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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Evaluation of biofilm formation activity of standard microorganism strains

Standart mikroorganizma suşlarının biyofilm formasyon aktivitelerinin değerlendirilmesi

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ABSTRACT

Objective: Biofilm is a structure formed by a group of microorganisms. Bacteria in the biofilm lead to much more serious problems in medical and industrial terms when compared to their planktonic forms. In this sense, it is important to know about the biofilm activities of the microorganisms. The ability of certain microorganism strains to form biofilms was shown, and the importance of this subject was tried to be emphasized in this study.

Methods: Fifteen bacteria and two yeast standard strains were used in the study. Microtiter plate method was used in order to determine the biofilm production capacities of standard strains. Biofilm formations were assessed as "nonadherent =0,weakly adherent = I, moderately adherent = II and strongly adherent =III."

Results: Biofilm formation was observed in all of the 17 standard strains following the study carried out. Among standard microorganisms, *Pseudomonas aeruginosa, Staphylococcus aureus*, *Corynebacterium pseudotuberculosis* and *Neisseria sicca* created a strong biofilm.

Conclusion: It is known that biofilms formed by microorganisms lead to negative consequences in human health. Therefore it's important to study on understanding biofilm formations. We believe that the biofilm formation data of the standard microorganisms we provide in our study will contribute to the researchers to conduct researches on this subject and the literature related to the subject. *J Clin Exp Invest 2015; 6 (2): 135-139*

Key words: Biofilm, microtiter plate method, antimicrobial resistance, standard strain

ÖZET

Amaç: Biyofilm mikroorganizmaların meydana getirdikleri bir yapıdır. Biyofilm içerisindeki bakteriler, planktonik formları ile kıyaslandığında tıbbi ve endüstriyel açıdan çok daha önemli sorunlara neden olmaktadır. Bu bakımdan mikroorganizmaların biyofilm aktivitelerinin bilinmesi önemlidir. Bu çalışmada bazı mikroorganizma suşlarının biyofilm oluşturabilme yetenekleri gösterilerek bu konunun önemi vurgulanmaya çalışılmıştır.

Yöntemler: Çalışmada 15 adet bakteri ve iki adet maya standart suşu kullanılmıştır. Standart suşların biyofilm üretim kapasitelerinin belirlenmesi amacıyla mikrotitre plak yöntemi kullanılmıştır. Biyofilm formasyonları negatif kontrol değeri baz alınarak 'biyofilm oluşturmayan =0, zayıf biyofilm = I, orta dereceli biyofilm = II ve güçlü biyofilm =III olarak değerlendirilmiştir.

Bulgular: Yapılan çalışma sonrasında 17 adet standart suşun tamamında biyofilm oluşumu gözlenmiştir. Standart mikroorganizmalardan *Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium pseudotuberculosis* ve *Neisseria sicca* güçlü biyofilm oluşturmuşlardır.

Sonuç: Mikroorganizmaların oluşturdukları biyofilmlerin insan sağlığı açısından olumsuz sonuçlara yol açtığı bilinmektedir. Bu bakımdan biyofilm formasyonlarının anlaşılması ile ilgili yapılacak çalışmalar önemlidir. Çalışmamızda sunduğumuz standart mikroorganizmalara ait biyofilm formasyon verilerinin bu konuda çalışma yapacak araştırıcılara ve konuyla ilgili literatüre katkı sağlayacağını düşünmekteyiz.

Anahtar kelimeler: Biyofilm, mikrotitre plak yöntemi, antimikrobiyal direnç, standart suş

INTRODUCTION

Biofilm is the structure formed by the microorganisms by sticking to each other in the extracellular matrix, also called the "glycocalyx", formed after their adhesion to biotic or abiotic surfaces in order to percept the environment by means of various signal molecules with a mechanism named as quorum

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sensing and ensure the communication between cells. This structure may exhibit different phenotype characteristics by the growth speed and gene transcriptions of the microorganisms [1-3].

By forming a biofilm, microorganisms protect themselves against physical and chemical stresses, as well as phagocytosis. Furthermore, it is seen that bacteria forming biofilm are much more resistant against antibiotics than planktonic cells [4-5]. The most important factor creating this resistance is that the biofilm decreases the effectiveness of antibacterial agents by preventing their penetration by forming a barrier [6].

The biofilm layer can be encountered in all surfaces coming in contact with water, such as industrial and domestic water systems. It is also seen that it can develop on many different surfaces such as medical implants; and lead to infections [7-9].

Certain studies carried out put forth that biofilms formed by microorganisms are responsible for approximately 65% of nosocomial infections, and this increases the costs of treatment significantly [10].

