The Journal of Antibiotics (2012) 65, 427–431

www.nature.com/ja

ORIGINAL ARTICLE

Luteimicrobium album sp. nov., a novel actinobacterium isolated from a lichen collected in Japan, and emended description of the genus *Luteimicrobium*

Moriyuki Hamada[1,2], Hideki Yamamura[2], Chiaki Komukai[1], Tomohiko Tamura[1], Ken-ichiro Suzuki[1] and Masayuki Hayakawa[2]

A novel Gram-stain-positive actinobacterium, designated RI148-Li105^T, was isolated from a lichen sample from Rishiri Island, Japan, and its taxonomic position was investigated by a polyphasic approach. 16S rRNA gene sequencing study indicated that strain RI148-Li105^T was related to the type strain of *Luteimicrobium subarcticum*, with a similarity of 97.8%. Cells of strain RI148-Li105^T exhibited a rod–coccus cycle. The diagnostic cell-wall diamino acid of this organism was lysine and the peptidoglycan type was found to be A4α. The predominant menaquinones were MK-8(H_2) and MK-9(H_2), and the major fatty acids were iso-$C_{16:0}$, $C_{17:1}$ ω9c and $C_{17:0}$. The DNA G + C content was 73.6 mol%. The major phenotypic characteristics of strain RI148-Li105^T basically corresponded to those of the genus *Luteimicrobium* excluding the fatty acid composition. These results suggest that strain RI148-Li105^T should be affiliated with the genus *Luteimicrobium*. Meanwhile, DNA–DNA hybridization and some phenotypic characteristics revealed that the strain differs from *L. subarcticum*. Therefore, strain RI148-Li105^T represents a novel species of the genus *Luteimicrobium*, for which the name *Luteimicrobium album* sp. nov. is proposed. The type strain of *Luteimicrobium album* is RI148-Li105^T (= NBRC 106348^T = DSM 24866^T).
The Journal of Antibiotics (2012) **65,** 427–431; doi:10.1038/ja.2012.45; published online 6 June 2012

Keywords: actinobacterium; lichen; *Luteimicrobium album* sp. nov.; polyphasic taxonomy.

INTRODUCTION

The genus *Luteimicrobium* was proposed by Hamada *et al.*[1] with a single species, *Luteimicrobium subarcticum*. The type strain of this species was isolated from a soil sample from Rishiri Island, Japan. The cells are Gram-stain-positive, non-motile and non-spore-forming, and exhibit a rod–coccus life cycle. This organism has peptidoglycan type A4α (with lysine as the diagnostic diamino acid).[2] The predominant menaquinone and the major fatty acid are MK-8(H_2) and anteiso-$C_{15:0}$, respectively. The genus *Luteimicrobium* is a member of the suborder *Micrococcineae* in the order *Actinomycetales*, but the family to which the genus *Luteimicrobium* belongs has not been specified.

Recently, lichens attracted attention as an isolation source of actinobacteria and, in fact, it has been reported that some actinobacteria were isolated from lichen samples and were proposed as novel taxa, namely *Actinomycetospora iriomotensis*, *A. rishiriensis*, *Frondihabitans cladoniiphilus*, *Leifsonia lichenia* and *Nocardioides exalbidus*.[3–7] During the screening of lichen-associated actinobacteria, we isolated a novel actinobacterium (strain RI148-Li105^T) from a foliaceous lichen sample collected from Rishiri Island, Hokkaido, Japan. Comparative 16S rRNA gene sequence analysis revealed that the isolate is related to the genus *Luteimicrobium*. In the current study, we aim to clarify the taxonomic position of strain RI148-Li105^T by using a polyphasic approach.

