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Research article

Effect of Soil Extracts on Ability of Clinical Isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to Produce Biofilm *in vitro*

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ABSTRACT

In present study the effect of soil extracts of different types of soil on ability of two clinical isolates, *Pseudomonas aeruginosa* and *Staphylococcus aureus* to form biofilm. The extract of soil was done by using sterile phosphate buffer saline and analyzed by Fourier Transform Infrared Spectroscopic (FTIR). Spectrophotometric method was used to check ability of the studied isolated bacteria to form biofilm on polystyrene microtiter plates. The data of FTIR showed very little difference was observed among extracts of three types of soil (soil contaminated with hydrocarbons; garden soil collected from gardens of al-jadrea, Baghdad and containers soil), but the highest difference was observed in the extract that obtained from peat moss clay soil. The results of current study showed that the extracts of soil contaminated with hydrocarbons and garden soil increased the biofilm that form by *P. aeruginosa* ($P<0.05$). While, the highest level of biofilm formation by *S. aureus* was observed after adding the extract of container soil ($P <0.05$). It can be concluded from present study that the soil extracts can enhance bacteria to form biofilm in vitro but that was dependent on the kind of soil.

Keywords: Biofilm**,** *Pseudomonas aeruginosa*, Soil extracts, *Staphylococcus aureus*

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INTRODUCTION

Over 5000 *antibiotics* have been identified from the culture of bacteria fungi, but only hundred antibiotics used to treat human, animal and plant disease [1]. Soil is the largest source of microorganisms [2] that can be used to produce antibiotics and these antibiotics can be check their antimicrobial activity against several infected bacteria to select the best. The highest number of antibiotics was produced from *Streptomycetes* that find normally in soil [3]. There are thousands of antibiotics which are produced by *Actinobacteria,* and fungi (*Penicillium* spp) and others by other bacteria [3].

Streptomycetes spp. Because the countenance modification in the genetic of bacteria, thousnasd of resistant isolates of bacteria isolated every year [4]. That is why; it is becoming necessary to find newer antibiotics to which the microorganism is sensitive [4]. *Pseudomonas aeruginosa*, a gram-negative bacillus that is widely distributed in nature, is commonly considered transitional opportunistic pathogens (Quinn *et al.*, 1994). It is well known resistant to wide range of antibiotics [4,5]. Several study focused on the ability of *P. aeruginosa* to produce biofilm and the role of biofilm formation in antibiotic

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resistance. Previous study showed that the role of biofilm on pathogenesis of *P. aeruginosa* [6].

Biofilm *formation* is an important aspect of many bacteria especially for *Staphylococcus aureus*. That creates complication of *S. aureus* infections including endocarditis, osteomyelitis, and infections of implanted medical devices. This finding not associate with these complication but also make the bacteria resistant to wide spectrum of antibiotic. Indeed, the presence of a biofilm limits the efficacy of antimicrobial therapy to the point that surgical intervention is often required to remove infected tissues and/or implanted devices [7,8]. That is why; biofilm formation by *S. aureus* creates big challenges for physicians to treat the patients infected with S. aureus. This study has focused on the role of extract of soli collected from different areas on the ability of *P. aeruginosa* and *S. aureus* to form biofilm onto polystyrene microtiter plates.

MATERIALS and METHODS

Bacterial isolates

Clinical isolates of *P. aeruginosa* and *S. aureus* were used in current study. The isolates were isolated from wound infection, diagnosed and identified in Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. Bacteria were routinely cultured at 37ºC on nutrient agar plates. Subcultures were made every two weeks.

Soil extract preparation

One gram of soil samples (peat moss clay soil; soil contaminated with hydrocarbons; garden soil collected from gardens of al-jadrea, Baghdad; containers soil) and was added to 9 ml of sterile distilled water and shacked to homogenize and then centrifuged (15,000 g, for 15 min), the suspension was filtered (0.2µ) by Millipore filter. Serial dilutions were prepared with phosphate buffer saline (PBS, 0.1 m, 7.2 pH).

Biofilm formation

Overnight cultures of *P. aeruginosa* and *S. aureus* in 5 ml of Tryptose soy broth (TSB) (Himedia) were washed three times with PBS (0.1 M; 7.2 pH) and once with fresh TSB, bacterial number was adjusted (standard inoculm) to be 10^7 c.f.u/ml (100 µl) and added to each well of sterile flat-bottom polystyrene tissue culture plates, and then 100 µl of sterile fresh TSB was added to the wells. The plates were incubated at 37°C for overnight in a closed and humidified container. The medium was discarded, and non adherent cells were removed by washing three times with sterile PBS (0.1 M, pH 7.2). Slime and adherent bacteria were fixed by incubating for 30 min at 60°C and then stained with Hucker crystal violet (0.4%) for five minutes. The excess stain was removed by washing with distilled water; the plate was 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 490 nm [9].

