JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2004, p. 5542–5548 Vol. 42, No. 12
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.12.5542–5548.2004

Nicoletella semolina gen. nov., sp. nov., a New Member of *Pasteurellaceae* Isolated from Horses with Airway Disease

Peter Kuhnert,[1]* Bożena Korczak,[1] Enevold Falsen,[2] Reto Straub,[3] Anneliese Hoops,[4] Patrick Boerlin,[1]† Joachim Frey,[1] and Reinier Mutters[4]

Institute of Veterinary Bacteriology[1] and Department of Equine Internal Medicine,[3] University of Bern, Bern, Switzerland; Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden[2]; and Institute for Medical Microbiology and Hygiene, Philipps University, Marburg, Germany[4]

Received 24 May 2004/Returned for modification 5 July 2004/Accepted 15 August 2004

Gram-negative, nonmotile bacteria that are catalase, oxidase, and urease positive are regularly isolated from the airways of horses with clinical signs of respiratory disease. On the basis of the findings by a polyphasic approach, we propose that these strains be classified as *Nicoletella semolina* gen. nov, sp. nov., a new member of the family *Pasteurellaceae*. *N. semolina* reduces nitrate to nitrite but is otherwise biochemically inert; this includes the lack of an ability to ferment glucose and other sugars. Growth is fastidious, and the isolates have a distinctive colony morphology, with the colonies being dry and waxy and looking like a semolina particle that can be moved around on an agar plate without losing their shape. DNA-DNA hybridization data and multilocus phylogenetic analysis, including 16S rRNA gene (rDNA), *rpoB*, and *infB* sequencing, clearly placed *N. semolina* as a new genus in the family *Pasteurellaceae*. In all the phylogenetic trees constructed, *N. semolina* is on a distinct branch displaying ~5% 16S rDNA, ~16% *rpoB*, and ~20% *infB* sequence divergence from its nearest relative within the family *Pasteurellaceae*. High degrees of conservation of the 16S rDNA (99.8%), *rpoB* (99.6%), and *infB* (99.7%) sequences exist within the species, indicating that *N. semolina* isolates not only are phenotypically homogeneous but also are genetically homogeneous. The type strain of *N. semolina* is CCUG43639^T (DSM16380^T).

Bacteria belonging to the family *Pasteurellaceae* are commonly isolated from a number of different animals, including horses, and most are regarded as commensals or opportunistic pathogens. To date, nine different genera (*Pasteurella*, *Actinobacillus*, *Haemophilus*, *Mannheimia*, *Lonepinella*, *Phocoenobacter*, *Histophilus, Gallibacterium*, and *Volucribacter*) have been described within the family *Pasteurellaceae*. The genus *Haemophilus* is very heterogeneous and certainly needs taxonomic reorganization (13, 14). Species of this genus are isolated from various animals as well as humans, in which some can act as important pathogens. *Mannheimia* species are predominantly isolated from ruminants (3). *Lonepinella koalarum* is the only species described for the genus *Lonepinella*, and no host besides the koala bear has been reported (25). Similarly, *Phocoenobacter uteri* is the only species of the genus *Phocoenobacter* and was isolated from a harbor porpoise (12). *Histophilus somni* has been proposed to include the three species *incertae sedis*: "*Haemophilus somnus*," "*Haemophilus agni*," and "*Histophilus ovis*" (2). Christensen et al. (6) have described the genus *Gallibacterium*, which presently consists of one species and one genomospecies. Finally, Bisgaard Taxon 33 has been recently classified as *Volucribacter* (5). On the basis of present phylogenetic classification studies, additional genera in the family *Pasteurellaceae* are expected to be described in the future (15, 22, 23).

Except for the genera *Pasteurella* and *Actinobacillus*, no host-adapted member of the family *Pasteurellaceae* has been described for horses. "*Pasteurella*" *caballi*, misclassified as a member of the genus *Pasteurella*, is host associated and is sporadically found to be involved, along with other species, in respiratory infections of horses (8, 27). Bacteria belonging to the genus *Actinobacillus* are the most common isolates from the mucosal membranes of the oropharynx and respiratory tract in horses (26). *Actinobacillus equuli* has recently been divided into the two subspecies *A. equuli* subsp. *equuli* and *A. equuli* subsp. *haemolyticus* (7). Here we report on the characterization of a bacterium that has been repeatedly isolated from horses with airway disease. These isolates are phenotypically and phylogenetically distinct from the other members of the family *Pasteurellaceae*, so we propose the establishment of a new genus and species, *Nicoletella semolina* gen. nov., sp. nov.

MATERIALS AND METHODS

Bacterial strains and biochemical characterization. All of the *N. semolina* strains used in this study are listed in Table 1. Strains used for DNA-DNA hybridization were *Pasteurella multocida* NCTC10322^T, *Actinobacillus lignieresii* NCTC4189^T, *Actinobacillus capsulatus* NCTC11408^T, *Actinobacillus equuli* NCTC8529^T, *Actinobacillus ureae* NCTC10220, *Actinobacillus hominis* SSI P575,

* Corresponding author. Mailing address: Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland. Phone: 41-31-6312485. Fax: 41-31-6312634. E-mail: peter.kuhnert@vbi.unibe.ch.

