Actinomadura meridiana sp. nov., isolated from mountain soil

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A novel actinomycete, designated strain DLS-45\textsuperscript{T}, was isolated from soil from the surface of a rock collected from the peak of Darangshi Oreum (Small Mountain) in Jeju, Republic of Korea. Substrate mycelium was well developed, but aerial mycelium was scant on most of the media tested. Spherical to subspherical spores on the aerial mycelium were in chains that were arranged in hooks and their surfaces were warty. The combination of morphological and chemical features supported the classification of the new isolate in the genus Actinomadura. The neighbour-joining tree based on 16S rRNA gene sequences showed that the strain belonged to the family Thermomonosporaceae and formed a coherent cluster with Actinomadura pelletieri IMSNU 22169\textsuperscript{T} (98.0% sequence similarity). The other closest relatives were Actinomadura bangladeshensis 3-46-b(3)\textsuperscript{T} (98.2%) and Actinomadura chokoreiensis 3-25-a(11)\textsuperscript{T} (97.9%). Levels of DNA–DNA relatedness between strain DLS-45\textsuperscript{T} and the type strains of the phylogenetic relatives were less than 17%. A battery of phenotypic, genotypic and DNA–DNA relatedness data indicated that strain DLS-45\textsuperscript{T} represented a novel species of the genus Actinomadura, for which the name Actinomadura meridiana sp. nov. is proposed. The type strain is strain DLS-45\textsuperscript{T} (=KCTC 19558\textsuperscript{T}=DSM 45252\textsuperscript{T}).

The family Thermomonosporaceae Rainey et al. 1997 contains the genera Actinomadura, Actinocorallia, Spirillospora and Thermomonospora. A fifth genus, Actinoallomorbus (Tamura et al., 2009), which includes Actinoallomorbus spadix (previously known as Actinomadura spadix), was recently added to the family. Members of these genera can be readily differentiated from one another using a battery of morphological, physiological and chemotaxonomic characteristics (Zhang et al., 1998, 2001; Miyadoh & Miyara, 2001; Trujillo & Goodfellow, 2003; Tamura et al., 2009). The emended genus Actinomadura (Zhang et al., 2001; Miyadoh & Miyara, 2001), at the time of writing, encompasses 45 species with validly published names, including Actinomadura rifamycini, previously considered a subspecies of Actinomadura cremae: Actinomadura cremae subsp. rifamycini (Prommuan et al., 2011). A polyphasic taxonomic characterization is presented of a mycelium-forming actinobacterium, isolated from soil from the surface of a rock, which possessed the phenotypic and genetic features typical of the genus Actinomadura.

Strain DLS-45\textsuperscript{T} was isolated from soil from the surface of a rock collected from the peak of Darangshi Oreum (Small Mountain) in Jeju, Republic of Korea. For bacterial isolation, the soil sample was treated and the medium prepared as described by Seo & Lee (2010). The isolate was maintained on ISP (International Streptomycyes Project) 2 medium (Shirling & Gottlieb, 1966) at 4°C and as 20% glycerol suspensions at −20 and −80°C. For phenotypic comparison and DNA–DNA hybridization, Actinomadura bangladeshensis DSM 45347\textsuperscript{T}, Actinomadura chokoreiensis DSM 45346\textsuperscript{T} and Actinomadura pelletieri IMSNU 22169\textsuperscript{T} were also grown on ISP 2 medium at 30°C.

Growth and cultural characteristics of strain DLS-45\textsuperscript{T} were examined by using various agar media (ISP 2–ISP 7) described by Shirling & Gottlieb (1966) and oatmeal-nitrate agar (Prauser & Bergholz, 1974), incubated for 21 days at 30°C. For scanning electron microscopy, the specimen was prepared using cells grown on ISP 4 medium for 21 days, as described by Seo & Lee (2010). The temperature range (4, 10, 20, 30, 37, 42 and 45°C), pH range (pH 4.0–10.0 at intervals of one pH unit) and tolerance of NaCl [0–6% (w/v), at intervals of 1.0%] for growth were determined by using ISP 2 medium. The temperature for growth was recorded with the plates incubated for 2 weeks. The pH and tolerance of NaCl for growth were observed after incubation for 2 weeks at 30°C. Urease activity was determined by a colour change in Bacto urea broth (Difco). Gram staining, oxidase and catalase activities, nitrate reductase, gelatinase and hydrolysis of aesculin were examined as described by MacFaddin et al.
Degradation of hypoxanthine, casein, DL-tyrosine and xanthine were assessed by the methods of Gordon et al. (1974). Hydrolysis of carboxymethylcellulose was examined on ISP 2 medium. Hydrolysis of DNA and starch was determined by using DNase test agar (Difco) and starch agar (Difco), respectively. Utilization of carbohydrates was examined on ISP 9 medium (Shirling & Gottlieb, 1966), with each filter-sterilized compound used at a final concentration of 1% (w/v) for sugars and 0.1% (w/v) for organic acids.

