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^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(H₄), MK-9(H₆) and MK-9(H₈) and the fatty acid profile was characterized by the predominance of iso-C_{16:0}, 10-methyl C_{17:0}, C_{17:1} *cis*9 and 10-methyl iso-C_{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^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^T (=DSM 19247^T =JCM 14718^T).

The suborder *Frankineae* Stackebrandt *et al.* 1997 currently accommodates 12 genera classified within six families (Garrity *et al.*, 2007): *Frankiaceae* Becking 1970 emend. Hahn *et al.* 1989 emend. Normand *et al.* 1996 emend. Stackebrandt *et al.* 1997 (genus *Frankia* Brunchorst 1886); *Geodermatophilaceae* Normand *et al.* 1996 emend. Stackebrandt *et al.* 1997 (genera *Geodermatophilus* Luedemann 1968, *Blastococcus* Ahrens and Moll 1970 and *Modestobacter* Mevs *et al.* 2000); *Nakamurellaceae* Tao *et al.* 2004 [substitute for the illegitimate name *Microsphaeraceae* Rainey *et al.* 1997 in Stackebrandt *et al.* (1997)] [genera *Nakamurella* Tao *et al.* 2004 (substitute for the illegitimate name *Microsphaera* Yoshimi *et al.* 1996), *Quadrisphaera* Maszenan *et al.* 2005 and *Humicoccus* Yoon *et al.* 2007]; *Sporichthyaceae* Rainey *et al.* 1997 in Stackebrandt *et al.* (1997) (genus *Sporichthya* Lechevalier *et al.* 1968);

Acidothermaceae Rainey *et al.* 1997 in Stackebrandt *et al.* (1997) (genus *Acidothermus* Mohagheghi *et al.* 1986); and 'Kineosporiaceae' (genera *Kineosporia* Pagani and Parenti 1978, *Cryptosporangium* Tamura *et al.* 1998 and *Kineococcus* Yokota *et al.* 1993).

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

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HKI 0501^T is EF490376.

Table 1. Characteristics that serve to differentiate strain HKI 0501^T from members of genera classified in the suborder *Frankineae*

Data for reference genera were taken from Lechevalier (1994) and Mirza *et al.* (1991) (*Frankia*), Luedemann & Fonseca (1989), Kroppenstedt (1985) and Collins *et al.* (1984) (*Geodermatophilus*), Urzì *et al.* (2004) (*Blastococcus*), Mevs *et al.* (2000) (*Modestobacter*), Yoshimi *et al.* (1996) and Yoon *et al.* (2007) (*Nakamurella*), Yoon *et al.* (2007) (*Humicoccus*), Maszenan *et al.* (2005) (*Quadrisphaera*), Tamura *et al.* (1999) and Rainey *et al.* (1993) (*Sporichthya*), Mohagheghi *et al.* (1986) (*Acidothermus*), Itoh *et al.* (1989) and Kudo *et al.* (1998) (*Kineosporia*), Tamura *et al.* (1998) (*Cryptosporangium*) and Yokota *et al.* (1993) (*Kineococcus*). +, Present; –, absent; ND, no data available.

