Dactylosporangium luridum sp. nov., *Dactylosporangium luteum* sp. nov. and *Dactylosporangium salmoneum* sp. nov., nom. rev., isolated from soil

Byung-Yong Kim,^{1,2} James E. M. Stach,¹ Hang-Yeon Weon,²
Soon-Wo Kwon² and Michael Goodfellow¹

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

¹Microbial Resources Laboratory, School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

²Korean Agricultural Culture Collection, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea

Forty strains isolated from soil taken from a hay meadow were assigned to the genus *Dactylosporangium* on the basis of colonial properties. 16S rRNA gene sequence analysis showed that the isolates formed a group that was most closely related to the type strain of *Dactylosporangium aurantiacum*, but well separated from other *Dactylosporangium* type strains and from '*Dactylosporangium salmoneum*' NRRL B-16294. Twelve of 13 representative isolates had identical 16S rRNA gene sequences and formed a subclade that was distinct from corresponding phyletic lines composed of the remaining isolate, strain BK63^T, the '*D. salmoneum*' strain and the type strains of recognized *Dactylosporangium* species. DNA–DNA relatedness data indicated that representatives of the multi-membered 16S rRNA gene subclade, isolate BK63^T and the '*D. salmoneum*' subclade formed distinct genomic species; all of these organisms had chemotaxonomic and morphological properties consistent with their classification in the genus *Dactylosporangium*. They were also distinguished from one another and from the type strains of recognized *Dactylosporangium* species based on a range of phenotypic properties. Combined genotypic and phenotypic data showed that isolate BK63^T, isolates BK51^T, BK53 and BK69, and strain NRRL B-16294^T should be classified in the genus *Dactylosporangium* as representing novel species. The names proposed for these species are *Dactylosporangium luridum* sp. nov. (type strain BK63^T = DSM 45324^T = KACC 20933^T = NRRL B-24775^T), *Dactylosporangium luteum* sp. nov. (type strain BK51^T = DSM 45323^T = KACC 20899^T = NRRL B-24774^T) and *Dactylosporangium salmoneum* sp. nov., nom. rev. (type strain NRRL B-16294^T = ATCC 31222^T = DSM 43910^T = JCM 3272^T = NBRC 14103^T).

The genus *Dactylosporangium* was proposed by Thiemann *et al.* (1967) to accommodate aerobic, filamentous actinomycetes that released motile spores formed in sporangia borne on short sporangiophores carried on the substrate mycelium. At the time of writing, the genus comprises six recognized species: *Dactylosporangium aurantiacum* (the type species; Thiemann *et al.*, 1967), *D. fulvum* (Shomura *et al.*, 1986), *D. matsuzakiense* (Shomura *et al.*, 1980), *D. roseum* (Shomura *et al.*, 1985), *D. thailandense* (Thiemann *et al.*, 1967) and *D. vinaceum* (Shomura *et al.*, 1983); two additional taxa, '*Dactylosporangium salmoneum*' and '*D. variesporum*', have been cited as species *incertae sedis* (Vobis,

1989). *Dactylosporangia* form a distinct phyletic branch in the *Micromonosporaceae* 16S rRNA gene sequence tree (Koch *et al.*, 1996; Thawai *et al.*, 2008), and can be distinguished from other genera classified in the family *Micromonosporaceae* by using a combination of chemotaxonomic, morphological and phylogenetic data (Vobis, 2006; Ara *et al.*, 2008). In contrast, members of *Dactylosporangium* species show minimal differences in chemical, morphological and physiological properties and are distinguished mainly on the basis of diffusible pigment and substrate mycelial colours (Vobis, 1989, 2006).

The primary aim of the present investigation was to determine the taxonomic status of a group of filamentous soil actinomycetes that had colonial and morphological properties characteristic of members of the genus

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains BK63^T, BK51^T and NRRL B-16294^T are FJ973605, FJ973604 and FJ973607, respectively.

Dactylosporangium. To this end, the isolates were compared with the type strains of recognized *Dactylosporangium* species and with '*D. salmoneum*' NRRL B-16294 in a polyphasic taxonomic study. The resultant genotypic and phenotypic data showed that the isolates represented two novel *Dactylosporangium* species. It is also proposed that '*D. salmoneum*' NRRL B-16294 be recognized as representing a novel species.

