Emended description of porcine [*Pasteurella*] *aerogenes*, [*Pasteurella*] *mairii* and [*Actinobacillus*] *rossii*

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The aim of this study was to improve the definition and identification of a group of veterinarily important bacteria referred to as the [*Pasteurella*] *aerogenes*–[*Pasteurella*] *mairii*–[*Actinobacillus*] *rossii* complex. These organisms have mainly been isolated from the reproductive and intestinal tracts of pigs and in most cases have been considered as opportunistic pathogens. A collection of 87 strains were characterized by phenotypic analysis from which 41 strains were selected for 16S rRNA gene sequence comparison, out of which 23 have been sequenced in the present study. One group of 21 strains phenotyped as biovars 1, 3–5, 9–11, 19 and 25–27, including the type strain of [*P.*] *aerogenes*, showed 16S rRNA gene sequence similarities of 99.6 % or higher; another group of 18 strains including biovars 2, 6–8, 12–15, 21, 23, 24 and 26A and the type strain of [*A.*] *rossii* showed 97.8 % or higher 16S rRNA gene sequence similarity. Between the two groups, 93.8–95.7 % 16S rRNA gene sequence similarity was observed. Strains of [*P.*] *mairii* showed 99.5 % similarity, with 95.5–97.2 and 93.8–95.5 % similarity to strains of [*P.*] *aerogenes* and [*A.*] *rossii*, respectively. Four strains could not be classified with any of these groups and belonged to other members of *Pasteurellaceae*. Comparisons were also made to DNA–DNA hybridization data. Biovars 1, 9, 10, 11 and 19, including the type strain of [*P.*] *aerogenes*, linked at 70 % DNA reassociation, whereas strains identified as biovars 2, 6, 7, 8, 12, 15 and 21 of [*P.*] *aerogenes* linked at 81 %. The latter group most likely represents [*A.*] *rossii* based on the 16S rRNA gene sequence comparisons. DNA reassociation between the [*P.*] *aerogenes* and [*A.*] *rossii* groups was at most 37 %, whereas 47 % was the highest DNA reassociation found between [*P.*] *aerogenes* and [*P.*] *mairii*. The study showed that [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii* can not be easily separated and may consequently be misidentified based on current knowledge of their phenotypic characteristics. In addition, these taxa are difficult to separate from other taxa of the *Pasteurellaceae*. A revised scheme for separation based upon phenotypic characters is suggested for the three species [*P.*] *aerogenes* emend., [*P.*] *mairii* emend. and [*A.*] *rossii* emend., with the respective type strains ATCC 27883^T, NCTC 10699^T and ATCC 27072^T.

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The GenBank/EMBL/DDBJ accession numbers of 16S rRNA gene sequences described in this report are AY465357–AY465374 and AY431030–AY431033, as listed in Table 1 and Fig. 2.

Supplementary tables showing the DNA reassociation data are available in IJSEM Online.

[*Pasteurella*] *aerogenes* was established as a species in 1974 (McAllister & Carter, 1974) and subsequently included in the Approved Lists (Skerman *et al.*, 1980). The species has been excluded from *Pasteurella sensu stricto* on the basis of genotypic studies, which is the reason for enclosing the genus name in brackets (Mutters *et al.*, 1989). Presently, [*P.*] *aerogenes* seems rather heterogeneous. Twenty-three biovars were reported by Bisgaard (1993) and five DNA clusters outlined by Böhme (1993). Four additional biovars

have subsequently been outlined. The species was initially isolated from the intestines, kidney and nose of necropsy specimens from piglets and young swine in the USA and found associated with diarrhoea and other bacterial infections (McAllister & Carter, 1974). Only a single isolate of [*P.*] *aerogenes* from a pig fetus was considered as a primary pathogen in the first report (McAllister & Carter, 1974). Later on, Fodor *et al.* (1991) also reported the bacterium isolated from a pig fetus in Hungary. The origins of pig isolates from Belgium were in accordance with the initial description; however, the bacteria were also reported from vaginal exudates of sows (Hommeze & Devriese, 1976). In the 1990s, it was concluded that most isolates of [*P.*] *aerogenes* were obtained from pathological lesions and that the ecology of the species was poorly known (Bisgaard, 1993). More recently [*P.*] *aerogenes* has been identified from human infections related to pig bites (Lindberg *et al.*, 1998).

rRNA–DNA hybridizations have shown that [*P.*] *aerogenes* was most closely related to *Pasteurella multocida* (De Ley *et al.*, 1990). This relationship was not confirmed by 16S rRNA phylogenetic analysis, since 16S rRNA phylogenetic analysis of the family *Pasteurellaceae* showed that [*P.*] *aerogenes*, [*Pasteurella*] *mairii* and [*Actinobacillus*] *seminis* formed a monophyletic unit defined as the *Seminis* cluster (cluster 14) (Olsen *et al.*, 2004).

[*P.*] *mairii* (Bisgaard taxon 19) was proposed as a separate species by Sneath & Stevens (1990). The species was named on basis of nine strains isolated from pigs and one strain isolated from a prairie marmot (Sneath & Stevens, 1985). Previous studies found [*P.*] *aerogenes* and [*P.*] *mairii* to be related by DNA–DNA hybridization at 46% DNA reassociation (Mutters *et al.*, 1985). [*P.*] *mairii* has also been suggested to be excluded from *Pasteurella sensu stricto* (Mutters *et al.*, 2004).

[*Actinobacillus*] *rossii* was established by Sneath & Stevens (1990) on the basis of an unnamed bacterial taxon represented by 10 strains isolated from pigs (Sneath & Stevens, 1985), including four strains originally described by Ross *et al.* (1972). [*A.*] *rossii* could only be separated unequivocally from [*P.*] *aerogenes* by acid production from sucrose according to their descriptions (Sneath & Stevens, 1985, 1990).

Misidentification of [*A.*] *rossii* and [*P.*] *aerogenes* occurs frequently when reference is made to 16S rRNA gene sequence comparison. The 16S rRNA gene comparison of Olsen *et al.* (2004) found strains MCCM 01550 and MCCM 01551 (listed as P.sp.2 and 1989-5477, respectively, in Table 1) to be related to the type strain of [*A.*] *rossii* in accordance with the present study despite their phenotypic identification as [*P.*] *aerogenes*. 16S rRNA phylogenetic analysis also showed that the type strain of [*A.*] *rossii* was closely related to [*Actinobacillus*] *porcinus* (Christensen *et al.*, 2003). Further studies of virulence and pathogenicity are dependent on an improved classification, including a correct identification of [*A.*] *rossii*.

The RTX toxin (*pax* gene) was found in four strains of [*P.*] *aerogenes* isolated from aborted or young piglets. All strains with the *pax* gene showed more than 99.6% similarity to the type strain of [*P.*] *aerogenes* as inferred by 16S rRNA gene sequence comparison. These strains were haemolytic on sheep-blood agar and CAMP-positive, whereas haemolytic activity was not observed in strains without the *pax* gene (Kuhnert *et al.*, 2000). Unfortunately, previous descriptions of [*P.*] *aerogenes* do not include CAMP-test data, and bovine blood was used rather than ovine blood. Another representative of the RTX toxin family was found in the type strain of [*A.*] *rossii* (Schaller *et al.*, 2000). Both genes showed similarity to the *apxII* and *apxIII* genes of *Actinobacillus pleuropneumoniae*. The RTX toxins of these organisms might represent a major virulence factor; however, further studies of their impact on pathogenicity and phylogenetic relationships remain to be performed. Such studies of virulence and pathogenicity have to await improved classification and identification of these organisms.

The aim of the present study consequently was to improve the classification of [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii* by comparing phenotypic results with genotypic characterization mainly obtained by 16S rRNA gene sequence analysis and DNA–DNA hybridizations.

