Fodinicola feengrottensis gen. nov., sp. nov., an actinomycete isolated from a medieval mine

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A filamentous, Gram-positive actinobacterium was isolated from acidic rocks in a medieval alum slate mine and was investigated by means of a polyphasic taxonomic approach. A 16S rRNA gene sequence similarity study indicated that strain HKI 0501\textsuperscript{T} forms an individual line of descent and is related to certain members of the suborder Frankineae, order Actinomycetales (~95% sequence similarity). Distance-matrix and neighbour-joining analyses set the branching point of the novel isolate between two clades, one being represented by members of the genus Cryptosporangium (family ‘Kineosporiaceae’) and the other by members of the genera Frankia and Acidothermus (family Frankiaceae and family Acidothermaceae, respectively). The organism had meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan and xylose as the characteristic cell-wall sugar. The muramic acid in the peptidoglycan was found to be N-acetylated. The major menaquinones were MK-9(H\textsubscript{4}), MK-9(H\textsubscript{6}) and MK-9(H\textsubscript{8}) and the fatty acid profile was characterized by the predominance of iso-C\textsubscript{16:0}, 10-methyl C\textsubscript{17:0} \textsubscript{cis}9 and 10-methyl iso-C\textsubscript{18:0}. The polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and several unknown phospholipids and glycolipids. Mycolic acids were absent. The DNA G+C content was 65 mol\%. The distinct phylogenetic position and the phenotypic markers that clearly separate the novel organism from all other members of the suborder Frankineae indicate that strain HKI 0501\textsuperscript{T} represents a novel genus and species, for which the name Fodinicola feengrottensis gen. nov., sp. nov. is proposed. The type strain of Fodinicola feengrottensis is HKI 0501\textsuperscript{T} (=DSM 19247\textsuperscript{T} =JCM 14718\textsuperscript{T}).


The members of the suborder Frankineae are morphologically and biochemically heterogeneous: the genera can be readily distinguished from one another by using a combination of chemotaxonomic and morphological properties (Table 1). Members of this suborder have been isolated from various specialized habitats (plants, hot springs, stone surfaces, activated sludge and geographically diverse soils). Most of the strains are characterized by low growth rates and fastidious growth requirements. At the time of writing, all of the genera affiliated to this suborder are grossly underspeciated.

The present polyphasic study was designed to determine the taxonomic position of a filamentous bacterial strain
Table 1. Characteristics that serve to differentiate strain HKI 0501^T from members of genera classified in the suborder Frankineae


