Discrimination of Members of the Family *Pasteurellaceae* Based on Polyamine Patterns

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In a study of the classification of members of the family *Pasteurellaceae*, the polyamine patterns of 101 strains were analyzed. These strains included the type strains of species belonging to the genera *Actinobacillus*, *Haemophilus*, and *Pasteurella* and additional strains of selected species, as well as numerous unnamed strains. Members of the genus *Actinobacillus* sensu stricto were characterized by the presence of 1,3-diaminopropane as the predominant compound. In the majority of the species of the genus *Haemophilus* sensu stricto 1,3-diaminopropane was also the major compound in the polyamine pattern. In contrast, *Haemophilus intermedius* subsp. *gazogenes* and *Haemophilus parainfluenzae* were characterized by high levels of 1,3-diaminopropane, cadaverine, and putrescine. These results confirmed the findings of Dewhirst et al. (F. E. Dewhirst, B. J. Paster, I. Olsen, and G. J. Fraser, Zentralbl. Bakteriologie, Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 279:35–44, 1993), who demonstrated that *H. parainfluenzae* is phylogenetically only distantly related to the type species of the genus *Haemophilus*, *Haemophilus influenzae*. The phylogenetic diversity of the genus *Pasteurella* sensu stricto determined by Dewhirst et al. was also reflected to some extent by different polyamine patterns. The common characteristics found in *Pasteurella multocida*, *Pasteurella canis*, *Pasteurella dagmatis*, *Pasteurella stomatis*, and *Pasteurella* sp. strain B were high levels of putrescine and spermidine and the presence of the unusual triamine sym-norspermidine. *Pasteurella avium*, *Pasteurella gallinarum*, and *Pasteurella volantium* contained high concentrations of 1,3-diaminopropane and spermidine. *Pasteurella langaa* contained only high concentrations of 1,3-diaminopropane, and *Pasteurella anatis* was characterized by the presence of 1,3-diaminopropane as the predominant compound and high levels of putrescine and spermidine. Our data demonstrate that polyamine patterns are useful for discrimination within the family *Pasteurellaceae*.

The family *Pasteurellaceae* accommodates species classified in the genera *Pasteurella* Trevisan 1887 (31), *Actinobacillus* Brumpt 1910 (3), *Haemophilus* Winslow et al. 1917 (32), and *Lonepinella* Osawa et al. 1995 (24), as well as numerous unnamed strains. All members of the family are parasites of vertebrates, such as mammals, birds, and reptiles. Phylogenetically, the family is located in the gamma subclass of the *Proteobacteria* and clusters with facultatively anaerobic, gram-negative, chemoorganotrophic bacteria which are members of the families *Enterobacteriaceae*, *Aeromonadaceae*, and *Vibrionaceae* (5, 6, 33).

Extensive DNA-DNA hybridization studies have demonstrated the genetic relatedness of members of the family *Pasteurellaceae* and have resulted in the definition of three genera, the genera *Actinobacillus*, *Pasteurella*, and *Haemophilus* (20). Recent DNA-rRNA hybridization studies and 16S rRNA sequence analyses of members of the family *Pasteurellaceae* have revealed that there is great heterogeneity within this family (6, 7). Many recognized species of the three genera are genetically more closely related to the type species of another genus than to the type species of their own genus. For example, *Actinobacillus actinomycetemcomitans* clusters phylogenetically with *Haemophilus influenzae*; *Pasteurella bettyae*, *Pasteurella haemolytica*, *Haemophilus paraphrohaemolyticus*, and *Haemophilus ducreyi* cluster phylogenetically with *Actinobacillus lignieresii*; and *Haemophilus paragallinarum*, *Haemophilus haemoglobinophilus*, and *Haemophilus paracuniculus* cluster phylogenetically with *Pasteurella multocida*.

Sometimes no phylogenetic relationship between a species and any of the genera is found. In some cases, the results of DNA reassociation and 16S rRNA analyses somewhat controversially indicate that an organism belongs to different groups. *Actinobacillus capsulatus*, *Haemophilus parainfluenzae*, *Pasteurella langaa*, and *Pasteurella anatis* are considered members of their respective genera sensu stricto based on DNA reassociation data (20), but each of these taxa is phylogenetically only distantly related to the type species of its genus (7). However, it is obvious from the results of these investigations that the family *Pasteurellaceae* encompasses more than the four genera that are recognized at this time.

Differentiation based on physiological and biochemical data often poses problems, as has been demonstrated for members of the genera *Actinobacillus* and *Pasteurella*, which are intermixed as determined by physiological and biochemical data (11, 30). Also, the present definition of the genus *Haemophilus* as a taxon whose members require X factor (protoporphyrin or protoheme) and/or V factor (NAD or NADP), combined with other phenotypic traits does not correlate with DNA reassociation data (16). Due to the lack of clear-cut definitions of the genera *Actinobacillus*, *Pasteurella*, and *Haemophilus* (including phenotypic and genotypic data), proposing new genera in the family is difficult. Thus, other approaches for classification of members of the family *Pasteurellaceae* are needed.

Attempts to define taxa in the family *Pasteurellaceae* based on analyses of fatty acids and polar lipids have failed due to the homogeneity of the fatty acids and polar lipids in representatives of the genera *Actinobacillus*, *Haemophilus*, and *Pasteurella* (8, 9, 21). Whole-cell sugar analysis revealed heterogeneous

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patterns which allowed discrimination between groups or species (8, 9, 21); the importance of this characteristic for genus discrimination, however, has not been demonstrated so far. Another important approach for differentiating taxa within the family is quinone content analysis, but there are problems in interpretation caused by contradictory results obtained for several species, such as *A. actinomycetemcomitans*, *Haemophilus aphrophilus*, and *Pasteurella pneumotropica* (9, 15, 21). Thus, well-established chemotaxonomic approaches do not appear to be suitable for reclassification of taxa within the family *Pasteurellaceae*.

Analysis of polyamine patterns has been shown to be a powerful method for classifying gram-negative bacteria (1, 4, 12, 13). In the recent proposal for establishment of the genus *Lonepinella*, promising results were obtained by using polyamine analysis for differentiation within the family *Pasteurellaceae* (24). However, the polyamine patterns of only the type species of the four genera were analyzed. Extension of the polyamine approach to more strains belonging to the family *Pasteurellaceae* will be necessary to evaluate the usefulness of polyamine patterns for classification within this family. In this study we examined polyamine data for the type strains of species belonging to the genera *Haemophilus*, *Actinobacillus*, and *Pasteurella*; the organisms used were selected mainly on the basis of their known positions in the family, as shown by DNA-DNA hybridization, DNA-rRNA hybridization, and/or 16S rRNA sequence analyses. Also included were additional strains of several species, as well as many unnamed strains considered to be members of the family *Pasteurellaceae*.

MATERIALS AND METHODS

Strains used and culture conditions. A total number of 101 strains were included in this study (Tables 1 through 4). The majority of the strains were provided by W. Mannheim (Marburg, Germany). *A. lignieresii* 9/91, *Actinobacillus suis* A209/83, *P. haemolytica* A2683/81, and *P. multocida* H983/87 and H1161/87 were provided by G. Amsberg (Hannover, Germany). *Actinobacillus pleuropneumoniae* ATCC 27088^T and 56153 were donated by R. Nielsen (Copenhagen, Denmark). *A. pleuropneumoniae* N-273 and N-282 were obtained from E. Molnar (Budapest, Hungary). *A. pleuropneumoniae* PL2 and PL10 were provided by P. Kielstein (Jena, Germany), and *H. paragallinarum* xx36 and xx37 were provided by K.-H. Hinz (Hannover, Germany).

The cells were cultivated aerobically at 37°C on PPLO broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.001% NAD (Sigma Chemical Co., St. Louis, Mo.), 0.001% hemin (Sigma), and 1% IsoVital X Enrichment (Becton Dickinson Microbiology Systems, Cockeysville, Md.). *H. ducreyi* MCCM 00729^T was grown at 30°C. Cells were harvested in the logarithmic phase of growth when the optical density at 600 nm was approximately 70% of the maximum optical density at 600 nm determined for each strain.