Taxon	Cellular morphology	Spore/bud formation	Motility	Cell-wall diamino acid(s)	Major menaquinone(s)	Polar lipid(s)*	Predominant fatty acid(s)	DNA G+C content (mol%)
<i>Fodinicola</i> gen. nov. (strain HKI 0501 ^T)	Substrate and aerial hyphae	– (Fragmentation of aerial hyphae)	–	meso-A ₂ pm	MK-9(H ₄), MK-9(H ₆), MK-9(H ₈)	DPG, PE, PS, PI, PL, GL	iso-C _{16:0} , 10-methyl C _{17:0} , C _{17:1} <i>cis</i> 9	65
Frankiaceae								
<i>Frankia</i>	Substrate hyphae; no aerial mycelium; multilocular sporangia	Sporangiospores	–	meso-A ₂ pm	MK-9(H ₄), MK-9(H ₆), MK-9(H ₈)	PI, PIM, DPG	iso-C _{15:0} , iso-C _{16:0} , C _{17:1}	66–71
Geodermatophilaceae								
<i>Geodermatophilus</i>	Thallus consisting of cuboid to oval cells; multilocular sporangia; rudimentary hyphae; no aerial mycelium	Zoospores	+ / –	meso-A ₂ pm	MK-9(H ₄)	PE, PIM, PI, DPG	iso-C _{16:0} , iso-C _{15:0} , iso-C _{17:0}	73–75
<i>Blastococcus</i>	Cocci, rods, vibrios; pairs, tetrads; clusters	Buds	+ / –	meso-A ₂ pm	MK-9(H ₄), MK-9	DPG, PG, PI, PE	iso-C _{16:0} , iso-C _{16:1} , iso-C _{15:0} , C _{18:1} <i>ω</i> 9c, C _{17:1} <i>ω</i> 8c, C _{17:0}	74
<i>Modestobacter</i>	Rods and cocci	Buds	+ / –	meso-A ₂ pm	MK-9(H ₄), MK-8(H ₄), MK-9(H ₆)	DPG, PE, PI, PG	C _{18:1} , iso-C _{16:0} , anteiso-C _{17:0}	68–70
Nakamurellaceae								
<i>Nakamurella</i>	Cocci; pairs; clusters	–	–	meso-A ₂ pm	MK-8(H ₄)	DPG, PE, PE-dimethyl	iso-C _{16:0} , iso-C _{15:0} , C _{18:1}	68
<i>Humicoccus</i>	Cocci	–	–	meso-A ₂ pm	MK-8(H ₄), MK-9(H ₄)	DPG, PE, PE-dimethyl	anteiso-C _{15:0} , iso-C _{15:0} , C _{17:0}	73
<i>Quadrisphaera</i>	Cocci; tetrads; clusters	–	–	meso-A ₂ pm	MK-8(H ₂)	DPG, PG, PI	anteiso-C _{15:0} , C _{16:0}	75
Sporichthyaceae								
<i>Sporichthya</i>	Short aerial hyphae; no substrate mycelium	Coccoid to rod-shaped spores	+	LL-A ₂ pm	MK-9(H ₈), MK-9(H ₆), MK-8(H ₆)	PI, PG, DPG, PL	C _{16:0} , iso-C _{16:0} , C _{17:1} , C _{17:0}	71
Acidothermaceae								
<i>Acidothermus</i>	Slender rods, filaments	–	–	A ₂ pm, Ser, Ala	ND	ND	ND	61
'Kineosporiaceae'								
<i>Kineosporia</i>	Substrate hyphae; no aerial mycelium; elongated, club-shaped sporangia	Spherical to ovoid spores	+	LL-A ₂ pm and meso-A ₂ pm	MK-9(H ₄), MK-8(H ₄), MK-9(H ₆)	PC, DPG, PI, PIM	C _{16:0} , C _{18:1} , 10-methyl C _{18:0}	69–71
<i>Cryptosporangium</i>	Substrate and aerial mycelia; sporangia	Sporangiospores	+	meso-A ₂ pm	MK-9(H ₆), MK-9(H ₄), MK-9(H ₈)	PE	iso-C _{16:0} , C _{17:1} , C _{18:1}	70
<i>Kineococcus</i>	Cocci; pairs, tetrads; clusters	–	+	meso-A ₂ pm	MK9(H ₂)	DPG, PG, GL	anteiso-C _{15:0}	74

*DPG, Diphosphatidylglycerol; 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 0501^T 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 0501^T 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 0501^T 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 0501^T 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 0501^T (see Fig. 1 for the neighbour-joining dendrogram); in view of its phylogenetically deep branching point within the family, HKI 0501^T could be considered as representing a novel genus. Members of the genera *Frankia* and *Acidothermus* are slightly less closely related to isolate HKI 0501^T (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 0501^T 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 0501^T 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 0501^T (Fig. 1) is confirmed by the moderate number of signatures shared with members of the *Frankiaceae*. Strain HKI 0501^T shares more signatures with members of the *Frankiaceae* (40–60 %) than with members of the genera *Kineosporia*, *Kineococcus* and *Quadrisphaera* (35–40 %).

For morphological and cultural studies, strain HKI 0501^T 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 (Axioscope 2; Zeiss) equipped with image-analysing software (AXIO VISION 2.05; Carl Zeiss).

