NOTE *Bacteriology*

The genetic organization of the capsular polysaccharide biosynthesis region of *Actinobacillus pleuropneumoniae* serotype 14

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ABSTRACT. The genetic organization of the gene involved in the capsular polysaccharide (CPS) biosynthesis of *Actinobacillus pleuropneumoniae* serotype 14 has been determined. The DNA region for the CPS biosynthesis of serotype 14 (*cps14*) comprised 9 open reading frames, designated as *cps14AB$_1$B$_2$B$_3$CDEFG* genes, encoding Cps14A to Cps14G protein, respectively. Cps14A was similar to CpsA of *A. pleuropneumoniae* serotypes 1, 4 and 12; the Cps14B$_1$ and Cps14B$_2$ were similar to CpsB of *A. pleuropneumoniae* serotypes 1, 4 and 12, suggesting that CPS structure of *A. pleuropneumoniae* serotype 14 would belong to Group I including *A. pleuropneumoniae* serotypes 1, 4, 12 and 15. Surprisingly, the overall nucleotide sequence, deduced amino acid sequence, and the genetic organization of the *cps14* were nearly identical to those of *Actinobacillus suis*. This study will provide the molecular basic knowledge for development of diagnostics and vaccine of *A. pleuropneumoniae* serotype 14.

KEY WORDS: *Actinobacillus pleuropneumoniae* serotype 14, *Actinobacillus suis*, capsular polysaccharide biosynthesis, genetic organization

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Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia [8]. To date, two biotypes and at least 15 serotypes are present in *A. pleuropneumoniae* on the basis of β-nicotinamide adenine dinucleotide (NAD) requirement for growth and the capsular polysaccharide (CPS) structure [4, 24]. *A. pleuropneumoniae* biotype 1 (NAD-dependent) has been divided into 14 serotypes (serotypes 1 to 13 and 15) and biotype 2 (NAD-independent) into 7 serotypes (serotypes 2, 4, 7, 9, 11, 13 and 14) [3, 19, 20, 23]. Isolation of biotype 2 strains from pleuropneumonic lungs of pigs is thought to be sporadic, and biotype 2 strains have been considered less virulent than biotype 1 [6, 8, 20]. The low rate of isolation of biotype 2 strains has been considered due to an underestimation by laboratories that are not familiar with this group, because of its NAD-independent growth [19]. Sporadic isolation of *A. pleuropneumoniae* biotype 2 serotype 14 strains has been reported only in Denmark [7, 20, 27]. However, Maldonado and other researchers have reported that biotype 2 serotype 14 could be implicated in non-sporadic outbreaks of fatal swine pleuropneumonia in Spain [18] as well as biotype 2 serotypes 2, 4, 7, 11 [19] and 13 [18], indicating that biotype 2 strains may have a potential to emerge, because of the current global movement of pigs.

The author reports here the nucleotide sequence and genetic organization of the genes involved in the capsular polysaccharide (CPS) biosynthesis of *A. pleuropneumoniae* biotype 2 serotype 14 (*cps14*). The first aim of this study is to obtain a molecular basic knowledge for development of the *cps14* gene-based diagnostic tools for *A. pleuropneumoniae* serotype 14, such as PCR typing methods [1, 5, 12, 13, 15, 25, 27]. The second aim of this study is to obtain a molecular basic knowledge for development of vaccines, such as a genetically modified capsule-deficient mutant vaccine for *A. pleuropneumoniae* serotype 14 [9].

A. pleuropneumoniae biotype 2 serotype 14 strain 3906, which was kindly provided by Dr. L. O. Andresen, National Veterinary Institute, Technical University of Denmark [20], was used in the present study. The strain was grown on tryptic soy agar (Difco Laboratories, Detroit, MI, U.S.A.) supplemented with 5% horse blood and 100 μg/m*l* β-NAD at 37°C and 5% CO_2.

