Analyses of Bordetella isolates collected from turkeys with respiratory disease using MALDI-TOF mass spectroscopy and comparison to a Bordetella avium vaccine

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ABSTRACT

Bordetella avium has been isolated from turkeys showing clinical signs of respiratory disease and increased mortality in Sanpete County Utah, despite receiving the B. avium vaccine. To determine if recent B. avium isolates are related, or unrelated to the vaccine strain, twenty-five isolates from different time periods and different locations in the U.S. were collected for comparison by MALDI-TOF mass spectroscopy. Spectra were evaluated by MALDI Biotyper software (Bruker Co.) to determine relationships among the clinical isolates. Cluster analysis of the spectra showed four major clusters using the principle component scores for the three spectral peaks in highest abundance. These clusters also accounted for >70% of the variability in the data based on identification score values. Four of five Utah isolates were in the same cluster as the vaccine strain. However, one isolate from Utah and isolates from other locations did not cluster with the vaccine strain.

INTRODUCTION

Tracheal swab cultures were used to isolate Bordetella avium from turkeys showing clinical signs of respiratory disease and increased mortality in Sanpete County Utah, despite receiving the B. avium vaccine. To determine if recent B. avium isolates are related, or unrelated to the vaccine strain, twenty-five isolates from different time periods and different locations in the U.S. were collected for comparison by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy.

MALDI-TOF mass spectroscopy is a powerful new tool for identification of pathogens in clinical specimens. Compared with other methods, including 16S rRNA and rpoB gene sequencing, reported accuracy of identification of bacteria, fungi or parasites has been 95.4% to 99.5% (Seng, et al., 2009).

Bacterial colonies are mixed with a matrix solution and placed and dried onto a target plate. The matrix solution (cinnamic acid) cocrystallizes with the bacterial sample on the target plate (96 sample plate). Samples are then exposed to short laser pulses under high vacuum, vaporizing the bacteria and the matrix. Bacterial proteins are ionized, and an electromagnetic field accelerates the ions as they enter the flight tube. The time of flight (TOF) required for analytes to reach the detector at the end of the flight tube is measured and recorded. A “characteristic spectrum” based on the TOF provides a specific sample fingerprint, considered unique for each bacterial species resulting in precise identification (Clark, et al., 2013). Software compares the collected spectra with a databank of reference spectra of bacterial isolates. A numerical score value of similarity to known isolates’ spectra is calculated. A score value above 2.0 is defined as a valid species level identification, while values between 2.0 and 1.7 represent reliable genus level identifications.

METHODS

Protocol for MALDI-TOF Mass Spectroscopy

Spectra were obtained from clinical isolates collected from turkeys, grown overnight on blood agar plates, and evaluated by MALDI-TOF mass spectroscopy. The MALDI-TOF spectra from the clinical isolates were evaluated using the Bruker MALDI Biotyper software (IVD MALDI Biotyper 2.3) to determine relationships among the clinical isolates. This was done by comparing the MALDI-TOF results to the database of microorganisms using the Biotyper software for peak-matching and by multivariate analyses using the three principal component scores (Ringner, 2008). After completion of the peak-matching algorithm, the score value was evaluated for the suggested matches. A score between 2.3 and 3.0 had a high degree of confidence that the correct species was identified. A score between 2.0 and 2.29 was also reliable, although indicates a lower level of confidence in the species identification. We manually selected samples with high scores and processed the data sets into both dendrograms and cluster charts. Types of analyses included: presumptive species, location of collection, and year of collection. In the cluster charts, the relative distance between points is indicative of bacterial similarity.

Process for Principal Component Analysis(PCA)

Raw data from the MALDI-TOF is imported into the software used for PCA. This data must be cleaned and transformed before proper analysis by performing batch processing which uses an algorithm to change deviations in mass peaks, therefore increasing accuracy. Both 3D scatter plots and PCA dendograms are then used to visualize how closely correlated the bacteria are based on the first three principal component scores of their protein signatures. An example is shown in Figure 6 to the right. Principal Component scores are labeled PC1, PC2, and PC3.

STATE

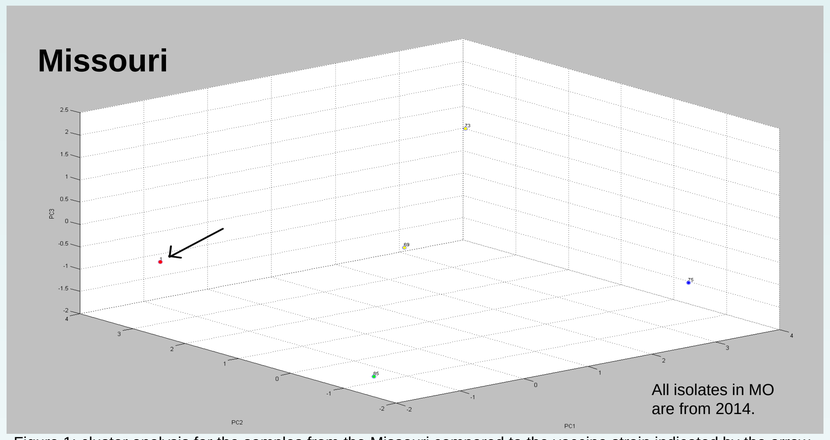

Figure 1: cluster analysis for the samples from the Missouri compared to the vaccine strain indicated by the arrow.