METHODS

Bacterial strains and isolation

A foliaceous lichen growing on a tree was collected from Rishiri Island, Hokkaido, Japan, and was then air dried for 7 days at room temperature. The lichen sample was homogenized with a sterilized mortar and pestle in the presence of sterilized distilled water, and the suspension was spread on humic acid–vitamin (HV) agar[8] containing nalidixic acid (20 mg^{-1}) and cycloheximide (50 mg^{-1}), which was then incubated at 30 °C for 2 weeks. Following the incubation period, strain RI148-Li105^T was isolated and transferred to a nutrient agar (NA) plate for purification. Yeast extract–Starch medium (NBRC medium 266; containing yeast extract 2 g, soluble starch 10 g in 1.0 liter tap water; agar 15 g, if required; pH 7.3) was used for general laboratory cultivation, morphological studies and determination of optimal growth parameters. Biomass for chemotaxonomic and molecular systematic studies was obtained by culturing the strain in shake flasks for 5 days at 28 °C at 100 r.p.m. *Luteimicrobium subarcticum* NBRC 105647^T (= R19-

[1]NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan and [2]Division of Applied Biological Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Kofu, Yamanashi, Japan
Correspondence: Dr M Hamada, NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan.
E-mail: hamada-moriyuki@nite.go.jp
Received 18 August 2011; revised 24 January 2012; accepted 29 April 2012; published online 6 June 2012

04^T) was used as the reference strain for comparison with the chemotaxonomic, physiological, biochemical and the DNA–DNA hybridization tests.

Morphological, physiological and biochemical tests

Colony appearance and pigment production were examined after incubation at 28 °C for 5 days on agar plate of NBRC medium 266. Morphological features were observed with age (up to 7 days) under a light microscope (model BX-51; Olympus, Tokyo, Japan) and a scanning electron microscope (model JSM-6060; JEOL, Tokyo, Japan). Cell motility was determined by observing cells suspended in a saline solution under a light microscope. The temperature range and optimum temperature for growth were determined by incubating the cultures at 5, 10, 15, 20, 25, 28, 37, 45 and 60 °C on agar plates of NBRC medium 266 after 5 days incubation. Growth at 5 and 10 °C was evaluated after 14 days incubation. The pH range and NaCl tolerance for growth were determined by measuring the turbidity (610 nm) of 5 ml of the culture medium in test tubes after 1–5 days of incubation at 28 °C. The pH range and optimum pH for growth were established by using a liquid NBRC medium 266 adjusted to pH 4–10 in 1.0 pH unit intervals. Tolerance to NaCl was tested using a liquid NBRC medium 266 adjusted to NaCl concentrations of 1, 3, 5, 7, 10 and 15% (w/v). Growth under anaerobic conditions was determined by incubating in an anaerobic chamber with an O_2-absorbing and CO_2-generating agent (Anaero-Pack; Mitsubishi Gas Chemical, Tokyo, Japan). Gram staining was performed using Hucker's modification.[9] Oxidase activity was determined using cytochrome oxidase paper (Nissui Pharmaceutical, Tokyo, Japan). Catalase activity was determined by production of bubbles after the addition of a drop of 3.0% H_2O_2. Other physiological and biochemical tests were performed using API ZYM, API Coryne, API 20E and API 50CH systems (bioMérieux, Tokyo, Japan) according to the manufacturer's instructions.

16S rRNA sequence determination and phylogenetic analysis

DNA was isolated and purified using a DNeasy Blood and Tissue kit (Qiagen, Tokyo, Japan) according to the manufacturer's instruction. 16S rRNA gene was amplified by PCR using *TaKaRa Ex Taq* (Takara Bio, Shiga, Japan) with the following pair of primers: 9 F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1541 R (5′-AAGGAGGTGATCCAGCC-3′). The amplified 16S rRNA gene was subjected to cycle sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the following primers: 9 F, 785 F (5'-GGATTAGATACCCTGGTAGTC-3′), 802R (5′-TACCA GGGTATCTAATCC-3′) and 1541R. The products were analyzed using an automated DNA sequencer (model ABI PRISM 3730 Genetic Analyzer; Applied Biosystems). The phylogenetic neighbors were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server.[10] The almost-complete 16S rRNA gene sequence determined in this study was aligned with reference sequences of the genus *Luteimicrobium* and some related taxa by using the CLUSTAL_X program.[11] Phylogenetic trees were constructed using the neighbor-joining and maximum-likelihood algorithms[12,13] using the MEGA 5.0 program.[14] The resultant tree topologies were evaluated by bootstrap analysis[15] based on 1000 replicates.

