Terracoccus luteus gen. nov., sp. nov., an LL-Diaminopimelic Acid-Containing Coccoid Actinomycete from Soil

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A gram-positive, aerobic actinomycete was isolated from soil. Spherical cells of this organism occur singly or form packets, which may cluster. The diagnostic diamino acid of the cell wall peptidoglycan is LL-diaminopimelic acid. The predominate menaquinone is MK-8 (H₄), and the main fatty acids are 13-methyl tetradecanoic acid and 12-methyl tetradecanoic acid. The diagnostic polar lipids are phosphatidylethanolamine and phosphatidylinositol. The DNA base composition is 73 mol% G+C. Comparison of 16S ribosomal DNA sequences showed that this isolate is a phylogenetic neighbor of *Terrabacter tumescens* and *Intrasporangium calvum*. Genotypic, chemotaxonomic, morphological, and physiological characteristics are used to describe a new genus and species, *Terracoccus luteus* gen. nov., sp. nov. The type strain is strain IMET 7848 (= DSM 44267).

The order *Actinomycetales* Buchanan 1917 was defined for gram-positive bacteria which produce mycelia or show at least a tendency to branch. The concept of the actinomycetes was extended on the basis of 16S ribosomal DNA (rDNA) comparisons, which revealed a major phylogenetic lineage that included coccoid, sarcinoid, rodlike, coryneform, and nocardioform organisms, as well as organisms forming aerial mycelia and spores. A combination of chemotaxonomic properties supported the phylogenetic clustering of species at the genus level (35). Recently, the order *Actinomycetales* was emended, and its genera were classified in a hierarchic system on the basis of 16S rDNA sequence comparisons (38).

Among the chemotaxonomic properties of the actinobacteria, the presence of particular diamino acids in position 3 of the peptide subunit of the peptidoglycan was shown to be a valuable taxonomic marker (31). One of these diagnostic diamino acids, LL-diaminopimelic acid (LL-A₂pm), was found to be characteristic of genera that had been described early, i.e., *Streptomyces* (44), *Intrasporangium* (12), and *Sporichthya* (17). Recent descriptions have considerably increased the number of LL-A₂pm-containing genera, which include *Nocardioides* (25, 26), *Terrabacter* (2), *Aeromicrobium* (21), *Luteococcus* (42), *Propioniferax* (47), *Microlunatus* (24), and *Friedmanniella* (32). These genera represent five phylogenetic lineages (32, 38), and in addition to their 16S rDNA sequence differences, they differ from each other in chemotaxonomic, morphological, and physiological properties (32).

In the course of studies directed at isolating novel coryneform and nocardioform actinomycetes for biotechnological exploitation, the strain described here, strain IMET 7848^T, was isolated among other strains from a soil sample during the summer of 1985. The coccoid LL-A₂pm-containing strain IMET 7848^T was studied with respect to its cultural, morphological, physiological, and chemotaxonomic characteristics, its 16S rDNA sequence, and its degree of DNA-DNA homology with related organisms. The organism was compared with previously described actinobacterial species and was found to represent a new species of a new genus.

MATERIALS AND METHODS

Bacterial strains. A soil sample was taken from the bank of a duck pond at Kloster (the island of Hiddensee, Baltic Sea, Germany) directly at the water-soil interface, and it was immediately subjected to an isolation procedure in its natural condition. One gram of soil was suspended in 100 ml of phosphate buffer, heavy particles were allowed to sediment, and the suspension was separated and then serially diluted with phosphate buffer; then 1 ml of an appropriate concentration was mixed with 6 ml of solubilized modified organic agar medium 79 (79m) (but with the agar concentration reduced to 0.6% [wt/vol]) at 48°C, and the mixture was poured onto 79m agar in petri dishes (diameter, 100 mm). Plates showing about 50 colonies after 4 to 6 days of incubation at 28°C were used for isolation under a binocular microscope at low magnification.

Strains used for the phenotypic comparisons were the following type strains of the type species of the genera which were included in this study: *Aeromicrobium erythreum* NRRL B-3381 (= DSM 8599), *Friedmanniella antarctica* DSM 11053, *Intrasporangium calvum* 7 KIP (= DSM 43043), *Janibacter limosus* DSM 11140, *Luteococcus japonicus* IFO 12422 (= DSM 10546), *Microlunatus phosphovorus* JCM 9379 (= DSM 10555), *Nocardioides albus* IMET 7807 (= DSM 43109), *Propioniferax innocua* NCTC 11082 (= DSM 8251), *Sanguibacter keddiei* NCFB 3025 (= DSM 10542), *Sporichthya polymorpha* IMRU 3913 (= DSM 43042), *Streptomyces albus* ATCC 3004 (= DSM 40313), and *Terrabacter tumescens* NCIB 8914 (= DSM 20308).