Polyamine analysis. Polyamines were extracted as described previously (2). Approximately 40 mg of lyophilized cells was hydrolyzed in 1 ml of 0.2 N perchloric acid for 30 min at 100°C with occasional shaking. Each mixture contained the internal standard 1,8-diaminooctane (25 µmol/40 mg of cells). After extraction the samples were centrifuged, 0.2 ml of each supernatant was transferred to a tube containing 0.3 ml of an Na₂CO₃ solution (100 mg/ml), and 0.8 ml of a dansyl chloride solution (7.5 µg/ml in acetone) was added. The tube was closed tightly, and dansylation was performed for 20 min at 60°C. Subsequently, 0.1 ml of a proline solution (50 mg/ml) was added to bind the excess dansyl chloride during incubation for 10 min at 60°C. After cooling to 5°C, the polyamines were extracted with 0.1 ml of toluene with shaking. The upper phase was used for a high-performance liquid chromatography analysis. The high-performance liquid chromatography apparatus was equipped with two Waters model 510 pumps, a model U6K injector, a column oven, a spectrofluorometric detector (Jasco model 821-FP), and a reversed-phase column (250 by 4 mm; Hypersil octyldecyl silane; 5-µm particles). To separate the polyamines, a linear 40 to 85% acetonitrile-water gradient was applied for 35 min, followed by a linear 85 to 100% acetonitrile-water gradient applied for 15 min at 40°C. To detect dansylated polyamines, an excitation wavelength of 360 nm was used, and the emitted light was measured at 520 nm. Polyamine concentrations were calculated by using internal standards. Repeated analyses of selected samples demonstrated that the deviation for major compounds was less than 10%.

RESULTS AND DISCUSSION

An extensive study of the phylogeny of members of the *Pasteurellaceae* based on 16S rRNA sequence analyses demonstrated that the family can be divided into seven clusters and several subclusters (7). The type species of the genera *Actinobacillus*, *Haemophilus*, *Pasteurella*, and *Lonepinella* are each in a separate group, which demonstrates the phylogenetic distinctness of these taxa (7, 24). Below, the results of the polyamine analyses (Tables 1 through 4) are discussed according to the phylogenetic clustering of the strains (7), and strains whose phylogenetic positions are not known are discussed separately (Table 4).

Cluster 1. Cluster 1 is subdivided into three subclusters. The only representative of subcluster 1A is *A. actinomycetemcomitans*, which includes four strains (7). This species has been reclassified several times (27, 28). The only significant compound in the polyamine pattern of the type strain, *A. actinomycetemcomitans* MCCM 00145, was 1,3-diaminopropane. This pattern was also found in species belonging to subcluster 1B (*H. aphrophilus* MCCM 00203^T and *Haemophilus segnis* MCCM 01957^T) and subcluster 1C (*H. influenzae* MCCM 00591^T and *Haemophilus aegyptius* MCCM 02771^T). DNA re-association studies (20) have demonstrated that the type strain of the type species of the genus *Haemophilus*, *H. influenzae* (MCCM 00591), and the type strain of *H. aegyptius* (MCCM 02772) are closely related and are considered members of the genus *Haemophilus* sensu stricto, whereas *H. aphrophilus* MCCM 00203^T and *H. segnis* MCCM 01957^T are not included in the genus *Haemophilus* sensu stricto (20). These data may provide evidence that members of the genus *Haemophilus* are characterized by the presence of 1,3-diaminopropane as the predominant compound, while a different polyamine pattern indicates that an organism does not belong to this genus. This homogeneity in the polyamine patterns of the species belonging to cluster 1, in addition to the similar quinone compositions of these organisms (9), indicates that there is a close phylogenetic relationship and may support inclusion of *A. actinomycetemcomitans*, *H. aphrophilus*, and *H. segnis* in the genus *Haemophilus* sensu stricto.

Cluster 2. The polyamine patterns of the cluster 2, subcluster 2A organisms *Actinobacillus seminis* MCCM 00565^T, *Pasteurella mairii* MCCM 00633^T, and *Pasteurella aerogenes* MCCM 00402^T were analyzed (7). The polyamine patterns of the first two organisms resembled each other, whereas *P. aerogenes* MCCM 00402^T had a different polyamine pattern (Tables 1 and 3). The differences may be explained by the deep phylogenetic branching of *P. aerogenes* within the subcluster (7). Thus, the grouping of *P. aerogenes* with *P. mairii* MCCM 00633^T and *A. seminis* MCCM 00565^T in a genuslike structure needs to be confirmed by other data. These observations confirm that *P. aerogenes* MCCM 00402^T (19) should be excluded from the genus *Pasteurella* sensu stricto (see below) and also are not consistent with classification of *P. mairii* MCCM 00633^T in the genus *Pasteurella* sensu stricto and classification of *A. seminis* MCCM 00565^T in the genus *Actinobacillus*. The polyamine compositions of two strains that were obtained as *P. aerogenes*-like strain MCCM 01523 and *P. aerogenes* complex strain MCCM 01544 differed significantly from the polyamine compositions of *P. aerogenes* MCCM 00402^T and the species of the genus *Pasteurella* sensu stricto (Table 3); this finding demonstrates that these two strains cannot be considered members of *P. aerogenes* or the genus *Pasteurella* sensu stricto.

Cluster 3. Species belonging to the genus *Pasteurella* sensu stricto (19) fall into three of the four subclusters of cluster 3. The type species of the genus, *P. multocida*, is located in sub-

