Janibacter limosus gen. nov., sp. nov., a New Actinomycete with *meso*-Diaminopimelic Acid in the Cell Wall

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New gram-positive bacteria were isolated from 1-year-old sludge from a wastewater treatment plant. The isolates are coccoid to rod-shaped, nonmotile aerobes that form neither spores nor mycelia. They are characterized by a peptidoglycan with directly cross-linked *meso*-diaminopimelic acid (type A1γ), by the presence of menaquinone MK-8(H₄), and by the lack of mycolic acids. The strains have complex fatty acid patterns with i-C_{16:0} and straight-chain saturated and unsaturated fatty acids as major components. The G+C content of the DNA is 70 mol%. The results of chemotaxonomic studies and a 16S ribosomal DNA sequence comparison support our proposal to assign these bacteria to a new genus, the genus *Janibacter* gen. nov.; the type species is *Janibacter limosus* sp. nov., and the type strain of *J. limosus* is strain HKI 83 (= DSM 11140).

Both spore-forming and asporogenous actinomycetes have been screened during the last few years for useful bioactive compounds (13, 39). The asporogenous organisms play important roles in mineralization of environmentally hazardous chemicals, such as polycyclic aromatic hydrocarbons (22), and in biotechnological production of natural products (23, 26, 29).

To isolate new actinomycetes with new biological activities, we investigated several samples of soil, sludge, and sewage waste. A total of 168 strains were isolated from a 1-year-old sludge sample collected from a wastewater treatment plant. Two of these isolates were found to be markedly different from the other isolates and from previously described genera in their chemotaxonomic and their physiological features. In this paper these two strains are characterized phenotypically and phylogenetically. Below we propose the creation of a new genus, the genus *Janibacter* gen. nov., with one species, *Janibacter limosus* sp. nov., for these organisms.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. Strains HKI 83^T (T = type strain) and HKI 84 were isolated from a 1-year-old sludge sample from the wastewater treatment plant near Jena, Thuringia, Germany, by the dilution plate technique on plate count agar (Difco Laboratories, Detroit, Mich.). General laboratory cultivation was performed at 28°C on solid rich medium (R medium) or in liquid R medium (43), which contained 1% (wt/vol) Bacto Peptone (Difco), 0.5% (wt/vol) yeast extract, 0.5% (wt/vol) Casamino Acids, 0.2% (wt/vol) meat extract, 0.5% (wt/vol) malt extract, 0.2% (wt/vol) glycerol, 0.1% (wt/vol) MgSO₄·7H₂O, and 0.005% (wt/vol) Tween 80 (pH 7.2). To determine the cellular fatty acids, strains were cultivated for 24 h in liquid tryptic soy broth (Difco) at 28°C.

Morphological and physiological characteristics. Colony morphology on R medium and cell morphology at different ages were determined by stereomicroscopy and phase-contrast microscopy (Olympus, Tokyo, Japan). Nitrate reductase activity, urease activity, indole production, methyl red and Voges-Proskauer reactions, hydrogen sulfide production, and hydrolysis of esculin and Tween 80 were determined as described by Lanyi (25). Acid production from carbohydrates was determined by using the method of Hugh and Leifson (16), as modified by Gledhill and Casida (10). Utilization of organic acids (2% wt/vol; sodium salts) was studied by the method of Gordon and Mihm (12). Decomposition of adenine, hypoxanthine, xanthine, and tyrosine was determined by using the method of Gordon et al. (11). Catalase production and hydrolysis of casein

and starch were determined by the methods of Gledhill and Casida (10). The cytochrome oxidase test was performed by using DrySlide Oxidase Test strips (Difco). The oxygen requirement was determined by using a Generbag Microaer incubation system (bioMerieux, Marcy l'Etoile, France). The test to determine hydrolysis of hippurate was performed as described by Cowan and Steel (5). The test to determine decomposition of gelatin was performed by using a modification of the method described by Lanyi (25). A UV-sterilized photographic film strip was immersed in phosphate buffer, which was inoculated with the test strains at a density of 6×10^8 cells per ml (McFarland no. 2 nephelometer standard). After 3 to 7 days of incubation, hydrolysis of the gelatin layer was checked. Sodium chloride tolerance and growth at 28, 37, and 50°C were tested on brain heart infusion (BHI) medium (Difco) and R medium. Resistance to antibiotics was tested by placing antibiotic discs (Oxoid, Hampshire, United Kingdom) on agar plates seeded with the test strains.

