Characterization of PaxA and Its Operon: a Cohemolytic RTX Toxin Determinant from Pathogenic Pasteurella aerogenes

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Pasteurella aerogenes is known as a commensal bacterium or as an opportunistic pathogen, as well as a primary pathogen found to be involved in abortion cases of humans, swine, and other mammals. Using broad-range DNA probes for bacterial RTX toxin genes, we cloned and subsequently sequenced a new operon named paxCABD encoding the RTX toxin PaxA in P. aerogenes. The pax operon is organized analogously to the classical RTX operons containing the activator gene paxC upstream of the structural toxin gene paxA, which is followed by the secretion protein genes paxB and paxD. The highest sequence similarity of paxA with known RTX toxin genes is found with apxIIIA (82%). PaxA is structurally similar to ApxIIIA and also shows functional analogy to ApxIII B, since it shows cohemolytic activity with the sphingomyelinase of Staphylococcus aureus, known as the CAMP effect, but is devoid of direct hemolytic activity. In addition, it shows to some extent immunological cross-reactions with ApxIII B. P. aerogenes isolated from various specimens showed that the pax operon was present in about one-third of the strains. All of the pax-positive strains were specifically related to swine abortion cases or septicemia of newborn pigs. These strains were also shown to produce the PaxA toxin as determined by the CAMP phenomenon, whereas none of the pax-negative strains did. This indicated that the PaxA toxin is involved in the pathogenic potential of P. aerogenes. The examined P. aerogenes isolates were phylogenetically analyzed by 16S rRNA gene (rrs) sequencing in order to confirm their species. Only a small heterogeneity (<0.5%) was observed between the rrs genes of the strains originating from geographically distant farms and isolated at different times.

The gram-negative bacterium Pasteurella aerogenes was first isolated from porcine intestine and described as a gas-producing Pasteurella-like organism (30). Reported cases of isolation in animals have included the buccal flora of wild boars (33), the urine of rabbit, or the ureteric cervix discharge of cow (3). In humans P. aerogenes has been isolated from lesions caused by cats, pigs, or wild boar (27, 30, 32).

Clinically, the isolation of P. aerogenes is mainly associated with abortion cases. The first case described in which P. aerogenes was directly involved as a pathogen was an abortion in swine, where it was isolated from several organs of the aborted fetuses (30). At least two additional cases of P. aerogenes-induced abortion in swine have been reported (13, 21). Abortion cases, where P. aerogenes could be responsible, were also reported in other mammals. It was isolated in pure culture from the uterus and peritoneal cavity of a rabbit which died 4 days after abortion (34). Also a human case is described where P. aerogenes could be isolated from a stillborn child and from its mother’s vaginal vault (P. Thorsen, B. R. Moller, M. Arpi, A. Bremmelgaard, and W. Fredericksen, Letter, Lancet 343: 485–486, 1994). During pregnancy, the mother had been working as an assistant on a pig farm. Other clinical cases are described in swine suffering from various diseases, where P. aerogenes was isolated from the lungs and respiratory system and quite often from intestines with gastroenteritis (3, 30), but its relevance as a primary pathogen in clinical findings other than abortion is doubtful. Despite the description of P. aerogenes as a potential pathogen, nothing is known about its possible virulence factors involved in pathogenicity.

RTX (repeats in the structural toxin) toxins, are a class of pore-forming protein toxins which are often found among various species of Pasteurellaceae and play an important role in pathogenicity (16). They were found in Actinobacillus pleuropneumoniae (ApxIa [20], ApxIIA [8], and ApxIIIA [7]), in Actinobacillus actinomycetemcomitans (AaltA [24]), Pasteurella haemolytica (LktA [28]), and P. haemolyticuss-like (PlktA [6]) and in Actinobacillus suis (Asha [5]). The operons are similarly organized in a CABS pattern where C codes for the activation protein, A encodes the structural toxin, and B and D code for proteins involved in the secretion of the toxin. We have therefore analyzed various strains of P. aerogenes, including strains from abortion cases in swine, for the presence of RTX genes by using a recently developed broad range detection system for this family of toxin genes (26). We describe a new RTX protein and its operon that was found in clinical P. aerogenes isolates and present a functional characterization of this toxin.