TABLE 1. Polyamine patterns of species of the genus *Actinobacillus*^a

Organism	Other designation(s)	Subcluster of Dewhurst's et al. ^b	Polyamine content (μmol/g [dry wt])							
			DAP	PUT	CAD	TYR	NSPD	SPD	HSPD	SPM
<i>A. actinomycetemcomitans</i> MCCM 00145 ^T	SSI P 229 ^T , NCTC 9710 ^T , ATCC 33384 ^T	1A	83.81 (97.6) ^c					1.05 (1.2)		0.99 (1.2)
<i>A. capsulatus</i> MCCM 02462 ^T	SSI P 243 ^T , NCTC 11408 ^T , CCUG 12396 ^T	3C	80.78 (97.1)	0.25 (0.3)			tr	0.71 (0.9)	tr	1.46 (1.8)
<i>A. equuli</i> MCCM 00142 ^T	SSI P 157 ^T , NCTC 8529 ^T , ATCC 19392 ^T	4A	110.87 (99.2)		0.09 (0.1)			0.06 (0.1)		0.79 (0.7)
<i>A. hominis</i> MCCM 00187 ^T	SSI P 578 ^T , NCTC 11529 ^T , CCUG 19800 ^T	4A	100.47 (92.8)	tr				4.07 (3.8)		3.67 (3.4)
<i>A. hominis</i> MCCM 00560			102.80 (95.9)				tr	1.23 (1.1)		3.11 (2.9)
<i>A. lignieresii</i> MCCM 00190 ^T	SSI P 151 ^T , NCTC 4189 ^T , CCUG 19795 ^T	4A	96.35 (96.7)	tr	tr		0.32 (0.3)	1.81 (1.8)		1.15 (1.2)
<i>A. lignieresii</i> 9/91			98.32 (99.8)	0.22 (0.2)				tr		
<i>A. pleuropneumoniae</i> MCCM 00339 ^T	ATCC 27088 ^T , CCM 5969 ^T , CCUG 12837 ^T	4A	71.89 (97.4)	tr				1.11 (1.5)		0.81 (1.1)
<i>A. pleuropneumoniae</i> (biovar 1, serotype 1)				tr		0.66 (2.0)		0.39 (1.2)		
<i>A. pleuropneumoniae</i> 56153 (biovar 1, serotype 11)			31.43 (96.8)	tr				tr		tr
<i>A. pleuropneumoniae</i> N-273 (biovar 2, serotype 1)			67.45 (100)					tr		tr
<i>A. pleuropneumoniae</i> N-282 (biovar 2, serotype 2)			58.05 (100)					tr		tr
<i>A. pleuropneumoniae</i> PL2 (biovar 2, serotype 2)			70.29 (100)					tr		
<i>A. pleuropneumoniae</i> PL10 (biovar 2, serotype 2)			91.12 (93.6)	tr				3.89 (4.0)		2.31
<i>A. rossii</i> MCCM 02461 ^T	SSI P 624 ^T , NCTC 10801 ^T , ATCC 27072 ^T		87.47 (94.7)	0.17 (0.2)	tr			1.63 (1.8)		3.10 (3.4)
" <i>A. salpingitidis</i> " MCCM 00280	NCTC 11314, CCM 5974, CCUG 23139	3D	40.77 (68.6)	0.92 (1.5)	0.35 (0.6)			14.29 (24.0)		3.11 (5.2)
" <i>A. salpingitidis</i> " MCCM 00924	M. Bisgaard 20.672/6		38.68 (72.2)	4.93 (9.2)				8.89 (16.6)		1.08 (2.0)
" <i>A. salpingitidis</i> " MCCM 00521	CCUG 28018, M. Bisgaard F 114		75.10 (75.8)	8.34 (8.4)	tr			11.56 (11.7)		4.10 (4.1)
<i>A. seminis</i> MCCM 00565 ^T	NCTC 10851 ^T , ATCC 15768 ^T	2A	10.80 (36.5)	8.79 (29.7)	4.14 (14.0)			1.67 (5.6)	tr	4.18 (14.1)
<i>A. suis</i> MCCM 02460 ^T	SSI P 635 ^T , CCUG 11624 ^T , CCM 5586 ^T	4A	78.42 (97.1)				tr	0.14 (0.2)	tr	2.17 (2.7)
<i>A. ureae</i> MCCM 00184 ^T	SSI P 161 ^T , NCTC 10219 ^T , ATCC 25976 ^T , CCUG 2139 ^T , Henriksen 3520/59 ^T	4A	62.50 (99.4)	tr		0.11 (0.2)		0.26 (0.4)	0.07 (0.1)	tr
<i>A. ureae</i> MCCM 02466	NCTC 10220		107.37 (99.5)	0.35 (0.3)				0.11 (0.1)		
<i>Actinobacillus</i> sp. strain MCCM 00398 (Bisgaard taxon 9)	ATCC 13376, CCM 5500, CCUG 24862	4B	31.13 (81.7)		0.33 (0.9)			5.70 (15.0)		0.94 (2.5)
Avian hemolytic <i>Actinobacillus</i> -like strain MCCM 01376 (Bisgaard taxon 26)	NCTC 11410, CCUG 28015, M. Bisgaard F-64		18.72 (75.8)	0.03 (0.1)				2.80 (11.3)		3.14 (12.7)
			89.39 (93.1)	3.12 (3.3)				3.49 (3.6)		

^a Abbreviations: DAP, 1,3-diaminopropane; PUT, putrescine; CAD, cadaverine; TYR, tyramine; NSPD, *syn*-homospemidine; SPD, spermidine; HSPD, *syn*-homospemidine; SPM, spermine; tr, trace (less than 0.05 mmol/g [dry weight]); ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; MCCM, Medical Culture Collection Marburg, Department of Medical Microbiology, Philips University, Marburg, Germany; NCTC, National Collection of Type Cultures, London, England; SSI, Statens Seruminstitut, Copenhagen, Denmark.

^b See reference 7.

^c The values in parentheses are percentages of the total polyamines.

TABLE 2. Variation in the polyamine patterns of selected members of the genus *Haemophilus*^a

Organism	Other designation(s)	Cluster or subcluster of Dewhirst's et al. ^b	Polyamine content (μmol/g [dry wt])						
			DAP	PUT	CAD	TYR	NSPD	SPD	SPM
<i>H. aegyptius</i> MCCM 02772 ^T	NCTC 8502 ^T , ATCC 11116 ^T	1C	35.56 (91.4) ^c	3.16 (8.2)	0.19 (0.5)			tr	tr
<i>H. aegyptius</i> MCCM 00678	NCTC 8134		28.51 (62.9)	10.11 (22.3)	6.67 (14.7)				
<i>H. aphrophilus</i> MCCM 00203 ^T	SSI P 801 ^T , NCTC 5906 ^T , ATCC 33389 ^T	1B	24.10 (98.2)	0.11 (0.4)	0.05 (0.2)			0.22 (0.9)	0.06 (0.2)
<i>H. ducreyi</i> MCCM 00729 ^T	NCTC 10945 ^T , ATCC 33940 ^T , CCUG 4438 ^T , CIP 54.2 ^T	4B	59.51 (79.1)	0.06 (0.1)	0.38 (0.5)		tr	8.65 (11.5)	6.68 (8.9)
" <i>H. felis</i> " MCCM 02738 ^T	ATCC 49733 ^T		0.43 (2.5)	12.03 (70.4)	0.95 (5.6)		3.03 (17.7)	0.64 (3.7)	tr
" <i>H. felis</i> " MCCM 02064	CCUG 30904		7.21 (33.4)	0.42 (1.9)			8.62 (40.0)	4.42 (20.5)	0.90 (4.2)
<i>H. influenzae</i> MCCM 00591 ^T (biovar II)	NCTC 8143 ^T , ATCC 33391 ^T , CCUG 15643 ^T	1C	55.08 (96.2)	0.76 (1.3)		0.54 (0.9)		0.79 (1.4)	0.08 (0.1)
<i>H. intermedius</i> subsp. <i>gazogenes</i> MCCM 02082	CCUG 15793		5.13 (29.1)	7.46 (42.4)	4.97 (28.2)			0.05 (0.3)	
<i>H. paracuniculus</i> MCCM 00703 ^T	ATCC 29986 ^T , CCUG 15644 ^T	3C	50.96 (71.6)	0.17 (0.2)	0.14 (0.2)		tr	6.61 (9.3)	0.6 (0.8)
<i>H. paragallinarum</i> MCCM 00300 ^T	NCTC 11296 ^T , ATCC 29545 ^T , CCUG 12835 ^T	3A	48.72 (84.9)	0.17 (0.3)	0.37 (0.6)		tr	5.21 (9.1)	tr
<i>H. paragallinarum</i> xx36			45.54 (73.7)	1.58 (2.6)	0.31 (0.5)			12.66 (20.5)	1.66 (2.7)
<i>H. paragallinarum</i> xx37			52.67 (70.6)	2.44 (3.3)	tr			18.40 (24.7)	1.09 (1.5)
<i>H. parahaemolyticus</i> MCCM 00504 ^T	NCTC 8479 ^T , ATCC 10014 ^T , CCUG 3716 ^T	4A	18.79 (86.7)					1.19 (5.5)	1.68 (7.8)
<i>H. parainfluenzae</i> MCCM 00486 ^T	NCTC 7857 ^T , ATCC 33392 ^T , CCUG 12836 ^T	6	14.85 (40.2)	7.87 (21.3)	14.21 (39.5)				
<i>H. parasuis</i> MCCM 02736 ^T	NCTC 4557 ^T , ATCC 19417 ^T , CCUG 3712 ^T	3C	41.01 (65.4)	20.33 (32.4)	0.27 (0.4)			1.06 (1.7)	
<i>H. segnis</i> MCCM 01957 ^T	NCTC 10977 ^T , ATCC 33393 ^T , CCUG 10787 ^T	1B	34.52 (99.7)					0.11 (0.3)	
Avian <i>Haemophilus</i> -like strain MCCM 01034			25.78 (62.2)	0.27 (0.7)	0.19 (0.5)			10.66 (25.7)	4.58 (11.0)
Avian <i>Haemophilus</i> -like strain MCCM 02795			0.14 (0.5)	3.96 (14.2)				21.68 (77.8)	2.10 (7.5)

^a CIP, Collection de l'Institut Pasteur, Paris, France. For other abbreviations see Table 1, footnote a.

