FT-IR spectroscopy as a tool for studying phenotypic changes in Moraxella bovis growing as biofilm

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Moraxella bovis is a gram-negative bacterium implicated in the pathogenesis of infectious bovine keratoconjunctivitis (IBK), a disease causing significant economic losses in cattle industries worldwide [1]. Some factors of the bacterium such as type IV pili have been proposed as responsible for playing an important role during infection. Moreover, it is known that administration of either piliated whole killed cells or purified pili protect cattle against ocular challenge with virulent strains. At industrial level *M. bovis* cells are produced in batch culture in stirred reactors. Under such conditions the expression of pili is lost yielding very low protection level vaccines. It is clear that this cultural condition does not represent a good approximation to the mode of life of bacteria *in vivo*. The biofilm growth is recognised as being of prime importance in the establishment and maintenance of infectious diseases on epithelial tissues. Therefore, we studied the phenotypic expression of *M. bovis* cells attached to abiotic surfaces (sessile populations) in an intent to resemble the *in vivo* bacterial growth.
M. bovis piliated and non-piliated cells were grown on glass beads (5 mm diameter) in column reactors (55 cm high - 4 cm diameter) with aeration (20 ml min^{-1}). To start each process, bacteria either piliated or non-piliated were seeded in reactors and incubated for 3h at 37^{o}C to allow cell attachment to beads. Then, the suspension was drained to remove unattached bacteria and 130 ml of BHI medium were added to columns. The medium was renewed every 48 hours. The processes were run for different periods of time until 120h. Sessile cells were detached from the beads at 24, 48, 72, 96 and 120h of growth and then analysed by FT-IR spectroscopy and colony morphology. Additionally, electrophoretic profiles of both outer membrane proteins (OMP) and lipopolysaccharides (LPS) were compared to those piliated and non-piliated cells selected from solid medium. Extracellular polymeric substance from biofilm was recovered and analysed by chemical methods and FT-IR spectroscopy [2]. We found the growth in biofilm induced several changes in the envelope of *M. bovis* cells (OMP profiles, hydrophobicity). Importantly, colony morphology and staining studies showed biofilm growing cells to be highly piliated. This phenotype was also obtained when the biofilm culture was started with a non-piliated population. These results were confirmed by FT-IR spectroscopy. The overlay of the IR spectrum of sessile cells of 120h of growth with the spectra of piliated and non-piliated cells from solid medium and planktonic cells of exponential and stationary phases, showed in each case important differences in the 1800-700 cm^{-1} region. The difference spectra showed conformational changes in the Amide I band due to pili expression and characteristic bands of biofilm markers (carboxylate group bands at 1627 and 1314 cm^{-1}, presence of N-acetyl groups by the bands at 1617, 1578 and 1541 cm^{-1}, and carbohydrates at 1115 and 1037 cm^{-1}) [3]. These results have shown that pili expression can be associated to growth adhered to surfaces and that FT-IR is a rapid an appropriate technique to monitor the phenotypic expression of *M. bovis* growing as biofilm.

[1] Brown, M. H., *et al.*, 1998. J. Am. Vet. Med. Assoc. 12:259-266. [2] Bosch, A., *et al.*, 2000. Physica Status Solidi (b) 220:635-640. [3] Naumann D., 2000, Infrared Spectroscopy in Microbiology. *In* Encyclopedia of Analytical Chemistry, R.A.Meyers (Ed).