MATERIALS AND METHODS

Bacterial strains. A total of 13 Pasteurella aerogenes strains consisting of the type strain ATCC 27883 and 12 field isolates were used in this study (Table 1). The field strains were freshly isolated at our diagnostic unit from clinical material of swine. Actinobacillus pleuropneumoniae serotype 2 reference strain ATCC 27089 (S1536) was included as control strain. For the analysis of the cohemolytic activity (CAMP) of RTX toxins (19) we used a beta-hemolytic Staphylococcus aureus expressing the sphingomyelinase and Actinobacillus pleuropneumoniae serotype 3 reference strain ATCC 27090 (S1421) secreting only ApxIIIA. Strains were grown either on Columbia Agar Base (Oxoid Unipath, Ltd., Basingstoke, Hampshire, England) or on 5% sheep blood agar plates at 37°C overnight.

Escherichia coli K-12 strains DH5a and HMS174 were used for gene cloning and expression, respectively. Strain JFS22 harboring the hhyB secretion genes on the plasmid pLG575 (29) was used in the CAMP test of recombinant pax constructs. All Escherichia coli strains were grown on Luria-Bertani broth supplemented, when necessary, with ampicillin (50 μg/ml), chloramphenicol (25 μg/ml), or a combination of both for selection and maintenance of plasmids.

Probe preparation. Broad-range probes for RTX gene detection, leading to the discovery of a potential RTX gene in P. aerogenes, are described elsewhere (26). The apxIIA and apxBIID probes from A. pleuropneumoniae were described previously (17). For generation of specific P. aerogenes paxCA, probe primers PAX14 (5′-ATTCCGGGATAACCATGAC-3′; positions 306 to 325

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on paco) and PAX10 (5'-CGCACAACCTAATTCCAG-3'; positions 2055 to 2035 on paco) were used. For the paxBD-specific probe, primers PAX4 (5'-GGGATTTAAAACTCTCAG-3'; positions 1077 to 1097 on paxB) and PAX15 (5'-TAAACGTAAAGCTTTTGCAG-3'; positions 925 to 945 on paxD) were used.

All probes were generated by PCR with digoxigenin-labeled dUTP (Roche Molecular Biochemicals, Rotkreuz, Switzerland). The labeling reaction was carried out in a 50-μl volume containing 5 μl of 10× PCR buffer, 20 pmol of primer (each), 1 mM deoxynucleoside triphosphate, 0.5 nmol of digoxigenin-11–dUTP, 2.5 U of Taq DNA polymerase (Roche Molecular Biochemicals), and 100 ng of genomic DNA. PCR conditions for the pax-specific probes were 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 60 s.

DNA extraction and Southern blot. Extraction of genomic DNA was done by using either the QIAamp Tissue Kit (Qiagen, Basel, Switzerland) or the method of Pitcher et al. (31). Chromosomal DNA was digested by restriction enzyme, size separated on a 0.7% agarose gel, and vacuum transferred to a positively charged nylon membrane (Roche Molecular Biochemicals) by using an LKB 2016 VacuGene Vacuum Blotting Pump (Pharmacia LKB Biotechnology AB, Bromma, Sweden).

Hybridization with digoxigenin-labeled probes was done according to the manufacturer's instructions (Roche Molecular Biochemicals) in a rotating hybridization oven. Posthybridization washing steps were performed at middle stringency, defined as twice for 5 min at room temperature in 2× SSC–0.1% sodium dodecyl sulfate (SDS) and twice for 15 min at room temperature in 0.2× SSC–0.1% SDS (1× SSC is 0.15 M NaCl plus 0.01 M sodium citrate, pH 7.0). Chemiluminescent detection with CDP Star (Roche Molecular Biochemicals) as a substrate was done by using X-ray films.

Cloning and DNA sequence analysis. Chromosomal DNA of P. aerogenes JF3139 and plasmid pBluescript II SK(−) were digested with corresponding restriction enzymes. Fragments were extracted from gel by using the Jetseq Kit according to the manufacturer’s instructions (Genomed, Bad Oeynhausen, Germany). After ligation, transformation of K-12 strain DH5α was done by using the CaCl2 procedure (2). Plasmid DNA was extracted by the alkaline lysis method (4), treated with RNase, and purified by phenol–chloroform extraction or by using the Qiagen MiniPrep Kit (Qiagen).

