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Actinobacillus minor sp. nov., *Actinobacillus porcinus* sp. nov., and *Actinobacillus indolicus* sp. nov., Three New V Factor-Dependent Species from the Respiratory Tract of Pigs

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The results of DNA-DNA relatedness experiments and comparisons of sequences of genes coding for 16S rRNA were used to determine the genetic relationships of selected V factor-dependent species belonging to the family *Pasteurellaceae* and obtained from the porcine respiratory tract. These results showed that the Minor group and taxa C, D plus E, and F are distinct phylogenetic groups that are separate from each other and from other members of the family *Pasteurellaceae*. On the basis of these results, three new species, corresponding to the Minor group, taxa D plus E, and taxon F, are proposed; the names of these new species are *Actinobacillus minor* (type strain, NM305), *Actinobacillus porcinus* (type strain, NM319), and *Actinobacillus indolicus* (type strain, 46KC2), respectively.

On the basis of extensive phenotypic characteristics, we identified V factor-dependent species belonging to the family *Pasteurellaceae* present in the porcine upper respiratory tract (14, 15). We found that in addition to *Actinobacillus pleuropneumoniae*, the causative agent of porcine pleuropneumonia, *Haemophilus parasuis*, the causative agent of Glässer's disease, and the Minor group (13), three other distinct taxa, previously designated taxa D, E, and F, colonize the porcine respiratory tract (15). Recent DNA-DNA hybridization studies of members of the family *Pasteurellaceae* revealed that the requirement for V factor is not an exclusive feature of the genus *Haemophilus*, but may be a feature of members of all three genera of this family (17, 18, 20). Comparative analysis of sequences of genes coding for 16S rRNA (16S rDNA) has been useful for differentiating species in the family *Pasteurellaceae* (6, 7). While several strains of *A. pleuropneumoniae* and *H. parasuis* and a few strains belonging to the Minor group have been analyzed by DNA-DNA hybridization (1, 16), no strains belonging to taxa D, E, and F have been included in such studies. Likewise, 16S rDNA sequence analysis has never been performed with isolates belonging to taxa D, E, and F. The purpose of this study was to use DNA-DNA relatedness and 16S rDNA sequence comparisons to determine the intra- and intergenetic relationships of the Minor group and taxa C, D, E, and F with selected V factor-dependent and V factor-independent members of the family *Pasteurellaceae*.

MATERIALS AND METHODS

Bacterial strains. A total of 28 strains were included in this study. Table 1 shows the sources of 22 V-factor dependent porcine strains belonging to the *Pasteurellaceae*, the type strains of the type species of the genera *Actinobacillus*, *Pasteurella*, and *Haemophilus* (*Actinobacillus lignieresii*, *Pasteurella multocida*, and *Haemophilus influenzae*), and the type strains of species commonly isolated from pigs (*A. pleuropneumoniae*, *Actinobacillus suis*, and *H. parasuis*). Two taxon C strains described by Kilian (12) were included because of their V factor dependence and their presumed porcine origin.

All of the strains were stored as lyophilized cultures. Phenotypic characteristics of the 22 porcine strains have been described elsewhere (15). The bacteria used for DNA preparation were propagated in 2.5-liter portions of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 10 mg of NAD per liter. Each inoculated broth preparation was incubated for 48 h in air at 37°C. The two strains belonging to taxon E failed to grow in brain heart infusion broth and consequently were grown on chocolate agar plates; for each of these strains 40 plates were incubated for 48 h at 37°C.

Preparation of DNA for DNA-DNA hybridization. The bacteria were harvested by centrifugation at 8,000 × *g* for 10 min. The pellet from each 2.5-liter culture was suspended in 120 ml of distilled water containing 0.05 M Tris, 0.05 M EDTA, and 0.1 M NaCl (pH 8.0). The bacteria harvested from each group of 40 chocolate agar plates were suspended in 120 ml of the same solution. The cells were lysed in 2.6 ml of a 25% (wt/vol) aqueous solution of sodium dodecyl sulfate (Sigma Chemical Co., St. Louis, Mo.) and 0.4 ml of a 2% (wt/vol) pronase (Calbiochem-Behring, La Jolla, Calif.) solution. Each mixture was vigorously shaken and incubated at 37°C overnight. DNA was extracted and purified from the cell lysate by a previously described procedure (3). The DNAs in the solutions were sheared by sonication, dialyzed overnight against 0.042 M NaCl, and stored at 4°C over a layer of chloroform.

