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Biofilm Formation by the Interaction of Candida Albicans and Pseudomonas Aeruginosa

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Abstract

Introduction. A biofilm is an aggregation of many cells of microorganisms on biotic or abiotic surfaces to form a community that enables microorganisms to survive for a long time and challenge abnormal environmental conditions.
Purpose. To evaluation of polymicrobial biofilms formation by more than one species from fungi and bacteria via determine the biofilms formation by the interaction of Candida albicans and Pseudomonas aeruginosa.
Materials and methods. All C. albicans and P. aeruginosa were isolated from various clinical samples including urine, sputum, diabetic feet, burns, and oral swabs. Biofilm formation assay by Crystal violet was performed to evaluate the ability of both P. aeruginosa and C. albicans isolates.
Results. High percent of P. aeruginosa isolates (53.33%) were not-producer of biofilm followed by (33.33%) of P. aeruginosa isolates were recorded as a weak biofilm producer and 6.67% of P. aeruginosa isolates were classified as moderate and strong biofilm producers respectively. High percent of C. albicans isolates (46.67%) was moderate-biofilm producers, and low percent (10%) was reported as non-biofilm producers. However, 43.33% of C. albicans isolates were non-biofilm producers. A Strong-biofilm producer was no registered among C. albicans isolates. In contrast to monospecies biofilms, the polymicrobial biofilm formation test indicated that the co-growth of both P. aeruginosa and C. albicans showed enable all isolates to become as a strong-biofilm producers.
Conclusion. The ability of a pathogen to form biofilms doubles if another organism present in the same environment contributes to the formation of biofilms that may lead to increase the virulence of these pathogens.
Keywords: Pseudomonas aeruginosa, Candida albicans, biofilms, polymicrobial biofilms

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Образование биопленок при взаимодействии Candida albicans и Pseudomonas aeruginosa

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Резюме

Введение. Биопленка – конгломерат (массив) клеток микроорганизмов на биотических или абиотических поверхностях, образующих устойчивую ассоциацию, благодаря которой микроорганизмы могут длительное время существовать и противостоять аномальным условиям окружающей среды.
Цель. Оценка способности к формированию полимикробных биопленок несколькими видами грибов и бактерий на примере пленкообразующей способности Candida albicans и Pseudomonas aeruginosa при их взаимодействии.
Материалы и методы. Все изоляты C. albicans и P. aeruginosa были получены из различных клинических образцов, включая мочу, мокроту, диабетические стопы, ожоги и мазки из полости рта. Оценку пленкообразующей способности изолятов P. aeruginosa и C. albicans в сформированных биопленках проводили методом окрашивания генцианом фиолетовым.
Результаты. Большинство изолятов P. aeruginosa (53,33%) не являлись продуцентами биопленки, 33,33% изолятов P. aeruginosa характеризовались низкой способностью к продуцированию биопленки, а 6,67% изолятов P. aeruginosa были классифицированы как продуценты биопленки умеренной и выраженной степени. Высокий процент изолятов C. albicans (46,67%) имел умеренную биопленкообразующую способность, в то время как незначительный процент (10%) был отнесен к небиопленкообразующим. При этом 43,33% изолятов C. albicans не являлись продуцентами биопленки. В группе изолятов C. albicans не было выявлено выраженных продуцентов биопленок. В отличие от моновидовых биопленок, тест на формирование полимикробной биопленки показал, что совместное культивирование P. aeruginosa и C. albicans способствовало значительному усилению способности всех изолятов к биопленкообразованию.
Заключение. Пленкообразующая активность патогена удваивается при наличии в этой же среде другого микроорганизма, участвующего в формировании биопленки, что в результате может привести к усилению вирулентности обоих патогенов.
Ключевые слова: Pseudomonas aeruginosa, Candida albicans, биопленки, полимикробные биопленки