† Present address: Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

TABLE 1. Strains used for the description of *N. semolina* isolated from diseased horses and assays performed

Strain	Isolation yr	Origin[a]	GenBank accession no. used for phylogenetic analysis			Assay performed[c]		
			16S rDNA	*rpoB*	*infB*	API NH test	Biochemical characteristics	DNA-DNA hybridization
CCUG43639^T	1998	CH	AY508816	AY508861	AY508835	+	+	+
CCUG43640	1999	CH	AY508817	AY508862	AY508836	+	+	+
CCUG43646	2000	CH	AY508818	AY508863	AY508837	+	+	+
CCUG43638	1997	CH	AY508819	AY508864	AY508838	+	+	−
CCUG43641	1999	CH	AY508820	AY508865	AY508839	+	+	−
JF2465[b]	2000	CH	AY508821	AY508866	AY508840	+	+	−
CCUG32135	1993	S	AY508822	AY508867	AY508841	+	−	−
CCUG39639	1998	S	AY508823	AY508868	AY508842	+	−	−
CCUG23468	1988	S	AY508824	AY508869		+	−	−
CCUG27342	1989	S	AY508825	AY508870		+	−	−
CCUG27497	1989	S	AY508826	AY508871		+	−	−
CCUG32179	1993	S	AY508827	AY508872		+	−	−
CCUG43643	1999	CH	AY508828	AY508873		+	−	−
CCUG43642	1999	CH	AY508829	AY508874		+	−	−
CCUG42978	1999	S	AY508830	AY508875		+	−	−
CCUG43647	2000	CH	AY508831	AY508876		+	−	−
CCUG43644	2000	CH	AY508832	AY508877		+	−	−
JF2408[b]	1999	CH	AY508833	AY508878		+	−	−
CCUG43645	2000	CH	AY508834	AY508879		+	−	−

[a] CH, Switzerland; S, Sweden.
[b] Strain number of the Institute of Veterinary Bacteriology, University of Bern.
[c] +, assay was performed; −, assay was not performed.

Actinobacillus suis CCM5586^T, *Actinobacillus actinomycetemcomitans* HIM946-2^T, *Pasteurella testudinis* ATCC 33688^T, *Pasteurella pneumotropica* ATCC 12555, *Histophilus somni* CCUG46774, and *Haemophilus influenzae* NCTC8143^T. The strains included in the phylogenetic analyses are listed in the figures. All *N. semolina* strains originated from horses with clinical cases of airway disease. Strains either were isolated at the Institute of Veterinary Bacteriology from tracheal-bronchial washes of horses admitted to the Department of Equine Internal Medicine, University of Bern, from different regions within Switzerland or were received from the Culture Collection of the University of Göteborg (CCUG) (Table 1). Isolates were grown on chocolate agar with PolyViteX plates (bioMérieux Suisse S.A., Geneva, Switzerland) in an atmosphere of 5% CO_2 for 24 to 48 h. Phenotypic characterization was done with API NH test strips (bioMérieux), according to the instructions of the supplier. As well, a limited number of isolates were also characterized by classical tube biochemical tests (23). For these tests, tubes were inoculated with a single colony, which was allowed to grow for 24 to 72 h, whereas in the API NH tests a large inoculum (turbidity equivalent to a 4 McFarland standard) of an overnight culture was used to inoculate the test strips for 2 h.

DNA-DNA hybridization. DNA-DNA hybridization was performed by the spectrophotometric method used by Mutters et al. (20). Renaturation rates of homologous and heterologous DNA solutions were determined with DNA at a concentration of 80 mg/ml in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 68°C.

Phylogenetic analyses. Genomic DNA was isolated with a PUREGENE DNA extraction kit (Gentra Systems, Minneapolis, Minn.). The sequence of a 1.4-kb fragment of the 16S rRNA gene (rDNA) was determined as previously described by Kuhnert et al. (16, 17). A 560-bp fragment of the *rpoB* gene was amplified by PCR and directly sequenced by the method of Korczak et al. (15). Primers infB-L (ATGGGNCACGTTGACCACGGTAAAAC) and infB-R (CCGATACCACA TTCCATACC) were designed for this study and were used for PCR amplification of a 1.3-kb fragment of the *infB* gene of all species except *Phocoenobacter uteri*, from which only a 0.5-kb fragment was obtained. The two PCR primers in combination with the internal primers infB-1 (CGTGAYGAGAARAAAGCA CGTGAAG) and infB-2 (CTTCACGTGCTTTYTTCTCRTCACG) were used for further sequencing. Reactions were carried out in MicroAmp tubes (Applied Biosystems, Foster City, Calif.) on a GeneAmp 9600 thermal cycler (Applied Biosystems). For all three PCRs the cycling conditions were an initial 3 min of denaturation at 96°C, followed by 35 cycles of 30 s at 96°C, 30 s at 54°C, and 1 min at 72°C. A final extension step of 7 min at 72°C was included. The PCR products were purified with a High Pure PCR Purification kit (Roche Applied Science, Rotkreuz, Switzerland) prior to sequencing with a BigDye terminator cycle sequencing kit (Applied Biosystems). After purification of the sequencing products by ethanol precipitation, the samples were run on an ABI 3100 genetic analyzer (Applied Biosystems). The sequences of both strands were determined and edited by using Sequencher software (GeneCodes, Ann Arbor, Mich.). Phylogenetic relationships and trees were established with Bionumerics software (version 3.0; Applied Maths, Kortrijk, Belgium).