DNA-DNA hybridization. Native DNA was labeled in vitro by nick translation (3). The S1 nuclease-trichloroacetic acid procedure was used for hybridization (9). Labeled DNA (about 10 ng) and unlabeled DNA (75 μg) were heat denatured, the NaCl concentration of the preparation (final volume, 0.5 ml) was adjusted to 0.42 M, and the preparation was incubated at 60°C for 16 h.

The T_m (the temperature at which 50% of the reassociated DNA became hydrolyzable by S1 enzyme treatment) was determined for some of the isolates. The ΔT_m (the difference between the T_m of a homologous reaction and the T_m of a heterologous reaction) was used to estimate the level of divergence between two organisms (2).

16S rDNA sequencing. Chromosomal DNA was extracted as described by Christensen et al. (5). The 16S rDNA was amplified by PCR and sequenced by the cycle sequencing method (10). Programs for data entry, editing, sequence alignment, secondary-structure comparison, similarity matrix generation, and phylogenetic tree construction were written in Microsoft QuickBASIC for use on IBM PC-AT and compatible computers (6). Similarity matrices were constructed from aligned sequences by using only those sequence positions for which 90% of strains had data. Similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (11). The neighbor-joining method of Saitou and Nei (21) was used for phylogenetic tree construction.

Determination of G+C content of the DNA. The G+C contents of three DNA samples were determined by O. Bouvet (Institut Pasteur, Paris, France). DNA was hydrolyzed with P1 nuclease (8). The nucleotides produced were separated by high-performance liquid chromatography as described by Perrone and Brown (19). The G+C content was determined from the nucleotide ratios by using hydrolyzed bacteriophage lambda DNA as the standard.

Nucleotide sequence accession numbers. The nucleotide sequences of the three bacterial strains examined in this study are available for electronic retrieval from the EMBL, GenBank, and DDBJ databases under the following accession numbers: strain NM305^T (T = type strain), U65582; strain NM319^T, U65583; and strain 46KC2,^{T} U65584.

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TABLE 1. Reference and field strains examined

Taxon	Strain(s)	Source or reference[a]
Minor group	565, 566, 52T3a	13
	202 (= HK 683)	Biberstein
	NM305^T, 562	Rosendal
	559, 273	Musser
Taxon D	596, 27Kb3	13
	NM319^T, K13	Rosendal
	K12	
Taxon E	20b	13
	30sp	Nielsen
Taxon F	K1, K4, 46KC2^T	13
	F6, F7	Heidt
Taxon C	5015, 5111	CAPM
A. pleuropneumoniae	ATCC 27088^T	ATCC
A. lignieresii	NCTC 4189^T	NCTC
A. suis	ATCC 15557^T	ATCC
P. multocida	NCTC 10322^T	NCTC
H. influenzae	ATCC 33391^T	ATCC
H. parasuis	ATCC 19417^T	ATCC

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RESULTS

DNA-DNA hybridization. The DNA-DNA relatedness data are shown in Table 2. Low levels of DNA relatedness were detected between representative strains of the five taxa examined in this study (the Minor group and taxa C, D, E, and F) and the type strains of the type species of the genera *Actinobacillus*, *Pasteurella*, and *Haemophilus*. Each of the five taxa exhibited only 6 to 33% DNA relatedness with *A. lignieresii*, 4 to 15% DNA relatedness with *P. multocida*, and 3 to 14% DNA relatedness with *H. influenzae*. Low levels of DNA relatedness were also detected between the five taxa and the type strains of *A. pleuropneumoniae* (levels of relatedness, 5 to 20%), *A. suis* (3 to 23%), and *H. parasuis* (6 to 39%).

Six of the eight strains in the Minor group formed a homogeneous group (DNA group I) (Table 2). When labeled DNA from one of the six strains (strain NM305^T) was used, these strains were 80 to 98% related, with ΔT_m values of 2.3°C or less. The remaining two strains (strains 202 and 565) were only distantly related to each other (levels of DNA relatedness, 39 to 49%) and were related to the DNA group I strains to varying degrees. Thus, strain 202 exhibited 58 to 77% DNA relatedness to the DNA group I strains (ΔT_m values, $\geq$5.6°C), and strain 565 exhibited 38 to 48% DNA relatedness to the DNA group I strains. The DNA group I strains, as well as strains 202 and 565, exhibited little or no DNA relatedness (levels of similarity, 1 to 26%) to members of taxa C, D, E, and F.

