEM Online). This clustering was in perfect agreement with the genomic variation observed among the isolates by using BOX-PCR (see Supplementary Fig. B in IJSEM Online).

The BOX-PCR grouping could also be distinguished by comparing the respiratory response to four Biolog substrates and chitosan (data available as Supplementary Table B in IJSEM Online).

Evaluation of phylogenetic position

Given the high degree of 16S rDNA sequence similarity to representatives of the genera *Herbaspirillum* (95·9–96·7 %) and *Janthinobacterium* (94·3–95·6 %), it could be argued that the Ter isolates belong to one or more novel species within one of these genera. However, as shown above, the Ter isolates do not match several basic biochemical and physiological characteristics of these genera (Tables 1 and 2). In addition, the Ter isolates differ from recognized *Herbaspirillum* species by lacking the ability to fix nitrogen (V. M. Reis, personal communication). Furthermore, unlike recognized *Herbaspirillum* species, the Ter isolates are soil bacteria with no apparent pathogenic or symbiotic relationship with plants (Baldani *et al.*, 1996; Kirchhof *et al.*, 2001; Valverde *et al.*, 2003). The Ter isolates have a smaller cell size (0·3–0·5 × 1·0–2·0 μm) than *Janthinobacterium* species (0·8–1·5 × 1·8–6·0 μm) (Sneath, 1984). None of the Ter isolates produced a purple pigment (violacein), which has been described as characteristic of the genus *Janthinobacterium* (Sneath, 1984). However, the species *Janthinobacterium agaricidamnosum* also does not produce this pigment (Lincoln *et al.*, 1999). The Ter isolates are negative or weakly positive for catalase, whereas *Janthinobacterium* strains are positive (Lincoln *et al.*, 1999). Given these differences with the genera *Herbaspirillum* and *Janthinobacterium*, it is proposed that the isolates should be assigned to a novel genus, *Collimonas* gen. nov. It should be noted that 16S rDNA sequence similarity between species of the genera *Herbaspirillum* and *Janthinobacterium* is also high (>96 %) but, as discussed by Lincoln *et al.* (1999), morphological, physiological, chemical and ecological characteristics are so different that it would be unrealistic to combine these two genera. The same holds true for the genus *Paucimonas* (formerly *Pseudomonas lemoignei*), which has 95·0–95·9 % 16S rDNA sequence similarity with *Herbaspirillum* species (Jendrossek, 2001).

The highest level of sequence similarity between *Collimonas* strains and previously described 16S rDNA sequences was found for GenBank entry AY154366 (98·9–99·6 %), which represents a naphthalene-degrading strain from the USA that was classified as *Herbaspirillum* sp. Hg (Wilson *et al.*, 2003). Given its close affinity with *Collimonas* and much lower similarity with *Herbaspirillum* species (<96·4 %), it would be interesting to determine whether other characteristics of this strain might support its placement within *Collimonas*, thereby extending the known range of this genus.

Although comparison of 16S rDNA sequences of the Ter isolates revealed that their interrelatedness was very high (98·6–99·9 % similarity), this is not sufficient to place them into a single species (Stackebrandt & Goebel, 1994). As shown above, four subclusters can be recognized on the basis of 16S rDNA sequences, genomic fingerprinting and substrate utilization (data available as supplementary material in IJSEM Online). This genomic and phenotypic variation forms the basis for establishing the centre and breadth of the novel genus (Christensen *et al.*, 2001). Subcluster A, which contains strains with colony type II morphology, is most distant from the other isolates and consists of four isolates with identical 16S rDNA sequences and BOX-PCR patterns. These *Collimonas* isolates are related most closely to the genus *Herbaspirillum* (96·2–96·7 % 16S rDNA sequence similarity). The other *Collimonas* subclusters are related more closely to each other and, as they represent the majority of isolates, it is from these subclusters that the type species should be chosen. As suggested by Christensen *et al.* (2001), the type species and strain is selected for pragmatic reasons, i.e. it should be most representative with respect to the characters that define the novel taxon. Based on the central position of the 16S rDNA sequences, the mean value of fatty acid contents and the most common metabolic profile, cluster C (four isolates) has been selected to represent *Collimonas fungivorans* gen. nov., sp. nov. Ter6^T (=NCCB 100033^T =LMG 21973^T) is the type strain.

Description of *Collimonas* gen. nov.

Collimonas (Col.li.mo′nas. L. masc. n. *collis* hill; Gr. n. *monas* a unit, monad; N.L. fem. n. *Collimonas* cell from the hill).

Cells are strictly aerobic, straight or slightly curved, Gram-negative rods, 0·3–0·5 × 1·0–2·0 μm. They occur singly and possess flagella (mostly one to three polar, but in some cases, several lateral) and pili when cultured in liquid media. Oxidase activity is positive; catalase activity is negative or weakly positive. Maximal growth is observed between 20 and 30 °C, without a sharp optimum. Maximum temperature that supports growth is approximately 35 °C. Optimal growth occurs at pH 6·5. Cells are able to hydrolyse colloidal chitin and milk proteins, but not lichenan or cellulose. Isolates vary in their ability to hydrolyse colloidal chitosan (deacetylated chitin). On minimal colloidal chitin/yeast agar, circular cleared haloes (final diameter, 4–10 mm) that support little translucent biomass are produced. Halo formation is completely repressed in chitin agar that contains TSB or glucose. On 0·1× TSB agar, colony morphology is variable. A wide range of sugars, alcohols, organic acids and amino acids can be metabolized. Several di- and trisaccharides cannot be used as substrates. In purified sand, cells proliferate upon introduction of intact, living fungal hyphae of various species of soil fungi. Major cellular fatty acids are $C_{16:0}$ and $C_{16:1}\omega7c$. DNA G+C content is 57–62 mol%. A member of the *β-Proteobacteria*, related most closely to the genera *Herbaspirillum* and *Janthinobacterium* in the family '*Oxalobacteraceae*', order '*Burkholderiales*'. Characteristics useful to differentiate the genus *Collimonas* from these related genera are given in Tables 1 and 2, as well as in the Results and Discussion. So far, the genus *Collimonas* is only known to occur in slightly acidic sandy dune soils. The type species is *Collimonas fungivorans*.

Description of *Collimonas fungivorans* sp. nov.

Collimonas fungivorans (fun.gi.vo′rans. L. n. *fungus* mould, mushroom, fungus; L. part. adj. *vorans* devouring, eating; N.L. part. adj. *fungivorans* fungus-eating).

General characteristics are the same as given above for the genus. On 0·1× TSB agar (3 days, 20 °C), the type strain forms flat, glossy, turbid, whitish colonies of 3–7 mm diameter with a layered structure (colony type I; see Results and Discussion). Catalase activity is negative. Colloidal chitosan is hydrolysed. The substrate-use profile of the type strain is given above. The G+C content of the type strain is 57·8 mol%.

The type strain is Ter6^T (=NCCB 100033^T=LMG 21973^T). Reference strains are Ter166, Ter300 and Ter330.

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