Proposal of *Umezawaea* gen. nov., a new genus of the *Actinosynnemataceae* related to *Saccharothrix*, and transfer of *Saccharothrix tangerinus* Kinoshita *et al.* 2000 as *Umezawaea tangerina* gen. nov., comb. nov.

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In the course of phylogenetic analyses of the taxa within the suborder *Pseudonocardineae*, it was observed that *Saccharothrix tangerinus* MK27-91F2^T was misplaced in the genus *Saccharothrix*. After a detailed examination of nucleotide signatures in the 16S rRNA gene sequence along with the morphological and chemotaxonomic characteristics of this strain, which are different from those of all species of *Saccharothrix* as well as the other genera within the suborder, it was concluded that this strain represents a new genus, for which the name *Umezawaea* gen. nov. is proposed. Pseudosporangia are produced on the aerial mycelium, the whole-cell sugar pattern consists of galactose, mannose and ribose, phosphatidylethanolamine, phosphatidylinositol and lyso-phosphatidylethanolamine are the predominant phospholipids and MK-9(H₄) is the predominant menaquinone. The type species of the proposed new genus is *Umezawaea tangerina* gen. nov., comb. nov., with the type strain MK27-91F2^T (=NRRL B-24463^T =DSM 44720^T =FERM P-16053^T =JCM 10302^T =NBRC 16184^T).

During phylogenetic analyses of the 16S rRNA gene sequences of all taxa within the suborder *Pseudonocardineae* using the ARB software package (Ludwig *et al.*, 2004), it was observed that *Saccharothrix tangerinus* MK27-91F2^T is misplaced within the genus *Saccharothrix*. Regardless of the phylogenetic algorithm used, this strain appears consistently as an outgroup from the species of *Saccharothrix* and is distinct from members of other genera in the family, including *Crossiella cryophila* NRRL B-16238^T and *Goodfellowia coeruleoviolacea* NRRL B-23058^T, formerly classified within the genus *Saccharothrix*, as can be observed in Fig. 1. The phylogenetic trees reconstructed using sequences for all taxa with validly published names within the suborder exhibit the same tree topography (data not shown). The 16S rRNA gene sequence similarity of MK27-91F2^T to all of the species of *Saccharothrix* is consistently below 96% and exhibits values quite similar to those observed between *Lechevalieria* species and members of the genus *Saccharothrix* (Supplementary Table S1, available in IJSEM Online). The 16S rRNA gene sequence similarity among species of the genus *Saccharothrix* ranges from 99.20% to a low of 96.96%, between *Saccharothrix*

texasensis NRRL B-16134^T and *Saccharothrix longispora* NRRL B-16116^T, another consistent, but not so distant, outlier in phylogenetic analyses of this genus.

An examination of the nucleotide signatures present in the 16S rRNA gene sequence (Fig. 2) compared with those previously described for *Saccharothrix* and closely related genera (Labeda *et al.*, 2001) revealed a pattern different from those diagnostic of the genera *Actinokineospora* (data not shown), *Actinosynnema*, *Crossiella*, *Goodfellowia*, *Lechevalieria*, *Lentzea* and *Saccharothrix*, providing further evidence of the uniqueness of *S. tangerinus*. It should be noted that *S. longispora* exhibits the 16S rRNA gene nucleotide signature pattern typical of the genus *Saccharothrix* (Fig. 2) as well as sequence similarity values of >97.5% to the vast majority of other species in this genus (Supplementary Table S1) and has a chemotaxonomic profile typical of *Saccharothrix*.

The original description of *S. tangerinus* (Kinoshita *et al.*, 1999) documented the fragmentation of the aerial mycelium into ovoid to cylindrical elements, typical of many genera within the suborder *Pseudonocardineae*, but also described the production of pseudosporangia on the aerial mycelia of this strain, unlike any of the other genera in the suborder. Moreover, the chemotaxonomic profile for

A 16S rRNA gene sequence similarity matrix is available as supplementary material with the online version of this paper.

Fig. 1. Phylogenetic dendrogram reconstructed for the genera of the family Actinosynnemataceae most related to *Saccharothrix* and two taxa originally classified as *Saccharothrix* species 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 *Umezawaea tangerina* gen. nov., comb. nov. MK27-91F2^T relative to the other taxa within the family. The taxa and sequences included in the genus groups are: *Actinosynnema mirum* DSM 43827^T (GenBank accession no. X84447), *Actinosynnema pretiosum* subsp. *pretiosum* NRRL B-16060^T (AF114800), *Lechevalieria aerocolonigenes* NRRL B-3298^T (AF114804), *Lechevalieria flava* NRRL B-16131^T (AF114808), *Lechevalieria fradiae* CGMCC 4.3506^T (AY114175), *Lentzea albida* NBRC 16102^T (AB006176), *Lentzea albidocapillata* IMMIB D-958^T (X84321), *Lentzea flaviverrucosa* AS 4.0578^T (AF183957), *Lentzea californiensis* NRRL B-16137^T (AF174435), *Lentzea koreensis* LM 121^T (Y09158), *Lentzea violacea* LM 036^T (AJ242633) and *Lentzea waywayandensis* NRRL B-16159^T (AF114813). Percentages at nodes represent levels of bootstrap support from 1000 resampled datasets; values less than 50% are not shown. Asterisks indicate that the corresponding branches that were also recovered in the maximum-likelihood (Felsenstein, 1993; Olsen *et al.*, 1994) and maximum-parsimony (Felsenstein, 1993) trees. Bar, 0.01 nucleotide substitutions per site.

