***Ornithinimicrobium humiphilum* gen. nov., sp. nov., a novel soil actinomycete with L-ornithine in the peptidoglycan**

Ingrid Groth,¹ Peter Schumann,² Norbert Weiss,² Barbara Schuetze,¹ Kurt Augsten³ and Erko Stackebrandt²

Author for correspondence: Ingrid Groth. Tel: +49 36 41 65 66 66. Fax: +49 36 41 65 66 60.
e-mail: igroth@pmail.hki-jena.de

¹ Hans-Knöll-Institut für
Naturstoff-Forschung e.V.,
D-07745 Jena, Germany

² DSMZ–Deutsche
Sammlung von
Mikroorganismen und
Zellkulturen GmbH,
D-38124 Braunschweig,
Germany

³ Institut für Molekulare
Biotechnologie e.V.,
D-07745 Jena, Germany

A Gram-positive bacterium originating from garden soil was taxonomically studied. Cells are non-motile, non-sporulating, irregular rods and cocci. The cell wall peptidoglycan contains L-ornithine as diagnostic diamino acid and an interpeptide bridge consisting of L-Orn←L-Ala←Gly←D-Asp. The major menaquinone is MK-8(H₄). 13-Methyl tetradecanoic acid and 14-methyl pentadecanoic acid are the predominant fatty acids. The polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, three unknown glycolipids and three unknown phospholipids. Mycolic acids are absent. The DNA G+C composition is 70 mol %. The acyl type of the glycan chain of peptidoglycan is acetyl. Glucose is the dominating whole cell sugar; arabinose, rhamnose and xylose are present in traces. Results of 16S rDNA sequence comparisons revealed that strain HKI 0124^T represents a novel lineage within the suborder *Micrococcineae* of the order *Actinomycetales* adjacent to the recently described genus *Ornithinicoccus*. On the basis of the clearly pronounced morphological, physiological, chemotaxonomic and phylogenetic differences between strain HKI 0124^T and all members of the suborder *Micrococcineae*, it is proposed to assign strain HKI 0124^T to a new genus and species, *Ornithinimicrobium humiphilum* gen. nov., sp. nov. The type and only strain is HKI 0124^T (= DSM 12362^T = CIP 106634^T).

Keywords: *Ornithinimicrobium humiphilum* gen. nov., sp. nov., L-ornithine-containing actinomycete

INTRODUCTION

Actinomycetes that contain ornithine as diagnostic diamino acid in their peptidoglycan are phylogenetically assigned to the families *Cellulomonadaceae* (*Cellulomonas*, *Rarobacter*) and *Microbacteriaceae* (*Microbacterium*, *Curtobacterium*) within the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997). The recently described genus *Ornithinicoccus*, however, still awaits a decision on a family affiliation (Groth *et al.*, 1999). The type strain of *Ornithinicoccus hortensis* possesses only 12 of the 17 family signature nucleotides described for the family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997), the members of which are

the closest phylogenetic neighbours of the genus *Ornithinicoccus*.

In this paper, characteristics of another isolate originating from the same soil sample as *Ornithinicoccus hortensis* are described. Despite the common occurrence of L-ornithine in the cell wall peptidoglycan and the predominant menaquinone MK-8(H₄) in both strains, remarkable differences in morphological, physiological and further chemotaxonomic features were found. Phylogenetically, strain HKI 0124^T forms a novel lineage within the suborder *Micrococcineae* adjacent to *Ornithinicoccus hortensis* but clearly distinct from this genus and from all other members of the order *Actinomycetales*.

Results presented in this study have revealed that strain HKI 0124^T should be placed in a new genus and species, for which the name *Ornithinimicrobium*

The EMBL accession number for the 16S rDNA sequence of *Ornithinimicrobium humiphilum* strain HKI 0124^T is AJ277650.

Fig. 1. 16S rDNA sequence-based phylogenetic dendrogram constructed from evolutionary distances (De Soete, 1983) showing the phylogenetic position of strain HKI 0124^T next to members of the family *Intrasporangiaceae* and other families of the suborder *Micrococineae* (Stackebrandt *et al.*, 1997). Numbers at branching points refer to bootstrap values (500 trees resampled); only values above 70% are indicated. Scale bar, two inferred nucleotide substitutions per 100 nucleotides.

humiphilum is proposed. The type strain of *Ornithinimicrobium humiphilum* is HKI 0124^T (= DSM 12362^T = CIP 106634^T).

