

Bacterial biofilms: Importance in animal diseases

F. Aguilar-Romero^{1,2}, N.A. Pérez-Romero¹, E. Díaz-Aparicio¹ and R. Hernández-Castro³

¹Centro Nacional de Investigación Disciplinaria en Microbiología Animal-Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Km. 15 ½ carretera libre México-Toluca, Cuajimalpa 05110, México, D.F. Mexico.

²Departamento de Microbiología e Inmunología. Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México. Coyoacán 04510, México, D.F. Mexico.

³Dirección de Investigación. Hospital Dr. Manuel Gea González-Secretaría de Salud. Tlalpan. 14080. México, D.F. Mexico.

Recent studies done by electron microscopy and molecular biology techniques have allowed to explain in detail aspects of microbial biology to establish that the majority of bacteria remain attached to surfaces within a structured ecosystem known as biofilm. This is defined as a community of microorganisms that grow inside a matrix of exopolysaccharides attached to an inert surface or living tissue, and may be comprised of a single bacterial species or a range of different species, including fungi.

There are many evidences that relate biofilm to different infectious processes, although the mechanisms that produce disease are not yet fully understood. It has also been observed the formation of these structures in the affected tissues and from which groups of bacteria are released into the bloodstream where they can evade the phagocytic cells of the immune system, besides being resistant to treatment with antibiotics. These issues are addressed in this review focused on bacteria of veterinary importance, in order to understand their participation in diseases such as pneumonia, myocarditis, orchitis, epididymitis, mastitis, abortion, otitis, conjunctivitis, polyarthritis and septicemia in domestic animals.

Keywords: Biofilm, Bacteria, animal, disease.

Throughout their evolution, bacteria have constantly modified their metabolism and physical characteristics, adapting to practically all environments of the planet. The generalized idea that bacteria have a unicellular way of life is not entirely accurate, given that pure planktonic growth is uncommon, bacteria frequently develop in complex communities [1]. One of the biggest paradigms in microbiology is the concept of the existence of bacteria as social organisms, whose only activity was to divide to generate a new bacterium, each one identical to the other. However, for more than 60 years it has been suggested that far from this isolated behavior, there may be a group behavior of bacteria [2]. Direct observations of an extensive variety of bacteria have allowed to establish that most microorganisms remain bounded to the surfaces inside a structured ecosystem named biofilm, and not as isolated organisms [3].

Biofilms are defined as communities of microorganisms that grow soaked up in a matrix of exopolysaccharides adhered to inert material or live tissue. These communities can be conformed by a single species or by different bacterial species and even different genera.

The composition of the biofilm varies depending on the system under study. In general, the main component is water, and can represent up to 97% of the total content. Besides water and bacterial cells, the matrix is a complex that is mainly formed of extracellular polymer and, in a lesser amount, of other macromolecules like proteins, DNA and diverse products derived from the lysis of bacteria [4,5].

There are numerous evidences that relate biofilms with different infectious processes, such as pneumonia, dermatitis and mastitis mainly [4]. Some of the mechanisms by which diseases are produced are still not well understood, but it has been suggested that bacteria can produce endotoxins once they are part of the biofilm. Bacteria can also be released into the bloodstream and become resistant to the phagocytic action of the immune system cells. This represents a niche for the appearance of antibiotic resistant bacteria, and it has even been mentioned that the bacteria that form biofilms can be up to 1000 times more resistant to chemotherapeutics than the ones that do not form any aggregates [6]. This last aspect can be particularly relevant, given that resistant bacteria originated in this aggregates can spread from animal to animal through workers or farm equipment such as feeders, water dispenser, salt blocks, etc. This may explain the presence of acute diseases that can later persist with a chronic course and become resistant to treatments with antimicrobial agents [7].