G + C content of DNA and DNA–DNA hybridization

DNA was obtained using the method of Saito and Miura.[16] The DNA G + C content was determined by the method of Tamaoka and Komagata[17] using a high-performance liquid chromatography (model LC-10A; Shimadzu, Kyoto, Japan) equipped with a Cosmosil 5C_{18}-MS-II column (100 × 4.6 mm i.d.; Nacalai Tesque, Kyoto, Japan). Acetonitrile–water containing 0.2 M ammonium phosphate was used as the mobile phase (acetonitrile, 5%, 15 min) at the flow rate of 1.0 ml min^{-1} with UV detection at 270 nm. The microplate hybridization method developed by Ezaki *et al.*[18,19] was used to determine DNA relatedness with minor modification.[20]

Chemotaxonomic tests

Cell-wall samples were prepared from ~1 g of wet cells by carrying out mechanical disruption with an ultrasonic oscillator. The cell walls were separated from unbroken cells by differential centrifugation in distilled water, and further purified in boiling 4% SDS (100 °C, 30 min), followed by several washings with distilled water. Molar ratios of the amino acids in cell-wall hydrolysates (4 M HCl, 16 h at 100 °C) were determined by high-performance liquid chromatography (model LC-10AD; Shimadzu) equipped with a Wakopak wakosil-PTC column (200 × 2.0 mm i.d.; Wako Pure Chemical Industries, Osaka, Japan), as their phenyl isothiocyanate derivatives (Wako Pure Chemical Industries). PTC-amino acids mobile phase A–PTC-amino acids mobile phase B (Wako Pure Chemical Industries) was used as the mobile phase under a linear gradient elution mode (solution B, 0–100%, 20 min) at the flow rate of 1.0 ml min^{-1} with UV detection at 254 nm. Amino-acid isomers in the cell-wall hydrolysates were analyzed according to the method described by Nozawa *et al.*[21] using a liquid chromatograph-mass spectrometer (LC-MS; model LCMS-2020 and LC-20AB; Shimadzu) equipped with a Shim-Pack FC-ODS column (150 × 2.0 mm i.d.; Shimadzu). Cell-wall sugars were analyzed as 1-phenyl-3-methyl-5-pyrazolone derivatives of the cell-wall hydrolysates (4 M HCl, 16 h at 100 °C), which were prepared according to the method described by Honda *et al.*[22] and Yang *et al.*[23] The 1-phenyl-3-methyl-5-pyrazolone-labeled hydrolysates were analyzed using a LC-MS (model LCMS-2020 and LC-20AB; Shimadzu), equipped with a Shim-Pack FC-ODS column (150 × 2.0 mm i.d.; Shimadzu). Acetonitrile–water containing 5 mM formic acid was used as the mobile phase under a linear gradient elution mode (acetonitrile, 15–30%, 30 min) at the flow rate of 0.2 ml min^{-1} with UV detection at 250 nm. Isoprenoid quinones were extracted from ~300 mg of dry cells by using chloroform–methanol (2:1, v/v). The menaquinone fractions were separated by TLC using hexane–diethyl ether (8.5:1.5, v/v) as the solvent. The menaquinone spot was detected under UV light, and the menaquinones were extracted with acetone, dried using a nitrogen stream and then analyzed using a LC-MS (model LCMS-8030 and LC-20AD; Shimadzu) equipped with a Senshu-Pak Pegasil ODS-SP-100 column (100 × 2.0 mm i.d.; Senshu Scientific, Tokyo, Japan). Methanol–isopropanol was used as the mobile phase (34% isopropanol, 60 min) at the flow rate of 0.2 ml min^{-1} with UV detection at 275 nm. The preparation and analysis of cellular fatty acid methyl esters were performed using the protocol of the MIDI Sherlock Microbial Identification System[24,25] and a gas chromatography (model 6890N; Agilent Technologies, Santa Clara, CA,