The two taxon C strains formed a homogeneous group and exhibited only 4 to 19% relatedness to members of the Minor group and taxa D, E, and F.

The two taxon E strains (strains 20b and 30sp) clustered together with three taxon D strains (DNA group II) (Table 2). The DNAs of these strains were 71 to 100% related, with ΔT_m values of less than 3.6°C. Two other taxon D strains (DNA group III) (Table 2), which were 84% related to each other with a ΔT_m of 3.9°C, exhibited 47 to 59% DNA relatedness to DNA group II strains. Taxon D and E strains exhibited little or no DNA relatedness (levels of DNA relatedness, 1 to 23%) to members of the Minor group and taxa C and F.

Four taxon F strains were 66 to 72% related to each other, with ΔT_m values of 4.0°C (DNA group IV) (Table 2). However, strain F7 was more distantly related to this group (levels of DNA relatedness, 38 to 50%). DNA group IV strains and strain F7 exhibited little or no DNA relatedness (levels of DNA relatedness, 5 to 26%) to members of the Minor group and taxa C, D, and E.

16S rDNA sequencing. On the basis of the DNA-DNA hybridization results (Table 2), the following three strains were selected for further investigation by 16S rDNA sequencing: strains NM305^T (Minor group, DNA group I), NM319^T (taxa D and E, DNA group II), and 46KC2^T (taxon F, DNA group IV). The sequence of approximately 95% of the gene encoding 16S rRNA was determined for each of these strains. Table 3 is a similarity matrix based on the 16S rDNA sequences of these strains, other V factor-dependent species, and representatives of five of the seven phylogenetic groups in the family *Pasteurellaceae* as described by Dewhirst et al. (7). The 16S rDNA sequences differed from each other at 54 to 73 base positions. A dendrogram was constructed from the similarity matrix (Fig. 1). The tree generated was in agreement with previously published phylogenetic trees consisting of seven groups (6, 7). Strain NM305^T fell into group 4B, which consisted of Bisgaard taxa 8 and 9, *Haemophilus* Minor group strain 202, *Haemophilus paraphrohaemolyticus*, *Haemophilus ducreyi*, *Pasteurella bettyae*, *Pasteurella haemolyticus*, and Bisgaard taxon 5 (7). The closest relative of strain NM305^T was Minor group strain 202 (level of sequence similarity, 97.8%). Strains NM319^T and 46KC2^T both fell into group 3C, which consisted of *Haemophilus haemoglobinophilus*, Bisgaard taxon 7, *Haemophilus paracuniculus*, *Actinobacillus capsulatus*, *Pasteurella trehalosii*, and two *H. parasuis* strains (6). The closest relatives of strain 46KC2^T were the two strains of *H. parasuis* (levels of sequence similarity, 97.4 to 97.7%). The closest relatives of strain NM319^T were one group consisting of strain 46KC2^T and the two *H. parasuis* strains and another group consisting of strain NM305^T and Minor group strain 202 (levels of sequence similarity, 95.5 to 96.6%).

G+C contents of DNAs. DNAs extracted from strains NM305^T, NM319^T, and 46KC2^T had G+C contents of 38.2, 41.4, and 35.5 mol%, respectively.

Biochemical characteristics. Table 4 shows the differential characteristics of the V factor-dependent porcine species; the data for these characteristics were obtained from reference 15.

DISCUSSION

On the basis of DNA-DNA hybridization and 16S rDNA sequencing results, the Minor group and taxa C, D plus E, and F clearly represent new species belonging to the family *Pasteurellaceae*. The results of the DNA-DNA hybridization experiments revealed that, with one exception, the five taxa previously delineated by biochemical characteristics (15) were only distantly related to each other. The single exception was the three taxon D strains and two taxon E strains which belonged to a single DNA relatedness group (DNA group II). The taxon E strains can be distinguished phenotypically from the taxon D strains by their negative or weak reactions in most biochemical tests and by their poor growth both on solid media and in liquid media (15). The high levels of DNA relatedness between the taxon E strains and some taxon D strains supported our previous assumption that taxon E strains are nearly identical to taxon D strains (15). Our results corroborate those of several previous studies. Borr et al. (1) found that the levels