Nucleotide accession numbers. The GenBank accession numbers for the 16S rDNA, *rpoB*, and *infB* sequences of the *N. semolina* strains determined in this study are listed in Table 1. The GenBank accession numbers for the *infB* sequences of the other species generated in this study are as follows: AY508843 for *Gallibacterium anatis* CCUG15563^T, AY508844 for *Pasteurella multocida* subsp. *multocida* CCUG17976^T, AY508845 for *Volucribacter psittacicida* JEO101, AY508846 for *Lonepinella koalarum* ATCC 700131^T, AY508847 for *Mannheimia haemolytica* NCTC9380^T, AY508848 for *Histophilus somni* HS8025^T, AY508849 for *Phocoenobacter uteri* NCTC12872^T, AY508850 for *Actinobacillus* genomospecies 1 CCUG22229, AY508851 for *Actinobacillus pleuropneumoniae* S4074^T, AY508852 for *Actinobacillus lignieresii* NCTC4189^T, AY508853 for *Actinobacillus* genomospecies 2 CCUG15571, AY508854 for *Actinobacillus arthritidis* CCUG24862^T, AY508855 for *Actinobacillus capsulatus* CCUG12396^T, AY508856 for *Actinobacillus hominis* CCUG19800^T, AY508857 for *Actinobacillus suis* ATCC 33415^T, AY508858 for *Actinobacillus equuli* subsp. *haemolyticus* CCUG19799^T, AY508859 for *Actinobacillus equuli* subsp. *equuli* ATCC 19392^T, and AY508860 for *Actinobacillus ureae* CCUG2139^T.

RESULTS AND DISCUSSION

Bacteria that are fastidious in growth were isolated from the airways of diseased horses of different ages, including foals. These animals presented mainly with chronic cough and in a few cases with pneumonia and/or nasal discharge. For primary isolation, strains originating from tracheal-bronchial washes were grown on chocolate agar plates at 37°C in an atmosphere of 5% CO_2. After 24 to 48 h of incubation, colonies resembling semolina particles could be observed. The gram-negative bacterium was a nonmotile, pleomorphic rod. It was the sole isolate or the predominant isolate and could be found at titers as high as 10^5/ml. The colonies, which were waxy, could be moved around the agar plate without losing their shape. These

TABLE 2. Phenotypic criteria that allow differentiation of *N. semolina* from other genera of the family *Pasteurellaceae*[a]

Test	Test result									
	Nicoletella	*Actinobacillus*	*Haemophilus*	*Pasteurella*	*Mannheimia*	*Histophilus*	*Lonepinella*	*Gallibacterium*	*Volucribacter*	*Phocoenobacter*
Catalase	+	+	+	+	+	−	−	+	v	−
Oxidase	+	+	+	+	+	+	−	+	v	+
Urease	+	+	v	v	−	−	−	−	−	−
Hemin (X factor)	−	−	+	−	−	−	−	−	−	−
NAD (V factor)	−	v	+	v	−	−	−	−	−	−
Indole	−	−	v	+	−	+	−	−	−	−
D-Galactose	−	+	+	+	+	−	+	+	+	−
D-Glucose	−	+	+	+	+	+	+	+	+	+

[a] Data for the genera *Mannheimia* (3), *Pasteurella* (19), *Lonepinella* (25), *Phocoenobacter* (12), *Haemophilus* (14), *Gallibacterium* (6), *Volucribacter* (5), *Histophilus* (2), and *Actinobacillus* (4,23) were obtained from previous reports. Symbols and abbreviations: +, 90 to 100%; −, 0 to 10%; v, variable.

bacteria were difficult to cultivate directly from clinical material on 5% sheep or 5% horse blood agar plates but could be subcultured on these media. The organism did not grow on MacConkey agar plates. The *N. semolina* colonies were gray, showed no hemolysis on sheep-blood agar plates, and were nonadherent. The strains examined in this study have been isolated over several years from different countries, thereby representing a temporally, geographically, and epidemiologically independent set of isolates.

All of the strains were tested with the API NH system, a rapid test routinely used in many diagnostic laboratories. With this system, all strains were positive for D-glucose, D-fructose, D-saccharose, and urease and negative for penicillinase, L-ornithine, lipase, proline arylamidase, gamma glutamyltransferase, and indole. Seventy-five percent of the strains were D-maltose positive, while 65% of the strains were beta-galactosidase negative and 90% were alkaline phosphatase negative.

A preliminary analysis of the 16S rDNA of a few isolates indicated that these strains belong to the family *Pasteurellaceae*. Six strains were therefore further extensively analyzed for their biochemical properties by using classical tube tests useful for the phenotypic identification and discrimination of isolates within the family *Pasteurellaceae* (23). All strains were positive for nitrate reduction, as well as oxidase, catalase, and urease activities. Beta-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside) and alkaline phosphatase reactions were variable but negative for most strains (for four and five of six strains, respectively). All strains were negative for arginine decarboxylase, lysine decarboxylase, and ornithine decarboxylase and for growth on citrate, adonitol, indole, H_2S, and gelatinase. No acid was produced from any of the sugars tested, including L-sorbose, D-glucose, D-galactose, D-mannose, D-fructose, L-rhamnose, D-xylose, L-arabinose, D-sucrose, trehalose, maltose, D-lactose, raffinose, D-mannitol, D-sorbitol, dulcitol, and *m*-inositol; nor was acid produced from salicin and esculin. Starch was not hydrolyzed, and neither hemin (X-factor) nor NAD (V-factor) was required for growth. The biochemical reactions which can be used to differentiate *N. semolina* from the other genera of the family *Pasteurellaceae* are shown in Table 2.