Bacterial strains and phenotypic characterization

Eighty-nine strains including reference strains were selected for the study based upon previous reports on phenotypic (Bisgaard, 1993) and DNA–DNA hybridization (Ursing, 1981; Böhme, 1993) data (Table 1). Further phenotypic characterization was performed according to Bisgaard *et al.* (1991) including separation into biovars based on 17 characters variable within bacteria previously recognized as [*P.*] *aerogenes* (Table 2). Only some of the biotyped strains were included in the present genotypic investigation. The type strain of [*A.*] *porcinus* was also phenotyped to allow comparison with [*P.*] *aerogenes*, [*P.*] *mairii*, [*A.*] *rossii* and [*A.*] *seminis*. [*A.*] *porcinus* was cultivated on chocolate agar.

RAPD characterization

Ten strains of [*P.*] *mairii* and 13 strains of [*A.*] *rossii* (Table 1) were characterized in order to select representative strains for 16S rRNA gene sequence comparison. For additional comparison, the type strains of the species [*P.*] *aerogenes*, [*P.*] *mairii*, [*A.*] *rossii* and [*A.*] *seminis* were included. Cells were cultivated in brain heart infusion broth (Difco) and DNA was isolated as described by Christensen *et al.* (1993). DNA was dried and redissolved in water of Millipore quality and the concentration was determined by spectrophotometry (GeneQuant; Pharmacia Biotech). PCR amplification was performed by the Ready-To-Go RAPD analysis kit (Amersham Biosciences) as described previously (Dziva *et al.*, 2001). Primer three (5'-GTAGACCCCGT) was selected for the study to give few and distinct bands on the basis of

Table 1. Strains and biovars of the [*P.*] *aerogenes*–[*P.*] *mairii*–[*A.*] *rossii* complex investigated

Accession numbers in bold indicate sequences determined in this study. In the column headed DNA–DNA, + indicates that DNA–DNA hybridization has been performed with the strain. In the column headed RAPD, I–VI refer to the RAPD profiles shown in Fig. 1. ND, Not determined.

Strain	Phenotype	Genotype (16S rRNA-based identification)	Origin/disease	Country*	16S rRNA gene accession no.	DNA–DNA	RAPD
ATCC 27883 ^T (= P634 ^T =CIP 80.14 ^T =CCUG 9995 ^T =CCUG 27904 ^T =DSM 10153 ^T =McAllister P172-71 ^T)	[<i>P.</i>] <i>aerogenes</i> biovar 1	[<i>P.</i>] <i>aerogenes</i>	Pig, intestine	USA	M75048, U66491	+	I
BA151.1 (= P.a.14)	[<i>P.</i>] <i>aerogenes</i> biovar 1	ND	Pig, lung	B	ND	+	ND
JF1319 (= P1290/94)	[<i>P.</i>] <i>mairii</i>	[<i>P.</i>] <i>aerogenes</i>	Pig, placenta/abortion	CH	U66492	ND	ND
JF2118 (= P325/98)	[<i>P.</i>] <i>mairii</i>	[<i>P.</i>] <i>aerogenes</i>	Pig, placenta/abortion	CH	AF139578	ND	ND
JF2032 (= 99/890)	Unclassified	[<i>P.</i>] <i>aerogenes</i>	Piglet/sepsis	CH	AF139580	ND	ND
P.a.9 (= BA27.5)	[<i>P.</i>] <i>aerogenes</i> biovar 3	ND	Pig, tonsil	B	ND	ND	ND
JF2185 (= P772/98)	[<i>P.</i>] <i>aerogenes</i> biovar 3	[<i>P.</i>] <i>aerogenes</i>	Pig, bronchus/pneumonia	CH	AF139586	ND	ND
F147	[<i>P.</i>] <i>aerogenes</i> biovar 4	ND	Rabbit, pneumonia	DK	ND	ND	ND
JF2011 (= P811/97)	[<i>P.</i>] <i>aerogenes</i> biovar 4	[<i>P.</i>] <i>aerogenes</i>	Pig, intestine/diarrhoea	CH	AF139577	ND	ND
JF2034 (= 99/968)	[<i>P.</i>] <i>aerogenes</i> biovar 4	[<i>P.</i>] <i>aerogenes</i>	Pig, intestine/diarrhoea	CH	AF139581	ND	ND
JF2039 (= P894/97)	[<i>P.</i>] <i>aerogenes</i> biovar 4	[<i>P.</i>] <i>aerogenes</i>	Pig, liver/sepsis	CH	AF139582	ND	ND
JF2101 (= 99/1449)	[<i>P.</i>] <i>aerogenes</i> biovar 4	[<i>P.</i>] <i>aerogenes</i>	Pig, intestine/diarrhoea	CH	AF139585	ND	ND
JF2154 (= P577/98)	[<i>P.</i>] <i>aerogenes</i> biovar 4	[<i>P.</i>] <i>aerogenes</i>	Pig, bronchus/pneumonia	CH	AF139587	ND	ND
C5527	[<i>P.</i>] <i>aerogenes</i> biovar 5	[<i>P.</i>] <i>aerogenes</i>	Pig, lymph node	DK	AY465371	ND	ND
3214/88	[<i>P.</i>] <i>aerogenes</i> biovar 9	ND	Human, pig bite	UK	ND	+	ND
JF2072 (= P28/98)	[<i>P.</i>] <i>aerogenes</i> biovar 9	[<i>P.</i>] <i>aerogenes</i>	Pig, liver/sepsis	CH	AF139584	ND	ND
P591 (= T748=McAllister SS-185-72)	[<i>P.</i>] <i>aerogenes</i> biovar 10	[<i>P.</i>] <i>aerogenes</i>	Pig	USA	AY465372	+	ND
G5223/1	[<i>P.</i>] <i>aerogenes</i> biovar 10	ND	Pig, lung	AU	ND	ND	ND
P592 (= T749=McAllister SS-7-73)	[<i>P.</i>] <i>aerogenes</i> biovar 11	[<i>P.</i>] <i>aerogenes</i>	Pig	USA	AY465373	+	ND
P593 (= T750=McAllister SS-68-73)	[<i>P.</i>] <i>aerogenes</i> biovar 11	ND	Pig	USA	ND	+	ND
BK1778/4 (= MCCM 01523)	[<i>P.</i>] <i>aerogenes</i> biovar 19	ND	Pig	B	ND	+	ND
JF2006 (= P787/97)	[<i>P.</i>] <i>aerogenes</i> biovar 19	[<i>P.</i>] <i>aerogenes</i>	Pig fetus, aborted	CH	AF139579	ND	ND
JF2419 (= B96/34)	[<i>P.</i>] <i>aerogenes</i> biovar 25	[<i>P.</i>] <i>aerogenes</i>	Dog, liver	B	AY465357	ND	ND
JF2420 (= 4-97=97AV121)	[<i>P.</i>] <i>aerogenes</i> biovar 26	[<i>P.</i>] <i>aerogenes</i>	Pig, joint	B	AY465358	ND	ND
JF2142 (= P542/98)	[<i>P.</i>] <i>aerogenes</i> biovar 27	[<i>P.</i>] <i>aerogenes</i>	Pig, intestines, diarrhoea	CH	AF139583	ND	ND
NCTC 10699 ^T (=CCUG 27189 ^T =P637 ^T †=Mair 5143/70 ^T)	[<i>P.</i>] <i>mairii</i> (Bisgaard taxon 19)	[<i>P.</i>] <i>mairii</i>	Pig fetus	UK	AF024532	+	II
P646 (= Mair 14679/70)	[<i>P.</i>] <i>mairii</i>	ND	Pig	UK	ND	ND	II
P639 (= Mair 18286/70)	[<i>P.</i>] <i>mairii</i>	ND	Pig	UK	ND	ND	II
P640 (= Mair 34152/70)	[<i>P.</i>] <i>mairii</i>	ND	Pig	UK	ND	ND	II
Past. 9	[<i>P.</i>] <i>mairii</i>	ND	Pig	B	ND	ND	ND
P638 (= NCTC 10700=Mair 7385/70)	[<i>P.</i>] <i>mairii</i>	ND	Pig uterus	UK	ND	ND	II

Table 1. cont.

