Conexibacter woesei gen. nov., sp. nov., a novel representative of a deep evolutionary line of descent within the class Actinobacteria

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A novel Gram-positive bacterial strain was isolated from forest soil. According to its 16S rRNA sequence, this strain is a deep-rooting member of the class Actinobacteria. The 16S rRNA sequence is most closely related (~ 94 % identity) to clones of uncultured bacteria detected in different terrestrial environments, while showing only a remote relationship (~ 90 % identity or less) to sequences of cultured species. Cells of the first cultured representative of this phylogenetic cluster are small, short rods that are motile by peritrichous flagella, catalase- and oxidase-positive and grow under aerobic conditions. In liquid culture, flagella from different cells can aggregate to form networks, clearly visible under the light microscope. The peptidoglycan contains meso-diaminopimelic acid and is directly cross-linked (type A1c). Mycolic acids are not present. The polar lipids are phosphatidylinositol and an unidentified phospholipid. Menaquinone MK-7(H4) was detected as the predominant isoprenoid quinone. Oleic, 14-methylpentadecanoic, hexadecanoic and 8c-heptadecenoic acids are the predominant components of the cellular fatty acid profile. The DNA G+C content is 71 mol%. The distinct phylogenetic position and the unusual combination of chemotaxonomic characteristics justify the proposal of a new genus and species, Conexibacter woesei gen. nov., sp. nov., with the type strain ID131577T (= DSM 14684T = JCM 11494T).

INTRODUCTION

The class Actinobacteria comprises bacteria that share > 80 % 16S rRNA sequence similarity and a characteristic set of 16S rRNA signature nucleotides (Stackebrandt et al., 1997). The vast majority of cultured members of this class belong to the subclass Actinobacteridae. Only a few strains have been isolated that form deeply branching lines of descent within this class and have given rise to the description of the subclasses Acidimicrobidae, Rubrobacteridae, Sphaerobacteridae and Coriobacteridae (Stackebrandt et al., 1997). Molecular analyses of environmental DNA from various locations have revealed the presence of several clones that are phylogenetically related to the deeply branching members of Actinobacteria (Stackebrandt et al., 1993; Rheims et al., 1996; Felske et al., 1997). In some instances, the phylogenetic depth of clusters represented by these clones corresponds to that of subclasses or orders (Rheims et al., 1999). However, attempts to isolate the corresponding microorganisms have so far been unsuccessful (Rheims et al., 1999).

A Gram-positive bacterium with a DNA G+C content of 71 mol% was isolated from forest soil. According to its 16S rDNA sequence, this strain belongs to a phylogenetic cluster consisting of clones of hitherto-uncultured bacteria that appear to be distributed worldwide in different soils (Rheims et al., 1996; Rheims & Stackebrandt, 1999). This isolate offers the first opportunity to study the phenotypic characteristics of one of these organisms. Because of the remote 16S rDNA similarity (< 84 %) to validly described taxa and the unusual combination of chemotaxonomic characteristics, we propose to accommodate the isolate in a new genus and species, Conexibacter woesei gen. nov., sp. nov., belonging to a deep evolutionary line of descent within the class Actinobacteria.

METHODS

Bacterial strain and cultivation conditions. Strain ID131577T was isolated from a soil sample collected from a wooded area in...
Gerenzano, Italy. Soil was plated on HV/2 (half-strength HV medium; Hayakawa & Nonomura, 1987) following dilution in water. Single colonies were transferred on ISP3 medium (Shirling & Gottlieb, 1966). Colonies were serially transferred on new HV/2, ISP3 and Todd–Hewitt (Difco) plates until a pure culture was obtained. Strain ID131577T was also cultured on brain heart infusion (BHI; Difco), Luria–Bertani (Difco), tryptic soy agar, R2A agar (Difco) and ISP2 (Shirling & Gottlieb, 1966) solid media. For liquid cultures, Todd–Hewitt medium, BHI, tryptone soy broth (Oxoid) and R2A medium were used.

