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Efficiency of Purified Biosurfactant (Pseudofactin) from P. Aeruginosa in Disruption of Biofilm Formation by Candida Albicans in Vaginal Candidiasis

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Abstract

Candidiasis caused by *Candida albicans* is one of the most common forms of hospital-acquired opportunistic infection. So that the biosurfactant was detected in *P. aeruginosa* isolated from burns and wounds infections and produced with different levels of productivity. The use of (2:1) of chloroform: acetone was the best for extraction of biosurfactant with 11.3 g/l while chloroform: propanol gave a low yield of 2.41 g/l of biosurfactant. *Candida albicans* causing vaginal candidiasis considered a stronger producer of biofilms. The effect of biosurfactant on *Candida* biofilm formation was inhibited with increasing incubation period and the percentage of biofilm inhibition reached 45-78% after 24 hours and increased to 65-84% after 48 hours.

Keywords: Pseudomonas aeruginosa, biosurfactant, vaginal candidiasis,

INTRODUCTION

Candida infections are becoming a major public health issue, resulting in high mortality rates and medical expenses for governments and hospitalized patients. The rising incidence of invasive systemic candidiasis and cases of septicemia, particularly in immunocompromised patients, can be blamed for increased mortality (1, 2). *Candida albicans* is the most common cause of candidiasis in most cases (3). In hospitalized patients, it is the third most usually isolated microbe from bloodstream infections (4). It's an opportunistic pathogen that can be found in the oral and conjunctival flora, as well as the gastrointestinal and urinary tracts (2).

Biofilms are three-dimensional formations made up of a community of core microbial cells (single or mixed species) that are attached to host tissue or abiotic surfaces and immersed in an extracellular polysaccharide material (EPS) that protects the microbes (5). Biofilm production makes therapy difficult and contributes to high rates of morbidity and mortality, making it one of the most important virulence factors in the pathogenesis of candidiasis(6).

Surfactant is a composite word made up of the words "surface active agent" and "surfactant." They are divided into four groups based on their hydrophilic properties: anionic, cationic, non-ionic, and amphoteric (7). Agriculture, pharmaceutics, and the petroleum

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industry all use biosurfactants (8). *Bacillus* and *Pseudomonas* are the most commonly found bacteria that produce biosurfactants. Biosurfactants have a wide range of chemical properties, including non-polar molecule solubility, heavy metal sequestration, antibacterial, and anti-adhesive qualities, all of which help to inhibit biofilm formation. Biosurfactants are also preferable to chemical surfactants because of their biodegradability (9). Because of their probiotic effects(10) and potential to prevent pathogenic bacteria and fungus, biosurfactant-producing bacteria in the human microbiota have piqued researchers' interest (11). As a result, the current work was aimed to investigate *P. aeruginosa* biosurfactant synthesis and its impact on *C. albicans* biofilm formation.

MATERIALS AND METHODS

Collection and Identification of bacterial isolates

Twenty- five clinical samples from burns and wounds were obtained from various hospitals. The samples were grown on brain heart infusion agar and incubated at 37°C for 24 hours. *Pseudomonas aeruginosa* with a mucous consistency and a green hue was streaked with selective media such as MacConkey and cetrimide agar and cultured at 37ºC for 24 hours. The diagnostic was then completed with cultural characteristics, microscopic characteristics, and a biochemical API test.

Screening of biosurfactant producers

The production of biosurfactants from bacterial isolates was screened by using two methods:

1- Spreading of oil activity

A 15 cm diameter culture dish was filled with 40 ml of water. The liquid paraffin was then added in 2–3 drops. 2–3 droplets of zymotic fluid were applied to the film's center after generating a stable oil film. The diameter of the oil spreading was then measured (12).

2- Emulsification index (E24%)

The growing layer of emulsifying was detected after equal volumes of crude extract and toluene were mixed for 2 minutes with a vortex and left at room temperature for 24 hours. The following formula was used to compute the emulsification index (13): **Emulsification index(%)= rising of emulsion layer/total rising of the liquid*100**

Extraction and purification of biosurfactant

The supernatant was applied to a separation funnel after culturing the selected isolate in brain heart infusion broth and centrifugation, and the extraction was performed using various solvents by modifying the method described by (14), which included (1:1 v/v) of chloroform: methanol, chloroform: propanol, and chloroform: acetone. The bottom aqueous layer was left intact, while the top emulsion layer was recovered and dried at room temperature. The weight of the produced powder was calculated to identify the best extraction process.

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Isolation of *Candida* spp. from vaginal candidiasis

Using sterile cotton swabs, 18 vaginal swabs from women with vaginal candidiasis were collected. These swabs were cultured on sabouraud dextrose agar and a selective medium of chromogenic *Candida* agar before being incubated aerobically for 48 hours at 37ºC. The fungal colonies were identified for the use of carbohydrate profiles using the Vitek 2 technology after being diagnosed based on their morphological properties (15).