The bacterial biofilm begins to form when any individual cell initially binds to a surface. The capacity of the cell to perform this initial reaction depends on environmental factors such as temperature and pH, and on the genetic factors that encode motor functions, sensitivity to the environment, adhesins and other proteins. Although the combination of factors influence the development of the biofilm, these factors initially depend on the species; some characteristics are common to most bacteria studied so far [8-10]. The formation process is also influenced by external factors such as the nutritional environment [11-13].

After the initial binding, the cell starts to grow and spread over the surface in a monolayer, while forming microcolonies. The cells change their behavior and give place to a complex architecture of the mature biofilm. The most evident change is the production of the matrix that will support the complete complex. While the biofilm continues to

grow, other events or changes occur, such as the spreading to other non infected areas or releasing some cells, that will recover the planktonic qualities and act as seeds from other similar structures on new surfaces [14].

Five stages have been determined: the reversible adsorption of the bacteria to the surface, an irreversible binding, a first maturity phase with growth and division, the second phase of exopolysaccharide production and the final development of the colony with dispersion of colonizing cells. The structure is permeabilized with a web of channels of water, bacterial residues, enzymes, nutrients, metabolites and oxygen. Most of the viable cells can be found in the periphery of the structure, and their numbers decrease with age; up to 80% of viable cells can be found in a young biofilm and only 50% on an old structure. An anaerobic environment can develop under the aerobic layer [15].

To adapt to the biofilm life, bacteria suffer radical changes in their physiology compared to planktonic cells [8,16]. As well as a different gene expression pattern, there is a repression of genes from the pili and flagellum, given that these are necessary for the initial formation, but they are probably not as necessary for the maturation of the structures, there is also an induction of genes that are related to antibiotic resistance [17]. The change of their environment and conditions, activates different genes that encode new structural proteins and enzymes. These genes and proteins are the ones that explain the fixation and antibiotic and disinfectant resistance of bacteria in biofilms. The recent advances since 2000 in proteomics and genomics have allowed to move forward in the study of complex systems, given that it has been possible to identify 800 proteins that change their concentration throughout the different phases of development [18].

A few days from the first colonization, other microorganisms are caught in the exopolysaccharide by physical catchment and electrostatic attraction. Fungus and bacteria that are not mobile on their own are capable of using residual materials from the first inhabitants and are able to produce their own residues that will be used by other microorganisms [19]. From the most simple biofilm to the most complex one, there is cooperation among the metabolic community, just like the live tissue in a multicellular organism. The different species live in a minimum niche, very specialized and tailored. If one species generates toxic residues, another will avidly devour them, this is how it is possible to coordinate the biochemical resources of all inhabitants; the different enzymes that numerous species of bacteria need to supply their nutrient provisioning are gathered here, because no single species could digest all of these enzymes alone. This will also be useful to respond or resist the attack of diverse chemical substances, for example biocides [20].

Antibiotic therapy usually eliminates bacteria in planktonic stage, but it cannot penetrate the biofilm. On the other hand, there have also been found hydrolytic enzymes of the β -lactamase type that are synthesized in small amounts but that are kept caught and concentrated in the matrix of the biofilm, and this contributes to its protection. There are evidences that demonstrate that these characteristics of bacteria can cause the presence of chronic diseases as a consequence. If bacteria can, in fact, have this behavior as a group, then the existence of a communication system among them is necessary [2], this is why in 1994 the term "quorum sensing" was created to describe a phenomenon dependant on cellular density. This process is based in the production of molecules that work as signals, whose concentration depends on the density of the organism that produces it. Once this molecules or autoinducers reach the threshold of detection, they induce different phenomena in the cell [24].