**Morphological and physiological characterization.** Cell morphology was examined by phase-contrast microscopy using a model Axioskop microscope (Zeiss) fitted with a model 3CCD camera (Sony). Negative staining of cells was performed prior to electron microscopic investigation. Bacteria were fixed in 2% glutaraldehyde for 30 min on ice and washed with TE buffer (20 mM Tris/HC1, 1 mM EDTA, pH 6–9). Bacteria were adsorbed onto freshly prepared thin carbon films, washed in TE buffer and negatively stained with 1% aqueous uranyl acetate. The carbon film was picked up with 200-mesh grids and air-dried. Samples were examined in a Zeiss EM910 transmission electron microscope at an acceleration voltage of 80 kV at calibrated magnifications.

Catalase and oxidase activity and NaCl tolerance were examined by standard methods as described by Lányi (1987). The API 20 NE test (bioMérieux) was used for investigation of additional physiological characteristics. Substrate utilization patterns were studied by using GP2 and GN2 microplates with panels of 95 carbon-containing compounds of the Biolog identification system. Inocula for the microplates were grown on tryptic soy agar. Enzyme activities were examined by using the API ZYM test (bioMérieux). For determination of the temperature range for growth, the bacteria were cultured on Todd–Hewitt and BHI plates. The pH growth range was determined using both agarized and liquid BHI medium, adjusted to pH values ranging between 3 and 11 with HCl or NaOH. Antibiotic susceptibility was determined by placement of antibiotic disks (bioMérieux and Oxoid) on BHI plates seeded with suspensions of strain ID131577T.

**Chemotaxonomic characterization.** Purified cell-wall preparations were obtained by the method of Schleifer & Kandler (1972). The isomer of diaminopimelic acid (Apm) was determined by one-dimensional TLC (Rhuland et al., 1955); other amino acids and peptides were analysed by two-dimensional TLC (Groth et al., 2001) of cell-wall hydrolysates on cellulose plates. Cellular fatty acid methyl esters were obtained from cells grown in tryptic soy broth (Oxoid) at 28°C by the method of Miller (1982). Identification and quantification of the fatty acid methyl esters, as well as the numerical analysis of the fatty acid profiles, were performed using the standard MSL Library Generation software (Microbial ID Inc.). Menaquinones were extracted as described by Collins et al. (1977) and analysed by HPLC (Groth et al., 1996) and electron-impact mass spectrometry using a QP 2000 mass spectrometer fitted with a direct sample inlet device (Shimadzu). Polar lipids extracted by the method of Minnikin et al. (1979) were identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). The absence of mycolic acids was demonstrated by TLC (Minnikin et al., 1975).

**DNA base composition.** DNA was isolated using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashon et al. (1977). The G+C content was determined by reverse-phase HPLC according to Mesbah et al. (1989).

**Sequencing of rDNA and phylogenetic analysis.** Genomic DNA was purified with the PUREGENE DNA isolation kit (Gentra Systems). The nearly complete 16S rRNA gene was amplified with primers F27 and R1492 (Heuer et al., 1997), as described previously (Degli Innocenti et al., 2002). The partial 23S rRNA gene was amplified under the same conditions with primers 23F2 (5′-GGAA-GTGAACATCTCAGTACCC-3′, annealing to positions 188–210 of the Escherichia coli 23S rRNA gene; Brosius et al., 1980) and 23R2 (5′-CGGAACTTCCCGACAAGG-3′, annealing to the complement of positions 1941–1959 of the E. coli 23S rRNA gene). For colony amplification, a loopful of bacteria was boiled for 5 min in 100 µl water and 5 µl was used as template in the PCR. PCR products, purified with the GFX PCR DNA and Gel Band purification kit (Amersham Pharmacia Biotech), were sequenced directly with the BigDye cycle sequencing kit (Applied Biosystems) according to the manufacturer’s instructions and run on an ABI Prism 310 automatic sequencer (Applied Biosystems). Sequences were compared with those maintained in the GenBank database through BLAST (Altschul et al., 1990). For phylogenetic analysis, sequences were aligned with those of reference strains with the program TOOL (Wisconsin package version 9.1 (Genetics Computer Group)). The aligned sequences were analysed with programs included in the PHYLIP package (Felsenstein, 1993). Distance matrices were calculated with DNADIST, using the maximum-likelihood method implemented in the program (Kishino & Hasegawa, 1989). Trees were derived from the distance matrices using neighbour-joining (Saitou & Nei, 1987). All analyses were performed on a bootstrapped dataset containing 100 replicates (generated by the program SEQBOOT).