METHODS

Bacterial strain and cultivation conditions. Strain HKI 0124^T was isolated from a sample of garden soil (Giessen, Germany). The isolation procedure and laboratory cultivation have been described by Groth *et al.* (1999).

Morphological and physiological characteristics. Microscopic studies including colony and cell morphology, motility, measurement of cell dimensions and preparation of cells for scanning electron microscopy were performed as described by Groth *et al.* (1997, 1999). Gram staining, acid-fast staining and growth dependence on different incubation temperatures, increasing concentrations of NaCl and oxygen supply, as well as the utilization of various organic compounds and selected enzyme activities have also been described previously (Groth *et al.*, 1997). The susceptibility to antibiotics was tested by placement of antibiotic discs (Oxoid) on R agar plates seeded with a suspension of strain HKI 0124^T (Groth *et al.*, 1997).

Cell wall analysis. Purified cell wall preparations were obtained by the method of Schleifer & Kandler (1972). The amino acids and peptides in cell wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). The amino-terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described by Schleifer (1985). Whole-cell sugars were determined as alditol acetates by GC as described by Groth *et al.* (1996). The molar ratios of amino acids were determined by GC and

GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987). Analysis of enantiomers of cell wall amino acids was performed by GC of *N*-pentafluoropropionyl amino acid isopropyl esters (Frank *et al.*, 1980) on an L-Chirasil-Val column (25 m × 0.25 mm i.d.; Macherey-Nagel) using helium as carrier gas at a linear velocity of 200 mm s⁻¹ and at a temperature programmed from 80 to 190 °C at a rate of 2 °C min⁻¹. Instruments used for GC and GC-MS were as specified previously (Groth *et al.*, 1996). The glycolate content of bacterial cell walls was determined by the colorimetric method of Uchida & Aida (1984).

Lipid analysis. Cellular fatty acid methyl esters obtained from cells grown in Bacto tryptic soy broth at 28 °C by the method of Stead *et al.* (1992) were separated by GC (Groth *et al.*, 1996) and identified as described previously (Schumann *et al.*, 1997). Menaquinones were extracted as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). 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 and its G + C content was determined by HPLC of deoxyribonucleosides as described by Groth *et al.* (1996).

16S rDNA sequence determination and phylogenetic analysis. Preparation of genomic DNA from strain HKI 0124^T was done as described previously (Rainey *et al.*, 1996). Sequences were aligned manually with previously published sequences. These were stored in the DSMZ database consisting of several thousand 16S rDNA sequence entries, including those from the Ribosomal Database Project (Maidak *et al.*, 1997) and EMBL. Similarity values were

transformed into phylogenetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of De Soete (Maidak *et al.*, 1997) and the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) were used in the construction of phylogenetic dendrograms. Bootstrap values were determined according to Felsenstein (1993). All analyses were done on a SUN SparcII workstation.

Nucleotide accession numbers. The accession numbers of the type strains used in the phylogenetic analysis are shown in Fig. 1.

RESULTS AND DISCUSSION

Phylogenetic analysis

The almost complete primary structure of 16S rDNA, consisting of 1489 nucleotides [96.4% of the *Escherichia coli* sequence (Brosius *et al.*, 1978)], has been determined and compared to the type strains of species of the order *Actinomycetales*. The highest binary similarity values were found with members of the family *Intrasporangiaceae* [excepting *Sanguibacter* (E. Stackebrandt and P. Schumann, unpublished results; 92.2–93.7%), *Janibacter* (93.2–93.9%) and *Ornithinococcus hortensis* (95.2%)]. The latter organism has recently been described to phylogenetically branch adjacent to members of the family *Intrasporangiaceae*. The decision for not including *Ornithinococcus hortensis* in the family *Intrasporangiaceae* was based on significant differences in the peptidoglycan type and the lack of five of the 17 signature nucleotides described as being specific for *Intrasporangiaceae*. Phylogenetic analysis applying neighbour-joining analysis and the algorithm of De Soete (1983) places strain HKI 0124^T adjacent to *Ornithinococcus hortensis* but the branching point is deep and not supported by high bootstrap values. Strain HKI 0124^T shares with *Ornithinococcus hortensis* 12 signature nucleotides specific for the family *Intrasporangiaceae*. However, only one out of the five signature nucleotides which differentiate *Ornithinococcus hortensis* and strain HKI 0124^T from members of the family *Intrasporangiaceae* has the same base composition in both ornithine-containing organisms.

The addition of a single new strain to the *Ornithinococcus* lineage of descent and the lack of high bootstrap values have prevented designation of a new family for these two strains.