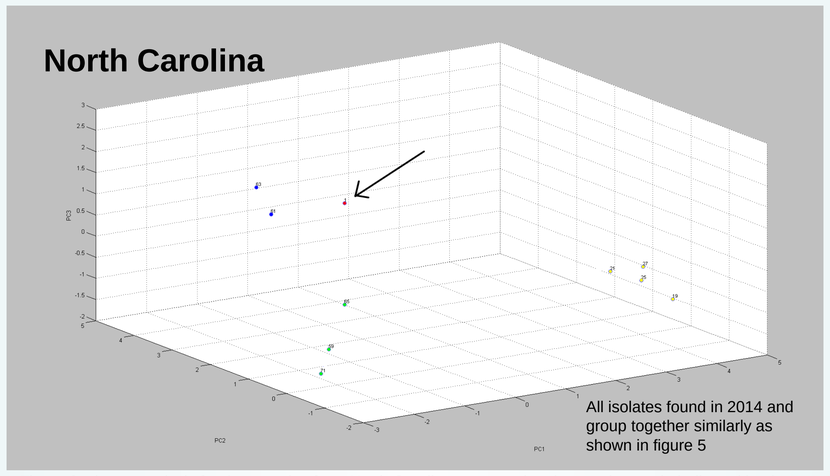

Figure 2: cluster analysis for the samples from North Carolina compared to the vaccine strain indicated by the arrow.

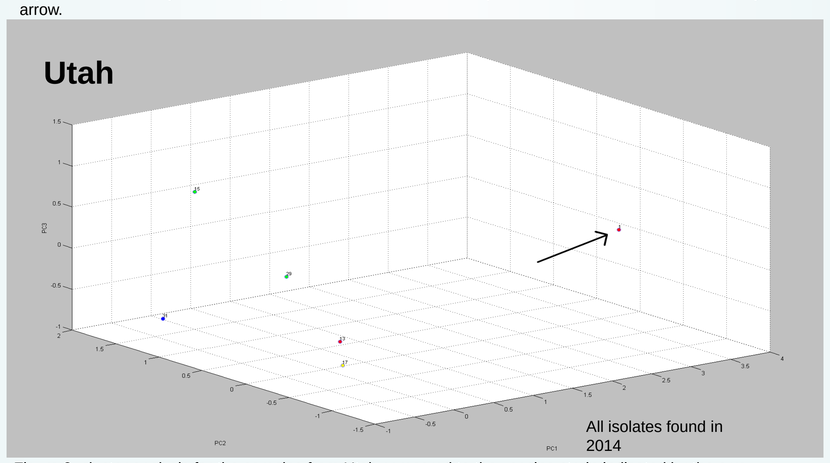

Figure 3: cluster analysis for the samples from Utah compared to the vaccine strain indicated by the arrow.

YEAR

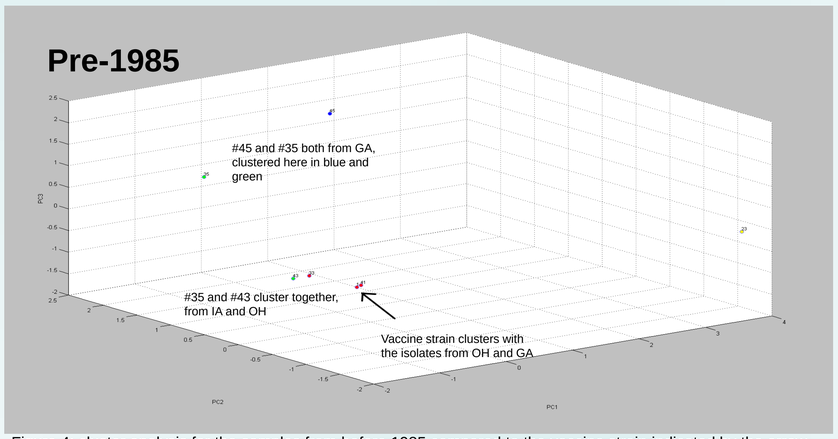

Figure 4: cluster analysis for the samples from before 1985 compared to the vaccine strain indicated by the arrow.

