Abstract

Background: The pleuropneumonia caused by Actinobacillus pleuropneumoniae is one of the most important swine respiratory diseases. Biochemical and serological tests are widely applied for the diagnosis and characterization of this bacterium. However, in some isolates, conflicting results are found. There are at least 15 serotypes with significant differences in virulence that have been identified until now. Moreover, cross reactions between serotypes are not uncommon. The serotype determination from isolates occurring in outbreaks is an important procedure in prophylaxis and control of the disease. The present work focuses on the application of an ERIC-PCR technique for genotyping and differentiating A. pleuropneumoniae isolates.

Materials, Methods & Results: Fifteen reference strains for the recognized A. pleuropneumoniae serotypes were analyzed in this work, alongside with 27 field isolates that had been previously characterized regarding biochemical, serological and molecular features. Total DNA from each sample was purified and subjected to PCR amplification using ERIC-specific primers (ERIC1R and ERIC2). The resulting amplicons were analyzed by agarose gel electrophoresis and their sizes were estimated from the gel images. Bands with similar sizes were identified and used to construct a binary matrix that took into account the presence or absence of individual bands in all lanes. Pair-wise similarity coefficients were computed from the binary matrix and the similarity matrices obtained were utilized to construct an UPGMA-based dendrogram. The amplicons obtained from the A. pleuropneumoniae reference strains generated a very distinctive pattern for each one of the tested strains. Moreover, all samples presented a large enough number of amplicons (bands) as to enable an unequivocal differentiation of each sample. Reproducibility of the developed ERIC-PCR method was assessed by means of duplicate PCR reactions. All duplicate reactions presented exactly the same pattern. Furthermore, amplicon abundance for bands with the same size in each duplicate was also very similar. When applied to A. pleuropneumoniae field isolates, collected from clinical cases of the disease, we were able to differentiate all samples from each other, even those belonging to the same serotype. Discussion: In the present work we have analyzed A. pleuropneumoniae strains isolated from a wide geographical area in Brazil, covering outbreaks that occurred over a period of more than a decade. The ERIC-PCR technique was standardized using DNA from the serotyped A. pleuropneumoniae reference strains, generating distinctive amplification patterns for each sample, which were not serotype specific. It is expected that the discriminatory power of the method would be enhanced by the large numbers of amplicons obtained for each sample. We have also analyzed the reproducibility of the ERIC-PCR method by performing several experiments where DNA from the same sample was amplified in duplicate independent PCR.
reactions and the PCR amplification patterns obtained were reproducible in all tested experiments. Also, very little variation in amplification efficiency was detected for the individual amplicons within a given sample. The application of the ERIC-PCR genotyping technique to A. pleuropneumoniae isolated from animals with clinical signs of the disease allowed the differentiation of each individual sample...

Keywords

Enterobacterial repetitive intergenic consensus, DNA fingerprinting, swine, respiratory diseases.