Morphological and physiological characteristics

Cells are irregular short rods and cocci, occurring singly, in pairs, short chains or clusters. Filamentous cells with swollen club-shaped ends or a tendency to primary branching and single spherical cysts were additionally observed subsequent to the isolation and later in a few cultures. Cell sizes varied depending on the culture conditions. Rods were about 1.4–3.2 µm in length and 0.6–1.0 µm wide. The cocci had diameters

Fig. 2. Micrograph of strain HKI 0124^T grown in liquid R medium for 24 h at 28 °C. Bar, 10 µm.

Fig. 3. Scanning electron micrographs of cells from a 3 d culture of strain HKI 0124^T grown on R agar at 28 °C. Bars, 1 µm.

of 0.7–1.4 µm. A clearly pronounced rod–coccus growth cycle was not observed. Cells were non-motile and formed no spores (Fig. 2 and Fig. 3). They stained

Table 1. Differential physiological properties of strains HKI 0124^T and *Ornithinococcus hortensis* HKI 0125^T

—, Negative; +, positive; w, weakly positive.

Property	HKI 0124 ^T	HKI 0125 ^T
Decomposition of:		
Hippurate	—	w
Potato starch	+	—
Tween 80	—	+
Acid produced from:		
L-Arabinose	+	—
D-Cellobiose	+	—
D-Fructose	—	+
D-Galactose	+	—
D-Glucitol	—	w
Glycerol	—	w
D-Mannitol	—	w
D-Mannose	—	w
D-Ribose	+	—
D-Xylose	w	—
Utilization of:		
Aconitate	—	+
Enzyme assay (API ZYM):		
Lipase (C14)	—	w
Trypsin	w	—
Growth at (°C):		
45	+	—
Growth in presence of NaCl (w/v):		
10 %	—	w

Gram-positive and were not acid-fast. Colonies were circular, convex, glistening and smooth with an entire margin. Colour changed within the yellow shade from whitish to bright yellow. The colony sizes were in the range 0.3–1.2 mm. Two colony types differing in size, colour and growth rate were observed on complex media. While colonies of the pale yellow, opaque type grew rapidly after their transfer on a fresh medium, the growth of the smaller transparent yellow colonies was delayed and somewhat reduced. Considering this heterogeneity in cell and colony morphology the purity of the culture was examined by separate cultivation of each colony type. The sequences of the 16S rDNA of both types were determined and compared. The results of the sequence comparisons confirmed the identity of the two cultures, which was equally supported by morphological studies. In subsequent passages of the separated colonies on R medium, a similar morphological differentiation of the colonies occurred.

After its isolation from soil, strain HKI 0124^T exhibited poor growth in the form of a thin confluent lawn on solid complex media. However, growth was improved by serial transfers in laboratory cultivation on complex organic media under aerobic conditions. In a microaerophilic atmosphere, growth was only slightly reduced but did not occur under anaerobic

conditions. Optimal temperatures for growth were between 37 and 42 °C. At a temperature of 50 °C, growth was inhibited. Addition of NaCl to R medium was tolerated up to a concentration of 6 % (w/v). At a concentration of 8 % (w/v) NaCl in the agar plates, traces of growth appeared after an incubation time of about 10 d.

Further physiological properties of strain HKI 0124^T are listed in Table 1 and compared with the characteristics of the closely related *Ornithinococcus hortensis*. The above-mentioned growth behaviour at temperatures in the range 40–50 °C is an obvious difference between strain HKI 0124^T and its phylogenetic neighbour *Ornithinococcus hortensis*. While strain HKI 0124^T exhibited good growth up to 45 °C, the growth of *Ornithinococcus hortensis* was completely inhibited at a temperature of 42 °C.

Strain HKI 0124^T was maintained in the form of liquid cultures at a temperature of –80 °C and in the vapour phase of liquid nitrogen. The latter is recommended for long-term storage.

