Goodfellowia gen. nov., a new genus of the *Pseudonocardineae* related to *Actinoalloteichus*, containing *Goodfellowia coeruleoviolacea* gen. nov., comb. nov.

D. P. Labeda¹ and R. M. Kroppenstedt²

Correspondence

D. P. Labeda
labedadp@ncaur.usda.gov

¹Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, 1815 N. University Street, Peoria, IL 61604 USA

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

During the course of a phylogenetic evaluation of *Saccharothrix* strains held in the ARS Culture Collection, it was discovered that *Saccharothrix coeruleoviolacea* NRRL B-24058^T is unrelated to other species within this genus, and a polyphasic study was undertaken to clarify its taxonomic position. Strain NRRL B-24058^T is observed to be phylogenetically separate from the genus *Saccharothrix* and is most closely related to the genus *Actinoalloteichus*. The strain exhibits chemotaxonomic properties that distinguish it from members of *Actinoalloteichus*, including a whole-cell sugar pattern consisting of galactose and ribose as diagnostic sugars, phosphatidylethanolamine, phosphatidylethanolamine containing 2-OH fatty acids and diphosphatidylglycerol as the predominant polar lipids and MK-9(H₄) and MK-10(H₄) as the only menaquinones observed. Strain NRRL B-24058^T is distinct from other taxa within the suborder *Pseudonocardineae* and a new genus to be named *Goodfellowia* gen. nov. is proposed. The type species of this new genus is *Goodfellowia coeruleoviolacea* gen. nov., comb. nov., and the type strain is NRRL B-24058^T (= DSM 43935^T = INA 3564^T = JCM 9110^T = NBRC 14988^T = VKM Ac-1083^T).

During the course of a phylogenetic evaluation of described species of the genus *Saccharothrix* based on 16S rRNA gene sequences (Labeda & Kroppenstedt, 2000), it was noted that *Saccharothrix coeruleoviolacea* NRRL B-24058^T clearly was not within the genus *Saccharothrix* and appeared to represent a new taxon in the suborder *Pseudonocardineae*. This strain was originally described as *Actinomadura coeruleoviolacea* by Preobrazhenskaya *et al.* (1976) and was subsequently transferred to the genus *Saccharothrix* as *Saccharothrix coeruleoviolacea* by Kroppenstedt *et al.* (1990). A polyphasic investigation was undertaken to characterize this strain fully and to compare it with the phylogenetically closely related genera *Actinoalloteichus*, *Crossiella*, *Kutzneria* and *Streptoalloteichus*.

The strain was cultivated on NZamine medium (DSMZ medium no. 554; DSMZ, 2001) at 28 °C. Morphological observations were made on the media of Shirling & Gottlieb

(1966) and DSMZ medium no. 554. Scanning electron microscopy was performed using a JEOL model JSM-4200 microscope on osmium tetroxide-fixed, dehydrated, critical point-dried and sputter-coated colonial growth.

Genomic DNA for sequencing of the 16S rRNA gene was isolated from growth on DSMZ medium no. 554 plates using UltraClean microbial DNA isolation kits (Mo Bio Laboratories), amplified and sequenced following previously described procedures (Labeda & Kroppenstedt, 2000). The sequence was aligned against sequences for taxa in the suborder *Pseudonocardineae* within ARB (Ludwig *et al.*, 2004) and a phylogenetic tree was constructed according to the neighbour-joining method of Saitou & Nei (1987) and the stability of the groupings was estimated by bootstrap analysis (Felsenstein, 1989). Genomic DNA for determination of G + C content was isolated by the method of Marmur (1961) from biomass grown for 5 days in DSMZ medium no. 554. The mol% G + C content of the DNA was calculated from the *T_m* value by the method of Marmur & Doty (1962).

For the analyses of the fatty acids, about 40 mg cells was scraped from agar plates whereas, for the other chemical analyses, the cells were grown in liquid medium and harvested

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NRRL B-24058^T is DQ093349.