this strain, as shown in Table 1, is different from those of the other genera in the Actinosynnemataceae as well as the closely related genera *Crossiella* and *Goodfellowia*. Moreover, the fatty acid profile of this strain is different from those of the other species of *Saccharothrix* (Table 2).

Strain MK27-91F2^T therefore appears distinct from members of the genus *Saccharothrix sensu stricto* as well as all of the other genera within the Pseudonocardineae, and a new genus, to be named *Umezawaea* gen. nov., is proposed to accommodate *Saccharothrix tangerinus* as the new combination *Umezawaea tangerina* gen. nov., comb. nov.

	601	611	621	841
<i>Lechevalieria aerocolonigenes</i> NRRL B-3298 ^T	ACTTGGGGCT	TAAACCCGAG	CCTGCGGTGG	ACGTTCTCCG
<i>Lentzea albidocapillata</i> DSM 44073 ^T		T..A	...T.....	...CC...T.
<i>Actinosynnema mirum</i> DSM 43827 ^T				...C.....
<i>S. australiensis</i> NRRL 11239 ^T	CAC.	GTG.		...C.....
<i>S. algeriensis</i> NRRL B-24137 ^T	CAC.	GTG.		...C.....
<i>S. coeruleofusca</i> NRRL B-16115 ^T	CAC.	GTG.		...C.....
<i>S. espanaensis</i> NRRL 15764 ^T	CAC.	GTG.		...C.....
<i>S. mutabilis</i> subsp. <i>mutabilis</i> DSM 43853 ^T	CAC.	...N...GTG.		...C.....
<i>S. mutabilis</i> subsp. <i>capreolus</i> DSM 40225 ^T	CAC.	...N...GTG.		...C.....
<i>S. longispora</i> NRRL B-16116 ^T	CAC.	GTG.	A.	...C.....
<i>S. syringae</i> NRRL B-16468 ^T	CAC.	GTG.		...C.....
<i>S. texasensis</i> NRRL B-16134 ^T	CAC.	GTG.	A.	...C.....
<i>S. xinjiangensis</i> AS 4.1731 ^T	CAC.	GTG.		...C.....
<i>Umezawaea tangerina</i> MK27-91F2^T	C.ACA.	TGT.	G..G...A..	...C.....
<i>Crossiella cryophila</i> NRRL B-16238 ^T	C.....	T..T.	...T...A..	...GC.....
<i>Goodfellowia coeruleoviolacea</i> NRRL B-24058 ^T	T.....	T.....	G..T.....	...GC.....
<i>Pseudonocardia thermophila</i> IMSNU 20112 ^T	TCA.	TGG.	...T.....	...GGT....

Fig. 2. Nucleotide signatures in the 16S rRNA gene in *Saccharothrix* and related taxa. N, Undetermined nucleotide.

Table 1. Chemotaxonomic characteristics of *Umezawaea* gen. nov. compared with other similar members of the family *Actinosynnemataceae*

All genera have *meso*-diaminopimelic acid as the cell-wall diamino acid and are of cell-wall chemotype III.

Genus	Whole-cell sugar pattern*	Phospholipid type	Phospholipid(s)†	Predominant menaquinone(s)
<i>Umezawaea</i>	Gal, Man, Rib, Rha (trace)	PII	PE, PI, OH-PE, lyso-PE, DPG, PIM	MK-9(H ₄); trace MK-10(H ₄)
<i>Actinokineospora</i>	Gal, Man, Rha	PII	PE	MK-9(H ₄)
<i>Actinosynnema</i>	Gal, Man	PII	PE, OH-PE, PI, PIM, DPG	MK-9(H ₄); MK-9(H ₆)
<i>Crossiella</i>	Gal, Rha, Rib	PII	PE, DPG, PI, PIM, PME	MK-9(H ₄)
<i>Goodfellowia</i>	Gal, Rib	PII	PE, OH-PE, DPG	MK-10(H ₄); MK-9(H ₄)
<i>Lechevalieria</i>	Gal, Man, Rha	PII	PE	MK-9(H ₄)
<i>Lentzea</i>	Gal, Man, Rib	PII	PE, DPG, PG, PI	MK-9(H ₄)
<i>Saccharothrix</i>	Gal, Rha, Man (trace)	PII, PIV	PE, OH-PE, PI, PIM, DPG, PG (v)	MK-10(H ₄); MK-9(H ₄)

*Gal, Galactose; Man, mannose; Rha, rhamnose; Rib, ribose.