Cell wall analysis. A cell wall analysis was performed as described previously (14). Purified cell wall preparations were obtained by the method of Schleifer and Kandler (34). The amino acids and peptides of cell wall hydrolysates were analyzed by thin-layer chromatography on cellulose plates by using the solvent systems described by Schleifer and Kandler (34). The molar ratios of amino acids were determined by gas chromatography-mass spectrometry of *N*-heptafluorobutyryl amino acid isobutyl esters as described previously (14). The whole-cell sugars were determined by gas chromatography of alditol acetates as described by Saddler et al. (33). The glycolate content of bacterial cells was determined by the colorimetric method of Uchida and Aida (41).

Lipid analysis. The cellular fatty acid methyl esters were determined by gas chromatography by using the method of Stead et al. (38). Menaquinones were extracted as described by Collins et al. (4) and were analyzed by high-performance liquid chromatography (14). Polar lipids were extracted and identified by the method of Minnikin et al. (31). The absence of mycolic acids was shown by thin-layer chromatography as described by Minnikin et al. (30).

Analysis of DNA base composition. DNA was isolated by using a modification of the Marmur method (27). DNA was purified by treatment with proteinase K and was degraded to nucleosides with P1 nuclease and bovine intestinal mucosa alkaline phosphatase as described by Mesbah et al. (28). The nucleosides were separated by reversed-phase high-performance liquid chromatography as described by Tamaoka and Komagata (40). G+C contents were calculated from the ratios of deoxyguanosine and thymidine.

DNA-DNA hybridization. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (1). DNA-DNA hybridization was carried out as described by De Ley et al. (6), with the modifications described by Huss et al. (17), using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (18).

16S rDNA sequence determination and analysis. Genomic DNAs were extracted from the strains investigated in this study and were used for PCR-mediated amplification of 16S ribosomal DNA (rDNA) as described by Rainey et al. (32). The purified PCR products were directly sequenced by using previously described protocols (32), and the sequence reaction mixtures were electrophoresed by using a model 373A automatic DNA sequencer (Applied Biosystems, Foster City, Calif.). The 16S rDNA sequences were manually aligned with the sequences of previously described members of the order *Actinomycetales*. Evolutionary distances were calculated by the method of Jukes and Cantor

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FIG. 1. (a) Micrograph of strain HKI 84 cells grown on solid R medium at 28°C for 24 h. Bar = 10 μ m. (b) Micrograph of strain HKI 83^T cells grown in liquid R medium at 28°C for 24 h. Bar = 10 μ m. (c) Micrograph of strain HKI 83^T cells grown on an agar slide at 28°C for 12 h. Bar = 10 μ m.

(20), and a phylogenetic dendrogram was constructed by using the least-squares method (7).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the EMBL database under the

following accession numbers: *J. limosus* DSM 11140^T, Y08539; *J. limosus* DSM 11141, Y08540; and *Microsphaera multipartita* JCM 9543^T, Y08541.

RESULTS

Morphological and cultural characteristics. Cells of strains HKI 83^T and HKI 84 are gram-positive and nonmotile and occur singly, in pairs, or occasionally in irregular clumps. While the cells of strain HKI 84 are always coccoid and vary in diameter from 0.4 to 1.1 μ m (Fig. 1a), the cells of strain HKI 83^T differ morphologically during the growth cycle. In liquid strain HKI 83^T cultures only coccoid cells are observed, and these cells vary from 0.3 to 1.2 μ m in diameter (Fig. 1b). After the cells are transferred to agar slides, the coccoid cells give rise to small irregular rods (0.4 by 1.2 μ m) (Fig. 1c), but the cells in older cultures are coccoid. Colonies of strain HKI 83^T on nutrient agar are white, opaque, and convex with glistening surfaces (Fig. 2a). The colony margins are entire. Colonies of strain HKI 84 on nutrient agar are yellow, opaque, and convex (Fig. 2b). The surfaces are matt, and the colony margins are entire.

Both strains grow aerobically and in presence of 4% (wt/vol) sodium chloride in the culture medium. The optimal growth temperature is 28°C. Growth occurs at 37°C on BHI medium but not on R medium. No growth occurs under anaerobic conditions. Spore formation is not observed.

Physiological characteristics. The physiological properties of isolates HKI 83^T and HKI 84 are shown in Table 1.