Sequence analysis. Sequence comparisons were done by using BLAST (1).

Analysis of 16S rRNA genes. All strains investigated were tested genetically for their phylogenetic relationship by sequencing a 1.4-kb fragment of the 16S rDNA gene (rs) as described previously (25). The rs gene was amplified by using the universal 16S primers 16SUNI-L (5'-AGAGTTTGATCATGCTTCAAG-3') and 16SUNI-R (5'-GTTGATCCGCTTGTCAG-3'). PCR was performed with a PE9600 automated thermal cycler with MicroAmp tubes (PE Biosystems) by using a polymerase with proofreading activity in order to avoid artifacts in the DNA sequences. The reaction was carried out in a 50-μl volume containing 5 μl of 10× PCR buffer, 20 pmol of primer (each), 1 mM deoxynucleoside triphosphate, 2.5 U of Pro DNA polymerase (Roche Molecular Biochemicals), and 100 ng of genomic DNA as a template. PCR conditions were as follows: 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 60 s. A final extension step for 7 min at 72°C was included. The PCR product was subsequently purified with the PCR Purification Kit (Qiagen) and sequenced as described above by using the set of primers described elsewhere (25).

Restricting pax clones and CAMP test. Restricting plasmids harboring either paxA genes or the entire paxCAB operon were generated by PCR with the Expand Long Template PCR System (Roche Molecular Biochemicals) by using genomic DNA of JF3139 as a template. Plasmid pPAXC was constructed by using primers paxCA-L (5'-GGGATCTAGTACGATCACATAAATAACACCTAAT-3'; positions 95 to 76 on paxC) and paxCA-R (5'-CCGCTCGAGATTAGTCTATTA-3'; positions 33 to 15 bp after the paxA stop codon). After amplification the PCR products were purified with Sephadex X50 and cloned into the corresponding sites of plasmid pBluescript II SK(−).

The CAMP test for cohemolytic activity (9) was performed on 5% sheep blood agar plates by using a beta-hemolytic Staphylococcus aureus strain as described previously (19). The CAMP reaction was done with erythrocytes from different species. Blood was aseptically taken from swine, rabbits, horses, and humans in the presence of Alsever’s solution. Agar plates were then prepared by overlaying Beta-Agar-Base (Oxoid, Hampshire, England) plates with Trypticase-Soy-Agar (Schaible, Augsburg, Germany) which was prewashed with 10 ml H2O and 10 ml of 6 M guanidinium-HCl–0.1 M NaH2PO4–0.01 M Tris (pH 8.0). The loaded plug was washed with 10 ml of 6 M guanidinium-HCl–0.1 M NaH2PO4–0.01 M Tris (pH 8.0) and incubated overnight with gentle shaking at 4°C. The dissolved sonication pellet was centrifuged 20 min at 10,000 rpm, and the pellet was dissolved in 20 μl of 6 M guanidinium-HCl–0.1 M NaH2PO4–0.01 M Tris (pH 8.0) and incubated overnight with gentle shaking at 4°C. The dissolved sonication pellet was centrifuged 20 min at 10,000 rpm, and the supernatant was loaded onto a 2.5-ml Ni-nitrilotriacetic acid-agarose column (Qiagen, Hilden, Germany) which was prewashed with 10 ml of H2O and 10 ml of 6 M guanidinium-HCl–0.2 M acetic acid and then equilibrated with 2 × 10 ml of 6 M guanidinium-HCl–0.1 M NaH2PO4–0.01 M Tris (pH 8.0). The loaded column was washed with 10 ml of 6 M guanidinium-HCl–0.1 M NaH2PO4–0.01 M Tris (pH 8.0) and then eluted with 10-ml aliquots of elution buffer (5 M urea, 0.1 M NaH2PO4, 0.01 M Tris at pH values of 8.0, 7.0, 6.0, 5.0, and 4.5. Fractions of 1 ml were collected and analyzed on an SDS-gel. Fractions containing