^b See reference 7.

^c The values in parentheses are percentages of the total polyamines.

TABLE 3. Variation in the polyamine patterns of selected members of the genus *Pasteurella*^a

Organism	Other designation(s)	Cluster or subcluster of Dewhurst et al. ^b	Polyamine content (μmol/g [dry wt])							
			DAP	PUT	CAD	TYR	NSPD	SPD	HSPD	SPM
<i>P. aerogenes</i> MCCM 00402 ^T	SSI P 634 ^T , ATCC 27883 ^T , CCUG 9995 ^T	2A	0.49 (1.6) ^c	6.52 (21.2)	1.76 (5.7)	0.51 (1.7)		19.74 (64.2)		1.72 (5.6)
<i>P. aerogenes</i> -like strain MCCM 01523			38.27 (88.6)	3.44 (8.0)	1.33 (3.1)		tr	0.13 (0.3)		tr
<i>P. aerogenes</i> complex strain MCCM 01544			40.34 (91.0)	0.12 (0.3)	0.23 (0.5)			2.49 (5.6)		1.15 (2.6)
<i>P. anatis</i> MCCM 00140 ^T	NCTC 11413 ^T , ATCC 43329 ^T , CCUG 15563 ^T , M. Bisgaard F 149 ^T	3D	50.84 (56.0)	22.48 (24.8)	0.09 (0.1)			14.05 (15.5)		3.26 (3.6)
<i>P. avium</i> MCCM 00272 ^T	NCTC 11297 ^T , ATCC 29546 ^T , CCUG 12833 ^T	3A	45.38 (74.0)	1.74 (2.8)				12.08 (19.7)		2.11 (3.4)
<i>P. bettyae</i> MCCM 00450 ^T	SSI P 843 ^T , NCTC 10535 ^T , ATCC 23273 ^T , CCUG 2042 ^T	4B	48.21 (94.0)	0.52 (1.0)	0.14 (0.3)			2.14 (4.2)		0.29 (0.6)
<i>P. caballi</i> MCCM 00841 ^T	ATCC 49197 ^T		67.72 (88.7)	5.98 (7.8)	2.59 (3.4)			0.07 (0.1)		
<i>P. canis</i> MCCM 00045 ^T	SSI P 293 ^T , NCTC 11621 ^T , ATCC 43326 ^T , CCUG 12400 ^T	3B	0.36 (1.0)	12.78 (34.8)	2.72 (7.4)		0.77 (2.1)	18.87 (51.3)		1.27 (3.5)
<i>P. canis</i> biotype 1 strain MCCM 00047			7.40 (12.2)	18.16 (29.9)	3.93 (6.5)		1.77 (2.9)	26.69 (44.0)		2.71 (4.5)
<i>P. canis</i> MCCM 00927			0.41 (1.5)	17.09 (63.0)	3.59 (13.2)		1.36 (5.0)	3.92 (14.4)		0.76 (2.8)
<i>P. dagmatis</i> MCCM 00024 ^T	SSI P 418 ^T , NCTC 11617 ^T , ATCC 43325 ^T , CCUG 12397 ^T	3B	19.65 (33.5)	26.95 (46.0)	0.27 (0.5)		3.45 (5.9)	8.19 (14.0)		0.07 (0.1)
<i>P. dagmatis</i> MCCM 00558			5.15 (6.6)	51.12 (65.0)	tr		4.52 (5.7)	17.08 (21.7)		0.74 (0.9)
<i>P. gallinarum</i> MCCM 00031 ^T	SSI P 287 ^T , NCTC 11188 ^T , ATCC 13361 ^T , CCUG 12391 ^T	3A	37.48 (76.2)	0.08 (0.2)	0.12 (0.2)			7.18 (14.6)		4.32 (8.8)
<i>P. gallinarum</i> MCCM 00033			4.69 (7.4)	18.73 (29.4)	0.61 (1.0)		5.48 (8.6)	31.40 (49.3)		2.75 (4.3)
<i>P. granulomatis</i> MCCM 00576 ^T	SSI P 1135 ^T		69.86 (95.2)	0.59 (0.8)	0.12 (0.2)			1.26 (1.7)		1.58 (2.2)
<i>P. haemolytica</i> MCCM 00395 ^T	SSI P 586 ^T , NCTC 9380 ^T , ATCC 33396 ^T , CCUG 408 ^T	4B	67.87 (93.9)	0.76 (1.1)				1.86 (2.6)		1.79 (2.5)
<i>P. haemolytica</i> MCCM 00388 (third taxon)	SSI P 731		61.74 (77.6)	1.48 (1.9)	0.25 (0.3)			9.20 (11.6)		6.87 (8.6)
<i>P. haemolytica</i> MCCM 00594 (fourth taxon)	SSI P 955		56.90 (78.9)	1.09 (1.5)	0.29 (0.4)			4.61 (6.4)		9.26 (12.8)
<i>P. haemolytica</i> A2683			90.34 (97.2)					1.16 (1.2)		1.45 (1.6)
<i>P. langaa</i> MCCM 00120 ^T	NCTC 11411 ^T , ATCC 43328 ^T , CCUG 15566 ^T	3D	53.08 (95.5)	1.49 (2.7)				1.04 (1.9)	tr	tr
" <i>P. leonis</i> " MCCM 00659	SSI P 876		15.24 (24.6)	29.27 (47.3)	0.60 (1.0)		11.08 (17.9)	5.20 (8.4)		0.53 (0.9)
<i>P. mairii</i> MCCM 00633 ^T	SSI P 637 ^T , NCTC 10699 ^T , CCUG 27189 ^T	2A	49.81 (47.7)	44.89 (43.0)	9.48 (9.10)			0.10 (0.1)		0.12 (0.1)
<i>P. multocida</i> subsp. <i>multocida</i> MCCM 00005 ^{Td}	SSI P 427 ^T , NCTC 10322 ^T , ATCC 43137 ^T , CCUG 17976 ^T	3B		52.71 (72.5)	6.68 (9.2)		1.38 (1.9)	11.57 (15.9)		0.35 (0.5)
<i>P. multocida</i> subsp. <i>gallicida</i> MCCM 00021 ^{Td}	SSI P 427 ^T , NCTC 10204 ^T , CCUG 17979 ^T		0.97 (1.2)	56.23 (67.0)	2.25 (2.7)		2.84 (3.4)	19.30 (23.0)		2.39 (2.8)
<i>P. multocida</i> subsp. <i>septica</i> MCCM 00008 ^{Td}	CCUG 19977 ^T , CIP A 125 ^T		1.19 (5.2)	10.82 (46.9)	0.31 (1.3)		1.71 (7.4)	7.20 (31.2)		1.82 (7.9)
<i>P. multocida</i> H 1161/87				23.30 (81.7)	0.11 (0.4)		0.26 (0.9)	4.31 (15.1)		0.55 (1.9)
<i>P. multocida</i> H 983/87			4.87 (7.4)	8.76 (13.3)	1.32 (2.0)		5.82 (8.8)	41.82 (63.5)		3.27 (5.0)
<i>P. pneumotropica</i> type Jawetz strain MCCM 00234 ^T	SSI P 421 ^T , NCTC 8141 ^T , ATCC 35149 ^T , CCUG 12398 ^T	5	45.04 (64.3)	22.71 (32.5)	2.09 (3.0)			0.12 (0.2)		0.10 (0.1)
<i>P. pneumotropica</i> complex strain MCCM 01911	M. Kilian HK 366		38.16 (87.2)	4.24 (9.7)	0.92 (2.1)			0.06 (0.1)		0.39 (0.9)
<i>P. pneumotropica</i> type Heyl strain MCCM 00237	SSI P 309, ATCC 12555, CCUG 998		51.07 (55.6)	9.20 (10.0)	31.52 (34.3)					tr
<i>P. pneumotropica</i> type Heyl strain MCCM 00253			33.13 (72.9)	1.67 (3.7)	10.25 (22.6)			0.10 (0.2)		0.30 (0.7)
<i>Pasteurella</i> species A strain MCCM 00072			32.60 (68.5)	0.17 (0.4)	0.05 (0.1)			11.09 (23.3)		3.66 (7.7)
<i>Pasteurella</i> species B strain MCCM 00102	SSI P 683, CCUG 19794	3B	3.02 (5.5)	10.64 (19.3)	1.32 (2.4)		2.20 (4.0)	33.31 (60.5)		4.61 (8.4)
<i>P. stomatis</i> MCCM 00100 ^T	NCTC 11623 ^T , ATCC 43327 ^T , CCUG 17979 ^T	3B	2.59 (4.4)	27.11 (45.7)	0.26 (0.4)	tr	3.55 (6.0)	23.34 (39.4)		2.43 (4.1)
<i>P. stomatis</i> MCCM 00104	NCTC 11624		tr	28.40 (58.1)	0.10 (0.2)		1.85 (3.8)	16.82 (34.4)		1.70 (3.5)
<i>P. stomatis</i> MCCM 00490	SSI P 294		1.74 (4.6)	18.93 (49.9)	0.10 (0.3)	tr	0.96 (2.5)	14.44 (38.1)		1.78 (4.7)
<i>P. testudinis</i> MCCM 00484 ^T	ATCC 33688 ^T , CCUG 19802 ^T	7	0.47 (0.7)	16.80 (26.5)	0.27 (0.4)			40.83 (64.5)		4.95 (7.8)
<i>P. testudinis</i> MCCM 00400			0.47 (0.9)	6.64 (12.6)	0.11 (0.2)			42.20 (80.4)		3.10 (5.9)
<i>P. trehalosi</i> MCCM 02084 ^T	NCTC 10370 ^T , ATCC 29703 ^T	3C	62.86 (96.7)					1.81 (2.8)		0.33 (0.5)
<i>P. volantium</i> MCCM 02204 ^T	NCTC 3438 ^T , ATCC 14385 ^T , CCUG 3713 ^T	3A	29.95 (74.8)		tr			6.79 (17.0)		3.30 (8.2)
<i>Pasteurella</i> -like strain MCCM 00658	NCTC 11051		31.00 (93.0)					1.26 (3.8)		1.06 (3.2)
<i>Pasteurella</i> -like strain MCCM 00466	Hamster group 1 I. Kunstyr 246		49.45 (92.9)	3.10 (5.8)	0.11 (0.2)			0.42 (0.8)	tr	0.17 (0.3)
<i>Pasteurella</i> -like strain MCCM 02120	Hamster group 2 I. Kunstyr 507		42.66 (94.3)	0.85 (1.9)				0.14 (0.3)		1.59 (3.5)
<i>Pasteurella</i> -like strain MCCM 01664	J. Perrin D-578		19.75 (24.9)	48.65 (61.5)	2.91 (3.7)			4.21 (5.3)		3.64 (4.6)

