Proposal for the new genus *Allokutzneria* gen. nov. within the suborder *Pseudonocardineae* and transfer of *Kibdelosporangium albatum* Tomita *et al.* 1993 as *Allokutzneria albata* comb. nov.

D. P. Labeda¹ and R. M. Kroppenstedt²

Correspondence

D. P. Labeda

david.labeda@ars.usda.gov

¹Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, IL 61604, USA

²DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

During the course of phylogenetic analyses based on 16S rRNA gene sequences for all currently described taxa within the family *Pseudonocardineae*, it became evident that *Kibdelosporangium albatum* DSM 44149^T was misplaced within the genus *Kibdelosporangium* and is phylogenetically most closely related to the genus *Kutzneria*. Chemotaxonomic analyses revealed that *Kibdelosporangium albatum* differed from *Kutzneria* in containing arabinose as well as galactose and mannose as diagnostic whole-cell sugars. The polar lipid pattern was distinct from both *Kibdelosporangium* and *Kutzneria* species in containing phosphatidylethanolamine containing 2-hydroxy fatty acids, lyso-phosphatidylethanolamine and lyso-phosphatidyl-methylethanolamine as well as phosphatidylethanolamine, phosphatidylmethylethanolamine, diphosphatidylglycerol and phosphatidylglycerol. These data preclude the inclusion of this strain within the genus *Kutzneria* or *Kibdelosporangium* and a new genus is proposed, to be named *Allokutzneria* gen. nov. The type species of this new genus is *Allokutzneria albata* gen. nov., comb. nov., the type strain of which is R761-7^T (=NRRL B-24461^T =DSM 44149^T =ATCC 55061^T).

Tomita *et al.* (1993) described the species *Kibdelosporangium albatum* for a strain producing the antiviral antibiotics cycloviracins based on morphological and chemotaxonomic characteristics. During the course of phylogenetic analyses based on 16S rRNA gene sequences for all currently described taxa within the family *Pseudonocardineae*, it became evident that this species was misplaced within the genus *Kibdelosporangium* and subsequent chemotaxonomic analyses indicated that it is distinct from the phylogenetically closest genus *Kutzneria*.

The 16S rRNA gene sequence for *Kibdelosporangium albatum* DSM 44149^T was obtained from GenBank (accession no. AJ512462) and aligned in the ARB package (Ludwig *et al.*, 2004) with sequences for all other taxa within the suborder *Pseudonocardineae* with validly published names. The accuracy of the deposited sequence was confirmed by isolating genomic DNA and amplifying and sequencing the 16S rRNA gene sequence of the equivalent strain NRRL B-24461^T from

the ARS Culture Collection using previously described methods (Labeda & Kroppenstedt, 2000). This confirmatory sequence was identical to that already available in GenBank and therefore was not submitted again. The sequence of the 16S rRNA gene of *Kutzneria albida* NRRL B-24060^T was found not to be available in any of the public databases; a sequence was obtained and deposited in GenBank under accession number EF543522. Phylogenetic relationships were calculated from an alignment consisting of a total of 1403 bp within ARB (Ludwig *et al.*, 2004) using Kimura's two-parameter evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987), as well as maximum-likelihood using AxML (Felsenstein, 1989; Stamatakis *et al.*, 2002) and maximum-parsimony using DNAPARS (Felsenstein, 1989) methods. Bootstrap support for the neighbour-joining and maximum-parsimony trees was evaluated from 1000 resamplings. Genomic DNA for determination of mol% G+C content by the method of Marmur & Doty (1962) was isolated from biomass grown for 5 days with shaking at 28 °C on DSMZ medium no. 554 broth using PowerMicrobial Maxi DNA isolation kits (Mo Bio Laboratories).

Biomass for chemotaxonomic analyses was grown on DSM medium no. 554 broth incubated with shaking at

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Kutzneria albida* NRRL B-24060^T is EF543522.

Details of additional sequences used in construction of Fig. 1 and a 16S rRNA gene sequence similarity matrix are available as supplementary material with the online version of this paper.