Phages used to study phage-host relationships were *Nocardioides* sensu stricto phages X1 (= DSM 49101), X3 (= DSM 49102), and X5 (= DSM 49103); *Nocardioides* sensu lato phages X6 (= DSM 49135), X10 (= DSM 49104), and X24 (= DSM 49105); *T. tumescens* phages Tb1 (= DSM 49116) and Tb2 (= DSM 49158); *Sporichthya polymorpha* phage Sp1 (= DSM 49146); and *Streptomyces* phages S6 (= DSM 49150) and S7 (= DSM 49151). Information on these phages has been given previously (6).

Media. The following media were used in a liquid or a semisolid state (the latter achieved by the addition of 1.5% [wt/vol] Kobo 1 agar [Serva]): 79m (32), R medium (46), enriched nutrient agar (Serva), Trypticase soy broth (TSB) medium (Difco), PYGV medium (39), and oatmeal agar (33). The first two media were used with and without the addition of vitamins according to a previously described prescription (39) at the concentration given for the PYGV medium.

Culture conditions. Growth and morphology on all of the media noted above were studied. The ranges of pH and of NaCl tolerance were determined on R agar, which was also used to prepare the inoculum for the physiological tests. The cell mass for the analyses of cell walls, mycolic acids, and polar lipids and for DNA extraction was produced on liquid R medium. For the study of menaquinones, cells were cultured on liquid 79m medium, and for the analysis of fatty acids, they were grown on TSB medium. The organisms were cultivated at 28°C, on a horizontal shaker at 220 rpm, in 100-ml bottles containing 20 ml of the medium. The cells were harvested after 24 h for the menaquinone and fatty acid analyses and after 48 h for the other chemotaxonomic studies.

Phage-host relationship studies. Susceptibility to phages was studied on 79m agar with double-layer agar plates by spotting high-titer phage suspensions (about 10¹⁰ phage/ml) onto the top layer, which had been charged with cells of the strains to be tested. A strain under study was registered as susceptible to a particular phage if lysis occurred on a second plate containing the same strain after the transfer of material from a lytic zone of the first plate.

Morphological studies. Colony shape, cell morphology, and aggregate morphology were studied by bright-field microscopy and by phase-contrast micros-

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copy (oil immersion objective; magnification, $\times 100$) of material obtained from surface growth on agar plates or from liquid shaken culture, as well as by direct observation of growing cultures on the agar surface.

Staining procedures. Gram staining and acid-fast staining were performed as described previously (16).

Physiological characterization. The temperature at which the physiological tests were performed was 28°C except for the studies of temperature range. The relationship to oxygen was studied by using stab cultures and agar slant cultures covered with paraffin oil. Oxidase activity was checked by examining the oxidation of N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride (34). Acid production from carbohydrates and alcohols was determined by the OF test as described previously (16). Utilization of organic acids as carbon sources and decomposition of xanthine, hypoxanthine, adenine, and DL-tyrosine were studied by previously described methods (8, 9). Catalase production was demonstrated on a slide by the production of bubbles immediately after a suspension of the organism was mixed with a drop of a 3% hydrogen peroxide solution. Urease activity, nitrate reduction, esculin hydrolysis, and hydrolysis of Tween 80 were studied by published methods (16). Decomposition of the emulsion of UV-sterilized cinefilm strips incubated in phosphate buffer was used to verify gelatin hydrolysis. Casein hydrolysis was determined on streak-inoculated agar (a mixture of 3 volumes of liquefied distilled-water agar and 1 volume of steamed skim milk). DNA hydrolysis was determined on DNase Test Agar (Difco), and hydrolysis of starch was determined on inorganic salts-starch agar (33) after flooding of the agar surface with Lugol's solution (0.1% [wt/vol] iodine and 0.2% [wt/vol] potassium iodide). H₂S production was determined as described previously (26). NaCl tolerance was checked by adding NaCl to R agar at final concentrations of 2, 4, and 10% (wt/vol). Additional carbon source utilization tests and enzyme tests were performed by previously described methods (13) which were modified as follows. The carbon source utilization test medium contained 6.7 g of yeast nitrogen base (Difco), 1.74 g of K₂HPO₄, and 0.36 g of KH₂PO₄ per liter. The enzyme test medium contained 0.05 M Tris-HCl buffer, 0.5 g of Casamino Acids (Difco) per liter, and 0.5 g of yeast extract (Difco) per liter. The panels were inoculated with standardized bacterial suspensions in 0.9% (wt/vol) NaCl with turbidities of 0.5 to 1.0 McFarland units and were subsequently incubated for 24 h at 28°C. Then, 50 μ l of the filter-sterilized indicator solution was added to each of the auxanographic test wells and control wells. The indicator solution contained 4 mM tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT; a redox dye) (Sigma) and 4 mM phenazine methosulfate (Sigma). The incubation was continued for 24 h in the dark. Formation of deep-blue-colored formazan indicated carbon source utilization (14). The auxanographic test results were read with a Multiscan MCC340 MKII photometer (Flow Laboratories, Meckenheim, Germany). The metabolization of a particular carbon source was considered to be positive if the following expression was fulfilled: E_{540} (substrate being tested) - E_{540} (control) > 0.129 (15). The results of the enzyme tests were visually evaluated.