Details of the reference sequences used in the generation of Fig. 1 are available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic dendrogram reconstructed for the genera of the suborder *Pseudonocardineae* calculated within ARB (Ludwig *et al.*, 2004) from almost-complete 16S rRNA gene sequences using Kimura's evolutionary-distance methods (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987) illustrating the taxonomic position of *Goodfellowia coeruleoviolacea* NRRL B-24058^T relative to the other taxa within the suborder. The taxa and sequences included in the genus groups are detailed in Supplementary Table S1 available in IJSEM Online. Bar, 0.01 nucleotide substitutions per site.

by centrifugation. Chemotaxonomic analysis of strains for polar lipids, menaquinones and fatty acids was performed using previously described methods (Grund & Kroppenstedt, 1989; Minnikin *et al.*, 1984; Sasser, 1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids and hydrolysis and decomposition of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, aesculin, urea and hippurate,

Table 1. Chemotaxonomic characteristics of *Goodfellowia* and related taxa

All strains contain *meso*-diaminopimelic acid as the cell-wall diamino acid. Data for reference strains were taken from Tamura *et al.* (2000) (*Actinoalloteichus cyanogriseus*), Labeda (2001) (*Crossiella cryophila*), Tomita *et al.* (1993) (*Kibdelosporangium albatum*), Stackebrandt *et al.* (1994) (*Kutzneria viridogrisea*) and Tomita *et al.* (1987) (*Streptoalloteichus hindustanus*).

Strain	Whole-cell sugar pattern*	Phospholipids†
<i>Goodfellowia coeruleoviolacea</i> NRRL B-24058 ^T	Gal, Rib	DPG, PE, HO-PE, methyl-PE
<i>Actinoalloteichus cyanogriseus</i> IFO 14455 ^T	Glc, Gal, Man, Rib	PIM, PI, PG, DPG, methyl-PE
<i>Crossiella cryophila</i> NRRL B-16238 ^T	Gal, Man, Rha, Rib	PE, DPG, PI, PIM, methyl-PE
<i>Kibdelosporangium albatum</i> ATCC 55061 ^T	Ara, Gal, Glc, Rha	PE, PME, PG, PI
<i>Kutzneria viridogrisea</i> DSM 43850 ^T	Gal, Rha	PI, DPG, PE, HO-PE, GluNU
<i>Streptoalloteichus hindustanus</i> IFO 15115 ^T	Gal, Man, Rha, Rib	PIM, PI, PG, DPG, PE, methyl-PE

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

†DPG, Diphosphatidylglycerol; GluNU, *N*-acetylglucosamine-containing phospholipid; HO-PE, phosphatidylethanolamine containing 2-hydroxy fatty acids; methyl-PE, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides.

were evaluated by using the media of Gordon *et al.* (1974). Phosphatase activity was evaluated by using the method of Kurup & Schmitt (1973). The temperature range for growth was determined on slants of DSMZ medium no. 554.

Strain NRRL B-24058^T is phylogenetically separate from the genus *Saccharothrix* and appears to be most closely related to the genus *Actinoalloteichus*, as can be seen in Fig. 1. The strain exhibits 16S rRNA gene sequence similarity of 95.5, 95.4, 94.6, 94.5, 94.3 and 97.2 %, respectively, to *Actinoalloteichus cyanogriseus* IFO 14455^T, *Actinoalloteichus spitiensis* MTCC 6194^T, *Kibdelosporangium aridum* subsp. *aridum* DSM 43828^T, *Kibdelosporangium aridum* subsp. *largum* DSM 44150^T, *Kibdelosporangium philippinense* DSM 44226^T and *Streptoalloteichus hindustanus* IFO 15115^T.

The chemotaxonomic properties of the strain that distinguish it from members of *Actinoalloteichus* and other related taxa can be seen in Table 1. The whole-cell sugar pattern of only galactose and ribose differs from those of the other four genera, as does the lack of phosphatidylinositol in the phospholipid pattern and the presence of both phosphatidylmonomethylethanolamine and phosphatidylethanolamine containing 2-hydroxy fatty acids. The menaquinone pattern (Table 2) also appears to be distinct from the other related genera in that substantial quantities of MK-10(H₄) are present. The fatty acid profile of strain NRRL B-24058^T (Table 3) clearly distinguishes it from its nearest phylogenetic neighbouring genera by the presence of significant quantities of 17:0 10-methyl fatty acid and various hydroxylated fatty acids.

Table 2. Menaquinone content of *Goodfellowia coeruleoviolacea* and related taxa

Strains: 1, *Goodfellowia coeruleoviolacea* DSM 43935^T; 2, *Actinoalloteichus cyanogriseus* IFO 14455^T (data from Tamura *et al.*, 2000); 3, *Crossiella cryophila* NRRL B-16238^T (Labeda, 2001); 4, *Kibdelosporangium albatum* ATCC 55061^T (Tomita *et al.*, 1993); 5, *Kutzneria viridogrisea* DSM 43850^T (Stackebrandt *et al.*, 1994); 6, *Streptoalloteichus hindustanus* IFO 15115^T (Tomita *et al.*, 1987). Values are percentages of the total menaquinones; —, not detected. Abbreviations are exemplified by MK-8(H₄), a menaquinone which has two of the eight isoprene units hydrogenated.