Actinomycetes were isolated on plates of *Streptomyces* isolation medium (per litre distilled water: 0.4 g casein, 1.0 g starch, 0.1 g CaCO₃, 0.2 g KH₂PO₄, 0.5 g KNO₃, 0.1 g MgSO₄, 15 g agar), supplemented with cycloheximide (20 µg ml⁻¹) and oxytetracycline (20 µg ml⁻¹), following inoculation with suspensions of soil taken from Palace Leas meadow hay plot 6 (Atalan *et al.*, 2000) at Cockle Park Experimental Farm, Northumberland, UK (National Grid Reference NZ 200913). Forty isolates that produced orange substrate mycelium characteristic of *Dactylosporangium* strains were purified and maintained on oatmeal agar (ISP medium 3; Shirling & Gottlieb, 1966) at room temperature and as suspensions of mycelial fragments in glycerol (20%, v/v) at -20 °C. rep-PCR, based on the use of BOX-, ERIC- and REP-PCR primers, was used to group and select isolates for the subsequent polyphasic study. Isolate BK63^T, '*D. salmoneum*' NRRL B-16294 and each of the *Dactylosporangium* marker strains were clearly separated from one another and from the remaining isolates on the basis of their rep-PCR profiles.

Thirteen strains were taken to represent the taxonomic variation shown by the isolates based on rep-PCR profiles. These isolates, together with the type strains of recognized *Dactylosporangium* species and '*D. salmoneum*' NRRL B-16194, were grown in shake flasks of modified Bennett's broth (Jones, 1949) at 28 °C for 14 days at 150 r.p.m. to obtain biomass for molecular systematic studies. The cultures were checked for purity and harvested by centrifugation. Biomass preparations were washed in NaCl/EDTA buffer (0.1 M EDTA, pH 8.0, 0.1 M NaCl) and stored at -20 °C until needed.

The phylogenetic positions of the strains were determined by 16S rRNA gene sequence analysis. Genomic DNA, PCR and direct sequencing of the purified products were carried out as described by Tan *et al.* (2006). The resultant, almost-complete 16S rRNA gene sequences (1430–1439 nt) were aligned manually, by using the jPHYDIT program (Jeon *et al.*, 2005), against corresponding sequences of representatives of genera classified in the family *Micromonosporaceae*, retrieved from the GenBank database. Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms from the MEGA version 3 program (Kumar *et al.*, 2004) and the maximum-likelihood method (Felsenstein, 1981) from the PHYLIP suite of programs (Felsenstein, 1993). The evolutionary distance model of Jukes & Cantor (1969) was used to generate evolutionary distance matrices for the neighbour-joining algorithm. The topologies of the

resultant trees were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset by using the CONSENSE and SEQBOOT options from the PHYLIP package.

In the 16S rRNA gene sequence tree of representative members of the *Micromonosporaceae*, the new isolates, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species formed a well-delineated clade (data not shown). The members of this taxon were most closely related to representatives of the genus *Virgisporangium*, a relationship that was supported by all of the tree-making algorithms (Fig. 1). All but one of the isolates had identical 16S rRNA gene sequences and formed a distinct subclade together with the type strain of *D. aurantiacum*; they shared 99.4% 16S rRNA gene sequence similarity with the latter, a value that corresponded to 9 nucleotide differences over 1435 locations. Relatively high 16S rRNA gene sequence similarities were shown with the type strains of *D. fulvum* (98.1%), *D. matsuzakiense* (98.3%), *D. roseum* (98.2%), *D. thailandense* (98.5%) and *D. vinaceum* (98.2%), as well as with '*D. salmoneum*' NRRL B-16294 (98.2%). The remaining isolate, strain BK63^T, also shared highest 16S rRNA gene sequence similarity with *D. aurantiacum* NRRL B-8018^T (98.9%; a value equivalent to 16 nucleotide differences across 1436 sites). The new isolates together with the type strain of *D. aurantiacum* formed a subclade that was supported by all of the tree-making algorithms.

A second subclade in the 16S rRNA gene sequence tree contained '*D. salmoneum*' NRRL B-16294 and the type strains of *D. matsuzakiense* and *D. vinaceum*. The close relationship between this taxon, the *D. matsuzakiense* subclade and the *D. aurantiacum* subclade was underpinned by all of the tree-making algorithms. '*D. salmoneum*' NRRL B-16294 and the type strains of *D. matsuzakiense* and *D. vinaceum* shared 16S rRNA gene sequence similarities of 98.9 and 98.8%, respectively, values that corresponded to 16 and 17 nucleotide differences over 1432 locations. *D. matsuzakiense* DSM 43810^T and *D. vinaceum* DSM 43823^T shared 99.6% 16S rRNA gene similarity, a value equivalent to 6 nucleotide differences over 1468 locations. *D. fulvum* DSM 43917^T and *D. roseum* DSM 43916^T, the two most closely related type strains in the genus, exhibited 99.7% 16S rRNA gene sequence similarity, equivalent to 5 nucleotide differences across 1471 sites. These strains, together with *D. thailandense* DSM 43158^T, formed a well-delineated taxon, the *D. thailandense* subclade, the integrity of which was underscored by all of the tree-making algorithms and by a bootstrap value of 94%.

