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Identification and Characterization of a DNA Region Involved in the Export of Capsular Polysaccharide by *Actinobacillus pleuropneumoniae* Serotype 5a

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Actinobacillus pleuropneumoniae **synthesizes a serotype-specific capsular polysaccharide that acts as a protective barrier to phagocytosis and complement-mediated killing. To begin understanding the role of *A. pleuropneumoniae* capsule in virulence, we sought to identify the genes involved in capsular polysaccharide export and biosynthesis. A 5.3-kb *Xba*I fragment of *A. pleuropneumoniae* serotype 5a J45 genomic DNA that hybridized with DNA probes specific for the *Haemophilus influenzae* type b *cap* export region was cloned and sequenced. This *A. pleuropneumoniae* DNA fragment encoded four open reading frames, designated *cpxDCBA*. The nucleotide and predicted amino acid sequences of *cpxDCBA* contained a high degree of homology to the capsule export genes of *H. influenzae* type b *bexDCBA*, *Neisseria meningitidis* group B *ctrABCD*, and, to a lesser extent, *Escherichia coli* K1 and K5 *kpsE* and *kpsMT*. When present in *trans*, the *cpxDCBA* gene cluster complemented *kpsM*::Tn*phoA* or *kpsT*::Tn*phoA* mutations, determined by enzyme immunoassay and by restored sensitivity to a K5-specific bacteriophage. A *cpxCB* probe hybridized to genomic DNA from all *A. pleuropneumoniae* serotypes tested, indicating that this DNA was conserved among serotypes. These data suggest that *A. pleuropneumoniae* produces a group II family capsule similar to those of related mucosal pathogens.**

Actinobacillus pleuropneumoniae is an economically important respiratory pathogen of swine. The serotype-specific, capsular polysaccharide protects it from phagocytosis and complement-mediated killing (14, 15, 46). Currently, 12 serotypes of *A. pleuropneumoniae*, which vary in virulence (36) and distribution around the world, are recognized (25). The capsular polysaccharide is therefore an important component for pathogenic, diagnostic, and epidemiologic studies. Nonencapsulated mutants isolated after ethyl methanesulfonate mutagenesis do not cause clinical symptoms or lung lesions in pigs, even after intratracheal challenge with a dose 10 times greater than the 50% lethal dose of the encapsulated parent (16). Furthermore, nonencapsulated *A. pleuropneumoniae* mutants induce protective immunity and are live vaccine candidates (16, 34). However, the genomic location, organization, and mechanism of cell surface expression of this important virulence determinant are unknown.

The genetic organization of the group II capsule gene loci of *Haemophilus influenzae* type b (20, 22), *Escherichia coli* K1 and K5 (4, 18, 33), and *Neisseria meningitidis* group B (10) is quite similar (3, 9). In each of these species, a central, serotype-specific DNA segment necessary for capsular polysaccharide biosynthesis is flanked by DNA encoding proteins for capsule export. Substantial homology occurs in the genes required for capsular polysaccharide export among these species, suggesting a common evolutionary origin (9). We report here the use of conserved DNA from an export region of the *H. influenzae* type b *cap* (*capb*) locus to identify, clone, and sequence a portion of the *A. pleuropneumoniae* serotype 5a capsulation locus involved in capsular polysaccharide export.

The bacterial strains, plasmids, and bacteriophage used in this study are described in Table 1. *A. pleuropneumoniae* strains were grown as described elsewhere (15). *E. coli* strains were grown in Luria-Bertani (LB) broth (38) for routine cultivation or in Terrific broth (44) for extraction of plasmids. Antibiotics were used in growth media for maintenance of plasmids at the following concentrations: ampicillin at 100 μg/ml, chloramphenicol at 30 μg/ml, streptomycin at 80 μg/ml, and tetracycline at 25 μg/ml.