**RESULTS AND DISCUSSION**

**Strain isolation and cultivation.** During isolation of filamentous actinomyces from soil, a putative Dactylosporangium colony, recovered on an HV/2 plate, was transferred to ISP3 medium. Direct sequencing of the 16S rRNA gene of this Dactylosporangium colony indicated the existence of a contaminating bacterium. Subsequent subculturing by serial transfer on HV/2, ISP3 and Todd–Hewitt plates clearly showed the presence of two different strains, a Dactylosporangium isolate and a non-filamentous bacterium. The latter, designated ID131577T, formed a transparent, hardly visible film in pure culture on ISP3 plates. Strain ID131577T was plated on different media and the best growth was obtained on Todd–Hewitt and BHI. In liquid media, growth was obtained under both shaking and static conditions. The strain was able to grow at 22, 28, 37 and to a lesser extent, at 4°C. No growth was obtained at 43 or 50°C. Growth occurred at pH values between 6 and 8, with an optimum at 7–7.5, both in liquid and agarized cultures. When cells maintained for 10 days in liquid cultures at pH values between 3 and 11 were transferred to BHI plates (pH 7–2), sustained growth was obtained only from cells that had been kept at pH values between 6 and 8. Cells that had been maintained at pH 5 grew poorly, while no growth was obtained from the other cultures. After dispersion of cells in liquefied R2A agar at 45°C and subsequent incubation at 28°C, strain ID131577T grew exclusively on the agar surface, indicating that aerobic conditions are required for growth. No growth was observed on BHI or Todd–Hewitt medium under anaerobic conditions (80 % N2, 10 % H2, 10 % CO2), while the strain was able to grow in the presence of 5 % CO2. When grown aerobically in liquid Todd–Hewitt medium at 28°C and at 200 r.p.m., the OD600 of the culture took over 50 h to double.
**Morphology**

On Todd–Hewitt agar, ID131577\(^{T}\) formed smooth, mucoid colonies with a whitish-creamy colour. Colonies were sticky and difficult to disintegrate. Cells were short rods (0·6–0·7 \(\times\) 0·9–1·2 \(\mu\)m), occurring singly or in pairs (Fig. 1). They stained Gram-positive. Cells were motile due to the presence of long peritrichous flagella (Fig. 2a). After about a week, in addition to cells, phase-contrast microscopy of liquid cultures revealed the presence of rigid, non-motile spiral bodies (Fig. 1). Electron microscopic investigations suggested that these bodies are formed by self-aggregation of flagella (Fig. 2e). Fig. 3(a, c and d) depicts self-aggregation of flagella originating from the same cell. Cells may become entangled (Fig. 3b) within flagellar networks (Fig. 2b–e). At higher densities, discarded flagella may form undulating or spiral structures with a regular periodicity of \(\sim 2–3\) \(\mu\)m (Fig. 2d, e). To our knowledge, this morphological feature has not been described before. However, interaction of bacterial cells along a self-produced network of filaments has recently been observed for a deep-branching \(\gamma\)-proteobacterium, strain F8 (Böckelmann et al., 2002).