### Table 1. Swine isolates analyzed in this study

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Isolation no.</th>
<th>RTX operon CAMP</th>
<th>Origin</th>
<th>Pathological finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 27883T</td>
<td></td>
<td>–</td>
<td>Intestine</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>JF2011</td>
<td></td>
<td>+</td>
<td>Intestine</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>JF2118</td>
<td>P1209/94</td>
<td>–</td>
<td>Placenta</td>
<td>Abortus</td>
</tr>
<tr>
<td>JF2006</td>
<td>P787/97</td>
<td>+</td>
<td>Placenta</td>
<td>Abortus</td>
</tr>
<tr>
<td>JF2032</td>
<td>99/890</td>
<td>+</td>
<td>Fetus</td>
<td>Abortus</td>
</tr>
<tr>
<td>JF2034</td>
<td>99/968</td>
<td>–</td>
<td>Intestine</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>JF2039</td>
<td>P894/97</td>
<td>–</td>
<td>Liver</td>
<td>Sepsis</td>
</tr>
<tr>
<td>JF2142</td>
<td>P542/98</td>
<td>–</td>
<td>Liver</td>
<td>Sepsis</td>
</tr>
<tr>
<td>JF2072</td>
<td>P78/98</td>
<td>–</td>
<td>Intestine</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>JF2101</td>
<td>99/1444</td>
<td>–</td>
<td>Bronchus</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>JF2185</td>
<td>P772/98</td>
<td>–</td>
<td>Bronchus</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>JF2154</td>
<td>P775/98</td>
<td>–</td>
<td>Bronchus</td>
<td>Pneumonia</td>
</tr>
</tbody>
</table>

* a Serotype 2 reference strain S1536.  
* b Serotype 3 reference strain S1421.
In the control strain *A. pleuropneumoniae* ATCC 27089 this results in the detection of two bands as expected from the sequence of the *apxIIIA* operon. One band at 730 bp resulted from a fragment containing the 3′ half of *apxIIC* and a short part of *apxIIIA*. The other band at 9 kb covers the 3′ part of *apxIIIA* as well as the two genes *apxIIIB* and *apxIIID*, coding for the secretion proteins. In *P. aerogenes* JF1319, two bands were observed which clearly differed from *apxIII*. The bands were located at 1.3 and ca. 11 kb, suggesting a different gene (Fig. 1). When an *apxIIIB*-specific probe was used, the 11-kb band of *P. aerogenes* also hybridized analogous to the 9-kb band of *A. pleuropneumoniae* (data not shown). This shows that *P. aerogenes* also contains RTX secretion genes *B* and *D* and suggests the presence of a complete RTX operon.

The genes of the putative RTX determinant of *P. aerogenes* field isolate JF1319 were cloned and sequenced. For this purpose a 3.3-kb *BglII* fragment hybridizing to the *apxIIICA* probe was cloned into the *BamHI* restriction site of plasmid pBlue-ScriptII SK(−) resulting in pJFFPAE1. This clone was used as a probe to find additional fragments covering the RTX operon. Thereby a 2.8-kb *PstI* fragment and a 5.5-kb *HindIII* fragment were cloned, resulting in pJFFPAE2 and pJFFPAE5, respectively. By using subclones of these basic clones and by using in addition the primer walking method we were able to sequence the complete operon in both directions. The new RTX operon was named *pax* (for *P. aerogenes* RTX toxin) in accordance with nomenclature of RTX toxins (18). Accordingly, the gene encoding the potential structural RTX toxin *paxA*, the gene for the activator *paxC*, and the two genes coding for putative secretion genes *paxB* and *paxD*.

A map of the *pax* operon, which shows the characteristic features of RTX operons, and the basic clones used for its sequence determination are shown in Fig. 2.