Strain	Phenotype	Genotype (16S rRNA-based identification)	Origin/disease	Country*	16S rRNA gene accession no.	DNA- DNA	RAPD
34152/71	[P.] <i>mairii</i>	ND	Pig	UK	ND	ND	II
27694/74	[P.] <i>mairii</i>	ND	Pig fetus	UK	ND	ND	II
34310/72	[P.] <i>mairii</i>	[P.] <i>mairii</i>	Piglet	UK	AY431033	ND	II
Past. 4	[P.] <i>mairii</i>	ND	Pig, liver	B	ND	ND	ND
9801/75	[P.] <i>mairii</i>	[P.] <i>mairii</i>	Pig	UK	AY431032	ND	II
JF2082 ^T (= A55 ^T = A68 ^T = Ross192 ^T = P624 ^T = ATCC 27072 ^T = CCUG 12395 ^T = CCUG 24851 ^T = CIP 102634 ^T = NCTC 10801 ^T = MCCM 02461 ^T)	[A.] <i>rossii</i>	[A.] <i>rossii</i>	Pig, vaginal exudate	USA	AF224289	ND	IV
P.sp.2 (= MCCM 01551)	[P.] <i>aerogenes</i> biovar 2	[A.] <i>rossii</i>	Pig bone	B	AF224290	+	ND
JF2424 (= P595 = T573 = McAllister SS-187-74)	[P.] <i>aerogenes</i> biovar 6	[A.] <i>rossii</i>	Pig	USA	AY465362	+	ND
A150	[P.] <i>aerogenes</i> biovar 7	ND	Pig	UK	ND	ND	ND
P.24 (= JF2167)	[P.] <i>aerogenes</i> biovar 7	[A.] <i>rossii</i>	Pig, brain	B	AY465365	+	ND
1989-5477 (= MCCM 01550)	[P.] <i>aerogenes</i> biovar 8	[A.] <i>rossii</i>	Pig abortion	C	AF224288	+	ND
X116-92	[P.] <i>aerogenes</i> biovar 8	ND	Goose, pneumonia/airsacculitis	ND	ND	ND	ND
266/1961	[P.] <i>aerogenes</i> biovar 8	ND	Pig lung	B	ND	ND	ND
1989-145	[P.] <i>aerogenes</i> biovar 8	ND	Pig vagina	C	ND	ND	ND
P.a.6 (= BA354.6)	[P.] <i>aerogenes</i> biovar 12	ND	Pig tonsil	B	ND	ND	ND
P.a.11 (= BA18M/4)	[P.] <i>aerogenes</i> biovar 12	ND	Pig tonsil	B	ND	ND	ND
BA657/3	[P.] <i>aerogenes</i> biovar 12	[A.] <i>rossii</i>	Pig	B	ND	+	ND
P.a.5 (= BA354.5)	[P.] <i>aerogenes</i> biovar 12	[A.] <i>rossii</i>	Pig tonsil	B	AY465369	ND	ND
3920.2	[P.] <i>aerogenes</i> biovar 13	[A.] <i>rossii</i>	Pig lung	B	Same as AY465360	ND	ND
P.a.12 (= BA84.11)	[P.] <i>aerogenes</i> biovar 14	[A.] <i>rossii</i>	Pig tonsil	B	AY465368	ND	ND
P.a.7 (= BA151.7)	[P.] <i>aerogenes</i> biovar 15	[A.] <i>rossii</i>	Pig liver	B	AY465367	+	ND
BK2661/4	[P.] <i>aerogenes</i> biovar 21	ND	Pig lung	B	ND	+	ND
JF2120	[P.] <i>aerogenes</i> biovar 21	[A.] <i>rossii</i>	Piglet	CH	AY465364	ND	ND
1 Hommez	[P.] <i>aerogenes</i> biovar 23	ND	ND	ND	ND		
JF2425 (= B96/45)	[P.] <i>aerogenes</i> biovar 23	[A.] <i>rossii</i>	Pig, intestine	B	AY465363	ND	ND
96AV2015 (= 4071.2 = 3-97 = JF2422)	[P.] <i>aerogenes</i> biovar 24	[A.] <i>rossii</i>	Pig, lung	B	AY465360	ND	ND
JF2073	[P.] <i>aerogenes</i> biovar 26A	[A.] <i>rossii</i>	Piglet	CH	AY465366	ND	ND
JF1390	[A.] <i>rossii</i>	[A.] <i>rossii</i>	Aborted pig fetus	CH	AF025839	ND	ND
JF1989	[A.] <i>rossii</i>	ND	Aborted pig fetus	CH	ND	ND	ND
JF2033	[A.] <i>rossii</i>	ND	Pig	CH	ND	ND	ND
JF2102	[A.] <i>rossii</i>	ND	Pig uterus	CH	ND	ND	ND
JF2163	[A.] <i>rossii</i>	ND	Aborted pig fetus	CH	ND	ND	ND
46377/76	[A.] <i>rossii</i>	ND	Pig	UK	ND	ND	V
41304/76	[A.] <i>rossii</i>	ND	Pig	UK	ND	ND	V

Table 1. cont.

Strain	Phenotype	Genotype (16S rRNA-based identification)	Origin/disease	Country*	16S rRNA gene accession no.	DNA-DNA	RAPD
P.a.1	[A.] <i>rossii</i>	ND	Pig, lung	B	ND	ND	V
6887/76	[A.] <i>rossii</i>	[A.] <i>rossii</i>	Aborted pig fetus	UK	AY431030	ND	V
32587/74	[A.] <i>rossii</i>	ND	Aborted pig fetus	UK	ND	ND	VI
48057/75	[A.] <i>rossii</i>	ND	Pig	UK	ND	ND	VI
16688/74	[A.] <i>rossii</i>	[A.] <i>rossii</i>	Pig	UK	AY431031	ND	VI
Ross 63 (= ATCC 27073)	[A.] <i>rossii</i>	ND	Pig	USA	ND	ND	IV
Ross 145	[A.] <i>rossii</i>	ND	Pig	USA	ND	ND	IV
BK2705.1	[A.] <i>rossii</i>	ND	Pig	ND	ND	ND	IV
P.a.8	[A.] <i>rossii</i>	ND	Porcine vaginal discharge	ND	ND	ND	IV
BL428.1	[A.] <i>rossii</i>	ND	Pig	ND	ND	ND	IV
Past. 22	[A.] <i>rossii</i>	ND	Pig, vaginal exudate	B	ND	ND	IV
Past. 1	[A.] <i>rossii</i>	ND	Pig, liver	B	ND	ND	ND
Past. 6	[A.] <i>rossii</i>	ND	Pig	B	ND	ND	ND
51811/78	[P.] <i>aerogenes</i> biovar 16	ND	Dog eye	UK	ND	+	ND
B96/12 (= JF2421)	[P.] <i>aerogenes</i> biovar 16	<i>A. equuli</i> subsp. <i>equuli</i>	Pig lung	B	AY465359	ND	ND
JF2423 (= P.a.3 = BA354.1)	[P.] <i>aerogenes</i> biovar 17	Taxon 10	Pig tonsil	B	AY465361	ND	ND
5-97	[P.] <i>aerogenes</i> biovar 17	ND	Horse intestine	DK	ND	ND	ND
BA543/4	[P.] <i>aerogenes</i> biovar 17	ND	Pig	B	ND	ND	ND
BA436.5	[P.] <i>aerogenes</i> biovar 18	Taxon 10	Pig	B	AY465374	+	ND
BA597.9	[P.] <i>aerogenes</i> biovar 20	<i>M. varigena</i> -like	Pig	B	AY465370	+	ND
20 pig	[P.] <i>aerogenes</i> biovar 22	ND	Pig	ND	ND	ND	ND
ATCC 15768 ^T	[A.] <i>seminis</i>	[A.] <i>seminis</i>	Ovine semen	AU	M75047	ND	III

*AU, Australia; B, Belgium; C, Canada; CH, Switzerland. DK, Denmark.