FIG. 2. (a) Single colonies of strain HKI 83^T grown on solid R medium at 28°C for 5 days. Bar = 1 mm. (b) Single colonies of strain HKI 84 grown on solid R medium at 28°C for 5 days. Bar = 1 mm.

TABLE 1. Morphological and biochemical characteristics of *J. limosus* HKI 83^T and HKI 84

Characteristic	Strain HKI 83 ^T	Strain HKI 84
Color of colonies	White	Yellow
Morphology	Coccoid, rod shaped	Coccoid
Motility	— ^a	—
Gram staining	+	+
Spore formation	—	—
Acid production from:		
L-Arabinose	—	—
D-Cellobiose	—	—
Dextrin	—	—
D-Fructose	—	—
D-Galactose	—	—
D-Glucose	—	—
Glycerol	—	—
Inulin	—	—
Lactose	—	—
Maltose	—	—
D-Mannitol	—	—
D-Mannose	—	—
D-Raffinose	—	—
L-Rhamnose	—	—
D-Ribose	—	—
Saccharose	—	—
Salicin	—	—
D-Sorbitol	—	—
Starch	—	—
Trehalose	—	—
D-Xylose	—	—
Utilization of:		
Acetate	+	—
Aconitate	—	—
Benzoate	—	—
Citrate	+	+
Formate	+	+
Malate	+	+
Succinate	—	—
Hippurate	—	—
DL-Tartrate	—	—
Decomposition of:		
Adenine	—	—
Casein	+	+
Esculin	—	—
Gelatin	+	+
Hypoxanthine	—	—
Potato starch	+	+
Tween 80	+	+
Tyrosine	+	+
Urea	—	—
Xanthine	—	—
Nitrate reduction	+	+
Production of H ₂ S	+	+
Production of indole	—	—
Voges-Proskauer test	—	—
Methyl red test	—	—
Oxidase test	+	+
Catalase reaction	+	+
Growth in the presence of:		
2% NaCl	+	+
4% NaCl	+	+
10% NaCl	+	+
Growth on BHI medium at:		
28°C	+	+
37°C	+	+
Growth on R medium at:		
28°C	+	+
37°C	—	—
Antibiotic susceptibility		
Ampicillin (10 µg)	+	+

Continued

TABLE 1—Continued

Characteristic	Strain HKI 83 ^T	Strain HKI 84
Chloramphenicol (30 µg)	+	+
Ciprofloxacin (5 µg)	+	+
Erythromycin (15 µg)	+	+
Gentamicin (10 µg)	+	+
Kanamycin (30 µg)	+	+
Lincomycin (2 µg)	+	+
Neomycin (30 µg)	+	+
Nitrofurantoin (300 µg)	+	+
Oxacillin (5 µg)	—	+
Oxytetracycline (30 µg)	+	+
Penicillin G (2 IU)	—	—
Polymyxin B (300 IU)	+	+
Rifampin (2 µg)	—	—
Streptomycin (10 µg)	—	+
Sulfonamide (300 µg)	—	—

^a —, negative; +, positive.

Chemotaxonomic characteristics. Diagnostic features that differentiate the new isolates from related genera are shown in Table 2. The peptidoglycan type is A1γ (directly cross-linked *meso*-diaminopimelic acid [*meso*-A₂pm]), the cell wall Ala-Glu-*meso*-A₂pm ratio for strain HKI 83^T is 1.6:1.0:1.1, and mycolic acids are absent. The acyl type is acetyl, the menaquinone is MK-8(H₄), and the polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Diagnostic cell wall sugars like arabinose and galactose are not detected, but mannose, ribose, and glucose are present. The complex fatty acid profile of strain HKI 83^T is characterized by C_{17:1}, C_{17:0}, and i-C_{16:0}, as well as minor amounts of straight-chain saturated and unsaturated fatty acids (Table 3). Strain HKI 84 has a complex fatty acid profile with i-C_{16:0} and C_{17:1} as its major components. In both strains branched-chain fatty acids other than i-C_{16:0} occur only in small amounts (<3%).

DNA base composition and DNA-DNA relatedness. The DNA base composition of both strains is 70 mol% G+C. The level of DNA-DNA relatedness between strains HKI 83^T and HKI 84 is 70.8%.

