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The Impressive Features of Swarming Motility on Antibiotics Resistance

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Abstract

Swarming motility is one of the most impressive features of microbial life and requires an extended investigations. Till now days, many studies have indicated that swarming is the most complex type of bacterial motility. It roles include the colonization of hydrated-viscous surfaces, the formation of biofilms and antibiotics resistance. Furthermore, among the human pathogene microbiota, *Pseudomonas aeruginosa* have attracted a significant interest because of their complexes swarming pattern. The direction of this movement is biased by chemotactic responses to several stimuli. Thus, the present review is focused on *Pseudomonas aeruginosa* swarming and their exhibition of adaptive antibiotics resistance.

Keywords: Swarming; Motility; *Pseudomonas aeruginosa*; Antibiotics resistance

Introduction

P. aeruginosa is the most common pathogen isolated from hospitalized patients and is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia. Nevertheless, attempts of treatment of *P. aeruginosa* from patients through intense antimicrobial therapy may lead to significant selection of resistance strains in care units of the hospitals [1]. Furthermore, *Pseudomonas aeruginosa* are opportunistic pathogens often associated with gastrointestinal infections, dermatitis, bacteremia, and a variety of systemic infections, particularly in patients with severe burns, cancer and AIDS [2].

Pseudomonas aeruginosa is a major nosocomial pathogen representing a critical threat for human health [3] because of its tolerance and rapid development of resistance towards almost all current antimicrobial therapies [4]. Moreover, its survival in the host in the early stages of infection is supported by the secretion of toxins and virulence factors, including pyocyanin and its proteases elastase and alkaline protease (AprA) [5,6]. Thus, infections by *P. aeruginosa* are notoriously difficult to treat because of its acquired resistance to antibiotics. All known mechanisms of antibiotics resistances can be displayed by this bacterium (intrinsic, acquired, and adaptive); sometimes all within the same isolate [3]. It is not surprising that these ubiquitous, Gram-negative aerobic rods with polar, monotrichous flagella and protein structures on the surface (pili) are responsible for adherence to respiratory epithelium [7]. Its adaptability and high intrinsic antibiotic resistance enable it to survive in a wide range of other natural and artificial settings, including surfaces in medical facilities [8]. With a defined adherence, motility and biofilm formation, host colonization is made. Biofilms are responsible for antimicrobial resistance [9] and persistent infections [10].

It is also noteworthy that, the bacterium *Pseudomonas aeruginosa* is capable of three types of motilities: swimming, twitching and swarming. The latter is characterized by a fast and coordinated group movement over a semi-solid surface resulting from intercellular interactions and morphological differentiation. A striking feature of swarming motility is the complex fractal-like patterns displayed by migrating bacteria while they move away from their inoculation point.

To the best of our knowledge, a review of the literature suggests that the first case of tendril-tendril communication was reported by O'Toole et al. [11] working with *P. aeruginosa*. This complex type of motility is usually defined as a rapid and coordinated translocation of a bacterial population across a semi-solid surface [12]. To our knowledge, the tendril-tendril communications are more related to the swarming pattern. Furthermore, bacterial swarming motility has been shown to be important to formation [13], where cells act not as individuals, but as coordinated groups to move across surfaces, often within a thin-liquid film [14].

The swarming communities of *P. aeruginosa* represent a complex intersection of physical, biological, and chemical phenomena. However, the branched tendril patterns that are often, but not always, observed in *P. aeruginosa* swarms [15,16] require production of rhamnolipid (RL) [17] witch reduce surface tension in bacterial suspensions. In addition to RL, a functional bacterial flagellum is also required for swarms to form tendrils [12].

Kohler et al. [16] reported that in addition to flagella, swarming of *P. aeruginosa* requires the release of two exoproducts, rhamnolipids (RLs) and 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), which act as wetting agents and chemotactic-like stimuli. According to Du et al. [17] *P. aeruginosa* uses the surfactant RL to control physical forces needed by swarms to efficiently expand over surfaces as a thin liquid film. Although it is well known that biological organisms respond to environmental cues, these swarming bacteria respond actively to alter their environment on a short timescale to greatly improve their colonization rate.

