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Cloning and Biological Analysis of Apx IVA Gene of Porcine *Actinobacillus pleuropneumoniae*

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Abstract

The aim of this study was to clone Apx IVA gene of *Actinobacillus pleuropneumoniae* (APP), to construct cloning vector PMD18-T-APXIV, and finally to perform a bioinformatical analysis after sequencing. This experiment was based on the Apx IVA gene sequence (FJ848574.1), and porcine contagious pleural pneumonia DNA was used template to amplify of Apx IVA fragment by PCR, constructed by TA cloning. The recombinant plasmid pMD18-T-Apx IV was sequenced and followed by Primer 5, DNAMAN and other software for a bioinformatical analysis. The results showed that the amplified length of Apx IVA was 972 bp and PMD-18-T-Apx IV (972 bp) was obtained, which shared the highest sequence similarity (98%) in nucleotide. This study provides a useful reference for the prokaryotic expression and antibody preparation of APP.

Keywords: ***Actinobacillus pleuropneumoniae; Apx IV; Biological information analysis cloning***

Introduction

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is a contagious disease reported to cause economic losses worldwide [1]. The main clinical signs of the acute disease are anorexia, depression, fever, coughing, dyspnea, and/or polypnea [2]. This disease causes substantial losses to the swine industry worldwide, both from the disease itself and the associated healthcare costs. Growing pigs from 2 to 6 months are particularly affected, even though all ages are sensitive to the pathogen. The bacteria present a series of actions coordinated with diverse virulence factors, such as capsular antigens [3,4], external membrane protein antigens [5,6], external membrane lipopolysaccharides [7,8], the Ca^{2+} dependent Apx I, II, III and IV toxins, with hemolytic and/or cytotoxic activity. Apx I, Apx II and Apx III are three different toxins; their virulence is strong but strong interspecific specificity and their secretion in different blood types. Apx IV, a fourth RTX toxin was identified from *A. pleuropneumoniae* recently, is expressed by all *A. pleuropneumoniae* regardless the serotypes only *in vivo* toxin, so it is optimal to develop species-specific and differentiated diagnostic assay [9].

However, there are few studies on the biological characteristics and cloning of Apx IV at present. The purpose of this study was to clone Apx IV gene of *A. pleuropneumoniae*, to construct cloning vector PMD18-T-ApxIV, and finally to perform a

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bioinformatical analysis after sequencing. Meanwhile, this experiment would provide the experimental basis for the rapid diagnosis of the test paper strip and the development of vaccine.

Materials and Methods

Bacterial strains

The bacterial was bought from The Inquiry Network for Microbial Strains of China and stored at -80°C.

Bioinformatics analysis of Apx IVA gene

The Hydrophobicity and hydrophilicity were predicted by Prot Scale (http://www.expasy.ch/tools/protparam.htm).

Extraction of total DNA

Total DNA extraction of APP was performed using Genomic DNA Isolation Kit (Sangon Biotech, Shanghai, China), under low temperature environment. Primers of Apx IVA (*BamHI*) (5'- CGCGGATCCTTATTAACTGTTACGCCTATT -3') and Apx IVA (*HindIII)* (5'- CCCAAGCTTGGTTAGATTAATTGTGCGA -3') were designed according to the DNA sequences (GenBank Accession No. FJ848574.1), to amplify the Apx IVA subunit protein gene (972 bp). The underscored parts were *BamHI* and *HindIII* restriction enzyme sites, respectively. The reaction mixture for PCR was composed of a total volume of 20 μL, containing 10 μL PCR Master Mix, 1 μL of each primer, 0.5 μL of DNA, and 7.5 μL of deionized water. The PCR cycle conditions were set as follows: 95°C for 5 min, followed by 35 cycles of 94°C, 50 s; 58°C, 50 s; and 72°C, 45 s, ending with an extension at 72°C for 10 min. The PCR product was separated by electrophoresis on 1% agarose and visualized by the GelDoc XR System (Bio-Rad, American).

Cloning of the Apx IVA subunit protein gene

The PCR product was purified with a commercially available kit (TaKaRa, Otsu, Japan) and then inserted into a pMD 18-T vector (TaKaRa, Otsu, Japan) according to the manufacturer's protocol and transformed into Top10 *Escherichia coli* competent cells (TIANGEN, Beijing). The cloning of the Apx IVA subunit protein gene was subjected to double digestion by *BamHI* and *HindIII* restriction enzymes. The product was separated by electrophoresis on 1% agarose and the inserts analyzed by sequencing analysis (Xiangyin Biotech, Shanghai).