Genomic DNA for PCR templates was extracted as described previously [10]. PCR was done in a total volume of 50μl. The primers 5′-TCT AAR AYC GCA STA TGG CTA GGR CCT GAR GT-3′ and 5′-AAY GCT TTA TCA AAA GCG TGC CAA TGR CGC T-3′, located in *cpxD* gene encoding capsule export protein and *lysA* gene encoding diaminopimelate decarboxylase, respectively, were designed from previously published sequences [26], since the two genes are conserved in *A. pleuropneumoniae* and serotype specific CPS biosynthesis region is usually flanked by *cpxD gene* in *Pasteurellaceae* [14, 26]. A touchdown PCR with DNA polymerase KOD-FX Neo (Toyobo, Osaka, Japan) was done for amplification of the *cps* region as recommended by the supplier. The PCR conditions were as follows: step 1: 94 for 2 min (1 cycle); step 2: 98°C for 10 sec and 74°C for 15 min (5 cycles); step 3: 98°C for 10 sec and 72°C for 15 min (5 cycles); step 4: 98°C for 10 sec and 70°C for 15 min (5 cycles); step 5: 98°C for 10 sec and 68°C for 15 min (20 cycles); final step: 68°C for 10 min (1 cycle). Amplified PCR products were analyzed by agarose gel electrophoresis, stained with ethidium bromide (10 μg/m*l*) and visualized under ultravio-

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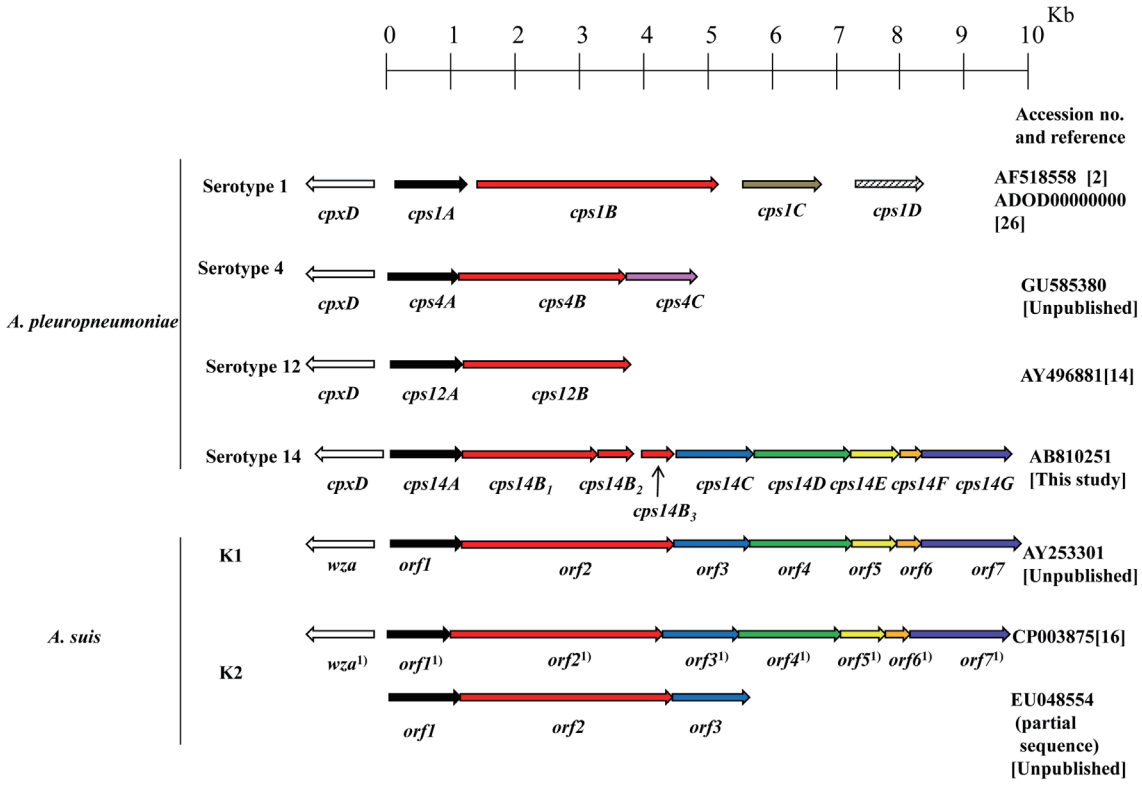