TABLE 2. Levels of DNA relatedness among porcine members of the *Pasteurellaceae* and the type species of the genera *Haemophilus*, *Actinobacillus*, and *Pasteurella*

Taxon	Homology group	Source of unlabeled DNA	% Relative binding at 60°C with labeled DNA from strain:											
			202	565	NM305^T	5015	NM319^T	30sp	20b	27Kb3	K13	46KC2^T	K1	F7
Minor group		202	100	39	66 (6.1)[a]	9	12			12		19		
		565	49	100	38	7	8			9		14		19
	I	52T3a	69 (6.6)	48	98 (2.3)	9	13			16		22		
	I	273	77 (5.6)	41	93	4	16			22		26		
	I	559	63	40	85	4	8			10		16		
	I	NM305^T	60	48	100	7	16			9		19		20
	I	562	62	38	86 (0.5)	6	10			8		20		
	I	566	58	42	80 (0.1)	9	13			12		16		
Taxon C		5015	7			100	9			11		10	6	7
		5111	5			111	7			13		9		
Taxa D and E	II	NM319^T	12			12	100	90 (3.6)	91	49 (7.8)	59	15	13	10
	II	30sp	1			12		100	79	39	52	8		
	II	20b	4			14			100	54		10		
	II	K12	14			10	71(0.8)	82			52	13		
	II	596	17			8	76(0.8)	108			48	8		
	III	27Kb3	13			19	50			100	84 (3.9)	11	23	11
	III	K13	11			17	47				100	5	17	10
Taxon F		F7	20			6	8			14		41	50	100
	IV	46KC2^T	14			13	11		11	10		100		41
	IV	F6	16			18	11		18	12		64 (4.0)	70	39
	IV	K1	23	5	8	10	6			6		66	100	38
	IV	K4	18			7	15			13		67	72(4.0)	42
		A. pleuropneumoniae	15	11	7	8	5		11	8		16	20	20
		A. lignieresii	16	16	33	9	6		7	11		13	19	15
		A. suis	18	18	3	11	6		6	7		18	23	16
		P. multocida	7	6	10	15	4		9	7		13	6	8
		H. influenzae	8	3	9	12	7		12	8		9	14	8
		H. parasuis	13		20	10	6		20	8		39		30

[a] The values in parentheses are ΔT_m values (in degrees Celsius).

of DNA relatedness between the Minor group and *A. pleuropneumoniae* and *H. parasuis* were less than 20%, and Morozumi et al. (16) reported that taxon C strains exhibited less than 13% DNA relatedness to *H. parasuis* and less than 10% DNA relatedness to *A. pleuropneumoniae*. Furthermore, Dewhirst et al. (6, 7) reported that the Minor group (strain 202) and taxon C could be clearly differentiated from other species of the family *Pasteurellaceae* on the basis of 16S rDNA sequence data.

According to recently published recommendations of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics (23), a species includes strains that exhibit approximately 70% or greater DNA-DNA relatedness and ΔT_m values of 5°C or less. In addition, phenotypic characteristics should be consistent with conclusions based on this definition, as stated previously by other workers (22). In the present work, intrageneric variations in DNA relatedness and ΔT_m values were observed for the Minor group, taxa D plus E, and taxon F (Table 2). The values obtained for DNA group I and Minor group strain 202 are at the borderline of species level values (levels of DNA relatedness, 58 to 77%; ΔT_m values, less than 6.6°C), and the values obtained for DNA group I and strain 565, for DNA groups II and III (taxa D and E), and for DNA group IV and taxon F strain F7 are less than species level values (levels of DNA relatedness, 38 to 50%). However, there were no phenotypic characteristics that delineated these strains, and thus, strains 202 and 565 were provisionally assigned to DNA group I, DNA group III was provisionally assigned to DNA group II, and strain F7 was provisionally assigned to DNA group IV.