<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cellular morphology</th>
<th>Spore/bud formation</th>
<th>Motility</th>
<th>Cell-wall diamino acid(s)</th>
<th>Major menaquinone(s)</th>
<th>Polar lipid(s)*</th>
<th>Predominant fatty acid(s)</th>
<th>DNA G + C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fodinicola gen. nov.</strong></td>
<td>Substrate and aerial hyphae</td>
<td>−</td>
<td>−</td>
<td>meso-A₂pm</td>
<td>MK-9(H₄), MK-9(H₆),</td>
<td>DPG, PE, PS, PI, PL, GL</td>
<td>iso-C₁₆:₀, C₁₇:₁ cis9</td>
<td>65</td>
</tr>
<tr>
<td>(strain HKI 0501^T)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MK-9(H₄)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Frankiaceae</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Frankia</strong></td>
<td>Substrate hyphae; no aerial mycelium; multilocular sporangia</td>
<td>Sporangiospores</td>
<td>−</td>
<td>meso-A₂pm</td>
<td>MK-9(H₄), MK-9(H₆),</td>
<td>PL, PIM, DPG</td>
<td>iso-C₁₅:₁, iso-C₁₆:₀, C₁₇:₁</td>
<td>66–71</td>
</tr>
<tr>
<td><strong>Geodermatophilaceae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MK-9(H₆), MK-9(H₈)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Geodermatophilus</strong></td>
<td>Thallus consisting of cuboid to oval cells; multilocular sporangia; rudimentary hyphae; no aerial mycelium</td>
<td>Zoospores</td>
<td>+/−</td>
<td>meso-A₂pm</td>
<td>MK-9(H₄)</td>
<td>PE, PIM, DPG</td>
<td>iso-C₁₆:₀, iso-C₁₅:₀, iso-C₁₇:₀</td>
<td>73–75</td>
</tr>
<tr>
<td><strong>Blastococcus</strong></td>
<td>Cocci, rods, vibrios; pairs, tetrads; clusters</td>
<td>Buds</td>
<td>+/−</td>
<td>meso-A₂pm</td>
<td>MK-9(H₄), MK-9(H₆),</td>
<td>DPG, PG, PI, PE</td>
<td>iso-C₁₅:₀, C₁₈:₁, C₁₇:₁, iso-C₁₆:₁, C₁₈:₁, C₁₉:₁</td>
<td>74</td>
</tr>
<tr>
<td><strong>Modestobacter</strong></td>
<td>Rods and cocci</td>
<td>Buds</td>
<td>+/−</td>
<td>meso-A₂pm</td>
<td>MK-9(H₆), MK-8(H₄),</td>
<td>DPG, PE, PG</td>
<td>iso-C₁₅:₀, C₁₆:₀, C₁₇:₀, anteiso-C₁₇:₀</td>
<td>68–70</td>
</tr>
<tr>
<td><strong>Nakamurellaceae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MK-9(H₆), MK-9(H₈)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nakamurella</strong></td>
<td>Cocci; pairs; clusters</td>
<td>−</td>
<td>−</td>
<td>meso-A₂pm</td>
<td>MK-8(H₄)</td>
<td>DPG, PE, PE-dimethyl</td>
<td>iso-C₁₆:₀, iso-C₁₅:₀, C₁₇:₁, C₁₈:₁, C₁₉:₁</td>
<td>68</td>
</tr>
<tr>
<td><strong>Humicoccus</strong></td>
<td>Cocci</td>
<td>−</td>
<td>−</td>
<td>meso-A₂pm</td>
<td>MK-8(H₄), MK-9(H₄)</td>
<td>DPG, PE, PE-dimethyl</td>
<td>iso-C₁₅:₀, C₁₇:₀, anteiso-C₁₇:₀</td>
<td>73</td>
</tr>
<tr>
<td><strong>Quadrisphaera</strong></td>
<td>Cocci; tetrads; clusters</td>
<td>−</td>
<td>−</td>
<td>meso-A₂pm</td>
<td>MK-8(H₂)</td>
<td>DPG, PG, PI</td>
<td>iso-C₁₅:₀, C₁₆:₀, C₁₇:₀</td>
<td>75</td>
</tr>
<tr>
<td><strong>Sporichthyaceae</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Sporichthya</strong></td>
<td>Short aerial hyphae; no substrate mycelium</td>
<td>Coccoid to rod-shaped spores</td>
<td>+</td>
<td>LL-A₂pm</td>
<td>MK-9(H₄), MK-9(H₆),</td>
<td>PI, PG, DPG, PL</td>
<td>iso-C₁₆:₀, iso-C₁₅:₀, C₁₇:₁, C₁₈:₁</td>
<td>71</td>
</tr>
<tr>
<td><strong>Acidothermaceae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MK-8(H₄), MK-8(H₆)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Acidothermus</strong></td>
<td>Slender rods, filaments</td>
<td>−</td>
<td>−</td>
<td>A₂pm, Ser, Ala</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>61</td>
</tr>
<tr>
<td><strong>Kineosoria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kineosoria</strong></td>
<td>Substrate hyphae; no aerial mycelium; elongated, club-shaped sporangia</td>
<td>Spherical to ovoid spores</td>
<td>+</td>
<td>LL-A₂pm and meso-A₂pm</td>
<td>MK-9(H₄), MK-8(H₄),</td>
<td>PC, DPG, PI, PIM</td>
<td>iso-C₁₆:₀, C₁₈:₁, 10-methyl C₁₈:₀</td>
<td>69–71</td>
</tr>
<tr>
<td><strong>Cryptosporangium</strong></td>
<td>Substrate and aerial mycelia; sporangiospores</td>
<td>Sporangiospores</td>
<td>+</td>
<td>meso-A₂pm</td>
<td>MK-9(H₄), MK-9(H₄),</td>
<td>PE</td>
<td>iso-C₁₆:₀, C₁₇:₁, C₁₈:₁</td>
<td>70</td>
</tr>
<tr>
<td><strong>Kineococcus</strong></td>
<td>Cocci; pairs, tetrads; clusters</td>
<td>−</td>
<td>+</td>
<td>meso-A₂pm</td>
<td>MK-9(H₄), MK-9(H₄),</td>
<td>DPG, PG, GL</td>
<td>anteiso-C₁₅:₀, C₁₈:₁, C₁₉:₁</td>
<td>74</td>
</tr>
</tbody>
</table>

*DPG, Diposphatidylglycerol; GL, unknown glycolipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE-dimethyl, phosphatidylmethylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid(s).
that had been isolated from a medieval alum slate mine. The resultant phylogenetic and phenotypic data showed that the novel organism should be classified in the suborder Frankineae within a novel genus and species.