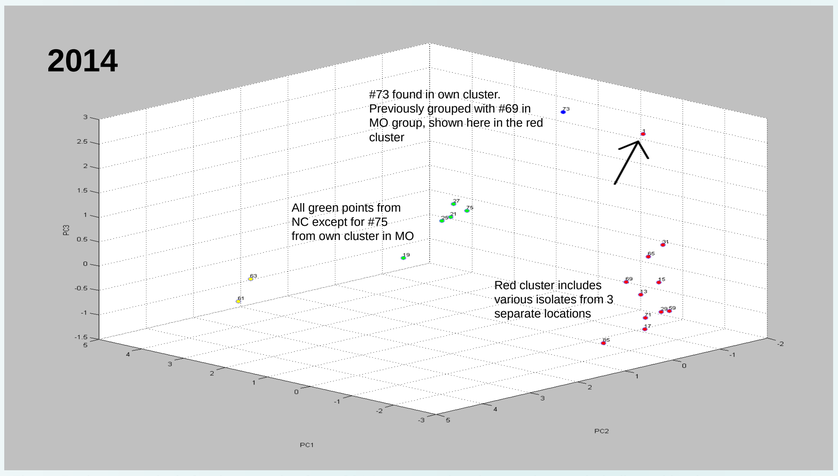

Figure 5: cluster analysis for the samples from 2014 compared to the vaccine strain indicated by the arrow.

Spectra of isolates were separated by the state and year in which they were collected and compared to the vaccine strain by PCA. Other isolates from Georgia, Iowa, Ohio, Virginia, and the year 2006 did not include enough samples required for PCA, but are included in the PCA performed in Figure 6. Some interesting details can be seen in these results. For example, in the results for 2014 shown in Figure 5 the vaccine strain is grouped with other isolates of B. avium indicated by red markers, however it is not included in the main cluster. The results from before 1985 show the vaccine strain not only grouped with other isolates of B. avium, but also included in the main cluster. The same phenomenon that occurs in the 2014 analysis can also be observed in the analysis from Utah where the two isolates determined as related are also significantly different from each other.

RESULTS

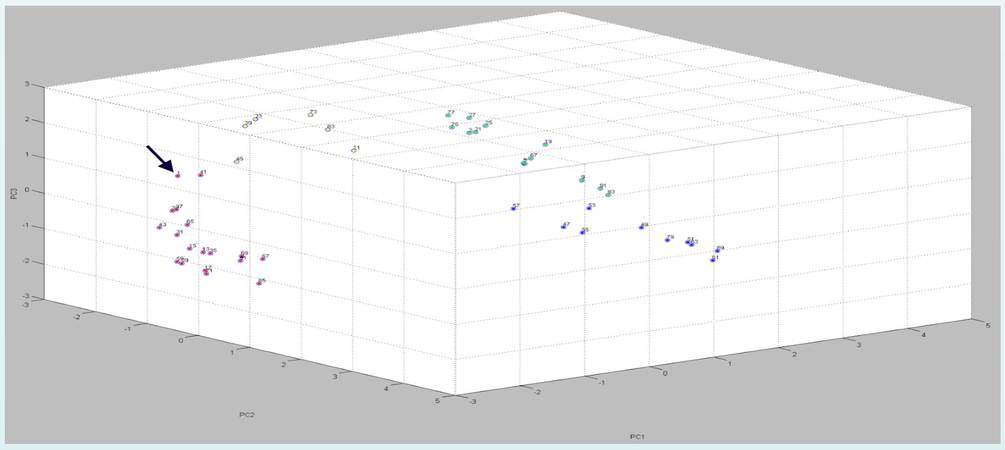

Figure 6: Cluster analysis for three spectral peaks in highest abundance from clinical isolates. Sample No. 1 (arrow) is the *B. avium* vaccine strain. Axis units indicate variance, with distances farthest from 0 being most different. Four major clusters, representing diverse samples, account for >70% of the variability in the data.

Spectra were evaluated by MALDI Biotyper software to determine relationships among clinical isolates. Cluster analysis of the spectra showed four major clusters using the principle component scores for the three spectral peaks in highest abundance. These clusters also accounted for >70% of the variability (considered significant) in the data based on identification score values (Figure 1). Four of five Utah isolates were in the same cluster as the vaccine strain. However, one isolate from Utah and isolates from other locations did not cluster with the vaccine strain.

FUTURE STUDIES

1. Hemagglutination (HA) assays will be done to identify species as *Bordetella avium* agglutinates red blood cells and other species of *Bordetella* do not.
2. Hemagglutination inhibition(HAI) assays will be done to find serological differences between the vaccine and other isolates to answer whether the serum from a vaccinated turkey will protect against other isolates.
3. Polymerase chain reaction(PCR) assays will be used to confirm the species that were tested.

REFERENCES

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