Chemotaxonomic characteristics

The peptidoglycan of strain HKI 0124^T contained Orn, Ala, Gly, Glu and Asp in a molar ratio of 0.8:3.5:1.6:1.0:0.9. Dinitrophenylated Asp and only traces of dinitrophenylated Gly and Ala were detected in hydrolysates of cell wall preparations treated with 1-fluoro-2,4-dinitrobenzene for labelling of the amino terminus of the interpeptide bridge. Enantiomeric analysis of cell wall amino acids resulted in detection of L-Orn, D-Ala, L-Ala, D-Glu, L-Glu and D-Asp. From these results and from two-dimensional TLC peptide patterns of partial hydrolysates of cell walls (data not shown), it was concluded that strain HKI 0124^T contains a peptidoglycan of type A4β (Schleifer & Kandler, 1972) with an L-Orn←L-Ala←Gly←D-Asp interpeptide bridge. According to the enantiomeric analysis, both enantiomers of Glu seem to occur in the peptide subunit. The acyl type of the glycan chain of peptidoglycan was acetyl. Glucose, xylose, rhamnose and arabinose were found as whole-cell sugars; the latter three sugars occurred only in traces.

The polar lipid pattern consisted of phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol, three unknown glycolipids and three unknown phospholipids. The cellular fatty acids (Table 2) were of the iso and anteiso branched type and were distinctly dominated by the occurrence of 13-methyl tetradecanoic acid and 14-methyl pentadecanoic acid.

The major menaquinone was MK-8(H₄). MK-8(H₂), MK-8 and MK-6 occurred in minor amounts. Mycolic acids were not present. Strain HKI 0124^T had a DNA G + C content of 70 mol %.

Based upon chemotaxonomic evidence, the presence of peptidoglycan of type A4β with ornithine and MK-

Table 2. Cellular fatty acid composition (%) of strain HKI 0124^T

The abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, hexadecanoic acid; C_{16:1}, hexadecenoic acid; i-C_{15:0}, 13-methyl tetradecanoic acid; ai-C_{17:0}, 14-methyl hexadecanoic acid.

Fatty acid	Composition (%)
i-C _{13:0}	0.6
ai-C _{13:0}	0.3
i-C _{14:0}	3.4
C _{14:0}	0.2
i-C _{15:0}	26.0
i-C _{15:1}	0.8
ai-C _{15:0}	10.6
C _{15:0}	1.2
i-C _{16:0}	27.0
i-C _{16:1}	2.7
C _{16:0}	1.3
C _{16:1}	0.4
i-C _{17:0}	4.2
ai-C _{17:0}	5.2
i-C _{17:1}	9.4
ai-C _{17:1}	0.2

8(H₄) as major menaquinone, and 16S rDNA similarity of 95.2%, strain HKI 0124^T is most closely related to *Ornithinococcus hortensis*. However, strain HKI 0124^T is differentiated from *Ornithinococcus hortensis* by its unique interpeptide bridge (L-Orn←L-Ala←Gly←D-Asp and L-Orn←Gly_(1,2)←D-Glu, respectively). The polar lipid pattern of strain HKI 0124^T differs from that of the genus *Ornithinococcus* in the absence of phosphatidylserine and the additional occurrence of unidentified glycolipids. The fatty acid profile of strain HKI 0124^T is characterized by a considerably higher content of 14-methyl pentadecanoic acid and lower amounts of 12-methyl tetradecanoic acid in comparison to that of *Ornithinococcus hortensis* (Table 2). Because of the differences in peptidoglycan structure, polar lipid pattern, fatty acid profile, and physiological (Table 1) and morphological features, as well as in the 16S rDNA sequence, strain HKI 0124^T should not be considered a member of the genus *Ornithinococcus*. Furthermore, strain HKI 0124^T is clearly separated from the other aforementioned genera with ornithine in the peptidoglycan as demonstrated by its position in the phylogenetic tree (Fig. 1) and its differentiating phenotypic characteristics (Table 3). Therefore, it is proposed that the genus *Ornithinimicrobium* should be established to harbour strain HKI 0124^T. The type species of the genus *Ornithinimicrobium* is *Ornithinimicrobium humiphilum* gen. nov., sp. nov., represented by the type strain HKI 0124^T (= DSM 12362^T = CIP 106634^T). As the proposed novel genus and species comprise only a single strain, the given descriptions cannot reflect the phylo-

genetic depth and phenotypic variability of the entire taxon.

Description of *Ornithinimicrobium* gen. nov.

Ornithinimicrobium (or'ni.thi.ni.mic.ro.bi.um. Gr. n. *ornithos* bird, ornithine an amino acid named after birds; Gr. adj. *micro* small; Gr. masc. n. *bios* life; N.L. neut. n. *Ornithinimicrobium* a microbe with ornithine).