Menaquinone	1	2	3	4	5	6
MK-8(H ₄)	—	10	—	—	1	—
MK-9	—	—	—	6	—	21
MK-9(H ₂)	—	9	9	13	9	12
MK-9(H ₄)	57	75	90	67	90	15
MK-9(H ₆)	—	2	—	10	4	3
MK-10	—	—	—	—	—	13
MK-10(H ₂)	—	—	tr	—	—	9
MK-10(H ₄)	43	3	1	—	2	23
MK-10(H ₆)	—	—	—	—	—	4

Scanning electron microscopic observations of colony growth on several different media have not revealed the presence of sporangia and the substrate mycelium appears to fragment into coccoidal rod elements. (Fig. 2). Spore chains typical of those observed for *Actinoalloteichus* species were not observed.

Strain NRRL B-24058^T therefore appears distinct from other taxa within the suborder *Pseudonocardineae* and a new genus is proposed, to be named *Goodfellowia* gen. nov., to accommodate *Saccharothrix coeruleoviolacea*.

Description of *Goodfellowia* gen. nov.

Goodfellowia (Good.fel.low'i.a. N.L. fem. n. *Goodfellowia* named for Michael Goodfellow, a microbiologist at the University of Newcastle, in recognition of his contributions to microbial systematics).

Table 3. Fatty acid content of *Goodfellowia coeruleoviolacea* DSM 43935^T (=NRRL B-24058^T) and phylogenetically most closely related taxa

Strains: 1, *Goodfellowia coeruleoviolacea* DSM 43935^T; 2, *Actinoalloteichus cyanogriseus* DSM 43889^T; 3, *Streptoalloteichus hindustanus* DSM 44523^T; 4, *Kibdelosporangium albatum* ATCC 55061^T (data from Tomita *et al.*, 1993). Values are percentages of total fatty acids; —, not detected. Abbreviations are exemplified by the following: 14:0 iso, isotetradecanoic acid or 12-methyl tridecanoic acid; 17:0 anteiso 2-OH, 2-hydroxy 14-methyl hexadecanoic acid.

Peak name	1	2	3	4
14:0 iso	—	4.62	—	3
15:0 iso	6.69	20.30	31.88	8
15:0 anteiso	4.97	12.00	11.51	2
15:1	—	1.83	—	—
15:1 <i>cis</i> 9	—	—	—	2
15:0	6.15	3.6	—	5
16:1 iso	4.52	4.99	—	1
16:0 iso	19.76	16.32	2.47	38
16:1 <i>cis</i> 9	—	2.33	0.69	—
16:0	—	1.95	—	10
16:1	—	—	—	2
16:1 10-methyl?	1.67	—	—	—
17:1 iso	—	—	0.68	—
17:1 anteiso	—	1.88	1.15	—
17:0 iso	1.66	3.95	11.08	3
17:0 anteiso	19.39	15.38	25.67	10
17:1 <i>cis</i> 9	—	6.61	0.81	—
17:0	3.08	2.84	—	13
17:0 10-methyl	9.44	—	—	—
18:1 <i>cis</i> 9	—	0.93	0.36	—
18:0 iso	—	—	—	1
18:0	—	—	—	4
15:0 iso 2-OH	1.67	—	—	—
16:0 2-OH	5.77	—	—	—
17:0 anteiso 2-OH	6.52	—	—	—

Fig. 2. Scanning electron micrograph of 21-day growth of *Goodfellowia coeruleoviolacea* NRRL B-24058^T on yeast extract-malt extract agar. Note that spores are produced by fragmentation of the vegetative mycelium. Bar, 10 μm.

Aerobic. Gram-positive, non-acid-fast, non-motile actinomycetes. Branched substrate mycelium (approx. 0.5 μm in diameter) and, on some media, aerial mycelia are produced. Ovoid conidia are produced by fragmentation of substrate mycelium. Catalase-positive. Contain *meso*-diaminopimelic acid as the diamino acid. The whole-cell sugar pattern consists of galactose and ribose. The phospholipid pattern consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine containing hydroxylated fatty acids and traces of phosphatidylinositol and phosphatidylinositol mannosides. The predominant menaquinones are MK-9(H₄) and MK-10(H₄). Have a fatty-acid profile rich in branched-chain and saturated components including 10-methyl-branched heptadecanoic acid and anteiso-branched 2-hydroxy fatty acids. Phylogenetically nearest neighbour is the genus *Actinoalloteichus*. Type species is *Goodfellowia coeruleoviolacea*.