Effect of soil extract on biofilm formation

Similar method was followed to check the effect of different soil extract on biofilm formation by *P. aeruginosa and S. aureus*. In this experiment, the different dilutions of different soil extract were mixed with TSB medium (final volume 100 *µl)* and mixed with standard inoculums of *P. aeruginosa* and *S. aureus* (10^7 c.f.u/ml). Similar procedure that mention above was followed to check the level of biofilm formation. The results of biofilm formation in presence of soil extract were compared with results of biofilm formation without soil extract to check the effect of soil extract on biofilm formation.

Fourier Transform Infrared Spectroscopic (FTIR) analysis

In Present study, FTIR technique was used to detect the degradation of components of different soil extracts that collected from different area. FTIR (Bruker, Germany) device was calibrated by the range of transmittance percents on the "Y" axes and the wave length (600-4000 cm) on the "x" axes [10].

Statistical Analysis

All values were taken as mean ± standard deviation (sd). The difference was detected by using Student's t test employing Origin 6.0 version Software. A value of $P<0.05$ was considered to be statistically significant.

RESULT

Fig 1 shows FTIR analysis to extracts of different soils collected from different places of Baghdad, Iraq. There are so many differences in terms of position of bonds and types but the major difference was observed in peat-moss. While the differences among the left three types of soil was miner.

In present study, ability of two species of bacteria *P. aeruginosa* and *S. aureus* to form biofilm and the effect of extracts of soils were on the ability of these bacterial isolates to form biofilm. It was found that two types of soil extracts increased the ability of *P. aeruginosa* to form biofilm significantly ($P <0.05$) more than control and these extracts were extracted from soil contaminated with hydrocarbons and soil collected from garden. While, the extract of soil that collected from containers coil increased the ability of bacteria to form biofilm but that was not significant. It was observed that the peat-moss did not affect on the ability of *P. aeruginosa* to form biofilm (**Fig. 2**). Fig. 3 shows that only extract of soil collected from containers soil increased ability of *S. aureus* to form biofilm significantly ($P <0.05$).

Discussion

Several study focused on the ability of bacteria to form biofilm [11,12] the most of bacteria on the earth can produce the biofilm [13]. Previous studies studied the ability of bacteria such as P. aeruginosa and S. aureus to adhere on biotic and abiotic surfaces [11,12]. This phenomenon is highly important to survive bacteria in the environment and in the host in case of pathogenic bacteria [14] as this mechanism help bacteria to adhere and invasive the host barriers to get the nutrients and to find a good habitat [15]. The most study found that the antimicrobial agents reduce the ability of bacteria to form biofilm [16].

In present study, the ability of clinical isolates of *P. aeruginosa* and *S. aureus* to form biofilm in presence of different extracts of soil. The results were highly different in case of each bacterial species. The extracts of soil contaminated with hydrocarbons, garden soil enhanced *P. aeruginosa* to form biofilm higher than control, while the extract of container soil enhanced S. aureus to from biofilm higher than control.

Before doing the experiment, it was thought that the soil will reduce the ability of bacteria to form biofilm, because the soil