A role for swarming motility during *in vivo* infection or colonization has not been established. However, transposon insertions that attenuate *P. aeruginosa* virulence in a rat chronic pulmonary infection model map to genes required for swarming [18]. Several cues required for swarming *in vitro*, namely rhamnolipids and elevated

glutamate levels are present in the sputum of cystic fibrosis (CF) patients [19].

Pseudomonas aeruginosa and Host Defenses

Many bacteria are capable of forming biofilms, and *Pseudomonas aeruginosa* is one of the most commonly studied. Recent work has begun to uncover some of the genetic and molecular mechanisms underlying biofilms production by this organism. Furthermore, biofilm-growing bacteria cause chronic infections [20] characterized by persistent inflammation and tissue damage [21].

Chronic infections, including foreign-body infections, are infections that (i) persist despite antibiotic therapy and the innate and adaptive immune and inflammatory responses of the host and (ii) in contrast to colonization, are characterized by immune response and persisting pathology. In a static system, during the early stages of biofilm development *P. aeruginosa* cells deficient in flagellar motility exhibit poor surface attachment, while cells lacking type IV pili are unable to form microcolonies [22].

The single polar flagellum of *P. aeruginosa* contributes to its nomadic lifestyle by exploring new niches in order to colonize and establish biofilms, since the flagellum dictates initial surface interactions [22].

Procaryotic flagella operate differently from eucaryotic flagella. The filament is in the shape of a rigid helix, and the cell moves when this helix rotates. Considerable evidence shows that flagella act just like propellers on a boat [23]. Furthermore, the direction of flagellar rotation determines the nature of bacterial movement, for *Pseudomonas monotrichous* polar flagella rotate counterclockwise (when viewed from outside the cell) during normal forward movement, whereas the cell itself rotates slowly clockwise.

The rotating helical flagellar filament thrusts the cell forward in a run with the flagellum trailing behind. For a few seconds, the bacterium will travel in a straight or slightly curved line called a run. When a bacterium is running, its flagella are organized into a coordinated, corkscrew-shaped bundle. Then the flagella "fly apart" and the bacterium will stop and tumble. The tumble results in the random reorientation of the bacterium so that it often is facing in a different direction. Therefore when it begins the next run, it usually goes in a different direction [23].

Bacteria lacking flagella caused less inflammation and death than wild-type counterparts in a murine model of acute pneumonia [24], possibly a reflection of flagellin's ability to trigger pro-inflammatory host responses via Toll-like receptor 5 rather than to a loss of motility per se [25].

To our knowledge, *P. aeruginosa* is one of the large component of the normal microbiota (outer ear, large intestine), in some stress conditions, malnutrition, immune deficiency, it become pathogenic and escape to immune system via a specific strategy.

It is interesting to point out, that the innate immune system distinguishes and recognizes SELF from microbial non-SELF via a set of specific and non-specific receptors. This recognition (non-specific immunity) strategy is based on the detection of conserved molecular structures that occur in patterns and are the essential products of normal microbial physiology.

These invariant structures are called Pathogen-Associated Molecular Patterns (PAMPs) (unique to microorganisms), invariant among microorganisms of a given class, and not produced by the host. Host recognition of PAMPs may have two entirely different consequences. An appropriate response leads to the eradication of a microorganism [26].

These PAMPs are recognized by receptors on phagocytic cells called pattern recognition receptors (PRRs) and more specifically the tool like receptor. In the case of *P. aeruginosa* the most well-known examples of PAMPs are the lipopolysaccharide (LPS) of Gram-negative bacteria. These and other PAMPs are recognized by receptors on phagocytic cells called pattern recognition receptors (PRRs). Because PAMPs are produced only by microorganisms, they are perceived by the phagocytic cells of the innate immune system as molecular signatures of infection.