Several differences in the sugar reactions (D-glucose, D-fructose, D-saccharose, and D-maltose) were observed between the commercial API NH system and the conventional tube format. These differences may be due to the fact that in the API NH system a large inoculum is used, and so no growth is required for the reaction. Therefore, the API NH test does not necessarily measure fermentation but, rather, measures only the acidification of sugars (e.g., indirectly by enzymatic sugar degradation). Therefore, the results obtained with the API NH test cannot be compared with those obtained by biochemical growth tests. For routine identification, the API NH system might give quick and reasonable results. However, for a scientifically sound discrimination of isolates within the *Pasteurellaceae*, the biochemical growth test results must be considered.

DNA-DNA hybridization studies were performed to investigate the relationship of *N. semolina* to other genera within the family *Pasteurellaceae*. Three strains of *N. semolina*, including the type strain, were hybridized against each other and showed binding values greater than 97%. As these were significant levels of DNA hybridization above the species level of 85% described for *Pasteurellaceae* (20), further hybridizations were done with only one representative of the group. Hybridization values for *N. semolina* with selected species of the *Pasteurellaceae* were highest with members of the genus *Actinobacillus* (from 54% with *A. lignieresii* to 66% with *A. capsulatus*). The genus *Actinobacillus* sensu stricto presently comprises the species *A. lignieresii*, *A. suis*, *A. equuli* subsp. *equuli*, *A. equuli* subsp. *haemolyticus*, *A. pleuropneumoniae*, *A. ureae*, *A. arthritidis*, *Actinobacillus* genomospecies 1 and 2, *A. hominis*, and *A. capsulatus* (21, 24). The position of *A. capsulatus* within the genus is still disputed, mainly because of its phylogenetic position within the 16S rDNA-derived tree (Fig. 1), but recent observations and the results presented here (Fig. 2 and 3) confirm the original classification by Mutters et al. (21). In order to further investigate whether the new taxon is a true member of *Actinobacillus* sensu stricto, additional phylogenetic investigations with species of this genus were undertaken to resolve the taxonomic position of this new taxon.

Phylogenetic analysis was carried out, including comparison of the sequences of 16S rDNA, the gene for the β subunit of the RNA polymerase (*rpoB*), and the gene for translation initiation factor 2 (*infB*). The usefulness of 16S rDNA sequences for the establishment of genetic relationships within the family *Pasteurellaceae* was previously shown by Dewhirst and coworkers (10, 11). The *rpoB* gene has also been successfully applied for the elaboration of phylogenetic relationships in several groups of bacteria, including the *Pasteurellaceae* (2, 9, 15, 18). The efficacy of *infB* sequences was shown for the *Pasteurellaceae* by the phylogenetic delineation of the genera *Haemophilus* and *Actinobacillus* (13, 22). 16S rDNA analysis of *N.*

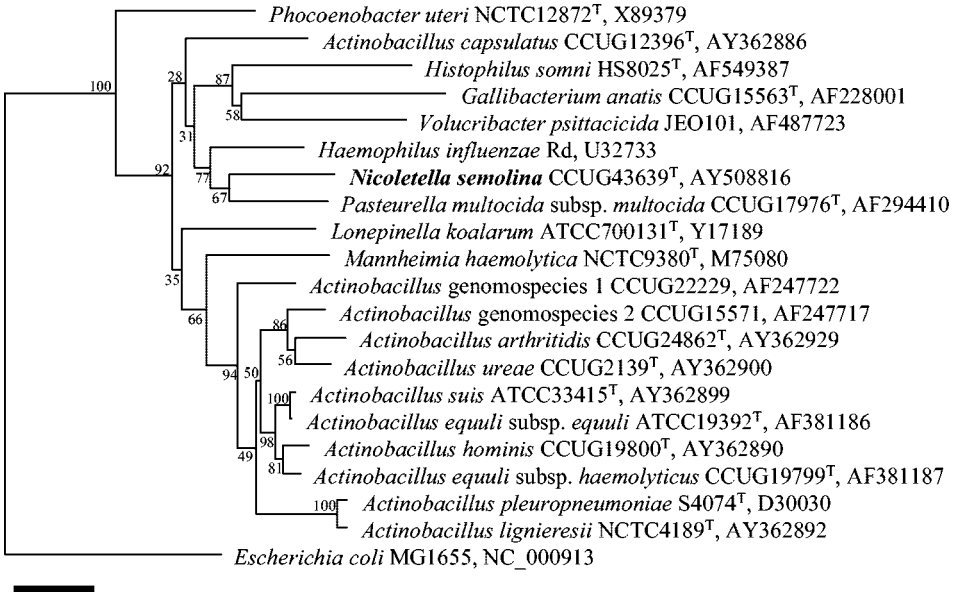

FIG. 1. Phylogenetic tree based on 16S rDNA sequences. The sequence of *N. semolina* was compared phylogenetically to those of the type species of the *Pasteurellaceae* as well as the members of *Actinobacillus* sensu stricto. *E. coli* was chosen as an outgroup. The tree was built with Bionumerics software (version 3.0) by using the Jukes-Cantor correction and neighbor joining for cluster analysis. The accession numbers of the sequences used are given. Bootstrap values for 500 simulated runs are given. The solid bar indicates 2% sequence divergence.

