Phenotypic Characterization of Biofilms Formed by Bordetella pertussis Clinical Isolates by FT-IR Spectroscopy

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Whooping cough is a highly contagious infectious disease of the upper respiratory tract caused by the Gram-negative pathogen *Bordetella pertussis* [1]. Despite widespread and efficient vaccination, in the last two decades there has been a worldwide resurgence of pertussis [2, 3]. The resurgence of the disease has been associated to various factors including suboptimal vaccines, waning immunity and pathogen adaptation to persist in vaccinated populations. Although traditionally known as a severe childhood disease recently, an increased incidence in adolescents and adults was reported. In the later groups the disease symptoms are mild or even not manifested, consequently, the infected individuals become silent carriers of the pathogen [4]. One proposed hypothesis to explain the survival and persistence of *B. pertussis* in human host is that this organism may adopt a biofilm lifestyle during respiratory infection as a strategy to survive and persist in their host [5].

The aim of this study was to get insights into the phenotypic diversity of *B. pertussis* local clinical isolates and a reference strain (*B.p.* Tohama I). Furthermore, we analyzed the ability of circulating isolates to grow as biofilm and compared their chemical composition with *B. pertussis* Tohama I strain biofilm by FT-IR spectroscopy.

We studied the reference strain *B. pertussis* Tohama I and a total of 15 clinical isolates recovered in the period 2001-2008 from pediatric patients in Argentina. Firstly, we analyzed the phenotypic diversity of the isolates after 48 h of culturing on Bordet-Gengou agar at 36 ºC by FT-IR spectroscopy. No significant differences were observed among the general features of first derivative spectra belonging to the clinical isolates and the reference strain along the whole spectral range. The maximum spectral variance among 3 independent replicates of the same isolates (Dmax = 23 ± 5) was similar to the spectral distance among different strains. Thus, the clinical isolates studied and the Tohama I strain seemed to be phenotypically indistinguishable by FT-IR spectroscopy. Nevertheless, when the biofilm formation ability of these bacteria was assessed on 6-well-microtiter dishes after 72 h of growth by quantifying the sessile biomass with crystal violet [6], all clinical isolates showed a significantly greater capacity to form biofilms, compared to the reference strain. Besides, the biofilms produced on the wells for all the strains was scraped from the microtiter walls and collected with distilled water for FT-IR spectroscopy analysis. All strains showed distinctive polysaccharide signals when growing as biofilms compared to the solid medium growth, may be due to the extracellular matrix composition. A semi-quantitative FT-IR analysis of the bands assigned to proteins, carbohydrates and lipids in clinical isolate biofilms showed an increase in the relative protein content. This result is in accordance with ongoing proteomic analysis showing an increased expression of several proteins associated to biofilm growth.

Overall, our results suggest a higher capability of clinical isolates than the reference strain to adopt the biofilm lifestyle, reinforcing the hypothesis that biofilm lifestyle can be considered as an “evolution trait” adopted by *B. pertussis* to persist in their host.

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