16S rDNA sequence analysis. Almost complete 16S rDNA sequences of strain HKI 83^T, strain HKI 84, and *M. multipartita* JCM 9543^T comprising between 1,466 and 1,473 nucleotides (>95% of the *Escherichia coli* sequence) were determined in this study. The phylogenetic dendrogram shown in Fig. 3 was constructed from evolutionary distances by the least-squares distance method (7). A total of 1,232 nucleotides present in all strains between positions 41 and 1448 (*E. coli* numbering) were used for this analysis. Figure 3 shows that strains HKI 83^T and HKI 84 fall within the radiation of the cluster comprising the genera *Intrasporangium*, *Terrabacter*, and *Sanguibacter*. The levels of 16S rDNA sequence similarity between organisms belonging to this cluster range from 94.8 to 97%. The level of 16S rDNA sequence similarity between strains HKI 83^T and HKI 84 when all 1,473 aligned nucleotide positions were compared was 99.4%. The levels of 16S rDNA sequence similarity to the other representatives of the actinomycetes included in the analysis and displayed in Fig. 3 ranged from 81.6 to 94.0%.

DISCUSSION

In the course of an isolation program designed to obtain new actinomycetes that produce useful bioactive compounds, we studied the chemotaxonomic and physiological properties of a large number of strains obtained from quite different sources. Two of these isolates attracted our attention because of their

TABLE 2. Differential characteristics of the genus *Janibacter* and other actinomycete genera^a

Genus	G+C content (mol%)	Diamino acid/murein type	Mycolic acids	Major menaquinone(s)	Polar lipids	Cellular fatty acid types
<i>Janibacter</i> gen. nov.	70	meso-A ₂ pm/A1γ	—	MK-8(H ₄)	DPG, PG, PI	S, I, U
<i>Microsphaera</i> ^b	67.5	meso-A ₂ pm/A1γ	—	MK-8(H ₄)	ND	S, A, I, U
<i>Brachybacterium</i> ^c	68–72	meso-A ₂ pm/A4γ	—	MK-7	DPG, PG, GL	S, A, I
<i>Dermabacter</i> ^d	62	meso-A ₂ pm/A1γ	—	MK-9, MK-8	PG, PL, GL	S, A, I
<i>Brevibacterium</i> ^e	60–67	meso-A ₂ pm/A1γ	—	MK-8(H ₂), MK-7(H ₂)	DPG, PG, GL	S, A, I
<i>Kineococcus</i> ^f	75	meso-A ₂ pm/A1γ	—	MK-9(H ₂)	DPG, PG, PGL	S, A, I
<i>Intrasporangium</i> ^g	68.2	LL-A ₂ pm/A3γ	—	MK-8	DPG, PG, PI, PIM	S, A, I
<i>Terrabacter</i> ^h	70–73	LL-A ₂ pm/A3γ	—	MK-8(H ₄)	DPG, PI, PE, PL	S, A, I
<i>Nocardioideis</i> ^g	66–72	LL-A ₂ pm/A3γ	—	MK-8(H ₄)	DPG, PG, PG-OH, PL	C
<i>Aeromicrobium</i> ^g	71–73	LL-A ₂ pm/A3γ	—	MK-9(H ₄)	PE, PG	S, U, T, 2OH-FA
<i>Luteococcus</i> ^g	66–68	LL-A ₂ pm/A3γ	—	MK-9(H ₄)	DPG, PG, PI, GL	S, U
<i>Microtholunatus</i> ^g	67.9	LL-A ₂ pm/A3γ	—	MK-9(H ₄)	DPG, PG, PI, PL	S, A, I
<i>Dermatophilus</i> ^h	57–59	meso-A ₂ pm/A1γ	—	MK-8(H ₄)	DPG, PG, PI	S, U
<i>Pseudonocardia</i> ⁱ	79	meso-A ₂ pm/A1γ	—	MK-8(H ₄)	DPG, PG, PC, PE, PI, GL	A, I
<i>Turicella</i> ^j	65–72	meso-A ₂ pm	—	MK-10, MK-11	ND	S, U, T
<i>Rhodococcus</i> ^k	63–73	meso-A ₂ pm/A1γ	+	MK-8(H ₂)	DPG, PI, PE, PIM	S, U, T
<i>Corynebacterium</i> ^k	51–67	meso-A ₂ pm/A1γ	+	MK-9(H ₂), MK-8(H ₂)	DPG, PI, PIM	S, U
<i>Sanguibacter</i> ^l	69–70	L-Lys/A4α	ND	MK-9(H ₄)	ND	S, A, I

^a Abbreviations: LL-A₂pm, LL-diaminopimelic acid; L-Lys, L-lysine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PC, phosphatidylcholine; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PL, unknown phospholipid; GL, unknown glycolipid; PGL, unknown phosphoglycolipid; S, straight chain saturated; I, iso branched; A, anteiso branched; U, monounsaturated; T, tuberculostearic acid; 2OH-FA, 2-hydroxylated fatty acid; C, complex (straight chain saturated, monounsaturated, iso branched, anteiso branched, tuberculostearic acid, and 2-hydroxylated fatty acid); ND, not determined. +, present; —, absent.