Strain DLS-45\(^{T}\) showed good growth on ISP 2, 3 and 6 media and oatmeal-nitrate agar, but poor growth on ISP 4 and 5 media and no growth on ISP 7 medium. Substrate mycelia were abundantly produced on most of the media tested and the colours were variable depending on the growth medium. Aerial mycelium was scant on most media, but moderately produced and white to grey on ISP 6 medium after incubation for 4 weeks. Diffusible pigments were produced on ISP 2 (dark brown) and ISP 6 (light yellow) media. Cultural characteristics are detailed in Supplementary Table S1 in IJSEM Online. Spherical to oval, non-motile, non-spore-forming, Gram-positive, aerobic, catalase-positive. The preparation and the analysis of fatty acid methyl esters were performed according to the method described by Minnikin et al. (1975). The 16S rRNA gene sequences were performed using the method described by Hopwood et al. (1985).

For genomic DNA extraction and chemotaxonomic analyses, biomass was obtained from cells grown in ISP 2 broth for 5 days at 30 °C with shaking. The isomer of diaminopimelic acid in the peptidoglycan was analysed according to the method described by Staneck & Roberts (1974). Whole-cell sugars were analysed by GC as described by Saddler et al. (1991). Isoprenoid quinones were analysed by HPLC as described by Kroppenstedt (1985). Polar lipids were analysed by TLC using the method of Minnikin et al. (1975). The analysis of mycolic acids was performed using the method of Minnikin et al. (1980). For analysis of cellular fatty acids, strain DLS-45\(^{T}\), A. bangladeshensis DSM 45346\(^{T}\) and A. pelletieri IMSNU 22169\(^{T}\) were grown in ISP 2 broth for 5 days at 30 °C at 170 r.p.m. Aliquots of each culture broth were transferred to ISP 2 medium and the plates were incubated for 7 days at 30 °C. The preparation and the analysis of fatty acid methyl esters were performed according to the instructions of the Microbial Identification System (version 2.11; MIDI), using the ACTINO database (version 3.8) for identification of fatty acids.

The whole-cell hydrolysates of strain DLS-45\(^{T}\) contained meso-diaminopimelic acid, glucose, galactose, mannose, madurose and ribose. The polar lipids contained diphasphatidylglycerol and phosphatidylinositol (see Supplementary Fig. S1 in IJSEM Online). The menaquinone content was MK-9(H\(_{6}\)) (61%), MK-9(H\(_{8}\)) (33%) and MK-9(H\(_{4}\)) (7%). The polar lipid and menaquinone profiles of A. pelletieri IMSNU 22169\(^{T}\) in this study were similar to those of strain DLS-45\(^{T}\). Mycolic acids were absent. The DNA G+C content of strain DLS-45\(^{T}\), as measured by HPLC (Mesbah et al., 1989), was 68.9 mol%.

The fatty acid profiles of strain DLS-45\(^{T}\), A. bangladeshensis DSM 45347\(^{T}\), A. chokoriensis DSM 45346\(^{T}\) and A. pelletieri IMSNU 22169\(^{T}\) consisted of a mixture of saturated, iso- and anteiso-branched, unsaturated and 10-methyl-branched fatty acids. The predominant fatty acids of strain DLS-45\(^{T}\) were C\(_{16:0}\) (55.3%), C\(_{18:1}\) cis\(_{9}\) (7.8%) and C\(_{18:0}\) (7.3%). Strain DLS-45\(^{T}\) could be differentiated from its phylogenetic relatives by the proportion of the major components and the presence/absence of C\(_{12:0}\), C\(_{16:0}\) 9-methyl and C\(_{17:0}\) 10-methyl acids. The fatty acid profiles of strain DLS-45\(^{T}\) and the phylogenetically related species of the genus Actinomadura are given in Supplementary Table S2.

