**Catenulispora acidiphila** gen. nov., sp. nov., a novel, mycelium-forming actinomycete, and proposal of **Catenulisporaceae** fam. nov.

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A novel, Gram-positive bacterial strain was isolated from forest soil. Among species with validly published names, the 16S rRNA gene sequence is related most closely (approx. 93 % similarity) to that of **Sporichthya polymorpha** DSM 43042T. However, differently from this species, it forms both vegetative and aerial mycelia. The aerial hyphae are straight to slightly flexuous, starting to septate to form chains of more than 20 cylindrical spores with a rugose surface. The strain is acidophilic, with a pH range for robust growth between 4·3 and 6·8 and an optimum around 6·0. The peptidoglycan type is A3γ: LL-Dpm–Gly. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and two unknown phospholipids. Predominant menaquinones are MK-9(H6) and -9(H4), and iso- and anteiso-branched C16:0 and C17:0 are the main cellular fatty acids. The DNA G+C content is 71·9 mol%. The distinct phylogenetic position and the unusual combination of chemotaxonomic characteristics justify the proposal of **Catenulispora** gen. nov., with the type species **Catenulispora acidiphila** sp. nov. (type strain, ID139908T = DSM 44928T = NRRL B-24433T).

**Catenulisporaceae** fam. nov. is also proposed.

### Isolation and cultivation of strain ID139908T

A soil sample collected from a wooded area in Gerenzano, Italy, was dried at 30°C under vacuum for 7 days, resuspended in 18·2 mM citric acid, 164 mM Na2HPO4 buffer (pH 7), and aliquots from serial dilutions were deposited onto GTV plates [GTV contains 500 ml soil extract 1⁻¹ (prepared by autoclaving 100 g fresh soil resuspended in 500 ml H2O, followed by filtering through sterile gauze), 10 g gellan gum ml⁻¹, 3 mM CaCl2 (resulting pH, 5) and was supplemented after autoclaving with 0·1 % (v/v) CMM vitamin solution (25 µg thiamin hydrochloride ml⁻¹, 250 µg calcium pantothenate ml⁻¹, 250 µg nicotinic acid ml⁻¹, 500 µg biotin ml⁻¹, 1·25 µg riboflavin ml⁻¹, 6 µg vitamin B12 ml⁻¹, 25 µg p-aminobenzoic acid ml⁻¹, 500 µg folic acid ml⁻¹ and 500 µg pyridoxal hydrochloride ml⁻¹) and 50 µg cycloheximide ml⁻¹] and incubated at 28°C. Seven strains, which appeared after an 8 week incubation, were related closely on the basis of their 16S rRNA gene sequences. One of these isolates was chosen for further studies and maintained on ISP2 agar (Shirling & Gottlieb, 1966) adjusted to pH 5·5–6·0 with HCl, and was designated strain ID139908T.

### Morphology

Morphology of aerial mass was examined directly on HSA5 agar plates [HSA5 consists of 0·5 g humatic acid l⁻¹,
Field emission scanning electron microscopy (FESEM) was performed as described elsewhere (Hammerschmidt et al., 2005) by fixing samples of 3-week-old HSA5 agar cultures of strain ID139908T with glutaraldehyde (2 %) and formaldehyde (5 %). FESEM revealed long filaments of aerial hyphae that showed marked septation, resulting in chains of more than 20 cylindrical arthrospores (see Supplementary Fig. S1, available in IJSEM Online). Spores show a rugose surface and are 0.4–1.0 μm long, with a mean diameter of 0.5 μm (Fig. 1).