Cells are irregular short rods and cocci occurring singly, in pairs, short chains or clusters. Cell diameters of the cocci vary from 0.7 to 1.4 µm. The rods are 1.4–3.2 × 0.6–1.0 µm in size. Gram-positive, not acid-fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. The peptidoglycan type is A4β with an L-Orn←L-Ala←Gly←D-Asp interpeptide bridge. The acyl type of the glycan chain of peptidoglycan is acetyl. The major menaquinone is MK-8(H₄). The predominant fatty acids are 13-methyl tetradecanoic and 14-methyl pentadecanoic acid. The polar lipids are phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol, three unknown glycolipids and three unknown phospholipids. The G + C content of the DNA is 70 mol%. Phylogenetically, this genus is a member of the suborder *Micrococcineae* in which it is closely related to members of the family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997). The type species is *Ornithinimicrobium humiphilum*.

Description of *Ornithinimicrobium humiphilum* sp. nov.

Ornithinimicrobium humiphilum (hu.mi'phil.um. L. adj. *humo* from soil; Gr. adj. *philus* liking, preferring; N.L. *humiphilum* liking soil).

Cells are irregular short rods and cocci occurring singly, in pairs, short chains or clusters. Cell diameters of the cocci vary from 0.7 to 1.4 µm. The rods are 1.4–3.2 × 0.6–1.0 µm. Gram-positive, not acid-fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. Colonies are 0.3–1.2 mm in diameter and vary in colour from whitish to bright yellow. Colony size, colour and growth rate change depending on the composition of the medium. Acids are produced from L-arabinose, D-cellobiose, D-galactose, D-glucose, maltose, D-ribose and starch and in a low amount from dextrin, sucrose, trehalose and D-xylose. No acid production from lactose, D-fructose, D-glucitol, glycerol, inulin, D-mannitol, D-mannose, D-raffinose, L-rhamnose or salicin. Formate is used as sole carbon source. Utilization of malate and succinate is delayed and weakly expressed. Acetate, aconitate, benzoate, citrate and DL-tartrate are not utilized. Nitrate is reduced to nitrite. H₂S and catalase are produced, indole and oxidase are not produced. Methyl red and Voges-Proskauer reactions are negative. Casein, gelatin, hypoxanthine, starch and xanthine are decomposed;

Table 3. Differential characteristics of strain HKI 0124^T and related taxa

Abbreviations: D-Orn, D-ornithine; L-Orn, L-ornithine; *meso*-A₂pm, *meso*-diaminopimelic acid; LL-A₂pm, LL-diaminopimelic acid; DPG, diphosphatidylglycerol; GL, unknown glycolipid(s); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; PSer, phosphatidylserine; PL, unknown phospholipid(s); A, anteiso methyl branched; I, iso methyl branched; S, straight chain saturated; U, monounsaturated.

Taxon	Cell morphology	Wall diamino acid	G + C content (mol %)	Murein type	Major menaquin one	Polar lipid type	Fatty acid type
HKI 0124 ^T	Irregular rods and cocci, non-motile	L-Orn	70	A4β	MK-8(H ₄)	PI, PG, DPG, PLs, GLs	S, A, I
<i>Ornithinococcus</i> *	Cocci, non-motile	L-Orn	72	A4β	MK-8(H ₄)	PI, PG, DPG, PSer, PLs	S, A, I
<i>Cellulomonas</i> †	Irregular rods, motile or non-motile	L-Orn	71–76	A4β	MK-9(H ₄)	DPG, PI, PGL	S, A, I
<i>Rarobacter</i> ‡§	Rods	L-Orn	65–66	A4β	MK-9	ND	S, A, I
<i>Curtobacterium</i> ‡	Irregular rods, motile	D-Orn	68–75	B2β	MK-9	DPG, PG, GLs	S, A, I
<i>Microbacterium</i> §	Irregular rods	D-Orn, L-Lys	67–70	B2β, B2α	MK-11, MK-12, MK-13, MK-14	DPG, PG, GL	S, A, I
<i>Intrasporangium</i> ¶	Hyphae	LL-A ₂ pm	68	A3γ	MK-8	PI, PIM, PG, DPG	S, A, I
<i>Terrabacter</i> #	Rod–coccus cycle	LL-A ₂ pm	70–73	A3γ	MK-8(H ₄)	DPG, PI, PE, PL	S, A, I
<i>Terracoccus</i> **	Cocci in packets, non-motile	LL-A ₂ pm	73	A3γ	MK-8(H ₄)	PE, PI, PG, DPG	S, A, I
<i>Janibacter</i> #	Coccoid to rod-shaped, non-motile	<i>meso</i> -A ₂ pm	70	A1γ	MK-8(H ₄)	DPG, PG, PI	S, I, U

* Data from Groth *et al.* (1999).