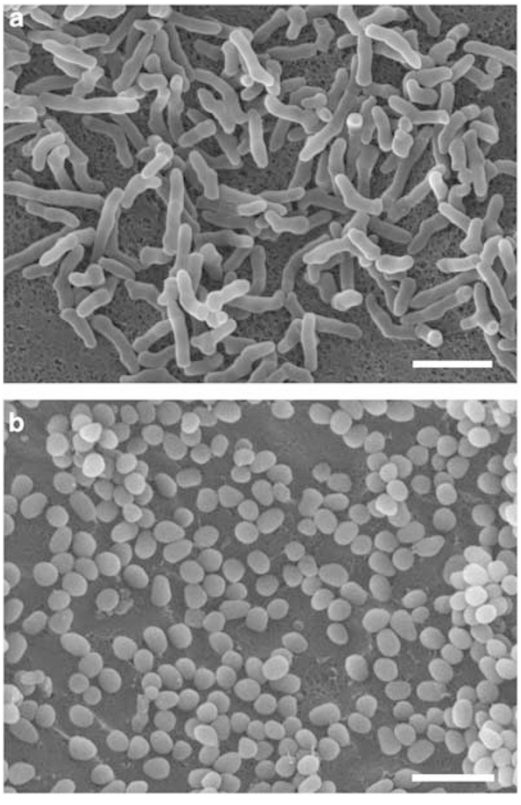

Figure 1 Scanning electron micrographs of strain RI148-Li105^T grown on NBRC medium 266 for 1 day (**a**) and 5 days (**b**) at 28 °C. Bar, 2 μm.

USA). The fatty acids were identified using the Microbial Identification Sherlock software package (ACTINO database, version 4.0). Polar lipid analysis was performed as described by Hamada *et al.*[26]

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RI148-Li105^T is AB646194.

RESULTS

Strain RI148-Li105^T formed circular, smooth and white colonies that were approximately 0.5 mm in diameter on NBRC medium 266 after 5 days of incubation at 28 °C. Pigment production was not observed. The cells of the strain were Gram-stain-positive, facultatively anaerobic, non-motile and non-spore-forming and exhibited a rod–coccus life cycle. Cells of exponentially growing cultures were irregular rods (0.5–0.7 × 1.5–4.0 μm), whereas stationary-phase cells were cocci (~0.5 μm in diameter) (Figure 1). Growth occurred at 10–37 °C and no growth was observed at 5, 45 or 60 °C. The pH range for growth was 6.0–8.0. Optimal growth was noted at 28 °C and pH 7.0. The strain exhibited good growth with NaCl concentrations of 0–3% (w/v) and moderate growth with 5%; no growth was observed with 7, 10 or 15% NaCl. The optimal NaCl concentration for growth was 1%. The results of other physiological and biochemical analyses are summarized in the species description below.

Phylogenetic analysis on the basis of the 16S rRNA gene sequence revealed that strain RI148-Li105^T belonged to the suborder *Micrococcineae*. However, this strain did not form a reliable clade with any members of the suborder. The highest similarity value was observed with *Luteimicrobium subarcticum* (97.8%), followed by *Cellulomonas chitinilytica* (97.2%), *C. denverensis* (97.0%) and *Cellulosimicrobium funkei* (97.0%). In the neighbor-joining tree, the phylogenetic lineage of strain RI148-Li105^T joined that of *L. subarcticum* R19-04^T (Figure 2). Although the bootstrap value of this branch was low in the neighbor-joining tree, this relationship was also recovered in the tree generated with the maximum-likelihood algorithm. The DNA–DNA relatedness between strain RI148-Li105^T and *L. subarcticum* NBRC 105647^T (= R19-04^T) was 1–9%. These values are well below the 70% cutoff point of DNA–DNA relatedness, which is a criterion for the assignment of bacterial strains to the same genomic species.[27]

The obtained peptidoglycan sample of strain RI148-Li105^T contained alanine (Ala), glutamic acid (Glu) and lysine (Lys) in a molar ratio of 2.0:2.0:1.1. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, L-Ala, D-Glu and L-Lys. These data strongly suggested that the cell-wall peptidoglycan of strain