**Cultural and physiological characteristics**

The media used were those recommended by Shirling & Gottlieb (1966), but acidified to pH 5.5–6.0 with HCl. Inocula for cultural and physiological analyses were obtained by growing strain ID139908T in ATSB medium (17 g casitone l⁻¹, 3 g soytone l⁻¹, 2.5 g glucose l⁻¹, 10 mM MES, adjusted to pH 5.5–6.0 with HCl) for 1 week at 28 °C on a rotary shaker at 200 r.p.m. Mycelium was washed with sterile H₂O and then diluted to a suitable inoculum. Aliquots of the suspension were streaked in a cross-hatched manner onto the media used. Unless stated otherwise, all evaluations were made after 3 weeks incubation at 28 °C. The pH range for growth was determined by using ISP2 as basal medium, adjusted to the desired values with HCl or NaOH. Temperature range for growth and NaCl and lysozyme tolerance were determined by using acidified (pH 5.5–6.0) ISP2 medium.

Table 1 shows the appearance of strain ID139908T on various agar media. It grew well on most of the tested media, with production of brownish pigments and whitish aerial mass, turning to yellow/green with age. However, the brownish pigments were not observed in tyrosine-supplemented Suter medium (Suter, 1978), suggesting that they are not melanin-related. Strain ID139908T grew well on media at initial pH between 4.3 and 6.8, with an optimum around pH 6–0, but scant growth was visible up to pH 7.5. This strain must thus be considered an acidophile. Optimal growth occurred between 22 and 28 °C and measurable growth also occurred at 11 and 32 °C.

Strain ID139908T could grow in the presence of up to 3 % (w/v) NaCl, albeit with a progressive reduction of pigmentation starting at 1 % NaCl, and was resistant to at least 100 μg lysozyme ml⁻¹. Among carbon sources, as recorded

### Table 1. Culture characteristics of strain ID139908T after 3 weeks growth at 28 °C

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth</th>
<th>Reverse*</th>
<th>Aerial mass</th>
<th>Diffusible pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISP2 (yeast extract–malt extract agar)</td>
<td>Abundant, convolute</td>
<td>Dark brown/nutmeg (15E8)</td>
<td>Absent</td>
<td>Abundant, brown</td>
</tr>
<tr>
<td>ISP3 (oatmeal agar)</td>
<td>Good, thin</td>
<td>Cream to light brown (12C6)</td>
<td>Moderate, whitish</td>
<td>Good, brownish</td>
</tr>
<tr>
<td>ISP4 (inorganic salts–starch agar)</td>
<td>Good</td>
<td>Cream to light orange (11F6)</td>
<td>Absent</td>
<td>Good, brownish</td>
</tr>
<tr>
<td>ISP5 (glycerol–asparagine agar)</td>
<td>Good</td>
<td>Brown/sienna (5G12)</td>
<td>Scant, whitish</td>
<td>Abundant, light brown</td>
</tr>
<tr>
<td>ISP6 (peptone–yeast extract–iron agar)</td>
<td>Absent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ISP7 (tyrosine agar)</td>
<td>Abundant</td>
<td>Brown/mahogany (6L12)</td>
<td>Moderate, whitish</td>
<td>Abundant, brown</td>
</tr>
</tbody>
</table>

*Colour codes in parentheses are according to Maerz & Paul (1950).
on acidified ISP9 medium (Shirling & Gottlieb, 1966), glucose, arabinose, xylose, mannitol, fructose and glycerol supported good growth, whereas no visible colonies were formed with sucrose, inositol, rhamnose or cellulose; scant growth was observed with raffinose. Aerobiosis-related properties were analysed on acidified ISP2 agar medium (plates were stored at 4°C and evaporated before use) under an aerobic, microaerophilic (GasPack system with a Gas Generating kit for Campylobacter; Oxoid BR056A) or anaerobic atmosphere. Strain ID139908T grew better under aerobic conditions, but reduced, non-pigmented growth was also obtained in microaerophilic and anaerobic atmospheres. The strain was positive for catalase (evaluated by mixing 0·8 ml of a 1-week-old ATSB culture with 0·5 ml freshly prepared 3% hydrogen peroxide), starch hydrolysis and gelatin liquefaction (Gottlieb, 1961), casein degradation [evaluated on acidified 1·5% (w/v) skimmed-milk agar] and H2S production (detected 2 days after inserting sterile lead acetate filter-paper strips into the necks of culture tubes containing acidified ISP6 medium). Strain ID139908T was negative for nitrate reduction [evaluated after 3 and 7 days in ATSB medium supplemented with 2 g KNO3 l−1, using Bacto-Nitrite test strips (Difco) to reveal the presence of nitrites]. When 15 μl of an antibiotic solution (10 μg ml−1) was spotted onto previously inoculated, acidified ISP2 plates, after 1 week at 28°C, strain ID139908T was found to be resistant to nalidixic acid, oxacillin, apramycin, daunomycin, GE2270, nisin, rifampicin and kanamycin, but sensitive to novobiocin, thiostrepton, A40926 and ramoplanin.