Chemotaxonomic characterization. Purified cell wall preparations were obtained as described previously (31). Amino acids and peptides of cell wall hydrolysates were analyzed by two-dimensional ascending thin-layer chromatography on cellulose plates, using solvent systems described previously (31). The molar ratios of cell wall amino acids were determined by gas chromatography (GC) and GC-mass spectrometry (GC-MS) of *n*-heptafluorobutyl amino acid isobutyl esters (18). Cell wall sugars were identified as alditol acetates by GC-MS (10). The acyl type of peptidoglycan was determined by a previously described colorimetric method (43). Cellular fatty acids were extracted according to a published procedure (40) and were analyzed by GC (10). Menaquinone profiles were examined by high-performance liquid chromatography (37). Polar lipids were extracted as described previously (23) and were identified by two-dimensional thin-layer chromatography followed by spraying with specific reagents (3). The absence of mycolic acids was demonstrated as described previously (22).

Analysis of DNA base composition and DNA-DNA hybridization. The G+C content of the DNA was determined by high-performance liquid chromatography as described previously (10). For DNA-DNA hybridization, previously described methods (20) were used.

16S rDNA sequence determination and phylogenetic analysis. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA, and sequencing of the PCR products were performed as described previously (29). Significant overlap in the regions of adjacent primer targets existed, which resulted in the determination of almost the complete 16S rDNA sequence. The sequence reaction products were electrophoresed by using a model 373A automatic DNA sequencer (Applied Biosystems, Foster City, Calif.). The 16S rDNA sequences were manually aligned with those of members of the order *Actinomycetales* by using the ac2 editor (19). Evolutionary distances for strain IMET 7848^T and for a selection of actinobacterial species (data not shown) were calculated by a previously described method (11). Phylogenetic dendrograms were reconstructed by published methods (5, 30).

Nucleotide sequence accession numbers. The 16S rDNA sequence of strain IMET 7848^T (= DSM 44267^T) has been deposited in the EMBL database under accession no. Y11928. The accession numbers for the sequences which were used in the construction of the phylogenetic tree are as follows: *Aeromicrobium erythrum* NRRL B-3381^T, M37200; *Arthrobacter globiformis* DSM 20124^T, M23411; *Cellulomonas flavigena* DSM 20109^T, X83799; *I. calvus* DSM 43043^T, Z78214; *I. limosus* DSM 11140^T, Y08539; *L. japonicus* DSM 10546^T, Z78208; *Microbac-*

terium lacticum DSM 20427^T, X77441; *Microlunatus phosphovorius* DSM 10555^T, Z78207; *N. albus* DSM 43109^T, Z78213; *Sanguibacter keddiei* NCFB 3025^T, X79450; *Sporichthya polymorpha* DSM 46113^T, X72377; *Streptomyces albidoflavus* DSM 40455^T, Z76676; and *T. tumescens* DSM 20308^T, X83812. The 16S rDNA sequence of *Dermatophilus congolensis* DSM 43037 was obtained from the Ribosomal Database Project (19).