Genomic DNA was isolated by suspending bacteria in 10 mM Tris–1 mM EDTA (pH 8.0) and incubating them with sodium dodecyl sulfate (0.66%) and RNase (100 μg/ml) for 1 h at 37°C. Proteinase K was added to a final concentration of 100 μg/ml, and the mixture was incubated at 56°C for 1 h. The mixture was then extracted once with buffered phenol and four times with buffered phenol-chloroform (Amresco, Inc., Solon, Ohio). The DNA was precipitated at −20°C by addition of 0.3 volumes of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 95% ethanol and resuspended in 10 mM Tris–1 mM EDTA (pH 8.0). Plasmid DNA was isolated by a rapid alkaline lysis method (17). Restriction fragments required for cloning and probe synthesis were eluted from agarose gels as described elsewhere (48). Restriction digests, agarose gel electrophoresis, and DNA ligations were performed as previously described (38). Plasmid DNA was transformed into *E. coli* strains by electroporation (7) with a BTX ECM 600 electroporator (BTX, Inc., San Diego, Calif.).

Knockout mutations in the *A. pleuropneumoniae* export region were attempted by homologous recombination. The procedure was identical to that used to obtain knockout mutants of the *A. pleuropneumoniae cap* biosynthesis region (47), except that the 2.1-kb *Cla*I region that spans part of *cpxC* and all of *cpxB* and *cpxA* of pCW-5E was deleted and ligated to the

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TABLE 1. Bacterial strains, plasmids, and bacteriophage used in this study

Strain, plasmid, or bacteriophage	Relevant genotype or characteristics	Source or reference
A. pleuropneumoniae strains		
4074	Serotype 1 strain (ATCC 27088)	ATCC[a]
1536	Serotype 2 strain (ATCC 27089)	ATCC
J45	Serotype 5a strain	B. Fenwick
K17	Serotype 5a strain (ATCC 33377)	ATCC
178	Serotype 5 strain	M. Mulks
29628	Serotype 7 strain	L. Hoffman
13261	Serotype 9 strain	J. Nicolet
E. coli strains		
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* (F^+ *proAB lacI*^{q} ZΔM15 Tn*10*); host for recombinant plasmids	Stratagene
MS101	*serA*$^+$ *rpsL* K5$^+$; Sm^r	43
Plasmids		
pGEM-3Z	Cloning vector, 2.74 kb; Amp^r	Promega Corp.
pCW-1C	5.3-kb *Xba*I fragment of J45 cloned into pGEM-3Z	This work
pCW-5E	Same insert as pCW-1C, except cloned in opposite orientation	This work
pSKH1	4.4-kb *Eco*RI fragment of *H. influenzae capb* locus cloned into pBR328; Amp^r Tet^r	13
pSKH2	9.0-kb *Eco*RI fragment of *H. influenzae capb* locus cloned into pBR328; Amp^r Tet^r	13
pPC6::6	Contains the entire *E. coli* K5 *kps* locus with a Tn*phoA* insertion in *kpsT*; Cm^r	28
pPC6::17	Contains the entire *E. coli* K5 *kps* locus with a Tn*phoA* insertion in *kpsM*; Cm^r	28
pKS1	3.8-kb *Bam*HI fragment of pUM24 containing the *nptI*[b]*-sacRB* cartridge[c]-cloned into the *Bam*HI site of pGEM-3Z; Amp^r Kan^r	S. M. Boyle
pCW1CK/S1	3.8-kb *Bam*HI fragment of pKS1 filled in and blunt end ligated to the 7.1-kb *Eco*RI-*Mlu*I fragment of pCW-1C; Amp^r Kan^r	This work
pCW11EΔ1KS1	pCW-11E with the 2.1-kb *Bgl*II-*Stu*I fragment deleted and the 3.8-kb *Bam*HI *nptI-sacRB* cartridge from pKS1 ligated in; Amp^r Kan^r	This work
pCW1C-20	pCW1C with 2.1-kb *Cla*I fragment deleted and the 1-kb *Nsp*V fragment of pBR325 ligated in; Amp^r Cm^r	This work
Bacteriophage K5	Specific for strains expressing the *E. coli* K5 capsular polysaccharide	I. S. Roberts

[a] ATCC, American Type Culture Collection.
[b] This marker was originally derived from the Tn*903 nptI* gene of pUC4K (Pharmacia Biotech, Piscataway, N.J.).
[c] This cartridge has been previously described (31).

