The genetic organization of the capsular polysaccharide biosynthesis region of *Actinobacillus pleuropneumoniae* serotype 15

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(Received 22 April 2014/Accepted 2 December 2014/Published online in J-STAGE 14 December 2014)

**ABSTRACT.** Nucleotide sequence determination and analysis of the *cps* gene involved in the capsular polysaccharide biosynthesis of *Actinobacillus pleuropneumoniae* serotype 15 revealed the presence of three open reading frames, designated as *cps15ABC* genes. At the protein level, Cps15A and Cps15B showed considerably high homology to CpsA (67.0 to 68.7%) and CpsB (31.7 to 36.8%), respectively, of *A. pleuropneumoniae* serotypes 1, 4 and 12, revealing the common genetic organization of the *cps* among serotypes 1, 4, 12 and 15. However, Cps15C showed no homology to any proteins of *A. pleuropneumoniae* serotypes, indicating that *cps15C* may be specific to serotype 15. This study will provide the basic molecular knowledge necessary for the development of diagnostics and a vaccine for *A. pleuropneumoniae* serotype 15.

**KEYWORDS:** *Actinobacillus pleuropneumoniae*, serotype 15 capsule

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in this study. Purified genomic DNA from serotype 15 strain HS143 was digested with restriction enzymes EcoRI and HindIII, religated with T4 DNA ligase in order to generate circular template DNAs and used for the following inverse touchdown PCR, respectively. The inverse touchdown PCR was performed in a total volume of 50 µl containing 1 X buffer (Toyobo, Otsu, Japan); 0.2 mM of each dNTP; 0.3 µM of each primer (invF and invR) and the template DNAs described above. The following amplification steps were used: 1 cycle at 94°C for 2 min (preheating); 5 cycles at 98°C for 10 sec and 74°C for 20 min (first step); 5 cycles at 98°C for 10 sec and 72°C for 20 min (second step); 5 cycles at 98°C for 10 sec and 70°C for 20 min (third step); 20 cycles at 98°C for 10 sec and 68°C for 20 min (forth step); 1 cycle at 68°C for 10 min (final step). Amplified DNAs (approximately 8 and 5 kilobase pairs) were purified by the QIA quick PCR amplification kit (Qiagen, Hilden, Germany) and submitted to nucleotide sequence determination with fluorescent dye terminators as described previously [11]. The nucleotide sequence determined has been deposited under accession number AB701753 in DDBJ/EMBL/GenBank.

The nucleotide sequence of the DNA (8,551 bp) comprising cps15 was determined. Three open reading frames (ORFs) were located between the cpx15D and lysA genes (the CPS export gene and the diaminopimelate decarboxylase genes, respectively), which are conserved in A. pleuropneumoniae and flanked by the cpx (Fig. 1). The ORFs were designated as cps15ABC genes (Fig.1) and encoded the Cps15A to Cps15C proteins, respectively. At the amino acid level, Cps15A showed considerably high homology to ORF1 of Actinobacillus suis [18] as well as did to Csp1A, Csp4A and Cps12A (CPS phosphotransferase) of A. pleuropneumoniae serotypes 1, 4 and 12, respectively [2, 16] (Table 1). Cps15B showed overall homology to a glycosyl tranferase of Mannheimia varigena and to ORF2 of A. suis [18] as well as did to Cps1B, Cps4B and Cps12B (glycosyl transferase family protein) of A. pleuropneumoniae [2, 16] (Table 1). Cps15C showed no homology to any proteins of A. pleuropneumoniae, whereas it showed homology to a hypothetical protein of Corynebacterium resistens [23] and to a protein involved in CPS biosynthesis of Neisseria meningitidis serogroup Z [8, 28] (Table 1). These findings suggested that a horizontal gene transfer of the cps gene across the taxonomically and phylogenetically unrelated bacterial classes, including Gram-positive bacteria C. resistens and N. meningitidis belonging to β-Proteobacteria, might have occurred during capsule evolution. The G+C contents of cps15A, cps15B and cps15C were 26.9, 26.8 and 34.2%, respectively (Table 1), which is lower than the 41% (overall G+C content of A. pleuropneumoniae) [26], indicating that the cps15ABC genes might have been acquired by horizontal gene transfer.

Serotype-specific enzymes that are involved in CPS biosynthesis are probably responsible for the dissimilarities among the CPS chemical structures [26]. However, it has been reported that the CPS structures produced by A. pleuropneumoniae serotypes 1 to 13 and 15 can be divided into three groups according to the basic differences in their chemical compositions and structures: Group I (serotypes 1, 4, 12 and 15), with CPS composed solely of repeating oligosaccharide units linked by phosphates; Group II (serotypes 5 and 10), with CPS composed of repeating oligosaccharide units; Group III (serotypes 2, 3, 6–9, 11 and 13), with CPS composed of teichoic acid polymers linked by phosphate diesters [16, 21, 26]. The genetic organization of the cps genes provided molecular evidence to support the CPS grouping of A. pleuropneumoniae serotypes [16, 26]. The present study...
Table 1. Identity of Cps protein of *Actinobacillus pleuropneumoniae* serotype 15 (Cps15) compared to those of *A. pleuropneumoniae* and other bacterial species

<table>
<thead>
<tr>
<th>Cps15 protein</th>
<th>Length of aa* of Cps15</th>
<th>G+C content (%) of cpx15 encoding Cps15</th>
<th>Bacterial species</th>
<th>Serotype</th>
<th>Homologous protein</th>
<th>Accession number</th>
<th>Reference</th>
<th>% Identity</th>
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Homologous proteins whose amino acid sequences show significant alignments to *A. pleuropneumoniae* and other bacterial species are shown. Lanes for *A. pleuropneumoniae* proteins are shaded. a) Amino acid; b) Partial sequence; c) Hypothetical protein; d) Protein name was designated in this study as named in *A. suis* serotype K1 [Accession no. AY253301]; e) *Mannheimia* varigena; f) Partial sequence; g) *Corynebacterium* resistens; h) Neisseria meningitidis; i) Serogroup; j) CapZD is a synonym for CszD.

revealed that *A. pleuropneumoniae* serotype 15 carries a gene for the CPS phosphotransferase (*cps 15A*) which may be involved in the chemical linkage of phosphates in the linear CPS backbone [26]. This, in turn, indicates that *A. pleuropneumoniae* serotype 15 belongs to Group I. This study also revealed that the genetic organization of the *cps* genes of *A. pleuropneumoniae* serotype 15 corresponds to the CPS structural classification, as do serotypes 1–13 [16, 21, 26].

As shown in Fig. 1, the genetic organization of the *cps* was essentially common among *A. pleuropneumoniae* serotypes 1, 4, 12 [16, 26] and 15 [this study]. However, the orientation of the *cps 15ABC* gene against *cps D* and *lysA* genes was different from that of other *A. pleuropneumoniae* serotypes [16, 26] (Fig. 1). The different orientation between the *cps 15ABC* genes and *cps* genes of other serotypes indicated that an inversion might have occurred only in *A. pleuropneumoniae* serotype 15.

In conclusion, the nucleotide sequence of the *cps 15* gene has been determined in this study. We believe that the present results will provide the basic molecular knowledge necessary to develop diagnostics and a vaccine for *A. pleuropneumoniae* serotype 15.

ACKNOWLEDGMENTS. This study was supported by grants from the National Agriculture and Food Research Organization, Japan. The authors thank Ms. K. Miyata for her help with the drawing in Fig. 1.

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