Final classification of Bisgaard taxon 9 as *Actinobacillus arthritidis* sp. nov. and recognition of a novel genomospecies for equine strains of *Actinobacillus lignieresii*

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Phenotypic characterization of bacteria from diseased and healthy horses identified 18 isolates as Bisgaard taxon 9 and 11 isolates as *Actinobacillus lignieresii*. All strains of taxon 9 were α -galactosidase- and raffinose-positive and showed variable fermentation of (+)L-arabinose and (–)D-sorbitol. Strains of *A. lignieresii* were negative for these characteristics, with the exception of raffinose. Two strains from the (–)D-sorbitol-negative group of taxon 9 showed a 16S rRNA similarity of 99.6%, while 99.5% similarity was found between two strains of the (–)D-sorbitol-positive group. DNA–DNA hybridization between the two strains representing the (–)D-sorbitol-negative group showed 98% binding, and their closest relationship was to a strain of *A. lignieresii* (64%). The two strains of the (–)D-sorbitol-positive group showed 83% binding and were related to the (–)D-sorbitol-negative group at a 76% DNA binding level. *Actinobacillus arthritidis* sp. nov. is proposed for 12 strains of the (–)D-sorbitol-positive group. *Actinobacillus* genomospecies 2 is suggested for the six strains of the (–)D-sorbitol-negative group. Phenotypically, strains of *A. arthritidis* and *Actinobacillus* genomospecies 2 differ in (–)D-sorbitol fermentation and can be separated from *Actinobacillus equuli* by being trehalose-negative, while a positive reaction for α -galactosidase separates the taxa from *A. lignieresii*. The type strain of *A. arthritidis*, CCUG 24862^T, was isolated from a joint of a horse. Three equine isolates of *A. lignieresii* that could not be separated from the type strain by means of phenotypic characteristics showed 98.6–100% 16S rRNA similarity, but only 96.4–96.7% similarity to the type strain. DNA–DNA hybridization between two strains of this group showed 92% binding but only 70% binding to the type strain of *A. lignieresii*. Consequently, these equine isolates of *A. lignieresii* represent a new genomospecies of *Actinobacillus*, suggested as genomospecies 1 because phenotypic characteristics are not presently available to separate it from the type strain of *A. lignieresii*.

Keywords: *Actinobacillus arthritidis*, *Actinobacillus lignieresii*, Bisgaard taxon 9, genomospecies, horse

INTRODUCTION

The redefinition of *Actinobacillus sensu stricto* has been discussed recently (Bisgaard, 1995). Mutters *et al.*

A table showing the DNA–DNA binding matrix is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

The GenBank accession numbers for the 16S rDNA sequences of strains CCUG 24862^T, F 141, H 4272/4, F 127, F 264 and 13/225/95 are respectively AF247712, AF247717, AF247719, AF247722, AF247723 and AY029174.

(1989) suggested that the genus be limited to include *Actinobacillus lignieresii*, *Actinobacillus pleuropneumoniae*, *Actinobacillus equuli*, *Actinobacillus capsulatus*, *Actinobacillus suis*, *Actinobacillus ureae*, *Actinobacillus hominis* and Bisgaard's taxa 5, 9 and 11. With the exception of *A. capsulatus* and Bisgaard's taxa 5 and 9, this was confirmed by 16S rRNA sequencing (Dewhirst *et al.*, 1993). For the present study, *Actinobacillus sensu stricto* has been restricted to include *A. equuli*, *A. hominis*, *A. lignieresii*, *A. pleuropneumoniae*, *A. suis*, *A.*

ureae and Bisgaard's taxa 8, 9 and 11, excluding *A. capsulatus*, *Actinobacillus delphinicola*, *Actinobacillus indolicus*, *Actinobacillus minor*, *Actinobacillus muris*, *Actinobacillus porcinus*, *Actinobacillus scotiae*, *Actinobacillus seminis* and Bisgaard taxon 5, since these taxa do not form a consistent monophyletic group with the first nine taxa on the basis of 16S rRNA sequence comparison (Dewhirst *et al.*, 1993; Foster *et al.*, 1996, 1998).

