Pseudokineococcus lusitanus gen. nov., sp. nov., and reclassification of *Kineococcus marinus* Lee 2006 as *Pseudokineococcus marinus* comb. nov.

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A Gram-reaction-positive, motile, coccus-shaped actinobacterium, designated strain T2A-S27^T, was isolated from a roof tile in Oporto (Portugal) and studied using a polyphasic approach. The 16S rRNA gene sequence of the novel isolate showed high similarity to that of *Kineococcus marinus* KST3-3^T (97.8 % sequence similarity). Strain T2A-S27^T showed lower 16S rRNA gene sequence similarities with other members of the genus *Kineococcus* and members of the family *Kineosporiaceae* (<94 %). A phylogenetic tree, based on 16S rRNA gene sequences, showed that strain T2A-S27^T formed a coherent clade with the type strain of *K. marinus* and *Quadrisphaera granulorum*. The isolate was characterized by the presence of *meso*-diaminopimelic acid in the cell-wall peptidoglycan, MK-9(H_2) as the predominant menaquinone and a polar lipid profile consisting of diphosphatidylglycerol and phosphatidylglycerol. The fatty acid profile was dominated by anteiso-$C_{15:0}$. The DNA G+C content was 76.9 mol%. The low level of DNA–DNA relatedness to *K. marinus* (46–47 %) and the results of the chemotaxonomic and physiological studies clearly distinguished strain T2A-S27^T from recognized species of the genus *Kineococcus*. On the basis of its phylogenetic position and phenotypic traits, strain T2A-S27^T (=LMG 24148^T =CECT 7306^T =DSM 23768^T) represents a novel species of a new genus in the family *Kineosporiaceae*, for which the name *Pseudokineococcus lusitanus* gen. nov., sp. nov. is proposed. The misclassified species *K. marinus* is transferred to the new genus as *Pseudokineococcus marinus* comb. nov. The type strain of *Pseudokineococcus marinus* is KST3-3^T (=KCCM 42250^T =NRRL B-24439^T).

Recently, the new suborder *Kineosporiineae* and the valid family *Kineosporiacae* have been described by Zhi *et al.* (2009). At the time of writing, the family *Kineosporiaceae* included the genera *Angustibacter* (Tamura *et al.*, 2010), *Kineococcus* (Yokota *et al.*, 1993), *Kineosporia* (Pagani & Parenti, 1978) and *Quadrisphaera* (Maszenan *et al.*, 2005).

In a survey on the colonization of roof tiles in Oporto, Portugal, a *Kineococcus*-like strain, designated T2A-S27^T, was isolated from a grey biofilm covering a roof tile, together with strains that were assigned to the genera *Streptomyces*, *Microbacterium*, *Pseudomonas*, *Azospirillum* and *Cellulomonas*. The aim of the present study was to determine the taxonomic position of strain T2A-S27^T. On the basis of the results presented below, the isolate represents a novel species of a new genus within the family *Kineosporiaceae*, which also incorporates the misclassified species *Kineococcus marinus*.

Strain T2A-S27^T was isolated on Tryptose Soy Agar (TSA) (Oxoid) after 4 weeks at 28 °C. The methods used in this study have been described previously (Jurado *et al.*, 2005a, b) except where indicated otherwise. Briefly, wet slide suspensions of cultures grown in TSA were observed by phase-contrast microscopy. Acid production from a variety of substrates was tested using the API 50 CH B/E kit (bioMérieux) and assimilation tests were carried out using the API 20 NE kit (bioMérieux); API tests were performed according to the manufacturer's instructions. Susceptibility to antibiotics was studied by placing antibiotic discs (Mast Diagnostics) on TSA plates inoculated with suspensions of the test strains. Oxidase activity was studied by monitoring oxidation on DrySlide oxidase (Becton Dickinson). For the Gram reaction, a 3 % solution of potassium hydroxide was used (Halebian *et al.*, 1981). Flagella were stained with flagella stain droppers (Becton Dickinson). Growth was determined at 4–40 °C. Tolerance to NaCl was studied on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T2A-S27^T is FN824365.