†Strain P637^T was received and characterized under three different numbering systems; Frederiksen (P637^T), Sneath (NCTC 10699^T) and Mair (5143/70^T).

Symbols: +, 90 % or more strains positive within 1–2 days; (+), 90 % or more strains positive within 3–14 days; –, 90 % or more strains negative within 14 days; d, 11–89 % positive, w, weakly positive.

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Fig. 1. RAPD analysis of strains of [*P.* *mairii* and *A.* *rossii*] in comparison with the type strains of [*P.* *aerogenes* and *A.* *seminis*]. Asterisks indicate groups that include the type strain of the stated species.

screening with the primers included in the kit against the type strain of [*A.* *rossii*] and a strain of [*P.* *aerogenes*]. The amplified fragments were resolved on agarose gels and stained in ethidium bromide for visualization.

Four groups, each with an identical profile, were found in addition to separate profiles for the type strains of [*P.* *aerogenes*] and [*A.* *seminis*] (Fig. 1). The distribution of strains within the four RAPD types is shown in Table 1. Between four (types IV–VI) and nine (type III) fragments were observed. No fragments were in common between the profiles II ([*P.* *mairii*]) and IV–VI ([*A.* *rossii*]), underlining the genotypic diversity of these taxa. Eleven strains of [*P.* *mairii*] including the type strain included under two different designations formed group II, while 14 strains representing [*A.* *rossii*] formed groups IV (seven strains including the type strain), V (four strains) and VI (three strains) (Table 1; Fig. 1). Up to four fragments with sizes of approx. 350, 600, 650, 700, 750, 800, 850, 900, 1000 and 1500 bp were shared between the profiles. Types IV–VI with strains of [*A.* *rossii*] shared fragments of 350, 600, 700 and 800 bp. None of the four type strains of [*P.* *aerogenes*], [*P.* *mairii*], [*A.* *rossii*] and [*A.* *seminis*] shared a profile. Three additional strains of [*A.* *seminis*] (CCUG 18730, CCUG 18731 and CCUG 23440) not included in the present study shared a profile and differed from the type strain in two fragments (data not shown).

Sequencing of 16S rRNA genes

The [*P.* *aerogenes*–*P.* *mairii*–*A.* *rossii*] complex was re-evaluated on the basis of genotypic comparison of 16S rRNA gene sequences and DNA–DNA hybridization. Strains were grouped based upon similarity to the type strains of [*P.* *aerogenes*], [*P.* *mairii*] and [*A.* *rossii*] as well as to other species of *Pasteurellaceae*. On the basis of this grouping, the phenotypic characteristics of the three species were re-evaluated (Tables 1–3). DNA–DNA reassociation data are available as two supplementary tables in IJSEM Online.

16S rRNA gene sequence analysis of 23 strains (Table 1) was performed as outlined previously (Angen *et al.*, 1998; Kuhnert *et al.*, 2000; Christensen *et al.*, 2002). These analyses were performed in different laboratories by common cycle-sequencing protocols. Strain BA436.5 was found difficult to analyse because of inter-operon differences.

Searches for 16S rRNA gene sequences were performed by BLAST (Altschul *et al.*, 1997) at GenBank. Pairwise sequence comparisons were performed by BESTFIT (Wisconsin Sequence Analysis Package, GCG) and multiple alignments made by PILEUP (GCG). Maximum-likelihood analysis was performed by fastDNAmL including bootstrap analysis (Felsenstein, 1981; Olsen *et al.*, 1994) on a Linux-compatible server. Maximum-parsimony analysis was performed by PHYLIP (Felsenstein, 1995).

Phylogenetic analysis based on 16S rRNA gene sequence comparison showed 17 strains to be closely related to the type strain of [*P.* *aerogenes*], with high support for their common node by bootstrap analysis and parsimony analysis (Fig. 2). These 17 strains represented organisms identified phenotypically as [*P.* *aerogenes*] biovars 3 ($n=1$), 4 (5), 5 (1), 9 (1), 10 (1), 11 (1), 19 (1), 25 (1), 26 (1) and 27 (1) in addition to [*P.* *mairii*] (2) and an unclassified strain, and showed 99.6% or higher 16S rRNA gene sequence similarity to the type strain of [*P.* *aerogenes*] (biovar 1). Strains JF2419 and JF2420 shared sequences with the previously deposited strains JF2011, JF2101, JF2154 and JF2039 under the accession numbers AF139577, AF139585, AF139587 and AF139582 (these strains had identical sequences). Strains P591 and P592 shared sequences with strain JF2072, previously deposited under accession no. AF139584. Strain C5527 shared a sequence with strain JF2034, previously deposited under accession no. AF139581.

Two strains, both of which were classified as [*P.* *mairii*] based upon phenotypic characters, showed 99.5 and 99.9% 16S rRNA gene similarity, respectively, to the type strain of [*P.* *mairii*], and this group was also well supported. The [*P.* *mairii*] group was closely related to the type strain of [*A.* *seminis*], and the three species [*P.* *aerogenes*], [*P.* *mairii*] and [*A.* *seminis*] formed a monophyletic group (Seminis) in accordance with the published phylogeny (Christensen *et al.*, 2003; Olsen *et al.*, 2004).

Fifteen strains were related to the type strain of [*A.* *rossii*] and formed a monophyletic group (Fig. 2). These strains represented organisms classified phenotypically as biovars 2, 6–8, 12–15, 21, 23, 24 and 26A of [*P.* *aerogenes*], each represented by a single isolate. Members of this group showed 97.8% or higher 16S rRNA gene sequence similarity to the type strain of [*A.* *rossii*] and the group was then slightly more diverse than was observed for the strains

Table 3. Phenotypic characters that separate [*P.*] *aerogenes*, [*P.*] *mairii*, [*A.*] *seminis*, [*A.*] *rossii* and [*A.*] *porcinus*

Symbols: +, 90 % or more strains positive within 1–2 days; (+), 90 % or more strains positive within 3–14 days; –, 90 % or more strains negative within 14 days; d, 11–89 % positive; w, weak reaction. Reactions of the type strains are given in brackets. For isolate-dependent characters, percentages of positive strains are given in parentheses.