Among the true species of *Actinobacillus*, *A. lignieresii*, *A. equuli* and Bisgaard's taxa 9 and 11 have been isolated from horses (Bisgaard, 1993). Other equine taxa belonging to *Actinobacillus* may exist (Blackall *et al.*, 1997; Sternberg & Brändström, 1999). These organisms are of clinical importance to both the veterinary and medical professions, since they may cause disease in horses and may be found in infected wounds of humans bitten by horses (Dibb *et al.*, 1981; Peel *et al.*, 1991; Ashhurst-Smith *et al.*, 1998).

Equine strains of organisms described previously as *A. suis*, or haemolytic variants of *A. equuli*, were classified as taxon 11 by Bisgaard *et al.* (1984). Subsequent investigations have confirmed the existence of taxon 11 and have shown that haemolytic strains of so-called *A. lignieresii* in horses have a whole-cell PAGE profile that is different from that of the bovine reference strain (Jang *et al.*, 1987; Samitz & Biberstein, 1991). Among the horses examined by Ward *et al.* (1998), lower-airway inflammation was associated significantly with taxon 11-like bacteria and *A. lignieresii*. 16S rRNA sequencing and DNA–DNA hybridizations have recently shown that *A. equuli* and Bisgaard taxon 11 represent two genotypes with different disease patterns and epidemiology (Christensen *et al.*, 2002).

A single horse-joint isolate (Wetmore 1706^T = CCUG 24862^T) received as *A. equuli* and identified phenotypically as *A. equuli* unexpectedly remained unclassified by DNA–DNA hybridization according to Escande *et al.* (1984). This strain was subsequently found to be identical to organisms obtained from the mucous membrane of the oropharynx in apparently normal horses in Denmark and tentatively designated as taxon 9, since they could be separated from *A. equuli sensu stricto* by means of differences in trehalose fermentation (Bisgaard, 1993). Taxon 9 has been obtained from diseased horses in Australia (Blackall *et al.*, 1997), Zimbabwe and Sweden (M. Bisgaard, unpublished).

A. lignieresii is normally associated in ruminants with pyogranulomatous lesions of the tissues of the upper alimentary tract and its draining lymph nodes (Biberstein, 1981). In addition, *A. lignieresii* can be obtained from the oropharyngeal mucosa and rumens of these animals (Bisgaard *et al.*, 1986). In horses, *A. lignieresii* has been isolated from an epidural abscess (Chladek & Ruth, 1976), an intermandibular phlegmon (Zaharija *et al.*, 1979), an enlarged tongue (Baum *et al.*, 1984) and in cases of stomatitis (Bisgaard, 1993). These

organisms can also be obtained from the cavum oris in apparently normal horses (Bisgaard, 1993). Although phenotypic variations have been reported for *A. lignieresii*, all bovine strains investigated fell into a single DNA hybridization group (Escande *et al.*, 1984). However, genetic investigations published so far have not included equine strains of *A. lignieresii*.

The aim of the present study, therefore, was to investigate the phylogenetic relationship between the bovine and equine isolates of *A. lignieresii* and to define the genetic borders of Bisgaard taxon 9 in order to be able to name this taxon.

METHODS

Bacterial strains and phenotypic characterization. The selection of strains (Table 1) was based on previous investigations (Dibb *et al.*, 1981; Peel *et al.*, 1991; Blackall *et al.*, 1997, 1998) and strains maintained in our laboratory. Phenotypic characterization was performed as reported previously (Bisgaard *et al.*, 1991; Angen *et al.*, 1997). For further genotypic characterization, four strains of Bisgaard taxon 9 were selected to represent differences in (+)L-arabinose, (–)D-sorbitol, maltose and (+)D-melibiose fermentation, source of isolation and geographical location. Three strains of *A. lignieresii* were selected to represent horse-associated isolates as well as isolates from horse bites of humans.

Serotyping. Equine isolates of *A. lignieresii* were serotyped using rabbit antisera raised against reference serotypes of *A. pleuropneumoniae*, using a latex agglutination test (Giese *et al.*, 1993).