RESULTS

Culture characteristics. Strain IMET 7848^T grew well on agar media and in liquid shaken cultures. R medium, medium 79m, and TSB medium supported the growth of this organism. Growth on oatmeal agar was poor, and only traces of growth occurred on PYGV agar. The addition of vitamins did not influence growth. Visible growth appeared on optimal media after 24 to 48 h of incubation at 28°C. Growth was less rapid at 20°C, was very poor at 15 and 37°C, and was lacking at 10 and 45°C. The optimal pH was 6.0 to 7.2. At pH 4.5 and 9.5, growth was sparse. In stab cultures, growth occurred only at the surface. No growth was observed on agar slant cultures covered with paraffin oil. The color of the colonies was yellow with a slight tendency toward orange. Two forms of colonies were seen on agar plates at various numerical proportions: (i) rough, irregularly raised, lobed colonies (Fig. 1A); and (ii) smooth, convex, circular colonies (Fig. 1B). The colonies varied from 0.05 mm (if densely grouped) to 2.0 mm (if widely spaced) in diameter.

Maintenance. Serial transfers at 4-week intervals followed by maintenance at 4°C, freeze-drying, and maintenance in the vapor phase above liquid nitrogen were suitable provisions for preservation.

Phage-host relationships. Strain IMET 7848^T was not susceptible to any of the phages used in this study.

Morphological characteristics. On R agar plates incubated at 28°C, the cells of strain IMET 7848^T were spherical or nearly spherical and ranged from 0.7 to 1.3 μ m in diameter (Fig. 1C). The cells of smooth colonies occurred singly, in pairs, in tetrads, or in small irregular clusters (Fig. 1C). Upon direct inspection, the cells of rough colonies on agar plates were found to be mostly arranged in more or less regular packets which resulted from cell divisions in three perpendicular planes (Fig. 1D). As a rule, the packets adhered to one another, forming clusters (Fig. 1D). Formation of spores and cell motility were not observed.

Staining reactions. Strain IMET 7848^T stained gram positive, and no lysis was observed following treatment with 3% (wt/vol) KOH. The cells were not acid fast.

Physiological characteristics. Strain IMET 7848^T grew only in the presence of air. The oxidase test was negative. Catalase, urease, and H₂S were produced. Nitrate was not reduced to nitrite. Acid was not produced from any of the tested substrates (D-ribose, L-arabinose, D-xylose, L-rhamnose, D-glucose, D-fructose, D-mannose, D-galactose, maltose, lactose, sucrose, D-trehalose, D-raffinose, adonitol, dulcitol, *myo*-inositol, D-mannitol, and D-sorbitol). Starch, casein, gelatin, esculin, and DNA were hydrolyzed. Tween 80 was not hydrolyzed. Sodium acetate, sodium formate, sodium aconitate, sodium benzoate, sodium citrate, disodium succinate, and potassium hydrogen tartrate were not utilized. Xanthine, hypoxanthine, adenine, DL-tyrosine, and sodium hippurate were not decomposed. The MTT reduction tests showed that strain IMET 7848^T utilized D-arabitol, gluconate, *N*-acetyl-D-glucosamine, *myo*-inositol, L-proline, succinate, sucrose, and D-turanose. Utilization of acetamide, L-alanine, 4-aminobutyrate, L-aspartate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, caprate, citrate, D-glucosaminic acid, D-glucuronate, 2-oxoglutarate, L-leucine, phenylacetate, pimelate, putrescine, quinate, L-rhamnose, D-ri-

bose, L-serine, tyramine, 2-hydroxyvalerate, and L-valine was not observed. Strain IMET 7848^T hydrolyzed all of the applied chromogenic substrates, i.e., *p*-nitrophenyl- β -D-xyloside, *p*-nitrophenylphosphorylcholine, and 2-deoxythymidine-5-*p*-nitrophenyl phosphate.

Chemotaxonomic characteristics. The peptidoglycan of strain IMET 7848^T contained LL-A₂pm. The molar ratio of the peptidoglycan amino acids Gly, Ala, Glu, and LL-A₂pm was 3.6:1.5:1.0:0.9. From this ratio and from the occurrence of typical peptides in partial hydrolysates (data not shown), it follows that the peptidoglycan represents the variation A3 γ with three glycine residues as an interpeptide bridge (31). On the same basis, it was concluded that a fourth glycine residue is bound to the α -carboxyl group of D-glutamic acid in position 2 of the peptide subunit (peptidoglycan type A41.2 [6]). In the present study, binding of glycine to glutamic acid in position 2 was also demonstrated for *I. calvum*, as had been seen previously (32). The acyl type was acetyl. The isoprenoid quinones were represented by MK-8(H₄) and MK-8(H₂) at a peak area ratio of 34:18. The fatty acid profile was distinctly dominated by 13-methyl tetradecanoic acid and 12-methyl tetradecanoic acid, with minor amounts of hexadecanoic acid and 14-methyl hexadecanoic acid (Table 1). Diagnostic polar lipids were phosphatidylinositol and phosphatidylethanolamine, in addition to phosphatidylglycerol and diphosphatidylglycerol. Mycolic acids were absent. Whole-cell sugars were, in order of decreasing amounts, rhamnose, ribose, galactose, glucose, and mannose.