Character	[<i>P.</i>] <i>aerogenes</i> (21 strains), biovars 1, 3–5, 9–11, 19, 25–27	[<i>P.</i>] <i>mairii</i> (8 strains)	[<i>A.</i>] <i>seminis</i> ATCC 15768 ^T	[<i>A.</i>] <i>rossii</i> (18 strains), biovars 2, 6–8, 12–15, 21, 23, 24, 26A	[<i>A.</i>] <i>porcinus</i> CCUG 38924 ^T
Catalase	+	+	+	d [+] (67)	–
Oxidase	d [+] (81)	d [–] (38)	–	d [+] (78)	w
β-Haemolysis (bovine blood)	–	– [w]	–	– [+] (6)	–
Methyl red, 37 °C	d [–] (19)	–	–	d [–] (39)	w
Ornithine decarboxylase	d [–] (81)	+	+	–	–
Indole	–	–	–	d [w] (50)	–
Phosphatase (NaOH detection)	– (5)	ND	–	–	+
Phosphatase (NH ₃ detection)	– (5)	+	–	– (6)	+
Growth on MacConkey agar	+	–	–	d [w] (28)	w
Glycerol	+/(+)	d [–] (13)	–	+/(+)	–
(+)-D-Arabitol	–	d [+] (75)	–	d [–] (17)	–
(+)-L-Arabinose	d [+] (81)	+	w	+	–
(–)-D-Arabinose	+/(+)	–	–	d [–] (83)	+
(+)-D-Xylose	d [+] (81)	+	w	d [+] (56)	+
meso-Inositol	+	+/(+)	w	d [+] (89)	+
(–)-D-Mannitol	d [–] (24)	+	w	+	+
(–)-D-Sorbitol	–	+	–	d [+] (78)	+
(+)-D-Glucose, gas	d [+] (86)	–	–	d [+] (78)	–
(+)-D-Mannose	+	+	–	d [–] (89)	+
(+)-L-Rhamnose	– (9)	–	–	d [+] (11)	–
NPG (β-glucosidase)	d [–] (24)	+	+	d [+] (22)	–
Lactose	+/(+)	–	–	+/(+)	+
Maltose	+	–	–	d [–] (78)	+
(+)-D-Melibiose	–	–	–	d [–] (39)	+
Sucrose	+/(+)	+	–	d [–] (83)	+
Raffinose	d [–] (29)	–	–	d [–] (78)	+
Dextrin	+	–	–	d [–] (78)	+
Aesculin, Fe	d [–] (24)	(+)	–	–	–
α-Galactosidase	–	–	–	d [–] (39)	+
PGUA (α-glucuronidase)	d [–] (24)	+	+	d [+] (22)	–
ONPX (β-xylosidase)	d [–] (24)	d [+] (88)	+	d [+] (41)	–

related to [*P.*] *aerogenes*. The species [*A.*] *rossii* and [*A.*] *porcinus* were related (Porcinus) (Christensen *et al.*, 2003) (Fig. 2). Strains representing the two groups [*P.*] *aerogenes* and [*A.*] *rossii* showed 93·8–95·2 % 16S rRNA gene sequence similarity. Between [*P.*] *aerogenes* and [*P.*] *mairii* strains, 95·3–97·2 % similarity was found, while strains of [*A.*] *rossii* and [*P.*] *mairii* showed 93·9–95·5 % similarity.

Strains representing biovars 16, 17, 18 and 20 of [*P.*] *aerogenes* were found to be unrelated to the [*P.*] *aerogenes*–[*P.*] *mairii*–[*A.*] *rossii* complex by 16S rRNA gene sequence comparison. The non-haemolytic but CAMP-positive strain B96/12 of biovar 16 was related to the type strain of *Actinobacillus equuli* subsp. *equuli* (accession no. M75072) with 99·9 % similarity. Strains JF2423 and BA436.5, respectively from biovars 17 and 18, showed 98·9 % 16S rRNA

gene sequence similarity to taxon 10 (strain CCUG 15572, accession no. AF024528), a taxon closely related to [*Actinobacillus*] *succinogenes* (Christensen *et al.*, 2003; Olsen *et al.*, 2004). Strain BA597.9 of biovar 20 was found to be variable in haemolysis, but showed a positive CAMP reaction and was 98–100 % similar in the 16S rRNA sequence to strains of *Mannheimia varigena* (accession nos AF053893, AF053899, U57076).

DNA–DNA hybridization

DNA–DNA hybridizations between 10 strains in nine pairs were performed according to the spectrophotometric method as described previously (Mutters *et al.*, 1985). Further data on DNA–DNA hybridization were reported by Böhme (1993) (spectrophotometric method)

Fig. 2. Phylogenetic relationships between the taxa of the *[P.] aerogenes*–*[P.] mairii*–*[A.] rossii* complex and representative members of the *Pasteurellaceae* based on maximum-likelihood analysis of 16S rRNA gene sequences. Support for monophyletic groups by bootstrap analysis is indicated as percentages for values higher than 50 and nodes also observed by maximum-parsimony are indicated by asterisks. Strains in bold have been sequenced in the present investigation and enclosure of strain names in brackets indicates sequence identity to the previous strain listed.

and Ursing (1981) (hydroxyapatite method). DNA reassociation values are shown in two supplementary tables in IJSEM Online, including published data. These data are illustrated as a single linkage dendrogram in Fig. 3. Strains of biovars 1, 9, 10, 11 and 19, including the type strain of *[P.] aerogenes*, linked at 70 % DNA reassociation, while members of biovars 2, 6, 7, 8, 12, 15 and 21 linked at 81 % reassociation. Between the *[P.] aerogenes* group and type strain of *[P.] mairii*, a maximum of 47 % DNA reassociation was observed, indicating that these taxa might be related at the genus level based on DNA–DNA hybridizations (Fig. 3).

Two species-like groups were outlined. One included biovars 1, 9, 10, 11 and 19 of *[P.] aerogenes*, while the other included biovars 2, 6, 7, 8, 12, 15 and 21. The second group was closely related to the type strain of *[A.] rossii*, since the strains analysed by both DNA–DNA hybridization and 16S rRNA gene sequence comparison showed at least 98.1 % 16S rRNA gene sequence similarity to the type strain of *[A.] rossii*. The DNA–DNA hybridization between the two groups was only 37 %, suggesting a genus-like difference (Fig. 3).

The relationship with biovars 16, 18 and 20 and the

relatedness between these biovars was at most 37 %, confirming the misidentification of these strains, since 16S rRNA gene sequence analysis showed that strains of these biovars were closely related to *A. equuli* subsp. *equuli*, taxon 10 of Bisgaard and *M. varigena*.

Emendation of the descriptions of *[P.] aerogenes*, *[P.] mairii* and *[A.] rossii*

Strains characterized by 16S rRNA gene sequence comparison, DNA–DNA hybridization as well as strains grouped by the RAPD analysis were all characterized phenotypically. Common characters for all strains of *[P.] aerogenes*, *[P.] mairii* and *[A.] rossii* investigated (Table 1) were Gram-negative staining and lack of motility at 22 and 37 °C. The Hugh & Leifson fermentation test with (+)-D-glucose was positive. Symbiotic growth (NAD requirement) was negative. The porphyrin test was positive. No growth was observed on Simmons' citrate agar and acid was not formed from mucate; an alkaline reaction was not seen in malonate broth. The H₂S/TSI and KCN tests were negative. The Voges–Proskauer test at 37 °C was also negative. Nitrate was reduced without gas formation. Urease and alanine aminopeptidase tests were positive. Tests for

Fig. 3. Relationships between strains of the [*P.*] *aerogenes*–[*P.*] *mairii*–[*A.*] *rossii* complex based on pairwise DNA–DNA hybridization experiments. Data were obtained from the present investigation by the spectrophotometric method and from Böhme (1993) and Ursing (1981). The dendrogram was constructed by single-linkage clustering based on the DNA reassociations shown in two supplementary tables in IJSEM Online.

arginine dehydrolase, lysine decarboxylase and phenylalanine deaminase were negative. The three species were gelatinase-negative and did not hydrolyse Tween 20 or 80. Pigment was not formed. Acid was not produced from *meso*-erythritol, adonitol, xylitol, (–)-L-xylose, dulcitol, (+)-D-fucose, (–)-L-sorbose, cellobiose, trehalose, (+)-D-melezitose, (+)-D-glycogen, inulin, aesculin, amygdalin, arbutin, gentiobiose, salicin, (+)-D-turanose and β -N-CH₃-glucosamid. Acid was produced from (–)-D-ribose, (–)-D-fructose, (–)-L-fucose and (+)-D-glucose. The ONGP test (β -galactosidase tested with *o*-nitrophenyl D-galactopyranoside) was positive. Tests for α -fucosidase and α -mannosidase were negative.