Until recently, the genus *Haemophilus* was differentiated from the rest of the family *Pasteurellaceae* by its requirement for either one or both of two growth factors, X factor (hemin) and V factor (NAD or NADP). However, the results of DNA relatedness studies and 16S rDNA sequence analyses have challenged this definition of the genus *Haemophilus*. On the basis of the results of DNA relatedness studies, *Haemophilus pleuropneumoniae*, which is composed of V factor-dependent and V factor-independent biotypes, has been transferred to the genus *Actinobacillus* as *A. pleuropneumoniae* (20). Similarly, *Haemophilus avium*, which is V factor dependent, has been transferred to the genus *Pasteurella* (18). In addition, it has been suggested that *H. parasuis* does not belong to any of the three genera now placed in the family *Pasteurellaceae* (1, 4) because of low DNA-DNA hybridization values. Furthermore, on the basis of 16S rDNA sequence analysis data, V factor-dependent species have been shown to be distributed throughout the family (6, 7). These rearrangements and the remaining questions concerning the taxonomic affiliations of existing species reflect the problems in defining natural genera in the family *Pasteurellaceae*. Although new genera may be warranted to accommodate species such as *H. parasuis*, *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus segnis*, and others, the natural boundaries for such genera are not easily defined. Without such a basis reorganization may cause unnecessary confusion and frustration in clinical micro-

