FT-IR and complementary techniques used to study microbial biofilms

Diego Serra[1], Alejandra Bosch[1], Genia Lücking[2], Monika Ehling-Schulz[2]
Juergen Schmitt[3], Dieter Naumann[4], and Osvaldo Yantorno[1*]

[1]Centro de Biotecnología Aplicada (CINDEFI, CONICET), Fac. de Cs. Exactas, UNLP, La Plata, Argentina; [2]Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL), Abteilung Mikrobiologie - Technische Universität München (TUM), Freising, Germany; [3]Synthon GmbH, Heidelberg, Germany; [4]Robert Koch Institute, Berlin, Germany.

Biofilms are defined as sessile communities composed of cells that are irreversibly attached to a surface or to each other, embedded in a matrix of extracellular polymeric substances (EPS), and that exhibit differential phenotypic characteristics with respect to their planktonic counterparts [(1)]. Being the predominant mode of microbial growth in nature and industrial settings, bacterial biofilms are particularly problematic in the context of microbial infections in humans accounting for up to 80% of all bacterial infections. Biofilms have been implicated in lung and urinary tract infections, catheter infections, middle-ear infections, dental plaque and gingivitis, and coating contact lenses. Biofilms can also be formed on the inert surfaces of implanted devices and prostheses such as catheters, prosthetic cardiac valves, joint prostheses, and intrauterine devices.

Particularly, in the case of *Bordetella pertussis*, a strictly human pathogen that colonizes the respiratory tract in not- or under-immunized infants and causes whooping cough has historically been associated with acute infections. Therefore, most of the therapeutic strategies and investigations have traditionally been based in results obtained from planktonic cultures. Nevertheless, it is becoming increasingly evident the appearance of persistent asymptomatic or milder infections in adolescents and adults[(2)]. Although little is known concerning the mechanisms by which *B. pertussis* causes these persistent infections, recent reports support the hypothesis that this pathogen may adopt a sessile biofilm lifestyle as a strategy to survive and persist in their host.

The aim of this presentation is to show how FT-IR spectroscopy in combination with multivariate statistical analysis and complementary techniques provides detailed molecular insight into bacterial biofilm lifestyle. We will discuss the robustness of these techniques together to show that *B. pertussis* is able to: *i)* adhere and grow on abiotic surfaces as biofilm, *ii)* overproduce EPS in such culture conditions and, *iii)* express a biofilm-specific phenotype.

B. pertusis cells were grown either in column bioreactors filled with polypropylene beads (when high amounts of sessile cells or EPS were required) or in continuous-flow chambers using borosilicate slides and ZnSe windows, for *in situ* and nondestructive measurements. Cell attachment and biofilm development were monitored by FT-IR spectroscopy, crystal violet stain, fluorescence microscopy and confocal laser scanning microscopy (CLSM). Sessile cells were analyzed and compared with planktonic cells by IR spectroscopy, 2D gel electrophoresis-based analysis of cytosolic and membrane-associated subproteomes, and chemical techniques. Likewise, chemical and spectroscopical characterization of purified EPS was performed [(3, 4, 5)].

By means of these complementary *in situ* techniques it was demonstrated that *B. pertussis* can grow and form biofilms on abiotic surfaces and that this cell growth is accompanied by significant phenotypic changes particularly in carbohydrate expression level providing a link between the polysaccharide overproduction and biofilm maturation. Further studies combining proteome analyses together with MALDI-TOF/MS identification of discrete proteins with whole cell FT-IR fingerprinting and multivariate statistical methods, revealed the biosynthesis of a putative acidic-type polysaccharide polymer.

Our studies demonstrated the potential of utilizing FT-IR and complementary techniques to illustrate that *B. pertussis* is capable to produce a mature biofilm that distinctively affects its phenotype and that could explain the long time persistence of the bacterium in the host.

References:

[1] Donlan and Costerton *Clin Microbiol Rev*, 2002; [2] Cherry et al., *Pediatrics*, 2005; [3] Bosch et al. *Appl Microbiol Biotechnol*, 2005; [4] Serra et al. *Anal Bioanal Chem*, 2007; [5] Serra et al. *Proteomics*, 2008.