Phylogenetic analyses based on partial RNA polymerase β-subunit (*rpoB*) gene sequences have provided valuable data in polyphasic studies designed to clarify relationships within and between genera of actinomycetes (Kim *et al.*, 1999, 2004; Goodfellow *et al.*, 2007). In the present study, partial *rpoB* gene sequences generated for four representative

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the relationships between strains BK51^T and BK63^T, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* and *Virgisorangium* species. Asterisks indicate branches of the tree that were also recovered by using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at nodes indicate levels of bootstrap support (%); only values >50 % are shown. The tested strains are given in bold. GenBank accession numbers are given in parentheses. The root position of the neighbour-joining tree was obtained by using *Micromonospora chalybeata* ATCC 12452^T as the outgroup. Bar, 0.01 substitutions per site.

isolates, strains BK51^T, BK53, BK63^T and BK69, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species were compared with corresponding genes of members of genera classified in the family *Micromonosporaceae*, drawn from GenBank, by using the same tree-making algorithms as above, but with *Streptomyces coelicolor* A3(2) as the outgroup.

Isolates BK51^T, BK53 and BK69 had identical *rpoB* gene sequences and formed a subclade with *D. aurantiacum* NRRL B-8018^T (Fig. 2); the integrity of this taxon was underpinned by all of the tree-making algorithms and by a bootstrap value of 95 %. The remaining isolate, strain BK63^T, formed a distinct, single-membered subclade at the periphery of the *Dactylosporangium rpoB* gene sequence tree.

Fig. 2. Neighbour-joining tree based on partial *rpoB* gene sequences showing the relationships between strains BK51^T, BK63^T, '*Dactylosporangium salmoneum*' NRRL B-16294, the type strains of recognized *Dactylosporangium* species and members of genera classified in the family *Micromonosporaceae*. The same tree-making algorithms and criteria were used as detailed in Fig. 1.

'*D. salmoneum*' NRRL B-16294 also formed a distinct subclade that was associated with *D. roseum* DSM 43916^T. These results are in good agreement with those from the 16S rRNA gene sequence analyses as they show that the isolates fall into one distinct multi-membered and one single-membered clade, although discrepancies are apparent between the topologies of the two trees (see Figs 1 and 2).

DNA–DNA relatedness studies were carried out to determine the finer taxonomic relationships between isolates BK51^T and BK63^T and '*D. salmoneum*' NRRL B-16294 and their closest phylogenetic neighbours (Table 1). Levels of DNA–DNA relatedness between the tested strains were established by measuring the divergence between the thermal denaturation midpoints of homologous and heterologous DNA (ΔT_m) following the procedure developed by Gonzalez & Saiz-Jimenez (2005). Confidence can be placed in the resultant data as comparable results have been obtained when the same strains have featured in thermal denaturation and spectrophotometric studies (Jurado *et al.*, 2005; Goodfellow *et al.*, 2007). ΔT_m values of 5.0 and 6.0 °C correspond to DNA–DNA relatedness levels of 60 and 70 %, respectively (Rosselló-Mora & Amann, 2001). DNA G + C contents were determined for all of the strains included in the DNA–DNA experiments by using the procedure described by Gonzalez & Saiz-Jimenez (2005). The strains had DNA G + C contents within the range 70–74 mol% (see Table 4).

Isolate BK51^T showed very low ΔT_m values (0.2–1.6 °C) with isolates BK53 and BK69 (Table 1), values well below the cut-off point recommended for the delineation of genomic species (ΔT_m >5.0 °C; Wayne *et al.*, 1987). In contrast, isolate BK51^T showed ΔT_m levels well above this cut-off point with isolate BK63^T, '*D. salmoneum*' NRRL B-16294 and the type strains of *D. aurantiacum*, *D. matsuzakiense* and *D. vinaceum*. Isolate BK63^T also exhibited relatively high ΔT_m values (5.8–6.2 °C) with the

Table 1. Levels of DNA–DNA relatedness between isolates BK51^T and BK63^T and '*D. salmoneum*' NRRL B-16294 and their closest phylogenetic neighbours based on ΔT_m values

Strains	ΔT_m (°C)	DNA–DNA relatedness (%)	Nucleotide base differences/total number of 16S rRNA nucleotides
Isolate BK51 ^T vs:			
Isolate BK53	1.6	82	0/1444
Isolate BK69	0.2	89	0/1444
Isolate BK63 ^T	7.2	53	11/1439
<i>D. aurantiacum</i> NRRL B-8018 ^T	7.2	53	9/1435
<i>D. matsuzakiense</i> NRRL B-16293 ^T	7.6	51	24/1430
' <i>D. salmoneum</i> ' NRRL B-16294	5.8	61	21/1431
<i>D. vinaceum</i> NRRL B-16297 ^T	9.5	42	26/1430
Isolate BK63 ^T vs:			
<i>D. aurantiacum</i> NRRL B-8018 ^T	6.2	59	16/1436
<i>D. matsuzakiense</i> NRRL B-16293 ^T	5.8	61	22/1432
' <i>D. salmoneum</i> ' NRRL B-16294	6.0	60	18/1433
<i>D. vinaceum</i> NRRL B-16297 ^T	5.8	61	23/1432
' <i>D. salmoneum</i> ' NRRL B-16294 vs:			
<i>D. aurantiacum</i> NRRL B-8018 ^T	7.5	52	23/1407
<i>D. matsuzakiense</i> NRRL B-16293 ^T	6.0	60	16/1468
<i>D. vinaceum</i> NRRL B-16297 ^T	6.5	57	17/1476