**Chemotaxonomic characteristics**

Freeze-dried biomass was obtained from a 1-week culture in ATSB medium. The peptidoglycan of strain ID139908T, analysed as described by Schleifer & Kandler (1972) and modified according to Willems et al. (1997), contained LL-diaminopimelic acid (Dpm), glycine, glutamic acid and alanine. From the two-dimensional TLC pattern (data not shown) of peptides in the partial peptidoglycan hydrolysate (4 M HCl, 100°C, 45 min), the peptidoglycan was assigned to type A3v LL-Dpm–Gly (A41.1 according to http://www.dsmz.de/species/murein.htm). Menaquinones [determined as described by Groth et al. (1997)] were MK-9(H6), -9(H4) and -9(H8) (ratio of peak areas, 4·5:2·8:1·0, respectively). Whole-cell sugars [determined according to Stanek & Roberts (1974)] contained large amounts of arabinose, together with xylose, ribose, rhamnose and glucose. The polar lipid pattern [determined according to Groth et al. (1997)] consists of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and two unknown phospholipids (see Supplementary Fig S2, available in IJSEM Online). The cellular fatty acid profile of strain ID139908T, analysed as described by Miller (1982), is composed of i-C16:0 (47·05%), ai-C17:0 (12·73%), i-C17:0 (5·73%), C16:0 (56·61%), i-C17:1<9c (5·89%), i-C15:0 (4·26%), i-C16:1 (3·41%), C16:1<7c (3·16%), ai-C17:1<9c (2·84%) and ai-C15:0 (2·25%), C16:1<9c (1·43%), C17:0 cyclo (1·17%) and C17:1<9c (1·06%) (fatty acids representing <1·00% of the total are not reported). The DNA G+C content of strain ID139908T, determined according to Mesbah et al. (1989), is 71·5 mol%.

Although strain ID139908T and members of the family Streptomycesaceae share the presence of LL-diaminopimelic acid in the peptidoglycan and type 2c fatty acids (sensu Kroppenstedt, 1985), their phospholipid and whole-cell sugar patterns, as well as the menaquinone composition, clearly differentiate them (Korn-Wendisch & Kutzner, 1992; Zhang et al., 1997; Kim et al., 2003).