One supplementary table is available with the online version of this paper.

TSA and in nutrient broth supplemented with 0–10 % (w/v) NaCl. Cellular fatty acids were determined as described previously (Jurado *et al.*, 2009). The polar lipid profile, whole-cell sugars and G + C content of the genomic DNA were determined at the DSMZ. For phylogenetic analysis, the almost complete 16S rRNA gene sequence of strain T2A-S27^T was aligned and compared with the corresponding sequences of members of the family *Kineosporiaceae* and other representatives of taxa of the order *Actinomycetales* using the multiple sequence alignment program CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura *et al.*, 2007) and PHYLO_WIN (Galtier *et al.*, 1996) with three treeing algorithms, the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods. The robustness of the resultant tree was assessed by bootstrap resampling (1000 replicates each). The degree of genomic relatedness between strain T2A-S27^T and *Kineococcus marinus* KCCM 42250^T (the most closely related species on the basis of 16S rRNA gene sequence similarity) was determined by DNA–DNA hybridization, as described by De Ley *et al.* (1970) and Rosselló-Mora & Amann (2001).

Cells of strain T2A-S27^T were aerobic, non-spore-forming, Gram-reaction-positive cocci that occurred singly or in pairs, tetrads and clusters and were motile due to flagella. Strain T2A-S27^T was oxidase-negative and catalase-positive. Other physiological characteristics of strain T2A-S27^T are shown in Table 1.

Analysis of 16S rRNA gene sequences revealed that strain T2A-S27^T formed a distinct cluster together with *K. marinus* (97.8 % sequence similarity, corresponding to 31 nt differences among 1381 nt positions) in the phylogenetic gene tree of the class *Actinobacteria*. Strain T2A-S27^T was loosely related to *Quadrisphaera granulorum* (93.8 % sequence similarity), from which it could be readily distinguished by the menaquinone type of MK8(H_2) and further phenotypic characteristics (Table 1). The 16S rRNA gene sequence similarities between strain T2A-S27^T and all species of the genus *Kineococcus* (except *K. marinus*) were <94 % (93.0 % for *Kineococcus aurantiacus*, 93.0 % for *Kineococcus radiotolerans*, 92.5 % for *Kineococcus gynurae*, 93.5 % for *Kineococcus xinjiangensis* and 93.2 % for *Kineococcus rhizosphaerae*). The 16S rRNA gene phylogenetic tree showed that the strain T2A-S27^T–*K. marinus*–*Q. granulorum* cluster obtained using the neighbour-joining, maximum-likelihood and maximum-parsimony treeing algorithms was supported by a bootstrap value of 76 %, showing that strain T2A-S27^T and *K. marinus* were phylogenetically distinct from the genus *Kineococcus* (Fig. 1). Furthermore, strain T2A-S27^T exhibited chemotaxonomic differences from all species of the genus *Kineococcus* and *Q. granulorum* (Table 2). The 16S rRNA gene nucleotide signature pattern was typical of the suborder described by Zhi *et al.* (2009), although strain T2A-S27^T and *K. marinus* shared the same pattern of 16S rRNA signature nucleotides at positions 75 (G), 79 (G), 139 (C), 191 (U), 203 (G), 381 (G), 589 (C), 591 (C), 610 (G), 630 (T), 648 (G), 837 (U), 839 (G), 849 (G), 1010 (U), 1020 (U), 1121 (G), 1152 (C) and 1252 (U) based on the numbering system of the *Escherichia coli* 16S rRNA gene sequence (GenBank accession number J01695). Thus, based on phylogenetic and chemotaxonomic data, strain T2A-S27^T did not belong to the genera *Kineococcus* or *Quadrisphaera*. The high sequence similarity between strain T2A-S27^T and *K. marinus* suggested that they belong to the same genus.