^a For abbreviations see Table 1, footnote a, and Table 2, footnote a.

^b See reference 7.

^c The values in parentheses are percentages of the total polyamines.

^d In addition, a small peak was detected in front of spermidine; this peak was assumed to be *sym*-norspermine.

TABLE 4. Polyamine patterns of "Histophilus ovis" and unnamed strains placed in the family Pasteurellaceae^a

Organism	Other designation(s)	Cluster or subcluster of Dewhirst et al. ^b	Polyamine content (μmol/g [dry wt])						
			DAP	POT	CAD	NSPD	SPD	SPM	
"Histophilus ovis" MCCM 00330	E. L. Biberstein 902	3D	50.78 (88.5) ^c	3.38 (5.9)	0.76 (1.3)		2.47 (4.3)	tr	
"Histophilus ovis" MCCM 02133	E. L. Biberstein 805		52.15 (78.7)	0.71 (1.1)	0.40 (0.6)		7.23 (10.9)	5.81 (8.8)	
Bisgaard taxon 2, biovar 1 strain MCCM 00886	NCTC 11414, CCUG 15564, M. Bisgaard F 150		0.49 (1.0)	11.87 (24.1)			36.04 (73.0)	0.94 (1.9)	
Bisgaard taxon 3, biovar 1 strain MCCM 02162		3C	0.16 (0.5)	7.67 (21.7)	8.53 (24.1)		17.96 (50.7)	1.09 (3.1)	
Bisgaard taxon 6 strain MCCM 02144	M. Bisgaard 7.5		61.33 (93.8)	0.44 (1.0)	0.30 (0.7)	0.08 (0.1)	2.14 (3.3)	1.83 (2.8)	
Bisgaard taxon 6 strain MCCM 02143	CCUG 24852		41.59 (90.2)	0.48 (0.8)	0.22 (0.4)		1.52 (3.3)	2.25 (4.9)	
Bisgaard taxon 7 strain MCCM 02309		7	51.93 (88.2)	7.28 (22.9)		23.23 (73.1)	2.44 (4.1)	3.82 (6.5)	
Bisgaard taxon 14, biovar 1 strain MCCM 02121	M. Bisgaard V6, CCUG 16499			9.42 (23.8)			1.25 (3.9)		
Bisgaard taxon 14, biovar 2 strain MCCM 02122	M. Bisgaard HPA 7		37.60 (79.3)	1.79 (3.8)	0.53 (1.1)		29.05 (73.3)	1.17 (3.0)	
Bisgaard taxon 18 strain MCCM 02527	M. Bisgaard HPA 96		41.18 (92.3)	0.27 (0.6)			5.45 (11.5)	2.03 (4.3)	
Bisgaard taxon 20 strain MCCM 00674	SSI P 411		46.75 (92.8)	0.25 (0.5)			1.88 (4.2)	1.30 (2.9)	
Bisgaard taxon 26 strain MCCM 01095	M. Bisgaard 20608/3		38.23 (92.4)	0.32 (0.8)			1.54 (3.7)	1.54 (3.1)	
SP group strain MCCM 00489	SSI P 603		36.97 (92.0)	0.49 (1.2)			2.76 (6.7)	0.06 (0.1)	
SP group strain MCCM 02119	SSI P 602		34.47 (85.2)	0.11 (0.3)			2.65 (6.6)	0.09 (0.2)	
Stewart and Letscher strain MCCM 02118	SSI P 625						5.67 (14.0)	0.18 (0.4)	

^a For abbreviations see Table 1, footnote a.^b See reference 7.^c The values in parentheses are percentages of the total polyamines.

cluster 3B. A characteristic polyamine pattern containing the unusual triamine sym-norspermidine was obtained for *P. multocida* subsp. *multocida* MCCM 00005^T, *P. multocida* subsp. *gallicida* MCCM 00021^T, *P. multocida* subsp. *septica* MCCM 00005^T, *Pasteurella canis* MCCM 00045^T, *Pasteurella dagmatis* MCCM 00024^T, *Pasteurella stomatis* MCCM 00100^T, and *Pasteurella* species B strain MCCM 00102^T (Table 3), which are grouped together in subcluster 3B. The polyamine pattern of *P. dagmatis* MCCM 00024^T was slightly different. The characteristic features of this group were also detected in *P. multocida* H 1161/87 and H 983/87, *P. canis* MCCM 00047, and MCCM 00927, *P. dagmatis* MCCM 00558, and *P. stomatis* MCCM 00104 and MCCM 00490. No other strain analyzed in this study and placed in another genetic group in the family Pasteurellaceae contained the triamine sym-norspermidine in significant amounts. Thus, the presence of sym-norspermidine might be a diagnostic feature of subcluster 3B.

Other species considered to be members of the genus *Pasteurella* sensu stricto, including *Pasteurella gallinarum*, *Pasteurella volantium*, *Pasteurella avium*, *P. langaa*, *P. anatis*, and *Pasteurella* species A (19), could be easily distinguished from the *P. multocida* group (subcluster 3B) based on a lack of sym-norspermidine. These species have been shown to belong to subcluster 3A (which includes *P. gallinarum* MCCM 00031^T, *P. volantium* MCCM 02204^T, *P. avium* MCCM 00272^T, *Pasteurella* species A strain MCCM 00072) and subcluster 3D (which includes *P. langaa* MCCM 00120^T and *P. anatis* MCCM 00140^T) (7).

