

# Does *Bordetella pertussis* have enough fitness to adopt the biofilm mode of life?

## Abstract

*Bordetella pertussis* is the causative agent of whooping cough or pertussis a highly contagious disease that affects respiratory tract. Although vaccination has reduced mortality due to pertussis infection, the efficacy of both current cellular and a cellular formulations produced from bacteria grown in stirred bioreactors is limited. Thus, the interest in the development of new vaccines increased. In this scenario, the hypothesis that *B. pertussis* colonizes and persists in their host through biofilm development started to be analyzed. The biofilm formation starts with the adhesion of free-floating bacteria to surfaces. Then, the production of extracellular polymeric substance is induced and this contributes to increase the anchor to the surface. The resultant mature biofilm is more resistant to the external physical, chemical or biological attack. We here reviewed the main results showing the fitness of *B. pertussis* reference strains and clinical isolates to adopt the biofilm lifestyle. We outline the implications of biofilm development in the proteomic of cells growing in liquid media (planktonic cells) and in biofilm (sessile cells). In particular an over-expression of the Bordetella intermediate protein A (BipA), a typical marker of intermediate phase, in the surface of biofilm cells is analyzed. Considering that bacterial biofilm are often immersed in liquid fluid and are subjected to hydrodynamic forces, we here highlight the importance of shear stress in initial adhesion of *B. pertussis* on surfaces. Finally, the differences in the capacity of biofilm formation between reference strains and clinical isolates recovered in Argentina and USA are reviewed. We conclude that elucidating the molecular mechanisms involved in the association of biofilm and pathogenesis of *Bordetella pertussis* will be important for the development of new and more effective vaccines to control the resurgence of whooping cough.

**Keywords:** *Bordetella pertussis*, biofilm, adhesion, shear stress, proteomic, clinical isolates

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## Introduction

It has been widely reported that biofilms represent the most widespread microbial mode of life in nature.<sup>1</sup> If this is the case, the question is: why is this way of life so fitting or attractive for bacteria? Different types of analysis performed on bacterial populations that adopt this form of growth showed that it provides bacteria with multiple protective advantages and allows them to remain within a niche for long time by resisting adverse environmental conditions.<sup>2</sup> The term biofilm is used to describe a structured community of microorganisms adhered or not to an inert or living surface embedded in an hydrated polymeric matrix which does not necessarily need to be produced by the bacteria of the biofilm.<sup>2</sup> Biofilms development takes place in a sequence of steps. Microorganisms adhere first reversibly, and then irreversibly to a surface and begin to produce an extracellular polymeric substance (EPS) that contributes to stick together and anchor the cells more firmly to the surface. In such conditions the mature biofilms becomes more protected from the action of external physical, chemical or biological attack. Finally, biofilms are either dispersed or planktonic bacterial cells are released from it.<sup>1</sup> Compared to their free-swimming counterpart, bacteria living in biofilms are better adapted to endure nutrient deprivation, pH changes, presence of oxygen radicals and antimicrobial agents.

*Bordetella pertussis* is a human-restricted bacterial pathogen that causes whooping cough, or pertussis a highly contagious respiratory disease particularly severe in infants and children.<sup>3</sup> Although

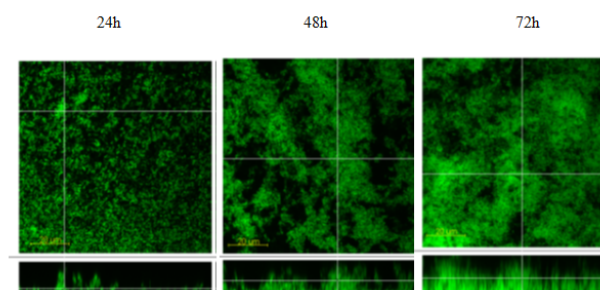
vaccination has reduced mortality due to *B. pertussis* infection in infants, whooping cough is still a major cause of vaccine-preventable deaths particularly in developing countries. Among the principal reasons suggested for the pertussis reemergence are the low efficacy of currently available a cellular pertussis vaccines and the antigenic and genetic changes in circulating strains.<sup>4,5</sup> Taking into account that current vaccines do not provide optimal control of pertussis, this has stimulated interest in the improvement of the existing formulations and in the development of new vaccines. Recent research has suggested reintroduction of less reactogenic whole cell pertussis vaccines as possible options to improve the capacity to fight against pertussis.<sup>6</sup> As with other pathogens both current cellular and a cellular pertussis vaccines are obtained from microorganisms growing in stirred bioreactors in rich culture media with the intention of obtaining high biomass yields, and in environmental conditions where the population reaches the maximum specific growth rate. However, this scenario most likely does not represent the environment which *B. pertussis* finds during the infection.