**Phylogenetic analysis**

The almost-complete 16S rRNA gene sequence for strain ID139908T [1441 nt, corresponding to 93·3% of the Escherichia coli sequence (Brosius et al., 1978)] was determined and compared with all GenBank entries as described previously (Monciardini et al., 2003). The partial 16S rRNA gene sequence of strain ID139908T is 98·6–99·5% identical to those of the actinomycete strains Ellin 5093, 5062, 5034, 5119 and 5116 (Joseph et al., 2003), recently isolated from a pasture soil in Ellinbank, Australia, and >99% identical to those of ‘ bacterium 12202’ (GenBank accession no. AY639903) and ‘ Actinobacterium Aac30’ (GenBank accession no. AB180773). No description of these strains has, to our knowledge, appeared in the literature. Among species with validly published names, the highest binary similarity values are with members of the suborder Frankineae, namely Sporichthya polymorpha DSM 43042T (93%) and Geodermatophilus obscurus DSM 43161 (92·5%). Representatives of several actinomycete lineages have 16S rRNA gene sequence similarities to ID139908T of between 91 and 92%. Although strain ID139908T clearly belongs to the order Actinomycetales, it does not seem to be affiliated to any of the described lineages within the order. Of the two described bacterial strains with the highest 16S rRNA gene sequence relatedness to strain ID139908T, S. polymorpha DSM 43042T also contains LL-diaminopimelic acid in its peptidoglycan, but the menaquinones differ, as strain ID139908T has MK-9(H6) and -9(H4) as major components, in contrast to DSM 43042T, which has MK-9(H6) and -9(H3) (Rainey et al., 1993). The fatty acid patterns also differentiate the two strains, as S. polymorpha is a 3c type, in contrast to ID139908T, which is of the 2c type (Kroppenstedt, 1985). Morphologically, the two strains are also completely different, as S. polymorpha exhibits a unique life cycle with the absence of a vegetative mycelium and the aerial mycelium dividing into rod-shaped to coccoid spores that are motile (Lechevalier & Lechevalier, 1989). G. obscurus DSM 43161 is even more different from ID139908T, as the two strains do not share any chemotaxonomic markers (Goodfellow, 1989) and G. obscurus does not show the growth-cycle characteristics of filamentous actinomycetes (Luedemann & Fonseca, 1989). Phenotypic characteristics of other members of the suborder Frankineae...
can be found elsewhere (Tamura et al., 1998; Maszenan et al., 2005).

When the sequence of strain ID139908T was aligned with those deposited for the Ellinbank isolates and with those obtained from similar strains isolated in our laboratory, we could identify the signature nucleotides of the order Actinomycetales with the exception of position 449, where a C is found instead of the A proposed for the order (Stackebrandt et al., 1997). However, we could not find the signature nucleotide pattern of any of the other described suborders (Stackebrandt et al., 1997). Of the six signatures described for the suborder Frankineae, three are not found in ID139908T and related strains, namely 141:222 (A–U instead of G–C), 371:390 (A–U instead of G–C) and 1003:1037 (G–C instead of G–G).

The 16S rRNA gene sequence of strain ID139908T was aligned with those of the type species of the major actinomycete lineages and analysed as described previously (Monciardini et al., 2003). The resulting phylogenetic tree is shown in Fig. 2. As no obvious relatives of the novel strain could be identified, we included in the analysis representatives of different families of the various suborders, with a particular focus on filamentous actinomycetes. Strain ID139908T, together with the Ellinbank isolates (Joseph et al., 2003), forms a coherent clade within the Actinomycetales lineage, clearly distinguished from other described strains. Although the closest relatives of the novel lineage appear to be members of the suborder Frankineae, bootstrap values are too low to allow definitive phylogenetic placement within this suborder, as also suggested by the low pairwise identity of 16S rRNA gene sequences and by the differences in the pattern of signature nucleotides. A phylogenetic tree obtained from the alignment of the 16S rRNA gene sequences of representatives of the described families of the suborder Frankineae (Tamura et al., 1998; Maszenan et al., 2005) with those of strain ID139908T and related strains further confirms that the two lineages are independent (see Supplementary Fig. S3, available in IJSEM Online). Additional phylogenetic analyses, performed with different sequences and treeing methods, confirm the high divergence of ID139908T and related strains from representatives of described families of the Actinomycetales (not shown). Altogether, phylogenetic data indicate that strain ID139908T is sufficiently divergent from known bacterial species as to be described as being representative of a novel genus.

