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Research article

Biofilm Formation by Environmental and Clinical Isolates of *Pseudomonas aeruginosa in vitro*

Ghada Mohammed Saleh[1*], Abass Mohammed[1]

ABSTRACT

Biofilm builds when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them to all kinds of material – such as metals, plastics, soil particles, medical implant materials, and most significantly, human or animal tissue. Most bacteria have a good ability to form biofilm. The studies that cover the compression of biofilm formation by environmental and clinical isolates of *Pseudomonas aeruginosa* are very scanty. The present study aimed to measure the biofilm formation by clinical and environmental isolates of *P. aeruginosa* to form biofilm onto polystyrene microtiter plates. Two clinical isolates of *P.* aeruginosa (CPa3 and CPa4) that were isolated from patients suffering from respiratory tract infection and two environmental isolates of *P. aeruginosa* (EPa1 and EP2) that were isolated from soil contaminated with oil wastes were included in the current study. The micro-dilution and spectrophotometric method was used to measure the biofilm formation of *P. aeruginosa* isolates to polystyrene microtiter plates. The optical density of the released crystal violates stain in the case of clinical isolates of *P. aeruginosa* was 0.28 ± 0.11, while this value was 0.13 ± 0.01 in the case of environmental isolates of *P. aeruginosa*. The present study provided the ability of clinical isolates to form biofilm higher than the ability of environmental isolates to form biofilm.

Keywords: Biofilm, Clinical isolates, Environmental isolates, *Pseudomonas aeruginosa*.

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1. INTRODUCTION

Pseudomonas aeruginosa is a common bacterium that can cause disease in animals, including humans and it can be found in water, soil, and waste ecosystems. It is an opportunistic human pathogen because it seldom infects healthy individuals. Instead, it often colonizes immunocompromised patients which is why, its 40-60% mortality rate. It uses a wide range of organic materials for food; in animals, the versatility enables the organism to infect damaged tissues or those with reduced immunity [1]. The symptoms of such infections are generalized inflammation and sepsis. If such colonization occurs in critical body organs, such as the lungs, the urinary tract, and the kidneys, the results can be fatal [2]. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is implicated in hot-tub rash [2].]. It is also able to decompose hydrocarbons and has been used to break down tar balls and oil from oil spills [3].

On 29 April 2013, scientists in Rensselaer Polytechnic Institute, funded by NASA, reported that, during spaceflight inside the International Space Station, *P. aeruginosa* bacteria seem to adapt to the microgravity and the biofilms formed during spaceflight exhibited a column-and-canopy structure that has "not been observed on Earth [4]. *P. aeruginosa* is an opportunistic human pathogen that infects immunocompro-mised individuals and people with cystic fibrosis. A major contributor to the pathogenesis of *P. aeruginosa* is its ability to secrete numerous virulent compounds and degradative enzymes [5]. *P. aeruginosa* is widespread in nature, inhabiting soil, water, plants, and animals (including humans). Identification of *P. aeruginosa* often includes identifying the production of both pyocyanin and fluorescein. Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C. *P. aeruginosa* has very simple nutritional requirements. *P. aeruginosa* is capable of growth in

* Correspondence: **Ghada Mohammed Saleh**. E. mail: ghada90m@gmail.com
Department of Biology, College Science, University of Baghdad, Baghdad, Iraq.
Full list of author information is available at the end of the article.

diesel and jet fuel, where it is known as a hydrocarbon-utilizing microorganism [3], causing microbial corrosion. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics [6]. Many appendages such as flagella and type IV pili play a central role in biofilm formation by *P. aeruginosa*. The wild-type biofilms were dynamic compositions with extensive motility, competition, and selection occurring during development. Bacterial migration prevented the formation of larger microcolonial structures in wild-type biofilms [7].

2. MATERIALS AND METHODS

2.1. Bacterial isolates

Clinical isolates of *P. aeruginosa* (CPa.1 and CPa.2) (isolated from infected wound) and environmental isolates of *P. aeruginosa* (EPa.3 and EPa.4) (isolated from freshwater) were procured from the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

2.2. Biofilm formation

The standard method of Zgair and Chhibber (2011) was followed in measuring the biofilm formation of different isolates of *P. aeruginosa* to polystyrene microtiter plates. Briefly, overnight cultures of *P. aeruginosa* strains grown in nutrient broth (containing 0.25% glucose) were diluted 1:200. The diluted cultures were transferred to wells of polystyrene microtiter plates (200 ml culture per well), and incubated at 37 °C for 24 h. The wells were then washed gently three times with 200 ml sterile phosphate buffer saline, air-dried, fixed at 60 ^{0}C for 30 min, and stained with 2% crystal violet for 5 min. Then, the plate was rinsed under running tap water, and air-dried, the crystal violet was resuspended in ethanol, and the OD 570 nm was determined [8].