The disintegration of biofilm can be influenced by the external environment. Some factors that affect this process are the changes in the environment, decrease in nutrient availability [25], electrochemical properties inside the structure [26] and limitation of the substrate [27]. It can also be affected by metabolism, since the regulating protein for the union to RNA, the CsrA (carbon storage regulator) acts as a repressor of the formation and activates its disintegration in different cultivation conditions [28]. The model proposed by O' Toole et al in 2000 [8] to describe all the aggregation and disintegration process, suggests that the cells in planktonic stage receive environmental signs that cause the initial adhesion to the substrate, after that, the bacteria begin to communicate with one another in the process called "quorum sensing" and these signs guide the formation of microcolonies that will form the mature biofilm, a three-dimensional structure soaked in an exopolysaccharide matrix. Finally, new signs indicate the disintegration to bacteria, to return to the planktonic stage and completing the cycle [2]. Diverse studies estimate that the formation of biofilm and some other predisponent factors are involved in up to 65% of the human bacterial diseases [29]. Besides, these studies show that the majority of the bacteria that form these bacterial aggregates are found viable. It is known that 80% of the cells that conform a biofilm are capable of emigrating to other places and form new groups [15,30]. In Veterinary Medicine, the relationship between the presence of biofilm and diverse diseases in different animal species has been reported, such as: *Acinetobacter baumannii* in infections of intravenous jugular catheters [31], *Actinobacillus pleuropneumoniae* and *Staphylococcus epidermidis* in postoperative infections [32-34], *Klebsiella spp.*, skeletal muscle infections in horses [35]; *Pseudomonas aeruginosa* in otitis [36], *Staphylococcus intermedius* in pyoderma in canines [37]; *Staphylococcus aureus* and *Staphylococcus epidermidis* in mastitis in bovines [38-41], *Haemophilus parasuis* in pneumonias in swine [42] and *Mycoplasma mycoides* subspecies *mycoides* SC in bovine pleuropneumonia [43]. In recent studies, it was shown that *Histophilus somni* is capable of forming a biofilm in conditions in vitro upon working with isolation of pathogens and commensals [44] as well as conditions in vitro on cardiac and pulmonary tissue, upon inoculating calves with a pathogenic strain [45]. In Mexico, the following study was carried out [46] and the objective was to determine the ability of biofilm production in 20 isolates of *P. multocida*, 20 of *M. haemolytica* and 10 *H. somni* obtained from pneumonic cattle lungs and sheep reproductive tract. In order to demonstrate the formation of biofilm, each of the

isolates were tested by the Christensen method, Congo red agar culture and assay in polyvinyl microplates of 96 wells. The results were evaluated by analysis of variance ($P < 0.05$) to determine the statistical difference between the tested isolates and strains of *E. coli* and *E. coli* K-12 used as positive and negative controls, respectively. In the case of *P. multocida* and *M. haemolytica* it was determined that the 40 isolates produced biofilm, of which four of *P. multocida* and three of *M. haemolytica* proved to be highly productive. For *H. somni* the 10 isolates were producers and two of them were highly productive. The highly productive isolates were subjected to scanning electron microscopy. In addition, the 50 isolates were tested by the Bauer *et al.* and MIC with gentamicin, tetracycline, ampicillin, penicillin and streptomycin. For of *P. multocida* isolates, the greatest producers of biofilm were resistant to gentamicin and tetracycline, those of *M. haemolytica*, were resistant to gentamicin and streptomycin and those of *H. somni*, to penicillin and streptomycin. The results were analyzed using the Kappa test, giving values of 0.61 to 1.0, which established that there was correlation between biofilm production and antimicrobial resistance [46].