Sequencing of 16S rRNA genes. Bacteria were cultured overnight in brain–heart infusion broth (Difco) at 37 °C. One ml culture was harvested by centrifugation and DNA extraction was performed according to Leisner *et al.* (1999). PCR amplification was performed as described by Vogel *et al.* (1997). Oligonucleotides for both PCR amplification and sequencing were synthesized according to the sequences and 16S rRNA positions given by Paster & Dewhirst (1988) and Dewhirst *et al.* (1989). PCR-amplified fragments were purified in Microspin columns (Amersham Pharmacia Biotech) and then cycle-sequenced (Thermo Sequenase fluorescent labelled primer cycle sequencing kit; Amersham Pharmacia Biotech) in an ALF sequencer (Amersham Pharmacia Biotech), using fluorescein-labelled primers.

Analysis of sequence data. Searches for 16S rRNA sequences were performed with FASTA and BLAST, using the Wisconsin sequence analysis package (Genetics Computer Group). Sequences were aligned and numbered according to the *Escherichia coli* 16S rRNA *rrnB* sequence. The region covering positions 28–1480 (*E. coli* numbering of *rrnB*) was aligned and 1374 positions remained after the removal of ambiguous positions (95 distinct data patterns were analysed). Maximum-likelihood analysis was performed by using fastDNAmI including bootstrap analysis (Felsenstein, 1981; Olsen *et al.*, 1994). Parsimony and neighbour-joining analyses were performed using PHYLIP (Felsenstein, 1995).

DNA–DNA hybridization. DNA similarities were determined according to the micro-well method (Christensen *et al.*, 2000).

Table 1. Strains investigated in this study

Strain	Source	Country	Reference
<i>Actinobacillus arthritidis</i> sp. nov. (taxon 9)			
CCUG 24862 ^T *	Horse, joint	Czech Republic	Escande <i>et al.</i> (1984)
(= Wetmore 1706 ^T = ATCC 13376 ^T = P 907 ^T)			
F 129	Horse, cavum oris	Denmark	
F 185	Horse, cavum oris	Denmark	
F 268	Horse, cavum oris	Denmark	
F 453	Horse, cavum oris	Denmark	
G 6562/4	Horse lung, septicaemia	Australia	Blackall <i>et al.</i> (1997, 1998)
I 5445/5	Horse brain, septicaemia	Australia	
13/225/95*	Horse, joint	Zimbabwe	Mohan <i>et al.</i> (1997)
KLM 91–229	Foal, sepsis	Sweden	M. Bisgaard (unpublished)
KLM 96–448	Foal, sepsis	Sweden	M. Bisgaard (unpublished)
CEH 14–99	Horse, oral cavity	Sweden	M. Bisgaard (unpublished)
Bd 937–94	Diseased foal	Sweden	M. Bisgaard (unpublished)
<i>Actinobacillus genomospecies 2</i> (taxon 9)			
F 141 (= CCUG 15571)*	Horse, cavum oris	Denmark	
H 4272/4*	Horse, spleen	Australia	Blackall <i>et al.</i> (1997, 1998)
KLM 96–146	Horse	Sweden	M. Bisgaard (unpublished)
F 124	Horse, cavum oris	Denmark	
F 192	Horse, cavum oris	Denmark	
F 193	Horse, cavum oris	Denmark	
<i>Actinobacillus lignieresii</i>			
NCTC 4189 ^T	Bovine, glands	UK	
<i>Actinobacillus genomospecies 1</i>			
F 126	Horse, cavum oris	Denmark	
F 127 (= CCUG 22229)*	Horse, cavum oris	Denmark	
F 128	Horse, cavum oris	Denmark	
F 167	Horse, cavum oris	Denmark	
F 258 (= CCUG 22230)	Horse, cavum oris	Denmark	
F 264 (= CCUG 22231)*	Horse, cavum oris	Denmark	
F 429	Horse, stomatitis	Denmark	
C 5309b	Horse, stomatitis	Denmark	
F 414 (= 6744/73)	Horse bite	Norway	Dibb <i>et al.</i> (1981)
T 354/87	Horse bite	Australia	Peel <i>et al.</i> (1991)
SSI P 1293*	Horse bite (finger)	Denmark	

* 16S rRNA sequence determined.