**Phylogenetic analysis**

The almost-complete sequence of the 16S rRNA gene, consisting of 1470 nt (95·2 % of the E. coli sequence; Brosius et al., 1978), was determined and compared to all GenBank entries. The highest binary similarity value (94·3 %) was found with clone TM220 (Rheims et al., 1996), derived from an uncultured bacterium. Clone TM220 belongs to a group of environmental sequences, retrieved from different locations, for which no cultured representative has so far been isolated (TM group I; Rheims et al., 1996). Sequence

**Fig. 1.** Phase-contrast micrograph showing cells of strain ID131577\(^{T}\) grown in tryptic soy broth at 28\(^\circ\)C for 10 days and a spiral body formed by self-aggregated discarded flagella. Bar, 5 \(\mu\)m.

**Fig. 2.** Electron micrographs of negatively stained cells of strain ID131577\(^{T}\). Individual bacteria exhibit peritrichous long flagella (a). After a longer period of growth, bacteria form aggregates (b) and, due to self-aggregation of flagella, they exhibit an undulating network-like structure with entangled bacteria (c–e). Bars, 5 (a), 3 (c, d) and 1 (b, e) \(\mu\)m.

**Conexibacter woesei gen. nov., sp. nov.**
analysis demonstrated that strain ID131577T clusters with the Gram-positive bacteria with high G+C content, but it is not closely related to any previously described reference species for which sequence data are available. The highest similarity values to validly described species are to *Thermoleiphilum minutum* (90%), *Thermoleiphilum album* (89·9%) (Zarilla & Perry, 1984, 1986), *Acidimicrobium ferrooxidans* (84·1%) (Clark & Norris, 1996) and *Rubrobacter xylanophilus* (83·8%) (Carreto et al., 1996). One cultured bacterium, strain B33D1, shows a higher 16S rRNA similarity (92·8%). Although strain B33D1 was subjected to 20 phenotypic tests together with other earthworm burrow isolates, only a statistical evaluation is available without individual data for the strain (Furlong et al., 2002). The 16S rRNA sequences of *T. album* and *T. minutum* have only recently been released and they clearly cluster with the *Actinobacteria*. This finding contrasts with the classification of this genus as a member of the *Pseudomonadaceae* (Garrity & Holt, 2001). The 16S rRNA sequence of strain ID131577T shares with the class *Actinobacteria* the signature A at position 906 (Stackebrandt et al., 1997), but diverges from most *Actinobacteria* in having a U (instead of A or C) at position 955. In this position, U is also found in members of the subclasses *Rubrobacteridae* and *Sphaerobacteridae* (Stackebrandt et al., 1997). According to the signature nucleotides and similarity values of >80% to other members of the class *Actinobacteria* (Stackebrandt et al., 1997), strain ID131577T can be considered a member of this class. However, the signature nucleotides of this strain do not correspond to those of any of the existing subclasses of *Actinobacteria*.

The 16S rRNA sequence was aligned with those of selected strains representing all subclasses of *Actinobacteria* and with sequences of environmental clones corresponding to the 16S rRNA of uncultured *Actinobacteria*. The resulting tree (Fig. 4) confirms that strain ID131577T, together with sequences from uncultured bacteria and strain B33D1 (Furlong et al., 2002), constitutes a coherent clade belonging to a deep evolutionary line of descent within the lineage of the high-G+C-content Gram-positives. The group clusters with the 16S rRNA sequences of *T. album* and *T. minutum*. The most similar of the described *Actinobacteria* lineages is represented by the order *Rubrobacterales*, as shown also by the common signature at position 955 (see above). However, the exact branching order of the two groups
remains uncertain due to low bootstrap values. Our data, therefore, would not support the affiliation of ID131577\textsuperscript{T} to any of the described Actinobacteria taxa. On the contrary, ID131577\textsuperscript{T} appears to be the first characterized representative of a novel taxon, the existence of which had been postulated on the basis of several 16S rRNA environmental clones (TM group I; Rheims et al., 1996).