type strains of related *Dactylosporangium* species and with '*D. salmoneum*' NRRL B-16294, indicating that it belongs to a distinct genomic species. '*D. salmoneum*' NRRL B-16294 could also be assigned to a novel genomic species as it exhibited high ΔT_m values (5.8–7.5 °C) with its nearest neighbours, namely *D. matsuzakiense* NRRL B-16293^T and *D. vinaceum* NRRL B-16297^T.

Biomass for chemotaxonomic studies was prepared by growing representative isolates BK51^T and BK63^T, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species in modified Bennett's broth at 150 r.p.m. for 21 days at 28 °C; cells were harvested by centrifugation, washed in distilled water, recentrifuged and freeze-dried. Standard procedures were used to extract and analyse the isomers of diaminopimelic acid (Staneck & Roberts, 1974), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida *et al.*, 1999), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Schaal, 1985), with appropriate controls. Cellular fatty acids were extracted, methylated and analysed by GC by using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990) and mycolic acids were identified by using the TLC procedure introduced by Minnikin *et al.* (1975).

All of the organisms contained mixtures of 3-hydroxy and *meso*-diaminopimelic acid, *N*-glycolylmuramic acid, hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H₆) and MK-9(H₈)] as predominant menaquinones and arabinose, galactose, glucose, mannose and xylose in whole-organism hydrolysates; mycolic acids were not found. The isolates contained iso-C_{15:0} and iso-C_{16:0} as predominant fatty acids with varying kinds and proportions of minor

compounds and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol as major polar lipids (phospholipid pattern type 2 *sensu* Lechevalier *et al.*, 1977) with a discontinuous distribution of phosphatidylinositol mannosides, an unknown amino-lipid and an unknown phospholipid (Table 2). These results are in good agreement with those of members of the genus *Dactylosporangium* (Kroppenstedt, 1985; Vobis, 2006).

Isolates BK51^T and BK63^T and '*D. salmoneum*' NRRL B-16294 were grown on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic salts-starch, glycerol-asparagine, peptone-yeast extract-iron and tyrosine agars (ISP media 1–7, respectively; Shirling & Gottlieb, 1966) for 21 days at 28 °C. Colonies growing on these media were examined by eye to determine substrate mycelial pigmentation and the colour of any diffusible pigments; colours were recorded by using National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1958; NBS, 1964). Peptone-yeast extract-iron and tyrosine agar plates were examined to determine whether the strains produced melanin pigments. The strains were also examined to establish whether they grew on Luria–Bertani, nutrient and trypticase soy agars (Sambrook *et al.*, 1989) after incubation at 28 °C for 3 weeks.

All of the strains grew well on inorganic salts-starch, oatmeal and tyrosine agar plates, producing a range of substrate mycelial colours but relatively few diffusible pigments (Table 3). The type strains of *D. roseum*, *D. thailandense* and *D. vinaceum* together with '*D. salmoneum*' NRRL B-16294 grew well on all of the media except glycerol-asparagine agar. In contrast, isolates BK51^T and BK63^T grew poorly on glycerol-asparagine, peptone-yeast

Table 2. Chemotaxonomic properties of strains BK51^T and BK63^T, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species

Strains: 1, BK51^T; 2, BK63^T; 3, *D. aurantiacum* NRRL B-8018^T; 4, *D. fulvum* DSM 43917^T; 5, *D. matsuzakiense* NRRL B-16293^T; 6, *D. roseum* DSM 43916^T; 7, '*D. salmoneum*' NRRL B-16294; 8, *D. thailandense* DSM 43158^T; 9, *D. vinaceum* NRRL B-16297^T. All data were acquired in the present study. —, Not detected.