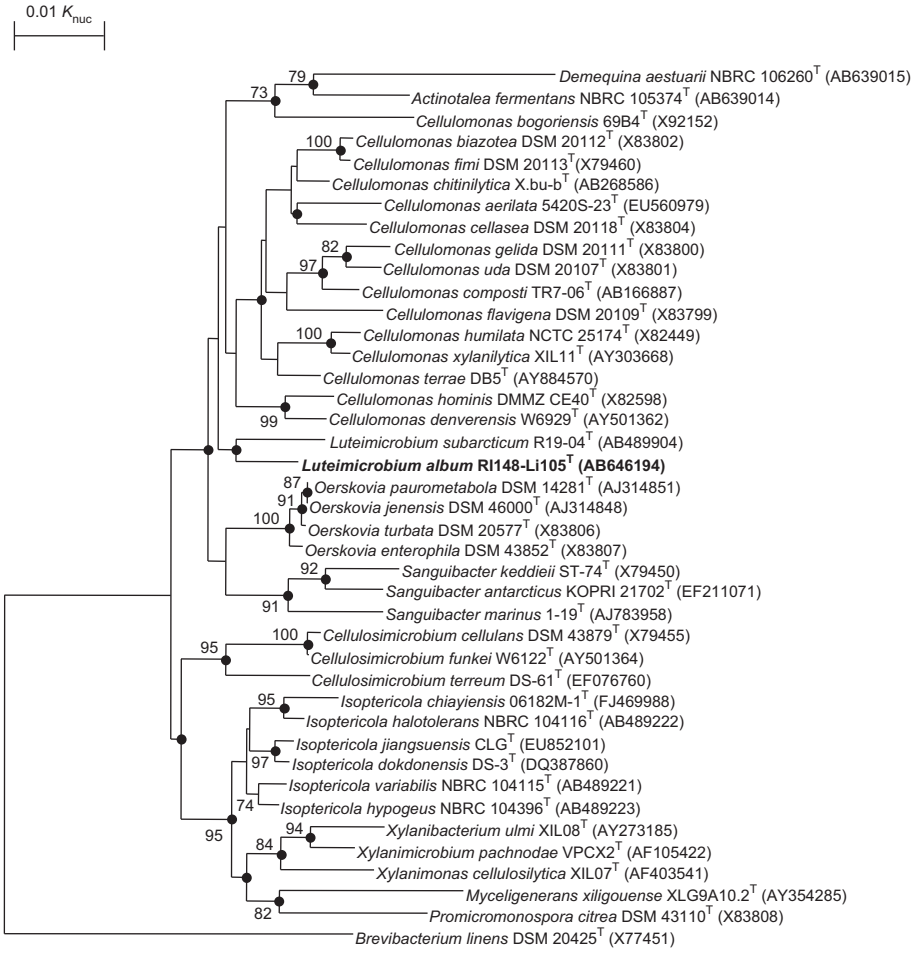

Figure 2 Phylogenetic tree derived from the 16S rRNA gene sequences of strain RI148-Li105^T and its taxonomic neighbors. The tree was constructed using the neighbor-joining method. The 16S rRNA gene sequence of *Brevibacterium linens* DSM 20425^T (X77451) was used as the out-group. The numbers at the branch nodes are bootstrap percentages (from 1000 replicates); only values of 70% or above are shown. Dots indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. Bar, 0.01 K_{nuc}.

RI148-Li105^T was of A4α type as described by Schleifer & Kandler,[2] with L-Lys as the diagnostic cell-wall diamino acid and an interpeptide bridge of the peptidoglycan comprising D-Glu. The major cell-wall sugar was mannose, whereas galactose was a trace component. The predominant menaquinones were MK-8(H_2) and MK-9(H_2) (65:35). The major cellular fatty acids were iso-$C_{16:0}$ (42.7%), $C_{17:1}$ ω9c (23.4%) and $C_{17:0}$ (12.2%) (Table 1). The major polar lipids determined were diphosphatidylglycerol, phosphatidylinositolmannoside, one glycolipid and four unidentified polar lipids, along with minor or trace amounts of phosphatydylinositol and one additional unidentified polar lipid (Supplementary Figure 1). The DNA G + C content was 73.6 mol%.