For scanning electron microscopy, samples were prepared by cutting agar blocks containing growing cells of strain HKI 0501^T (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 0501^T 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 0501^T 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 0501^T 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 0501^T; 2, *Frankiaceae*; 3, *Geodermatophilaceae*; 4, *Nakamurella*; 5, *Humicoccus*; 6, *Sporichthyaceae*; 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
66:104	G–C	G–C	A–U	A–U	A–U	A–U	G–C	A–U	G–C	A–U
139:224	C–G	G–C	C–G	C–G	U–A	U–A	C–G	U–A	U–A	U–G
140:223	A–U	A–U	A–U	A–U	A–U	A–U	U–U	G–C	C–G	G–Y
157:164	G–U	G–C	A–U	G–C	G–C	G–C	G–C	G–G	G–C	G–C
158:163	G–C	G–C	A–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C
186:191	G–C	G–C	C–G	C–G	C–G	G–C	G–C	var.	G–C	var.
293:304	G–C	G–C	G–U	G–C	G–C	G–C	G–C	G–C	G–C	G–Y
600:638	G–C	G–C	G–C	G–C	G–C	U–G	C–G	G–C	G–C	U–G
602:636	U–G	C–G	C–G	A–U	A–U	C–G	C–G	C–G	C–G	C–G
839:847	C–G	A–G	Y–R	U–A	U–A	U–A	A–U	a–u/u–a	Y–G	var.
986:1219	A–U	A–U	U–A	A–U	A–U	A–U	U–U	A–U	A–U	A–U
987:1218	A–U	G–C	A–U	A–U	A–U	A–U	G–C	A–U	A–U	A–U
998:1043	G–C	G–C	G–C	G–C	G–C	A–U	G–C	G–C	A–U	G–Y
999:1042	G–U	C–G	C–G	C–G	C–G	U–A	C–G	G–U	C–G	C–G
1308:1329	U–A	C–G	U–A	U–A	U–A	U–A	C–G	C–G	C–G	Y–R
1059:1198	C–G	C–G	U–A	U–A	U–A	U–A	C–G	U–A	U–A	U–A

Fig. 2. Scanning electron micrograph of cells of strain HKI 0501^T from cultures grown on humic acid agar for 21 days. Fragmentation of aerial hyphae is visible. Bar, 1 μm.

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 *Kineosporia* (which are characterized by the common occurrence of both LL-A₂pm and *meso*-A₂pm). In *Acidothermus cellulolyticus*, the A₂pm isomer has not been defined (Mohagheghi *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

Table 3. Cultural characteristics of strain HKI 0501^T

The strain was grown at 28 °C for 21 days.

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

*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^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 xylose 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_{16:0} (32.2 %), 10-methyl C_{17:0} (10.7 %), C_{17:1} *cis*9 (8.9 %) and 10-methyl iso-C_{18:0} (8.5 %) and the presence of smaller proportions of C_{17:0} (7.4 %), iso-C_{17:0} (5.8 %), C_{18:1} *cis*9 (4.8 %), 10-methyl C_{18:0} (4.7 %), iso-C_{18:0} (3.2 %), anteiso-C_{17:0} (3.0 %), iso-C_{16:0} 2-OH (2.7 %) and C_{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^T in the 16S rRNA gene sequence-based phylogenetic tree for the suborder *Frankineae*. It is evident from both the phylogenetic and phenotypic studies that strain HKI 0501^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 (Fo.di.ni'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 mycelium and sparse to abundant, white aerial mycelium. The aerial hyphae break up into irregular rod-like elements. The cell-wall peptidoglycan contains *meso*-A₂pm, alanine, glycine and glutamic acid. The muramic acid in the peptidoglycan is *N*-acetylated. The cell-wall sugars are xylose and minor amounts of an unknown compound. The predominant menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈). The polar lipids comprise diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and several unknown phospholipids and glycolipids together with unknown ninhydrin-positive compounds. Mycolic acids are absent. The cellular fatty acid profile is characterized by the predominance of iso-C_{16:0}, 10-methyl C_{17:0}, C_{17:1} *cis*9, 10-methyl iso-C_{18:0} and C_{17:0}. The G+C content of the genomic DNA of the type strain of the type species is 65 mol%. Phylogenetically, the genus is affiliated to the suborder *Frankineae*. The type species is *Fodinicola feengrottensis*.

Description of *Fodinicola feengrottensis* sp. nov.

Fodinicola feengrottensis (fe.en.grot.ten'sis. N.L. masc. adj. *feengrottensis* pertaining to the Thuringian cave Feengrotten, the origin of the type strain).

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-xylose 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 (C8), α-galactosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-glucosidase, lipase (C14) (weakly), α-mannosidase, naphthol-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^T (=DSM 19247^T =JCM 14718^T), was isolated from rocks from a medieval alum slate mine in Thuringia, Germany.

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