Strain HKI 0501T was isolated from acidic and heavy metal-containing rocks in the ‘Barbara Grotto’ of the Feengrotten medieval alum slate mine in Saalfeld, Thuringia, Germany. Material from the rock surface was scraped off with a sterile cotton swab and the adhering bacteria were dispersed in about 1 ml sterile distilled water. Aliquots of the resultant suspension were spread over starch-casein agar plates (Küster & Williams, 1964) supplemented with cycloheximide (50 μg ml⁻¹). The agar plates were incubated at 28 °C for about 4 weeks. Subcultivation of the isolate was done on solidified organic medium 79 (Prauser & Falta, 1968; http://www.dsmz.de/microorganisms/html/media/medium000426.html) and ISP 2 medium (Difco; Shirling & Gottlieb, 1966). Pure cultures of strain HKI 0501T were preserved at −80 °C as a mixture of well-growing cultures in organic medium 79 broth and glycerol medium that consisted of K₂HPO₄ (1.26 %), KH₂PO₄ (0.36 %), MgSO₄ (0.01 %), sodium citrate (0.09 %), (NH₄)₂SO₄ (0.18 %) and glycerol (8.8 %). Stock cultures of the novel isolate in liquid organic medium 79 supplemented with 5 % DMSO were also maintained in the vapour phase of liquid nitrogen.

Bacterial growth for chemotaxonomic and molecular systematic studies was prepared by cultivating strain HKI 0501T at 28 °C for 2–7 days in liquid organic medium 79 or Bacto tryptic soy broth (Sigma-Aldrich). Chromosomal DNA was extracted from the isolate by using a slightly modified version of the method of Pospiech & Neumann (1995). Amplification of the 16S rRNA gene and subsequent purification and direct sequencing of the respective PCR products were performed as described by Carlsohn et al. (2007).

The 16S rRNA gene sequence (1441 bp) was aligned to actinobacterial sequences from the database of the DSMZ using the ae2 editor (Maidak et al., 1997). Evolutionary distances were calculated by using the Jukes–Cantor method (Jukes & Cantor, 1969). Dendrograms were constructed by using the neighbour-joining and maximum-likelihood algorithms (Felsenstein, 1993) and by using the algorithm of De Soete (1983). Bootstrap analysis (500 resamplings) was used to evaluate the tree topology (Felsenstein, 1985).

Similarity values calculated for isolate HKI 0501T indicated a remote relationship (<95 % similarity) with members of the suborder Frankineae. Although similar values were shared with respect to type strains of both Cryptosporangium (family ‘Kineosporiaceae’) and Sporichthya (family Sporichthyaceae) (92.9–94.8 and 93.9–94.5 %, respectively), the two treeing algorithms applied to the Jukes–Cantor-corrected dissimilarity values clearly indicated an individual line of descent for strain HKI 0501T (see Fig. 1 for the neighbour-joining dendrogram); in view of its phylogenetically deep branching point within the family, HKI 0501T could be considered as representing a novel genus. Members of the genera Frankia and Acidothermus are slightly less closely related to isolate HKI 0501T (92.5–93.0 %) than members of the genus Cryptosporangium. According to the neighbour-joining Fig. 1. Phylogenetic dendrogram, based on 16S rRNA gene sequences and constructed from evolutionary distances (De Soete, 1983), showing the position of strain HKI 0501T within the radiation of members of the suborder Frankineae, order Actinomycetales (Stackebrandt et al., 1997). Numbers at branching points refer to bootstrap percentages (based on 500 resamplings); only values above 60 % are shown. Members of the family Microbacteriaceae were used to root the tree. Bar, 5 inferred nucleotide substitutions per 100 nucleotides.
dendrogram, the family ‘Kineosporiaceae’ does not emerge as a phylogenetically coherent family, perhaps because of novel entries into Frankineae that were not present when the suborder was originally defined (Garrity et al., 2007; Stackebrandt et al., 1997).