Although strain T2A-S27^T was most closely related to *K. marinus*, both organisms showed numerous differences in their physiological and chemotaxonomic characteristics (Tables 1 and 2). In addition to the differences presented in Table 1, strain T2A-S27^T was positive for acid production from L-sorbose, while *K. marinus* was negative for this trait;

Table 1. Phenotypic characteristics of strain T2A-S27^T and related species

Strains: 1, T2A-S27^T; 2, *K. marinus* KCCM 42250^T; 3, *K. aurantiacus* IFO 15268^T; 4, *K. radiotolerans* ATCC BAA-149^T; 5, *K. gynurae* NRRL B-24568^T; 6, *K. xinjiangensis* CCTCC AB 207179^T; 7, *K. rhizosphaerae* DSM 19711^T; 8, *Q. granulorum* DSM 44889^T. For phenotypic tests, strain T2A-S27^T and *K. marinus* were grown under the same conditions in this study. Data for the reference strains *K. aurantiacus*, *K. radiotolerans*, *K. gynurae*, *K. xinjiangensis*, *K. rhizosphaerae* and *Q. granulorum* were taken from Lee (2009), Duangmal *et al.* (2008), Liu *et al.* (2009) and Maszenan *et al.* (2005). +, positive; −, negative; (+) weakly positive; +/−, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Acid produced from:								
D-Arabitol	−	+	ND	ND	+	ND	ND	+
D-Galactose	+	+	−	+	+	ND	+	−
Lactose	(+)	+	−	−	+	ND	−	−
Maltose	+	+	−	−	+	+	+	−
D-Mannitol	+	+	−	+	+	−	ND	−
D-Mannose	+	+	−	+	+	ND	+	−
Melezitose	−	+	ND	ND	+	ND	+	−
Melibiose	+/−	−	ND	ND	+	ND	+	ND
Raffinose	−	+	−	−	−	ND	+	−
D-Ribose	−	+	−	−	−	−	−	ND
Trehalose	+	+	−	ND	+	ND	−	ND
Gentiobiose	−	+	ND	ND	+	ND	ND	ND
Glycerol	+	+	−	+	−	ND	−	+
Inositol	−	(+)	−	+	−	+	ND	ND
L-Rhamnose	+	+	−	−	+	+	−	−
N-Acetylglucosamine	+/−	−	ND	ND	−	ND	ND	−
Growth at/in:								
7 % (w/v) NaCl	(+)	+	−	−	(+)	−	−	ND
5 °C	−	+	−	−	−	−	−	−
Hydrolysis of:								
Aesculin	+	+	−	ND	ND	+	+	+
Gelatin	+	+	−	ND	+	+	+	−
Starch	V	+	−	−	ND	−	−	ND
Urea	−	−	+	−	+	−	+	−

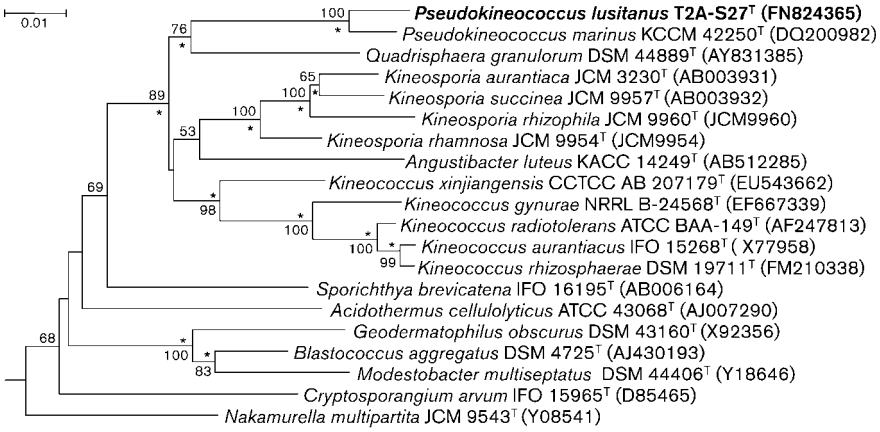

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain T2A-S27^T and species belonging to the suborders *Frankineae* and *Kineosporiineae.* The tree was reconstructed by using the neighbour-joining method and was based on a comparison of 1407 nt. The tree was rooted by using *Mycobacterium alvei* DSM 44176^T (accession no. AF023664) as the outgroup (not shown). Bootstrap values are expressed as percentages of 1000 replications. Asterisks indicate branches of the tree that were also recovered using maximum-likelihood and maximum-parsimony treeing algorithms. Bar, 0.01 substitutions per nucleotide position.

