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Research article

Role of water taken from different environments on the ability of *Pseudomonas aeruginosa* to form biofilm on abiotic surfaces

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ABSTRACT

Pseudomonas aeruginosa readily binds to different kind of abiotic surfaces and form biofilm. The ability of the bacterial species to form biofilm onto polyvinyl chloride (PVC) is associated with several economic, health and environmental problems. The effect of kind of water on ability of this bacterium to form biofilm is scanty in literature. In present study, the ability of different environmental isolates of *P. aeruginosa* to form biofilm onto polystyrene microtiter plate was evaluated. Furthermore, the effect of waters that collected from different sources on biofilm formation of this bacterium onto PVC was studied. Spectrophotometric method was used to check the ability of bacteria to form biofilm and evaluated the role of waters onto ability of *P. aeruginosa* to form biofilm. The current study showed that all environmental isolates of *P. aeruginosa* had a good ability to form biofilm onto polystyrene microtiter plate. PAE1 showed the maximum ability of biofilm formation onto polystyrene microtiter plate. The water that collected from different places such as well water, river water, sewage water, distilled water, tap water and lake water showed negative effect ($P<0.05$) on the ability of PAE1 to form biofilm onto polystyrene microtiter plate and PVC as compared to normal saline. From present study, it can be concluded that all isolates of *P. aeruginosa* that isolated from soil had a good ability to biofilm formation. The waters that collected from different environmental areas affected negatively on ability of *P. aeruginosa* to form biofilm onto polystyrene and PVC.

Keywords: Biofilm, *Pseudomonas aeruginosa*, polystyrene, polyvinyl chloride.

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INTRODUCTION

Pseudomonas aeruginosa, a ubiquitous Gram-negative bacterium, is widespread in nature, inhabiting soil, water, plants, and animals [1,2]. It is an opportunistic pathogen with a high incidence of hospital infections that represents a threat to immune compromised patients [2].

Biofilm is an aggregation of microorganisms which are irreversibly associated with a surface and enclosed in a hydrated matrix [4]. The fundamental unit of a biofilm is a micro colony, wherein close contact of cells provides a perfect environment for the creation of nutrient gradients, genetic exchange and signaling [5,6]. Channels present in biofilm favor exchange of water, bacterial waste, nutrients, enzymes, metabolites and oxygen. Biofilm-related infections may be caused by a single monopolized species or a mixture of species. The biofilm on device is triggered by bacteria present on the skin of the host, cross-contamination of healthcare workers, tap water to which entry ports are exposed, or other sources within the local environment [7]. The occurrence of a biofilm on a medical device may also hamper the function of the device itself [8]. Increasing use of medical devices in healthcare systems is always associated with a definitive risk of bacterial infections [9].

Both clinical and environmental isolates of *P. aeruginosa* have a good ability to adhere to biotic and abiotic surfaces [6]. Both kind of isolates characterized by biofilm formation on different kinds of abiotic surfaces [10,11]. This behavior provides the priority of *P. aeruginosa* to outbreak in different environmental areas [6]. The formation of biofilm by *P. aeruginosa* can prolong the disease status in cystic fibrosis patients and favours the growth of diverse microorganisms leading to secondary infections [12]. The production of extracellular polymeric substance (EPS) serves as a backbone for biofilm establishment and plays a vital role in initial attachment, cell-cell interactions, tolerance and exchange of genetic materials [12].It is well known that bacterial appendixes such as flagella and pili play the central role in bacterial adhesion and biofilm formation [13,14]. Similarly, these appendixes are responsible for *P. aeruginosa* adhesion and biofilm formation [15,16].

Several study showed the ability of *P. aeruginosa* to adhere to different surfaces such as stainless steel polyvinyl chloride (PVC), polyurethane (PU) and silicones latex [17]. But there is no study showed the effect of water that obtained from different sources on ability of *P. aeruginosa* to adhere to polystyrene and polyvinyl chloride (PVC). The present study aimed to demonstrate the ability of different environmental isolates of *P. aeruginosa* to adhere and biofilm formation onto polystyrene microtiter plate and polyvinyl chloride (PVC). Moreover the current study aimed to check the effect of water that collected from different sources on the ability of *P. aeruginosa* to form biofilm on polystyrene microtiter plate and polyvinyl chloride (PVC).

MATERIALS AND METHODS

Bacterial isolates

P. aeruginosa isolates (PAE1, PAE2, PAE3, PAE4 and PAE5) were used in this study. The isolates were isolated from soil of different areas in Baghdad, Iraq. The isolates were diagnosed and identified in department of Biology, college of science, University of Baghdad, Baghdad, Iraq. Bacterial isolates were preserved by lyophilization and were routinely cultured at 37^{o}C on Luria-Bertani agar plates. Subcultures were made every two weeks [18].