Strain HKI 0501T possesses most of the 16S rRNA gene sequence signatures defined for the suborder Frankineae (Stackebrandt et al., 1997), the exception being a U residue instead of a C residue at position 222. Several new genera have been described since the definition of the signatures for Frankineae, but no families have been proposed for these genera. Table 2 provides an update of the signatures for families described by Stackebrandt et al. (1997) and for novel genera that are not yet assigned to families. The intermediate branching position of strain HKI 0501T (Fig. 1) is confirmed by the moderate number of signatures shared with members of the Frankineae. Strain HKI 0501T shares more signatures with members of the Frankineae (40–60 %) than with members of the genera Kineosporia, Kineococcus and Quadrisphaera (35–40 %).

For morphological and cultural studies, strain HKI 0501T was cultivated on agar plates containing ISP media 2, 3, 4 and 5 (Difco; Shirling & Gottlieb, 1966), humic acid agar (Hayakawa & Nonomura, 1987), Bennett’s agar (Jones, 1949) and organic medium 79 for up to 21 days at 28 °C. Cell morphology and cell dimensions were examined using a phase-contrast microscope (Axioskop 2; Zeiss) equipped with image-analysing software (AXIOVISION 2.05; Carl Zeiss).

For scanning electron microscopy, samples were prepared by cutting agar blocks containing growing cells of strain HKI 0501T (humic acid agar, 21 days) and fixing them in a solution of 2.5 % glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2, for about 20 h at room temperature. Subsequently, samples were rinsed with 0.1 M sodium cacodylate solution and dehydrated through a graded ethanol series (30–100 %); this was followed by critical-point drying and sputter-coating with gold before observation under a LEO 1450 VP scanning electron microscope at an acceleration voltage of 15 kV and a working distance of 12 mm. Motility was checked as recommended by Tamura et al. (1999). Growth parameters (temperature, pH and tolerance of NaCl) were determined using organic medium 79. The pH range for growth was established in shake flasks of liquid medium adjusted to pH values between 4.5 and 10.0 with either 1 M HCl or 20 % (w/v) Na2CO3 solution after sterilization. The cultures were incubated at 28 °C for up to 7 days. Physiological tests, including the determination of enzyme activities and antibiotic susceptibilities, were carried out as described previously (Groth et al., 2003).

Strain HKI 0501T formed a branched substrate mycelium and sparse to abundant, short, white aerial hyphae that fragmented into irregular rod-like elements (Fig. 2). Spore chains and motility of the fragments, as reported for the coccoid to rod-shaped spores of the closely related Sporichthya strains (Lechevalier et al., 1968; Tamura et al., 1999), were not observed. Furthermore, the unique morphological properties of Sporichthya strains (lack of a substrate mycelium and the presence of a basal cell as a holdfast in solid medium) serve to distinguish strain HKI 0501T from members of that genus. Representatives of the equally closely related genus Cryptosporangium (Tamura et al., 1998) can be also readily distinguished from isolate HKI 0501T as they produce spherical to irregularly shaped sporangia with spores that show motility when they are

### Table 2. 16S rRNA signature nucleotides for families of the suborder Frankineae and some related genera

| Taxa: 1, strain HKI 0501T; 2, Frankineae; 3, Geodermatophilaceae; 4, Nakamuraella; 5, Humicoccus; 6, Sporichthyaaceae; 7, Acidothermaceae; 8, Kineosporia; 9, Cryptosporangium; 10, Kineococcus and Quadrisphaera. var., Variable sequence observed at these positions. |
|---------------------------------|---|---|---|---|---|---|---|---|---|---|
| **Positions** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
suspended in water. As shown in Table 1, the less closely related members of the genus *Frankia*, which comprise nitrogen-fixing root symbionts of dicotyledonous plants, and the only strain of the monospecific thermophilic genus *Acidothermus* can also be differentiated easily from strain HKI 0501 T on the basis of their typical morphological properties. The cultural characteristics of strain HKI 0501 T on different media are listed in Table 3. For laboratory cultivation, growth was generally improved when the culture media were acidified with HCl (after sterilization) to about pH 6.0–6.4. The physiological properties of strain HKI 0501 T are given in the species description.

Standard HPLC and TLC procedures were used to determine the isomers of diaminopimelic acid (A2pm) present in whole-organism hydrolysates (Hasegawa *et al.* 1983), the predominant cell-wall sugars (Becker *et al.*, 1965; Schön & Groth, 2006), the muramic acid type (Uchida & Aida, 1984), the fatty acid composition (MIDI system; http://www.midi-inc.com/), the predominant menaquinones (Collins *et al.*, 1977; Groth *et al.*, 1996), the presence of mycolic acids (Minnikin *et al.*, 1975) and the polar lipid pattern (Minnikin *et al.*, 1979; Collins & Jones, 1980). For determination of the molar ratios of the cell-wall amino acids, highly purified peptidoglycan preparations were obtained according to the method of Schleifer & Seidl (1985). The amino acid composition of the peptidoglycan hydrolysate (4 M HCl, 16 h, 100 °C) was determined by one-dimensional TLC on cellulose plates (Merck) using the solvent system of Rhuland *et al.* (1955) and by GC and GC/MS of amino acids (Schumann *et al.*, 1997) after derivatization according to MacKenzie (1987).