RESULTS AND DISCUSSION

Phenotypic characterization

All strains of Bisgaard taxon 9 and *A. lignieresii* were found to be positive in the following tests: catalase, oxidase, fermentative reaction in Hugh–Leifson medium with (+)D-glucose, porphyrin test, nitrate reduction, urease, alanine aminopeptidase, phosphatase, ONPG and production of acid from (–)D-ribose, (+)D-xylose, (–)D-mannitol, (–)D-fructose, (+)D-galactose, (+)D-glucose, (+)D-mannose, lactose and sucrose.

Gram-staining was negative and negative results were obtained in the following tests: motility at 22 and 37 °C, symbiotic growth, β-haemolysis on bovine blood agar, Simmons citrate, mucate-acid, malonate-base, H₂S/tri-sugar iron (TSI), growth in the presence of KCN, methyl red, Voges–Proskauer at 37 °C,

production of gas from nitrate, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, indole production, gelatinase, hydrolysis of Tweens 20 and 80, pigment production, α-glucosidase (*p*-nitrophenyl α-D-glucopyranoside; PNPG), β-glucosidase (*p*-nitrophenyl β-D-glucopyranoside; NPG), α-fucosidase (*o*-nitrophenyl α-L-fucopyranoside; ONPF), α-glucuronidase (*p*-nitrophenyl β-D-glucopyranosiduronic acid; PGUA), α-mannosidase, production of acid from *m*-erythritol, adonitol, (+)D-arabitol, xylitol, (–)L-xylose, dulcitol, *m*-inositol, (+)D-fucose, (+)L-rhamnose, (–)L-sorbose, cellobiose, trehalose, (+)D-melezitose, (+)D-glycogen, inulin, aesculin, amygdalin, arbutin, gentiobiose, salicin, (+)D-turanose and β-*N*-CH₃-glucosamid and formation of gas from (+)D-glucose.

Characteristics that separate the taxa investigated are shown in Table 2. All strains identified as taxon 9 gave

Table 2. Variable phenotypic characteristics of the equine isolates investigated

+, All strains positive within 1–2 days; –, all strains negative after 14 days; (+), all strains positive within 3–14 days; d, some strains positive; w, weakly positive. ONPX, *o*-Nitrophenyl β -D-xylanopyranoside.

Characteristic	<i>A. lignieresii</i>		Bisgaard taxon 9		
	NCTC 4189 ^T	11 isolates	<i>Actinobacillus</i> genomospecies 2 (6 isolates)	<i>A. arthritidis</i> sp. nov.	
				CCUG 24862 ^T	11 isolates
Growth on MacConkey's agar	–	d	d	–	d
Fermentation of:					
Glycerol	w	+/(+)	d	(+)	d
(+)-L-Arabinose	–	–	–	–	d
(–)-D-Arabinose	–	(+)	d	–	d
(–)-D-Sorbitol	–	–	–	+	+
(–)-L-Fucose	+	d	d	–	d
Maltose	+	+/(+)	d	+	+/(+)
(+)-D-Melibiose	–	–	d	(+)	+/(+)
Raffinose	–	(+)	+	+	+
Dextrin	+	+	+/(+)	+	d
α -Galactosidase	–	–	+	+	+
β -Xylosidase (ONPX)	–	–	d	–	–

positive results in the α -galactosidase and raffinose tests. With the exception of H 4272/4, these strains were also positive in the (+)-D-melibiose test, and only two strains were found to be negative in the maltose test. Among the (–)-D-sorbitol-positive strains of taxon 9, some strains were (+)-L-arabinose-positive, while all strains of *A. lignieresii* were negative for the characteristics listed in Table 2 with the exception of raffinose and maltose.

Serotyping

The three *A. lignieresii* strains F 127, SSI P 1293 and F 264 were typable as serotype 3 by using reference sera for *A. pleuropneumoniae*, while the other equine strains of *A. lignieresii* were untypable by this method.

16S rRNA sequence comparison

For the four strains of taxon 9 investigated (Table 1), the sequences from *E. coli* *rrnB* positions 29–1481 were obtained, with lengths of 1461 nt. The 16S rRNA sequence of strain CCUG 24862^T was found to be 100% similar to the published sequence (accession no. L06084). For strains F 127 and F 264 of *A. lignieresii*, the sequences at positions 17–1507 and 28–1500 were obtained with respective lengths of 1489 and 1470 nt. The sequence of strain SSI P 1293 was identical to that of strain F 127, and only the latter strain was included in the analysis.

