BBD-PP-26

TITLE
MYCOPLASMA HYOPNEUMONIAE STRAIN GENOTYPING USING SANGER SEQUENCING OF FOUR OR TWO LOCI

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CONTENT
Mycoplasma hyopneumoniae (Mhyo) genetic variability has been described by different molecular techniques. Most methods are based on the characterization of different loci containing variable number of tandem repeats (VNTRs). Nevertheless, there is no information reporting if the number of analyzed loci can affect the variability detected. Therefore, this study aimed to describe Mhyo genetic variability analyzing four or two loci by Sanger sequencing.

Forty-six Mhyo real time PCR positive lung samples were selected. Additionally, two lungs spiked with reference strains (RF) 11 (ATCC® 25095™) and J (ATCC® 25934™) were included as controls. Samples with cycle threshold (Ct)?30 were selected to be genotyped by Sanger sequencing and defining the VNTR of loci P97, P146, H1 and H5. Obtained sequences per each loci were aligned with MUSCLE v3.8.31. Afterwards, sequences from the four loci were concatenated to obtain a unique typing profile (TP, TP4). The same analysis was done using two loci (P97, P146) obtaining a TP2.

From the 48 tested samples (including the two reference strains), 45 (94%) had Ct?30 and were positive to all studied loci. P97 and P146 loci were successfully sequenced from 40 (88%) samples, but only in 33 of them (73%) all locus sequences were obtained. From the aligned sequences, a total of 26 and 19 different TP4 and TP2 were obtained, respectively.

Obtained TPs from lung samples were different from RF using 2 or 4 loci. Mhyo genetic variability varied according to the number of loci analyzed. This suggests that Mhyo characterization using four loci, or even more, can detect higher variability than when only two loci are studied. Further research is needed to investigate the optimal number of loci needed to ascertain Mhyo variability.