Kineosporia babensis sp. nov., isolated from plant litter in Vietnam

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Three actinomycetes, designated strains VN05A0342, VN05A0351 and VN05A0415^T, were isolated from plant-litter samples collected in the north of Vietnam and examined in a polyphasic taxonomic study. Phylogenetic analysis based on the 16S rRNA gene sequences showed that these isolates were most closely related to the type strain of *Kineosporia mikuniensis* (98.5% sequence similarity). Morphological properties (the formation of spore domes and motile spores) and chemotaxonomic data supported the assignment of the three isolates to the genus *Kineosporia*. The isolates all contained the following: meso-diaminopimelic acid in the peptidoglycan (with small amounts of the LL isomer); ribose, mannose, galactose and glucose as the whole-cell sugars; MK-9(H₄) as the predominant isoprenoid quinone; C_{18:1} and C_{16:0} as the major cellular fatty acids; and phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol as the phospholipids. The high DNA–DNA relatedness (>71%) among the three isolates showed that they represented a single species. On the other hand, the DNA–DNA relatedness between the novel isolates and all type strains of *Kineosporia* species was less than 46%. The physiological properties of our isolates were distinct from those of all of the *Kineosporia* species with validly published names, e.g. decomposition of L-tyrosine and aesculin and the utilization of raffinose and D-arabitol. Therefore, strains VN05A0342, VN05A0351 and VN05A0415^T represent a novel species of the genus *Kineosporia*, for which the name *Kineosporia babensis* sp. nov. is proposed. The type strain is VN05A0415^T (=VTCC-A-0961^T =NBRC 104154^T).

The genus *Kineosporia* was first reported by Pagani & Parenti (1978). The genus was described as comprising organisms that form sporangia (each containing a single zoospore) at the edge of the substrate mycelium and contain only LL-diaminopimelic acid (LL-A₂pm) in the peptidoglycan. Subsequently, Itoh *et al.* (1989) and Kudo *et al.* (1998) emended the description of the genus on the basis of the presence of both LL-A₂pm and meso-A₂pm and the similarity of the colonial morphology to ‘spore-dome actinomycetes’ (Willoughby, 1969). At the time of writing, the genus *Kineosporia* comprises five species with validly

published names. In this study, we used a polyphasic approach to classify three novel actinomycete isolates. On the basis of the data from this study, these three isolates represent a novel species of the genus *Kineosporia*.

The samples of plant litter were collected in 2005 from the mountainside at Ba Be National Park, Bac Kan Province, in northern Vietnam. The samples were dried at room temperature for 5–7 days and then inoculated using the rehydration–centrifugation method (Hayakawa *et al.*, 2000) on humic acid-vitamin agar (Hayakawa & Nonomura, 1987) containing nalidixic acid (20 mg l⁻¹) and kabicidin (0.75 mg l⁻¹). Our isolates, designated VN05A0342, VN05A0351 and VN05A0415^T, were isolated after incubation for more than 10 days at room temperature.

Strains VN05A0342, VN05A0351 and VN05A0415^T were incubated on yeast extract-soluble starch medium (YS medium; 2 g yeast extract, 10 g soluble starch and 15 g agar l⁻¹; pH 7.3) at 28 °C for 10–14 days. The orange colonies that formed appeared moist and were raised, like

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene sequences of strains VN05A0415^T, VN05A0342 and VN05A0351 are AB377116, AB377118 and AB377119, respectively.

Cultural characteristics and fatty acid compositions of strains VN05A0342, VN05A0351 and VN05A0415^T and all of the type strains of *Kineosporia* species are available as supplementary material with the online version of this paper.

Fig. 1. Scanning electron micrograph of strain VN05A0415^T grown on water agar for 10 days at 28 °C. Bar, 5 μm.

'spore-dome actinomycetes' (Willoughby, 1969), from the surface of the YS medium. Aerial mycelium was absent. The spore-domes were formed by bunches of single spores borne on sporophores similar to those described by Itoh *et al.* (1989). Scanning electron microscopy showed that the spores were globular and/or ovoid (1.0–2.0 μm in diameter) with a smooth surface. The spores seemed to be enveloped in a club-shaped sporangium (Fig. 1), as reported by Pagani & Parenti (1978). Light-microscopic observation of cells suspended in phosphate buffer (pH 7.0, 1 mM) showed the spores to be motile. The cultural characteristics of our isolates and all type strains of *Kineosporia* species were observed on ISP media 2–7 (Shirling & Gottlieb, 1966) and YS medium after incubation at 28 °C for 3 weeks (see Supplementary Table S1, available in IJSEM Online). Our isolates and all type strains of *Kineosporia* species showed good growth on YS medium, ISP 2 and ISP 3. Only our isolates and the type strain of *Kineosporia aurantiaca* grew on ISP 6.