National Institutes of Health (NIH) emphasizes that 70% of world microbial infections are related to biofilm. In this sense, important studies have been carried out recently on biofilm formations and antibiofilm agents [7].

As we believe the studies to be carried out with the aim of understanding the biofilm formation mechanisms of microorganisms well and preventing their formation would be important, we wanted to show the biofilm formation data of standard strains of the microorganisms threatening the environment and human health in our study altogether. Thus, we hope our study will contribute to the researches to be carried out on this subject and the literature.

METHODS

The microtiter plate method was used in order to determine the biofilm production capacities of standard strains. 15 bacteria and two yeast standard strains were used in the study (Table 2). The standard bacteria strains were incubated for 24 hours at 37ºC by means of passaging to the blood agar plate, and yeast strains were incubated for 48 hours at 37ºC by means of passaging to the Sabouraud Dextrose Agar (SDA) plates. Strains to be used in the study were suspended in tryptic soy broth (TSB) which was added 0.25% glucose, and incubated for one night. The suspensions were prepared in accordance with 0.5 Mcfarland sliding scale, and it was ensured that each of them is 10^8 CFU/ml. 200 µl was taken from the suspensions and transferred to the microtiter plate with 96 wells. TSB without the addition of 200 µl bacteria suspension was used as a negative control. The wells were slowly emptied in order to eliminate planktonic cells in the wells following the one-night incubation at 37ºC, and washed with phosphate- buffer saline (PBS) twice. The biofilms formed were dyed by adding 200 µl crystal violet of 0.1% to the wells dried at room temperature. After waiting for 30 minutes, the wells were emptied by washing twice with PBS once again. The absorbance values at 550 nm were read in Triturus microelisa (Norcross, GA, USA) device by adding ethanol of 95% to the wells dried at room temperature. Biofilm formations were evaluated in accordance with the scale reported by Csuhri et al. based on the negative control absorbance value [11] (Table 1).

Table 1. Assessment scale for the biofilm formation of microorganisms

OD cont > OD MB	Nonadherent	0
OD cont < OD MB < 2 OD cont	Weakly adherent	I
2 OD cont < OD MB < 4 OD cont	Moderately adherent	II
4 OD cont < OD MB	Strongly adherent	III

OD: Optical Density, OD MB: Optical Density of microorganisms biofilm, Adherent: Create a level of biofilm,

The study was performed in three repetitions. Biofilm formation activities of each microorganism were graded by the above mentioned scale by calculating the arithmetic mean and standard deviation of the values obtained. Our research was carried out with the suitability decision of Cumhuriyet University, Faculty of Medicine, Head of the Ethics Committee.

RESULTS

As a result of the study carried out, biofilm formations of 17 standard strains were graded by their absorbance values (Table 2). While five of these strains created weak and eight created medium, *Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium pseudotuberculosis* and *Neisseria sicca* created a strong biofilm.

Table 2. Biofilm formation activities of standard microorganism strains

Microorganisms	OD Control	OD MB			Mean OD ± SD	Adherent
		I	II	III		
Proteus vulgaris (ATCC 7829)	0,289	0,682	0,702	0,752	0,712 ± 0,036	II
*Salmonella typhi (*ATCC 14028)	0,289	0,436	0,424	0,461	0,440 ± 0,018	I
*Shigella dysenteriae (*ATCC 11835)	0,289	0,651	0,802	0,681	0,711 ± 0,079	II
*Shigella boydii (*ATCC 9905)	0,289	0,690	0,560	0,596	0,615 ± 0,067	II
*Bacillus cereus (*ATCC 10987)	0,289	0,491	0,431	0,616	0,512 ± 0,094	I
Neisseria sicca (ATCC 9913)	0,289	1,639	1,202	1,828	1,556 ± 0,321	III
Bacillus subtilis (ATCC 6633)	0,289	0,523	0,499	0,561	0,527 ± 0,031	I
Corynebacterium pseudotuberculosis (ATCC 19410)	0,289	1,583	1,920	1,428	1,643 ± 0,251	III
Streptococcus pyogenes (ATCC 19615)	0,289	0,561	0,610	0,575	0,582 ± 0,025	II
Streptococcus mutans (ATCC 21752)	0,289	1,072	0,998	0,885	0,985 ± 0,094	II
Streptococcus sanguinis (ATCC 10557)	0,289	0,905	0,927	0,943	0,925 ± 0,019	II
Klebsiella pneumoniae (ATCC 10031)	0,289	0,419	0,434	0,669	0,507 ± 0,140	I
Escherichia coli (ATCC 11229)	0,289	0,851	1,005	1,112	0,989 ± 0,131	II
Pseudomonas aeruginosa (ATCC 27853)	0,289	2,268	2,264	2,331	2,287 ± 0,037	III
Staphylococcus aureus (ATCC 25923)	0,289	1,639	1,402	1,327	1,456 ± 0,162	III
Candida albicans (ATCC 10231)	0,289	0,499	0,427	0,501	0,475 ± 0,042	I
Candida tropicalis (ATCC 750)	0,289	0,822	0,915	1,087	0,941 ± 0,134	II