^b Data from reference 45.

^c Data from references 3 and 15.

^d Data from reference 19.

^e Data from reference 2.

^f Data from reference 44.

^g Data from reference 35.

^h Data from reference 36.

ⁱ Data from reference 42.

^j Data from reference 9.

^k Data from references 24 and 37.

^l Data from reference 8.

unique taxonomic characteristics. These strains were isolated only from a sludge sample that resulted from wastewater treatment. Strains of this chemotaxonomic type were not found in the other habitats examined.

A phylogenetic analysis based on a 16S rDNA sequence comparison revealed that the two isolates are closely related and that their sequences are phylogenetically distinct from all actinomycete sequences available in databases. The genera *Terrabacter*, *Intrasporangium*, and *Sanguibacter* are the closest phylogenetic neighbors of the new isolates (Fig. 3). The genera *Terrabacter* and *Intrasporangium* differ from the new isolates in peptidoglycan type because they have type A3γ peptidoglycans with LL-diaminopimelic acid, a Gly₃ interpeptide bridge, and a glycine residue bound to the α-carboxyl group of D-glutamic acid in the peptide subunit (34, 35). Differences in the fatty acid patterns were also found, with the predominant fatty acid i-C_{15:0} (35) distinguishing the genera *Terrabacter* and *Intrasporangium* from the strains described here. Additional differences which characterize the genus *Intrasporangium* are my-

celial growth and the presence of completely unsaturated menaquinone MK-8 (21). In contrast to strains HKI 83^T and HKI 84, members of the genus *Sanguibacter* are motile and can be distinguished by the presence of major menaquinone MK-9(H₄), by having peptidoglycan type A4α with a L-Lys-Ser-D-Glu interpeptide bridge, and by a fatty acid composition whose predominant components are anteiso-C_{15:0} and C_{16:0} (8).

While peptidoglycan type A1γ is characteristic for mycolic acid-containing genera (e.g., the genera *Corynebacterium* and *Rhodococcus*), the following six coryneform genera are known to contain meso-A₂pm and to lack mycolic acids: the genera *Brevibacterium* (2), *Brachybacterium* (3, 15), *Dermabacter* (19), *Kineococcus* (44), *Microsphaera* (45), and *Turicella* (9). Members of these genera exhibit coccoid or rod-shaped morphology similar to the morphology of the strains described here. In contrast to members of the genera *Brachybacterium* (3), *Dermabacter* (19), and *Kineococcus* (44), members of the genus *Brevibacterium* (2) and new isolates HKI 83^T and HKI 84 are not able to produce acid from sugars in a peptone medium.

TABLE 3. Fatty acid compositions of *J. limosus* HKI 83^T and HKI 84

Strain	Fatty acid composition (%) ^a																		
	i-C _{14:0}	C _{14:0}	i-C _{15:0}	ai-C _{15:0}	C _{15:0}	C _{15:1}	i-C _{16:0}	i-C _{16:1}	C _{16:0}	C _{16:1}	i-C _{17:0}	i-C _{17:1}	ai-C _{17:1}	C _{17:0}	C _{17:1}	i-C _{18:0}	i-C _{18:1}	C _{18:0}	C _{18:1}
HKI 83 ^T	0.5	0.4	1.7	0.3	3.8	0.3	17.5	0	3.9	2.4	1.3	0	0.2	20.5	29.7	1.4	0.7	2.4	8.1
HKI 84	0.7	0.2	0.7	0.1	0.9	0.3	40.8	0.3	1.3	2.6	1.0	0.2	0.1	5.0	27.6	0.3	2.9	0.7	8.5

^a The abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, hexadecanoic acid; C_{18:1}, octadecenoic acid; i-C_{15:0}, 13-methyltetradecanoic acid; ai-C_{15:0}, 12-methyltetradecanoic acid. i, iso; ai, anteiso.