TABLE 3. Similarity matrix

Taxon	% Similarity^a																									
	Actinobacillus actinomycetemcomitans	*Haemophilus aphrophilus*	*Haemophilus segnis*	*Haemophilus haemolyticus*	*Haemophilus influenzae*	*Haemophilus aegypticus*	*Actinobacillus equuli*	*Actinobacillus suis*	*Actinobacillus ureae*	*Actinobacillus pleuropneumoniae*	*Actinobacillus lignieresii*	*Actinobacillus minor*	*Haemophilus* "Minor group" sp. strain 202	*Actinobacillus porcinus*	*Actinobacillus indolicus*	*Haemophilus parasuis* NCTC 4557^T	*Haemophilus parasuis* Nagasaki	*Pasteurella* species A	*Haemophilus* taxon C	*Pasteurella gallinarum*	*Haemophilus paragallinarum*	*Pasteurella canis*	*Pasteurella dagmatis*	*Pasteurella multocida*	Bisgaard taxon 14	*Pasteurella testudinis*
Actinobacillus actinomycetemcomitans		94.4	94.2	94.4	95.1	95.2	92.2	92.3	92.5	92.4	92.4	93.0	93.7	93.9	93.1	93.6	93.6	93.3	93.4	92.1	92.4	93.3	93.5	93.7	91.4	91.1
Haemophilus aphrophilus	94.2		97.1	95.7	94.8	94.9	94.3	94.5	94.9	94.2	94.2	94.7	93.9	94.2	94.4	94.6	94.7	93.2	93.1	93.5	93.5	93.8	94.7	93.6	91.2	91.2
Haemophilus segnis	94.0	97.0		96.6	94.8	95.0	94.7	94.6	95.0	94.9	94.9	94.7	94.4	94.2	94.8	94.3	94.8	93.3	93.1	93.6	93.4	93.8	94.8	94.2	92.3	91.3
Haemophilus haemolyticus	94.2	95.5	96.5		96.3	96.5	95.8	95.7	96.2	95.4	95.4	95.5	94.9	95.5	95.3	95.2	94.2	94.2	94.6	94.5	94.5	95.4	95.2	91.8	91.5	
Haemophilus influenzae	95.0	94.7	94.6	96.2		99.4	94.0	94.1	94.3	94.0	95.1	95.3	95.4	94.3	95.4	95.6	94.2	94.3	93.6	94.0	94.9	94.5	95.2	92.4	92.4	
Haemophilus aegyptius	95.1	94.7	94.8	96.5	99.4		93.9	93.9	94.1	93.8	93.8	94.9	95.1	95.3	94.4	95.3	95.7	94.1	94.1	93.5	94.0	94.6	94.4	95.1	92.4	92.2
Actinobacillus equuli	91.8	94.1	94.5	95.7	93.8	93.6		99.8	98.9	98.2	98.3	95.7	95.7	93.7	93.9	94.0	94.0	92.7	92.6	93.0	93.0	93.6	94.2	93.9	91.7	91.8
Actinobacillus suis	91.9	94.3	94.4	95.6	93.8	93.7	99.8		98.9	98.1	98.3	95.7	95.7	93.8	93.9	93.8	93.8	92.7	92.6	92.9	93.0	93.7	94.3	93.9	91.7	91.8
Actinobacillus ureae	92.1	94.8	94.8	96.1	94.0	93.8	98.9	98.9		98.2	98.3	96.2	96.1	94.4	94.3	94.1	94.1	93.0	93.0	93.3	93.3	93.8	94.5	94.2	91.6	92.0
Actinobacillus pleuropneumoniae	92.0	93.9	94.7	95.2	93.7	93.5	98.2	98.1	98.2		99.9	96.0	95.7	94.9	93.9	93.5	94.3	93.1	93.0	93.4	93.7	93.6	94.5	94.2	91.5	92.3
Actinobacillus lignieresii	92.0	94.0	94.7	95.3	93.8	93.6	98.3	98.2	98.2	99.9		96.0	95.7	94.9	93.9	93.4	94.3	93.1	93.1	93.4	93.8	93.7	94.6	94.2	91.6	92.3
Actinobacillus minor	92.6	94.5	94.5	95.3	94.9	94.7	95.6	95.6	96.1	95.9	95.9		97.8	96.7	96.6	96.4	96.7	94.1	94.4	94.4	94.3	94.8	95.8	95.1	91.6	92.4
Haemophilus "Minor group" sp. strain 202	93.4	93.7	94.1	95.3	95.1	94.9	95.6	95.6	96.0	95.6	95.6	97.8		96.3	95.9	96.3	96.2	95.0	94.8	94.0	94.0	95.1	95.2	95.7	92.2	93.1
Actinobacillus porcinus	93.7	94.0	94.0	94.7	95.3	95.2	93.5	93.5	94.1	94.7	94.7	96.6	96.2		95.6	96.6	96.6	94.7	95.0	94.2	94.5	95.4	95.2	95.6	92.1	92.9
Actinobacillus indolicus	92.8	94.2	94.6	95.3	94.1	94.2	93.7	93.7	94.1	93.7	93.6	96.5	95.7	95.5		97.4	97.7	94.5	94.3	94.5	94.3	94.6	95.8	95.3	91.4	91.1
Haemophilus parasuis NCTC 4557^T	93.3	94.4	94.1	95.1	95.2	95.2	93.7	93.5	93.9	93.2	93.1	96.3	96.3	96.5	97.4		98.4	95.1	94.9	94.2	94.3	95.4	95.2	95.8	91.7	92.2
Haemophilus parasuis Nagasaki	93.3	94.5	94.6	95.1	95.5	95.6	93.7	93.5	93.9	94.1	94.0	96.6	96.1	96.5	97.6	98.4		95.1	95.0	94.8	94.5	95.7	95.6	96.0	91.9	92.1
Pasteurella species A	93.0	92.9	93.0	94.0	94.0	93.9	92.3	92.3	92.7	92.8	92.8	93.8	94.8	94.5	94.3	94.9	94.9		99.3	98.3	97.5	94.1	94.3	94.7	91.1	92.3
Haemophilus taxon C	93.1	92.8	92.8	93.9	94.0	93.9	92.3	92.3	92.6	92.7	92.8	94.2	94.6	94.8	94.1	94.7	94.8	99.3		98.6	97.4	94.2	94.4	94.6	91.1	92.6
Pasteurella gallinarum	91.7	93.2	93.3	94.4	93.3	93.2	92.6	92.6	93.0	93.1	93.1	94.1	93.7	93.9	94.3	94.0	94.6	98.2	98.6		98.2	93.8	94.4	94.1	90.8	91.9
Haemophilus paragallinarum	92.0	93.2	93.1	94.3	93.7	93.7	92.6	92.6	93.0	94.5	94.5	94.1	93.7	94.3	94.1	94.1	94.3	97.5	97.3	98.2		93.7	94.4	94.2	90.7	91.9
Pasteurella canis	92.9	93.5	93.5	94.3	94.7	94.4	93.4	93.4	93.5	93.3	93.5	94.7	94.9	95.3	94.4	95.2	95.6	93.9	94.0	93.5	93.5		98.4	98.3	92.9	92.4
Pasteurella dagmatis	93.2	94.5	94.6	95.2	94.3	94.1	94.0	94.1	94.3	94.3	94.4	95.7	95.0	95.1	95.7	95.0	95.4	94.1	94.1	94.2	94.2	98.4		98.0	92.8	92.0
Pasteurella multocida	93.4	93.4	93.9	95.1	95.0	94.9	93.7	93.6	93.9	93.9	94.0	94.9	95.5	95.4	95.2	95.6	95.9	94.5	94.4	93.9	93.9	98.2	98.0		93.5	92.8
Bisgaard taxon 14	90.9	90.7	91.9	91.3	92.0	92.0	91.2	91.2	91.1	91.0	91.1	91.1	91.8	91.7	90.8	91.2	91.4	90.6	90.6	90.1	90.1	92.6	92.6	93.2		94.9
Pasteurella testudinis	90.5	90.7	90.7	91.0	92.0	91.8	91.3	91.3	91.6	91.9	91.8	92.0	92.7	92.5	90.6	91.8	91.7	91.9	92.2	91.4	91.5	91.9	91.6	92.5	94.7	

