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-S27T, 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 KST3-3T (97.8 % sequence similarity). Strain T2A-S27T 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-S27T 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(H2) as the predominant menaquinone and a polar lipid profile consisting of diphosphatidylglycerol and phosphatidylglycerol. The fatty acid profile was dominated by anteiso-C15 : 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-S27T from recognized species of the genus Kineococcus. On the basis of its phylogenetic position and phenotypic traits, strain T2A-S27T (5 LMG 24148T 5 CECT 7306T 5 DSM 23768T) 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 KST3-3T (5 KCCM 42250T 5 NRRL B-24439T).

Recently, the new suborder Kineosporineae and the valid family Kineosporiaceae 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-S27T, 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-S27T. 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-S27T 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...
strain T2A-S27T was aligned and compared with the analysis, the almost complete 16S rRNA gene sequence of DNA were determined at the DSMZ. For phylogenetic analysis, the almost complete 16S rRNA gene nucleotide signature pattern was typical of relatedness between strain T2A-S27T and other representatives of taxa 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-S27T and *Kineococcus marinus* KCCM 42250T (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-S27T were aerobic, non-spore-forming, Gram-positive cocci that occurred singly or in pairs, tetrads and clusters and were motile due to flagella. Strain T2A-S27T was oxidase-negative and catalase-positive.

Analysis of 16S rRNA gene sequences revealed that strain T2A-S27T 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-S27T was loosely related to *Quadrisphaera granulorum* (93.8% sequence similarity), from which it could be readily distinguished by the menaquinone type of MK8(H2) and further phenotypic characteristics (Table 1). The 16S rRNA gene sequence similarities between strain T2A-S27T 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 rhizophaerae*). The 16S rRNA gene phylogenetic tree showed that the strain T2A-S27T–*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-S27T and *K. marinus* were phylogenetically distinct from the genus *Kineococcus* (Fig. 1). Furthermore, strain T2A-S27T 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-S27T 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-S27T did not belong to the genera *Kineococcus* or *Quadrisphaera*. The high sequence similarity between strain T2A-S27T and *K. marinus* suggested that they belong to the same genus.

Although strain T2A-S27T 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-S27T was positive for acid production from L-sorbose, while *K. marinus* was negative for this trait;

### Table 1. Phenotypic characteristics of strain T2A-S27T and related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of:</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Aesculin</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Gelatin</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Starch</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>- Urea</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

the opposite was seen for D-adonitol. Also, strain T2A-S27T produced alkaline phosphatase, acid phosphatase and N-acetyl-b-glucosaminidase unlike K. marinus which produced a-galactosidase. While K. marinus assimilated mannose, potassium gluconate and DL-malic acid, strain T2A-S27T did not. Strain T2A-S27T 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-S27T 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-S27T contained glucose, ribose and rhamnose as characteristic sugars in the whole-cell hydrolasates, 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-S27T, which was absent in K. marinus. Phosphatidylinositol was present

**Table 2.** Phenotypic characteristics of strain T2A-S27T and related taxa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain T2A-S27T</th>
<th>K. marinus</th>
<th>Kineococcus</th>
<th>Quadrisphaera</th>
<th>Kineosporia</th>
<th>Angustibacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Cocci in pairs, tetrads and clusters</td>
<td>Cocci singly, in pairs or in clusters</td>
<td>Cocci in tetrad arrangements</td>
<td>Cocci in tetrad arrangements</td>
<td>Single spores borne at tips of substrate hyphae and spore clusters on a sporophore</td>
<td>Irregular rods and cocci</td>
</tr>
<tr>
<td>Motility</td>
<td>Motile meso-A2pm</td>
<td>Motile meso-A2pm</td>
<td>Motile meso-A2pm</td>
<td>Non-motile meso-A2pm</td>
<td>Motile meso- and LL-A2pm</td>
<td>Non-motile meso-A2pm</td>
</tr>
<tr>
<td>Predominant menaquinone</td>
<td>MK-9(H2)</td>
<td>MK-9(H2)</td>
<td>MK-9(H2)</td>
<td>MK-8(H2)</td>
<td>MK-9(H4)</td>
<td>MK-9(H4)</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>DPG, PG, PL, GL, PGL</td>
<td>PG, PI</td>
<td>DPG, PG, GL</td>
<td>DPG, PG, PI</td>
<td>PC, DPG, PI, PIM</td>
<td>DPG, PG, PI, PIM</td>
</tr>
<tr>
<td>Characteristic sugars</td>
<td>Glu, Rib, Rha 76.6</td>
<td>Gal, Ara 76.6</td>
<td>Gal, Ara 73–77</td>
<td>ND</td>
<td>Gal, Glu, Man, Rib 69–71</td>
<td>Gal, Glu, Rib 71</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>76.9</td>
<td>76.6</td>
<td>75</td>
<td>ND</td>
<td>75</td>
<td>71</td>
</tr>
</tbody>
</table>

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain T2A-S27T and species belonging to the suborders Frankineae and Kineosporineae. 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 44176T (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.
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.kine.o.co cus) Gr. adj. pseudēs 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, 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 cystine arylamidase, -diaminopimelic acid. The major menaquinone is MK-9(H2). Whole-cell hydrolysates contain glucose, ribose, rhamnose and traces of galactose, mannose and xylose. The major menaquinone is MK-9(H2). Poly polar lipids comprise diphasphatidylglycerol, 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).


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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**References**