The *Pasteurella* species belonging to subcluster 3A contained the predominant compound 1,3-diaminopropane (68 to 76% of the total polyamines) and high levels of spermidine (14 to 23%) (Table 3). *H. paragallinarum* MCCM 00300^T, another member of subcluster 3A, had a similar pattern (Table 2). The relative levels of the single polyamines differed slightly in the type strain of *H. paragallinarum*, whereas the polyamine contents of two other *H. paragallinarum* isolates, xx36 and xx37, were within the ranges found for the *Pasteurella* species that grouped in cluster 3A. *P. gallinarum* MCCM 00033 had a polyamine pattern that was characteristic for members of subcluster 3B but different from the pattern found for members of subcluster 3A. The results of a biochemical and physiological characterization of *P. gallinarum* MCCM 00033 and a comparison with *P. gallinarum* MCCM 00031^T did not indicate relatedness at the species level (data not shown). Thus, the identity of *P. gallinarum* MCCM 00033 should be checked, and the relationship of this organism to members of subcluster 3B should be investigated.

Subcluster 3D consists of *P. anatis*, *P. langaa*, "Actinobacillus salpingitidis," Bisgaard taxon 2, and Bisgaard taxon 3. *P. anatis* MCCM 00140^T and *P. langaa* MCCM 00120^T are phylogenetically related only at a low level and differ in quinone composition (8). The quinone composition of *P. anatis* MCCM 00140^T is very similar to the quinone composition of species belonging to subclusters 3A and 3B. In this respect, *P. langaa* MCCM 00120^T is not similar to other members of the genus *Pasteurella* sensu stricto. A close phylogenetic relationship between *P. anatis* MCCM 00140^T and subcluster 3A has been demonstrated recently, whereas *P. langaa* is located on a separate branch (10). The polyamine pattern of *P. anatis* MCCM 00140^T (Table 3) distinguished this species from members of subcluster 3A only because a high concentration of putrescine was found. *P. langaa* MCCM 00120^T could be distinguished from the other species of the genus *Pasteurella* sensu stricto based on the presence of the predominant compound 1,3-diaminopropane. Thus, the relationship of *P. langaa* to the genus *Pasteurella* sensu stricto needs to be reinvestigated. "A.

salpingitidis" MCCM 00280 is not considered a member of the genus *Actinobacillus* sensu stricto (20). Phylogenetically, this organism is the organism that is most closely related to *P. anatis*. The suggested type strain of "*A. salpingitidis*" MCCM 00280 (25), had a polyamine pattern similar to the polyamine patterns found for strains belonging to subcluster 3A. In two strains obtained as "*A. salpingitidis*", MCCM 00924 and MCCM 00521, the putrescine content was somewhat higher than the putrescine content of the suggested type strain. The latter strain has been shown not to belong to the species "*A. salpingitidis*" (25). However, the polyamine patterns clearly differentiate the three strains from the genus *Actinobacillus* sensu stricto (see below) and indicate that the strains might be classified with the subcluster 3A strains. Bisgaard taxon 2 strain MCCM 00886 and Bisgaard taxon 3 strain MCCM 02162 were characterized by polyamine patterns which distinguished them from the other members of subcluster 3D (Table 4). The heterogeneity of the polyamine patterns of the subcluster 3D strains indicates that this subcluster does not represent a group of strains that might be classified in one genus.

H. paracuniculus MCCM 00703^T, which grouped in subcluster 3C, was characterized by a polyamine pattern (Table 2) which was similar to the polyamine pattern found in members of subcluster 3A. Other strains placed in subcluster 3C, including *A. capsulatus* MCCM 02462^T, *Haemophilus parasuis* MCCM 02736^T, *Pasteurella trehalosi* MCCM 02084^T, and Bisgaard taxon 7 strain MCCM 02309, could be clearly distinguished from strain MCCM 00703^T. The polyamine pattern of *P. trehalosi* MCCM 2084^T distinguished this organism from members of the genus *Pasteurella* sensu stricto (Table 3). A similar pattern was obtained for Bisgaard taxon 7 strain MCCM 02309 (Table 4) and *A. capsulatus* MCCM 02462^T (Table 1). The predominant compound in the polyamine patterns, 1,3-diaminopropane, is common to the vast majority of the members of the family *Pasteurellaceae* and does not indicate relatedness to a certain cluster. On the other hand, based on the polyamine pattern, it cannot be assumed that Bisgaard taxon 7 strain MCCM 02309 is a member of the genus *Pasteurella* sensu stricto. *H. parasuis* MCCM 02736^T had a polyamine pattern that was similar to the polyamine pattern obtained for *P. pneumotropica* type Jawetz strain MCCM 00234^T, which is a member of cluster 5 (see below). A close relationship between these two organisms is not supported by other data.

Cluster 4. Cluster 4 is subdivided into two subclusters. Most of the species belonging to *Actinobacillus* sensu stricto as defined by DNA relatedness data (20, 25) are members of subcluster 4A. Members of the genus *Actinobacillus*, including *A. lignieresii* MCCM 00190^T, *Actinobacillus equuli* MCCM 00142^T, *A. capsulatus* MCCM 02462^T, *A. suis* MCCM 02460^T, *Actinobacillus ureae* MCCM 00184^T, *Actinobacillus hominis* MCCM 00187^T and MCCM 00560, *A. pleuropneumoniae* MCCM 00339^T, and *Actinobacillus* sp. strain MCCM 00398 (Bisgaard taxon 9), had a polyamine pattern in which 1,3-diaminopropane was the predominant polyamine. Other polyamines were not present or were found in minor amounts. This pattern was also detected in other strains of *A. pleuropneumoniae* belonging to biovars 1 and 2, in *A. lignieresii* 9/91, and in an isolate of *A. suis* (Table 1). The other strain of *A. ureae* analyzed in this study, MCCM 02466, exhibited 100% DNA reassociation with the type strain (18, 23); it contained 1,3-diaminopropane as its major polyamine, but high concentrations of spermidine were also detected. This observation indicates that some variability in polyamine patterns occurs in *A. ureae*. A pattern characteristic of the genus *Actinobacillus* was detected in avian hemolytic *Actinobacillus*-like strain MCCM 01376 (Bisgaard taxon 26), which is considered an authentic

member of the genus *Actinobacillus* (25), and in Bisgaard taxon 26 strain MCCM 01095. *Haemophilus parahaemolyticus* MCCM 00504^T, which clusters with the *Actinobacillus* group in subcluster 4A, was found to possess this pattern as well.

Similar polyamine patterns were obtained for the three representatives of subcluster 4B, *H. ducreyi* MCCM 00729^T, *P. bettyae* MCCM 00450^T, and *P. haemolytica* MCCM 00395^T (Tables 2 and 3). However, in addition to the major compound 1,3-diaminopropane, significant amounts of spermidine and spermine were detected in *H. ducreyi* MCCM 00729^T. These patterns allowed discrimination of *H. ducreyi* MCCM 00729^T from the genus *Haemophilus* sensu stricto and discrimination of the two *Pasteurella* species from the genus *Pasteurella* sensu stricto.

P. haemolytica A2683 had a polyamine pattern similar to that of the type strain. *P. haemolytica*-like strain MCCM 00388 (Frederiksen's third taxon) and *P. haemolytica*-like strain MCCM 00594 (Frederiksen's fourth taxon) contained 1,3-diaminopropane as the major polyamine, but the spermidine and spermine contents of these organisms were significantly higher than the spermidine and spermine contents of *P. haemolytica* MCCM 00395^T. This resemblance in polyamine patterns indicates that there is some degree of relatedness between strains MCCM 00388 and MCCM 00594, although it has been demonstrated that these organisms belong to different species (17). A close relationship to members of other clusters, which might be indicated by the similarity in the polyamine patterns, is not supported by other data.