Description of *Goodfellowia coeruleoviolacea* comb. nov.

Basonym: *Actinomadura coeruleoviolacea* Preobrazhenskaya and Terekhova 1987.

Other synonym: *Saccharothrix coeruleoviolacea* (Preobrazhenskaya and Terekhova 1987) Kroppenstedt *et al.* 1991.

Vegetative mycelium is pale yellow to dark brownish yellow, depending on medium; white aerial hyphae are produced on most media, becoming blue in colour on several media including inorganic salts-starch (ISP-4) agar and yeast extract-malt extract (ISP-2) agar. Pale-violet soluble pigment produced on inorganic salts-starch agar and blue-green soluble pigment produced on yeast extract-malt extract agar. Degrades or hydrolyses casein, aesculin, gelatin, hypoxanthine, starch, tyrosine and urea. No degradation of adenine, allantoin or xanthine. Weakly reduces nitrates.

Assimilates acetate, citrate, malate, oxalate, propionate and succinate; does not assimilate benzoate, lactate, mucate or tartrate. Acid is produced from adonitol, arabinose, cellobiose, dextrin, erythritol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, methyl α-D-glucoside, methyl β-xyloside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose; no acid produced from dulcitol or melezitose. Temperature range for growth 15–45 °C with an optimum around 30 °C. Grows weakly in the presence of 4 % NaCl and not at all at higher salt concentrations. G + C content of the DNA is 68.2 mol% (*T_m* method).

The type strain is NRRL B-24058^T (= DSM 43935^T = INA 3564^T = JCM 9110^T = NBRC 14988^T = VKM Ac-1083^T), isolated from a soil sample from Russia.

Acknowledgements

The able technical assistance of E. N. Hoekstra for physiological characterization, DNA isolation and purification and 16S rRNA sequencing 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

- DSMZ (2001). *Catalogue of Strains*. Braunschweig: Deutsche Sammlung von Mikroorganismen und Zellkulturen.
- Felsenstein, J. (1989). PHYLIP – Phylogeny Inference Package, version 3.5.1. Distributed by the author. University of Washington, Seattle, USA.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- 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.
- Kroppenstedt, R. M., Stackebrandt, E. & Goodfellow, M. (1990). Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetraspora*. *Syst Appl Microbiol* **13**, 148–160.
- Kurup, P. V. & Schmitt, J. A. (1973). Numerical taxonomy of *Nocardia*. *Can J Microbiol* **19**, 1035–1048.
- Labeda, D. P. (2001). *Crossiella* gen. nov., a new genus related to *Streptoalloteichus*. *Int J Syst Evol Microbiol* **51**, 1575–1579.
- 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. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

- 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, A., Athalye, M., Schaal, K. & Parlett, J. H. (1984).** An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Preobrazhenskaya, T. P., Terekhova, L. P., Laiko, A. V., Selezneva, T. I., Zenkova, V. A. & Blinov, N. O. (1976).** *Actinomadura coeruleoviolacea* sp. nov. and its antagonistic properties. *Antibiotiki* **21**, 779–784 (in Russian).
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 1–6.
- 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 & Omura 1969), and *Streptosporangium albidum* (Furumai et al. 1968) to *Kutzneria* gen. nov. as *Kutzneria viridogrisea* comb. nov., *Kutzneria kofuensis* comb. nov., and *Kutzneria* comb. nov., respectively, and emendation of the genus *Streptosporangium*. *Int J Syst Bacteriol* **44**, 265–269.
- Tamura, T., Liu, Z., Zhang, Y. & Hatano, K. (2000).** *Actinoalloteichus cyanogriseus* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **50**, 1435–1440.
- Tomita, K., Nakakita, Y., Hoshino, Y., Numata, K. & Kawaguchi, H. (1987).** New genus of the *Actinomycetales*: *Streptoalloteichus hindustanus* gen. nov., nom. rev., sp. nov., nom. rev. *Int J Syst Bacteriol* **37**, 211–213.
- Tomita, K., Hoshino, Y. & Miyaki, T. (1993).** *Kibdelosporangium albatum* sp. nov., producer of the antiviral antibiotics cycloviracins. *Int J Syst Bacteriol* **43**, 297–301.