The G+C content of the DNA was estimated by monitoring the fluorescence intensity during DNA denaturation according to Xu *et al.* (2000) but using a MiniOpticon real-time PCR system (Bio-Rad) at a ramping rate of 0.1 °C s⁻¹ and genomic DNA from *Bacillus subtilis* subsp. *subtilis* strain 168 (DSM 402; G+C content 42 mol%; Kunst *et al.*, 1997) as the reference. The resulting value (65 mol%) was in good agreement with the 65.3 mol% obtained with the HPLC method (Mesbah *et al.*, 1989).

The cell-wall peptidoglycan of strain HKI 0501 T contained meso-A2pm, alanine, glycine and glutamic acid in a molar ratio of 1:0.9:2.4:1, respectively. The presence of meso-A2pm is a feature shared by most of the genera of the *Frankineae* (Table 1), the exceptions being the strains of the phylogenetically close genus *Sporichthya* (which possess LL-A2pm) and those of the distantly related genus *Kinesporia* (which are characterized by the common occurrence of both LL-A2pm and meso-A2pm). In *Acidothermus cellulolyticus*, the A2pm isomer has not been defined (Mohaghegh *et al.*, 1986). The main menaquinones in the novel isolate were MK-9(H4), MK-9(H6) and MK-9(H8) (similar proportions; ratio of HPLC peak areas, 31:29:29), this profile being shared with the majority of the members of the *Frankineae*. Comparable menaquinone profiles are not present in the coccoid genera *Nakamurella* and *Quadrisphaera* (family *Nakamurellaceae*). The predominant menaquinones in strains of these genera possess eight isoprene units. However, the type strain of the only species of the genus *Humicoccus*, which belongs to the same family, contains both MK-8(H4) and MK-9(H4) (similar amounts) as the main menaquinones. No data on

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### Table 3. Cultural characteristics of strain HKI 0501 T

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth</th>
<th>Substrate mycelium</th>
<th>Aerial mycelium</th>
<th>Soluble pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract-malt extract agar (ISP 2)</td>
<td>Good</td>
<td>Wrinkled, pale orange</td>
<td>Short, white</td>
<td>None</td>
</tr>
<tr>
<td>Oatmeal agar (ISP 3)</td>
<td>Poor</td>
<td>Wrinkled, orange</td>
<td>Sparse, short, white</td>
<td>None</td>
</tr>
<tr>
<td>Inorganic salts-starch agar (ISP 4)</td>
<td>Good, flat</td>
<td>Wrinkled, pale orange</td>
<td>Abundant, short, white</td>
<td>None</td>
</tr>
<tr>
<td>Glycerol-asparagine agar (ISP 5)</td>
<td>Poor, flat</td>
<td>Wrinkled, beige</td>
<td>Sparse, short, white</td>
<td>None*</td>
</tr>
<tr>
<td>Bennett’s agar</td>
<td>Good</td>
<td>Wrinkled, pale orange</td>
<td>Abundant, short, white</td>
<td>None</td>
</tr>
<tr>
<td>Humic acid agar</td>
<td>Good, flat</td>
<td>Orange-brown</td>
<td>Abundant, short, white</td>
<td>None</td>
</tr>
<tr>
<td>Organic medium 79 agar</td>
<td>Good</td>
<td>Wrinkled, beige to pale orange</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*After storage at room temperature for about a further 6 weeks, growth increased and a soluble yellow pigment was observed.*
menaquinones are available for A. cellulolyticus. Although these two chemotaxonomic traits are shared with other organisms, strain HKI 0501\textsuperscript{T} can be readily distinguished from its closest phylogenetic neighbours and from all other representatives of the suborder Frankineae on the basis of its cellular fatty acid profile, its polar lipid composition, the presence of xyllose as its diagnostic cell-wall sugar and its genomic DNA G+C content (65 mol%). The fatty acid profile (analysed from freeze-dried biomass grown in shake flasks of Bacto tryptic soy broth for 48 h at 28 °C) was characterized by a predominance of iso-C\textsubscript{16:0} (32.2 %), 10-methyl C\textsubscript{17:0} (10.7 %), C\textsubscript{17:1} \textit{cis}9 (8.9 %) and 10-methyl iso-C\textsubscript{18:0} (8.5 %) and the presence of smaller proportions of C\textsubscript{17:0} (7.4 %), iso-C\textsubscript{17:0} (5.8 %), C\textsubscript{18:1} \textit{cis}9 (4.8 %), 10-methyl C\textsubscript{18:0} (4.7 %), iso-C\textsubscript{18:0} (3.2 %), anteiso-C\textsubscript{17:0} (3.0 %), iso-C\textsubscript{16:0} \textit{2-}OH (2.7 %) and C\textsubscript{16:0} (2.6 %). The phospholipids comprise diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and several unknown phospholipids and glycolipids together with unknown ninhydrin-positive compounds.