Fig. 1. Phylogenetic tree for taxa of the suborder *Pseudonocardineae* calculated within ARB (Ludwig *et al.*, 2004) from almost-complete 16S rRNA gene sequences using Kimura's two-parameter evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987) illustrating the taxonomic position of *Allokutzneria albata* DSM 44149^T (=NRRL B-24661^T) relative to species of *Kibdelosporangium*, *Kutzneria* and the other taxa within the suborder. Percentages at nodes represent levels of bootstrap support from 1000 resampled datasets; values less than 60% are not shown. Asterisks indicate corresponding branches that were also recovered in the maximum-likelihood (Felsenstein, 1989; Stamatakis *et al.*, 2002) and maximum-parsimony (Felsenstein, 1989) trees. Bar, 0.01 nucleotide substitutions per site. The taxa and sequences included in the genus groups are detailed in Supplementary Table S1.

200 r.p.m. for 5 days. The biomass was killed by autoclaving, collected by centrifugation and washed with sterile deionized water before freeze drying. The analyses were done using methods described previously (Grund & Kroppenstedt, 1989; Minnikin *et al.*, 1984; Saddler *et al.*, 1991; Sasser, 1990).

As can be seen in Fig. 1, strain DSM 44149^T is more closely related phylogenetically to species of the genus *Kutzneria* than to the genus *Kibdelosporangium*, and the tree topography in this region is supported by DNA distance-

neighbour-joining, maximum-parsimony and maximum-likelihood methods. The 16S rRNA gene sequence of strain DSM 44149^T exhibits 94.7–95.6% similarity to those of all species of *Kibdelosporangium* and 95.6–97.0% similarity to *Kutzneria* species (see Supplementary Table S2, available in IJSEM Online).

The whole-cell sugar pattern of strain DSM 44149^T consisted of arabinose, galactose and mannose; the presence of arabinose is absolutely distinct from *Kutzneria*, the phylogenetically nearest neighbouring genus, and confirms the

Table 1. Comparison of the chemotaxonomic profile of *Allokutzneria albata* gen. nov., comb. nov. with those of the phylogenetically nearest genera and *Kibdelosporangium*

Components are listed in descending order of abundance. ND, No data available.

Taxon	Whole-cell sugar pattern*	Phospholipids†	Predominant menaquinone(s)	DNA G + C content (mol%)
<i>Allokutzneria albata</i>	Ara, Gal, Man	PE, PME, OH-PE, PI, lyso-PME, DPG, PG, lyso-PE	MK-9(H ₄)	71.6
<i>Crossiella</i>	Gal, Man, Rha, Rib	PE, DPG, PI, PIM, PME	MK-9(H ₄)	71.4
<i>Actinoalloteichus</i>	Glc, Gal, Man, Rib	PIM, PI, PG, DPG, PME	MK-9(H ₄)	72–72.5
<i>Goodfellowiella</i>	Gal, Rib	PE, DPG, OH-PE, PME	MK-9(H ₄), MK-10(H ₄)	69.2
<i>Kibdelosporangium</i>	Ara, Gal, Glc, Rha	PE, PME, PG, PI	MK-9(H ₄)	66
<i>Kutzneria</i> (all species)	Gal, Rha	PE, DPG, PI, PG, PME	MK-9(H ₄)	70.3–70.7
<i>Streptoalloteichus</i>	Gal, Man, Rha, Rib	PE, DPG, PI, PIM, DPG, PME	MK-9(H ₆), MK-10(H ₆)	ND

*Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; lyso-PE, PE where one fatty acid chain is missing from the glycerol backbone; OH-PE, PE with hydroxy fatty acids; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, PI mannosides; PME, phosphatidylmethylethanolamine; lyso-PME, PME where one fatty acid chain is missing from the glycerol backbone.

chemotaxonomic data originally reported by Tomita *et al.* (1993). The phospholipid profile observed for this strain (Supplementary Fig. S1) is quite distinct from those of described species of *Kibdelosporangium* and *Kutzneria* and contains both *lyso*-phosphatidylmethylethanolamine and *lyso*-phosphatidylethanolamine in addition to phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylethanolamine containing 2-hydroxy fatty acids, diphosphatidylglycerol and phosphatidylglycerol. A comparison of the chemotaxonomic properties of strain DSM 44149^T with those of the genus *Kibdelosporangium* and phylogenetically closely related genera is shown in Table 1. The fatty acid profile of strain DSM 44149^T is also distinct from those of members of *Kibdelosporangium* and the phylogenetically nearest genera (Table 2).