All except a single isolate of [*A.*] *rossii* produced acid from (+)-D-galactose, while only a single isolate was PNPG positive (α -glucosidase activity tested by 4-nitrophenyl α -D-glucopyranoside as substrate). Using the limits that positive or negative reactions refer to 90% of strains, these tests were excluded from Table 3, in which characters that separate the three taxa are stated. From this table, it is noted that at least four characters separate [*P.*] *aerogenes* from [*P.*] *mairii* and [*P.*] *mairii* from [*A.*] *rossii*, while not even a single character allows a clear-cut separation of

[*P.*] *aerogenes* from [*A.*] *rossii*. However, a combination of ornithine decarboxylase, production of indole and acid from (–)-D-mannitol, (–)-D-sorbitol, (+)-D-melibiose and raffinose separates these taxa.

The present study showed that phenotypic characterization was insufficient for classification and identification of porcine members of the [*P.*] *aerogenes*–[*P.*] *mairii*–[*A.*] *rossii* complex. Comparison of 16S rRNA gene sequences showed that [*P.*] *aerogenes* should be restricted to only 11 biovars, whereas 12 other biovars showed high 16S rRNA gene sequence similarity to the type strain of [*A.*] *rossii*. Finally, four biovars were found to be unrelated to [*P.*] *aerogenes* and [*A.*] *rossii* by 16S rRNA gene sequence comparison, but related to other taxa of the *Pasteurellaceae* including *Actinobacillus sensu stricto*, *Mannheimia* and taxon 10 of Bisgaard. Both *A. equuli* subsp. *equuli* and *M. varigena* have been isolated from pigs. [*P.*] *aerogenes* biovar 16 was recorded as producing gas from (+)-D-glucose. However, reinvestigations showed that gas production could not be reproduced and misidentification is explained as the presence of gas in the Durham tube before inoculation. Biovar 18 was (–)-D-mannitol- and phosphatase-negative and (–)-L-fucose-positive, characters not previously

reported for *A. equuli*. Only differences in phosphatase and formation of gas from glucose separate taxon 10 from *A. equuli sensu stricto*. For the same reasons, biovar 18 might be regarded as a (–)-D-mannitol-negative taxon 10 (M. Bisgaard, unpublished results). Biovar 17 differs from taxon 10 (CCUG 15572) only in (–)-D-mannitol and from biovar 18 in (+)-L-rhamnose. Similar phenotypic characters were, however, observed for biovar 17 and taxon 6 of Bisgaard. DNA–DNA hybridizations are needed to resolve the taxonomic positions of these taxa. Several phenotypic characters [β -haemolysis, methyl red, urease, (+)-D-mannose, PNPG and ONPF] separate biovar 20 from *M. varigena* biovar 1. ONPF is testing for α -fucosidase activity and was performed with 2-nitrophenyl α -L-fucopyranoside.

Strains JF2006, JF1319, JF2032 and JF2118 of [*P.*] *aerogenes* with the RTX toxin (*pax* gene) (Kuhnert *et al.*, 2000) could not be separated from the other strains based on 16S rRNA gene sequence comparison.

Polyamine profiles have been reported for two strains of [*P.*] *aerogenes*; however, strain BK1778/4 of biovar 19 differed significantly from the type strain. In the type strain, spermidine occurred in the highest proportion followed by putrescine, but in the other strain, BK1778/4, 1,3-diaminopropane was dominant, making up around 90 % of all polyamines (Busse *et al.*, 1997). The results might be explained if the strains had been misidentified; however, strain BK1778/4 was included in the present study and found to give a DNA–DNA reassociation value of 87 % with the type strain of [*P.*] *aerogenes*. Further investigations are needed to solve this problem. In the type strain of [*A.*] *rossii* and in strain BK2661.4 of biovar 21, 1,3-diaminopropane dominated, with 94.7 and 91.0 % of polyamines, respectively. This similarity was in accordance with a 16S rRNA gene sequence similarity of 99.1 % between these two strains. For the type strain of [*P.*] *mairii*, 1,3-diaminopropane and putrescine constituted 47.7 and 43 % of the total polyamine content, respectively, diverging significantly from the strains of [*P.*] *aerogenes* and [*A.*] *rossii* characterized.

Human isolates obtained from infections related to pig bites seem to be related to biovar 17 of [*P.*] *aerogenes* (Lester *et al.*, 1993; Lindberg *et al.*, 1998), although their phenotype was also reportedly related to that of taxon 6 of Bisgaard (Bisgaard *et al.*, 1983), a taxon otherwise isolated only from guinea pigs. In addition, taxon 6 of Bisgaard could not be separated phenotypically from biovar 17 in the present study. Finally, the SP group strains MCCM 00489 and MCCM 02119, which are phenotypically related to Bisgaard taxon 6 (M. Bisgaard, unpublished results), showed polyamine patterns related to [*A.*] *rossii*, with 1,3-diaminopropane dominance of around 92 % (Busse *et al.*, 1997). The identification of a strain of biovar 17 of [*P.*] *aerogenes* by 16S rRNA gene sequence comparison as a misidentified strain of taxon 10 of Bisgaard could indicate that the human isolates might also belong to this taxon, since 98.9 % similarity was reported between strain JF2423

(=P.a.3) of the present study and taxon 6 of Bisgaard. Exactly the same 16S rRNA gene sequence similarity was found between taxa 6 and 10 of Bisgaard.

Ribotyping (RFLP with *EcoRI* probed by 16S–23S *Escherichia coli* rRNA) of 20 of the strains in the present study was able to separate the strains into groups with similarity to [*P.*] *aerogenes* or [*A.*] *rossii* as well as separating the divergent biovars 16, 17, 18 and 20 (M. Bisgaard, unpublished results). Previously, ribotyping with the application of *EcoRI* was reported by Lester *et al.* (1993). Five of the strains investigated in the present study (P591 of biovar 10, P592 and P593 of biovar 11, P595 of biovar 6 and the type strain P634^T of [*P.*] *aerogenes*) belonged to two clusters related at 57 % similarity only. Strains of biovar 10 and 11 were related to the type strain of [*P.*] *aerogenes* at 92 % similarity, while the strain of biovar 6 (shown to represent [*A.*] *rossii* in the present study) clustered at 57 % (Lester *et al.*, 1993). In the present study, strains of biovars 10 and 11 showed 99.8 % 16S rRNA gene sequence similarity to the type strain of [*P.*] *aerogenes*, whereas biovar 6 was related to the type strain of [*P.*] *aerogenes* with 95.1 % similarity only. It can be concluded that ribotyping with *EcoRI* is a reliable tool for separation of isolates related to either [*P.*] *aerogenes* or [*A.*] *rossii*.

Based on the present emended description of the three species [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii*, it would be tempting to re-evaluate published phenotypic data by Ross *et al.* (1972), Sneath & Stevens (1985), McAllister & Carter (1974) and Fodor *et al.* (1991) on these species. However, such a comparison is difficult for several reasons. First of all, methods might differ slightly. As examples, an important character such as β -haemolysis depends on the type of blood (bovine or ovine) and medium used, including pH (M. Bisgaard, unpublished observation). In addition, the outcome of the phosphatase test seems to depend on the type of test used (see Table 3). Another problem is that the original data do not include results for single strains, which is a precondition for clear-cut comparisons.