Biofilm formation

Overnight cultures of *P. aeruginosa* isolates (PAE1, PAE2, PAE3, PAE4 and PAE5) in 3 ml of Tryptose soy broth (TSB) (Himedia) were washed three times with fresh TSB, and bacterial count adjusted to 10^7 c.f.u/ml. 200 µl of standardized inoculums were added to the wells of sterile flat-bottom polystyrene tissue culture plates, and incubated at 37°C for 24 h in a closed and humidified plastic container. The medium was then discarded, and non adherent cells were removed by washing three times with sterile PBS (0.1 M, pH 7.2). Spectrophotometric method with minor modifications was used to quantitation of biofilm. Slime and adherent organisms were fixed by incubating them for 30 min at 60°C and then stained with crystal violet (0.4%) for 5 min. After thorough washing with water to remove excess stain, the plates were dried for 30 min at 37°C. The extent of biofilm was determined by measuring the absorbance of stained adherent film upon treatment with acetone:ethanol (30 : 70) at a wavelength of 492 nm [19-21].

Similar method was done for the bacterial isolate that gave the maximum biofilm formation in presence of 50 µl of different kind of water (Well water, river water, sewage water, distilled water, tap water, lake water, normal saline).

In another experiment 1 cm 2 of polyvinyl chloride was incubated with standard inoculum (mentioned above) of *P. aeruginosa* that formed the maximum biofilm for 24 h at 37 ^{o}C. After that, the pieces of PVC were washed three times with PBS (0.1 M, pH 7.2). The pieces were submerged into crystal violet (0.4%) for 5 min. After thorough washing with water to remove excess stain, the pieces were dried for 30 min at 37°C. The extent of biofilm was determined by measuring the absorbance of stained adherent film upon treatment with 3 ml of acetone:ethanol (30 : 70) at a wavelength of 492 nm.

Statistical analysis

All the experiments were carried out in triplicate and all values have been taken as mean value and standard deviation calculated. The differences between test and control were analyzed by using Student's t test by employing Origin 8.0 version Software. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Fig. 1 showed the ability of different isolates of *P. aeruginosa* to adhere to polystyrene microtiter plate. *P. aeruginosa* (PAE1) showed the maximum ability to adhere to polystyrene microtiter plate followed by PAE5. *P. aeruginosa* (PAE4) showed the minimum ability of bacterial isolate to adhere to polystyrene microtiter plate. From this results the isolate PAE1 was used in further experiment to evaluate the effect of kind of water to stimulate *P. aeruginosa* to form biofilm on polystyrene and PVC.

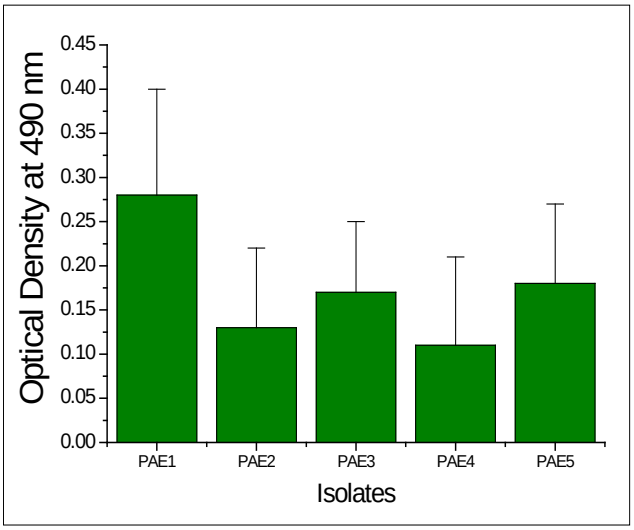

Fig.1. Biofilm formation of different isolates of *P. aeruginosa* that isolated from different area of soil on polystyrene microtiter pale after 24 h of incubation. The maximum biofilm formation was found in PAE1 while the minimum was found in case of PAE4.

Effect of kind of water on biofilm formation

In present study, the effect of water that collected from different sources on the ability of *P. aeruginosa* (PAE1) to form biofilm was evaluated. The highest biofilm formation was found when the bacterial isolated incubated with 50 µl of normal saline. The lowest biofilm formation was observed when this bacterial isolated incubated with 50 µl of distilled water (**Fig. 2**). Similar result obtained when the effect of different water on biofilm formation onto the PVC was evaluated (**Fig. 3**).