[a] The values on the upper right are levels of similarity, and the values on the lower left are levels of similarity corrected for multiple base changes.

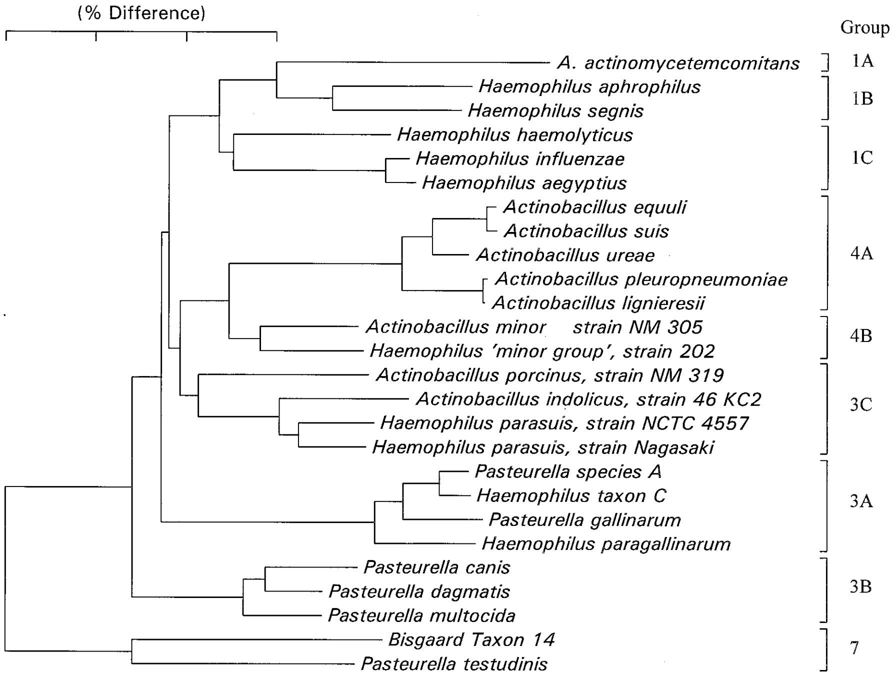

FIG. 1. Dendrogram showing the phylogenetic relationships derived from levels of 16S rRNA sequence similarity for porcine species belonging to the family *Pasteurellaceae* and representatives of each of the seven clusters described previously (6). Bar = 3% difference in nucleotide sequence.

biology. As we doubt that new developments in technology will solve this problem, which is basically a reflection of the population structure of the family *Pasteurellaceae*, we chose to describe the taxa examined in this study as three new species of the genus *Actinobacillus*. The genus allocation is based on the 16S rDNA sequence data (Table 3 and Fig. 1), which show that the three new species are more closely related to the type species of the genus *Actinobacillus* than to the type species of the two other genera in the family. This is a pragmatic solution based on the considerations discussed above. Because of the limited number of taxon C strains (four strains), we will not formally name this species until more strains are isolated and characterized.

