Development of a cps-Based Multiplex PCR for Typing of Actinobacillus pleuropneumoniae Serotypes 1, 2 and 5

Hiroya ITO1)

1)National Institute of Animal Health, National Agriculture and Food Research Organization, 3–1–5 Kannondai, Tsukuba, Ibaraki 305–0856, Japan

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ABSTRACT. A cps-based multiplex PCR for typing of Actinobacillus pleuropneumoniae serotypes 1, 2 and 5 was developed. This method should be specific and practical in Japan where more than 88% of isolates are serotypes 1, 2 or 5.

KEY WORDS: Actinobacillus pleuropneumoniae, cps, multiplex PCR, serotypes 1, 2 and 5, typing.

Actinobacillus pleuropneumoniae is the etiologic agent of porcine pleuropneumonia that causes serious economic losses to pig-rearing industry [5]. To date, 15 serotypes are recognized mainly based on antigenic diversity of capsular polysaccharides in the organisms [2, 16]. Prevalent serotypes differ in countries, regions and farms [3, 5]. In Japan, serotype 2 is the most common; serotype 5 and 1 follows; more than 88% of A. pleuropneumoniae isolates belong to serotypes 1, 2 and 5 [12, 13, 19]. Serotyping is widely performed in veterinary diagnostic laboratories since virulence differs in serotypes [5] and vaccines for A. pleuropneumoniae are serotype-specific [14, 18]. However, only a few veterinary diagnostic laboratories can prepare a full set of serotype-specific antisera for serotyping. Furthermore, cross-reactions are often observed among different serotypes in a number of methods, which prevent accurate and rapid typing of field isolates [4, 7–10, 11, 15].

In the last decade, the genes involved in biosynthesis of capsular polysaccharides (cps), have been described for A. pleuropneumoniae serotypes 1, 2, 3, 5, 6, 7, 8 and 12 [1, 6, 7, 17, 20, 21]. Subsequently, serotype-specific PCR typing methods based on the cps gene have been developed for the serotypes [1, 6, 7, 17, 20, 21]. However, no multiplex PCR for serotypes 1, 2 and 5, which are the most prevalent in Japan, in a single tube, has been developed yet. In this study, the author developed a cps-based multiplex PCR for typing of A. pleuropneumoniae serotypes 1, 2 and 5.

A. pleuropneumoniae serotype strains (serotype 1, 4074; serotype 2, S1536; serotype 3, S1421; serotype 4, M62; serotype 5a, K17; serotype 6, Femo, serotype 7, WF83; serotype 8, 405; serotype 9; CVJ13261; serotype 10, D13039; serotype 11; 56153; serotype 12, 8329; serotype 13, N273; serotype 14, 4906; serotype 15, HS143) were used for determination of suitable conditions for the multiplex PCR. Japanese field isolates of A. pleuropneumoniae serotype 1 (n=15), serotype 2 (n=53), serotype 5 (n=15), serotype 7 (n=11), serotype 8 (n=3), serotype 12 (n=3), untypable (n=4) and serotype 10 Canadian isolates (n=2) were examined for assessing field feasibility. These strains were isolated from various area and in diverse years (from 1976 to 2008). The organisms were cultivated with tryptic soy agar (Difco, U.S.A.) and 10% defibrinated horse blood and 2% fresh yeast extracts at 37°C. Template DNA was purified with High Pure PCR Template Preparation Kit and High Pure PCR Product Purification Kit as instructed by suppliers (Roche Diagnostics, Switzerland). PCR was done in a total volume of 50 µL containing 1 × PCR gold buffer (Applied Biosystems, U.S.A); 1.5 mM MgCl2, 0.2 mM of each dNTP; 0.5 µM of each serotype-specific primers Ap1F and Ap1R [1]; Ap2F & Ap2R [6]; Ap5A & Ap5B [6] (primer combination A); 2.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, U.S.A); 10 ng of template DNA. The amplification steps used were as follows: 95°C for 10 min (preheating); 35 cycles of 95°C for 1 min (heat denature), 56°C for 1 min (annealing), and 72°C for 2 min (extension); and final step at 72°C for 5 min. Another multiplex PCR with other primer pairs (Ap1U1 & Ap1L1) [17], instead of Ap1F and Ap1R, (primer combination B) was also tried under identical conditions except serotype 1-specific primers. Ten µL of each reaction was analyzed by electrophoresis with a 1.5% agarose gel.

Reference strains of A. pleuropneumoniae serotypes 1, 2, and 5 produced 0.75-, 0.5- and 1.1-kb DNA, respectively, when the primer combination A was used (Fig. 1). On the contrary, reference of serotypes 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 did not yield any PCR products (Fig. 1). No difference was observed in results even when annealing temperature was 46, 48, 50, 52 and 54°C, respectively, indicating specificity of the primers used were very high (data not shown). Detection limits of the multiplex PCR in this study were 50, 50 and 500 pg DNAs for serotypes 1, 2 and 5, respectively. Another multiplex PCR with the primer combination B amplified specific 1.6-, 0.5- and 1.1-kb DNA in serotypes 1, 2 and 5, respectively, but also did 0.7-kb DNA in serotype 8 (Fig. 2). Thus, specificity depends on primer combination in the multiplex PCR for the serotypes.

NOTE
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*Correspondence to: ITO, H., National Institute of Animal Health, 3–1–5 Kannondai, Tsukuba, Ibaraki 305–0856, Japan.

e-mail: itohiroy@affrc.go.jp
Consequently, the multiplex PCR with the primer combination A was used for further analysis for field isolates. All field strains of *A. pleuropneumoniae* serotypes 1, 2 and 5 produced 0.75-, 0.5- and 1.1-kb DNA in the multiplex PCR with primer combination A (data not shown) as well as serotype reference strains. On the contrary, all field strains of serotypes 7, 8, 10, 12 and untypable field strains did not yield any PCR products (data not shown). Thus, the multiplex PCR developed in this study enables veterinary diagnostic laboratories to perform specific and rapid typing of *A. pleuropneumoniae* field isolates without any specific antisera for serotyping.

In conclusion, the multiplex PCR with the primer combination A should be specific and practical in Japan where serotypes 1, 2 and 5 are the most prevalent [12, 13, 19].

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