DISCUSSION

Phylogenetic analysis based on 16S rRNA gene sequence suggested that strain RI148-Li105^T was phylogenetically related to the member of the genus *Luteimicrobium*. In addition, strain RI148-Li105^T exhibited a rod–coccus life cycle, and the peptidoglycan of the strain was of A4α type. These phenotypic characteristics corresponded to those of the genus *Luteimicrobium*. The predominant menaquinones of the strain were MK-8(H_2) and MK-9(H_2) (molar ratio, 65: 35), and that of *L. subarcticum* was MK-8(H_2) with MK-9(H_2) as the minor component (molar ratio, 90:10). Therefore, it can be considered that strain RI148-Li105^T and *L. subarcticum* have a similar isoprenoid quinone system. This characteristic permits the distinction among the genus *Luteimicrobium* and its phylogenetic neighbors (Table 2). Considering the result of the phylogenetic analysis, the menaquinone composition and the peptidoglycan structure, it is suitable that strain RI148-Li105^T is regarded as a member of the genus *Luteimicrobium*. The major fatty acid of *Luteimicrobium subarcticum* and the members of the genera *Cellulomonas*, *Cellulosimicrobium*, *Isoptericola* and *Oerskovia* was reported to be anteiso-$C_{15:0}$. Meanwhile, the major fatty acid of strain RI148-Li105^T was iso-$C_{16:0}$, but anteiso-branched fatty acids were not detected (Table 2). Also, it is interesting that monounsaturated fatty acid ($C_{17:1}$ ω9c) was detected as the major component of strain RI148-Li105^T. At this time, the difference of cellular fatty acid profile may be considered as a interspecies diversity within the genus *Luteimicrobium* as well. If many strains closely related to the genus *Luteimicrobium* are discovered in the future, strain RI148-Li105^T may be reclassified in a separate genus.

On the other hand, the DNA–DNA relatedness between strain RI148-Li105^T and *L. subarcticum* was low, and the results of the physiological and biochemical tests distinguished strain RI148-Li105^T from *L. subarcticum* (Table 3). Therefore, a novel species of the genus *Luteimicrobium*, namely, *Luteimicrobium album* sp. nov., is proposed for strain RI148-Li105^T. In addition, the description of the genus *Luteimicrobium* Hamada *et al.*[1] should be emended to reflect the variations in the fatty acid profile, menaquinone composition, polar lipids and oxygen relationship.

Table 1 Cellular fatty acid compositions (%) of strain RI148-Li105^T and *L. subarcticum* NBRC 105647^T

Fatty acid	*RI148-Li105^T*	*L. subarcticum NBRC 105647^T*
Straight-chain saturated fatty acids		
$C_{14:0}$	—	1.3
$C_{15:0}$	2.4	—
$C_{16:0}$	3.0	**10.9**
$C_{17:0}$	**12.2**	—
Branched saturated fatty acids		
iso-$C_{14:0}$	1.1	1.0
iso-$C_{15:0}$	—	2.3
iso-$C_{16:0}$	**42.7**	5.1
iso-$C_{18:0}$	1.4	—
anteiso-$C_{15:0}$	—	**71.4**
anteiso-$C_{17:0}$	—	8.0
Mono-unsaturated fatty acids		
$C_{16:1}$ ω9c	2.5	—
$C_{17:1}$ ω9c	**23.4**	—
$C_{18:1}$ ω9c	5.2	—
10-Methyl fatty acids		
10-Methyl $C_{17:0}$	4.4	—

Bold type shows the major components (>10%). –, Not detected or detected in trace amounts (<1%).

Emended description of the genus *Luteimicrobium*

The description is as given by Hamada *et al.*[1] with the following modifications. Cells are aerobic or facultatively anaerobic. The predominant menaquinones are MK-8(H_2) and/or MK-9(H_2). The major cellular fatty acids consist of iso- or anteiso- branched fatty acids. Straight-chain and monounsaturated fatty acids may be contained as major components. The major polar lipid is diphosphatidylglycerol. Phosphatidylglycerol, phsphatidylinositolmannoside and glycolipid may be contained as the major components.

Description of *Luteimicrobium album* sp. nov.

Luteimicrobium album (al'bum. L. neut. adj. album, white). Cells exhibit a rod–coccus cycle and are Gram-stain-positive, facultatively anaerobic, non-motile and non-spore-forming. Rods are 0.5–0.7 × 1.5–4.0 μm and cocci are ~0.5 μm in diameter. Colonies are circular, smooth and white. Catalase- and oxidase-negative. The temperature range for growth is 10–37 °C, and the optimal temperature is 28 °C. The

Table 2 Major chemotaxonomic characteristics of strain RI148-Li105^T and the genera *Luteimicrobium*, *Cellulomonas*, *Cellulosimicrobium*, *Isoptericola* and *Oerskovia*