The phylogenetic tree (Fig. 1) is based on the seven sequences determined in the present study, seven

unpublished sequences of Bisgaard taxon 11 and *A. equuli* (deposited with GenBank) and published sequences of genuine actinobacilli. The sequence with accession number M75073 was left out, since it was found previously not to represent the strain reported (Hedegaard *et al.*, 2001). The tree was found to be highly consistent across the different methods used for phylogenetic reconstruction. Of the 19 nodes of the maximum-likelihood tree, 17, 11 and 10 nodes were also supported by parsimony, neighbour-joining and by bootstrap values higher than 50%, respectively. *A. lignieresii*, the type species of the genus, and *A. pleuropneumoniae* were closely related and more distantly related to taxon 8. This group did not contain equine strains. Two strains of taxon 9 representing the (–)-D-sorbitol-negative group (F 141 and H 4272/4) were closely related, having 99.5% 16S rRNA sequence similarity. A high degree of consistency was found for the node joining these strains and *A. hominis*. Strains F 141 and H 4272/4 were related to the type strain of *A. hominis*, showing 98.2–98.5% similarity. The similarity between strains CCUG 24862^T and 13/225/95 of the (–)-D-sorbitol-positive group was 99.6%, and these strains were related to the (–)-D-sorbitol-negative group, showing 97.7–97.9% similarity. The two strains of the (–)-D-sorbitol-positive group were related to *A. ureae*, having 98.0–98.3% similarity and a common node in the phylogenetic analysis.

The similarity between strains F 127 and F 264 of *A. lignieresii* was 98.5%. Surprisingly, a high degree of divergence was found between strains F 127, SSI P 1293 and F 264 of *A. lignieresii* and the type strain of

Fig. 1. Phylogenetic relationships between strains of selected taxa associated with horses and published sequences of genuine actinobacilli, with *Actinobacillus minor* included as an outgroup, based on maximum-likelihood analysis of 16S rRNA sequences. Significances of specific nodes obtained in bootstrap analysis are indicated for values higher than 50%. Nodes supported in phylogenetic trees obtained by neighbour-joining and parsimony methods are indicated by + and *, respectively. Strains sequenced in the present study are shown in bold. bv., Biovar.

this species, there being only 96.4–96.7% similarity. The highest similarities observed between strains F 127, SSI P 1293 and F 264 and the strains of *Actinobacillus* investigated were 98.6–98.8%, to strains F 92, F 154 and T 355/87 of Bisgaard taxon 11. The lowest similarities observed between strains F 127, SSI P 1293 and F 264 and the strains of *Actinobacillus* investigated were 96.0–96.2%, to the type strain of *A. pleuropneumoniae*, strain CCUG 16494 of Bisgaard taxon 8 and strains CCUG 24862^T and 13/225/95 of Bisgaard taxon 9.

Comparison of 16S rRNA sequences showed that equine actinobacilli tentatively classified as *A. lignieresii* and taxon 9 of Bisgaard were located in three novel groups among the genuine actinobacilli. These organisms were formerly included in cluster 4A and partly in cluster 4B by Dewhirst *et al.* (1993). According to

Mutters *et al.* (1989), genuine actinobacilli are defined as including *A. equuli*, *A. hominis*, *A. lignieresii*, *A. pleuropneumoniae*, *A. suis*, *A. ureae* and taxa 9 and 11 of Bisgaard, and 16S rRNA sequence data include Bisgaard taxon 8 but exclude *A. capsulatus*, *A. minor* and Bisgaard taxon 5, as these taxa do not form a consistent monophyletic group with the first eight taxa (Dewhirst *et al.*, 1993; Foster *et al.*, 1996, 1998).