References

- [1] March PD. Microbiologic aspects of dental plaque and dental caries. *Dent Clin N Am* 1999;43:599-614.
- [2] Colón-González M, Membrillo-Hernández J. Comunicación entre bacterias. <http://microbiologia.org.mx/microbiosenlinea/capitulo04>. Consultado febrero 2008.
- [3] Costerton J, Cheng G, Gersey, Nickel M, Marrie T. Bacterial biofilms in nature and disease. *Annu Rev Microbiol.* 1987;41:435-464.
- [4] Watnick P, Kolter R. Biofilm, City of Microbes. *Journal of Bacteriology.* 2000; 182:2675–2679.
- [5] Betancourth M, Botero J, Rivera S. Biopelículas: Una comunidad microscópica en desarrollo. *Colombia Médica.* 2004;35:34-39.
- [6] Gilbert P, Das J, Foley I. Biofilms susceptibility to antimicrobials. *Science.* 2007; 11:160-167.
- [7] Maestre M, Vera M. Biofilm: Modelo de comunicación bacteriana y resistencia a los antimicrobianos. *Prous Science.* 2004;17:26-28.
- [8] Costerton J. Overview of microbial biofilms. *Indus Microbiol.* 1995;15:137-140.
- [9] O'Toole G, Kaplan H, Kolter R. Biofilm formation as microbial development. *Annu Rev Microbiol.* 2000;54:49-79.
- [10] Hydrodynamic influences donde biofilm formation and and growth. <http://carambola.usc.edu/research/biophysics/biofilms.html>
- [11] Pratt L, Kolter R. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Mol Microbiol.* 1998;30:285-293.
- [12] Corona-Izquierdo F, Membrillo-Hernández J. Biofilm formation in *Escherichia coli* is affected by 3-(N-morpholino) propane sulfonate (MOPS). *Res Microbiol.* 2002; 153:181-185.
- [13] Corona-Izquierdo F, Membrillo-Hernández J. A mutation in rpoS enhances biofilm formation in *Escherichia coli* during exponential phase of growth. *FEMS Microbiol.* 2002;211:105-110.
- [14] Danese P, Pratt L, Kolter R. Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. *Bacteriol.* 2000;182:3593-3596.
- [15] Wimpenny J. Heterogeneity in biofilms. *FEMS Microbiol Rev,* 2000;24:661-671.
- [16] O'Toole GA, Pratt LA, Watnick PI, Newman DK, Weaver VB, Kolter R. Genetic approaches to study of biofilms. *Methods Enzymol.* 1999;310:91-109.
- [17] Whiteley M. Gene expresión in *Pseudomonas aeruginosa* biofilms. *Nature reviews microbiology.* 2001;413:860-864.
- [18] Stewart SP, Franklin JM. Physiological heterogeneity in biofilms. *Nature reviews microbiology.* 2008;6:199.
- [19] Mandell G, Bennett J, Dolin R. *Staphylococcus*, in Principles and practice of infectious diseases. *Panamericana.* 2000;20:96.
- [20] Brown MR, Allison DG, Gilbert P. Resitance of bacterial films to antibiotics: related effect? *J Antimicrob Chemother.* 1988;22:777-780.
- [21] Winder A, Frei R, Rajcic Z, Zimmerli W. Correlation between in vivo and in vitro efficacy of antimicrobial agents agains foreing body infections. *J Infect Dis.* 1990; 162:96-102.
- [22] Merle Olson, Howard Ceri, Douglas Morck, Andre Buret, Ronald Read. Biofilm bacteria: formation and comparative susceptibility to antibiotics. *The Canadian Journal of Veterinary Research.* 2002;66:86-92.
- [23] Costerton JW, Veeh R. The application of biofilm science to study and control of chronic bacterial infections. *J Clin Invest.* 2003;112:1466-1477.
- [24] Fuqua WC, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional activators. *J Bacteriol.* 1994;176:269-275.
- [25] Stoodley PJ, Jørgensen F, Williams P, Lappin-Scott HM. The role of hydrodynamics and AHL signaling molecules as determinants of the structure of *Pseudomonas aeruginosa* biofilms.
- [26] Characklis WG, McFeters GS, Marshall KC. Physiological ecology in biofilm systems. *Biofilms wiley.* 1990;12:341-394.
- [27] Peyton BM, Characklis WG. A statistical analysis of the effect of substrate utilization and shear-stress on the kinetics of biofilm detachment. *Biotechnol Bioeng.* 1993;41:728-735.
- [28] Jackson DW, Suzuki K, Oakford L, Simecka JW, Hart ME, Romeo T. Biofilm formation and dispersal under the influence of the global regulator CsrA of *Escherichia coli*. *J Bacteriol.* 2002;184:290-301.
- [29] Potera C. Forging a link between biofilms and disease. *Science.* 1999;284:1837-1839.
- [30] Singh R, Stine OC, Smith DL. Microbial diversity of biofilms in dental unit wáter systems. *Appl Environ Microbiol.* 2003;69:3412-3420.
- [31] Vaneechoutte M, Devriese LA, Dijkshoorn L, Lamote B, Deprez P. *Acinetobacter baumannii* infected vascular catéter collected from horses in an equine clinic. *J Clin Microbiol.* 2000;38:4280-4281.
- [32] Kaplan J, Mulks M. Biofilm formation is prevalent among field isolates of *Actinobacillus pleuropneumoniae*. *Veterinary Microbiology.* 2005;108:89-94.

