Development of a new technique for the detection of Moraxella bovis piliated cells using spectroscopic markers of type IV pili

Alejandra Bosch[1], Claudia Prieto[1], José Cúneo[1], Diego Serra[1], Dieter Naumann[2] and Osvaldo Yantorno[1]

[1]Centro de Investigación y Desarrollo de Fermentaciones Industriales (CINDEFI-CONICET), Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata (1900), Argentina;
[2]Robert Koch-Institute, Berlin, Nordufer 20, 13353 Berlin, Germany

Type IV pili (tfp) are bacterial appendages that are widely spread among Gram-negative bacteria (*Pseudomonas aeruginosa,* pathogenic *Neisseria, Vibrio cholerae*, enterotoxigenic *Escherichia coli, Moraxella bovis*) and have been shown to be the key elements for the interactions of bacteria with host cells[1]. *M. bovis*, in particular, is implicated in infectious bovine keratoconjunctivitis (IBK), the most common ocular disease which causes significant economic losses in cattle raising worldwide[2]. Effective immunization against IBK is achieved using highly piliated cells of *M. bovis* or purified pili. At industrial level, it is grown in batch culture in stirred bioreactors. Under such conditions, the cell-bound pili are lost and as a consequence, the protection conferred by such vaccines is very low. The control of vaccines and the differentiation between piliated (p+) and non-piliated (p-) populations is generally performed by ELISA techniques and analysis of colony morphology[3]. However, these methodologies require time-consuming processes and in particular ELISA techniques have additional problems due to the presence of seven different pilus serogroups. The aim of this work was to find pili specific spectroscopic markers for *M. bovis* cells which could be applied in the discrimination between p+ and p- bacteria independently of pilus serogroups, growth phase or type of culture used.

Piliated and non-piliated colonies of 12 different strains of *M. bovis*, as well as cells recovered from liquid cultures at lag, early exponential and stationary phase, were analysed by FT-IR spectroscopy and an ELISA technique for cell-bound pili quantification [4].

When the normalized first derivatives spectra of the different cells were compared, 3 spectral windows were found to be highly characteristic of piliation (1416-1411, 1383-1377 and 1697-1682 cm^{-1}) in all samples. Such infrared pili markers were then used as input data for cluster analysis. A good discrimination in 2 clusters, p+ and p-, were obtained from all the samples analysed. In order to confirm that the spectral windows selected were actually due to the presence of pili, we included in our analyses spectra of cells which had been previously sheared and washed to remove cell-bond pili. The first derivatives of these spectra were clustered with the p- cells.

A good agreement between the signal intensity in these 3 spectral windows and the piliation level estimated by ELISA was observed. This result provides the bases to develop a quantitative multivariate model capable of predicting the piliation percentage in *M. bovis* populations. In addition, preliminary analysis with other Gram-negative piliated bacteria of this group showed that the same markers were also present. We conclude that FT-IR spectroscopy might provide a simple and reliable alternative in the evaluation of pili in tfp-containing bacteria.

References:

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