Property	1	2	3	4	5	6	7	8	9
Fatty acids (% of total)									
iso-C _{14:0}	3.3	4.0	4.9	2.8	1.5	1.2	1.4	1.9	6.8
anteiso-C _{15:0}	8.1	9.7	3.5	4.2	3.4	2.2	4.7	4.0	10.0
iso-C _{15:0}	38.7	45.0	27.1	32.0	18.4	27.8	33.5	30.1	17.5
C _{16:0}	2.0	0.7	1.6	4.6	1.1	3.3	0.4	0.5	—
iso-C _{16:0}	21.1	13.9	42.3	25.6	43.0	32.9	31.3	36.7	54.6
iso-C _{16:1} G	2.2	2.0	0.3	—	7.9	—	3.0	7.1	—
C _{17:0}	2.1	5.8	1.4	1.8	1.1	—	0.3	0.9	2.4
10-Methyl C _{17:0}	2.1	4.0	1.8	0.6	2.7	4.8	1.2	1.8	—
anteiso-C _{17:0}	5.8	3.4	4.1	5.7	7.2	4.7	9.1	6.5	8.8
iso-C _{17:0}	4.0	2.0	4.3	5.1	3.5	4.4	7.0	4.0	—
C _{17:1} ω8c	1.9	5.4	0.5	—	2.1	—	0.2	0.6	—
C _{18:0}	1.4	0.2	1.6	5.7	1.1	2.4	0.5	0.4	—
C _{18:1} ω9c	2.7	0.4	2.4	2.1	1.9	5.4	0.4	0.4	—
Menaquinones (% of total)									
MK-9(H ₂)	—	15	—	—	—	—	—	—	—
MK-9(H ₄)	10	25	10	—	—	—	14	—	—
MK-9(H ₆)	23	50	20	28	10	15	47	10	16
MK-9(H ₈)	66	5	69	71	90	82	36	88	80
Major polar lipids*	DPG, PE, PG, PI, PL	DPG, PE, PG, PI, PL	DPG, PE, PG, PI, PIM, PL	DPG, PE, PG, PI	DPG, PE, PG, PI, PL	DPG, PE, PG, PI	DPG, PE, PG, PI, PL	DPG, PE, PG, PI, PIM, PL	DPG, PE, PG, PI, PL

*DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid.

extract-iron, tryptone-yeast extract and yeast extract-malt extract agar plates. In general, few of the organisms produced soluble pigments on the agar media, although *D. vinaceum* NRRL B-16297^T formed soluble pigments on all of these media. It is also interesting that isolates BK51^T and BK63^T could be distinguished by the colour of the substrate mycelium that they produced on the various media.

Isolates BK51^T and BK63^T together with '*D. salmoneum*' NRRL B-16294 were examined for micromorphological properties on 3-week-old glycerol-asparagine, oatmeal, tyrosine and yeast extract-malt extract agar plates by using a Nikon Optiphot light microscope fitted with a long-working-distance objective. In addition, gold-coated, dehydrated preparations from the plates were examined by using a Cambridge Stereoscan 240 scanning electron microscope following the procedure described by O'Donnell *et al.* (1993). Preparations from oatmeal agar plates were flooded with sterile water, left for 60 min and then examined for the presence of motile spores with a light microscope. All of the strains formed irregular branched substrate hyphae (0.3–0.7 µm in diameter), which penetrated the agar. Similarly, all three strains

formed sporangia, which released motile spores. Motile spores were formed in finger-like, short, narrow sporangia produced directly on the substrate mycelium (Fig. 3). They also formed globose bodies with smooth surfaces at the top of short sporophores on substrate hyphae.

Isolates BK51^T, BK53, BK63^T and BK69, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species were examined for a broad range of phenotypic properties by using the media and methods described by Goodfellow *et al.* (1997). All of the strains were positive for catalase, degraded aesculin and xylan, utilized glycogen, (–)-D-glucose and (–)-sucrose as sole carbon sources, and were sensitive to novobiocin (8 µg ml⁻¹) and rifampicin (16 µg ml⁻¹). All were negative for nitrite reduction, did not degrade adenine, cellulose, chitin, guanine, hypoxanthine, pectin, tributyrin, Tween 20, uric acid or xanthine, did not use (+)-L-lactic acid, (–)-L-sorbose, oxalic acid or (–)-D-ribose as sole carbon sources and did not grow in the presence of 3 % (w/v) NaCl or 0.05 % (w/v) lysozyme.

Isolates BK51^T, BK53 and BK69 had identical phenotypic profiles that served to distinguish them from isolate BK63^T,

Table 3. Growth and cultural characteristics of strains BK51^T and BK63^T, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species

Strains: 1, BK51^T; 2, BK63^T; 3, *D. aurantiacum* NRRL B-8018^T; 4, *D. fulvum* DSM 43917^T; 5, *D. matsuzakiense* NRRL B-16293^T; 6, *D. roseum* DSM 43916^T; 7, '*D. salmoneum*' NRRL B-16294; 8, *D. thailandense* DSM 43158^T; 9, *D. vinaceum* NRRL B-16297^T. Data were obtained in this study.