1-kb *Nsp*V (chloramphenicol resistance gene) fragment of pBR325. This plasmid was designated pCW1C-20. Another deletion in pCW-1C was generated in the *Eco*RI-*Mlu*I site, which spans the 3′ end of *cpxD* to the 5′ end of *cpxB*. The 3.8-kb *Bam*HI fragment of pUM24 containing the *nptI-sacRB* cartridge from pKS1 was then filled in with the Klenow fragment of DNA polymerase I and blunt end ligated. This plasmid was designated pCW1CK/S1. Both plasmids were electroporated into *A. pleuropneumoniae* J45 as described previously (47).

Restriction endonuclease-digested DNA was transferred to MagnaGraph nylon membranes (Micron Separations, Inc., Westboro, Mass.) with 20× saline sodium citrate (SSC; 20× SSC is 3 M NaCl plus 300 mM sodium citrate, pH 7) as previously described (38, 42). DNA was covalently linked to nylon membranes by UV irradiation with a UV Stratalinker (Stratagene, La Jolla, Calif.). Digoxigenin-labeled probes for DNA hybridizations were synthesized by the random primer method with the Genius System nonradioactive labeling and detection kit (Boehringer Mannheim Corp., Indianapolis, Ind.). *E. coli* colonies containing recombinant plasmids were screened by colony hybridization with digoxigenin-labeled DNA probes according to the instructions of the manufacturer (Boehringer Mannheim Corp.). DNA hybridizations were performed at either 55 or 68°C (as indicated) in solutions containing 5× SSC. The membranes were washed and developed according to the Genius System directions for colorimetric detection.

The nucleotide sequence of both strands of a 4.6-kb *Xba*I-*Cla*I DNA fragment of pCW-1C was determined by the dideoxy chain termination method (39) with the Sequenase version 2.0 DNA sequencing kit (United States Biochemical Corp., Cleveland, Ohio) with ^{35}S-dATP (DuPont/NEN Research Products, Boston, Mass.). Double-stranded DNA templates were sequenced by using custom oligonucleotide primers (DNAgency, Inc., Malvern, Pa.) to continue reading along each strand. The nucleotide sequence obtained was analyzed with DNASTAR analysis software (DNASTAR, Inc., Madison, Wis.). Sequence similarity searches of the EMBL/GenBank/DDBJ databases were performed with BLAST software (1) at the National Center for Biotechnology Information (Bethesda, Md.).

E. coli capsular polysaccharide on recombinant strains was identified by plaque formation by using K5 capsule-specific bacteriophage and enzyme-linked immunosorbent assay (ELISA). For plaque analysis, 200 μl of mid-log-phase culture was combined with 200 μg of IPTG (isopropyl-β-D-thiogalactopyranoside) per ml–10 μl of diluted phage stock (approximately 200 PFU total)–3 ml of LB top agar (38) containing antibiotics and IPTG. This mixture was poured onto prewarmed (37°C) LB agar plates and allowed to solidify. The plates were incubated

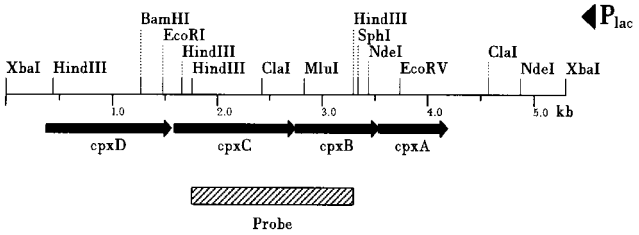

FIG. 1. Physical map of pCW-1C insert DNA from *A. pleuropneumoniae* J45. The locations and directions of transcription of the four major open reading frames (*cpxDCBA*) identified by dideoxy sequencing are indicated. The direction of transcription from the *lac* promoter (P_{lac}) located on the plasmid vector is indicated by the arrowhead. The 1.5-kb *Hin*dIII fragment used as the DNA probe in Fig. 3 is shown. pCW-5E is identical to pCW-1C, but with the insert in the opposite orientation.