The data obtained from the phenotypic characterization underline the separate position of strain HKI 0501\textsuperscript{T} in the 16S RNA gene sequence-based phylogenetic tree for the suborder Frankineae. It is evident from both the phylogenetic and phenotypic studies that strain HKI 0501\textsuperscript{T} cannot be classified within any genus of the suborder Frankineae and thus merits classification within a novel genus and species, for which the name Fodinicola feengrottensis gen. nov., sp. nov. is proposed.

**Description of Fodinicola gen. nov.**

_Fodinicola_ (Fod.in'i.co.la. L. n. _fodina_ a pit, mine; L. suff. -cola from L. n. _incola_ dweller; N.L. masc. n. _Fodinicola_ a mine dweller).

Gram-positive, aerobic, non-motile, catalase-positive, oxidase-negative actinomycetes that form branched substrate-(30). Displays the following properties in addition to those given in the genus description. Hyphal diameter is 0.35–0.52 μm. Diffusible pigments may be produced. Colonies are wrinkled and beige to orange in colour. Good growth occurs between 20 and 28 °C, but growth is not evident below 10 °C or above 32 °C. Grows well between pH 5.0 and 6.0, but does not grow at pH 4.0 or 8.0. Growth at pH 7.0 is delayed and reduced. 1 % NaCl in combination with organic medium 79 is tolerated, but 2 % NaCl is not. Aesculin, casein, gelatin, potato starch and urea are hydrolysed. Nitrate is not reduced to nitrite. Adenine, hypoxanthine and tyrosine are not degraded. L-Arabinose, D-fructose, D-glucose (weakly), D-mannitol, raffinose, L-rhamnose, sucrose and D-xyllose are used as sole carbon sources for energy and growth, but _myo_-inositol and cellulose are not (all at 1 %, w/v). Produces α-chymotrypsin (weakly), cystine arylamidase, leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (CB), _β_-galactosidase, _β_-galactosidase, N-acetyl-β-glucosaminidase, _α_-glucosidase, lipase (C14) (weakly), _α_-mannosidase, naphtho-AS-BI-phosphohydrolase, acid phosphatase and alkaline phosphatase, but not _α_ -fucosidase, _β_-glucosidase or _β_-glucuronidase. Production of trypsin is variable (API ZYM tests). Susceptible to the following antibiotics (µg per disc): chloramphenicol (30), ciprofloxacin (5), imipenem (10), kanamycin sulphate (30), norfloxacin (10), novobiocin (5), oxytetracycline hydrochloride (30), streptomycin sulphate (10), sulfonamide (200) and vancomycin hydrochloride (30). Resistant to the following antibiotics (µg per disc, unless otherwise indicated): ampicillin (10), lincomycin hydrochloride (2), meticillin (5), nalidixic acid (30), penicillin G (10 IU), polymyxin B (300 IU) and rifampicin (30).

The type strain, HKI 0501\textsuperscript{T} (=DSM 19247\textsuperscript{T} =JCM 14718\textsuperscript{T}), was isolated from rocks from a medieval alum slate mine in Thuringia, Germany.

**Acknowledgements**

The authors are grateful to Carmen Schult and Christiane Weigel for excellent technical assistance, to Dr Walter Richter (Friedrich Schiller University, Jena, Germany) for performing the scanning electron microscopy studies, to Gerald Lackner for assistance with the HPLC-based DNA G+C content measurements, to Bernd Lochner (Saalfelder Feengrott und Tourismus GmbH, Saalfeld, Germany) for his support in the sampling campaigns and to Jean P. Euzéby for his valuable suggestions regarding the naming of the novel organism.

**References**