Culture medium	1	2	3	4	5	6	7	8	9
Glycerol-asparagine agar									
Growth	Poor	Poor	Poor	Poor	Poor	Poor	Moderate	Moderate	Poor
Substrate mycelium	Orange	Light yellow	Light yellow	Light yellow	Orange	Light yellow	Light yellow	Light yellow	Orange
Soluble pigment	None	None	None	None	None	None	None	None	Light brown
Inorganic salts-starch agar									
Growth	Abundant	Moderate	Abundant	Abundant	Abundant	Abundant	Abundant	Abundant	Abundant
Substrate mycelium	Orange	Light yellow	Light yellow	Brown	Reddish brown	Light yellow	Light orange	Light brown	Deep purple
Soluble pigment	None	None	None	Light brown	None	None	None	Light brown	Black purple
Oatmeal agar									
Growth	Abundant	Moderate	Abundant	Abundant	Abundant	Abundant	Abundant	Abundant	Abundant
Substrate mycelium	Orange	Light yellow	Orange	Yellow	Reddish orange	Orange	Deep orange	Light brown	Purple
Soluble pigment	None	None	None	Light brown	None	None	None	Light brown	Black purple
Peptone-yeast extract-iron agar									
Growth	Poor	Poor	Poor	Poor	Poor	Moderate	Moderate	Moderate	Moderate
Substrate mycelium	Orange	Light yellow	Yellow	Yellow	Orange	Orange	Yellow	Orange	Reddish orange
Soluble pigment	None	None	None	None	None	None	None	None	Brown
Tryptone-yeast extract agar									
Growth	Poor	Poor	Moderate	Moderate	Moderate	Moderate	Abundant	Abundant	Moderate
Substrate mycelium	Orange	Light yellow	Orange	Light brown	Orange	Orange	Yellow	Orange	Reddish brown
Soluble pigment	None	None	None	None	None	None	None	None	Brown
Tyrosine agar									
Growth	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Substrate mycelium	Orange	Light yellow	Orange	Reddish yellow	Light brown	Light yellow	Light brown	Yellow	Reddish orange
Soluble pigment	Light brown	None	Light brown	None	Light brown	None	None	None	Light brown
Yeast extract-malt extract agar									
Growth	Poor	Poor	Moderate	Abundant	Moderate	Moderate	Abundant	Abundant	Moderate
Substrate mycelium	Orange	Light yellow	Orange	Light brown	Orange	Orange	Yellow	Orange	Reddish brown
Soluble pigment	None	None	None	None	None	None	None	Light yellow	Light brown

Fig. 3. Scanning electron micrographs of isolate BK51^T grown on ISP 2 medium for 2 weeks (a), isolate BK63^T grown on ISP 7 medium for 2 weeks (b) and '*D. salmoneum*' NRRL B-16294 grown on ISP 7 medium for 8 weeks (c). Bars, 1.0 µm.

'*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species (Table 4). Indeed, these three isolates were the only strains to use (+)-D-arabitol as a sole carbon source and to be unable to produce H₂S or degrade Tween 80. Similarly, unlike most of the other strains, they utilized adonitol, (+)-L-arabinose, dextrin, (–)-D-fructose and (+)-melibiose as sole carbon sources, but were unable to hydrolyse gelatin or to grow at 37 °C. Isolate BK63^T and '*D. salmoneum*' NRRL B-16294 could also readily be separated from one another and from the *Dactylosporangium* type strains, notably by the inability of strain BK63^T to metabolize most of the sole carbon sources and by the activity of '*D. salmoneum*' NRRL B-16294 in the degradation tests.

The genotypic and phenotypic data clearly indicate that isolates BK51^T, BK53 and BK69, isolate BK63^T and '*D. salmoneum*' NRRL B-16294 represent three new centres of

taxonomic variation in the genus *Dactylosporangium*. It is therefore proposed that these strains be classified in the genus *Dactylosporangium* as representing *Dactylosporangium luteum* sp. nov., *Dactylosporangium luridum* sp. nov. and *Dactylosporangium salmoneum*, respectively. The genotypic data acquired on isolates BK50, BK52, BK54–BK57, BK60, BK67 and BK68 are consistent with their assignment to *D. luteum*.

Description of *Dactylosporangium luridum* sp. nov.

Dactylosporangium luridum (lu'ri.dum. L. neut. adj. *luridum* pale yellow).

Aerobic, Gram-stain-positive, motile actinomycete that forms branched, pale-yellow mycelium on inorganic salts-starch and oatmeal agars. Short, narrow finger-like sporangia (1.0–1.2 × 2.0–2.5 µm), which release motile spores, are formed on glycerol-asparagine and tyrosine agars. Globose bodies with smooth surfaces are produced on short sporophores arising from substrate hyphae. Melanin pigments are not formed on either peptone-yeast extract-iron or tyrosine agars. Grows well on inorganic salts-starch, oatmeal and tyrosine agars, but poorly on glycerol-asparagine, peptone-yeast extract-iron, tryptone-yeast extract and yeast extract-malt extract agars. Grows well between 20 and 30 °C (optimally around 28 °C) and at pH 4–9 (optimally around pH 7). Additional phenotypic properties are given in the main text and in Table 4. Has chemotaxonomic properties consistent with its classification in the genus *Dactylosporangium*, and forms a distinct phyletic line in the *Dactylosporangium* 16S rRNA gene sequence tree. The DNA G + C content of the type strain is 70 mol%.