Description of *Actinobacillus minor* sp. nov. *Actinobacillus minor* (mi'nor. L. comp. adj. *minor*, less, smaller, referring to the name of this taxon used previously [15]). Cells are gram-negative small rods (length, 1.6 to 2.4 μm). They are V factor dependent, X factor independent, nonmotile, nonhemolytic, urease positive, and indole negative. Lysine and ornithine are not decarboxylated. Ferments glucose, mannose, maltose, sucrose, and raffinose. Does not ferment arabinose, inulin, esculin, mannitol, sorbitol, and inositol. Colonies on chocolate agar

TABLE 4. Differential characteristics of porcine V factor-dependent members of the *Pasteurellaceae* species[a]

Characteristic	*A. minor* (*n* = 23)	*A. porcinus* (*n* = 32)	*A. indolicus* (*n* = 28)	*A. pleuropneumoniae* (*n* = 17)	*H. parasuis* (*n* = 28)	Taxon C (*n* = 4)
V factor requirement	+ (+)[b]	d (+)[c]	+ (+)	+ (+)	+ (+)	+
Hemolysis	− (−)	− (−)	− (−)	d (+)	− (−)	−
CAMP	− (−)	− (−)	− (−)	+ (+)	− (−)	−
Urease	+ (+)	− (−)	− (−)	+ (+)	− (−)	−
Indole	− (−)	− (−)	+ (+)	− (−)	− (−)	−
Catalase	− (−)	− (−)	+ (+)	d (−)	+ (+)	+
ONPG[d]	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	−
α-Fucosidase	− (−)	d (+)	d (+)	− (−)	+ (+)	−
Fermentation of:						
Arabinose	− (−)	d (−)	− (−)	− (−)	− (−)	+
Raffinose	+ (+)	d (+)	+ (+)	− (−)	− (−)	+
Lactose	+ (+)	d (+)	d (−)	− (−)	− (−)	−
Mannitol	− (−)	d (+)	− (−)	+ (+)	− (−)	−

[a] Data from reference 15.

[b] +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; d, 11 to 89% of the strains are positive. The reactions in parentheses are the reactions of the type strains (*A. minor* NM305, *A. porcinus* NM319, *A. indolicus* 46KC2, *A. pleuropneumoniae* Shope 4074, and *H. parasuis* NCTC 4557.

[c] Four of the *A. porcinus* strains did not grow on agar plates, and thus V factor dependence was not determined. All of the remaining *A. porcinus* strains are V factor dependent.

[d] ONPG, *o* nitrophenyl-β-D-galactopyranoside.

are smooth, greyish, and approximately 0.8 mm in diameter after 48 h of incubation. Biochemical characteristics important for identification are shown in Table 4. Belongs to the resident microflora of the porcine upper respiratory tract. The G+C content of the DNA is 38.2 mol%. The type strain is strain NM305.

Description of *Actinobacillus porcinus* sp. nov. *Actinobacillus porcinus* (por.cin′ us. M. L. adj. *porcinus*, pertaining to pigs, hogs). Cells are gram-negative small rods (length, 0.2 to 2.4 μm). They are V factor dependent, X factor independent, nonmotile, nonhemolytic, and urease, catalase, and indole negative. Lysine and ornithine are not decarboxylated. Fermentation of most carbohydrates is variable. Does not ferment inulin, esculin, and salicin. Colonies on chocolate agar are smooth and translucent to greyish. While some isolates grow poorly on chocolate agar and their colonies are only 0.2 mm in diameter, others grow readily on chocolate agar and form colonies that are approximately 0.6 mm in diameter after 48 h of incubation. Biochemical characteristics important for identification are shown in Table 4. Belongs to the resident microflora of the porcine upper respiratory tract. The G+C content of the DNA is 41.4 mol%. The type strain is strain NM319.

Description of *Actinobacillus indolicus* sp. nov. *Actinobacillus indolicus* (in.dol′i.cus. M.L. adj. *indolicus*, pertaining to indole, which is formed by the organism). Cells are gram-negative small rods (length, 0.8 to 2.4 μm). They are V factor dependent, X factor independent, nonmotile, nonhemolytic, urease negative, and catalase and indole positive. Lysine and ornithine are not decarboxylated. Ferments galactose, glucose, mannose, maltose, and sucrose. Does not ferment arabinose, inulin, esculin, salicin, mannitol, sorbitol, and inositol. Colonies are greyish and opaque on chocolate agar and are 2 mm in diameter after 48 h of incubation. Cultures have a characteristic pungent smell. Biochemical characteristics important for identification are shown in Table 4. Belongs to the resident microflora of the porcine upper respiratory tract. The G+C content of the DNA is 35.5 mol%. The type strain is strain 46KC2.

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