Chromosomal DNA was extracted and purified using the method described by Hopwood et al. (1985). The 16S rRNA gene of strain DLS-45\(^{T}\) was amplified by PCR, as described by Lee et al. (2000), and purified by using the Wizard PCR Preps DNA Purification System (Promega). The PCR product was sequenced using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl, Applied Biosystems). The almost complete 16S rRNA gene sequence (1433 nt) of strain DLS-45\(^{T}\) determined in this study was compared with the corresponding sequences of members of the family Thermomonosporaceae. Multiple alignments of the 16S rRNA gene sequences were performed using the CLUSTAL_X program (Thompson et al., 1997). Phylogenetic relationships were analysed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed, by using the NEIGHBOR program in the PHYLIP package, version 3.6 (Felsenstein, 2002), from evolutionary distances computed as described by Jukes & Cantor (1969), with bootstrap values based on 1000 replications (Felsenstein, 1985).

The neighbour-joining tree (Fig. 2) based on 16S rRNA gene sequences showed that strain DLS-45\(^{T}\) belonged to the family Thermomonosporaceae and formed a tight cluster with...

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**Fig. 1.** Field-emission scanning electron micrograph of strain DLS-45\(^{T}\), showing hooked chains of warty ornamented spores, after 3 weeks’ growth at 30 °C on ISP 4 medium. Bar, 1 μm.
A. pelletieri IMSNU 22169T. This relationship was supported by a bootstrap value of 74% and was also found in the trees created by the maximum-parsimony and maximum-likelihood treeing algorithms. Sequence similarity calculations after neighbour-joining analysis showed that the closest relatives of strain DLS-45T were A. bangladeshensis 3-46-b(3)T (98.2%), A. pelletieri IMSNU 22169T (98.0%), and A. chokoriensis 3-25-a(11)T (97.9%). Levels of 16S rRNA gene sequence similarity between strain DLS-45T and the other species of the genus Actinomadura were less than 97.7%.

DNA–DNA hybridization experiments between strain DLS-45T and the related three type strains of the genus Actinomadura were performed by using a photobiotin-labelled DNA probe and microplate wells, as described by Ezaki et al. (1989). DNA–DNA relatedness was fluorometrically determined using five replications for each sample, as described by Yoon et al. (2010). Strain DLS-45T shared DNA–DNA relatedness values of 16.9, 14.9 and 2.0% with A. bangladeshensis DSM 45347T, A. chokoriensis DSM 45346T and A. pelletieri IMSNU 22169T, respectively, showing that it represents a different genomic species (Wayne et al., 1987). Strain DLS-45T could be readily distinguished from its phylogenetically closest relatives by using a range of morphological and physiological properties (Table 1) and cellular fatty acid profiles. The combination of the phenotypic, genotypic and DNA–DNA relatedness data indicated that strain DLS-45T represents a novel species of the genus Actinomadura, for which the name Actinomadura meridiana sp. nov. is proposed.

**Description of Actinomadura meridiana sp. nov.**

Actinomadura meridiana (me.ri.di.a’na. L. fem. adj. meridiana southern, indicating that the type strain was isolated from the south of the Republic of Korea).

Gram-positive-staining. Good growth occurs on ISP 2, 3 and 6 media and oatmeal-nitrate agar. Poor growth occurs

### Table 1. Differential characteristics of strain DLS-45T and phylogenetic neighbours of the genus Actinomadura

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
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<tr>
<td>Morphology</td>
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<tr>
<td>Spore chain arrangement</td>
<td>Hooked</td>
<td>Curved to hooked</td>
<td>Spiral</td>
<td>Hooked, spiral</td>
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<tr>
<td>Spore surface ornamentation</td>
<td>Warty</td>
<td>Rough</td>
<td>Rough</td>
<td>Warty</td>
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<tr>
<td>Temperature range for growth (°C)</td>
<td>20–30</td>
<td>20–45</td>
<td>20–45</td>
<td>20–42</td>
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<tr>
<td>pH range for growth</td>
<td>6.0–9.0</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
<td>6.0–9.0</td>
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<tr>
<td>Growth at 4% NaCl (w/v)</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Nitrate reduction</td>
<td>–</td>
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<td>–</td>
<td>+</td>
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<td>Hydrolysis</td>
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<td>Aesculin</td>
<td>W</td>
<td>W</td>
<td>–</td>
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<tr>
<td>DNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Gelatin</td>
<td>+</td>
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<td>Starch</td>
<td>–</td>
<td>+</td>
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<td>Utilization</td>
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<tr>
<td>L-Arabinose</td>
<td>–</td>
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<td>+</td>
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<td>D-Fructose</td>
<td>–</td>
<td>–*</td>
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<td>Lactose</td>
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<td>Maltose</td>
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<td>Raffinose</td>
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<td>+</td>
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<tr>
<td>L-Rhamnose</td>
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<td>Sucrose</td>
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<td>Trehalose</td>
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<td>D-Xylose</td>
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<td>Adonitol</td>
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<tr>
<td>Glycerol</td>
<td>W</td>
<td>+</td>
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<td>–</td>
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<td>Acetate</td>
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<td>+</td>
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<td>DL-Tartrate</td>
<td>–</td>
<td>+</td>
<td>–</td>
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*Response differs from that described by Ara et al. (2008).
on ISP 4 and 5 media. Growth does not occur on ISP 7 medium. Forms a well-developed, branched, non-fragmenting substrate mycelium. The aerial mycelium is sparse on ISP 3 medium, but moderate and white to grey-coloured on ISP 6 medium after incubation for 4 weeks. The vegetative mycelium is dark brown to black on ISP 2 medium.