- [33] Smith MA, Ross MW. Postoperative infection with *Actinobacillus* spp I n horses: 10 cases (1995-200). *J Am Vet Med Assoc.* 2002;221:1306-1310.
- [34] Trostle S, Peavey CL, King DS. Treatment of methicillin resistant *Staphylococcus epidermidis* infection following repair of an ulnar fracture and humeroradial joint luxation in a horse. *J Am Vet Med Assoc.* 2001;218:554-559.
- [35] Moore RM, Schneider RK, Kowalski J. Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979-1989. *J Equine Vet.* 1992;24:450-456.
- [36] Hariharan H, McPhee L, Heaney S, Bryenton J. Antimicrobial drug susceptibility of clinical isolates *Pseudomonas aeruginosa.* *Can Vet.* 1995;36:166-168.
- [37] Lloyd DH, Lamport A, Noble WC, Howell SA. Fluoroquinolone resistance in *Staphylococcus intermedius.* *Vet Dermatol.* 1999;10:249-251.
- [38] San Martín B, Cruze J, Morales MA, Agüero H, León B, Espinoza S. Bacterial resistance of mastitis pathogens isolated from dairy cows in the Vth región, Metropolitan región and Xth región Chile. *Arch Med Vet.* 2003;34:221-234.
- [39] Vasudevan P, Kumar M, Nair M, Annamalai T, Venkitanarayanan K. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. *Veterinary Microbiology.* 2003;92:179-185.
- [40] Fox L, Zadoks R, Gaskins C. Biofilm production by *Staphylococcus aureus* associated with intramammary infection. *Veterinary Microbiology.* 2005;107:295-299.
- [41] Olivera M, Bexiga R, Nunes S, Carneiro C, Cavaco L, Bernardo F, Vilela C. Biofilm-forming ability profiling of *Staphylococcus aureus* and *Staphylococcus epidermidis* mastitis isolates. *Veterinary Microbiology.* 2006;118:133-140.
- [42] Jin H, Zhou R, Kang M, Luo R, Cai X, Chen H. Biofilm formation by field isolates and reference strain of *Haemophilus parasuis.* *Veterinary Microbiology.* 2006;118:117-123.
- [43] McAuliffe L, Ayling R, Ellis R, Nicholas R. Biofilm-grown *Mycoplasma mycoides* subsp. *mycoides* SC exhibit both phenotypic and genotypic variation compared with planktonic cells. *Veterinary Microbiology.* 2008;129:315-324.
- [44] Sandal I, Wenzhou H, Swords W, Inzana J. Characterization and comparison of biofilm development by pathogenic and comensal isolates of *Histophilus somni.* *Bacteriol.* 2007; (189):8179-8185.
- [45] Sandal I, Shao J, Annadata S, Apicella M, Boye M, Jensen T, Inzana J. *Histophilus somni* biofilm formation in cardiopulmonary tissue of the bovine host following respiratory challenge. *Microbes and Infection.* 2009;(11):254-263.
- [46] Pérez-Romero NA. Estudio de la capacidad de producción de biopelícula y Resistencia a antimicrobianos en cepas de *Pasteurella multocida*, *Mannheimia haemolytica* e *Histophilus somni.* Tesis. Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México. 2010.