overnight at 37°C before evaluation of plaque formation. Antiserum to purified K5 capsule could not be raised because it is identical in structure to the first intermediate component of heparin (19). Therefore, antiserum to whole cells was raised in Swiss Webster mice by immunization with 10^8 CFU of encapsulated, formalin-killed *E. coli* K5 strain MS101 mixed 1:1 with Freund's complete adjuvant. Immunizations were repeated without adjuvant until an immune response to the K5 capsule was detected by ELISA. Antibodies specific for the K5 capsule were purified by affinity chromatography (24). An ELISA to measure capsule on whole cells was done as described previously (2).

Statistical analysis was performed by the unpaired *t* test and two-tailed *P* value with InStat statistical software (GraphPad Software, San Diego, Calif.).

Identification and cloning of a DNA region involved in *A. pleuropneumoniae* encapsulation. Probes specific for the export regions of the *H. influenzae* type b capsulation (*capb*) locus hybridized to *A. pleuropneumoniae* DNA under conditions of medium to low stringency (55°C, 5× SSC) (data not shown). A 4.4-kb *Eco*RI fragment from pSKH1 (13), containing the region 1 *bexD* gene involved in *H. influenzae* type b capsular polysaccharide export (21), hybridized to 1.2-kb *Hin*dIII and 5.3-kb *Xba*I fragments of strain J45 genomic DNA. A 9.0-kb *Eco*RI fragment of pSKH2 (13) from the *H. influenzae capb* locus, containing the region 1 capsule export *bexCBA* genes (21), hybridized to 1.5-kb *Hin*dIII, 5.3-kb *Xba*I, and 2.4-kb *Xho*I fragments of J45 genomic DNA. These data indicated that *A. pleuropneumoniae* serotype 5a DNA had regions of homology with *H. influenzae* type b *cap* genes, most likely region 1 export genes, as both probes bound the strain J45 5.3-kb *Xba*I DNA fragment. The 5.3-kb *Xba*I fragment was cloned into the *Xba*I site of pGEM-3Z in both orientations. The resulting plasmids were designated pCW-1C (Fig. 1), and those with the insert in the opposite orientation were designated pCW-5E. Probes specific for *H. influenzae* type b *bexD*, *bexC*, *bexB*, and *bexA* hybridized by Southern blotting to adjacent restriction fragments of pCW-1C in the same order in which *bexDCBA* occur in *H. influenzae* type b (data not shown).

Nucleotide sequence and analysis of *A. pleuropneumoniae* capsulation DNA. The nucleotide sequence of the 4.6-kb *Xba*I-*Cla*I restriction fragment of pCW-1C was determined. Four open reading frames designated *cpxDCBA* (*cpx* for capsular polysaccharide export) were detected in close proximity on the same DNA strand. The AUG initiation codon of *cpxC* was 26 nucleotides downstream from the UAA termination codon of *cpxD*, whereas the AUG initiation codon of *cpxB* overlapped the UAA termination codon of *cpxC*, and the AUG initiation codon of *cpxA* overlapped the UGA termination codon of *cpxB*. Shine-Dalgarno ribosome-binding consensus sequences (40) were identified within 17 bases upstream of each AUG initiation codon. A putative promoter, containing sequences similar to *E. coli* σ^{70} −10 (TATAAT) and −35 (TTGACA) consensus sequences (11), was identified upstream of *cpxD*. A palindromic sequence, which may function as a rho-independent transcription termination signal (35), was identified downstream from *cpxA*. BLAST searches (1) of the combined, nonredundant nucleotide and protein databases at the National Center for Biotechnology Information indicated that *A. pleuropneumoniae cpxDCBA* were highly homologous at both the nucleotide and amino acid levels to *H. influenzae bexDCBA* (region 1 capsule locus) (21) and *N. meningitidis ctrABCD* (region C capsule locus) (9) (Table 2). Lesser homology was also detected between CpxC, CpxB, and CpxA and *E. coli* K1 and K5 KpsE (region 1) (6, 27, 37), KpsM (region 3), and KpsT (region 3) (26, 41), respectively (Table 2). Substantial homology to other *E. coli* region 1 genes (*kpsDUCS*) was not evident.