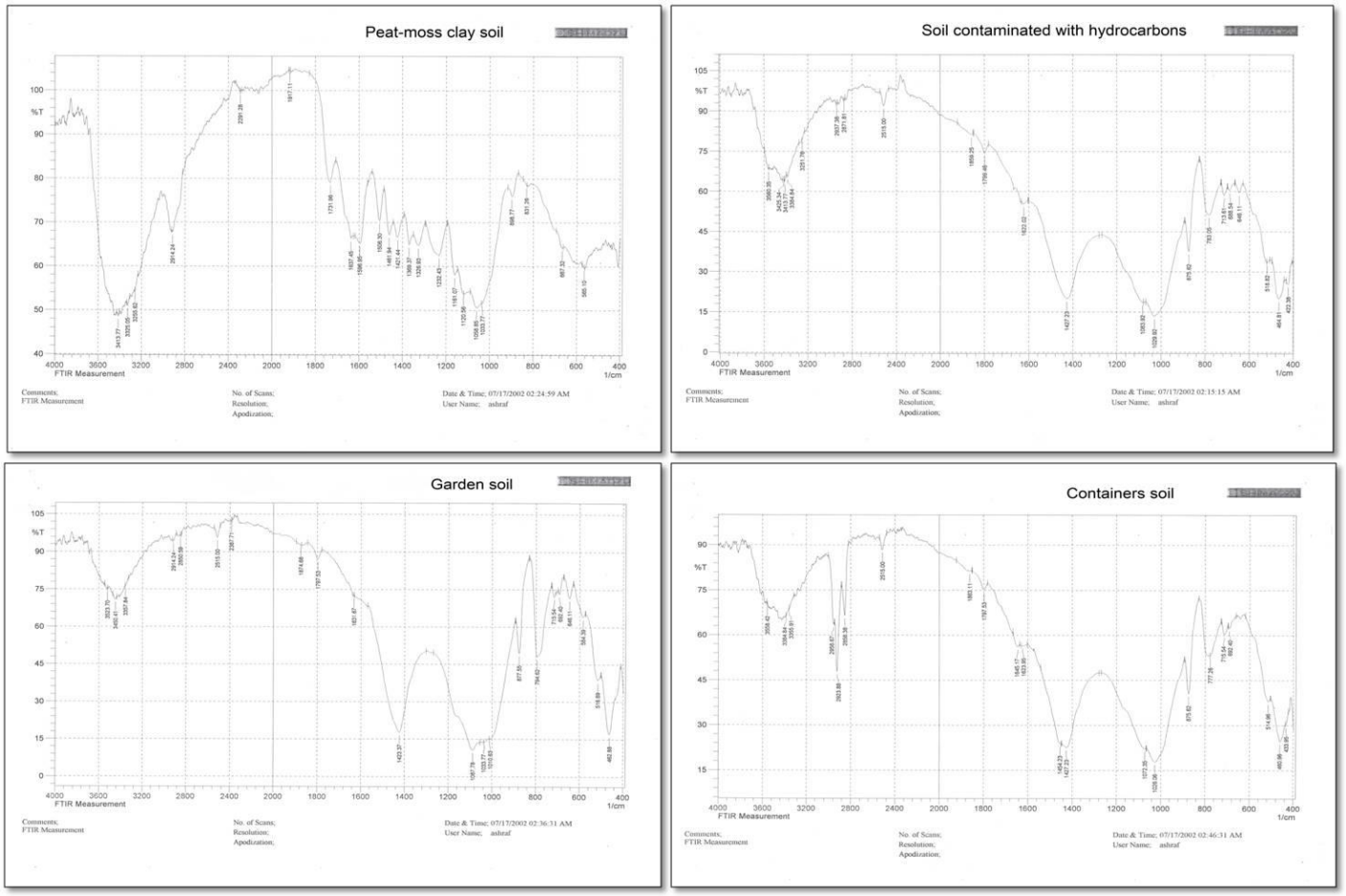

Fig 1. FTIR of extracts of different soil that collected from different places of Baghdad city.

composed of different kinds of actinomycetes and other microorganisms that produce antibacterial agents [17]. But the results yielded were not matching with what I thought before starting the experiments. Because some soil extracts enhanced the bacteria to form biofilm but no one of spil extracts reduced the biofilm formation by any one of both species of bacteria.

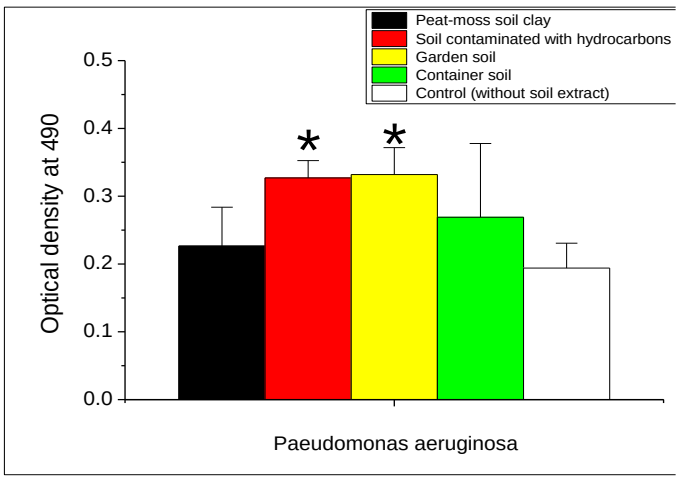

Fig 2. Effect of peat-moss soil, soil contaminated with hydrocarbons, garden soil and container soil on biofilm formation by *P. aeruginosa*. *P<0.05 versus control group.

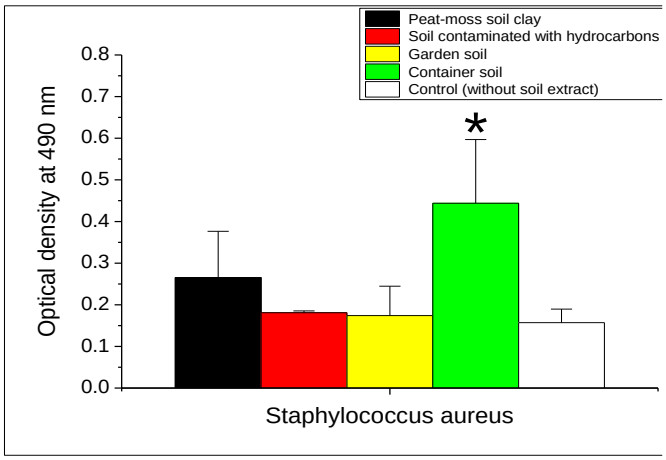

Fig 3. Effect of peat-moss soil, soil contaminated with hydrocarbons, garden soil and container soil on biofilm formation by S. aureus. *P<0.05 versus control group

The catch results can be explain by the positive effect of soil extract on biofilm formation, as the soil composed of several materials important in biofilm formation such as sugars and minerals. Ramli et al (2012) fond that the role of several component such as glucose on biofilm formation [18]. Previous study support that there several minerals elements play an important role in biofilm formation in vitro [19]. From previous

study, it can be concluded that the soil extracts have several elements that can enhance of biofilm formation in vitro.

Conflict of interest

The author declares that she has no conflict of interests.

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