Fig. 1. Schematic diagram of the genetic organization of the DNA region involved in the CPS of *Actinobacillus pleuropneumoniae* serotypes 1 (Accession number (no.) AF518558 [2] and ADOD00000000 [26]), 4 (Accsession no. GU585380 [unpublished]), 12 (Accession no. AY496881 [14]), 14 (AB810251 [this study]) and *A. suis* serotypes K1 (Accession no. AY253301 [unpublished]) and K2 (Accession no. CP003875 [16] and EU048554 [unpublished]). The arows in the same colors indicate open reading frames, of which encoding proteins show amino acid sequence homology. [1)]Since the gene names are not designated by the author (Accession no. CP003875 [16]), the eight genes are named as described in *A. suis* serotype K1 (Accession no. AY253301) and serotype K2 (Accession no. EU048554). Length of *orf1* under accession no. CP003875 was slightly smaller than that under accession no. EU048554, althogh the same strain H91-380 was used. It is unknown why identical or nearly identical nucleotide sequence of genes for CPS synthesis region and the common genetic organization are observed between *A. suis* serotpes K1 and K2 [16].

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The PCR products were purified and sequenced as described previously [11]. Homology searches of the DDBJ /EMBL/Genbank databases were performed using the BLAST server at National Institute of Genetics, Japan. The nucleotide sequence of the CPS biosynthesis genes of *A. pleuropneumoniae* serotype 14 strain 3906 has been deposited in DDBJ/EMBL/Genbank under accession number AB810251.

Comparison of overall nucleotide sequence determined in this study (11,497 nucleotides (nt)) revealed the highest similarity to the *cps* region of *Actinobacillus suis* (98% identity over 10,588 nt) [16]. Nine open reading frames (ORFs) were located between *cpx14D* and *lysA* genes (Fig. 1). The ORFs were designated as *cps14AB$_1$B$_2$B$_3$CDEFG* genes (Fig. 1), encoding Cps14A to Cps14G protein, respectively. Cps14B$_1$ showed homology to the N-terminal region of Cps1B, Cps4B and Cps12B from *A. pleuropneumoniae* serotypes 1, 4 and 12 and ORF 2 of *A. suis*, respectively; Cps14B$_2$ showed homology to the central or C-terminal region of Cps1B, Cps4B and Cps12B from *A. pleuropneumoniae* serotypes 1, 4 and 12 and ORF2 from *A. suis*, respectively; Cps14B$_3$ showed homology to C-terminal region of *A. suis*, indicating that three ORFs, *cpsB$_1$B$_2$B$_3$,* seem to be generated due to the existence of the stop codons in *A. pleuropneumoniae* serotype 14 which are absent in the *cpsB* of *A. pleuropneumoniae* serotypes 1, 4, 12 and ORF2 of *A. suis*. The G+C content of the *cps14AB$_1$B$_2$B$_3$CDEFG* ranged from 25% (in *cps14B$_3$*) to 33% (in *cps14G*) (Table 1), which is lower than the 41 and 40% (overall G+C content of the *A. pleuropneumoniae* [26] *and A. suis* [16] genome, respectively), indicating that the region may be acquired by horizontal gene transfer.

At the amino acid level, Cps14A to Cps14G showed a homology to proteins of *A. pleuropneumoniae* serotypes 1, 4, 12 and *A. suis* [2, 14, 16] and did not show any significant homology to CPS proteins of *A. pleuropneumoniae* other serotypes. Their identities are shown in Table 1. Serotype-specific enzymes that are involved in the CPS biosynthesis are probably responsible for the dissimilarities of the CPS chemical structures [26]. However, *A. pleuropneumoniae*

Table 1. Identity of Cps proteins of *Actinobacillus pleuropneumoniae* seotype 14 (Cps14) to that of *A. pleuropneumoniae* and *Actinobacillus suis* serotypes