The type strain, BK63^T (=DSM 45324^T =KACC 20933^T =NRRL B-24775^T), was isolated from a soil sample taken from Palace Leas meadow hay plot 6 at Cockle Park Experimental Farm, Northumberland, UK.

Description of *Dactylosporangium luteum* sp. nov.

Dactylosporangium luteum (lu'te.um. L. neut. adj. *luteum* orange-yellow, flame-coloured).

Aerobic, Gram-stain-positive, motile actinomycete that forms branched, orange-yellow mycelium on oatmeal and inorganic salts-starch agars. Short, narrow finger-like sporangia (1.0–1.2 × 2.0–2.5 µm), which release motile spores, are formed on glycerol-asparagine, tyrosine and yeast extract-malt extract agars. Globose bodies with smooth surfaces are produced on short sporophores arising from substrate hyphae. Melanin pigments are not formed on either peptone-yeast extract-iron or tyrosine agars. Grows well on inorganic salts-starch, oatmeal and tyrosine agars, but poorly on glycerol-asparagine, peptone-yeast extract-iron, tryptone-yeast extract and yeast extract-malt extract agars. Grows well between 15 and 30 °C (optimally around

Table 4. Differential phenotypic characteristics between strains BK51^T and BK63^T, '*D. salmoneum*' NRRL B-16294 and the type strains of recognized *Dactylosporangium* species

Strains: 1, BK51 (identical results for strains BK53 and BK69); 2, BK63^T; 3, *D. aurantiacum* NRRL B-8018^T; 4, *D. fulvum* DSM 43917^T; 5, *D. matsuzakiense* NRRL B-16293^T; 6, *D. roseum* DSM 43916^T; 7, '*D. salmoneum*' NRRL B-16294; 8, *D. thailandense* DSM 43158^T; 9, *D. vinaceum* NRRL B16297^T. All data were acquired in the present study.

Characteristic	1	2	3	4	5	6	7	8	9
Biochemical tests									
Allantoin hydrolysis	—	—	—	—	—	+	—	+	—
Coagulation and peptonization of milk	—	—	—	—	—	—	—	—	+
Hydrogen sulfide production	—	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	—	—	+	+	—	—
Urease production	—	—	+	+	—	—	—	+	+
Degradation tests									
Arbutin	+	—	+	+	+	+	+	+	+
Casein	—	—	—	—	—	+	+	—	+
DNA	—	—	+	+	—	—	+	—	—
Elastin	—	—	+	—	—	+	—	—	—
Gelatin	—	—	+	+	—	+	+	+	+
RNA	—	—	—	—	—	—	+	—	—
Starch	+	+	—	+	+	—	+	+	+
Tween 40	—	—	—	+	+	+	+	+	+
Tween 60	—	+	+	+	+	+	+	+	+
Tween 80	—	—	—	+	+	—	+	+	—
Sole carbon source utilization (1.0 %, w/v)									
Adonitol	+	—	—	+	—	—	+	—	—
(+)-L-Arabinose	+	—	—	—	—	—	+	—	—
(+)-D-Arabitol	+	—	—	—	—	—	—	—	—
(+)-Cellobiose	+	—	+	+	—	+	+	+	—
Dextrin	+	—	—	+	—	—	+	—	—
(-)-D-Fructose	+	—	—	—	—	—	+	+	—
(+)-D-Galactose	+	—	—	+	—	—	+	+	—
Glycerol	+	—	+	+	—	—	+	—	—
myo-Inositol	—	—	—	—	—	—	+	—	—
Inulin	+	—	+	+	—	+	+	+	+
(+)-Lactose	+	—	+	—	—	—	+	+	—
(+)-Maltose	+	—	—	—	—	+	+	+	—
(+)-D-Mannitol	+	—	—	+	—	—	+	+	—
(+)-D-Mannose	+	—	—	—	—	+	+	+	—
(+)-Melibiose	+	—	+	—	—	—	+	—	—
Methyl α -D-glucoside	+	—	+	+	—	—	+	+	—
(+)-Raffinose	+	—	+	+	—	+	+	+	—
(-)-L-Rhamnose	+	—	+	—	—	—	+	+	—
Salicin	—	—	+	+	—	—	+	—	—
Starch	+	+	+	+	+	—	+	+	+
(+)-Trehalose	+	+	+	+	—	—	+	+	—
(+)-D-Xylose	+	+	+	+	—	—	+	+	—
Sole carbon source utilization (0.1 %, w/v)									
Citric acid	+	—	—	—	—	—	+	+	—
(+)-L-Lactic acid	+	—	—	+	—	—	—	+	—
Malic acid	+	—	—	+	—	+	+	+	—
Propionic acid	+	—	—	+	—	+	+	—	—
Pyruvic acid	+	—	+	+	+	+	+	+	—
(+)-L-Tartaric acid	+	—	—	+	—	—	+	+	—
Growth at:									
15 °C	+	—	+	—	+	—	—	+	—
37 °C	—	—	+	+	+	+	+	+	—
pH 4.0	—	+	—	—	+	+	+	+	—
pH 10.0	+	—	—	+	—	—	+	+	—

Table 4. cont.