Results

Hydrophobicity/hydrophilicity prediction of the Apx IVA protein

The hydrophobicity and hydrophilicity of the target protein sequence were predicted by DNAMAN 8.0 software (Lynnon Biosoft, American). As shown in FIG. 1, the highest hydrophobicity was 2.34 (FIG. 1a) and the lowest hydrophilicity was 2 (FIG. 1b), the score of entire polypeptide chain of most amino acids was low. The results showed that the hydrophilic amino acids were more evenly distributed than the hydrophobic amino acids. The entire polypeptide chain was hydrophilic amino acid, thus inferring that Apx IVA is hydrophilic protein.

Cloning of the Apx IVA subunit protein gene

In order to clone the coding sequence of Apx IVA subunit protein gene, the DNA of APP was extracted and synthesized. After PCR amplification, the expected sizes of 972 bp products were successfully obtained (FIG. 2a). This recombinant plasmid and PMD-18-T-Apx IVA vector were then double-enzyme digested to release the Apx IVA subunit protein insert and prepare the vector for ligation and transformation (FIG. 2b).

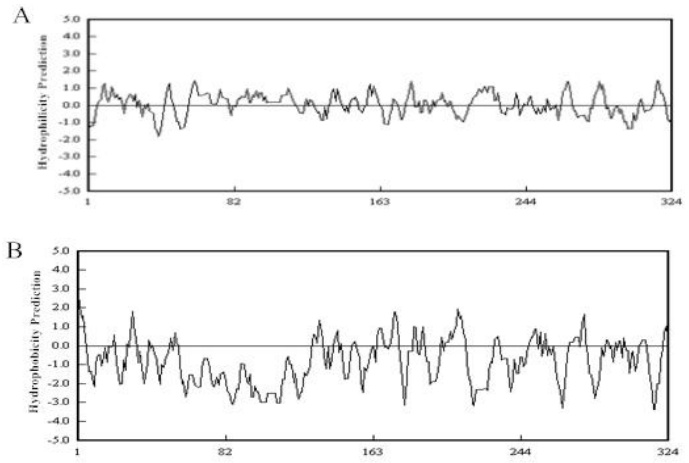

FIG. 1. **Hydrophilicity/hydrophobicity prediction of the Apx IVA protein.**

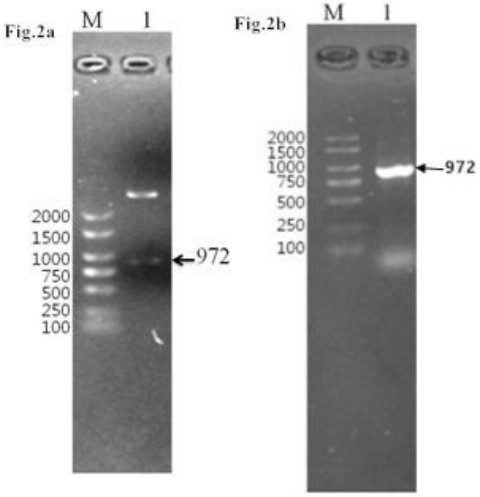

FIG. 2. **The results of Apx IVA gene PCR amplification and PMD-18-T-Apx IVA vector after restriction enzyme digestion. a) The results of Apx IVA gene PCR amplification, the coding sequence of Apx IVA subunit protein gene was successfully obtained. Lane 1: PCR products of Apx IVA subunit protein gene; Lane M: DNA Marker/DL2000; b) The results of PMD-18-T-Apx IVA vector after restriction enzyme digestion. Line1: PMD-18-T-Apx IVA vector digested with *Bam HI* and *HindIII*; Line M: DNA Marker/DL2000.**

DNA sequencing

The DNA Star result suggested that the length of segment was 972 bp, and the sequence was more than 98% identity with the Apx IVA gene.

Discussion

App was detected with neither the PCR nor bacteriological techniques in some of seropositive pigs. In fact, serology and bacteriology or PCR give different types of information. Furthermore, PCR tests are now frequently developed to detect or characterize App from pure or mixed cultures [10-12].

The composition of hydrophilic and hydrophobic amino acids is the main driving force of protein folding, which can reflect the folding of protein and hydrophobic region can be used as a reference for evaluating potential trans membrane regions [13]. Therefore, the hydrophobicity and hydrophilicity can clearly express whether the protein is a transmembrane protein.

Conclusion

In this study, we successfully amplified the Apx IVA gene and cloned the gene into the prokaryotic expression vector, and constructed the prokaryotic expression vector (PMD18-T-Apx IVA) successfully. Also, this study provides a useful reference for the prokaryotic expression and antibody preparation of APP.

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