OD: Optical Density, OD MB: Optical Density of microorganisms biofilm, Adherent: Create a level of biofilm, SD: Standard deviation

DISCUSSION

At the present time, it is known that the biofilms can be formed in many natural ecosystems [12]. While people used to think that biofilms only lead to industrial problems, it is now known that they lead to significant problems affecting the environment and public health and play a part in many chronic infections [13]. The communication system from cell to cell named as quorum sensing is held responsible for the formation of biofilm [14].

In the study we carried out, it was detected that standard microorganisms create biofilms at different levels (Table 2). We saw that the *Pseudomonas aeruginosa* ATCC 27853 strain created a strong biofilm. *P.aeruginosa* is an opportunist pathogen that is frequently isolated from serious infections. In a study carried out, it was expressed that these bacteria form biofilms in water treatment facility units, and pose a significant threat in terms of the environment and human health in this sense [15]. *P.aeruginosa* may show a “suitable” pathology for the microenvironmental conditions, and is one of the most significant pathogenity criteria in order for the biofilm to form [16]. This bacteria lead to the contamination of the medical tools-equipment in the hospitals and the formation of hospital infections with its ability to easily proliferate in aqueous environments. Furthermore, it was also stated that it can lead to infections related to the use of swimming pool, jacuzzi and contact lenses in healthy people outside the hospital [17]. As we detected in our study, the standard strain of *P.aeruginosa* can form a strong biofilm. In this sense, we believe the necessary precautions should be sensitively taken in consideration of the biofilm formation ability in the environments.

Pathogenic *Staphylococcus* species which are significantly threaten human health. *S.aureus* and *Staphylococcus epidermidis* is a factor in the formation of many infections in human such as respiratory tract infections, catheter infections, meningitis, septicemia, arthritis, dermatitis, endocarditis, thrombophlebitis [18,19]. Furthermore, these bacteria can also form a biofilm by sticking to the surfaces where food is produced, and thus cause the continuity of the contamination in the production line. In different studies carried out, it was stated that the biofilm formation rates of *S. aureus* isolates are between 50% and 68.6% [20-22]. It was reported that the biofilm created by *S. aureus* may pose a risk for public

health by generating resistance against antibiotics, disinfectants and immune-defense elements, and may also lead to economic losses by causing deterioration in food [23,24]. According to the result of our study, it is seen that the *S.aureus* ATCC 25923 strain may create a strong biofilm. It is especially important for the existence of this bacterium to be detected in hospital and food production areas, and foresee the negative consequences it may lead to with the effect of the biofilm it forms.

It is seen that *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750 strains we included in our study form weak and medium level biofilms, respectively. In the studies carried out, it was reported that the biofilm rates detected in *Candida* species vary between 8-85% [25,26]. It was stated that the Candida isolates that can form biofilms have a high amphotericin B MIC level; and that this medicine shows 100 times less effectiveness against the cells in the biofilm when compared to planktonic cells. It was expressed that this constitutes a significant problem in the treatment, and may lead to an increase in Candida infections related to catheter [27].

It is known that many microorganisms become more effective by creating biofilms [12]. For example, it is expressed that *Streptococcus mutans* increases its virulence by creating a biofilm in its external surfaces [28], and *N.sicca* can develop pathologies such as metaplasia, abscess and endometritis in human and animals [29]. It was emphasized that *Proteus vulgaris* can form biofilms by clinging even on steel surfaces, whereby both threatening the hospital hygiene in the field of health and also leading to the contamination of the food substances produced in the food industry [30,31]. In the studies carried out, it was reported that bacteria such as *Klebsiella pneumoniae, Escherichia coli* can create resistant strains in hospitals as a result of their ability to generate biofilms [32,33], *Salmonella* lead to new contaminations by means of the biofilms they form by clinging to various surfaces [34], and *Bacillus* species lead to significant economic losses in food industry as a result of the biofilms they create [35].

Considering the structures, effects and consequences of the biofilms, we believe the studies to be carried out on the detection of biofilm formations of the microorganisms, the determination of how and under what conditions the biofilms form, their general structure, and biofilm formation will be very significant.

We believe the biofilm formation activities we determined at different levels in 17 standard microorganism strains we provided in our study will contribute to the researchers who will carry out studies on this subject and the relevant literature.

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