the opposite was seen for D-adonitol. Also, strain T2A-S27^T produced alkaline phosphatase, acid phosphatase and *N*-acetyl-β-glucosaminidase unlike *K. marinus* which produced α-galactosidase. While *K. marinus* assimilated mannose, potassium gluconate and DL-malic acid, strain T2A-S27^T did not. Strain T2A-S27^T grew well on TSA at concentrations of NaCl below 8 % (w/v) with an optimum at 0–3 % (w/v), while *K. marinus* tolerated up to 9 % (w/v) NaCl with optimal growth at 1–4 % (w/v). Growth of strain T2A-S27^T occurred at 6–37 °C with an optimum at 28–30 °C, while the temperature range for growth of *K. marinus* was 4–37 °C. Strain T2A-S27^T contained glucose, ribose and rhamnose as characteristic sugars in the whole-cell hydrolysates, while arabinose and galactose were found in *K. marinus*. Further differences were noticed in the compositions of polar lipids and fatty acids. Diphosphatidylglycerol was the main polar lipid in strain T2A-S27^T, which was absent in *K. marinus*. Phosphatidylinositol was present

Table 2. Phenotypic characteristics of strain T2A-S27^T and related taxa

Data from Tamura *et al.* (2010), Lee (2006) and this study. A_2pm, diaminopimelic acid; A, anteiso-methyl-branched; I, iso-methyl-branched; M, 9- 10-methyl-branched; S, straight-chain saturated; U, monounsaturated; DPG, diphosphatidylglycerol; GL, unknown glycolipid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipids; PGL, unknown phosphoglycolipid; Ara, arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; ND, no data.

Characteristic	Strain T2A-S27^T	*K. marinus*	*Kineococcus*	*Quadrisphaera*	*Kineosporia*	*Angustibacter*
Cell morphology	Cocci in pairs, tetrads and clusters	Cocci singly, in pairs or in clusters	Cocci in tetrad arrangements	Cocci in tetrad arrangements	Single spores borne at tips of substrate hyphae and spore clusters on a sporophore	Irregular rods and cocci
Motility	Motile	Motile	Motile	Non-motile	Motile	Non-motile
Cell-wall diamino acid(s)	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	*meso*- and LL-A_2pm	*meso*-A_2pm
Fatty acid type	S, I, A	S, I, A	S, I, A	S, I, A, U	S, U, M	S, I, A, U, M
Predominant menaquinone	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-8(H_2)	MK-9(H_4)	MK-9(H_4)
Polar lipids	DPG, PG, PL, GL, PGL	PG, PI	DPG, PG, GL	DPG, PG, PI	PC, DPG, PI, PIM	DPG, PG, PI, PIM
Characteristic sugars	Glu, Rib, Rha	Gal, Ara	Gal, Ara	ND	Gal, Glu, Man, Rib	Gal, Glu, Rib
DNA G+C content (mol%)	76.9	76.6	73–77	75	69–71	71

only in *K. marinus*. In both species *anteiso*-$C_{15:0}$, was the predominant fatty acid, which is a typical characteristic of the genus *Kineococcus* (Yokota *et al.*, 1993); however, there were differences in the pattern of other fatty acids between strain T2A-S27^T and *K. marinus* as shown in the Supplementary Table S1 (available in IJSEM Online).

The DNA G+C content of strain T2A-S27^T was 76.9 mol%. The level of DNA–DNA relatedness between strain T2A-S27^T and *K. marinus* KCCM 42250^T was 46.6±0.8.

The phenotypic and genotypic characteristics described above and the observed differences between strain T2A-S27^T and previously described species of the genus *Kineococcus* revealed that strain T2A-S27^T represents a novel species of a new genus, for which the name *Pseudokineococcus lusitanus* gen. nov., sp. nov., is proposed. *K. marinus* is transferred to the new genus as *Pseudokineococcus marinus* comb. nov.