**Characterization of the *pax* operon.** The *paxC* gene is 510 bp long coding for a putative 169-aa (17.5-kDa) activator protein. It shows 82% similarity to the *apxIIIC* gene. The *paxA* gene is 3.15 kb long, encodes a presumed 1,069-aa protein of 107.5 kDa and also shows 82% similarity to *apxIIIA*. The deduced protein sequence of *PaxA* (Fig. 3) contains seven characteristic glycine-rich nonapeptide repeats based on the consensus L/I-F-X-G-G-X-G-N/D-D-X (36). Four similar repeats precede these classical patterns. The potential secretion protein genes *paxB*, which spans 2,136 bp and codes for a calculated 711-aa protein of 73 kDa, and *paxD*, which encodes a presumed 477-aa protein (49 kDa), are also present on the operon and show typical features of ABC transporters. The *paxB* gene is 83% similar to *apxIIIB*, the *paxD* gene 82% similar to *apxIIID*. Table 2 summarizes the similarities of the *pax* DNA and amino acid

**RESULTS**

Cloning and sequencing the *pax* operon. Based on the observation that a *P. aerogenes* field isolate (JF1319) hybridized with a set of broad-range DNA probes for the detection of RTX toxin genes (26), we characterized the hybridization signal in more detail. The strongest hybridization was seen with a clone consisting of the *apxIIICA*-derived gene probe. Genomic DNA of *P. aerogenes* JF1319 and of the *A. pleuropneumoniae* serotype 2 reference strain ATCC 27089 (S1536), used as a control for the *apxIII*, was digested with *EcoRI* and blotted onto nylon membranes. Southern blots were subsequently hybridized with a probe specific for *apxIIICA* (Fig. 1).

![FIG. 1. Southern blot of *P. aerogenes* and *A. pleuropneumoniae* with *apxIIICA* as probe. Genomic DNA of *P. aerogenes* JF1319 from a swine abortion case and *A. pleuropneumoniae* serotype 2 reference strain ATCC 27089 (S1536) was digested with *EcoRI*. After electrophoresis on a 1% agarose gel and transfer to nylon membrane, the filter was hybridized with the digoxigenin-labeled *apxIIICA*-derived probe.](image1)

![FIG. 2. Restriction map of *pax* operon from *P. aerogenes* JF 1319 and positions of the different clones used for determination of its sequence. The black arrows represent the four genes in the *pax* operon, with the arrowheads showing the direction of transcription.](image2)
sequences with RTX genes described in other Pasteurellaceae and the alpha-hemolysin of E. coli.

**Presence of pax in P. aerogenes strains.** In order to determine the prevalence of pax in this species, all P. aerogenes strains (Table 1) were screened for pax by Southern blots with PstI-digested genomic DNA and probes specific for paxCA and paxBD. The results in Fig. 4 show the characteristic bands for the pax operon in four P. aerogenes strains isolated from abortus cases or from a young piglet with septicemia originating from geographically distant farms and isolated in different years (JF1319, JF2118, JF2006, and JF2032). The P. aerogenes type strain, as well as the resting eight strains that were isolated from clinical material of pathologies other than abortus, did not show any signal with the pax-derived gene probes (Table 1).

**Identification of P. aerogenes by sequence analysis of the 16S rRNA gene (rrs).** Since the phenotypic identification of P. aerogenes has shown to be sometimes ambiguous, we further identified all strains selected for this study by sequencing of the 16S rRNA gene (rrs). A few strains which were initially identified by phenotypic methods to be P. aerogenes and were thus not included in this study. Among the

**TABLE 2. Similarity of the nucleotide and amino acid sequence of Pax proteins and their genes to other RTX determinants in Pasteurellaceae and to Hly of E. coli.**

<table>
<thead>
<tr>
<th>Locus</th>
<th>DNA or protein</th>
<th>% Identity (DNA) or % similarity (protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApxI</td>
<td>DNA</td>
<td>63 63 74 70</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>72 76 94 80</td>
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<td></td>
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<tr>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
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</tr>
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<td>DNA</td>
<td>66 62 71 61</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>74 78 94 78</td>
</tr>
</tbody>
</table>

**FIG. 3.** Amino acid sequence of PaxA. The seven consensus glycine-rich nonapeptide sequences are double underlined. The four preceding similar nonapeptide repeats are underlined.