The predicted molecular masses of CpxD, CpxC, CpxB, and CpxA were 42.1, 43.4, 29.9, and 24.6 kDa, respectively. The first 20 amino acids of CpxD were characteristic of an N-terminal prokaryotic lipoprotein signal peptide (29). The hydropathy plots of CpxD, CpxC, CpxB, and CpxA, as well as their structural homology to their homologs from *H. influenzae* type b, *N. meningitidis* group B, and *E. coli* K5 (23), are shown in Fig. 2. CpxD may be an outer membrane lipoprotein involved in capsular polysaccharide transport across the outer membrane. CpxC was predicted to be a relatively hydrophilic protein with hydrophobic domains near the N and C termini that may serve as membrane anchors. The CpxC homologs are cytoplasmic membrane proteins that have a substantial periplasmic domain (6, 9, 21, 37) and are proposed to serve as the second component of a protein complex involved in polysaccharide transport across the cytoplasmic membrane (30). CpxB was predicted to be a very hydrophobic protein containing at least six potential membrane-spanning α-helical domains, suggesting that CpxB may be an integral membrane protein. CpxA was predicted to be a relatively small, hydrophilic protein containing amino acid sequences matching the A (GRXGXGK ST) and B (XXXXD) motif consensus sequences characteristic

TABLE 2. Pairwise comparison of nucleotide and deduced amino acid (in parentheses) sequences of *A. pleuropneumoniae* J45 *cpxDCBA* to sequences involved in capsular polysaccharide export in *H. influenzae* type b (*bexDCBA*), *N. meningitidis* group B (*ctrABCD*), and *E. coli* K1 and K5 (*kpsE* and *kpsMT*)

Gene	% Similarity[a] with gene:			
	cpxD	*cpxC*	*cpxA*	*cpxA*
bexD	66.5 (74.4)			
ctrA	50.6 (50.3)			
bexC		65.5 (75.1)		
ctrB		52.5 (56.2)		
kpsE		27.0 (23.0)		
bexB			66.9 (75.9)	
ctrC			64.5 (72.9)	
kpsM			26.9 (26.3)	
bexA				75.0 (82.9)
ctrD				70.0 (82.5)
kpsT				46.1 (41.5)

[a] Percent similarity was determined by analyzing distances of aligned sequences by the Clustal algorithm of DNASTAR sequence analysis software (DNASTAR, Inc.) with the PAM250 residue weight table (protein alignments) and the Weighted residue weight table (nucleotide alignments).