FIG. 3. Phylogenetic dendrogram based on the results of a 16S rDNA sequence comparison. Bar = 5 nucleotide substitutions per 100 nucleotides.

Strains HKI 83^T and HKI 84 can be easily distinguished from members of the genera *Brevibacterium*, *Brachybacterium*, *Dermabacter*, *Kineococcus*, and *Turicella* on the basis of the presence of menaquinone MK-8(H₄) and differences in the fatty acid profiles. Compared with strains HKI 83^T and HKI 84, members of the genera *Brevibacterium* and *Dermabacter* are characterized by lower DNA G+C contents and members of the genus *Kineococcus* are characterized by higher DNA G+C contents (Table 2). Strains HKI 83^T and HKI 84 have the same peptidoglycan type, (type A1γ) and the same menaquinone [menaquinone MK-8(H₄)] as members of the genera *Microsphaera*, *Dermatophilus*, and *Pseudonocardia* but are phylogenetically unrelated to these taxa (Fig. 3). *M. multipartita* also differs in its predominant fatty acids (iso-C_{16:0}, iso-C_{15:0}, and C_{18:1}) and in having a lower G+C content (67.5 mol%). Representatives of the genus *Dermatophilus* are characterized by

the formation of mycelia and by low G+C contents (57 to 59 mol%) compared with strains HKI 83^T and HKI 84 (36).

The genus *Pseudonocardia* is characterized by the presence of arabinose and galactose as diagnostic cell wall sugars; these sugars could not be detected in our isolates. The polar lipids phosphatidylcholine and phosphatidylethanolamine, which are present in members of the genus *Pseudonocardia*, were not found in strains HKI 83^T and HKI 84 (42).

On the basis of the results of our 16S rDNA sequence analysis and chemotaxonomic comparisons, we propose that the new genus *Janibacter* should be established for the strains described in this study. The type species of the genus *Janibacter* is *Janibacter limosus* sp. nov., which is represented by type strain HKI 83 and strain HKI 84.

Description of *Janibacter* gen. nov. *Janibacter* (Ja.ni.bac'ter. L. n. Janus, a god in roman mythology, who is said to have had two faces; M. L. masc. n. bacter, a rod; L. n. Janibacter, referring to the changing morphology of the microorganisms). Cells are gram positive, coccoid to rod shaped, and nonmotile and occur singly, in pairs, or occasionally in irregular clumps. No spores are formed. Not acid fast. Aerobic chemoorganotroph. Catalase is produced. Oxidase negative. The habitat of the strains is sludge and sewage waste. The cell wall type is A1γ (directly cross-linked meso-A₂pm). Mycolic acids are absent; the menaquinone is MK-8(H₄). The fatty acid profile is complex with straight-chain saturated and monounsaturated fatty acids as the major components, and the polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. The DNA base composition is 70 mol% G+C. The type species is *Janibacter limosus*.

Description of *Janibacter limosus* sp. nov. *Janibacter limosus* (li.mo'sus. L. adj. limosus, muddy, pertaining to sludge, the natural habitat of the species). Cells are gram-positive and nonmotile and occur singly, in pairs, or occasionally in irregular clumps. In liquid cultures cells are coccoid (diameter, 0.3 to 1.2 μm). On solid medium strains vary in cell morphology. The cells of some strains are short rods (0.4 by 1.4 μm), which change shape and become coccoid in older cultures. Colonies on nutrient agar are white or yellow, opaque, and convex; the colony surfaces are glistening or matt; and the colony margins are entire. *J. limosus* grows aerobically and tolerates 4% (wt/vol) NaCl in the medium. The optimal growth temperature is 28°C; growth occurs at 37°C on BHI medium but not on R medium. No growth occurs under anaerobic conditions. Catalase, indole, and H₂S are produced; urease and oxidase are not produced. Gelatin, starch, Tween 80, and casein are hydrolyzed. Citrate, formate, malate, and tyrosine are decomposed; aconitate, benzoate, succinate, tartrate, hippurate, esculin, adenine, xanthine, and hypoxanthine not decomposed. Nitrate is reduced to nitrite. Voges-Proskauer and methyl red reactions are negative. No acid is produced from L-arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, *m*-inositol, inulin, lactose, maltose, mannitol, mannose, raffinose, L-rhamnose, ribose, salicin, D-sorbitol, sucrose, potato starch, trehalose, and D-xylose. Has the chemotaxonomic characteristics of the genus. The DNA base composition is 70 mol% G+C. The type strain is strain HKI 83 (= DSM 11140).