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

Table 2. Comparison of fatty acid patterns of *Umezawaea tangerina* gen. nov., comb. nov. with those of *Saccharothrix* type strains

Values are percentages of total fatty acids. Data for *Saccharothrix* were taken from Grund & Kroppenstedt (1990) and are given as means and as ranges of values for *Saccharothrix syringae* DSM 43886^T, *S. mutabilis* subsp. *mutabilis* DSM 43853^T, *S. longispora* DSM 43749^T and *S. coeruleofusca* DSM 43679^T. Values that differ significantly are highlighted in bold. Fatty acid concentrations <1 % may not be significant. —, Not detected.

Fatty acid	<i>Saccharothrix</i> species		<i>U. tangerina</i> DSM 44270 ^T
	Range	Mean	
iso-14:0	0.93–6.43	2.52	9.33
14:0	—	—	0.41
iso-15:1	—	—	0.77
iso-15:0	4.12–21.02	12.73	10.80
anteiso-15:0	1.35–6.36	3.16	3.42
15:1 (c9)	0.00–0.06	0.53	1.03
15:0	0.47–0.92	0.90	3.55
iso-16:1	0.00–11.94	5.54	5.90
iso-16:0	30.67–60.56	37.01	40.18
16:1 (c9)	0.00–3.71	1.49	3.82
iso-15:0 2-OH	0.00–3.07	1.2	—
16:0	0.47–4.00	1.74	3.47
iso-16:0 10-methyl	—	—	0.39
16:0 9-methyl	0.00–6.10	4.49	—
16:0 10-methyl	—	—	3.10
anteiso-17:1	—	—	0.39
iso-17:0	0.83–7.63	3.48	0.52
anteiso-17:0	2.99–13.52	6.48	1.90
17:1 (c9)	0.91–8.36	4.40	3.03
iso-16:0 2-OH	1.93–5.94	4.03	4.02
17:0	0.40–2.66	1.58	2.11
iso-17:0 10-methyl	—	—	0.43
17:0 10-methyl	0.00–1.71	1.28	0.83
iso-18:0	0.26–1.31	0.55	—
18:1 (c9)	0.00–3.43	0.67	—
anteiso-17:0 2-OH	0.29–1.80	0.78	—
18:0 10-methyl	0.00–0.39	0.18	—

Description of *Umezawaea* gen. nov.

Umezawaea (U.me.za'wa.e.a. N.L. fem. n. *Umezawaea* named for the late Hamao Umezawa, of the Institute of Microbial Chemistry, Tokyo, in recognition of his leadership and contributions to the study of the biology and natural products of actinomycetes).

Aerobic. Gram-positive, non-acid-fast, non-motile actinomycetes. Branched substrate mycelium (approx. 0.3–0.5 µm in diameter) and, on some media, aerial mycelia are produced. Ovoid or cylindrical conidia (0.3–0.5 × 1.1–1.9 µm) are produced by fragmentation of substrate mycelium. Pseudosporangia are produced on some media. Contains *meso*-diaminopimelic acid as the diamino acid. The muramic acid in the cell wall peptidoglycan is acetyl type. The whole-cell sugar pattern consists of galactose, mannose and ribose, with a trace of rhamnose. The phospholipid pattern consists predominantly of phosphatidylethanolamine, phosphatidylinositol, phosphatidylethanolamine containing hydroxylated fatty acids and lyso-phosphatidylethanolamine. The predominant menaquinone is MK-9(H₄), with a trace of MK-10(H₄). Mycolic acids are absent. The G + C content of the DNA is 74 mol% (HPLC). Has a fatty-acid profile consisting predominantly of iso-16:0 fatty acids, with iso-14:0, iso-15:0, 16:0, 16:1, 17:1 and iso-16:1 as minor components. The phylogenetically nearest neighbour is the genus *Saccharothrix*. The type species is *Umezawaea tangerina*.

Description of *Umezawaea tangerina* comb. nov.

Umezawaea tangerina (tan.ge.ri'na. N.L. fem. adj. *tangerina* tangerine-coloured, referring to the colour of the vegetative growth).

Basonym: *Saccharothrix tangerinus* Kinoshita *et al.* 2000.

The description matches that published previously for *Saccharothrix tangerinus* by Kinoshita *et al.* (1999). The type strain is MK27-91F2^T (=NRRL B-24463^T =DSM 44720^T =FERM P-16053^T =JCM 10302^T =NBRC 16184^T).

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