Lentzea kentuckyensis sp. nov., of equine origin

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A novel actinomycete, designated strain LDDC 2876-05^T, was isolated from an equine placenta during the course of routine diagnostic tests for nocardioform placentitis. In a preliminary study, the strain was observed to be phylogenetically distinct from the genera *Crossiella* and *Amycolatopsis* and probably a member of the genus *Lentzea*. A polyphasic study of strain LDDC 2876-05^T confirmed its identification as a member of *Lentzea* on the basis of its chemotaxonomic and morphological similarity to all of the known species of the genus. Moreover, the strain could be distinguished from other species with validly published names on the basis of its phylogenetic and physiological characteristics and its fatty acid profile. Therefore strain LDDC 2876-05^T represents a novel species of the genus *Lentzea*, for which the name *Lentzea kentuckyensis* sp. nov. is proposed. The type strain is LDDC 2876-05^T (=NRRL B-24416^T =DSM 44909^T).

Nocardioform placentitis is a distinctive type of placentitis in horses, in which lesions observed on the chorionic surface of the placenta are associated with Gram-positive, branching micro-organisms that can be recovered upon culture (Donahue & Williams, 2000; Giles *et al.*, 1993; Hong *et al.*, 1993). The actinomycetes isolated from these lesions are predominantly identified as either *Crossiella equi* (Donahue *et al.*, 2002) or various *Amycolatopsis* species (Labeda *et al.*, 2003), but representatives of other actinomycete genera have also been isolated. During the course of evaluating isolates from diagnostic studies of equine placentas during the 2005 breeding season, an actinomycete placental isolate, designated strain LDDC 2876-05^T, was obtained that did not belong to the genera *Crossiella* or *Amycolatopsis* according to preliminary molecular systematic identification. No gross or histopathological lesions were seen in the placenta, and it was believed that the isolate was a contaminant picked up from the mare's environment during the birthing process. The mare produced a live, healthy foal. A polyphasic study of this strain was undertaken to clarify its taxonomic identity.

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.

Genomic DNA for sequencing was isolated from cells grown on plates containing DSMZ medium no. 554, using

UltraClean microbial DNA isolation kits (Mo Bio Laboratories), amplified, sequenced according to previously described procedures (Labeda & Kroppenstedt, 2000) and then deposited in GenBank. This sequence was aligned, within ARB (Ludwig *et al.*, 2004), against those for taxa in the suborder *Pseudonocardineae*. The program PHYLO_WIN (Galtier *et al.*, 1996) was used to calculate evolutionary distances according to the method of Kimura (1980) and linkages according to the neighbour-joining method of Saitou & Nei (1987) and to perform maximum-parsimony and maximum-likelihood analyses. The topographies of the trees resulting from the neighbour-joining and maximum-parsimony analyses were evaluated by bootstrap analysis of the data on the basis of 500 resamplings (Felsenstein, 1989).

Genomic DNA for DNA relatedness determinations was isolated from biomass of *Lentzea albidocapillata* NRRL B-24057^T, *Lentzea waywayandensis* NRRL B-16159^T and strain LDDC 2876-05^T grown for 5 days with shaking at 28 °C on DSMZ medium no. 554 broth, using Power-Microbial Maxi DNA isolation kits (Mo Bio Laboratories). The purified DNA was then sheared to 300–500 bp by passage twice through a French pressure cell (Thermo Fisher Scientific) and levels of DNA relatedness were determined in 5 × SSC (1 × SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) supplemented with 20% DMSO as described by De Ley *et al.* (1970).

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

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain NRRL B-24416^T (=LDDC 2876-05^T) is DQ291145.

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

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

Phylogenetic analyses clearly demonstrated that strain LDDC 2876-05^T represents a species of the genus *Lentzea*, closely related to *Lentzea albida* and *L. waywayandensis* (Fig. 1). Tree topographies for the other algorithms evaluated were very similar. The levels of 16S rRNA gene sequence similarity with respect to the type strains of *L. albida*, *L. albidocapillata*, *Lentzea californiensis*, *Lentzea flaviverrucosa*, *Lentzea violacea* and *L. waywayandensis*, as determined using the GAP algorithm in GCG 11 (Accelrys), were 98.9, 97.0, 96.8, 96.9, 97.4 and 98.4 %, respectively. The levels of DNA relatedness between the phylogenetically nearest and most distant *Lentzea* type strains, *L. albidocapillata* NRRL B-24057^T and *L. waywayandensis* NRRL B-16159^T, were determined as 61 and 49 %, respectively. Further relatedness determinations were not considered necessary, because both of these values were below 70 % and therefore support the proposal that strain LDDC 2876-05^T represents a novel species of the genus *Lentzea*.