semolina clearly showed that phylogenetically it belongs to the family *Pasteurellaceae*. Figure 1 shows the 16S rDNA-based tree for *N. semolina*, the nine presently described genera of this family, and the species of *Actinobacillus* sensu stricto in relation to *Escherichia coli*. *N. semolina* is positioned within the family *Pasteurellaceae*, yet it forms a branch of its own. This also holds true when *N. semolina* is included in the full 16S rDNA phylogeny (15). In order to compare the 16S rDNA sequence of *N. semolina* with all known 16S rDNA sequences, a search of the GenBank database was carried out with the BLAST algorithm (1). None of the 16S rDNA entries gave a greater than 95% match to *N. semolina*. Analysis of the *rpoB* genes resulted in phylogenetic relationships similar and complementary to those obtained with 16S rDNA. The results of *rpoB* sequence analysis are presented in the phylogenetic tree in Fig. 2, which again shows that *N. semolina* is clearly distinct from the other phyla. This also held true when *N. semolina* was included in the full *rpoB* phylogeny, since comparison of the *N.*

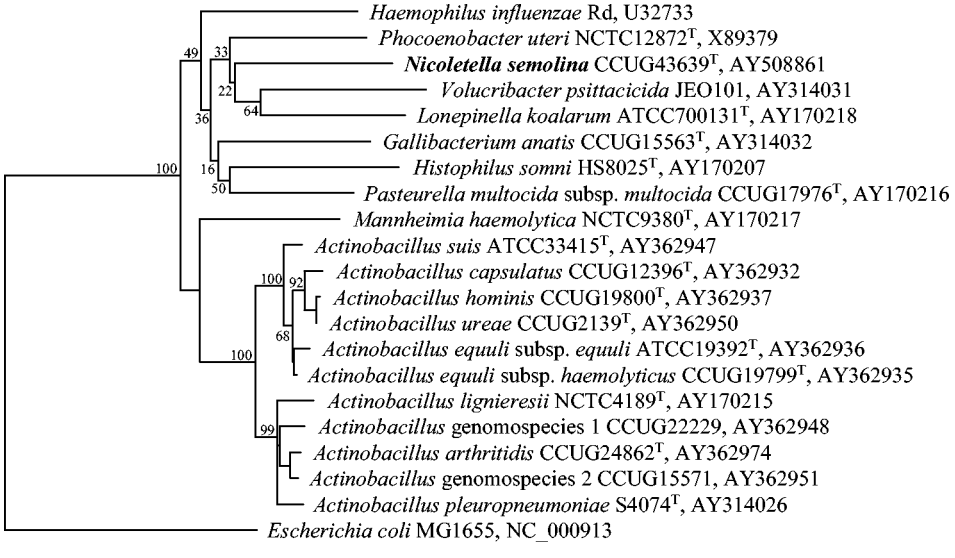

FIG. 2. Phylogenetic tree based on *rpoB* gene sequences. The sequence of *N. semolina* was compared phylogenetically to those of the type species of the *Pasteurellaceae* as well as the members of *Actinobacillus* sensu stricto. *E. coli* was chosen as an outgroup. The tree was built with Bionumerics software (version 3.0) by using the Jukes-Cantor correction and neighbor joining for cluster analysis. The accession numbers of the sequences used are given. Bootstrap values for 500 simulated runs are given. The solid bar indicates 2% sequence divergence.