For the chemotaxonomic analysis, biomass from each strain was obtained by centrifugation and lyophilization after incubation in yeast extract-glucose broth (10 g yeast extract and 10 g glucose l⁻¹; pH 7.3) for 7–10 days at 28 °C. The whole-cell sugars, isoprenoid quinones, phospholipids and cellular fatty acids were analysed as described by Stanek & Roberts (1974), Minnikin *et al.* (1984) and Tamura *et al.* (1994). The A₂pm isomer in the peptidoglycan was analysed as described by Nozawa *et al.* (2007). Kudo *et al.* (1998) reported that *Kineosporia* strains exhibit heterogeneity of the A₂pm isomer because of the presence of different isomers in mycelium and spores. Our isolates, strains VN05A0342, VN05A0351 and VN05A0415^T, also contained both of the A₂pm isomers: a small amount of LL-A₂pm was present, but the main isomer was *meso*-A₂pm. The whole-cell sugars were ribose, mannose, galactose and glucose. The isoprenoid quinone was MK-9(H₄). Phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol were detected, but

phosphatidylethanolamine and phosphatidyl-*N*-methyl-ethanolamine were absent. The major cellular fatty acids were C_{18:1} and C_{16:0}, but iso- and/or anteiso-branched fatty acids were not detected (Supplementary Table S2). The chemotaxonomic data for our isolates were consistent with the characteristics described for the genus *Kineosporia*.

The DNA was extracted as described by Marmur (1961) and Saito & Miura (1963), but with a slight modification: after lysis, we used 20 % SDS and protease K to denature proteins, and phenol/chloroform/isoamyl alcohol (25:24:1, by vol.) to remove denatured proteins. 16S rRNA gene sequences were analysed as described by Tamura & Hatano (2001). Sequence analysis was performed with an ABI Prism BigDye Terminator cycle sequencing kit (PE Applied Biosystems) and an automatic DNA sequencer (model 3130 Genetic Analyzer; PE Applied Biosystems). The CLUSTAL_X program (Thompson *et al.*, 1997) was used to align the 16S rRNA gene sequences with corresponding sequences (available in the GenBank/EMBL/DBJ databases) from all of the type strains of *Kineosporia* species and some related actinomycetes of the suborder *Frankineae*. Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. The topology of the trees was evaluated by means of bootstrap analysis based on 1000 replicates (Felsenstein, 1985). DNA–DNA hybridization was carried out using the method of Ezaki *et al.* (1989). The G + C content of the DNA was determined using the method of Mesbah *et al.* (1989).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that our isolates and all type strains of the genus *Kineosporia* formed a monophyletic cluster (Fig. 2). The cluster had bootstrap support in both neighbour-joining

Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, for strains VN05A0342, VN05A0351 and VN05A0415^T, all type strains of *Kineosporia* species and some actinomycetes in suborder *Frankineae*. Numbers at branch points are confidence limits estimated by means of bootstrap analysis based on 1000 replicates; only values >500 are presented. Bar, 0.01 *K_{nuc}* in nucleotide sequences.

Table 1. DNA–DNA hybridization among strains VN05A0342, VN05A0351 and VN05A0415^T and all type strains of *Kineosporia* species

Source of unlabelled DNA	DNA–DNA relatedness (%) with labelled DNA from:							
	1	2	3	4	5	6	7	8
1. VN05A0342	100	92	108	13	19	23	23	30
2. VN05A0351	82	100	98	13	21	21	23	30
3. VN05A415 ^T	76	71	100	13	21	26	25	32
4. <i>K. aurantiaca</i> NBRC 14067 ^T	20	26	17	100	19	20	14	28
5. <i>K. rhamnosa</i> NBRC 16231 ^T	5	7	6	9	100	8	7	11
6. <i>K. succinea</i> NBRC 16232 ^T	28	40	21	16	22	100	19	23
7. <i>K. rhizophila</i> NBRC 16233 ^T	34	46	27	17	22	25	100	33
8. <i>K. mikuniensis</i> NBRC 16234 ^T	28	33	19	15	19	19	22	100

and maximum-parsimony phylogenetic trees. Although our isolates and the type strain of *Kineosporia rhizophila* formed a clade in the phylogenetic tree, the tree topology was not supported by bootstrapping analysis (52.2 %). Our isolates shared 16S rRNA gene sequence similarity of 99.8–100 %. The nucleotide sequence similarity between our isolates and all type strains of the genus *Kineosporia* ranged from 96.1 to 98.5 %. Our isolates showed the greatest similarity with respect to the type strain of *Kineosporia mikuniensis*.