The morphological characteristics of strain LDDC 2876-05^T, namely the production of aerial mycelium that fragments into rod-shaped elements, are also typical of those of

the genus *Lentzea*. The strain grew well on all of the media evaluated and was observed to produce substrate mycelium that was yellow to deep yellow in colour; no soluble pigments were produced on any of the test media, except peptone-iron agar. White to yellowish-white aerial mycelium was produced on all of the growth media tested.

The cell wall of strain LDDC 2876-05^T was observed to contain *meso*-diaminopimelic acid, and the whole-cell sugars consisted of galactose and ribose. The polar lipids present were phosphatidylethanolamine, hydroxyphosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, traces of phosphatidylinositol mannosides and phosphatidylglycerol. Additionally, two unknown glycolipids were observed. The only menaquinone present was MK-9(H₄). As expected, mycolic acids could not be detected. This chemotaxonomic profile is consistent with assignment of strain LDDC 2876-05^T to the genus *Lentzea*. The fatty acid pattern mainly comprises iso/anteiso-branched fatty acids and small, but diagnostic, amounts of 2-hydroxy fatty acids. The detailed fatty acid profile for LDDC 2876-05^T, shown in Table 1, can be used to distinguish the strain from described species of this genus.

The physiological characteristics of strain LDDC 2876-05^T are summarized in the formal species description below and the physiological characteristics that serve to distinguish between the species of the genus *Lentzea* are shown in Table 2.

The chemotaxonomic, morphological and phylogenetic data clearly support the assignment of LDDC 2876-05^T to the genus *Lentzea*, and the physiological and phylogenetic data demonstrate that the strain can be differentiated from described species of the genus *Lentzea*. Therefore, strain LDDC 2876-05^T represents a novel species of the genus *Lentzea*, for which the name *Lentzea kentuckyensis* sp. nov. is proposed.

Fig. 1. Phylogenetic tree for strain NRRL B-24416^T (=LDDC 2876-05^T), members of the genus *Lentzea* and representative neighbouring taxa, calculated from 16S rRNA gene sequences using Kimura's evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987). Bar, 0.01 nucleotide substitutions per site.

Table 1. Fatty acid compositions (%) for strain LDDC 2876-05^T and type strains of *Lentzea* species

Strains: 1, LDDC 2876-05^T; 2, *L. albidocapillata* NRRL B-24057^T; 3, *L. albida* NRRL B-24073^T; 4, *L. californiensis* NRRL B-16137^T; 5, *L. flaviverrucosa* JCM 113873^T; 6, *L. violacea* IMSNU 50388^T; 7, *L. waywayandensis* NRRL B-16159^T. Data were obtained in this study except for *L. flaviverrucosa* (data from Xie *et al.*, 2002) and *L. violacea* (data from Lee *et al.*, 2000).

Fatty acid	1	2	3	4	5	6	7
13:0 iso	0.19	—	0.46	—	—	—	—
13:0 anteiso	0.13	—	—	—	—	—	—
13:0	—	—	0.44	—	—	—	—
14:0 iso	8.88	8.00	3.28	10.28	5.87	8.90	12.70
14:1 <i>cis</i> 9	—	—	—	—	—	0.21	—
14:0	0.52	0.56	1.91	1.85	1.55	1.27	—
15:1 iso	0.23	—	—	—	—	—	—
15:0 iso	10.07	3.58	14.45	9.90	5.63	6.15	6.55
15:0 anteiso	8.20	2.57	11.33	16.21	6.25	3.79	4.26
15:1 <i>cis</i> 9	0.79	1.24	1.38	—	0.84	1.47	0.93
15:0	1.01	0.75	7.00	2.21	2.53	1.00	0.58
16:1 iso	3.83	9.27	0.62	—	1.85	4.05	10.07
16:0 iso	47.40	55.63	19.29	23.47	31.97	45.03	45.60
16:1 <i>cis</i> 9	4.64	9.66	8.42	8.70	16.26	15.52	8.87
15:0 anteiso	—	—	1.12	—	—	—	—
2-OH							
16:0	1.9	2.21	9.91	17.08	12.01	4.01	2.07
16:0 10-methyl	—	3.04	—	4.14	5.48	1.40	4.10
17:1 iso	1.39	—	1.12	—	—	—	—
17:1 anteiso	0.68	—	—	—	—	—	—
17:0 iso	1.21	—	2.22	—	0.77	0.58	—
17:0 anteiso	4.61	0.97	5.64	4.01	2.32	1.18	1.42
17:1 <i>cis</i> 9	1.95	1.57	4.14	—	2.09	1.87	1.48
17:1 <i>cis</i> 11	1.03	—	—	—	—	2.49	—
16:0 iso 2-OH	—	0.95	0.63	—	1.08	—	1.35
17:0	0.65	—	4.75	2.16	1.62	0.39	—
17:0 10-methyl	0.18	—	—	—	—	—	—
18:0 iso	0.34	—	—	—	—	—	—
18:1 <i>cis</i> 9	0.17	—	0.75	—	1.03	0.69	—
18:0	—	—	1.05	—	0.84	—	—