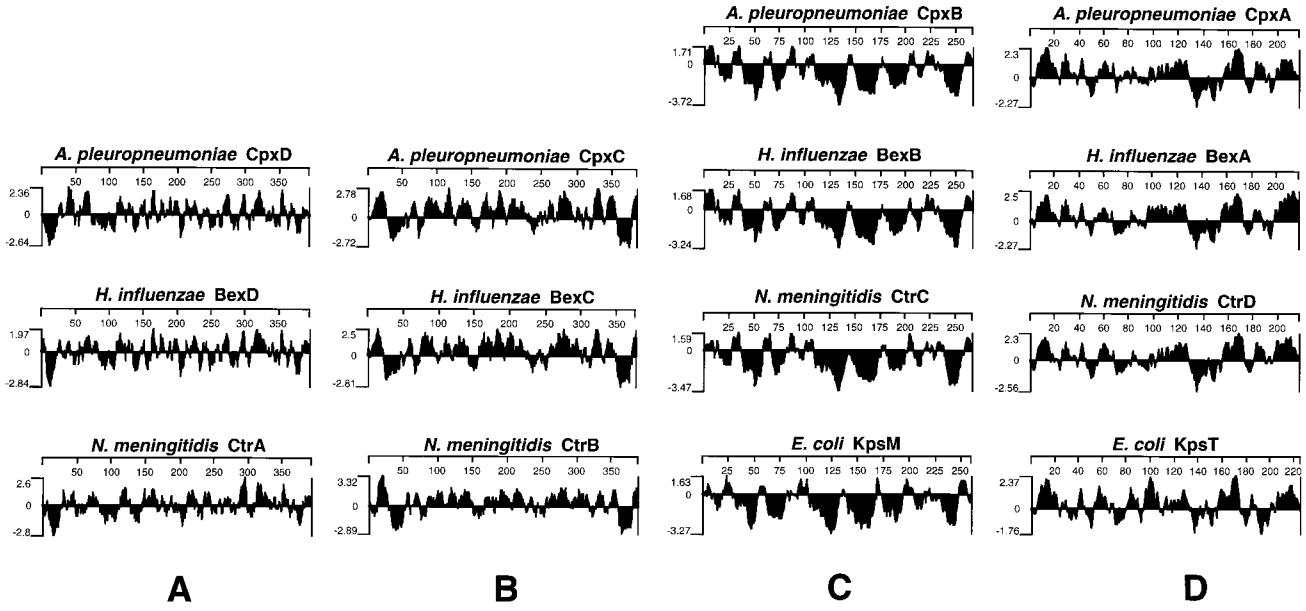

FIG. 2. Comparison of hydropathy profiles of the predicted amino acid sequences of *A. pleuropneumoniae* J45 *cpxDCBA*, *H. influenzae* type b *bexDCBA*, *N. meningitidis* group B *ctrABCD*, and *E. coli* K5 *kpsMT* involved in capsular polysaccharide export. Profiles were determined by the algorithm developed by Kyte and Doolittle (23) with a window size of 9 amino acids. The vertical axis displays relative hydrophilicity with negative scores indicating relative hydrophobicity. The horizontal axis displays a scale of the numbered amino acid residues from the N to C terminus of each protein. (A, B, C, and D) Display profiles of CpxD, CpxC, CpxB, and CpxA and their homologs, respectively.

of ATP-binding proteins (8, 12, 45). Thus, based on predicted functions of their homologs, CpxC, CpxB, and CpxA may be components of a group of ATP-binding cassette transporters (8, 12), known as the ATP-binding cassette 2 subfamily (30), involved in polysaccharide export across the cytoplasmic membrane. Similar types of transporters that are involved in the export of other bacterial cell surface polysaccharides across the cytoplasmic membrane have been identified elsewhere (5, 8, 9, 21, 26, 41).

Complementation of *E. coli* K5 *kpsM*::Tn*phoA* and *kpsT*::Tn*phoA*. To confirm the role of *cpxDCBA* in capsular polysaccharide export, attempts were made to generate nonencapsulated *A. pleuropneumoniae cpx* mutants by allelic replacement with a *cpxBA* deletion derivative of pCW-1C containing kanamycin. However, after repeated attempts no kanamycin-resistant *A. pleuropneumoniae* organisms were isolated following electroporation (data not shown). In contrast, similar allelic exchange experiments have been successful in obtaining nonencapsulated mutants containing deletions in DNA downstream of *cpxD*, which would correspond to the region containing capsule biosynthesis genes (47). Following these experiments, it was noted that Kroll et al. (21) obtained similar results when attempts were made to mutate *bexB* in *H. influenzae* type b. Since deletions in *bexC* did result in viable, nonencapsulated cells, the authors concluded that mutations in *bexB* were lethal.