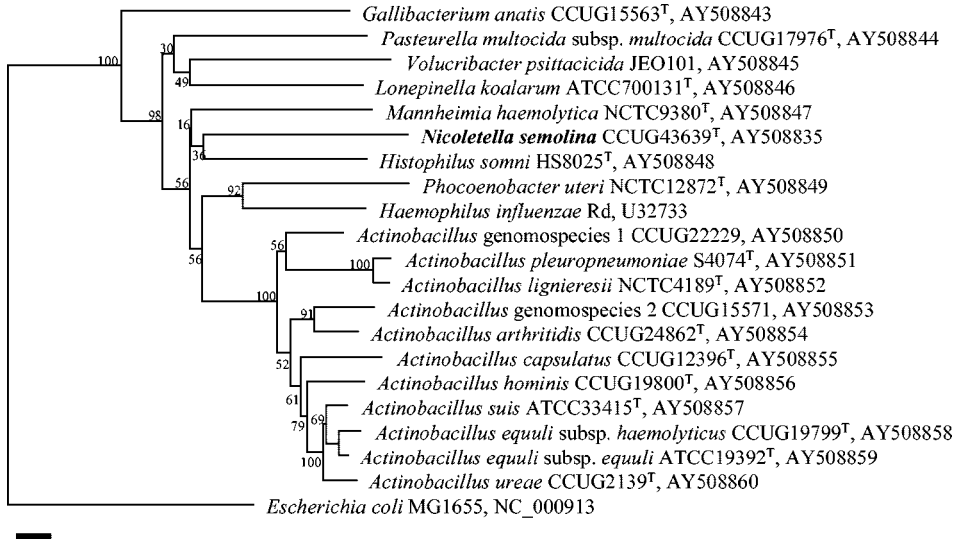

FIG. 3. Phylogenetic tree based on *infB* gene sequences. The sequence of *N. semolina* was compared phylogenetically to those of the type species of the *Pasteurellaceae* as well as the members of *Actinobacillus* sensu stricto. *E. coli* was chosen as an outgroup. The tree was built with Bionumerics software (version 3.0) by using the Jukes-Cantor correction and neighbor joining for cluster analysis. The accession numbers of the sequences used are given. Bootstrap values for 500 simulated runs are given. The solid bar indicates 2% sequence divergence.

semolina rpoB sequence with those from the entire family showed matches not greater than 85% (15). Finally, analysis of *infB* sequence-based phylogeny (Fig. 3) also showed a separate branching of *N. semolina*. The similarities of the *N. semolina infB* sequence to those of other known representatives were not greater than 80%.

The DNA-DNA hybridization values observed for the *N. semolina* strains (~60%) with members of the genus *Actinobacillus* sensu stricto would allow classification of this new taxon in the genus *Actinobacillus* (20). However, the phenotypic and phylogenetic characteristics presented here argue for the classification of these strains as a new genus. First, the distinctive culture morphology and the inability to ferment D-glucose and other carbohydrates is so unusual (23) that these strains were at first not recognized as belonging to the family *Pasteurellaceae* (M. Bisgaard and J. Nicolet, personal communication). Nevertheless, the phylogenetic analyses of the strains as well as the DNA-DNA hybridization results clearly show that they belong to the family *Pasteurellaceae*. Second, the tree topologies obtained with the three genes analyzed did not show cobranching of *N. semolina* with the genus *Actinobacillus*. All species presently belonging to *Actinobacillus* sensu stricto were included in the phylogenetic analyses to emphasize this fact. Third, the sequence divergence of the genes of *N. semolina* analyzed to those of other species of the *Pasteurellaceae* and especially to the genus *Actinobacillus* was high enough to support a new genus (15, 22). In the case of 16S rDNA, this divergence from members of *Actinobacillus* sensu stricto was more than 5%. For *rpoB*, the divergence from *Actinobacillus* sensu stricto was about 18%, which is above the threshold value of 12% (15). Finally, in the case of *infB*, the divergence between *N. semolina* and members of the genus *Actinobacillus* sensu stricto was about 20%, which is again higher than that of the threshold value for this genus, which is 15% (22). On the other hand, phylogenetic analysis of numerous strains isolated from clinically, geographically and epidemiologically unrelated cases (Table 1) showed a high degree of conservation of the species genome, as represented by the three genes analyzed (Fig. 1 to 3). The intraspecies variabilities were very low: less than 0.2% for 16S rDNA, less than 0.4% for the *rpoB* gene, and 0.3% for the *infB* gene. This genetic conservation is also confirmed by the high DNA binding values of more than 97% between the three *N. semolina* strains analyzed; within other species of the *Pasteurellaceae* this value can be as low as 85% (20). *N. semolina* is therefore a highly homogeneous genetic group for which easy molecular confirmation of the preliminary phenotypic identification is possible, especially since identification by conventional biochemical growth tests is difficult. Furthermore, no differences in the major biochemical reactions or phenotypic criteria used to separate *N. semolina* from the other genera of *Pasteurellaceae* were observed (Table 2). Finally, the taxonomy of *Pasteurellaceae* is under constant revision since it contains many misclassified species. Phylogenetic analysis indicates that the number of genera at present must certainly be increased in order to structure the family reasonably. Placement of *N. semolina* into the genus *Actinobacillus* now would certainly make a future reclassification necessary, as we have shown by our phylogenetic analyses using accepted marker genes for the *Pasteurellaceae*.

Isolation of *N. semolina* in our experience is not uncommon. It remains to be determined whether *N. semolina* is a new emerging species or one that simply was not recognized in routine laboratory diagnostics in the past. Moreover, it will be important to determine whether *N. semolina* is a primary pathogen, is part of the normal flora that can occasionally cause disease, or is merely coincidentally present when there is airway disease. A retrospective analysis of putative *N. semolina* strains (strains of unknown designation which fit the description) that might reside in several laboratories, together with a prospective study of the prevalence of *N. semolina* in horse populations from various geographical regions, would help to clarify this point. A PCR approach based on one of the phy-

logenetic markers analyzed in this study could be helpful for that purpose. Future research will certainly be necessary to investigate the virulence potential of this new species and the pathogenesis of the disease associated with it.

Description of *Nicoletella* gen. nov. *Nicoletella* (Ni.co.le.te′lla. N.L. fem. n. *Nicoletella*, named in tribute to Jacques Nicolet, a Swiss microbiologist, for his contribution to research on *Pasteurellaceae*).