Description of *Pseudokineococcus* gen. nov.

Pseudokineococcus (Pseu.do.ki.ne.o.coc′cus. Gr. adj. *pseudês* false; NL. masc. n. *kineococcus* a bacterial genus name; N.L. masc. n. *Pseudokineococcus* the false *Kineococcus*).

Cells are spherical, 1.0–1.5 μm in diameter and occur in pairs, in tetrads, or in clusters. Cells are motile and have tufts of flagella. Endospores are not formed. Gram-positive. Colonies are circular, rough and orange-coloured. Strictly aerobic. Catalase-positive and oxidase-negative. Do not reduce nitrate to nitrite. Acid is produced from glucose and some other sugars. Aesculin is hydrolysed. The diagnostic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. The major menaquinone is MK-9(H_2). Mycolic acids are not present. The characteristic phospholipid of the genus is phosphatidylglycerol. Diphosphatidylglycerol and phosphatidylinositol may be present. The major cellular fatty acid is anteiso-$C_{15:0}$. The type species of the genus is *Pseudokineococcus lusitanus*.

Description of *Pseudokineococcus lusitanus* sp. nov.

Pseudokineococcus lusitanus (lu.si.ta′nus. L. masc. adj. belonging to Lusitania the Latin name for Portugal, where the organism was isolated).

Cells are Gram-reaction-positive cocci that are 1.0–1.5 μm in diameter and occur singly or in pairs, tetrads or clusters. Colonies are circular, rough and orange-coloured. Cells are aerobic, oxidase-negative, urease-negative and catalase-positive. Growth occurs at 6–37 °C (optimum 28–30 °C). Grows well at 0–3 % (w/v) NaCl, moderately at 5–6 % and poorly at 7–8 %. Produces acid from D-and L-arabinose, D-xylose, D-glucose, D-fructose, L-sorbose, D-sorbitol, methyl-α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, cellobiose, sucrose, starch, glycogen, xylitol, turanose and D-lyxose but not from erythritol, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, dulcitol, methyl-α-D-mannopyranoside, inulin, L-arabitol, D-fucose, potassium gluconate, 2-ketogluconate, 5-ketogluconate or L-fucose. Acid production from D-tagatose is variable. Produces alkaline phosphatase, esterase (C1), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase but not lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase or α-fucosidase. Assimilates glucose, L-arabinose, mannitol, *N*-acetylglucosamine and maltose but does not assimilate mannose, gluconate, capric acid, adipic acid, DL-malic acid, citrate or phenylacetic acid. Negative for glucose fermentation and arginine dihydrolase activities. Nitrate reduction and indole tests are negative. Sensitive to (μg per disc) chloramphenicol (30), rifampicin (5), tetracycline (30), novobiocin (30), streptomycin (10), carbenicillin (100), framycetin (50), erythromycin (15), doxycycline (30), vancomycin (30), gentamicin (10) and kanamycin (30) but resistant to norfloxacin (10), ampicillin (10) and nalidixic acid (30). The predominant fatty acid is anteiso-$C_{15:0}$. Whole-cell hydrolysates contain glucose, ribose, rhamnose and traces of galactose, mannose and xylose. The major menaquinone is MK-9(H_2). Polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid, an unidentified glycolipid and an unidentified phosphoglycolipid.

The type strain, T2A-S27^T (=LMG 24148^T =CECT 7306^T =DSM 23768^T) was isolated from a roof tile in Oporto, Portugal. The DNA G+C content of the type strain is 76.9 mol%.

Description of *Pseudokineococcus marinus* comb. nov.

Pseudokineococcus marinus (ma.ri′nus. L. masc. adj. *marinus* of the sea, the origin of the sample from which the type strain was isolated).

Basonym: *Kineococcus marinus* Lee (2006).

The description is identical to that given for *Kineococcus marinus* by Lee (2006). The type strain is KST3-3^T (=KCCM 42250^T =NRRL B-24439^T).

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