Raman microspectroscopy as an alternative diagnostic for porcine circovirus type 2

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Introduction

Porcine circovirus type 2 (PCV2) is a small non-enveloped virus with single-stranded circular DNA and has been associated with different disease syndromes collectively named PCV diseases (PCVD). The detection of PCV2 specific DNA and/or protein in histological lesions is considered a hallmark of PCV2 diagnosis (3). Although these methods are sensitive and specific, they are costly and time-consuming. Raman microspectroscopy (RMS) has been used in virology for rapid and specific identification of viruses such as Influenza, Adenovirus, Hepatitis B and Dengue (1, 2, 4, 5). RMS is a non-destructive fingerprinting technique, which requires no sample preparation, and it is based on the interaction of laser light with the vibrational modes of molecules, thus causing detectable inelastic scattering. This study aimed to demonstrate the primary classification of porcine cells infected with PCV2 and submitted to RMS, presenting promising outputs for future diagnostic applications.

Materials and Methods

Swine testis cells (ST) in 6-well plates were prepared and inoculated in triplicate with PCV2 virus strain (BRMSA 01351). After adsorption, plates were incubated for five days (37ºC, 5% CO_2). Uninfected ST cells were kept as a negative control. D-glucosamine treatment was conducted on day 2 and, on day 3, ST cells were submitted to a synchronization protocol. On day 5, ST cells were fixed with paraformaldehyde (4%) and analyzed in a Raman confocal microscope (InViaTM Renishaw®) equipped with a 633 nm laser. A total of 150 spectra centered at 1300 cm^{-1} was obtained for each sample (5 spectra/cell) of PCV2 infected and uninfected cells, with the acquisition time of 5 seconds. Data were pre-processed for baseline correction, noise reduction, and spectral intensity normalization. A multivariate analysis was performed through Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), followed by a leave-one-out cross-validation (LOOCV) test.

Results

The averaged spectra obtained from biological replicates of PCV2-infected and uninfected cells analyzed are presented in Figure 1. The spectra are characterized by peaks due to protein, lipid and DNA vibrations. Figure 2 demonstrates the statistical clusterization obtained with the PCA/LDA analysis. The LOOCV test showed 100% of sensitivity.

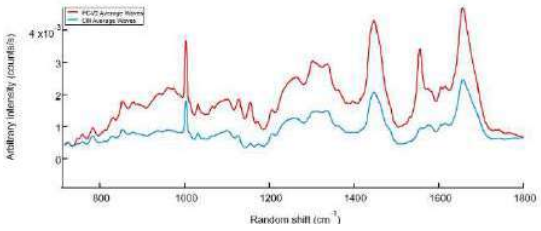

Figure 1. Average spectra from PCV2 infected (red) and uninfected cells (blue).

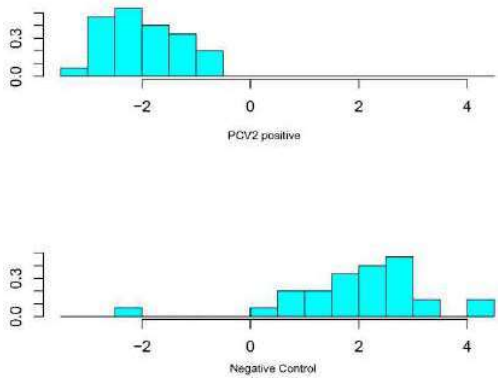

Figure 2. Histogram of sample clusterization obtained with the PCA/LDA applied to RMS data.

Conclusions and Discussion

Based on the high sensitivity observed in our results, we conclude that RMS combined with PCA-LDA analysis is an effective strategy to discriminate PCV2 infected from uninfected cells. It is a promising foundation for diagnostic methods development on preventive veterinary medicine.

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