DNA–DNA hybridization among our isolates and all type strains of *Kineosporia* species was determined (Table 1). The DNA relatedness among strains VN05A0342, VN05A0351 and VN05A0415^T ranged from 71 to 108 %. Consequently, our isolates were identified as representing a single species. The DNA relatedness between our isolates and all *Kineosporia* type strains was less than 46 %, being below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). Therefore, strains VN05A0342, VN05A0351 and VN05A0415^T were different from all type strains of *Kineosporia* species. The G+C contents of their DNAs were in the range 69–70 mol%.

Physiological and biochemical characteristics of our isolates were tested after incubation at 28 °C for 3 weeks. NaCl tolerance was examined on YS medium prepared with 0, 1, 2, 3, 4, 5 and 6 % NaCl (w/v). ISP 8 (Gordon & Mihm, 1957) was used to test for nitrate reduction. Decomposition of urea was determined on Christensen urea agar containing 2 % urea (Gordon *et al.*, 1974). Degradation of casein and other compounds (final concentration 0.5 %) was determined using nutrient agar as the basal medium (Gordon *et al.*, 1974). Aesculin hydrolysis and utilization of citrate were examined according to the methods of Gordon *et al.* (1974). The utilization of other carbohydrates was tested on yeast nitrogen base without amino acids (Bacto), as described by Goodfellow (1971). The utilization of organic acids was determined on a medium composed of (l⁻¹) 1.0 g NH₄NO₃, 1.0 g KH₂PO₄, 0.5 g MgSO₄·7H₂O and 0.2 g KCl (pH 7.2), containing 20 ml 0.04 % phenol red and

supplemented with 1 % organic salts or 0.2 % benzoic acid. An acid-production test was performed on basal medium composed of (l⁻¹) 10 g peptone and 5 g NaCl (pH 7.2) plus the test compound (1 %).

The features that served to differentiate strains VN05A0342, VN05A0351 and VN05A0415^T from known species of the genus *Kineosporia* were the decomposition of L-tyrosine and aesculin and the utilization of raffinose and D-arabitol (Table 2).

On the basis of the results of the polyphasic taxonomic study presented here, strains VN05A0342, VN05A0351 and VN05A0415^T represent a novel species of the genus *Kineosporia*, for which the name *Kineosporia babensis* sp. nov. is proposed.

Description of *Kineosporia babensis* sp. nov.

Kineosporia babensis (ba.ben'sis. N.L. fem. adj. *babensis* referring to Ba Be National Park, Vietnam, from which the first strains were isolated).

The orange-coloured colonies grow prolifically on YS medium and appear moist and raised. Each raised colony produces clusters of single spores. The spore surface is smooth and the spores are globular and/or ovoid (1.0–2.0 µm in diameter). Grows at 10–28 °C, but not at 5 or 37 °C. Grows in the presence of 3 % NaCl (w/v). Melanin is not produced on ISP 6 or ISP 7. Negative for nitrate reduction. Decomposes aesculin, arbutin, casein, testosterone, L-tyrosine and urea, but not adenine, hypoxanthine or xanthine. Utilizes carbon sources such as L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, maltose, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, starch, sucrose, trehalose and D-xylose, but not adonitol, D-arabitol, dulcitol, methyl α-D-glucoside, L-sorbose or xylitol. Utilizes organic acids such as fumarate, malate and succinate, but not benzoate, mucate, oxalate or L-tartrate. Produces acid from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, L-rhamnose, sucrose and D-xylose, but not from adonitol, i-erythritol, *myo*-inositol, melezitose and D-sorbitol. The cell wall contains major

Table 2. Differential physiological characteristics among strains VN05A0342, VN05A0351 and VN05A0415^T and type strains of all *Kineosporia* species

Strains: 1, VN05A415^T (strains VN05A0342 and VN05A0351 showed identical results unless indicated); 2, *K. aurantiaca* NBRC 14067^T; 3, *K. rhamnosa* NBRC 16231^T; 4, *K. succinea* NBRC 16232^T; 5, *K. rhizophila* NBRC 16233^T; 6, *K. mikuniensis* NBRC 16234^T. Data for reference type strains were taken from Kudo *et al.* (1998).

Strain	1	2	3	4	5	6
Decomposition of: L-Tyrosine	+	–	–	–	–	–
Aesculin	+	+	–	–	–	–
NaCl tolerance (% v/v)	4*	<3	<2	<5	<5	<1
Utilization of: Raffinose	+	–	±	–	+	–
D-Arabitol	–	+	–	+	+	+

*Strain VN05A0351 tolerated 3 % NaCl but not 4 %.

amounts of meso-A₂pm and small amounts of LL-A₂pm. The whole-cell sugars are ribose, mannose, galactose and glucose. The predominant menaquinone is MK-9(H₄). The phospholipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The major cellular fatty acids are C_{18:1} and C_{16:0}. The DNA G + C content is 69–70 mol%.

The type strain, VN05A0415^T (=VTCC-A-0961^T =NBRC 104154^T), was isolated from plant litter.

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