Description of *Lentzea kentuckyensis* sp. nov.

Lentzea kentuckyensis (ken.tuc.ky.en'sis. N.L. fem. adj. *kentuckyensis* from Kentucky, named after the place of origin of the type strain, the state of Kentucky, USA).

Yellow to strong-yellow substrate mycelium is produced on most media. Aerial mycelium ranging in colour from white to yellowish white is produced on most media. A faint-brown, soluble pigment is produced on some media. Micromorphological and chemotaxonomic characteristics are typical for the genus *Lentzea*. Casein, aesculin, gelatin, hypoxanthine, tyrosine, urea and xanthine are hydrolysed or decomposed. Adenine and allantoin are not hydrolysed or decomposed. Nitrate is not reduced. Phosphatase is not produced. Malate, oxalate, and succinate are assimilated; citrate and lactate are assimilated weakly. Acetate,

Table 2. Differential physiological properties of strain LDDC 2876-05^T and type strains of *Lentzea* species

Strains: 1, LDDC 2876-05^T; 2, *L. albidocapillata* NRRL B-24057^T; 3, *L. albida* NRRL B-24073^T; 4, *L. californiensis* NRRL B-16137^T; 5, *L. flaviverrucosa* JCM 113873^T; 6, *L. violacea* IMSNU 50388^T; 7, *L. waywayandensis* NRRL B-16159^T. Data obtained in this study except for *L. flaviverrucosa* (data from Xie *et al.*, 2002) and *L. violacea* (data from Lee *et al.*, 2000). +, Positive; w, weakly positive; —, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Hydrolysis of:							
Casein	+	+	+	+	—	+	+
Urea	+	w	w	+	—	+	+
Nitrate reductase	—	—	—	+	+	—	+
Assimilation of:							
Acetate	—	—	+	+	+	+	+
Citrate	w	—	+	+	ND	—	+
Lactate	w	—	—	—	—	+	+
Malate	+	+	+	+	+	—	+
Acid from:							
Adonitol	—	+	+	—	—	—	+
Cellobiose	+	+	+	+	+	—	+
Inositol	+	+	+	+	+	—	+
Maltose	+	+	+	+	+	—	+
Mannitol	+	+	+	+	+	—	+
Raffinose	+	w	—	+	+	+	+
Rhamnose	+	+	+	+	—	—	+
Sucrose	+	+	+	+	+	—	+
Trehalose	+	+	+	+	—	—	+
Xylose	+	+	+	+	—	—	+
Growth at:							
10 °C	+	+	—	+	ND	+	+
37 °C	+	+	+	+	+	+	w
42 °C	—	—	+	—	+	—	—
45 °C	—	—	+	—	—	—	—

benzoate, mucate, propionate and DL-tartrate are not assimilated. Acid is produced from arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, mannitol, D-mannose, melibiose, raffinose, rhamnose, salicin, sucrose and xylose. Acid is produced weakly from methyl α -D-glucoside and D-sorbitol. Acid is not produced from adonitol, dulcitol, *meso*-erythritol, melezitose or methyl β -xyloside. Growth occurs in the presence of NaCl concentrations up to 7% (w/v). Temperature range for growth is 10–37 °C.

The type strain, LDDC 2876-05^T (=NRRL B-24416^T =DSM 44909^T), was isolated from an equine placenta in Lexington, KY, USA.

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use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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