Alternatively, complementation of plasmid-encoded *E. coli* K5 *kps* loci containing Tn*phoA* insertions in *kpsM* (pPC6::17) or in *kpsT* (pPC6::6) (28) was performed with pCW-1C and pCW-5E. Expression of the *E. coli* K5 capsular polysaccharide was determined by examining plaque formation with a K5-specific bacteriophage and by ELISA. The bacteriophage did not form plaques on host *E. coli* XL1-Blue(pPC6::6) or XL1-Blue(pPC6::17) but did form plaques on encapsulated control strain MS101 (43). The K5-specific bacteriophage formed faint, but clearly defined, plaques on XL1-Blue(pPC6::6/pCW-5E) and XL1-Blue(pPC6::17/pCW-5E) (data not shown due to poor photographic reproduction of plaques). Although the plaques formed were faint, they were more defined on XL1-Blue (pPC6::6/pCW-5E), in which sequence homology between *kpsT* and *cpxA* was greater than that between *kpsM* and *cpxB*. Complementation of *kpsM*::Tn*phoA* or *kpsT*::Tn*phoA* mutations with *cpxDCBA* was dependent on the orientation of these genes relative to the *lac* promoter on pGEM-3Z because the bacteriophage did not form plaques on *E. coli* XL1-Blue (pPC6::6/pCW-1C) or XL1-Blue(pPC6::17/pCW-1C) (data not shown). Therefore, it was probable either that the putative promoter sequence identified upstream from *cpxD* was not functional in *E. coli* or that the promoter sequence necessary for transcription of *cpxDCBA* was not located on the 5.3-kb *Xba*I DNA fragment originally cloned in pCW-1C. The incomplete plaques may have been due to poor complementation or translational efficiency, resulting in a low level of exported capsular polysaccharide. Alternatively, expression of capsule on XL1-Blue by genes encoded in *trans* may have resulted in inefficient entry and lysis of the cells by the bacteriophage.

To directly demonstrate and quantitate capsule on the cell surface of XL1-Blue(pPC6::6/pCW-5E) and XL1-Blue(pPC6::17/pCW-5E), complementation of capsule export was confirmed by ELISA. Affinity-purified antibody to K5 capsule diluted 1:400 had a mean absorbance of 0.4440 ± 0.0475 for MS101 cells, 0.3683 ± 0.0251 for XL1-Blue(pPC6::6/pCW-5E) cells, 0.3033 ± 0.0207 for XL1-Blue(pPC6::17/pCW-5E) cells, 0.1497 ± 0.0211 for XL1-Blue(pPC6::6) cells, and 0.0563 ± 0.0525 for XL1-Blue(pPC6::17) cells. The difference in absorbance between MS101 cells and XL1-Blue(pPC6::6/pCW-5E) cells was considered not quite significant ($P = 0.0713$) but was significant between MS101 cells and XL1-Blue(pPC6::17/pCW-5E) cells ($P = 0.0093$). However, the absorbance difference was considered extremely significant between XL1-Blue(pPC6::6/pCW-5E) cells and XL1-Blue(pPC6::6) cells ($P = 0.0003$) and very significant between XL1-Blue(pPC6::17/pCW-5E) cells and XL1-Blue(pPC6::17) cells ($P = 0.0016$).

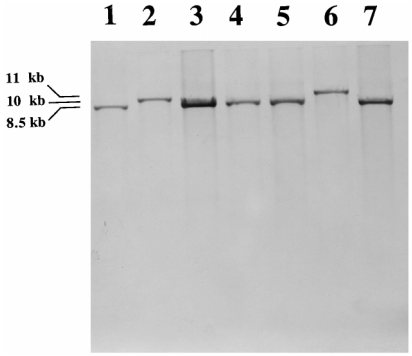

FIG. 3. Southern blot analysis of *A. pleuropneumoniae* genomic DNA hybridized to a digoxigenin-labeled probe specific for *A. pleuropneumoniae* J45 *cpxCB*. *Bgl*II-digested genomic DNAs from serotype 1 strain 4074 (lane 1), serotype 2 strain 1536 (lane 2), serotype 5a strain J45 (lane 3), serotype 5a strain K17 (lane 4), serotype 5 strain 178 (lane 5), serotype 7 strain 29628 (lane 6), and serotype 9 strain 13261 (lane 7) were hybridized with the 1.5-kb *Hin*dIII fragment of pCW-1C (Fig. 1) at 68°C. The approximate molecular size of each band that hybridized with the probe is indicated in kilobases.