DNA base composition. The DNA base composition was 73 mol% G+C.

DNA-DNA hybridization. The percent DNA-DNA homology was 38.8% for strains IMET 7848^T and *I. calvum* DSM 43043, 36.45% for strains IMET 7848^T and *T. tumescens* DSM 20308, and 52.7% for the *I. calvum* and *T. tumescens* strains.

16S rDNA sequence analysis. An almost complete 16S rDNA sequence comprising 1,476 nucleotides (>95% of the *Escherichia coli* sequence [1]) was determined for strain IMET 7848^T. A total of 1,322 nucleotides present in all of the strains between positions 41 and 1458 (*E. coli* positions [1]) were used for comparative analysis. The 16S rDNA sequence correspondence values were 97.6% for the strain pair IMET 7848^T and *T. tumescens*, 97.1% for the strain pair IMET 7848^T and *I. calvum*, and 97.1% for the strain pair *T. tumescens* and *I. calvum*. The phylogenetic dendrogram (Fig. 2) shows that *J. limosus* and *Sanguibacter keddii* are less closely related to the aforementioned three organisms than they are to each other. The cluster of strain IMET 7848^T, *T. tumescens*, *I. calvum*, *J. limosus*, and *Sanguibacter keddii* is supported by bootstrap values of greater than 83%. The relationship between strain IMET 7848^T and *T. tumescens* is supported by a bootstrap value of 100%.

Two colony forms of strain IMET 7848^T exist. Initially only one form of colony, a rough one, was observed for strain IMET 7848^T (Fig. 1A and 1D). During the detailed studies, smooth colonies occurred (Fig. 1B) together with the rough ones. To

FIG. 1. (A and B) Bright-field photomicrographs, taken without a cover slide, of growing, densely located colonies on the surface of R agar in petri dishes after 4 days of incubation at 28°C. Bar = 50 μ m. (A) Strain IMET 7848-r—rough colonies. (B) Strain IMET 7848-s—smooth colonies. (C) Phase-contrast photomicrograph of strain IMET 7848^T cultivated for 2 days at 28°C in shaken culture with R medium. Cells occur singly, in pairs, in tetrads, or in little clusters. Bar = 10 μ m. (D) Phase-contrast photomicrograph of strain IMET 7848^T, taken after putting a cover slide onto growth on R agar in ring culture (diameter, 45 mm; thickness of agar layer, 2.5 mm) after 2 days of incubation at 28°C. Cells are in regular packets which form large, irregular clusters. Bar = 10 μ m.

TABLE 1. Percentages of cellular fatty acids for strains IMET 7848^T, IMET 7848-r, and IMET 7848-s and for related organisms

Organism	Fatty acid (% of total) ^a													
	i-C _{13:0}	ai-C _{13:0}	i-C _{14:0}	i-C _{14:1}	C _{14:0}	i-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}	ai-C _{17:0}
Strain IMET 7848 ^T	0.7	0.7	2.9	2.1	2.1	32.2	32.2	26.8	1.3	2.9	13.3	1.0	1.5	5.4
Strain IMET 7848-r	1.1	1.1	3.8	1.7	1.7	35.9	33.2	33.2	2.5	2.4	7.6	1.2	0.7	3.8
Strain IMET 7848-s	1.1	1.1	3.5	1.8	1.8	34.3	34.3	30.4	1.9	0.1	10.0	0.9	1.0	4.1
<i>T. tumescens</i> ^b			24.2			35.4	35.4	1.5	1.1	13.4	8.6	1.4	1.7	3.9
<i>I. calvum</i> ^b			5.0			37.8	37.8	3.5	2.7	1.5	12.3	3.1	1.7	3.7

^a i, iso; ai, anteiso. Examples of further characterization: C_{16:0}, hexadecanoic acid; i-C_{16:1}, 14-methyl pentadecenoic acid; ai-C_{15:0}, 12-methyl tetradecanoic acid.

^b Data are from reference 32.