Outer membrane lipoproteins, LPS, flagellin, and nucleic acids all serve as ligands for TLR2, -4, -5, and -9, respectively. These TLRs and their respective downstream effectors molecules have proven critical to the host response to *P. aeruginosa*, although the protective effects of TLRs may be impaired and in some cases, enhanced in the CF patient, contributing to the particular susceptibility of individuals with this disease to *P. aeruginosa* infection [27].

In *P. aeruginosa*, one other possible TLR ligand is flagellin, the known TLR5 ligand, which has been implicated in a pathogenic role in acute pneumonia [28] and which has been demonstrated to cause inflammation when instilled into the lungs [29]. As reported in the scientific literature, the studies of TLR-Pseudomonas interactions have been limited to acute infections. Certain of these interactions may fail to control the infection because of microbial factors (virulent such as formation of biofilm and EPS.

Furthermore, Worgall et al. [30] analyzed the capacity of PA to induce cell death in human alveolar macrophages (AM) and murine dendritic cells (DC), antigen presenting cells that play a central role in the initiation of pulmonary host defenses against pathogens.

It is of interest that phagocytes are important in resistance to Pseudomonas infections. Antibodies to somatic antigens and exotoxins also contribute to recovery. Humoral immunity is normally the primary immune mechanism against Pseudomonas infection but does not seem to resolve infection in certain patients despite high levels of circulating antibodies.

Swarming Motility and Antibiotics Resistance

Swarming is one of the two important systems of bacterial motility and probably related with the pathogenic process in certain pathologies. An elevated resistance to multiple antibiotics has been reported for swarming populations of many bacterial species in the case of *Salmonella enterica* [31], *Pseudomonas aeruginosa* [32], and a variety of other medium-agar swarmers, including *Serratia marcescens* and *Bacillus subtilis* [33].

For the purpose of this review, our attention will be focused on swarming motility. Swarming allows a colony to migrate collectively over soft agar surfaces and travel distances that are several orders of magnitude longer than their cell length within a few hours. *P. aeruginosa* swarms can have flat, two-dimensional (2D) branches that are approximately 2–5 mm wide and less than 1mm thick, with branching points typically approximately 1 cm from each other [34].

When the effect of antibiotics in these motility types was explored, clear differences were observed among the different antibacterial as

reported by Linares et al. [35]. These authors did not detect any effect on motility in the case of bacteria growing in the presence of tetracycline, whereas a reduction in both types of motility was observed in the case of ciprofloxacin. Noteworthy, the aminoglycoside tobramycin induced both swimming and swarming of *P. aeruginosa*. Again, this finding indicates that sub-inhibitory antibiotic concentrations do not necessarily produce a burden on bacterial physiology but in some occasions may enhance some potentially adaptive characteristics useful for colonization of specific environments [35].

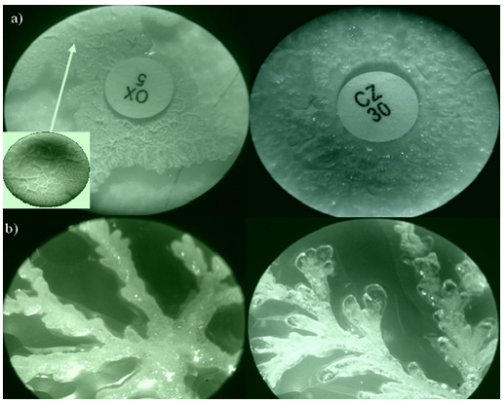

Figure 1: Macroscopic views of swarming phenotype of *P. aeruginosa* inoculated on Mueller-Hinton agar (a) and on tryptic soy agar (TSA) (b) and in the presence of oxacillin and cefazolin. Swarming expression is dependent on the microbial medium.

It is also noteworthy in our ongoing study that a branched tendril pattern was observed in the case of Oxacillin and Cefazolin with a resistance phenotype (Figure 1). Thus, and consistent with these observations a question remains open if the branched tendril pattern is induced by the presence of certain class of antibiotics.