**Fig. 2.** A neighbour-joining tree showing the position of strain DLS-45\(^T\) within the radiation of the family Thermomonomosporaceae. Asterisks represent the corresponding branches that were also found in the trees created by both the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values (>50\%) based on 1000 replications are shown at the nodes. Bar, 1 substitution per 100 nt positions.
and 6 media, pale yellow to brown on ISP 3 and 4 media and oatmeal-nitrate agar, and white to cream on ISP 5 medium. Warty spores are formed on the aerial mycelium, in hooked chains. Diffusible pigments are produced on ISP 2 (dark brown) and ISP 6 (light yellow) media. The temperature range for growth is 20–30 °C. Growth does not occur below 10 °C or above 37 °C. The pH range for growth is pH 6.0–9.0. Growth does not occur at pH 5.0 or 10.0. Casein is hydrolysed, but carboxymethylcellulose, DNA, starch and urea are not. Hypoxanthine, DL-tyrosine and xanthine are not decomposed. Gelatin liquefaction is observed. Nitrate is not reduced to nitrite. Growth does not occur at or above 1% NaCl (w/v). Cellobiose, dextrin, L-rhamnose (weak) and glycerol (weak) are utilized as sole carbon sources, but L-arabinose, D-arabinose, D-fructose, D-galactose, lactose, maltose, D-mannose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, L-rutose, sucrose, trehalose, D-xylitol, L-sorbose, adonitol, meso-erythritol, myo-inositol, D-mannitol, D-sorbitol and D-xylitol are not utilized. Organic acids (acetate, citrate, formate, DL-malate, succinate and DL-tartrate) are not assimilated. meso-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan. The whole-cell sugars are glucose, galactose, mannose, madurose and ribose. The major menaquinones are MK-9(H₈) and MK-9(H₄). The polar lipid profile contains diphosphatidylglycerol and phosphatidylglycinositol. Mycolic acids are not present. The predominant fatty acids are C₁₂:0, C₁₆:0, C₁₇:1 i₁₈:0 and C₁₈:0.

The type strain, DLS-45T (=KCTC 19558T = DSM 45252T), was isolated from soil from the surface of a rock collected from the peak of Darangshi Oreum (Small Mountain) in Jeju, Republic of Korea. The G+C content of the DNA of the type strain is 68.9 mol%.

Acknowledgement

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References


Actinoallomurus coprocola sp. nov., Actinoallomurus fulvus sp. nov.,
Actinoallomurus iriomotensis sp. nov., Actinoallomurus luridus sp.
ov., Actinoallomurus purpureus sp. nov. and Actinoallomurus

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. &
strategies for multiple sequence alignment aided by quality analysis

classification of clinically significant aerobic sporoactinomycetes and
related organisms. Antonie van Leeuwenhoek 84, 39–68.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O.,
Krivelchsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other
authors (1987). International Committee on Systematic Bacteriology.
Report of the ad hoc committee on reconciliation of approaches to

(2010). Nocardoides daedukensis sp. nov., a halotolerant bacterium

Zhang, Z. S., Wang, Y. & Ruan, J. S. (1998). Reclassification of

Zhang, Z., Kudo, T., Nakajima, Y. & Wang, Y. (2001). Clarification of
the relationship between the members of the family Thermomono-
sporaceae on the basis of 16S rDNA, 16S-23S rRNA internal transcribed
spacer and 23S rDNA sequences and chemotaxonomic analyses. Int J