***Nicoletella* is a new genus in the family *Pasteurellaceae*.** The genus *Nicoletella* consists of gram-negative, nonmotile, pleomorphic rods. Isolates do not grow on MacConkey agar and do not require hemin or NAD. They reduce nitrate to nitrite and are catalase, oxidase, and urease positive. Otherwise, the members of the genus are biochemically inert. They are negative for arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, adonitol, indole, H_2S, and gelatinase and do not grow on citrate. No acid is produced from L-sorbose, D-glucose, D-galactose, D-mannose, D-fructose, L-rhamnose, D-xylose, L-arabinose, D-sucrose, trehalose, maltose, D-lactose, raffinose, D-mannitol, D-sorbitol, dulcitol, *m*-inositol, salicin, or esculin. Starch is not hydrolyzed. The type species is *Nicoletella semolina*.

Description of *Nicoletella semolina* sp. nov. *Nicoletella semolina* (se.mo.li′na. N.L. fem. n. *semolina*, indicating the typical semolina-like colony characteristic).

Cells are gram-negative, nonmotile, pleomorphic rods. The species is capnophilic, and optimal growth conditions are on chocolate agar in a 5% CO_2 atmosphere, on which after 24 to 48 h incubation it forms typical colonies of 0.5 to 2 mm in diameter which can be moved around on the plate like a semolina particle without losing their shape. The colony morphology is so typical that laboratory personnel can easily make a preliminary identification of this species. This is therefore an important diagnostic marker. The colonies are gray, odorless, and nonhemolytic and show positive reactions for catalase, oxidase, and urease. The species is biochemically inert when physiological characteristics are tested during growth. The species is negative for arginine decarboxylase, lysine decarboxylase, and ornithine decarboxylase and for growth on citrate, adonitol, indole, H_2S, and gelatinase. No acid is produced from L-sorbose, D-glucose, D-galactose, D-mannose, D-fructose, L-rhamnose, D-xylose, L-arabinose, D-sucrose, trehalose, maltose, D-lactose, raffinose, D-mannitol, D-sorbitol, dulcitol, *m*-inositol, salicin, or esculin. Starch is not hydrolyzed.

With API NH test strips, *N. semolina* is positive for urease and acidification of glucose, fructose, and saccharose. Maltose, beta-galactosidase, and alkaline phosphatase reactions are variable. Tests for penicillinase, ornithine decarboxylase, lipase, proline amidase, gamma glutamyltransferase and indole are negative. Strains are isolated from the trachea of horses. The type strain is CCUG43639^T (DSM 16380^T).

ACKNOWLEDGMENTS

We thank André Burnens for valuable discussions; Yvonne Schlatter, Carol Suter, Sandra Zumwald, and Margrit Krawinkler for excellent technical help; and Janet MacInnes and Sarah Burr for critical reading of the manuscript.