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REFERENCES

- Cashion, P., M. A. Holder-Franklin, J. McCully, and M. Franklin. 1977. A rapid method for the base ratio determination of bacterial DNA. *Anal. Biochem.* **81**:461-466.
- Collins, M. D. 1992. The genus *Brevibacterium*, p. 1351-1354. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*, 2nd ed. Springer-Verlag, New York, N.Y.
- Collins, M. D., J. Brown, and D. Jones. 1988. *Brachybacterium faecium* gen. nov., sp. nov., a coryneform bacterium from poultry deep litter. *Int. J. Syst. Bacteriol.* **38**:45-48.
- Collins, M. D., T. Pirouz, M. Goodfellow, and D. E. Minnikin. 1977. Distribution of menaquinones in actinomycetes and corynebacteria. *J. Gen. Microbiol.* **100**:221-230.
- Cowan, S. T., and K. J. Steel. 1965. *Manual for the identification of medical bacteria*. Cambridge University Press, Cambridge, United Kingdom.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* **12**:133-142.
- De Soete, G. 1983. A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**:621-626.
- Fernandez-Garayzabal, J. F., L. Dominguez, C. Pascual, D. Jones, and M. Collins. 1995. Phenotypic and phylogenetic characterization of some unknown coryneform bacteria isolated from bovine blood and milk: description of *Sanguibacter* gen. nov. *Lett. Appl. Microbiol.* **20**:69-75.
- Funke, G., S. Stubbs, M. Altwegg, A. Carlotti, and M. D. Collins. 1994. *Turicella otidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. *Int. J. Syst. Bacteriol.* **44**:270-273.
- Gledhill, W. E., and L. E. Casida, Jr. 1969. Predominant catalase-negative soil bacteria. III. *Agromyces*, gen. n., microorganisms intermediary to *Actinomyces* and *Nocardia*. *Appl. Microbiol.* **18**:340-349.
- Gordon, R. E., D. A. Barnett, J. E. Handerhan, and C. H.-N. Pang. 1974. *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int. J. Syst. Bacteriol.* **24**:54-63.
- Gordon, R. E., and J. M. Mihm. 1957. A comparative study of some strains received as nocardiae. *J. Bacteriol.* **73**:15-27.
- Gräfe, U., M. Ritzau, W. Ihn, U. Möllmann, W. F. Fleck, I. Groth, and R. Reissbrodt. 1994. A new microbial isoquinoline iron chelator from *Streptomyces* spec. 2002-104. *J. Basic Microbiol.* **34**:351-355.
- Groth, I., P. Schumann, N. Weiss, K. Martin, and F. Rainey. 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.
- Gvozdyak, O. R., T. M. Nogina, and P. Schumann. 1992. Taxonomic study of the genus *Brachybacterium*: *Brachybacterium nesterenkovi* sp. nov. *Int. J. Syst. Bacteriol.* **42**:74-78.
- Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *J. Bacteriol.* **66**:24-26.
- Huss, V. A. R., H. Festl, and K. H. Schleifer. 1983. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst. Appl. Microbiol.* **4**:184-192.
- Janke, K.-D. 1992. BASIC computer program for evaluation of spectroscopic DNA renaturation data from GILFORD SYSTEM 2600 spectrophotometer on a PC/XT/AT type personal computer. *J. Microbiol. Methods* **15**:61-73.
- Jones, D., and M. D. Collins. 1988. Taxonomic studies on some human cutaneous coryneform bacteria: description of *Dermabacter hominis* gen. nov., sp. nov. *FEMS Microbiol. Lett.* **51**:51-56.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21-132. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, New York, N.Y.
- Kalakoutskii, L. V. 1989. Genus *Intrasporangium* Kalakoutskii, Kirillova and Krasil'nikov 1967, 79^{AL}, p. 2395-2397. In S. T. Williams, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 4. The Williams and Wilkins Co., Baltimore, Md.
- Kästner, M., M. Breuer-Jammali, and B. Mahro. 1994. Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). *Appl. Microbiol. Biotechnol.* **41**:267-273.
- Kramer, R. 1996. Genetic and physiological approaches for the production of amino acids. *J. Biotechnol.* **45**:1-21.