FIG. 2. 16S rDNA sequence-based phylogenetic dendrogram reconstructed from evolutionary distances by the neighbor-joining method (30). Scale bar, 5 inferred nucleotide substitutions per 100 nucleotides. Bootstrap values are represented by the numbers given at the branching points. Bootstrap values below 80% have been omitted. All organisms which are not members of the family Intrasporangiaceae serve as outgroup organisms for this family.

exclude the possibility of contamination, cells were isolated from both colony forms. The colony appearances of the resulting two strains proved to be stable on all applied media. After 5 days of incubation on R agar plates at 28°C, the mats of the irregularly raised and lobed colonies of IMET 7848-r were dull while those of the circular, convex colonies of strain IMET 7848-s were shiny, and those of strain IMET 7848^T, which exhibited both forms of colonies, were less shiny. There were no differences in color. The fatty acid profiles of the three strains were similar with regard to the quality and quantity of the components (Table 1). The partial 16S rDNA sequences of these strains were identical over the 5'-terminal 350 nucleotides. The DNA-DNA hybridization studies revealed DNA similarity values of 100% for the strain pair IMET 7848^T and IMET 7848-r and for the strain pair IMET 7848^T and IMET 7848-s; i.e., the three strains, which originated from the same original culture, are identical from a taxonomic point of view. The organism under study has the capacity to develop rough as well as smooth colonies. The strains designated IMET 7848-r (for the rough form) and IMET 7848-s (for the smooth form) have been deposited at the DSMZ-German Collection of Microorganisms and Cell Cultures as DSM 44275 and DSM 44274, respectively.

DISCUSSION

The phylogenetic analysis places strain IMET 7848^T in a cluster together with *T. tumescens* and *I. calvum* (Fig. 2). *T. tumescens* was previously affiliated with the genera *Arthrobacter* and *Pimelobacter* (41), but the organism was shown to represent a separate phylogenetic lineage (2). *I. calvum*, originally (and mistakenly) thought to form sporangia (12), is a phylogenetic neighbor of *T. tumescens* (32). All of the other LL-A₂pm-containing actinobacterial genera have been recognized as members of phylogenetic lineages which are separate from the *Terrabacter-Intrasporangium* lineage (2, 21, 28, 32, 38, 42, 47).

Two recently described organisms, *Sanguibacter keddiei* (7)

and *J. limosus* (20), were shown to be the nearest phylogenetic neighbors of *T. tumescens* and *I. calvum* (Fig. 2) (20, 38). The genera *Intrasporangium*, *Terrabacter*, and *Sanguibacter* were placed in the recently introduced family *Intrasporangiaceae* Rainey et al. (38), which was affiliated with the suborder *Micrococccineae* (Prévot) Stackebrandt et al. of the order *Actinomycetales* (Buchanan) Stackebrandt et al. (38). According to the conception of the latter authors (38), the genus *Janibacter* (20) is also a member of the family *Intrasporangiaceae*. However, *Sanguibacter keddiei* and *J. limosus* differ significantly from strain IMET 7848^T and its relatives, and from each other, with regard to phenotypic characteristics (Table 2). *J. limosus* contains meso-diaminopimelic acid in its directly cross-linked peptidoglycan and shows a complex fatty acid profile that includes straight-chain, iso-branched, saturated, and unsaturated fatty acids (Table 2). *Sanguibacter keddiei* has lysine as the diamino acid in the peptidoglycan, has predominately straight-chain saturated fatty acids (Table 2), and has menaquinone MK-9(H₄) instead of menaquinone MK-8(H₄), which is present in strain IMET 7848^T and in *T. tumescens* (Table 2).

Strain IMET 7848^T, *T. tumescens*, and *I. calvum* share an LL-A₂pm-containing peptidoglycan structure which is unique among the organisms of the class *Actinobacteria* Stackebrandt et al. (38) in that three glycine residues form the interpeptide bridge and another glycine residue is bound to the glutamic acid of the peptide subunit.

Since the published fatty acid profiles of one and the same strain frequently differ to some degree, e.g., the profiles reported for the type strain of *T. tumescens* (4, 25, 32, 41), the type strains of all LL-A₂pm-containing actinobacteria were examined under strictly standardized conditions in a previous study (32). The strains IMET 7848^T, IMET 7848-r, and IMET 7848-s were subjected to the same procedure, and the results were compared with the previous ones (Table 1). *T. tumescens*, *I. calvum*, and strain IMET 7848^T all have large amounts of 13-methyl tetradecanoic acid (iso-C_{15:0}). However, strain IMET 7848^T differs significantly from both *T. tumescens* and *I. calvum* in having comparably large amounts of 12-methyl tetradecanoic acid (anteiso-C_{15:0}) and of hexadecanoic acid (C_{16:0}) and a small amount of 14-methyl pentadecanoic acid (iso-C_{16:0}) and in the presence of five and the absence of two minor fatty acids, respectively (Table 1). Additionally, strain IMET 7848^T differs from *T. tumescens* in having a small amount of 12-methyl tridecanoic acid (iso-C₁₄) (Table 1). In other words, strain IMET 7848^T is the most divergent organism with regard to fatty acid profile within the group consisting of the genera *Terrabacter* and *Intrasporangium* and strain IMET 7848^T. Concerning the polar lipids, strain IMET 7848^T differs from *T. tumescens* in the presence of phosphatidylglycerol and the absence of unidentified phospholipids, and it differs from *I. calvum* in the presence of phosphatidylethanolamine and the absence of phosphatidylinositol mannosides (Table 2).