2.3. Statistical analysis

Utilizing Origin 8 software, the statistical analysis and graphs were created. The information was presented as means SE. The differences were assessed using a one-way ANOVA and a student t-test. P values less than 0.05 were regarded as statistically significant.

3. RESULT

The present study was carried out to check the ability of clinical isolates and environmental isolates of *P. aeruginosa*. The comparison in terms of biofilm formation was made between clinical isolates and environmental isolates of *P. aeruginosa*. The environment isolates of *P. aeruginosa* (EPa 1 and EPa 2) should have low biofilm formation as compared with clinical isolates (CPa 3 and CPa 4) (Fig. 1).

Fig. 2 shows the mean of biofilm formation in terms of optical density of release crystal violet by clinical isolate was higher than Biofilm formation by environmental isolate significantly ($P< 0.005$).

The mean of optical density of crystal violet uptake in the case of clinical isolate was 0.28 ± 0.11, while the Biofilm formation in terms of crystal violet uptake by environmental isolate was 0.13 ± 0.13. The current study provides the ability of clinical isolate of *P. aeruginosa* was higher than biofilm formation by environmental isolate.

4. DISCUSSION

Bacteria grown in the biofilm have greatly enhanced tolerance to stress, antimicrobial agents, and host immunological defenses [9]. Biofilm-related infections pose serious health problems for hospital patients with indwelling medical devices [10]. Thus, developing effective anti-biofilm strategies for the treatment of biofilm-related infections is critical.

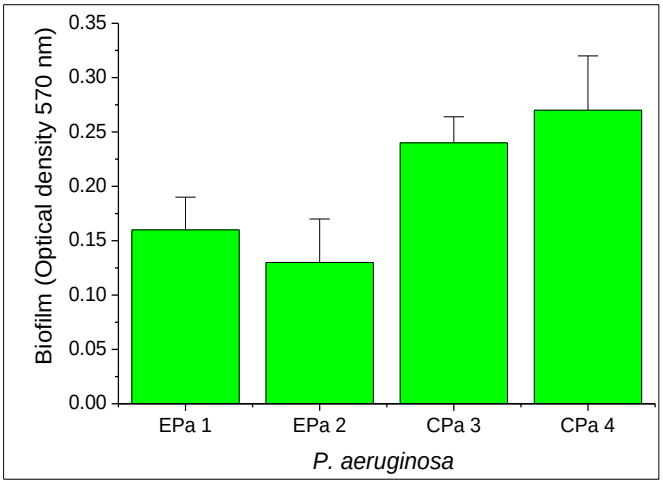

Fig. 1 Biofilm formation of two environmental and clinical isolates of *P. aeruginosa*. The biofilm formation was measured with optical density at 570 nm. The results are expressed in mean and standard deviation.

In the current study, we checked the ability of clinical and environmental isolates of *P. aeruginosa* to form biofilm. The present study proved that the ability of clinical isolates was higher than environmental isolates to form biofilm. This finding brings to our mind a conclusion that the clinical isolates may be more resistant to antibiotics than environmental isolates. That is why, the clinical isolates that were isolated from the respiratory tract of patients had higher pathogenicity than environmental isolates thus they can persist in the lungs of patients. Thus it can be concluded that the clinical isolates have a higher ability to form biofilm than environmental.

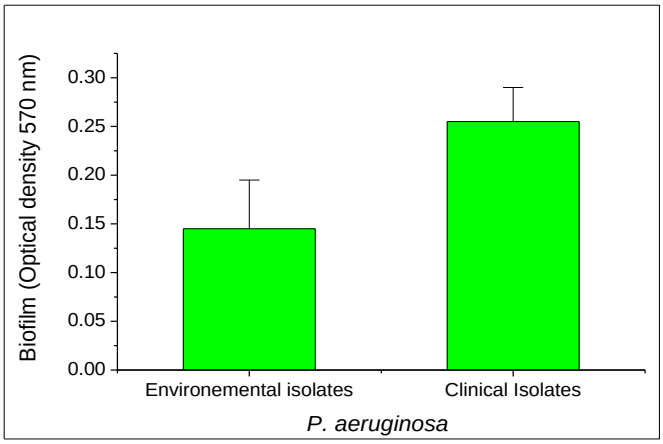

Fig. 2. Mean of biofilm formation of environmental and clinical isolates of *P. aeruginosa*. The biofilm formation was measured with optical density of 570 nm. Asterisk indicates of significant difference from the biofilm formation by environmental isolates. The results are expressed in mean and standard deviation.

5. CONCLUSION

The current study showed that Pseudomonas aeruginosa bacteria isolated from clinical samples (infected wound) have a higher ability to produce biofilm than Pseudomonas aeruginosa isolates isolated from environmental samples (freshwater).

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the Scientific Committee of the University of Baghdad, Baghdad, Iraq (No 174, 2019).

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Author affiliation

1. *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*