Thus, both assays demonstrated the presence of extracellular K5 capsule on recombinant XL1-Blue cells containing pCW-5E. However, the amount of extracellular capsule on XL1-Blue (pPC6::6/pCW-5E) cells was more similar to the capsule content on MS101 cells than plaque analysis indicated. The difference between plaque intensity and ELISA absorbance may therefore be due to differences in bacteriophage entry and lysis between bacteria in which all the genes necessary for capsule formation, export, and attachment are located on the chromosome and bacteria in which the genes for a single capsule locus are located on a plasmid and expressed in *trans*. Furthermore, greater homology between CpxA and KpsT than between CpxB and KpsM could result in more efficient export of capsule, explaining the enhanced plaque formation and ELISA absorbance by XL1-Blue(pPC6::6/pCW-5E) compared to XL1-Blue(pPC6::17/pCW-5E). Nonetheless, since pPC6::6 and pPC6::17 contain all the genes necessary for K5 capsule production, except for a mutation in export gene *kpsT* or *kpsM*, respectively, it can be concluded that one of the genes in pCW-5E (presumably *cpxA* or *cpxB*) complemented *kpsT* and *kpsM*, respectively, and enabled export of K5 capsule. The complementation of defective capsular polysaccharide export genes with homologous genes from another capsular type of the same species has been described elsewhere (32, 33). However, to the best of our knowledge, this is the first report describing the complementation of a defective capsular polysaccharide export gene by a homologous gene from a different bacterial species.

Hybridization of *cpxCB* with DNA of heterologous *A. pleuropneumoniae* serotypes. The group II capsule export genes are known to be highly conserved (9, 22, 33). To determine if *cpxDCBA* was conserved among *A. pleuropneumoniae* serotypes, the 1.5-kb *Hin*dIII fragment of pCW-1C specific for *cpxCB* (Fig. 1) was used to probe *Bgl*II-digested genomic DNA from *A. pleuropneumoniae* serotypes 1, 2, 5, 7, and 9 (Fig. 3). The *cpxCB* probe hybridized under conditions of high stringency (68°C, 5× SSC) to an approximately 8.5-kb fragment from serotypes 1, 5, and 9; an approximately 10-kb fragment from serotype 2; and an approximately 11-kb fragment from serotype 7. Therefore, *cpxCB* was conserved among all *A. pleuropneumoniae* serotypes examined, although a restriction fragment length polymorphism was observed between some serotypes. Since J45 *cpxDCBA* were contiguous and *cpxCB* hybridized to genomic DNA from several *A. pleuropneumoniae* serotypes, it is likely that *cpxDCBA* comprise a single region required for capsular polysaccharide export that flanks a serotype-specific region required for capsular polysaccharide biosynthesis. The presence of the capsule biosynthesis DNA region has recently been confirmed by sequencing and knockout mutagenesis by allelic exchange in the DNA upstream of *cpxDCBA* (47). We propose to designate the *cpxDCBA* gene cluster as region 1 of the *A. pleuropneumoniae* serotype 5a capsule gene locus, since *cpxDCBA* were most homologous to the region 1 *bexDCBA* gene cluster of the *H. influenzae* type b *cap* locus. The identification of this region is an important first step toward incorporating defined mutations in this locus and evaluating *A. pleuropneumoniae* capsule mutants in pathogenic and vaccine studies.

Nucleotide sequence accession number. The nucleotide sequence of the 4.6-kb *Xba*I-*Cla*I restriction fragment of pCW-1C was submitted to GenBank and assigned accession no. U36397.

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