**FIG. 4.** Southern blot of P. aerogenes strains. Genomic DNA of the type strain ATCC 27883T and 12 clinical isolates was digested with PstI, electrophoresed on 1% agarose gel, and transferred to nylon membranes. (A) Hybridization with digoxigenin-labeled paxCA derived probe. (B) Hybridization with digoxigenin-labeled paxBD-derived probe. M, HindIII marker, showing the 2.3- and the 2.0-kb fragments.

P. aerogenes strains isolated from clinical material, variations in the rrs sequences ranged from two to six different nucleotides (<0.5% variation) compared to the type strain rrs sequence. Some strains showed ambiguous bases at a few positions, indicating the presence of more than one rrs operon. Strains JF2111, JF2154, and JF2039, as well as strains JF1319 and JF2118, had identical sequences. By comparison of our sequence of the type strain with the one previously deposited by others (accession number M75048), we could resolve all of the unidentified bases in the latter. In addition, we detected three differences between sequence M75048 and our sequence of the type strain (U66491).

**Functional analysis of the PaxA toxin.** Since paxA-containing P. aerogenes did not show direct hemolytic activity on sheep and swine erythrocytes, we performed the CAMP test (9) for cohemolytic activity of this new RTX protein. Cohemolytic activity is known to be associated with Apx toxins, including ApxIII (19, 22). For this purpose all P. aerogenes strains were grown in the vicinity of a beta-hemolytic S. aureus. Whereas all four pax-positive isolates produced a clear hemolytic zone comparable to the ApxIII control, none of the pax-negative strains showed a CAMP effect (Fig. 5 and Table 1).
lytic if paxCA genes are also present (Fig. 5, lane 11). The less intensive cohemolysis of this last construct might be due to the fact that the hlyBD gene products secrete PaxA less efficiently than does the endogenous paxCA-encoded secretion machinery.

In order to determine whether the cohemolytic CAMP effect was specific to a given host, blood-agar plates prepared with erythrocytes from different species were used. For both RTX toxins, PaxA and ApxIII A, the cohemolytic zone differed on erythrocytes from different species (Table 3). Whereas on sheep blood the cohemolysis was generally more intense, it was less strong on human and rabbit erythrocytes and weak on pig and horse erythrocytes. On pig erythrocytes both PaxA and ApxIII A showed about the same strength of cohemolysis, while on erythrocytes of the other species PaxA was less active than ApxIII A.

Purified recombinant polyhistidine-tailed PaxA protein showed a serological cross-reaction with the ApxIIIA toxin from A. pleuropneumoniae, thus giving further evidence of the close relationship between these two toxins (data not shown).

**DISCUSSION**

*P. aerogenes* is known to belong to the normal intestinal flora of swine, as well as to act as an opportunistic pathogen (21, 30). Human cases of infection are rare and may occur after being bitten or gored by swine or via dog and cat bites or scratches. Due to its dual role as normal flora and an opportunistic pathogen, its pathogenicity is poorly understood and difficult to investigate. One study was done by inoculating mice with different strains of *P. aerogenes*. The various strains affected the mice heterogeneously, and two of the ten strains tested led to death after 24 h (33).

Despite its recognition as a mainly opportunistic pathogen, there are sporadic reports of *P. aerogenes* as a pathogen in abortion cases. Already in the first description of *P. aerogenes*, McAllister and Carter (30) describe an abortion case as the only clinical finding where *P. aerogenes* was involved as a primary pathogen. Other cases of abortion in swine were later reported by Hommez and Devriese (21), as well as by Fodor et al. (13). Thorsen et al. (Letter, Lancet 343:485–486, 1994) recently published a case report of human abortion due to *P. aerogenes*.

We report here the identification of a new RTX toxin gene, paxA, and its corresponding cohemolytic phenotype, which associates with specific *P. aerogenes* strains isolated from abortion cases in swine or from septicemia of young piglets. Other *P. aerogenes* strains which were isolated from different clinical samples of pigs with uneven pathological findings were devoid of the pax operon and did not produce CAMP cohemolysis.