The growth cycle of *T. tumescens* includes cocci, irregular rods, initial branching, and fragmentation to cocci; i.e., the organism is typically coryneform. *I. calvum* develops an extended substrate mycelium which fragments into irregular propagative elements; i.e., the organism is typically nocardioform. Strain IMET 7848^T is invariably coccoid and propagates by cell divisions in three perpendicular planes; i.e., it has a morphology and a life cycle like those of a sarcina (Fig. 1D).

The absence of susceptibility of strain IMET 7848^T to any of the applied phages is not unexpected. It has been shown (27) that actinophages in general, and phages that are active against LL-A₂pm-containing actinomycetes in particular, are highly specific at the species or genus level or for closely related

genera (6). The absence of phage susceptibility of strain IMET 7848^T, particularly to the *T. tumescens* phages Tb1 and Tb2, supports a separate taxonomic placement of strain IMET 7848^T.

The 73 mol% G+C of the DNA of strain IMET 7848^T corresponds to the values reported for *T. tumescens* (32) and differs from those of *I. calvum* by 5 mol% (Table 2).

DNA-DNA hybridization was performed, since 16S rDNA sequence homologies above 97.5% can correspond to DNA-DNA homology values that may be low or as high as 100% (36). In contrast to the delineation of species, for which DNA-DNA similarity values have been suggested to be higher than 70% (45), no guidelines have been published for the delineation of genera. In those cases in which discriminating chemotaxonomic and morphological properties that have been used in the discrimination of other actinobacterial genera are present, even moderate DNA-DNA similarity values, as shown in the present study, should not exclude the description of novel genera.

Taking into account the phylogenetic data, the DNA-DNA homology values, and the chemotaxonomic and morphological characteristics, including the growth cycles, there are two possibilities for classification of strain IMET 7848^T and its relatives: (i) emendation of the genus *Intrasporangium*, reclassification of *T. tumescens* as *Intrasporangium tumescens*, and description of strain IMET 7848^T as the type strain of a new species of the genus *Intrasporangium*; or (ii) maintenance of *I. calvum* and *T. tumescens* as representatives of individual genera and description of a new genus and new species for strain IMET 7848^T. Considering the present kind of taxonomic reasoning concerning the genus level within the order *Actinomycetales*, we assume the latter procedure to be the more adequate one. Additional isolates of relevant organisms will contribute to a better understanding of the relationships within this taxonomic area. However, these strains are presently not available. Thus, we propose that strain IMET 7848 be the type strain of the type species of a new genus, *Terracoccus*, which is described below.

Description of *Terracoccus* gen. nov. *Terracoccus* (Ter.ra.coc'cus. M. L. fem. n. *terra*, soil; Gr. masc. n. *coccus*, berry; M. L. masc. n. *Terracoccus*, coccus isolated from soil). Spherical to slightly ellipsoidal cells existing singly, in pairs, in tetrads, in irregular small clusters, or in more or less regular sarcinoid packets which may form large irregular clusters. Gram positive. Not acid fast. Nonmotile. No formation of spores.

Strictly aerobic. Chemoorganotrophic. Oxidase negative. Catalase positive.

The LL-diaminopimelic acid-containing peptidoglycan of group A3γ has three glycine residues as an interpeptide bridge and a fourth glycine residue bound to the α-carboxyl group of the D-glutamic acid in position 2 of the peptide subunit. The major menaquinone is MK-8(H₄). The fatty acid profile represents the iso, anteiso type with predominately 13-methyl tetradecanoic acid followed by 12-methyl tetradecanoic acid. Minor straight-chain fatty acids occur. The polar lipids are phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol. Mycolic acids are absent.

The DNA base composition is 73 mol% G+C.