■ INTRODUCTION

The interaction between bacteria and fungi was discovered by Alexander Fleming as a result of a coincidence when an agar plate containing staphylococci was contaminated by an unknown mold that was later named Penicillium spp. This discovery and extraction of the antibiotic penicillin, which has significant antibacterial effects, led to numerous important discoveries and discoveries since that time [1]. Nature offers numerous instances of prokaryote-eukaryote interaction [2, 3]. These interactions can result in a variety of results and may benefit one side (for example, through the other's animosity) or both parties (mutualism). Fungus and bacteria commonly interact with one another in numerous habitats, including the human body [4–6]. These interactions have been studied using culture-dependent or culture-independent approaches, particularly in the context of the human host. The latter, however, has the advantage of being able to detect microbial invaders at low cell densities that may be important in infection. The gastrointestinal system is one of the biological systems where polymicrobial interaction happens, which is home to a diversity of microbial species [7]. Additionally, polymicrobial infections are common in the lungs, urinary system, and wounds, where they can impair the healing process. This is particularly true for people with cystic fibrosis, whose weakened immune makes them more vulnerable to infection (CF). In a polymicrobial infection, altered host immunity, altered metabolism, and altered expression of virulence factors can all be caused by the competition brought on by close proximity and nutritional limitations [7, 8]. This interaction between fungus and bacteria can greatly enhance morbidity and death when compared to an infection brought on by a single species [9, 10]. P. aeruginosa is a gram-negative bacteria with a single mobilization flagellum that measures (0.5–0.8 m) in diameter and (1.5–3 m) in length, was identified in 1882 by the French bacteriologist and chemist Carle Gessard. By exhibiting a positive oxidase reaction, P. aeruginosa sets itself apart from the majority of gram-negative bacteria. Furthermore, P. aeruginosa is not able to continuously digest lactose [11]. In nature, it is typically observed as plankton swimming through water or as a biofilm, which is a microbial assemblage with a common phenotypic and chemical composition. P. aeruginosa is exceptional in that it can survive and thrive in a variety of temperature and nutrient environments. Previous studies have demonstrated that P. aeruginosa may flourish in distilled water, giving it an advantage in adapting to different environments [12]. A single polar flagellum is used for movement by the majority of P. aeruginosa strains. The optimal temperature P. aeruginosa can grow at a temperature of 37 °C, although its maximum growth temperature is 42 °C [13]. Infections in the bloodstream, urinary tract, burn, wounds, and surgical sites are only a few of the ailments that can be contracted by people after contracting the common opportunistic bacterium P. aeruginosa [14]. In immunocompromised persons, due to its incredible ability to resist antibiotics, repeated infections are the main cause of morbidity and mortality, which frequently makes it difficult to prevent and cure its infections [15, 16]. The polymorphic fungus C. albicans can take on a variety of morphological forms depending on its environment, including yeast, hyphae, and pseudohyphae [17]. This yeast normally causes no harm and is a common component of the healthy microbiota in the female vaginal canal, gastrointestinal tract, and epidermis [18]. C. albicans, however, can prey on those with impaired immune systems and cause a number of opportunistic infections [19]. Three crucial characteristics that C. albicans and P. aeruginosa have in common

for sickness are the capacity to switch the release of virulence proteins, the creation of biofilms, and the interaction between the bacterium and filamentous development by C. albicans and vice versa [20].

■ PURPOSE OF THE STUDY

To evaluation of polymicrobial biofilms formation by more than one species from fungi and bacteria via determine the biofilms formation by the interaction of C. albicans and P. aeruginosa.

■ MATERIALS AND METHODS

Samples collection

A total of 180 samples were collected from different sources such as; urine, sputum, diabetic feet, burns, and oral swabs from patients attendance at hospitals and private clinics in Thi-Qar proving. The samples were immediately cultured on bacteriological and mycological media and incubated at 37 °C for 24–72 hrs.

Biofilm formation assay by Crystal violet

A 96-well microtiter plates with Brain heart infusion broth (HiMedia) were employed to detect the development of biofilms. Semi-quantitative evaluations of biofilm development were made according to Hemati et al. (2016). Each P. aeruginosa isolate was grown in triplicate wells of 96-well plates containing Brain heart infusion broth medium with 1g of glucose at 37 °C for 24 hrs. The plates were vigorously washed three times with regular saline to get rid of any free-floating bacteria. After that, 15 minutes of staining at room temperature with 100 ml of 0.1 percent (w/v) crystal violet solution. The crystal violet was then removed from the wells with 150 µl of 95% ethanol and acetone [8:2 (v/v)]. Using a microplate reader, the plates were measured at 630 nm for bacteria and at 570 for yeast. The results were classified as following: non-biofilm, weak biofilm, moderate biofilm, and strong biofilm according to optical density of microorganisms' cells. The results were interpreted as follows: if OD ≤ODc had no biofilm development, ODc < OD ≤2 ×ODc had weak biofilm, 2 ×ODc < OD ≤4 ×ODc had moderate biofilm and OD > 4 × ODc had high biofilm [21].

Co-culture growth by P. aeruginosa and C. albicans

P. aeruginosa was inoculated onto preformed culture of C. albicans by using a spotting 10 µl of overnight Pseudomonas growth, including a 7×10^7 CFU/µl onto the surface of the plate-grown Candida culture. C. albicans were prepared by spreading 350 µl of Brain heart infusion broth grown overnight onto the plate contain Sabouraud agar and incubated at 30 °C for 48 hrs. After incubation period, spotting 10 µl of overnight Pseudomonas growth, including a 7×10^7 CFU/µl were spotted onto the surface of the plate-grown Candida culture. The co-cultures plates were incubated at 37 °C for an additional 24 to 96 hrs [22].