The pax operon shows high similarity to the apxIII operon of *A. pleuropneumoniae*. Due to its high similarity (94%) to ApxIII A and due to its immunological relatedness to ApxIII A the activity of PaxA could be similar. ApxIII A is nonhemolytic but strongly cytotoxic for alveolar macrophages and neutrophils (16) and shows a cohemolysis with the *S. aureus* sphingomyelinase known as the CAMP reaction (19). The same cohemolytic effect was observed with *P. aerogenes* harboring the pax operon, whereas none of the pax-negative isolates showed the CAMP effect. The cohemolytic CAMP reaction of PaxA was observed on erythrocytes from different hosts. This finding is in agreement with other hemolytic or cohemolytic RTX toxins for which also no host specificity as determined by erythrocyte lysis was found.

We could demonstrate that the cohemolytic activity in *P. aerogenes* is specifically caused by the presence of the pax operon (Fig. 5). Transforming *E. coli* K-12 with the entire pax operon was sufficient to convert this CAMP-negative strain into a CAMP-positive one. The same phenotype conversion was observed when transforming only the paxCA genes into a K-12 strain containing functional genes for the hlyBD genes. This could be the result of a less-efficient secretion of paxA via the hly secretion pathway compared to its own specific pathway.

**TABLE 3. CAMP cohemolytic activity of PaxA and ApxIII A on erythrocytes of different species**

<table>
<thead>
<tr>
<th>Blood source</th>
<th>Reactiona with:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aerogenes</em></td>
<td>PaxA&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>Horse</td>
<td>−</td>
</tr>
<tr>
<td>Pig</td>
<td>(+)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>−</td>
</tr>
<tr>
<td>Sheep</td>
<td>++</td>
</tr>
</tbody>
</table>

<sup>a</sup> + + +, strong CAMP reaction; (+), distinct CAMP cohemolysis still visible.
P. aerogenes
uous identification of the species and were thus excluded. This revealed the importance of ge-

P. aerogenes producing

13
P. aerogenes from other, probably less virulent

virulence factor described in

virulent representatives of this

P. aerogenes. Since PaxA-toxigenic strains and

as a bacterium of low epidemiologic impact in many other cir-

born piglets in certain cases due to PaxA-toxigenic strains and

virulence factor described in

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the moment. Nevertheless, since RTX toxins are known induc-

sRRs

oral DNA. It could thereby be lost or

hypothesis that

haemolytica (15) (now Mannheimia haemolytica). Actinobacil-

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hysical effects of the species P. aerogenes, since unambig-

identification of the species P. aerogenes by phenotypic

means seems to be hampered by certain biochemical reac-

tions. Comparison of the 16S rRNA (rs) gene sequences of the

13 P. aerogenes strains included in this study revealed only

minor variation in its rs genes, i.e., <0.5%. This is within the

range of intraspecies variation (10, 14). Based on their rs

sequence, all strains map at the very same position on the phy-

logenetic tree of Pasteurellaceae described by Dewhirst et al.

(12). There was no correlation between the presence of the pax

operon and the 16S rDNA sequence analysis, which raises the

hypothesis that pax might not be clonal and therefore could

be located on a relatively mobile DNA. It could thereby be lost or

acquired by certain P. aerogenes strains. This would help to ex-

plain the ambiguous role of P. aerogenes as a pathogen leading to severe complications such as abortus or septicemia of new-

born piglets in certain cases due to PaxA-toxigenic strains and

as a bacterium of low epidemiologic impact in many other cir-

cumstances (nontoxigenic strains). In this respect the detection of

pax could be an indicator for virulent representatives of this

species. The role of PaxA in abortion remains speculative for the

moment. Nevertheless, since RTX toxins are known induc-

ers of cytokines such as interleukin-1 and tumor necrosis fac-

tor, they are thought to have an immunomodulating effect (11).

Therefore, it is conceivable that in the special immune status of

pregnancy this modulating effect could finally lead to abortion.

In summary, the new RTX toxin PaxA is the first potential

virulence factor described in P. aerogenes. PaxA showed cohe-

molytic activity in the CAMP test. This simple diagnostic test

allows researchers to differentiate PaxA-toxigenic P. aerogenes

from other, probably less virulent P. aerogenes strains. Since PaxA

and its operon paxCABD was specifically found in P. aerogenes

isolated from cases of abortion or septicemia in newborn pig-

lets, we speculate that PaxA is involved in the virulence of

P. aerogenes.

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