FT-IR Spectroscopy as a Method for Monitoring Phenotypic Changes of Moraxella bovis Growing in Batch Cultures

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Moraxella bovis -the etiological agent of Infectious Bovine Keratoconjunctivitis (IBK)- produces different virulence factors such as pili and hemolysin, implicated in the pathogenesis of the disease. It was demonstrated that pili are important protective antigens against IBK [1]. While *M. bovis* produces pili readily when grown on solid media, this ability could be lost when the bacteria are cultivated in liquid media, the preferred industrial method for vaccine production. As this expression could be associated to the environmental conditions then, monitoring and controlling the phenotypic expression of *M. bovis* during growth in bioreactor is crucial for the quality of the vaccines and indeed a difficult task. Most of the current monitoring processes are based on the measurement of simple physico-chemical variables (pH, pO_2, rpm), and they try to correlate these to the immunogenic status of the bacteria.

For a known microbial strain FT-IR allows to obtain direct and immediate analyses of the main biochemical composition and cell components [2, 3]. In this study we investigated the feasibility of applying FT-IR spectroscopy to monitoring macromolecular composition changes during batch fermentation of *M. bovis*, underlying the possibility of evaluation the phenotypic changes during growth specially in pili expression.

Batch cultivations of *M. bovis* were performed in 4 L bioreactor using BHI medium . During the processes biomass samples were withdrawn at different times. Spectra of washed cells were measured with FT-IR spectrometer (Bruker 113 V and Perkin-Elmer, Spectrum One). The concentration of biomass in the samples was determined in order to monitor the growth rate of the culture. The four main biochemical cell components (protein, carbohydrate, lipids and nucleic acids) were determined by using analytical chemistry and pili expression was evaluated by an ELISA method.

Important differences between the FT-IR spectra of *M. bovis* biomass during batch fermentation could be observed. Evaluating the biomass spectra in the region 1250-900 by cluster analysis, growth phases could be differentiated in three different clusters. FT-IR spectra were also processed by using second derivative, curve fitting and peak area integration techniques [3]. The evolution of the macromolecular composition during growth was determined by comparing the peak areas of specific spectral regions as a function of time. These results were also compared to the data obtained applying a Vierordt system equations [4], and data obtained by chemical methods and immunological techniques .

We conclude that FT-IR might be used for monitoring *M. bovis* biomass biochemical changes by analysing the macromolecular composition and the expression of pili during the fermentation process for vaccine production.

References:

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