The phylogenetic analysis relates this genus to the genera *Terrabacter* and *Intrasporangium*, which are, along with the genus *Terracoccus*, members of the family *Intrasporangiaceae* of the *Actinomycetales* suborder *Micrococccineae*. All signature nucleotides defined for the family *Intrasporangiaceae* are also found in strain IMET 7848^T except for base pair positions 1134 to 1140, in which strain IMET 7848^T has an A-U rather than a G-C pair.

TABLE 2. Differential characteristics of strain IMET 7848^T and its relatives^a

Organism	Morphology	A ₂ pm isomer	Inter-peptide bridge	Major menaquinone ^b	Polar lipids	G+C mol% of DNA	Profile of cellular fatty acid (%): ^c											
							i-C _{14:0}	C _{14:0}	i-C _{15:0}	ai-C _{15:0}	i-C _{16:0}	i-C _{16:1}	C _{16:0}	ai-C _{17:0}	C _{17:0}	C _{17:1}	C _{18:0}	C _{18:1}
Strain IMET 7848 ^T	Cocci in packets	LL	Gly ₃	MK-8(H ₄)	PE, PL, PG, DPG	73			32	27			13		5			
<i>T. tumescens</i> ^d	Rod/coccus cycle	LL	Gly ₃	MK-8(H ₄)	PE, PI, DPG, PL	70-73	24		35		13	9						
<i>I. calum</i> ^d	nf-mycelium	LL	Gly ₃	MK-8	PI, PIM, PG, DPG	68	5		38	13	12							
<i>T. limosus</i> ^e	Cocci, rods	meso	None	MK-8(H ₄)	DPG, PG, PI	70					18				21	30		8
<i>Sanguibacter keddii</i> ^f	Motile rods	None (lysine)	ND	MK-9(H ₄)	ND	69-70	6			11			53			10		

^a Abbreviations: Gly, glycine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylmannosides; PE, phosphatidylethanolamine; PL, unidentified glycolipid(s); ND, not determined; nf, nocardioform fragmentation of the hyphae.

^b Menaquinone nomenclature: e.g., MK-8(H₄) = menaquinone with two of eight isoprene units hydrogenated.

^c Values less than 5% are not shown. Percentages are rounded off to units. For abbreviations and for detailed fatty acid profiles, see Table 1.

^d Data are from reference 32.

^e Data are from reference 20.

^f Data are from reference 7.

The type species is *Terracoccus luteus*.

Description of *Terracoccus luteus* sp. nov. *Terracoccus luteus* (lu'te.us M. L. adj. *luteus*, yellow). The cells are spherical to slightly ellipsoidal, 0.7 to 1.3 µm in diameter, gram positive, not acid fast, and nonmotile. The colonies, 0.05 to 2.0 mm in diameter, may be rough or smooth. Rough colonies are lobed and irregularly raised; smooth colonies are circular and convex. The cells of rough colonies are mostly arranged in more or less regular sarcinoid packets, which may form large irregular clusters. The cells of smooth colonies occur singly, in pairs, in tetrads, or in small irregular clusters.

Aerobic. Chemoorganotrophic. Oxidase negative. Catalase positive. Urease and H₂S are produced. Nitrate is not reduced to nitrite. Formation of acids from sugars and alcohols is not observed. Only a few of the tested compounds are utilized for growth, i.e., D-arabitol, gluconate, N-acetyl-D-glucosamine, myo-inositol, L-proline, succinate, sucrose, and D-turanose. Starch, casein, gelatin, esculin, and DNA are hydrolyzed; Tween 80 is not hydrolyzed. Sodium acetate, formate, aconitate, benzoate, citrate, hippurate, disodium succinate, and potassium tartrate are not utilized. Xanthine, hypoxanthine, adenine, and DL-tyrosine are not decomposed.

The LL-A₂pm-containing cell wall peptidoglycan contains three glycine residues as an interpeptide bridge and another glycine residue bound to glutamic acid of the peptide subunit. The major menaquinone is MK-8(H₄). The major fatty acids are 13-methyl tetradecanoic acid and 12-methyl tetradecanoic acid. Diagnostic polar lipids are phosphatidylethanolamine and phosphatidylinositol. Mycolic acids are absent.

The DNA base composition is 73 mol% G+C.

The only known isolation site is soil at the water-soil interface of a duck pond at the island of Hiddensee, Germany.

The type strain is strain IMET 7848. It has been deposited in the DSMZ-German Collection of Microorganisms and Cell Cultures GmbH as strain DSM 44267^T.

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