Statistical Analysis

This data was statistically analysis by SPSS version 26, based in using both descriptive and non-parametric chi-square at p. value <0.05.

■ RESULTS

Thirty isolates of both P. aeruginosa and C. albicans were randomly isolated and identified out of 180 samples. All P. aeruginosa and C. albicans isolates were evaluated to form biofilm. The ability of single and polymicrobial species (P. aeruginosa and C. albicans) biofilms formation were assessed. P. aeruginosa and C. albicans isolates were diverse in their ability to form biofilms as monospecies or multispecies in broth culture. The most of P. aeruginosa isolates (53.33%) did not form biofilms as a monospecies followed by group of isolates formed a weak biofilm (33.33%). However, four isolates were classified as Moderate and strong biofilm producers (Table 1).

When assessing the ability of C. albicans isolates to form a biofilm, no isolates showed the ability to form a strong biofilm. The highest percentage of isolates (46.67%) was recorded as moderate biofilm producers. Few isolates were classified as weak biofilm producers (10%). High percent (43.33%) of isolates was characterized with non-biofilm producers (Table 2). In contrast, the mixed growth of C. albicans and P. aeruginosa showed a higher proportion of Biofilm production in all isolates (Table 3), also higher biomass within mixed culture.

Table 1
Biofilm formation by P. aeruginosa

Optical Density of P. aeruginosa	Categories of Biofilm Production	No.	%
$OD \leq OD_C$	Non-Biofilm	16	53.33
$OD_C < OD \leq 2\times OD$	Weak-Biofilm	10	33.33
$2\times OD_C < OD \leq 4\times OD$	Moderate-Biofilm	2	6.67
$OD > 4\times OD_C$	Strong-Biofilm	2	6.67
Total		30	100

Table 2
Biofilm formation by C. albicans

Optical Density of Candida Spp.	Categories of Biofilm Production	No.	%
$OD \leq OD_C$	Non-Biofilm	13	43.33
$OD_C < OD \leq 2\times OD$	Weak-Biofilm	3	10.00
$2\times OD_C < OD \leq 4\times OD$	Moderate-Biofilm	14	46.67
$OD > 4\times OD_C$	Strong-Biofilm	0	0.00
Total		30	100

Table 3
Biofilm formation by mixed growth (C. albicans and P. aeruginosa)

Optical Density of Mix	Biofilm Production	No.	%
$OD \leq OD_C$	Non-Biofilm	0	0.0
$OD_C < OD \leq 2\times OD$	Weak-Biofilm	0	0.0
$2\times OD_C < OD \leq 4\times OD$	Moderate-Biofilm	0	0.0
$OD > 4\times OD_C$	Strong-Biofilm	10	100

In order to support the above result, the growth of both organisms was tested on the solid culture medium to clarify what happened in the liquid culture medium. It was found that the growth levels for P. aeruginosa were either increased or decreased according to C. albicans isolates (Figure 1 and 2), such as P. aeruginosa isolates no. 17 had clearly increased its growth with C. albicans isolates (no. 30, 26, 18, 14), while its growth was decreased with C. albicans isolate no. 16, as well as other isolates (Figure 1 and 2).

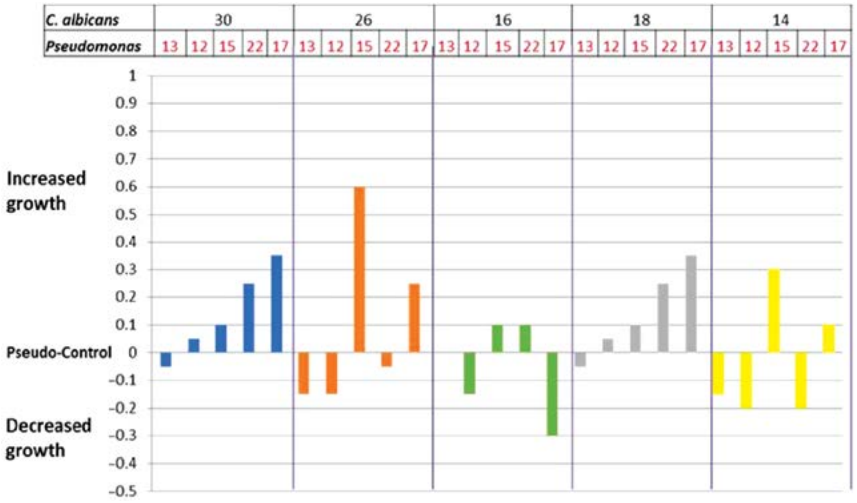

Fig. 1. Co-culture growth by P. aeruginosa and C. albicans Each C. albicans isolate was grown with five P. aeruginosa isolates. Blue color: C. alb isolate No. 30, Brown color: C. alb isolate No. 26, Green color: C. alb isolate No. 16, Grey color: C. alb isolate No. 18, Yellow color: C. alb isolate No. 14