Cluster 5. *P. pneumotropica* type Jawetz groups with *Actinobacillus muris* in cluster 5, and *P. pneumotropica* complex strain MCCM 01911 represents a distinct species that is closely related to *P. pneumotropica* MCCM 00234^T (type Jawetz) (29). The phylogenetic position of *P. pneumotropica* type Heyl, which is also considered a distinct species (29), is still unclear. *P. pneumotropica* MCCM 00234^T, MCCM 01911, and MCCM 00237 could be distinguished from each other on the basis of differences in their polyamine patterns, whereas *P. pneumotropica* type Heyl strain MCCM 00253 had the same characteristic polyamine pattern as strain MCCM 00237 (Table 3). These observations indicate that the strains of *P. pneumotropica* type Jawetz and *P. pneumotropica* type Heyl may be distinguished by differences in their polyamine patterns. However, the polyamine patterns of the four strains studied are clearly different from the polyamine patterns which are representative of the genus *Pasteurella* sensu stricto, demonstrating that these organisms should be classified separately from the genus *Pasteurella* sensu stricto.

Cluster 6. *H. parainfluenzae*, which is considered a member of the genus *Haemophilus* sensu stricto (20), is the only species in cluster 6. This cluster is phylogenetically distant from *H. influenzae*, the type species of the genus, and the distance was supported by the distinct polyamine pattern (Table 2) obtained for *H. parainfluenzae* MCCM 00486^T. The phylogenetic position and the results of the polyamine analysis indicate that the taxonomic position of *H. parainfluenzae* MCCM 00486^T should be reassessed.

Cluster 7. *Pasteurella testudinis* and Bisgaard taxon 14 strains are grouped in cluster 7. *P. testudinis* MCCM 00484^T and MCCM 00400 had polyamine patterns in which spermidine was the predominant compound, and there were also relatively high concentrations of putrescine. The polyamine patterns clearly distinguished *P. testudinis* MCCM 00484^T from the genus *Pasteurella* sensu stricto (Table 3). The similar polyamine pattern obtained for the two Bisgaard taxon 14 strains, MCCM 02121 and MCCM 02122 (Table 4), which are considered members of one species (2), is consistent with the close genetic

relationship between these organisms and *P. testudinis* MCCM 00484^T. Osawa et al. (24) reported a similar polyamine pattern for *Lonepinella koalarum*, but in addition, they detected significant amounts of an unknown compound which we were not able to find in cluster 7 strains. The suggested genuslike structure consisting of *P. testudinis* MCCM 00484^T and *P. haemolytica* MCCM 00395^T and MCCM 00388 (Frederiksen's third taxon) (17) is not supported by the differences in polyamine patterns (Tables 2 and 3) and the results of phylogenetic clustering based on 16S rRNA sequence analyses (7).

Phylogenetically unassigned strains. So far, *Haemophilus intermedius* is considered a species that belongs to the genus *Haemophilus* sensu stricto (20). This species has not been included in 16S rRNA sequence analyses. *H. intermedius* subsp. *gazogenes* MCCM 02082 was found to have a polyamine pattern similar to that obtained for *H. parainfluenzae* MCCM 00486^T (cluster 6). This observation indicates that *H. intermedius* subsp. *gazogenes* MCCM 02082 and *H. parainfluenzae* MCCM 00486^T may be closely related and does not support a close phylogenetic relationship to the genus *Haemophilus* sensu stricto. The distant relationship of *H. intermedius* to *H. influenzae* is also indicated by significant differences in quinone composition (9). Thus, reclassification of *H. intermedius* should be considered.

"*Haemophilus felis*" (14) and "*Pasteurella leonis*" have not been included in 16S rRNA studies. However, no relationship between "*H. felis*" and some representatives of the genus *Haemophilus* was demonstrated by the results of DNA reassociation studies (14). The presence of significant amounts of symnorspermidine in the polyamine patterns (Tables 2 and 3) indicates that "*H. felis*" MCCM 02738^T and MCCM 02064 and "*P. leonis*" might be members of subcluster 3B, the *P. multocida* group.

The results of DNA-rRNA studies have demonstrated that "*Histophilus ovis*" is a member of the family *Pasteurellaceae* (6). Similar polyamine patterns which varied only in the amounts of the minor compounds (Table 4) were obtained for "*Histophilus ovis*" MCCM 00330 (formerly "*Haemophilus agni*") and "*Histophilus ovis*" MCCM 02133 (formerly "*Haemophilus somnus*"), which can be considered strains of the same species (26). These patterns were similar to those obtained for subcluster 3A and 3C strains. A high level of phylogenetic relatedness of "*Histophilus ovis*" to one of these two subclusters was not supported by the results of DNA-rRNA studies which included representatives of subcluster 3A (*Pasteurella* species A) and subcluster 3C (*H. paracuniculus*) (6). Also, the results of DNA-DNA hybridization studies which included "*Histophilus ovis*," *P. avium*, *P. volantium*, and *Pasteurella* species A did not reveal a relationship at the species level (26). The avian *Haemophilus*-like organism strain MCCM 01034 had a polyamine pattern (Table 4) which was most similar to the polyamine patterns obtained for subcluster 3A and 3C strains and "*Histophilus ovis*" but the relationship of this organism to previously established taxa remains to be investigated.

The results of DNA reassociation studies have demonstrated that *Actinobacillus rossii* MCCM 02461^T cannot be considered a member of the genus *Actinobacillus* (20). However, its polyamine pattern (Table 1) did not distinguish this species from *Actinobacillus* species. It can be assumed that this organism is not closely related to the genus *Haemophilus* sensu stricto because of differences in quinone composition (21).

The polyamine patterns of *Pasteurella granulomatis* MCCM 00576^T, *Pasteurella caballi* MCCM 00841^T, *Pasteurella*-like strain MCCM 00658, *Pasteurella*-like strain MCCM 00466 (hamster group 1), and *Pasteurella*-like strain MCCM 02120 (hamster group 2) clearly distinguished these organisms from

the genus *Pasteurella* sensu stricto (Table 3), indicating that these strains should not be included in the genus *Pasteurella* sensu stricto. Relatedness to the genus *Actinobacillus* or *Haemophilus* or to other groups in the family characterized by the presence of 1,3-diaminopropane as the single polyamine in the polyamine pattern should be examined. *Pasteurella*-like strain MCCM 01664 had a polyamine pattern which contained putrescine as the major compound and high levels of 1,3-diaminopropane. This pattern was different from the patterns of species belonging to the genus *Pasteurella* sensu stricto, and it can be assumed that *Pasteurella*-like strain MCCM 01664 occupies a separate taxonomic position within the *Pasteurellaceae*.

Because the major compound 1,3-diaminopropane was detected in Bisgaard taxon 6 strains MCCM 02144 and MCCM 02143, Bisgaard taxon 20 strain MCCM 00674, and SP group strains MCCM 00489 and MCCM 02119 (Table 4), the possible relationship of these organisms to the genera *Actinobacillus* and *Haemophilus* should be investigated. Steward and Letscher strain MCCM 02118 and Bisgaard taxon 18 strain MCCM 02527 were characterized by a polyamine pattern (Table 4) which was similar to that obtained for members of subclusters 3A and 3C, but there are no other data which support a close relationship to one of these subclusters.

The polyamine pattern obtained for avian *Haemophilus*-like strain MCCM 02795 clearly distinguished this strain from the genus *Haemophilus* sensu stricto (Table 2). This polyamine pattern was most similar to the pattern obtained for *P. aerogenes* MCCM 00402^T, Bisgaard taxon 2 strain MCCM 00886, and the two Bisgaard taxon 14 strains (see above). A possible relationship to any of these strains awaits investigation.