Ward *et al.* (1998) reported on equine isolates of [*P.*] *mairii*; however, the strains differed in trehalose, lactose and salicin according to the present description of [*P.*] *mairii*. For the same reasons, identification of isolates of [*P.*] *mairii* from horses should be questioned unless genetic data confirm their classification.

The accuracy of the present characterization was evaluated by the inclusion of three different designations of the type strain of [*P.*] *mairii*. Both phenotypic and RAPD characterization of strains P637^T, Mair5143/70^T and NCTC 10699^T showed identical results.

The three taxa [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii* can probably not be separated on the basis of DNA G+C content or genome size. The DNA G+C content of the type strain of [*P.*] *aerogenes* was 41.8 mol% (Mutters *et al.*, 1985), with a genome size of 1.6–2.0 GDa (Mutters *et al.*,

2004). The DNA G+C content of the type strain of [*P.*] *mairii* was 43.4 mol% and the genome size 1.9 GDa (Mutters *et al.*, 1985; Piechulla *et al.*, 1985), and the DNA G+C content of the type strain of [*A.*] *rossii* was 41.9 mol% with a genome size of 1.8 GDa (Piechulla *et al.*, 1985). For two other strains related to [*A.*] *rossii* by 16S rRNA gene sequence comparison, slightly lower DNA G+C contents but larger genome sizes were found in the present study. The DNA G+C content of strain P.24 of [*A.*] *rossii* ([*P.*] *aerogenes* biovar 7) was 40.5 mol%, with a genome size of 2.5 GDa, and the DNA G+C content of strain BA657/3 of [*A.*] *rossii* ([*P.*] *aerogenes* biovar 12) was 39 mol% and the genome size was 2.2 GDa.

Based on the unique phylogenetic position of [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *seminis*, making up the *Seminis* group inferred from 16S rRNA gene sequence comparison (Christensen *et al.*, 2003; Olsen *et al.*, 2004), this group might deserve genus rank. With the emended description of [*P.*] *aerogenes* in the present study, this species showed 95.5–97.2% 16S rRNA gene sequence similarity to the emended [*P.*] *mairii*, while this taxon showed 97.6–97.7% similarity to the type strain of [*A.*] *seminis*. Unfortunately data are missing to document fully the relationships between [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *seminis*, since [*A.*] *seminis* has not been extensively characterized phenotypically and DNA–DNA hybridizations have not been made between this taxon and [*P.*] *aerogenes* and [*P.*] *mairii*. With the exception of a negative reaction in urease and ONPG and differences in colonial morphology, [*A.*] *seminis* shares the common phenotypic properties for [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii* given in the present paper (M. Bisgaard, unpublished data). Characters that separate all four taxa are stated in Table 3. In addition, RAPD profiling seems to be a valuable tool to confirm the separation of the three species and their separation from [*A.*] *seminis* (see Fig. 1). However, until further information is provided, it is only possible to change the circumscription of the three species [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii* to improve the identification of these taxa and not to name a new genus. The major changes compared to the original descriptions (McAllister & Carter, 1974; Kilian & Frederiksen, 1981; Sneath & Stevens, 1990) have been the extended number of strains characterized and different kinds of phenotypic properties evaluated. Few characters have changed compared with the original description; however, new characters useful for differentiation of the organisms have been added to the descriptions. Twelve characters (negative Gram stain, lack of motility at 22 and 37 °C, lack of growth in Simmons' citrate agar and acid formation from glucose, nitrate reduction, positive urease, negative lysine decarboxylase and gelatinase reactions and lack of acid production from dulcitol and salicin) were conserved between all three species and were in accordance with the original descriptions of McAllister & Carter (1974) and Sneath & Stevens (1990). A further 14, 28 and 31 reactions were in accordance with the original descriptions for each of the three species [*P.*] *aerogenes*, [*P.*] *mairii* and

[*A.*] *rossii*, respectively. However, 10, 18 and 15 reactions diverged from the original descriptions. This might be related to different numbers of strains investigated changing the positive and negative signs in relation to the tolerated limits of 10% variation allowed for a reaction. Different methods or variation in methods might also account for the variation and, finally, phenotypic characterization might have diverged from the 16S rRNA-based identification in previous studies, as already discussed. For 44, 22 and 22 characters, respectively, new information was provided for the three species [*P.*] *aerogenes*, [*P.*] *mairii* and [*A.*] *rossii*.

16S rRNA gene sequence-based phylogenetic analysis has indicated that [*A.*] *rossii* and [*A.*] *porcinus* form a genus-like group (Christensen *et al.*, 2003; Olsen *et al.*, 2004). Comparison of phenotypic characteristics of [*A.*] *rossii* with the type strain of [*A.*] *porcinus* showed divergence in seven characters. Reactions of three divergent characters [phosphatase, glycerol and (+)-L-arabinose] are shown in Table 3. In addition, the type strain of [*A.*] *porcinus* was NAD-requiring, urease-negative, without ability to form acid from (–)-D-ribose and ONPF-positive compared with [*A.*] *rossii* and also [*P.*] *aerogenes* and [*P.*] *mairii*, being without NAD requirement, urease-positive, with ability to form acid from (–)-D-ribose and ONPF-negative. Since only the type strain of [*A.*] *porcinus* has been characterized in detail, it is not currently possible to consider whether the *Rossii* 16S rRNA group can be named as a new genus; however, the distinctiveness of [*A.*] *rossii* and [*A.*] *porcinus* at the species level has been verified.

The type strain of [*A.*] *porcinus* has previously been characterized by Møller *et al.* (1996) and Kielstein *et al.* (2001) and agreement was found for 30 of 31 characters; for (–)-D-fructose, we determined a positive reaction compared with the negative result determined by Kielstein *et al.* (2001).

Emended description of [*Pasteurella*] *aerogenes* McAllister and Carter 1974

[*Pasteurella*] *aerogenes* (ae.ro.ge'nes. Gr. subst. masc. *aeros* air; Gr. subst. neut. *genos* generating; N.L. subst. *aerogenes* air-generating).

Cellular and colonial morphologies are as reported by McAllister & Carter (1974). Cells on blood agar are 0.5–1.0 µm wide and 1.1–2.0 µm long with filaments seen, especially in older cultures. After 24 h incubation on bovine blood agar, colonies are circular, smooth, convex, regular and greyish, 0.5–1.0 mm in diameter. Haemolysis is not observed on bovine blood agar. Cells are Gram-negatively stained and do not show motility at 22 or 37 °C. Catalase reaction is positive. Hugh & Leifson fermentation test with (+)-D-glucose is positive. Symbiotic growth (NAD requirement) is negative. Porphyrin test is positive. No growth is observed on Simmons' citrate agar and acid is not formed from mucate; alkaline reaction is not seen in malonate broth. H₂S/TSI and KCN tests are negative. Voges–Proskauer test at 37 °C is also negative. Nitrate is