This research was supported by a grant (grant 6041.1 KTS) from KTI and by the Research Fund of the Institute of Veterinary Bacteriology, Bern, Switzerland.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic logical alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Angen, O., P. Ahrens, P. Kuhnert, H. Christensen, and R. Mutters.** 2003. Proposal of *Histophilus somni* gen. nov., sp. nov. for the three species incertae sedis '*Haemophilus somnus*,' '*Haemophilus agni*' and '*Histophilus ovis.*' Int. J. Syst. Evol. Microbiol. **53:**1449–1456.
3. **Angen, O., R. Mutters, D. A. Caugant, J. E. Olsen, and M. Bisgaard.** 1999. Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov., *Mannheimia ruminalis* sp. nov., and *Mannheimia varigena* sp. nov. Int. J. Syst. Bacteriol. **49:**67–86.
4. **Christensen, H., and M. Bisgaard.** 2004. Revised definition of *Actinobacillus sensu stricto* isolated from animals. A review with special emphasis on diagnosis. Vet. Microbiol. **99:**13–30.
5. **Christensen, H., M. Bisgaard, B. Aalbaek, and J. E. Olsen.** 2004. Reclassification of Bisgaard taxon 33 with proposal of *Volucribacter psittacicida* gen. nov., sp. nov. and *Volucribacter amazonae* sp. nov. as new members of *Pasteurellaceae*. Int. J. Syst. Evol. Microbiol. **54:**813–818.
6. **Christensen, H., M. Bisgaard, A. M. Bojesen, R. Mutters, and J. E. Olsen.** 2003. Genetic relationships among avian isolates classified as *Pasteurella haemolytica*, '*Actinobacillus salpingitidis*' or *Pasteurella anatis* with proposal of *Gallibacterium anatis* gen. nov., comb. nov. and description of additional genomospecies within *Gallibacterium* gen. nov. Int. J. Syst. Evol. Microbiol. **53:**275–287.
7. **Christensen, H., M. Bisgaard, and J. E. Olsen.** 2002. Reclassification of equine isolates previously reported as *Actinobacillus equuli*, variants of *A. equuli*, *A. suis* or taxon 11 of Bisgaard and proposal of two subspecies of *A. equuli*: *A. equuli* subsp. *equuli* and *A. equuli* subsp. *haemolyticus*. Int. J. Syst. Evol. Microbiol. **52:**1569–1576.
8. **Church, S., K. E. Harrigan, A. E. Irving, and M. M. Peel.** 1998. Endocarditis caused by *Pasteurella caballi* in a horse. Aust. Vet. J. **76:**528–530.
9. **Dahllof, I., H. Baillie, and S. Kjelleberg.** 2000. *rpoB*-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. Appl. Environ. Microbiol. **66:**3376–3380.
10. **Dewhirst, F. E., B. J. Paster, I. Olsen, and G. J. Fraser.** 1992. Phylogeny of 54 representative strains of species in the family *Pasteurellaceae* as determined by comparison of 16S rRNA sequences. J. Bacteriol. **174:**2002–2013.
11. **Dewhirst, F. E., B. J. Paster, I. Olsen, and G. J. Fraser.** 1993. Phylogeny of the *Pasteurellaceae* as determined by comparison of 16S ribosomal ribonucleic acid sequences. Zentbl. Bakteriol. Parasitenkd. Infektkrankh. Hyg. Abt. 1 Orig. **279:**35–44.
12. **Foster, G., H. M. Ross, H. Malnick, A. Willems, R. A. Hutson, R. J. Reid, and M. D. Collins.** 2000. *Phocoenobacter uteri* gen. nov., sp. nov., a new member of the family *Pasteurellaceae* Pohl (1979) 1981 isolated from a harbour porpoise (*Phocoena phocoena*). Int. J. Syst. Evol. Microbiol. **50:**135–139.
13. **Hedegaard, J., H. Okkels, B. Bruun, M. Kilian, K. K. Mortensen, and N. Norskov-Lauritsen.** 2001. Phylogeny of the genus *Haemophilus* as determined by comparison of partial *infB* sequences. Microbiology **147:**2599–2609.
14. **Kilian, M.** 2004. Genus *Haemophilus* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1917, 561^{AL}. *In* R. Garrity (ed.), Bergey's manual of systematic bacteriology. Springer, New York, N.Y.
15. **Korczak, B., H. Christensen, S. Emler, J. Frey, and P. Kuhnert.** 2004. Phylogeny of the family *Pasteurellaceae* based on *rpoB* sequences. Int. J. Syst. Evol. Microbiol. **54:**1393–1399.
16. **Kuhnert, P., S. E. Capaul, J. Nicolet, and J. Frey.** 1996. Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. **46:**1174–1176.
17. **Kuhnert, P., J. Frey, N. P. Lang, and L. Mayfield.** 2002. A phylogenetic analysis of *Prevotella nigrescens*, *Prevotella intermedia* and *Porphyromonas gingivalis* field strains reveals a clear species clustering. Int. J. Syst. Evol. Microbiol. **52:**1391–1395.
18. **Mollet, C., M. Drancourt, and D. Raoult.** 1997. *rpoB* sequence analysis as a novel basis for bacterial identification. Mol. Microbiol. **26:**1005–1011.
19. **Mutters, R., H. Christensen, and M. Bisgaard.** 2003. Genus *Pasteurella* Trevisian 1887, 94^{AL}, *Nom. cons.* Opin. 13, Jud. Comm. 1954. *In* R. Garrity (ed.), Bergey's manual of systematic bacteriology. Springer, New York, N.Y.
20. **Mutters, R., P. Ihm, S. Pohl, W. Frederiksen, and W. Mannheim.** 1985. Reclassification of the genus *Pasteurella* Trevisan 1887 on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis*, *Pasteurella canis*, *Pasteurella stomatis*, *Pasteurella anatis*, and *Pastuerella langaa*. Int. J. Syst. Bacteriol. **35:**309–322.
21. **Mutters, R., W. Mannheim, and M. Bisgaard.** 1989. Taxonomy of the group, p. 3–34. *In* C. Adlam and J. M. Rutter (ed.), *Pasteurella* and pasteurellosis. Academic Press, London, United Kingdom.
22. **Norskov-Lauritsen, N., H. Christensen, H. Okkels, M. Kilian, and B. Bruun.** 2004. Delineation of the genus *Actinobacillus* by comparison of partial *infB* sequences. Int. J. Syst. Evol. Microbiol. **54:**635–644.

23. **Olsen, I., F. E. Dewhirst, B. J. Paster, and H. J. Busse.** 2004. Family *Pasteurellaceae*. *In* R. Garrity (ed.), Bergey's manual of systematic bacteriology. Springer, New York, N.Y.
24. **Olsen, I., and K. Moller.** 2004. Genus *Actinobacillus* Brumpt 1919, 849^{AL}. *In* R. Garrity (ed.), Bergey's manual of systematic bacteriology. Springer, New York, N.Y.
25. **Osawa, R., F. A. Rainey, T. Fujisawa, E. Lang, H. J. Busse, T. Walsh, and E. Stackebrandt.** 1995. *Lonepinella koalarum* gen. nov., sp. nov., a new tannin-protein complex degrading bacterium. Syst. Appl. Microbiol. **18:**368–373.
26. **Rycroft, A. N., and L. H. Garside.** 2000. *Actinobacillus* species and their role in animal disease. Vet. J. **159:**18–36.
27. **Schlater, L. K., D. J. Brenner, A. G. Steigerwalt, C. W. Moss, M. A. Lambert, and R. A. Packer.** 1989. *Pasteurella caballi*, a new species from equine clinical specimens. J. Clin. Microbiol. **27:**2169–2174.