Characteristic	1	2	3	4	5	6	7	8	9
Production of melanin pigments on tyrosine agar	+	+	+	–	–	–	–	–	+
Growth in the presence of 1.5 % NaCl (w/v)	+	+	–	–	+	–	+	–	–
Resistance to antibiotics ($\mu\text{g ml}^{-1}$)									
Ampicillin (4)	–	–	–	–	+	–	+	+	+
Cephaloridine (2)	–	–	–	–	+	–	+	+	–
Chloramphenicol (8)	–	–	–	+	–	–	–	+	–
Ciprofloxacin (2)	–	–	+	+	+	+	+	+	–
Clindamycin (8)	+	–	+	+	+	–	+	+	–
Gentamicin (8)	+	–	+	+	+	–	+	+	+
Kanamycin (8)	–	–	–	–	+	–	+	–	+
Lincomycin (8)	+	–	+	+	+	–	+	+	+
Oxytetracycline (8)	–	–	+	+	+	–	+	+	–
Penicillin G (2)	+	–	–	–	+	+	+	+	+
Streptomycin (4)	–	–	–	+	–	–	–	–	–
Tetracycline (8)	–	–	+	+	–	–	–	–	–
Tylosin (8)	–	–	+	+	+	–	+	+	–
Vancomycin (2)	–	–	–	–	+	–	–	+	+
DNA G + C content (mol%)	74	70	73	71	73	73	73	71	73

28 °C) and at pH 5–10 (optimally around pH 7). Additional phenotypic properties are detailed in the main text and in Table 4. Has chemotaxonomic properties consistent with its classification in the genus *Dactylosporangium*, and forms a distinct phyletic line in the *Dactylosporangium* 16S rRNA gene sequence tree. The DNA G + C content of the type strain is 74 mol%.

The type strain, BK51^T (=DSM 45323^T =KACC 20899^T =NRRL B-24774^T), was isolated from a soil sample taken from Palace Leas meadow hay plot 6 at Cockle Park Experimental Farm, Northumberland, UK. BK50, BK52, BK54–BK57, BK60, BK67 and BK68 are additional strains of the species.

Description of *Dactylosporangium salmoneum* (ex Celmer *et al.* 1978) sp. nov., nom. rev.

Dactylosporangium salmoneum (sal.mo.ne'um. L. n. *salmo* -onis salmon; L. adj. suff. -eus -a -um suffix used with various meanings; N.L. neut. adj. *salmoneum* salmon-coloured).

The description is based on data from the present study and from Celmer *et al.* (1978). Aerobic, Gram-stain-positive, motile actinomycete that forms branched, orange mycelium on inorganic salts-starch and oatmeal agars. Numerous sporangia are formed on calcium malate plates. Sporangia are enlarged slightly towards the apex (1.5 × 5.5 μm), are dactyloform and contain three to four spores. Spores are mainly elliptical (1.1–1.6 × 2.2–2.7 μm) and are motile. Globose bodies with smooth surfaces are formed at the top of short sporophores on substrate hyphae. Melanin pigments are not produced on either peptone-yeast extract-iron or tyrosine agars. Good growth is shown on glycerol-asparagine, inorganic salts-starch,

oatmeal, peptone-yeast extract-iron, tryptone-yeast extract, tyrosine and yeast extract-malt extract agars; grows poorly on Luria–Bertani, nutrient and trypticase soy agars. Grows well between 20 and 37 °C (optimally around 28 °C) and at pH 4–10 (optimally around pH 7). Additional phenotypic properties are detailed in the main text and in Table 4. Has chemotaxonomic properties consistent with its classification in the genus *Dactylosporangium*, and forms a distinct phyletic line in the *Dactylosporangium* 16S rRNA gene sequence tree. The DNA G + C content of the type strain is 73 mol%.

The type strain, NRRL B-16294^T (=ATCC 31222^T =DSM 43910^T =JCM 3272^T =NBRC 14103^T), was isolated from soil in Japan.

Acknowledgements

B.-Y.K gratefully acknowledges receipt of an Overseas Research Scholarship, a Newcastle University International Postgraduate Scholarship and funding from the National Institute for International Education, Korea. We are indebted to the ARS Culture Collection, Peoria, IL, USA, for providing the *Dactylosporangium* type strains and the '*D. salmoneum*' strain and to Dr Jean Euzéby for his invaluable advice on naming the novel species.

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