The data presented in Drenkard and Ausubel [36] investigations indicate that *P. aeruginosa* is capable of undergoing transient phenotypic changes, which allow the bacteria to increase their antibiotic resistance both *in vitro* and *in vivo*.

These authors speculate that resistant phenotypic variants present in *P. aeruginosa* biofilms are responsible for the increased resistance to antimicrobial agents observed in CF infections by *P. aeruginosa*. However, Mah and O'Toole [37] found that phenotypes in PA14 RSCV have been associated with the emergence of antibiotic resistance in bacterial biofilms. The same authors propose that variant phenotypes selected inside mature biofilms by antibiotic treatment and other conditions present in the lung of CF patients or in the biofilm itself (such as nutrient limitation) constitute the so-called resistant biofilm phenotype.

It seems that the appearance of phenotypic variants in response to antibiotic treatment has been reported in both Gram-negative and Gram-positive bacteria as reported by McNamara and Proctor [38] data. Butler et al. [39] reported that the analysis of this swarming motility has revealed the protective power of high cell densities to withstand exposure to otherwise lethal antibiotic concentrations. These authors find that high densities promote bacterial survival, even in a non-swarming state, but that the ability to move, as well as the speed of movement, confers an added advantage, making swarming an effective strategy for prevailing against antimicrobials.

Overview the Branched Tendril Patterns of *P. aeruginosa*

P. aeruginosa is not a multicellular organism but has social traits resembling multi-cellularity, such as biofilm formation [11,40], cell-to-cell communication [41] and swarming motility [17,42]. Thus, swarming communities of *P. aeruginosa* represent a complex intersection of physical, biological, and chemical phenomena [18].

The branched tendril patterns that are often, but not always, observed in *P. aeruginosa* swarms [16] require production of rhamnolipid (RL) [17]. In addition to RL, a functional bacterial flagellum is also required for swarms to form tendrils [12]. Thus, bacterial swarming motility has been shown to be important to biofilm formation [43,44] and lifecycle (Figure 2), where cells act not as individuals, but as coordinated groups to move across surfaces, often within a thin-liquid film [15].

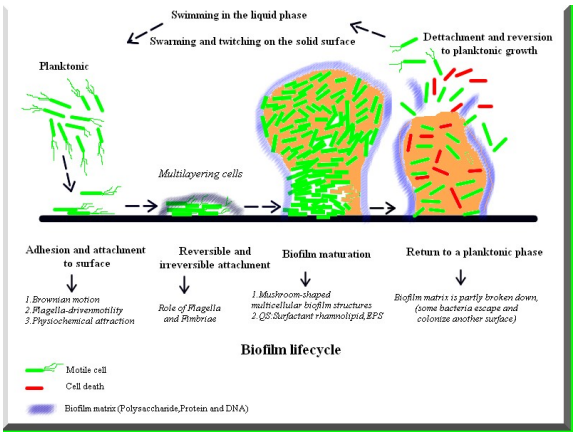

Figure 2: Biofilm lifecycle occurs in several stages, comprising the initial attachment where bacteria adhere via Brownian motion, flagella-driven motility and physiochemical attraction (van der Waals interactions). During stage 2-3, reversible and irreversible attachment, Flagella and Fimbriae permanently anchor the bacteria to the surface. During stage 4, biofilm maturation through cell division and an extracellular matrix composed primarily of polysaccharides holds the biofilm together. During stage 5, dispersal, the biofilm matrix is partly broken down and returns to planktonic phase, some bacteria escape to colonize another surface [42].

Conclusion

Another line of research is devoted to understand the link between swarming patterns motility and antibiotics resistance. To improve upon the current situation, attempts are being made to grasp the complex kind of motility where certain species of Pseudomonas maintain high cell density circulating within the multilayered colony to minimize exposure to antibiotics. Exploring the molecular interactions may eventually lead to novel strategies to control Immune dysfunction, infections induced by this bacteria and answer to antibiotic therapy.

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