Cps14 protein	Lengh of aa[a] of Cps14	G+C% of *cps14* gene	Bacterial species	Serotype	Homologous protein	Accession number	Reference	% identity	Length over homologous aa
Cps14A	370	30	Ap[b)]	1	Cps1A	AF518558	[2]	98.8	339
			Ap	4	Cps4A	GU585380	Unpublished	98.1	370
			As[c)]	K1	ORF1	AY253301	Unpublished	95.9	368
			As	K2	ORF1	EU048554	Unpublished	95.9	368
			As	K2	ORF1[d)]	CP003875	[16]	95.5	332
			Ap	12	Cps12A	AY496881	[14]	93.2	367
$Cps14B_1$	709	28	As	K1	ORF2	AY253301	Unpublished	99.3	706
			As	K2	ORF2[d)]	CP003875	[16]	99.3	706
			As	K2	ORF2	EU048554	Unpublished	99.3	706
			Ap	4	Cps4B	GU585380	Unpublished	52.5	713
			Ap	1	Cps1B	AF518558	[2]	49.9	717
			Ap	12	Cps12B	AY496881	[14]	35.5	713
$Cps14B_2$	190	28	As	K1	ORF2	AY253301	Unpublished	99.5	189
			As	K2	ORF2[d)]	CP003875	[16]	99.5	189
			As	K2	ORF2	EU048554	Unpublished	99.5	189
			Ap	4	Cps4B	GU585380	Unpublished	55.8	147
			Ap	1	Cps1B	AF518558	[2]	58.7	75
			Ap	12	Cps12B	AY496881	[14]	33.1	151
$Cps14B_3$	152	25	As	K1	ORF2	AY253301	Unpublished	100	152
			As	K2	ORF2[d)]	CP003875	[16]	100	152
			As	K2	ORF2	EU048554	Unpublished	100	152
Cps14C	410	27	As	K1	ORF3	AY253301	Unpublished	99.5	410
			As	K2	ORF3[d)]	CP003875	[16]	99.5	410
			As	K2	ORF3	EU048554	Unpublished	99.5	410
Cps14D	549	29	As	K1	ORF4	AY253301	Unpublished	99.8	549
			As	K2	ORF4[d)]	CP003875	[16]	99.8	549
Cps14E	238	29	As	K1	ORF5	AY253301	Unpublished	99.6	238
			As	K2	ORF5[d)]	CP003875	[16]	99.6	238
Cps14F	128	30	As	K1	ORF6	AY253301	Unpublished	97.7	128
			As	K2	ORF6[d)]	CP003875	[16]	97.7	128
Cps14G	522	33	As	K1	ORF7	AY253301	Unpublished	99.4	522
			As	K2	ORF7[d)]	CP003875	[16]	99.4	522

a) aa=Amino acid; b) *A. pleuropneumoniae*; c) *A. suis*; d) Protein name was designated in this study as named in *A. suis* serotype K1 [Accession number AY253301].

serotypes 1 to 13 and 15 can be divided into three groups based on basic differences of their chemical compositions and the structures of the CPS: Group I (serotypes 1, 4, 12 and 15), with CPS composed solely of repeating oligosaccharide units joined through phosphate linkages; Group II (serotypes 5 and 10), with CPS composed of repeating oligosaccharide units; Group III (serotypes 2, 3, 6, 7, 8, 9, 11 and 13), with CPS composed of teichoic acid polymers joined through phosphate diester linkages [14, 17, 21, 22]. The genetic organization of the *cps* genes provided molecular evidence to support the grouping of *A. pleuropneumoniae* serotypes described above [26]. *A. pleuropneumoniae* serotype 14 carried the *cps14A* gene encoding putative CPS phosphotransferase (Table 1). This enzyme shared by serotypes 1, 4 and 12 may be involved in the chemical linkage of phosphate in the linear CPS backbone [26]. Therefore, it is suggested that the CPS of serotype 14 belongs to Group I including serotypes 1, 4 and 12 [14, 26], although the CPS chemical structure of serotypes 14 should be determined by the chemical structural characterization. Surprisingly, the overall nucleotide sequence, deduced amino acid sequence and the genetic organization of the *cps14* were nearly identical to those of *A. suis* (Table 1 and Fig. 1). High similarities in the nucleotide sequence of the *cps* genes of some *A. pleuropneumoniae* serotypes may be suggestive evidences for chemical structural similarity, but not for antigenic similarity of CPS [14, 26]. Therefore, the antigenic similarity of the CPS between *A. pleuropneumoniae* serotype 14 and *A. suis* should be determined for development of serological diagnostics and vaccine for the 2 organisms.

In conclusion, the author believes that this study will provide the molecular basic knowledge for diagnostics and vaccine development of *A. pleuropneumoniae* serotype 14.

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