The mean 16S rRNA sequence difference between type strains of species belonging to the genera *Pasteurella* and *Actinobacillus*, as defined by DNA–DNA hybridizations, is respectively 7.0 and 5.0% (Angen *et al.*, 1999). The mean sequence difference between a representative from each of the five 16S rRNA clusters of the newly proposed genus *Mannheimia* was 3.4%. The exclusion of *A. capsulatus* and Bisgaard taxon 5 from the genus *Actinobacillus*, as defined by DNA–DNA hybridization (Mutters *et al.*, 1989), and of *A. minor*, on the basis of 16S rRNA analysis (Foster *et al.*, 1998), would reduce the mean sequence difference between species of *Actinobacillus* to 1.7%. Redefinition of the genus *Pasteurella* to include only cluster 3B of Dewhirst *et al.* (1993), and the formation of a new genus to include the avian taxa of cluster 3A, would result in mean sequence differences for these tentative new genera (respectively 2.9 and 1.7%) that are at approximately the same level as for *Actinobacillus*. The mean variation between 16S rRNA sequences of genuine actinobacilli increases to 2.3% with inclusion of the three taxa described in the present study. The variations between 16S rRNA sequences within species of genuine actinobacilli would then vary from 0.3% (type strains of *A. pleuropneumoniae* and *A. lignieresii*) to 4.0% (strain 13/225/95 of Bisgaard taxon 9 and strain F 127 of *A. lignieresii*). Low variation has also been reported for other species (Vandamme *et al.*, 1996), and no definitive limits for the separation of species on the basis of 16S rRNA sequence variation can be drawn (Stackebrandt & Goebel, 1994; Vandamme *et al.*, 1996).

DNA–DNA hybridization

DNA–DNA hybridization between the two strains representing the (–)D-sorbitol-negative group of taxon 9 showed 98% binding; the closest relationship (64%) was to a strain of *A. lignieresii*. A full DNA-binding matrix is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>). The two strains of the (–)D-sorbitol-positive group of taxon 9 showed 83% binding and were related to the (–)D-sorbitol-negative group (72–76% DNA binding). DNA–DNA hybridization between the two equine strains of *A. lignieresii* showed 92% binding, but there was only 70–72% binding to the type strain of *A. lignieresii* (see supplementary material). Consequently, these equine isolates of *A. lignieresii* represent a novel genomospecies of *Actinobacillus*, since phenotypic characteristics are not presently available to separate them from the type strain of *A. lignieresii*.

Species identification and separation is based on DNA–DNA hybridizations and sufficient phenotypic variation to allow identification by biochemical characterization (Vandamme *et al.*, 1996). The final decision about the classification of *Actinobacillus sensu stricto* must await further DNA–DNA hybridization experiments. For the same reason, precise limits of DNA binding for separation of *Actinobacillus* species cannot be given at present. The results of our study have shown a limit of around 80%, which is slightly lower than that for the genus *Mannheimia* and for other species of the family, for which a limit of 85% has been set (Angen *et al.*, 1999; Muters *et al.*, 1989).

In the present work, it has been shown that Bisgaard taxon 9 represents two novel species, supported by DNA–DNA hybridization. The 16S rRNA sequence comparison showed the (–)D-sorbitol-positive group to be related to *A. ureae*; however, DNA binding between strain CCUG 24862^T of the (–)D-sorbitol-positive group and the type strain of *A. ureae* was only 44% (Escande *et al.*, 1984), indicating that the close relationship obtained by the phylogenetic analysis of 16S rRNA sequences was not reflected in the level of total DNA similarity. Further DNA–DNA hybridization experiments were not performed because of the low degree of DNA binding to the taxa most closely related in terms of 16S rRNA sequence. The (–)D-sorbitol-negative group was related to *A. hominis* in the 16S rRNA sequence comparison; however, DNA–DNA binding between the type strain of *A. hominis* and strain CCUG 15571 of Bisgaard taxon 9 has recently been reported to be only 50% (Friis-Møller *et al.*, 2001).