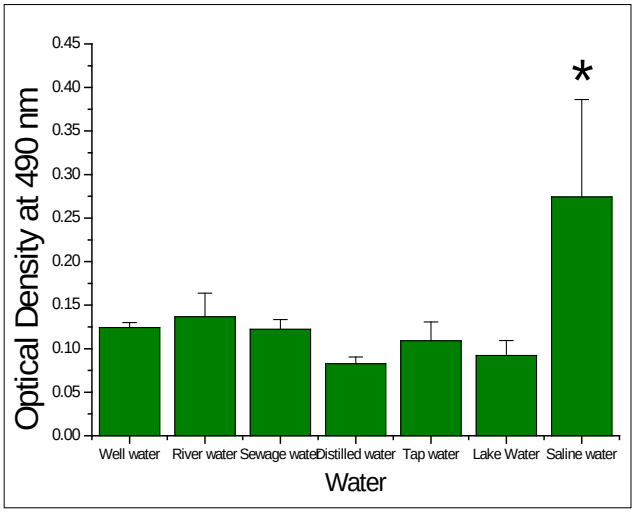

Fig.2. The effect of kind of water (well water, river water, sewage water, distilled water, tap water, lake water and normal saline) on the biofilm formation of *P. aeruginosa* (PAE1) onto polystyrene microtiter plate. Asterisk indicates the significant difference from other kinds of water.

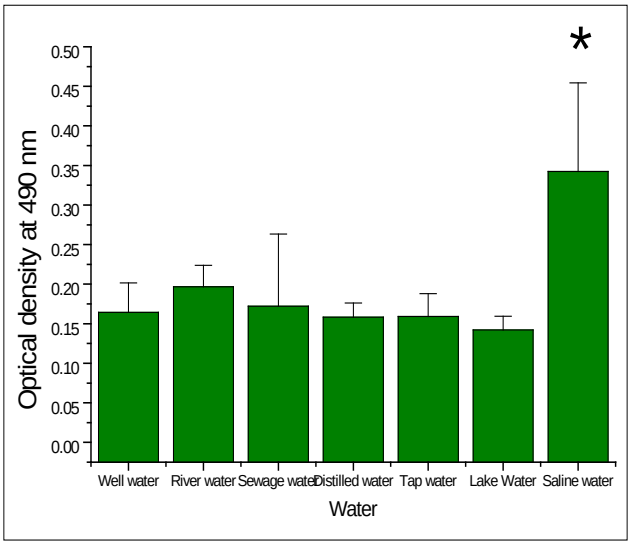

Fig.3. The role of kind of water (well water, river water, sewage water, distilled water, tap water, lake water and normal saline) in biofilm formation of *P. aeruginosa* (PAE1) onto polyvinyl chloride (PVC). Asterisk indicates the significant difference from other kinds of water.

DISCUSSION

Colonization of abiotic surfaces such as medical devices and plastic tubes by *P. aeruginosa* is likely to depend on the ability to adhere to solid surfaces [22], which then allows micro-organisms to form biofilm in which they are protected from harmful environmental factor [23]. Most studies of microbial factors involved in adherence to inert surfaces have focused on physical forces such as surface hydrophobicity and

role of appendix to help bacteria to adhere to biotic or abiotic surfaces [14, 15, 20, 23,24].

In present study we focused on the ability of different isolates of *P. aeruginosa* to adhere to polystyrene microtiter plates and PVC. Moreover the effect of different kinds of water that collected from different environmental areas on the ability of *P. aeruginosa* to adhere to polystyrene microtiter plates and PVC. *P. aeruginosa* (PAE1) showed the highest ability of adhesion. Furthermore, all waters that collected from different areas and distilled water affected negatively on ability of *P. aeruginosa* to adhere to abiotic surfaces as compared with normal saline.

Several previous study highlighted on ability of *P. aeruginosa* to adhere to polystyrene and PVC [17, 25]. The main force that was found in the adhesion area between *P. aeruginosa* and PVC is hydrophobicity [17]. The electronmicrographs confirmed that synthesis of a slime-like material was involved in adherence of non-mucoid strains. This slime-like material is known to consist of three chromatographic fractions, one of them being uronic acid, as in the slime produced by mucoid strains. Adherence values were also influenced by the nature of the material especially with non-mucoid strains. These differences may justify in-vitro studies of microorganism- material interactions when evaluating the application of new materials for clinical use [17]. The literature that covers the role of kind of water and source of water on the ability of bacteria to adhere to abiotic surfaces is very scanty, that is why; our study represent the pioneer study deals with the effect of kind of water on the ability of *P. aeruginosa* to adhere to abiotic surfaces. Thus in our laboratory several studies that deal with the effect of kind of water on ability of bacteria to adhere on biotic and abiotic surfaces.

The results of this study proved that *P. aeruginosa* isolates have a good ability to adhere to polystyrene and PVC. Furthermore the normal saline is the best water for adhesion of *P. aeruginosa* to polystyrene and PVC.

Conflict of interest

The authors declare that they have no conflict of interests.

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