Nuclear Magnetic Resonance in metabolomics of sheep infected with Corynebacterium pseudotuberculosis

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Abstract
This research seeks to understand the mechanisms of action of Corynebacterium pseudotuberculosis, a bacterium that affects many creations of sheep and goats around the world causing caseous lymphadenitis (CL). The CL disease brings drastic losses in agribusiness, provoked with sacrifice of animals, and losses in the production of wool, milk and meat due to animal weight loss and devaluation of the skin injured with scars. And with the aims to early detect and understand this severe infection, we have employed metabolomics on animal blood serum samples combining Nuclear Magnetic Resonance (NMR) Spectroscopy and Principal Component Analysis (PCA). Two groups of animal serum samples were compared, infected and healthy, and very interesting results were obtained.

Key words: Nuclear Magnetic Resonance, Metabolomics, Corynebacterium pseudotuberculosis.

Introduction

The C. pseudotuberculosis is an etiological agent of caseous lymphadenitis (CL) that shows a high resistance to the environment and is easily transmitted.1 Aiming to gain insights into the changes that occur in animals when infected with C. pseudotuberculosis we propose metabolomics2 by NMR spectroscopy on blood serum samples from two groups of animals: healthy and infected. Then, using the PCA in NMR data processing, all information obtained on samples grouping, and also on anomalous samples, were used for choosing the NMR spectral regions for posterior 2D NMR analysis.

Results and Discussion

The blood serum samples from the healthy and infected sheep were obtained from our collaborators, totaling 60 samples. Among these, samples from two sheep breeds were analyzed: Santa Inês and Dorper.

All animal serum samples were collected according to all ethical rules under guide of professor Vasco Azevedo from Cellular and Molecular Genetics Laboratory of the UFMG, and professor Ricardo Portela from the UFBA. These were kept at -80°C for not more then 14 days before being analyzed. For the NMR analysis, the samples were prepared using simple dilution of 250 μL of the blood serum sample with 250 μL of deuterium oxide (D2O). The 1H NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer at 25°C. We have used the same experiment conditions, processing and spectra transporting into the data-matrix for the posterior chemometrics. Some of the results are shown in Figures 1 and 2.

Conclusions and Perspectives

Until the present moment, metabolomics by 1H NMR were executed on 60 animal blood serum samples. The PCA results have shown good separation between two classes of sera and it is expected, through 2D NMR analyses, that possible biomarkers for CL disease can be identified and greatly improve our knowledge on CL.

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Figures 1 and 2.

Example 1. Examples of 1H NMR spectra (ns = 128) of blood serum samples from: healthy (blue), and infected sheep (red).

Figure 2. PCA obtained from animal blood samples. Health (blue circle) and infected sheep (red triangle).