Characteristic	*1*	*2*	*3*	*4*	*5*	*6*
Peptidoglycan type	A4α	A4α	A4β	A4α	A4α	A4α
Diamino acid	L-Lys	L-Lys	L-Orn	L-Lys	L-Lys	L-Lys
Interpeptide bridge	D-Glu	D-Glu	D-Asp or D-Glu	D-Ser–D-Asp or L-Thr–L-Asp	D-Asp or D-Glu	L-Thr–D-Asp or L-Thr–D-Glu
Major menaquinone(s)	MK-8(H_2), MK-9(H_2)	MK-8(H_2)	MK-9(H_4), MK-8(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)
Major fatty acids	i-$C_{16:0}$, $C_{17:1}$ ω9c, $C_{17:0}$	ai-$C_{15:0}$, $C_{16:0}$	ai-$C_{15:0}$, $C_{14:0}$	ai-$C_{15:0}$, i-$C_{15:0}$	ai-$C_{15:0}$, i-$C_{15:0}$	ai-$C_{15:0}$, $C_{16:0}$

Taxa: 1, strain RI148-Li105^T; 2, *Luteimicrobium*; 3, *Cellulomonas*; 4, *Cellulosimicrobium*; 5, *Isoptericola*; 6, *Oerskovia*. Data for reference genera were taken from Hamada *et al.*[1] +, Positive; –, negative; ai, anteiso-branched; i, iso-branched.

Table 3 Differential phenotypic characteristics of strain RI148-Li105^T and *Luteimicrobium subarcticum*

Characteristic	*RI148-Li105^T*	*L. subarcticum*
Colony color	White	Yellow
Growth at 37 °C	+	−
pH range for growth	6.0–8.0	5.0–9.0
Catalase	−	+
Acid phosphatase	+	−
β-Galactosidase	−	+
α-Glucosidase	−	+
Nitrate reduction	−	+
Acid production from (API 50CH):		
N-acetyl-glucosamine	−	+
Amygdalin	−	+
L-arabinose	−	+
Arbutin	−	+
Gentiobiose	−	+
Salicin	−	+
D-turanose	+	−
Major fatty acids (>10%)	i-$C_{16:0}$, $C_{17:1}$ ω9c, $C_{17:0}$	ai-$C_{15:0}$, $C_{16:0}$
Polar lipids	DPG, PI, PIM, GL	DPG, PG
DNA G+C content (mol%)	73.6	72.9

Abbreviations: +, positive; –, negative; ai, anteiso-branched; i, iso-branched; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; GL, glycolipid.

pH range for growth is 6.0–8.0, and the optimal pH is 7.0. Growth occurs at NaCl concentrations of 0–5% (w/v), but not at concentrations higher than 7%. The optimal NaCl concentration for growth is 1%. Acid is produced from D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, D-sucrose and D-xylose. *N*-acetyl-β-glucosaminidase, acid phosphatase, β-glucosidase, leucine arylamidase are present, whereas alkaline phosphatase, arginine dihydrolase, α-chymotrypsin, cysteine arylamidase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase, ornithine decarboxylase, phosphohydrolase, pyrazinamidase, trypsin, tryptophan deaminase, urease and valine arylamidase are absent. Aesculin is hydrolyzed, but gelatin is not. H_2S and indole are not produced. Nitrate is not reduced. The peptidoglycan is of the A4α type with an interpeptide bridge comprising D-Glu. The major cell-wall sugar is mannose. The predominant menaquinones are MK-8(H_2) and MK-9(H_2). The major cellular fatty acids are iso-$C_{16:0}$, $C_{17:1}$ ω9c and $C_{17:0}$, followed by $C_{18:1}$ ω9c, 10-methyl $C_{17:0}$, $C_{16:0}$, $C_{16:1}$ ω9c, $C_{15:0}$, iso-$C_{18:0}$, iso-$C_{18:0}$ and $C_{18:0}$. The polar lipids are diphosphatidylglycerol, phosphatidylinositolmannoside, one glycolipid and four unidentified polar lipids. The DNA G+C content of the type strain is 73.6 mol%.

The type strain RI148-Li105^T (=NBRC 106348^T=DSM 24866^T) was isolated from a lichen sample from Rishiri Island, Japan.

ACKNOWLEDGEMENTS

We are grateful to Mr Yuya Sakuraki for excellent technical assistance. This study was partly supported by a research grant from the Institute for Fermentation, Osaka (IFO), Japan.