High-G+C-content Gram-positive bacteria are characterized by the presence of an insertion of ~100 nt within domain III of their 23S rRNA genes (Roller et al., 1992), which is present in all members of the subclass Actinobacteridae analysed so far (Embley & Stackebrandt, 1994). No data are available for the other Actinobacteria subclasses. However, according to unpublished observations reported by Embley & Stackebrandt (1994), this feature is lacking in Atopobium minutum. The 23S rRNA gene of strain ID131577\textsuperscript{T} was partially amplified by PCR and sequenced (1821 nt, corresponding to 62.7% of the E. coli 23S rRNA; Brosius et al., 1980). Sequence analysis revealed that the 100 nt insertion is not present in strain ID131577\textsuperscript{T}, suggesting that deep-branching lineages within the class might not share this feature with the Actinobacteridae.

Similarity with available sequences is low and limited to conserved regions within the 23S rRNA gene (not shown). This finding was expected because, within the class Actinobacteria, 23S rRNA sequences are available only for members of the subclass Actinobacteridae.

### Chemotaxonomic characteristics

The peptidoglycan of strain ID131577\textsuperscript{T} contained meso-A\textsubscript{2}pm and is directly cross-linked (type A1\textsubscript{G}; Schleifer & Kandler, 1972). Mycolic acids were not present. Strain ID131577\textsuperscript{T} contains the menaquinone MK-7(H\textsubscript{4}) as the only component. The polar lipid pattern consisted of phosphatidylinositol and an additional but unknown phospholipid component, amino-functional lipids or glycolipids could not be detected. The fatty acid profile of strain ID131577\textsuperscript{T} was composed of C\textsubscript{12}:0(10.9%), iso-C\textsubscript{16}:0 (16.3%), C\textsubscript{17}:1\textsubscript{\omega}6c (13.9%), C\textsubscript{16}:0 (12.7%), C\textsubscript{16}:1\textsubscript{\omega}7c (1.9%), C\textsubscript{16}:0 (1.6%), C\textsubscript{17}:1\textsubscript{\omega}8c (1.5%), C\textsubscript{19}:1\textsubscript{\omega}6c (1.5%) and iso-C\textsubscript{17}:0 (1.2%) fatty acids representing less than 1% of the total fatty acids are not reported). There was no match to any entry in the TSB40 MIS library. The DNA G+C content of strain ID131577\textsuperscript{T} was 71 mol%.

Although strain ID131577\textsuperscript{T} is sufficiently differentiated by its distant phylogenetic position, its chemotaxonomic features were compared to the data available for representatives of deeply branching lineages of descent of the class Actinobacteria for informative purposes. The peptidoglycan type A1\textsubscript{G}; based on meso-A\textsubscript{2}pm, of strain ID131577\textsuperscript{T} is not found in cultured members of the subclasses Rubrobacteridae, Sphaerobacteridae or Coriobacteridae. Lysine was reported for Rubrobacter radiotolerans (Suzuki et al., 1988), R. xylanophilus (peptidoglycan type A3\textsubscript{\alpha}, L-Lys⇒L-Ala; Carreto et al., 1996) and Coriobacterium glomerans (peptidoglycan type A4\textsubscript{\alpha}, L-Lys⇒Asp; Haas & König, 1988). The peptidoglycan of the genus Atopobium contains either ornithine (Atopobium minutum, L-Orn⇒L-Ser⇒D-Glu; N. Weiss, personal communication) or lysine (Atopobium parvulum, L-Lys⇒D-Asp; Weiss, 1981). Sphaerobacter thermophilus shows the peptidoglycan type A3\textsubscript{B} L-Orn⇒β-Ala (Demharter et al., 1989). A unique feature of strain ID131577\textsuperscript{T} is the occurrence of the tetrahydrogenated menaquinone MK-7(H\textsubscript{4}), which has not been found as a major component in other bacteria before. The genera Rubrobacter and Sphaerobacter contain the completely unsaturated menaquinone MK-8 (Suzuki et al., 1988; Carreto et al., 1996; Demharter et al., 1989). 12-Methylhexadecanoic, 12-methylheptadecanoic and 12-methyl-16-methyloctadecanoic acids, characteristic of Rubrobacter species (Suzuki et al., 1988; Carreto et al., 1996), could not be detected in strain ID131577\textsuperscript{T}. Unfortunately, no chemotaxonomic data are available for Acidimicrobium ferrooxidans. Strain ID131577\textsuperscript{T} belongs to the high-G+C group of deeply branching
members of the class Actinobacteria represented by the genera 
Rubrobacter (G+C content 68 mol%; Suzuki et al., 1988; Carreto et al., 1996), Sphaerobacter (66 mol%; Demharter et al., 1989), Coriobacterium (60–61 mol%; Haas & König, 1988) and Acidimicrobium (67–68.5 mol%; Clark & Norris, 1996). The genus Atopobium displays considerably lower G+C values, of 35–46 mol% (Collins & Wallbanks, 1992).