Although whooping cough has been considered an acute disease, mainly affecting unvaccinated infants, and the infections are cleared by host immunity and/or antibiotic treatments, the only ecological niche recognized so far for these bacteria are humans. In addition, in the last decades, a shift in incidence towards vaccinated children, adolescents, and adults has become increasingly evident.<sup>7</sup> In this new scenario it was recently demonstrated that *B. pertussis* could persist

and be transmitted via airborne droplets -as it was recently described in a baboon model of *B. pertussis* infection.<sup>8</sup> In order to better understand the colonization, survival and persistence of these bacteria into the host, we and other authors hypothesized that *B. pertussis* could adopt the mode of biofilm growth during interaction with its natural host. Here we summarized some of our experimental results that support this hypothesis. Adhesion of microorganisms to host cells is assumed to be the first and most critical step in the development of many infectious diseases. In our initial investigations we reported the capacity of *B. pertussis* to adhere and grow in a biofilm form on different abiotic surfaces, such as glass and polypropylene.<sup>9</sup> We analyzed *B. pertussis* adhesion incubating bacterial suspensions in contact with surfaces at 37°C under static conditions for different intervals of time. It is interesting to note that although this way of evaluating adhesion is still widely used, it does not take into account that *in vivo* bacteria must adhere to the upper respiratory tract overcoming shear forces that seek to remove them. We therefore went further and could also analyze the biofilms produced on biotic surfaces as respiratory tract tissue using mice models.<sup>10,11</sup> More recently, we begin to analyze the adhesion of *B. pertussis* Tohama I strain in virulent and avirulent phases under shear stress. We are using a shear value of 0.9 dynes/cm<sup>2</sup>, similar to that described in the human respiratory tract. Under this hydrodynamic condition a reduction of 60% of the adhesion was detected for the virulent phase, but the remaining 40% of bacteria resist the shear forces and continue adhered to the surface after 4 h of hydrodynamics conveys effects (unpublished data). Taking into account these early results and reported data showing that for some pathogenic bacteria, shear stress stimulates the synthesis of adhesins, we are actually carrying out investigations trying to get insights into the effect of different hydrodynamic regimes in *B. pertussis* biofilm. To achieve permanent adhesion under stress conditions, bacterial cells develop a series of adhesins able to assist the attachment. In *B. pertussis* efficient biofilm formation has been associated with the virulent phenotype. Most virulence factors in *Bordetella* -including adhesins- are regulated by the two-component signal transduction system BvgAS (*Bordetella* virulence gene activator/sensor) in response to environmental signals. *B. pertussis* exhibits three different virulent phases: i) virulent (Bvg<sup>+</sup>); avirulent (Bvg<sup>-</sup>), and an intermediate virulent phase (Bvg<sup>i</sup>). We reported that Filamentous Haemagglutinin (FHA), the principal adhesin of *B. pertussis* expressed in Bvg<sup>+</sup> phase, promotes an efficient biofilm formation mediated by cell-substrate and inter-bacterial adhesions.<sup>12</sup> FHA also plays a critical role in the formation and maintenance of biofilms in the mouse respiratory tract.<sup>12</sup> The expression of exo polysaccharides was also reported as critical for the stability and maintenance of the complex biofilms architecture. While this polymer was not crucial for initial attachment to artificial surfaces, it was critical for the formation of mature biofilms.<sup>13</sup>

Proteomic analysis of *B. pertussis* biofilm showed that biofilm formation seems to have a more pronounced effect for the membrane subproteome (49.5%) than for the cytosolic one (27.8%).<sup>14</sup> Besides, although the biofilm formation was associated to the expression of the Bvg<sup>+</sup> phenotype interestingly, De Gow et al.<sup>15</sup> reported that *Bordetella* intermediate protein A (BipA), typical marker of Bvg<sup>i</sup> phase, was the most abundant surface-exposed protein, for bacteria growing in biofilm under non modulating conditions. Therefore, it could be assumed that biofilm growth imposes to *B. pertussis* sessile cells some kind of stress that could induce a change of phase. We could also confirm that *bipA* is expressed during respiratory tract infection of mice, and

that anti-BipA antibodies are present in the serum of convalescent whooping cough patients, suggesting that biofilm formation represents an important aspect of *B. pertussis* infection, and antigens expressed during this growth may therefore be potential targets for vaccination.<sup>15</sup> Recent studies of the biofilm-forming abilities between *B. pertussis* Tohama I strains and currently circulating isolates from Argentina and USA, two countries with different vaccination programs, showed that compared to reference strain, all clinical isolates analyzed formed biofilms at high levels (Figure 1). In this work the higher biofilm formation in circulating isolates was proposed to be associated to the persistence, transmission, and continued circulation of *B. pertussis* strains in the population.<sup>16-18</sup> Despite the large amount of information on *B. pertussis* biofilms formed on artificial surfaces and in animal model, it is still unclear how biofilms may develop in the host during infections. Elucidating the molecular mechanisms involved in the association of biofilm and pathogenesis of *B. pertussis* currently circulating clinical isolates will be important for the development of new and more effective vaccines to control the resurgence of whooping cough.



**Figure 1** Biofilms of a *B. pertussis* clinical isolate. Confocal Laser Scanning Microscopy (CLSM) of the biofilms produced on coverslips placed in a 96-well plates for 24, 48 and 72 h. The Green Florescence protein (GFP)-labeled isolate Images are represented in planes xy and xz.

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## Conflict of interest

The author declares no conflict of interest.

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