In conclusion, from the results obtained in this study it is obvious that the variability in polyamine patterns in the family *Pasteurellaceae* is much greater than the variability expected from studies of other gram-negative bacteria, in which polyamine patterns appear to be conserved and characteristic for higher taxa (1, 2, 12, 13). In the family *Pasteurellaceae*, at least seven different polyamine patterns were detected. Each of these patterns might be characteristic for a reclassified genus within the family *Pasteurellaceae* or for certain species of a genus. The variability does not seem to depend on the host from which a strain was isolated since members of the *P. multocida* group (subcluster 3B) characterized by similar polyamine patterns have been isolated from different mammalian species, including porcine, bovine, dog, and cat species, as well as humans (19). A similar observation was made for members of subcluster 3A. This subcluster consists of strains with a common polyamine pattern which have been isolated from several bird species (19, 22). Other isolates from mammals (*P. bettyae* and *P. caballi*) (16a) and birds (*P. anatis* and *P. langaa*) (19) had distinguishing polyamine patterns. These results demonstrate that the possible adaptation of a species belonging to the *Pasteurellaceae* to a host does not necessarily result in one particular polyamine pattern.

The importance of some of the polyamine patterns obtained for members of the family *Pasteurellaceae* has not been evaluated yet, since the phylogenetic relationships are not yet clear. Often, the phylogenetic branching of the strains within the family is rather deep, and different phylogenetic neighbors are found in different trees (7, 10, 24). Closer phylogenetic relatives have to be found and analyzed to determine their polyamine patterns in order to evaluate the reliability of such patterns for use in the classification of these groups.

Some variability in the polyamine patterns of strains belonging to the same species appears to be possible, as shown for "*Histophilus ovis*" and *A. ureae*. This observation demonstrates that concentrations of certain polyamines that are less than 10

TABLE 5. Phylogenetic clusters within the family *Pasteurellaceae* and the corresponding polyamine patterns

Organism ^a	Cluster of Dewhirst et al. ^b	Subcluster of Dewhirst et al. ^b	Polyamine patterns ^c		
			Major compound(s) ^d	Characteristic minor compound(s) ^d	Diagnostic compound
<i>A. actinomycetemcomitans</i>	1	1A	DAP		
<i>H. aphrophilus</i>	1	1B	DAP		
<i>H. segnis</i>	1	1B	DAP		
<i>H. influenzae</i>	1	1C	DAP		
<i>H. aegyptius</i>	1	1C	DAP		
<i>A. seminis</i>	2	2A	DAP, PUT	SPM, CAD	
<i>P. mairii</i>	2	2A	DAP, PUT	CAD	
<i>P. aerogenes</i>	2	2A	SPD	PUT	
<i>H. paragallinarum</i>	3	3A	DAP	SPD	
<i>P. avium</i>	3	3A	DAP	SPD	
<i>P. volantium</i>	3	3A	DAP	SPD	
<i>P. gallinarum</i>	3	3A	DAP	SPD	
<i>P. multocida</i>	3	3B	PUT	SPD	NSPD
<i>P. canis</i>	3	3B	SPD, PUT		NSPD
<i>P. dagmatis</i>	3	3B	PUT, DAP	SPD	NSPD
<i>P. stomatis</i>	3	3B	PUT, SPD		NSPD
<i>Pasteurella</i> species B	3	3B	SPD	PUT	NSPD
Bisgaard taxon 7	3	3C	DAP		
<i>H. paracuniculus</i>	3	3C	DAP	SPD	
<i>A. capsulatus</i>	3	3C	DAP		
<i>P. trehalosi</i>	3	3C	DAP		
<i>H. parasuis</i>	3	3C	DAP, PUT		
<i>P. langaa</i>	3	3D	DAP		
Bisgaard taxon 2	3	3D	SPD	PUT	
<i>P. anatis</i>	3	3D	DAP	PUT, SPD	
" <i>A. salpingitidis</i> "	3	3D	DAP	SPD	
<i>A. equuli</i>	4	4A	DAP		
<i>A. suis</i>	4	4A	DAP		
<i>A. hominis</i>	4	4A	DAP		
<i>A. ureae</i>	4	4A	DAP		
<i>A. lignieresii</i>	4	4A	DAP		
<i>A. pleuropneumoniae</i>	4	4A	DAP		
<i>H. parahaemolyticus</i>	4	4A	DAP		
Bisgaard taxon 9	4	4B	DAP	SPD	
<i>H. ducreyi</i>	4	4B	DAP	SPD	
<i>P. bettii</i>	4	4B	DAP		
<i>P. haemolytica</i>	4	4B	DAP		
<i>P. pneumotropica</i>	5		DAP, PUT		
<i>P. parainfluenzae</i>	6		DAP, CAD	PUT	
<i>P. testudinis</i>	7		SPD, PUT		
Bisgaard taxon 14	7		SPD, PUT		

^a Organisms which have been phylogenetically investigated (7) and analyzed to determine their polyamine patterns are listed.

^b See reference 7.

^c For abbreviations see Table 1, footnote a. Compounds are listed in order of decreasing concentration.

^d Polyamines which account for more than 25% of the total polyamines are considered major compounds, whereas polyamines which account for 9 to 25% of the total polyamines are considered characteristic minor compounds.

μmol/g (dry weight) of cells are sometimes difficult to interpret. An increase in the amount of a polyamine, such as the amount of spermidine in "*Histophilus ovis*" MCCM 00330 and *A. ureae* MCCM 02466, can lead to a change in the polyamine pattern to a pattern that is characteristic for other clusters. Thus, the results of polyamine analyses should be integrated in a polyphasic approach for classification of members of the family *Pasteurellaceae*. Nevertheless, some findings important for classification of members of the *Pasteurellaceae* are ob-

tained solely from analyses of the polyamine patterns (Table 5).

The type species of the genera *Actinobacillus* and *Haemophilus* and their phylogenetic neighbors have a polyamine pattern in which 1,3-diaminopropane is the predominant compound. This pattern was found in many members of the family belonging to different clusters. Thus, the value of this characteristic for classification within the family is limited. However, species that are considered members of the genus *Actinobacillus* or

Haemophilus should contain 1,3-diaminopropane as the predominant compound. Species and subspecies of these genera which are characterized by other patterns should be investigated to determine whether they can be considered members of the genera to which they are assigned; examples of such taxa are *A. seminis*, *H. ducreyi*, *H. intermedius* subsp. *gazogenes*, *H. paracuniculus*, *H. paragallinarum*, *H. parainfluenzae*, and *H. parasuis*, which have distinguishing polyamine patterns.

The differences in the polyamine patterns of members of the genus *Pasteurella* sensu stricto support the suggestion of Dewhirst et al. (7) that taxa included in the genus *Pasteurella* sensu stricto as defined by Mutters et al. (20) represent more than one genus. The following four genera are indicated based solely on different polyamine patterns: genus 1, which is characterized by high levels of putrescine and spermidine and the presence of sym-norspermidine (subcluster 3B, possibly including "*P. leonis*" and "*H. felis*"); genus 2, which is characterized by high levels of 1,3-diaminopropane and spermidine (subcluster 3A); genus 3, which is characterized by the predominance of 1,3-diaminopropane (*P. langaa*); and genus 4, which is characterized by high levels of 1,3-diaminopropane, putrescine, and spermidine (*P. anatis*).

Another genuslike structure may be created for members of cluster 7 (*P. testudinis* and strains belonging to Bisgaard taxon 14), which are characterized by a polyamine pattern in which the predominant compound is spermidine and high levels of putrescine are present.

The results of our investigations of polyamine patterns agree better with the groups based on the results of 16S rRNA sequence analyses (7) than with the taxa based on DNA reassociation values. On the other hand, the polyamine patterns of the representatives of some subclusters, such as subclusters 2A, 3C, 3D, and 4B, are not homogeneous. This may be an indication that within these groups the polyamine approach is not suitable for classification which is in agreement with the phylogenetic clusters or that the phylogenetic branching needs to be reassessed. However, more genetic and chemotaxonomic data have to be obtained for reliable reclassification of the genera within the family and for descriptions of the new taxa which were revealed by the different polyamine patterns obtained for members of the family *Pasteurellaceae*.

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