Physiological properties

Strain ID131577T is catalase- and oxidase-positive and reduces nitrate to nitrite. It does not decompose urea and does not grow on media containing 2% (w/v) NaCl or more. Gelatin and aesculin are hydrolysed. Strain ID131577T is able to utilize L-arabinose, D-ribose, D-xylene, acetic acid, z-ketovaleric acid, propionic acid, pyruvic acid, glycerol (Biolog GP microplate), methylpyruvate, β-hydroxybutyric acid, z-ketoglutaric acid and z-ketovaleric acid (Biolog GN microplate). Strain ID131577T showed the following enzyme activities: esterase for 2-naphthyl caprylate and 2-naphthyl butyrate, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase (API ZYM).

All other tests of the Biolog GP and GN microplate substrate panels, of the API 20 NE test and of the API ZYM enzyme assay were negative, as indicated in the species description.

The data reported here show that strain ID131577T is indeed divergent from known bacterial species. The two strains with higher 16S rRNA sequence relatedness, T. album and T. minutum, were described as Gram-negative, non-motile and obligately thermophilic, growing only on n-alkanes containing between 13 and 20 carbon atoms (Zarilla & Perry, 1984, 1986), and are clearly different from strain ID131577T. Albeit not isolated hitherto, the strain is relatively easy to cultivate with standard techniques and does not require unusual media. We cannot exclude the possibility that strain ID131577T was recovered only because of its physical association with a Dactylosporangium isolate. However, we are inclined to believe that this was only a fortuitous event, and ID131577T may be obtained directly after plating on HV/2 medium.

Due to the divergence of the isolate from known genera, it is proposed that a new genus, Conexibacter gen. nov., should be established in order to harbour strain ID131577T. As none of the closest phylogenetic relatives of strain ID131577T has been cultured, we are aware that the description based on a single isolate cannot reflect the phenotypic diversity of the genus. Despite its phylogenetically remote position, we refrain from proposing a novel family, as the 16S rRNA signature nucleotides of a single strain cannot define the phylogenetic depth and width of a higher taxon. The type species of the genus is Conexibacter woesei gen. nov., sp. nov., represented by the type strain ID131577T (= DSM 14684T = JCM 11494T).

Description of Conexibacter gen. nov.

Conexibacter (Co.nex.i.bacter. L. part. adj. conexus bound, tied; N.L. masc. n. bacter from Gr. n. baktron rod; N.L. masc. n. Conexibacter a rod that is bound).