The name *Actinobacillus arthritidis* sp. nov. is proposed in the final classification of Bisgaard taxon 9. *A. arthritidis* is positive for fermentation of (–)D-sorbitol, while *Actinobacillus* genomospecies 2 is negative. Characteristics exist for the phenotypic separation of *Actinobacillus* genomospecies 2 from other phenotypically related species of actinobacilli. For example, *A. arthritidis* and *A. lignieresii* are trehalose-negative, while *A. equuli* and taxon 11 are positive. *A. arthritidis*, *A. equuli* and taxon 11 are α -galactosidase-positive, while *A. lignieresii* is negative. Most strains of *A. arthritidis* are (–)L-fucose-positive, like *A. lignieresii*, while *A. equuli* and taxon 11 are (–)L-fucose-negative. Finally, most strains of the novel species are (+)D-melibiose-positive, like *A. equuli* and taxon 11, while all strains of *A. lignieresii* are (+)D-melibiose-negative (see Table 2 and Bisgaard *et al.*, 1984).

Three equine strains of *A. lignieresii* were serotyped as *A. pleuropneumoniae* serotype 3. This could have been related to the high similarity between these two species, since *A. lignieresii* can only be separated phenotypically from *A. pleuropneumoniae* biovar 2 by being non-haemolytic. However, the 16S rRNA sequences of these strains show low similarities to the type strains of *A. lignieresii* and *A. pleuropneumoniae*, and it is concluded that serotyping is not a reliable method for

the separation of these species. These equine isolates of *A. lignieresii* represent a novel species with respect to both 16S rRNA sequence data and DNA binding; however, phenotypic characteristics needed for identification were not found. For this reason, we refrain from naming this taxon. The low degree of 16S rRNA sequence similarity between bovine and equine strains of *A. lignieresii* was also reflected by DNA–DNA hybridization. Earlier investigations have shown 95% DNA binding between two bovine strains of *A. lignieresii* (Borr *et al.*, 1991; Pohl *et al.*, 1983), while only 70% DNA binding was found between an equine isolate and a bovine isolate in the present study.

Description of *Actinobacillus arthritidis* sp. nov.

Actinobacillus arthritidis (ar.thri'ti.dis. N.L. fem. gen. n. *arthritidis* of arthritis, from Gr. n. *árrhron* joint; L. suffix *-itis* used in names of inflammations).

The species is positive in the following tests: catalase, oxidase, fermentative reaction in Hugh–Leifson medium with (+)D-glucose, porphyrin test, nitrate reduction, urease, alanine aminopeptidase, phosphatase, ONPG, α -galactosidase and production of acid from (–)D-ribose, (+)D-xylose, (–)D-mannitol, (–)D-sorbitol, (–)D-fructose, (+)D-galactose, (+)D-glucose, (+)D-mannose, lactose, sucrose and raffinose. Negative results are obtained in the following tests: Gram-staining (pleomorphic without distinctive morphology), motility at 22 and 37 °C, symbiotic growth, β -haemolysis, Simmons citrate, mucate-acid, malonate-base, H₂S/TSI, growth in the presence of KCN, methyl red and Voges–Proskauer at 37 °C, production of gas from nitrate, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, indole production, gelatinase, hydrolysis of Tweens 20 and 80, pigment production, α -glucosidase (PNPG), β -glucosidase (NPG), α -fucosidase (ONPF), α -glucuronidase (PGUA), α -mannosidase, β -xylosidase, production of acid from *m*-erythritol, adonitol, (+)D-arabitol, xylitol, (–)L-xylose, dulcitol, *m*-inositol, (+)D-fucose, (+)L-rhamnose, (–)L-sorbose, cellobiose, trehalose, (+)D-melezitose, (+)D-glycogen, inulin, aesculin, amygdalin, arbutin, gentiobiose, salicin, (+)D-turanose and β -N-CH₃-glucosamid and formation of gas from (+)D-glucose. Characteristics that show variation are listed in Table 2. On bovine-blood agar, the colonies are non-haemolytic, circular, raised and regular with entire margins. The surface of the colonies is generally smooth, shiny, greyish and non-transparent. Mucoid or watery cultures also exist. A diameter of 2–3 mm is often observed after 24 h aerobic incubation at 37 °C. Strains have been isolated from the oral cavities of apparently healthy horses, as well as from diseased foals or horses (mainly those with septicaemia and arthritis) (Table 1).

The type strain, CCUG 24862^T (= ATCC 13376^T = Wetmore 1706^T), was isolated from a joint of a horse.

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