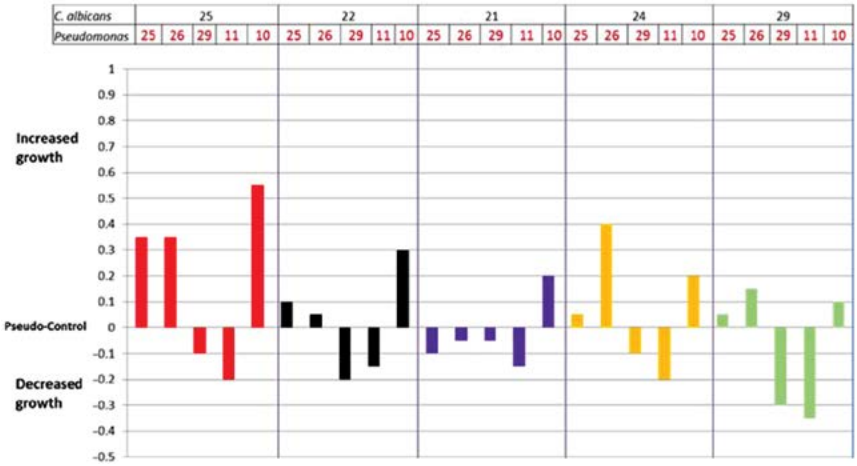

Fig. 2. Co-culture growth by P. aeruginosa and C. albicans Each C. albicans isolate was grown with five P. aeruginosa isolates. Red color: C. alb isolate No. 25, Black color: C. alb isolate No. 22, Purble color: C. alb isolate No. 21, Orange color: C. alb isolate No. 24, Light green color: C. alb isolate No. 29

■ DISCUSSION

Pathogenic agents that have the ability to form biofilm causes chronic and recurrent infections due to difficult treatment and their antibiotic resistance as well as biofilm enable the pathogen escape from immune system [23, 24]. The current study isolated both P. aeruginosa and C. albicans from clinical sources and 30 isolates from both above microorganisms were randomly selected for biofilm formation experiments. Previous studies confirmed infection by synergistic of P. aeruginosa and C. albicans [25]. C. albicans may help P. aeruginosa to adhere and began to form biofilm. Which consider a virulence factor in many pathogens [26]. However, other studies reported the antagonism between P. aeruginosa and C. albicans [27].

In this study 30 isolates of each P. aeruginosa and C. albicans were tested using the microtiter culture plate method (TCP) assay with crystal violet dye, which previously described as an easy and quick way to measure the production of biofilms [28]. The main result of this study was both P. aeruginosa and C. albicans could be considered as biofilm producers in infections that occur via a monospecies or multispecies pathogens. This finding was consistent with the findings of Halstead et al. (2015) [29], who noted that P. aeruginosa isolate exhibited variable biofilm production as well as C. albicans [30].

The surprising result was that both organisms significantly increased their ability to produce biofilms when grown mixed. This was in agreement with previous studies. An experiment on agar culture was made to confirm the biofilm production, where it was found that the growth levels increased and decreased according to isolate type for both C. albicans and P. aeruginosa. The complexity of interactions may differ according to various genotypes [22], or P. aeruginosa interacts with fungus depending on its morphology [31].

An increase in bacterial growth may indicate that the bacteria are benefiting from the presence of yeast in two cases: first is that P. aeruginosa compete the yeast for nutrition. The interactions may be affected by the environment since different species are present in the same place [32]. Second is that yeast may help bacteria in adhesion process throughout hyphal formation. The adhesion consider the first step to form biofilms [33]. Another study observed that C. albicans made as a base layer for P. aeruginosa when they build their own biofilms [34]. In other words, the interaction that occurs between bacterial and fungal isolates may be either antagonistic or synergistic, depending on the compatibility between different isolates [35].

■ CONCLUSION

In polymicrobial infections, bacteria frequently engage in synergistic interactions that enhance pathogenesis. Both pathogens (P. aeruginosa and C. albicans) are able to form a biofilm, when they are a single. But biofilms formation increases when they are together. A relationship between P. aeruginosa and C. albicans occurred, regardless of whether this relationship is antagonistic or synergistic. This relationship may be affected by different strains. Thus increasing the pathogenicity and making it difficult to treat because it may be the complexity of biofilm structure in case of polymicrobial infection.

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