Cells are small rods (0.6–0.7×0.9–1.2 μm), occurring singly or in pairs. Gram-positive and non-sporulating. Motile by long, peritrichous flagella. Aerobic. Catalase- and oxidase-positive. The peptidoglycan is of the A1γ type (based on meso-diaminopimelic acid, direct cross-linkage). Mycolic acids are absent. The major isoprenoid quinate is menaquinone MK-7 (H4). The polar lipid pattern consists of phosphatidylglycerol and an unidentified phospholipid; amino-functional lipids and glycolipids are absent. The fatty acid profile is dominated by oleic, 14-methyl-pentadecanoic, hexadecanoic and 9cis-heptadecenoic acid. The G+C content of the DNA is 71 mol%. Phylogenetically, the genus is a member of the class Actinobacteria. The type species is Conexibacter woesei.

Description of Conexibacter woesei sp. nov.

Conexibacter woesei (woe’sei. N.L. gen. n. woesei of Woese, named to honour Carl R. Woese, for his pioneering work on the use of 16S rRNA in phylogenetic analysis).

In addition to the properties described for the genus, colonies on Todd–Hewitt agar are smooth, mucoid to sticky and of white to cream colour. Nitrate is reduced to nitrite. Gelatin and aesculin are hydrolysed, urea is not decomposed. NaCl is not tolerated at concentrations of 2% (w/v) or above. The type strain is able to utilize the following substrates: glycerol, L-arabinose, D-ribose, D-xylene, acetic acid, z-ketovaleric acid, propionic acid, pyruvic acid (Biolog GP microplate), methylpyruvate, β-hydroxybutyric acid, z-ketoglutaric acid and z-ketovaleric acid (Biolog GN microplate). The type strain shows the following enzyme activities: esterase for 2-naphthyl caprylate and 2-naphthyl butyrate, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase (API ZYM test). The following tests of the Biolog substrate panels, API 20 NE gallery and API ZYM enzyme assay are negative: mannann, α-cyclodextrin, dextrin, glycogen, Tween 40, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, z-D-glucose, m-inositol, z-lactose, z-D-lactose lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xyitol, monomethyl succinate, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluciconic acid, D-glucosaminic acid, D-glucuronic acid, z-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, itaconic acid, z-ketobutyric acid, DL-lactic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyL L-aspartic acid, glycyL L-glutamic acid, L-histidine, hydroxy-L-proline,
l-leucine, l-ornithine, l-phenylalanine, l-proline, l-pyroglutamic acid, D-serine, l-serine, l-threonine, Dl-carnitine, γ-amino butyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, β-cyclodextrin, inulin, N-acetylmannosamine, amygdalin, arbutin, lactulose, maltotriose, D-melezitose, methyl α-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl α-D-mannoside, palatinose, salicin, sedoheptulose, stachyose, D-tagatose, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, l-malic acid, methyl succinate, N-acetyl-l-glutamic acid, adenosine, 2′-deoxyadenosine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, fructose 6-phosphate, caprate, adipate, phenylacetate, degradation of tryptophan, fermentation of glucose, arginine dihydrolase, urease, alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, N-acetyl-α-glucosaminidase and α-fucosidase. Good growth occurs at pH 7–7.5 and at 28–37°C. The type strain is susceptible to amikacin (30 μg), gentamicin (10 μg), nitrofurantoin (300 μg), novobiocin (30 μg), polymyxin B (300 IU) and teicoplanin (30 μg) and only weakly susceptible to chloramphenicol (30 μg), erythromycin (15 μg), tetracycline (30 μg) and vancomycin (30 μg). Cells are resistant to ampicillin (10 μg), aztreonam (100 μg), cefazidime (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), kanamycin (30 μg), methicillin (5 μg), norfloxacin (10 μg), oxacillin (1 μg), rifampicin (30 μg), streptomycin (10 μg), trimethoprim (5 μg) and tobramycin (10 μg). Chemotaxonomic characteristics are as given in the genus description. The G+C content of the DNA is 71 mol%. Habitat: temperate forest soil. The type strain is ID131577T (= DSM 14684T = JCM 11949T).

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