1 Hamada, M. *et al. Luteimicrobium subarcticum* gen. nov., sp. nov., a new member of the suborder *Micrococcineae*. *Int. J. Syst. Evol. Microbiol.* **60,** 796–800 (2010).
2 Schleifer, K. H. & Kandler, O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36,** 407–477 (1972).
3 Yamamura, H. *et al. Actinomycetospora iriomotensis* sp. nov., a novel actinomycete isolated from a lichen sample. *J. Antibiot.* **64,** 289–292 (2011).
4 Yamamura, H. *et al. Actinomycetospora rishiriensis* sp. nov., an actinomycete isolated from a lichen. *Int. J. Syst. Evol. Microbiol.* **61,** 2621–2625 (2011).
5 Cardinale, M., Grube, M. & Berg, G. *Frondihabitans cladoniiphilus* sp. nov., a novel actinobacterium of the family *Microbacteriaceae* isolated from lichen, and emended description of the genus *Frondihabitans*. *Int. J. Syst. Evol. Microbiol.* **61,** 3033–3038 (2011).
6 An, S. Y., Xiao, T. & Yokota, A. *Leifsonia lichenia* sp. nov., isolated from lichen in Japan. *J. Gen. Appl. Microbiol.* **55,** 339–343 (2009).
7 Li, B., Xie, C. H. & Yokota, A. *Nocardioides exalbidus* sp. nov., a novel actinomycete isolated from lichen in Izu-Oshima Island, Japan. *Actinomycetologica* **21,** 22–26 (2007).
8 Hayakawa, M. & Nonomura, H. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J. Ferment. Technol.* **65,** 501–509 (1987).
9 Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. *Methods for General and Molecular Bacteriology* (Edited by American Society for Microbiology) (Washington, DC, 1994).
10 Chun, J. *et al.* EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **57,** 2259–2261 (2007).
11 Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25,** 4876–4882 (1997).
12 Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4,** 406–425 (1987).
13 Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17,** 368–376 (1981).
14 Tamura, K. *et al.* MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28,** 2731–2739 (2011).
15 Felsenstein, J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39,** 738–791 (1985).
16 Saito, H. & Miura, K. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta.* **72,** 619–629 (1963).
17 Tamaoka, J. & Komagata, K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS. Microbiol. Lett.* **25,** 125–128 (1984).
18 Ezaki, T. *et al.* Simple genetic identification method of viridans group streptococci by colorimetric dot hybridization and quantitative fluorometric hybridization in microdilution wells. *J. Clin. Microbiol.* **26,** 1708–1713 (1988).
19 Ezaki, T., Hashimoto, Y. & Yabuuchi, E. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39,** 224–229 (1989).
20 Tamura, T., Nakagawa, Y. & Kawasaki, H. DNA–DNA hybridization method. In *Identification Manual of Actinomycetes* (Edited by The Society for Actinomycetes Japan) 134–137 (The Business Center for Academic Societies in Japanese, Tokyo, 2001).
21 Nozawa, Y., Sakai, N., Arai, K., Kawasaki, Y. & Harada, K. Reliable and sensitive analysis of amino acids in the peptidoglycan of actinomycetes using the advanced Marfey's method. *J. Microbiol. Methods* **70,** 306–311 (2007).
22 Honda, S. *et al.* High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl-5-pyrazolone derivatives. *Anal. Biochem.* **180,** 351–357 (1989).
23 Yang, X., Zhao, Y., Wang, Q., Wang, H. & Mei, Q. Analysis of the monosaccharide components in *Angelica* polysaccharides by high performance liquid chromatography. *Anal. Sci.* **21,** 1177–1180 (2005).
24 Sasser, M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids, MIDI Technical Note 101. MIDI Inc.* Newark, Delaware (1990).
25 MIDI *MIS operating manual, version 4.5. MIDI, Inc.* Newark, Delaware (2002).
26 Hamada, M. *et al. Mobilicoccus pelagius* gen. nov., sp. nov. and *Piscicoccus intestinalis* gen. nov., sp. nov., two new members of the family *Dermatophilaceae*, and reclassification of *Dermatophilus chelonae* (Masters *et al.* 1995) as *Austwickia chelonae* gen. nov., comb. nov. *J. Gen. Appl. Microbiol.* **56,** 427–436 (2010).
27 Wayne, L. G. *et al.* Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37,** 463–464 (1987).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)