reduced without gas formation. Urease and alanine aminopeptidase tests are positive. Tests for arginine dehydrolase, lysine decarboxylase and phenylalanine deaminase are negative and so are tests for indole and phosphatase. The species is gelatinase-negative and does not hydrolyse Tween 20 or 80. Growth on MacConkey is positive. Pigment is not formed. Acid is not produced from *meso*-erythritol, adonitol, (+)-D-arabitol, xylitol, (–)-L-xylose, dulcitol, (–)-D-sorbitol, (+)-D-fucose, (+)-L-rhamnose, (–)-L-sorbose, cellobiose, (+)-D-melibiose, trehalose, (+)-D-melezitose, (+)-D-glycogen, inulin, aesculin, amygdalin, arbutin, gentiobiose, salicin, (+)-D-turanose or β -N-CH₃-glucosamid. Acid is produced from glycerol, (–)-D-arabinose, (–)-D-ribose, *meso*-inositol, (–)-D-fructose, (–)-L-fucose, (+)-D-galactose, (+)-D-glucose, (+)-D-mannose, lactose, maltose, sucrose and dextrin. The ONGP test is positive. Tests for α -fucosidase, α -mannosidase and α -galactosidase and PNPG are negative. The oxidase, methyl red, ornithine decarboxylase, NPG (β -glucosidase test determined by 4-nitrophenyl β -D-glucopyranoside), PGUA (β -glucuronidase reaction determined by 4-nitrophenyl β -D-glucopyranosiduronic acid) and ONPX (β -xylosidase test performed with 2-nitrophenyl β -D-xylopyranoside) reactions are isolate-dependent in addition to production of acid from (+)-L-arabinose, (+)-D-xylose, (–)-D-mannitol, raffinose, hydrolysis of aesculin and gas formation from glucose. It is recommended to use supplementary genotyping methods in the identification of this species, e.g. 16S rRNA gene sequencing or ribotyping. The bacteria are mostly reported from pigs and infrequently from rabbit, dog and humans bitten by pigs. The bacteria have been isolated from tonsils, lung, bronchia, intestines, placenta, stomach of aborted pigs, joint, liver and lymph nodes and associated with sepsis, pneumonia and diarrhoea.

The type strain is ATCC 27883^T (=CCUG 9995^T=CIP 80.14^T=DSM 10153^T), isolated from pig intestine in USA. The DNA G+C content of the type strain is 41.8 mol% (Mutters *et al.*, 1985) and the genome size is 1.6–2.0 GDa (Mutters *et al.*, 2004).

Emended description of [*Pasteurella*] *mairii* Sneath and Stevens 1990

[*Pasteurella*] *mairii* (mair'i.i. N.L. gen. n. *mairii* of Mair).

Cellular and colony morphologies are mostly as previously reported by Sneath & Stevens (1990). Cells are small, non-motile bacilli or coccobacilli, mostly shorter than 1 μ m and not more than 0.5 μ m wide, occurring singly or as filaments. Colonies on bovine blood agar are regular, circular and convex, greyish, semi-transparent and approx. 1–1.5 mm in diameter after 24 h incubation at 37 °C. Haemolysis is normally not seen but may be observed, mainly under the colonies. Cells are Gram-negative and do not show motility at 22 or 37 °C. Catalase reaction is positive. Hugh & Leifson fermentation test with (+)-D-glucose is positive. Symbiotic growth (NAD requirement) is negative. Porphyrin test is positive. No growth is observed

on Simmons' citrate agar and acid is not formed from mucate; an alkaline reaction is not seen in malonate broth. H₂S/TSI and KCN tests are negative. Methyl red and Voges–Proskauer tests at 37 °C are also negative. Nitrate is reduced without gas formation. Urease and alanine aminopeptidase test are positive. Tests for arginine dehydrolase, lysine decarboxylase and phenylalanine deaminase are negative. Ornithine decarboxylase and phosphatase tests are positive. Indole and gelatinase tests are negative and members of the species do not hydrolyse Tween 20 or 80. Growth on MacConkey is negative and pigment is not formed. Acid is not produced from *meso*-erythritol, adonitol, xylitol, (–)-D-arabinose, (–)-L-xylose, dulcitol, (+)-D-fucose, (+)-L-rhamnose, (–)-L-sorbose, cellobiose, lactose, maltose, (+)-D-melibiose, trehalose, (+)-D-melezitose, raffinose, dextrin, (+)-D-glycogen, inulin, aesculin, amygdalin, arbutin, gentiobiose, salicin, (+)-D-turanose or β -N-CH₃-glucosamid. Acid is produced from (+)-L-arabinose, (+)-D-xylose, (–)-D-ribose, *meso*-inositol, (–)-D-mannitol, (–)-D-sorbitol, (–)-D-fructose, (–)-L-fucose, (+)-D-galactose, (+)-D-glucose (without gas), (+)-D-mannose and sucrose. ONGP, NPG and PGUA tests are positive. Tests for α -fucosidase, α -mannosidase, α -galactosidase and PNPG are negative. Aesculin is hydrolysed. Oxidase and ONPX reactions are isolate-dependent and so is acid formation from glycerol and (+)-D-arabitol. It is recommended to use supplementary genotyping methods in the identification of this species, e.g. 16S rRNA gene sequencing and RAPD DNA fingerprinting. The bacteria have been isolated from the reproductive tract and from aborted pigs.

The type strain is NCTC 10699^T (=CCUG 27189^T), isolated from a pig fetus in the UK. The DNA G+C content of the type strain is 43.4 mol% and the genome size is 1.9 GDa (Mutters *et al.*, 1985; Piechulla *et al.*, 1985).

Emended description [*Actinobacillus*] *rossii* Sneath and Stevens 1990

[*Actinobacillus*] *rossii* (ross'i.i. N.L. gen. n. *rossii* of Ross).

Cellular and colony morphologies are mostly as reported previously by Sneath & Stevens (1990). Cells are mostly small bacilli, rarely coccobacilli, less than 2 μ m. Colonies on bovine agar are circular, regular, convex and greyish and semi-transparent and about 1–2 mm in diameter after 24 h incubation at 37 °C. β -Haemolysis is not commonly observed. Cells are Gram-negatively stained and do not show motility at 22 or 37 °C. Hugh & Leifson fermentation test with (+)-D-glucose is positive. Symbiotic growth (NAD requirement) is negative. Porphyrin test is positive. No growth is observed on Simmons' citrate agar and acid is not formed from mucate; an alkaline reaction is not seen in malonate broth. H₂S/TSI and KCN tests are negative. Voges–Proskauer test at 37 °C is also negative. Nitrate is reduced without gas formation. Urease and alanine aminopeptidase test are positive. Tests for arginine dehydrolase, lysine decarboxylase, ornithine decarboxylase and phenylalanine deaminase are negative. Phosphatase test is negative.

The species is gelatinase-negative and does not hydrolyse Tween 20 or 80. Pigment is not formed. Acid is not produced from *meso*-erythritol, adonitol, xylitol, (–)-L-xylose, dulcitol, (+)-D-fucose, (–)-L-sorbose, cellobiose, trehalose, (+)-D-melezitose, (+)-D-glycogen, inulin, aesculin, amygdalin, arbutin, gentiobiose, salicin, (+)-D-turanose or β -N-CH₃-glucosamid. Acid is produced from glycerol, (+)-L-arabinose, (–)-D-ribose, (–)-D-mannitol, (–)-D-fructose, (–)-L-fucose, (+)-D-galactose, (+)-D-glucose and lactose. ONPG test is positive. Tests for α -fucosidase, α -glucosidase, PNPG and α -mannosidase are negative. Catalase, oxidase, methyl red, indole, growth on MacConkey agar, NPG, α -galactosidase, PGUA and ONPX reactions are isolate-dependent, as are acid formation from (+)-L-arabitol, (–)-D-arabinose, (+)-D-xylose, *meso*-inositol, (–)-sorbitol, (+)-mannose, (+)-L-rhamnose, maltose, (+)-D-melibiose, sucrose, raffinose and dextrin and gas formation from glucose. It is recommended to use supplementary genotyping methods in the identification of this species, e.g. 16S rRNA gene sequencing, RAPD and ribotyping. The bacteria have been isolated from vaginal exudate, abortion, tonsils, lungs, liver, intestines, bone and brain of pigs.

The type strain is ATCC 27072^T (= CCUG 12395^T = NCTC 10801^T = CIP 102634^T), isolated from vaginal exudates of a pig in the USA. The DNA G + C content is 39–41.9 mol% and the genome size is 2.2–2.5 GDa.

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