![Fig. 2. Neighbour-joining tree based on 1324 aligned positions within the 16S rRNA gene. GenBank accession numbers for the sequences are in parentheses. The tree was rooted by using the 16S rRNA gene sequence of *Bifidobacterium bifidum* NBRC 14252T (GenBank accession no. S83624). Numbers at nodes are bootstrap values based on 100 resamplings; only values >65 are shown. Bar, 1 inferred nucleotide substitution per 100 nt.](image-url)
addition, these data support the hypothesis that strain ID139908, along with the closely related Ellinbank strains, represents a novel family within the order Actinomycetales. Thus, it is proposed that Catenulispora gen. nov. should be established in order to harbour strain ID139908. Following the guidelines for the affiliation to higher hierarchical taxa in the class Actinobacteria proposed by Stackebrandt et al. (1997), which base the clustering solely on phylogenetic relationships, we propose the description of Catenulisporaceae fam. nov. to accommodate the proposed genus Catenulispora.

Description of Catenulisporaceae fam. nov.

(Ca.te.nu.li.spo.ra.ceae. N.L. fem. n. Catenulispora type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. Catenulisporaceae the Catenulispora family).


Description of Catenulispora gen. nov.

Catenulispora (Ca.te.nu.li.spo.ra. L. fem. n. catenula small chain; Gr. fem. n. spora seed; N.L. fem. n. Catenulispora a thin chain of spores).

Gram-positive, acidophilic, non-acid-fast, aerobic organisms forming branching hyphae. Non-fragmentary vegetative mycelium and aerial hyphae starting to septate in chains of cylindrical arthrospores are produced. Motile elements are not produced. Peptidoglycan contains LL-diaminopimelic acid, glycine, glutamic acid and alanine. Glucose, xylose, ribose, rhamnose and arabinose (the latter in large amounts) are detected as whole-cell sugars. i-C16:0 and ai-C17:0 are present as major cellular fatty acids. Menaquinones MK-9(H6) and MK-9(H4) are predominant, but MK-9(H8) is also detected. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidyl-inositol mannosides and two unknown phospholipids. The G+C content of the DNA is 71 mol%. The type species is Catenulispora acidiphila.

Description of Catenulispora acidiphila sp. nov.

Catenulispora acidiphila (a.c.i.di’phi.la. N.L. neut. n. acidum acid; Gr. adj. philos loving; N.L. fem. adj. acidiphila acid-loving).

Chemotaxonomic and general characteristics are the same as given above for the genus. Acidophilic: grows well in a pH range from 4·3 to 6·8, optimal around 6·0. Mesophilic: best growth occurs at 22–28 °C, but significant growth can also be observed at 11 and 37 °C; no growth occurs at 4 or 40 °C. Aerial hyphae are relatively short, straight to flexuous and produce chains of more than 20 cylindrical arthrospores. Spores have a length in the range 0·4–1·0 μm and a mean diameter of around 0·5 μm. Spore surface is rugose. The organism grows well on various media, better on acidic yeast extract–malt extract agar and acidic tyrosine agar. In general, the soluble pigments produced by the type strain, as well as the vegetative mycelium, are brownish, whilst the aerial mass is whitish, turning to yellow/green with ageing. Brown mahogany soluble pigment is produced on tyrosine agar; no pigmentation is observed on tyrosine-supplemented Suter synthetic medium. H2S is produced. Nitrates are not reduced. Starch and casein are hydrolysed. Gelatin is liquefied. Catalase-positive. Up to 3 % (w/v) NaCl is tolerated, as well as 100 μg lysozyme ml−1. Glucose, arabinose, xylose, mannitol, fructose and glycerol are utilized, whereas sucrose, inositol, rhamnose and cellulose are not. Resistant to nalidixic acid, oxacillin, apramycin, daunomycin, GE2270, nisin, rifampicin and kanamycin at 10 μg ml−1, but sensitive to the same concentration of novobiocin, thiostrpton, A40926 and ramoplanin. The G+C content of the DNA is 71·5 mol%.

The type strain, ID139908T (= DSM 44928T = NRRL B-24433T), was isolated from temperate forest soil.

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References