- Kroppenstedt, R. M. 1985. Fatty acids and menaquinone analysis of actinomycetes and related organisms, p. 173-189. In M. Goodfellow and D. E. Minnikin (ed.), *Chemical methods in bacterial systematics*. Academic Press, London, United Kingdom.
- Lanyi, B. 1987. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol.* **19**:1-67.
- Maisnierpatin, S., and J. Richard. 1995. Activity and purification of linenscin OC2, an antibacterial substance produced by *Brevibacterium linens* OC2, an orange cheese coryneform bacterium. *Appl. Environ. Microbiol.* **61**:1847-1852.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* **3**:208-218.
- Mesbah, M., U. Premachandran, and W. B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**:159-167.
- Miller, E. S., C. R. Woese, and S. Brenner. 1991. Description of the erythromycin-producing bacterium *Arthrobacter* sp. strain NRRL B-3381 as *Aeromicrobium erythreum* gen. nov., sp. nov. *Int. J. Syst. Bacteriol.* **41**:363-368.
- Minnikin, D. E., L. Alshamaony, and M. Goodfellow. 1975. Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolsolysates. *J. Gen. Microbiol.* **88**:200-204.
- Minnikin, D. E., M. D. Collins, and M. Goodfellow. 1979. Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J. Appl. Bacteriol.* **47**:87-95.
- Rainey, F. A., N. Ward-Rainey, R. M. Kroppenstedt, and E. Stackebrandt. 1996. The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsiaceae* fam. nov. *Int. J. Syst. Bacteriol.* **46**:1088-1092.
- Saddler, G. S., P. Tavecchia, S. Locuro, M. Zanol, L. Colombo, and E. Selva. 1991. Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J. Microbiol. Methods* **14**:185-191.
- Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**:407-477.
- Schumann, P., H. Prauser, F. A. Rainey, E. Stackebrandt, and P. Hirsch. 1997. *Friedmanniella antarctica* gen. nov., sp. nov., an L-diaminopimelic acid-containing actinomycete from Antarctic sandstone. *Int. J. Syst. Bacteriol.* **47**:278-283.
- Stackebrandt, E., R. M. Kroppenstedt, and V. J. Fowler. 1983. A phylogenetic analysis of the family *Dermatophilaceae*. *J. Gen. Microbiol.* **129**:1831-1838.
- Stackebrandt, E., J. Smida, and M. D. Collins. 1988. Evidence of phylogenetic heterogeneity within the genus *Rhodococcus*: revival of the genus *Gordona* (Tsukamura). *J. Gen. Appl. Microbiol.* **34**:341-348.
- Stead, D. E., J. E. Sellwood, J. Wilson, and I. Viney. 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.
- Suzuki, K., K. Nagai, Y. Shimizu, and Y. Suzuki. 1994. Search for actinomycetes in screening for new bioactive compounds. *Actinomycetologica* **8**:122-127.
- Tamaoka, J., and K. Komagata. 1984. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**:125-128.
- Uchida, K., and K. Aida. 1984. An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J. Gen. Appl. Microbiol.* **29**:131-134.
- Warwick, S., T. Bowen, H. McVeigh, and T. M. Embley. 1994. A phylogenetic analysis of the family *Pseudonocardiaceae* and the genera *Actinokineospira* and *Saccharothrix* with 16S rRNA sequences and a proposal to combine the genera *Amycolata* and *Pseudoamycolata* in an emended genus *Pseudonocardia*. *Int. J. Syst. Bacteriol.* **44**:293-299.
- Yamada, K., and K. Komagata. 1972. Taxonomic studies on coryneform bacteria. IV. Morphological, cultural, biochemical, and physiological characteristics. *J. Gen. Appl. Microbiol.* **18**:399-416.
- Yokota, A., T. Tamura, T. Nishi, and T. Hasegawa. 1993. *Kineococcus aurantiacus* gen. nov., sp. nov., a new gram-positive motile coccus with meso-diaminopimelic acid and arabinogalactan in the cell wall. *Int. J. Syst. Bacteriol.* **43**:52-57.
- Yoshimi, Y., A. Hiraishi, and K. Nakamura. 1996. Isolation and characterization of *Microsphaera multipartita* gen. nov., sp. nov., a polysaccharide-accumulating gram-positive bacterium from activated sludge. *Int. J. Syst. Bacteriol.* **46**:519-525.