Observation of growth of strain DSM 44149^T on various media typically used for characterization of actinomycetes (Shirling & Gottlieb, 1966) using the light microscope also confirmed the morphological description of Tomita *et al.* (1993); large sporangial bodies typical of either the genus *Kutzneria* or *Kibdelosporangium* were observed on relatively minimal growth medium (i.e. Czapek's sucrose agar).

Strong consideration was given to the transfer of this species to *Kutzneria* as a new combination, since this strain appears phylogenetically most closely related to the species of that genus. However, the chemotaxonomic properties of strain DSM 44149^T and, in particular, the presence of arabinose as a whole-cell sugar and *lyso*-phosphatidylethanolamine and *lyso*-phosphatidylmethylethanolamine as diagnostic phospholipids preclude its inclusion in the genus *Kutzneria* or in any of the phylogenetically closely related genera. On the basis of this substantial difference in chemotaxonomic properties, the creation of a new genus, *Allokutzneria* gen. nov., is therefore proposed, with *Allokutzneria albata* comb. nov. as the type species.

Description of *Allokutzneria* gen. nov.

Allokutzneria (Al.lo.kutz.ne'ri.a. Gr. adj. *allos* other; N.L. fem. n. *Kutzneria* a bacterial generic name; N.L. fem. n. *Allokutzneria* the other *Kutzneria*, referring to the fact that it is phylogenetically close to *Kutzneria* but chemotaxonically distinct).

The description is generally consistent with the original description of *Kibdelosporangium albatum* by Tomita *et al.* (1993). Form sporangium-like bodies containing hyphae but not spores and also produce aerial chains of spores. Substrate mycelium is subject to varying degrees of fragmentation. Cell-wall diamino acid is *meso*-diaminopimelic acid. Whole-cell hydrolysates contain arabinose, galactose and mannose. Cell-wall acyl type is acetyl. Principal phospholipids include phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylethanolamine containing 2-hydroxy fatty acids, *lyso*-phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and *lyso*-phosphatidylethanolamine.

Table 2. Fatty acid content of strain DSM 44149^T, members of phylogenetically closely related genera and *Kibdelosporangium aridum* subsp. *aridum*

Strains: 1, *Allokutzneria albata* DSM 44149^T; 2, *Actinoalloteichus cyanogriseus* DSM 43889^T (data from Tamura *et al.*, 2000); 3, *Crossiella cryophila* DSM 44230^T; 4, *Kibdelosporangium aridum* subsp. *aridum* DSM 43828^T; 5, *Kutzneria viridogrisea* DSM 43828^T (data from Stackebrandt *et al.*, 1994); 6, *Streptoalloteichus hindustanus* DSM 44523^T. Data were obtained in this study unless indicated. Values are percentages of total fatty acids. tr, Trace; —, not detected/not reported. Major components (≥10%) are highlighted in bold.

Fatty acid	1	2	3	4	5	6
iso-14:0	6.4	5.0	0.7	5.2	—	—
14:0	—	—	—	1.8	—	—
iso-15:0	6.0	15.0	40.0	8.7	2.7	34.2
anteiso-15:0	0.5	10.0	2.2	12.7	—	10.7
15:1(c9)	3.2	—	3.8	—	—	—
15:0	2.0	tr	2.6	0.7	1.6	—
iso-16:1	10.5	8.0	3.2	6.0	0.6	—
iso-16:0	42.5	19.0	10.7	34.5	30.3	2.6
16:1(c9)	1.5	2.0	3.9	0.3	3.8	0.6
iso-15:0 2-OH	—	—	—	4.2	—	—
anteiso-15:0 2-OH	—	—	—	2.5	—	—
16:0	1.6	2.0	2.2	1.5	—	—
iso-16:0 10-methyl	—	—	0.9	—	—	—
16:0 10-methyl	—	—	—	0.6	8.6	—
iso-17:1	—	—	—	—	—	14.5
anteiso-17:1	—	—	—	0.4	—	1.2
iso-17:0	2.3	3.0	10.3	1.0	6.2	10.6
anteiso-17:0	0.8	20.0	1.2	10.8	7.5	22.8
17:1(c9)	8.0	—	2.3	—	2.1	1.0
iso-16:0 2-OH	—	—	—	5.4	11.7	—
17:0	3.8	tr	1.8	0.4	4.3	—
17:0 10-methyl	—	—	—	—	5.9	—
iso-16:0 3-OH	3.0	—	—	—	—	—
iso-18:0	1.0	—	—	0.3	0.9	—
18:1(c9)	1.0	—	—	—	0.8	—
anteiso-17:0 2-OH	—	—	—	—	4.5	—
iso-17:0 3-OH	3.8	—	—	0.9	—	—
anteiso-17:0 3-OH	—	—	4.1	—	—	0.3
17:0 3-OH	0.7	—	—	2.1	—	—
18:0 10-methyl	—	—	—	—	1.5	—
anteiso-19:0	—	—	—	—	—	0.5