† Data from Stackebrandt *et al.* (1995).

‡ Data from Yokota *et al.* (1993).

§ Data from Goto-Yamamoto *et al.* (1993).

|| Data from Takeuchi & Hatano (1998).

¶ Data from Schumann *et al.* (1997).

Data from Martin *et al.* (1997).

** Data from Prauser *et al.* (1997).

adenine, aesculin, hippurate, Tween 80, tyrosine and urea are not. Addition of NaCl to R medium is well tolerated up to a concentration of 6% (w/v). Good growth occurs between 37 and 42 °C; no growth at 50 °C. Cells are susceptible to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (2 µg), neomycin (30 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg), penicillin G (2 IU), polymyxin B (300 IU), rifampicin (2 µg) and streptomycin (10 µg). No susceptibility to oxacillin (5 µg) or sulfonamide (300 µg). Tested by the API ZYM enzyme assay, strain HKI 0124^T is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase and negative for lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, β-glu-

cosidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase. The enzyme assay showed a weak reaction for trypsin and chymotrypsin. The peptidoglycan is of the A4β type with an L-Orn←L-Ala←Gly←D-Asp interpeptide bridge. The acyl type of the glycan chain of peptidoglycan is acetyl. The fatty acids are of the iso and anteiso branched type with predominant amounts of 13-methyl tetradecanoic acid and 14-methyl pentadecanoic acid. The major menaquinone is MK-8(H₄). The polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, three unknown glycolipids and three unknown phospholipids. Glucose is the dominating whole cell sugar; arabinose, rhamnose and xylose are found in traces. The G + C content of the DNA is 70 mol%. Mycolic acids are absent. The habitat is soil. The type strain is HKI 0124^T (= DSM 12362^T = CIP 106634^T).

ACKNOWLEDGEMENTS

The authors thank Ina Kramer, Carmen Schult and Christiane Weigel for excellent technical assistance.

REFERENCES

- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of the 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- De Soete, G. (1983). A least square algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5.1. Department of Genetics, University of Washington, Seattle, USA.
- Frank, H., Rettenmeier, A., Weicker, H., Nicholson, G. J. & Bayer, E. (1980). A new gas chromatographic method for determination of amino acid levels in human serum. *Clin Chim Acta* **105**, 201–211.
- Goto-Yamamoto, N., Sato, S.-I., Miki, H., Park, Y. K. & Tadenuma, M. (1993). Taxonomic studies on yeast-lysing bacteria, and a new species *Rarobacter incanus*. *J Gen Appl Microbiol* **39**, 261–272.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terragena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.
- Groth, I., Schumann, P., Martin, K., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Ornithinococcus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. *Int J Syst Bacteriol* **49**, 1717–1724.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J. & Woese, C. R. (1997). The Ribosomal Database Project. *Nucleic Acids Res* **25**, 109–111.
- Martin, K., Schumann, P., Rainey, F. A., Schuetze, B. & Groth, I. (1997). *Janibacter limosus* gen. nov., sp. nov., a new actinomycete with meso-diaminopimelic acid in the cell wall. *Int J Syst Bacteriol* **47**, 529–534.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolsates. *J Gen Microbiol* **88**, 200–204.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.
- Prauser, H., Schumann, P., Rainey, F. A., Kroppenstedt, R. M. & Stackebrandt, E. (1997). *Terracoccus luteus* gen. nov., sp. nov., an LL-diaminopimelic acid-containing coccoid actinomycete from soil. *Int J Syst Bacteriol* **47**, 1218–1224.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schumann, P., Prauser, H., Rainey, F. A., Stackebrandt, E. & Hirsch, P. (1997). *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Stackebrandt, E., Koch, C., Gvozdiak, O. & Schumann, P. (1995). Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* **45**, 682–692.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N.-L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Stead, D. E., Sellwood, J. E., Wilson, J. & Viney, I. (1992). Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* **72**, 315–321.
- Takeuchi, M. & Hatano, K. (1998). Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium*. *Int J Syst Bacteriol* **48**, 739–747.
- Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.
- Yokota, A., Takeuchi, M., Sakane, T. & Weiss, N. (1993). Proposal of six new species of the genus *Aureobacterium* and transfer of *Flavobacterium esteraromaticum* Omelianski to the genus *Aureobacterium* as *Aureobacterium esteraromaticum* comb. nov. *Int J Syst Bacteriol* **43**, 555–564.