Menaquinones are predominantly MK-9(H₄) with traces of MK-9(H₂), MK-9(H₆) and MK-10(H₄). Mycolic acids are not present. Major fatty acid components are iso- and anteiso-branched fatty acids. The type species is *Allokutzneria albata*.

Description of *Allokutzneria albata* (Tomita *et al.* 1993) comb. nov.

Allokutzneria albata (al.ba'ta. N.L. fem. adj. *albata* clothed in white, referring to the colour of the aerial mycelium and the non-chromogenicity of the organism).

Basonym: *Kibdelosporangium albatu* Tomita *et al.* 1993.

A description of the physiological and morphological characteristics of this taxon has been published previously by Tomita *et al.* (1993). In addition to the properties described above for the genus, the G+C content of the DNA of the type strain is 71.7 mol% (T_m). The type strain is R761-7^T (=NRRL B-24461^T =DSM 44149^T =ATCC 55061^T).

Acknowledgements

The able technical assistance of E. N. Hoekstra for physiological characterization and G. Poetter for chemotaxonomic analyses is gratefully acknowledged. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

References

- Felsenstein, J. (1989).** PHYLIP (phylogeny inference package) version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Grund, E. & Kroppenstedt, R. M. (1989).** Transfer of five *Nocardiopsis* species to the genus *Saccharothrix* Labeda *et al.* 1984. *Syst Appl Microbiol* **12**, 267–274.
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Labeda, D. P. & Kroppenstedt, R. M. (2000).** Phylogenetic analysis of *Saccharothrix* and related taxa: proposal for *Actinosynnemataceae* fam. nov. *Int J Syst Evol Microbiol* **50**, 331–336.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004).** ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Saddler, G. S., Tavecchia, P., Lociuo, S., Zanol, M., Colombo, E. & Selva, E. (1991).** Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J Microbiol Methods* **14**, 186–191.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–426.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E., Kroppenstedt, R. M., Jahnke, K.-D., Kemmerling, C. & Gürtler, H. (1994).** Transfer of *Streptosporangium viridogriseum* (Okuda *et al.* 1966), *Streptosporangium viridogriseum* subsp. *kofuense* (Nonomura and Ohara 1969), and *Streptosporangium albidum* (Furumai *et al.* 1968) to *Kutzneria* gen. nov. as *Kutzneria viridogrisea* comb. nov., *Kutzneria kofuensis* comb. nov., and *Kutzneria albida* comb. nov., respectively, and emendation of the genus *Streptosporangium*. *Int J Syst Bacteriol* **44**, 265–269.
- Stamatakis, A. P., Ludwig, T., Meier, H. & Wolf, M. J. (2002).** AxML: a fast program for sequential and parallel phylogenetic tree calculations based on the maximum likelihood method. *Proc IEEE Comput Soc Bioinform Conf* **1**, 21–28.
- Tamura, T., Liu, Z., Yamei, Z. & Hatano, K. (2000).** *Actinoalloteichus cyanogriseus* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **50**, 1435–1440.
- Tomita, K., Hoshino, Y. & Miyaki, T. (1993).** *Kibdelosporangium albatu* sp. nov., producer of the antiviral antibiotics cycloviracins. *Int J Syst Bacteriol* **43**, 297–301.