Screening of biofilm production

Each isolate's colony was put into brain heart infusion broth and cultured for 24 hours at 37°C. Following the incubation period, each isolate was diluted at a ratio of 1:20 using new brain heart infusion broth at a concentration of 200 l in a microtiter plate and incubated at 37°C for 24 hours. The microtiter was then emptied, cleaned with distilled water, and inverted to dry. For 15 minutes, each well was filled with 200 l of 1 percent crystal violet and rinsed with distilled water. About 200 l of ethanol: acetone (80:20) was poured into each well, and the absorbency at 450 nm was measured using an Elisa reader. As a cut-off value, the average optical density of the control well containing sterile brain heart infusion broth was employed. According to, samples with absorbance more than the cut-off value are deemed positive, while those with absorbance less than the cut-off value are considered negative (15).

Biosurfactant as biofilm inhibitor

In a microtiter plate method, 125 µl of purified biosurfactant in 50 mM sodium acetate buffer, pH 5.0, was combined with 125 µl of chosen cells suspension to determine biosurfactant activity against biofilm formation. After a 24- and 48-hour incubation period at 37°C, the biofilm experiment was done as described above. As previously stated, the biofilm activity was repeated. (16) was used to calculate the percentage of biofilm inhibition:

Percentage of biofilm inhibition(%) =[O.D control-O.D treatment]/O.D control x100

RESULTS AND DISCUSSION

Collection and Identification of bacterial isolates

Only 9 isolates with typical morphological traits, biochemical tests, and molecular identification belonged to *Pseudomonas aeruginosa* among 25 samples acquired from various clinical sites (burns and wounds) of patients, whereas the remaining 16 isolates belonged to distinct infective bacteria. Cetrimide agar is a differential/selective media for identifying *P. aeruginosa*. The findings of bacterial identification utilizing biochemical tests such as cetrimide agar and oxidase tests, among others. Except for *Pseudomonas aeruginosa*, bacterial growth is inhibited by the cultivation of Cetrimide agar (N-acetyl-NNN-trimethyl-ammonium-bromide). Except for *P. aeruginosa*, it functions as a quaternary structure cationic ammonium cleaner, releasing phosphorus and nitrogen from bacteria (17).

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Screening of biosurfactant producers

The results showed that 9 *P. aeruginosa* isolates produced the surface-active substance (biosurfactant), but at different levels of productivity, as measured by emulsification indexes (E24%) and oil spreading diameters, which ranged from 37 to 82 % emulsification indexes and 5-17.5 mm oil spreading diameters, as shown in figure (1). As a result, *P. aeruginosa* 8 was chosen as the top producer for biosurfactant purification. *Pseudomonas* strains obtained from soil contaminated with petroleum wastes produced biosurfactant by creating clearing zones on mineral salt agar plates that were sprayed with polycyclic aromatic hydrocarbon (18).

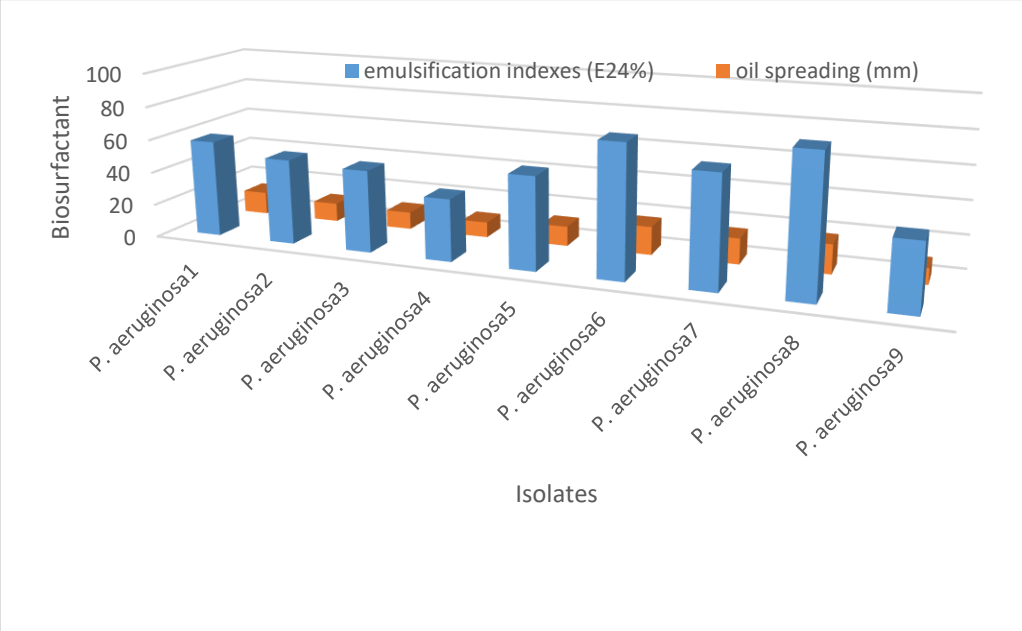

Figure 1: Detection of biosurfactant production by *P. aeruginosa* isolates

Extraction and purification of biosurfactant

The biosurfactant from *P. aeruginosa* was extracted using a variety of solvents and ratios. Figure (2) shows that extraction of biosurfactant using (2:1) chloroform: acetone yielded the highest yield (11.3 g/l), whereas chloroform: propanol yielded the lowest yield (2.41 g/l). *Pseudomonas* sp. was grown in the heart infusion broth medium to create a biosurfactant, which was extracted using two solvent systems: hexane and a 2:1(v/v) mixture of chloroform and MeOH (19). Another study (20) utilized chloroform: and methanol and found that biosurfactants precipitated as white sediments.

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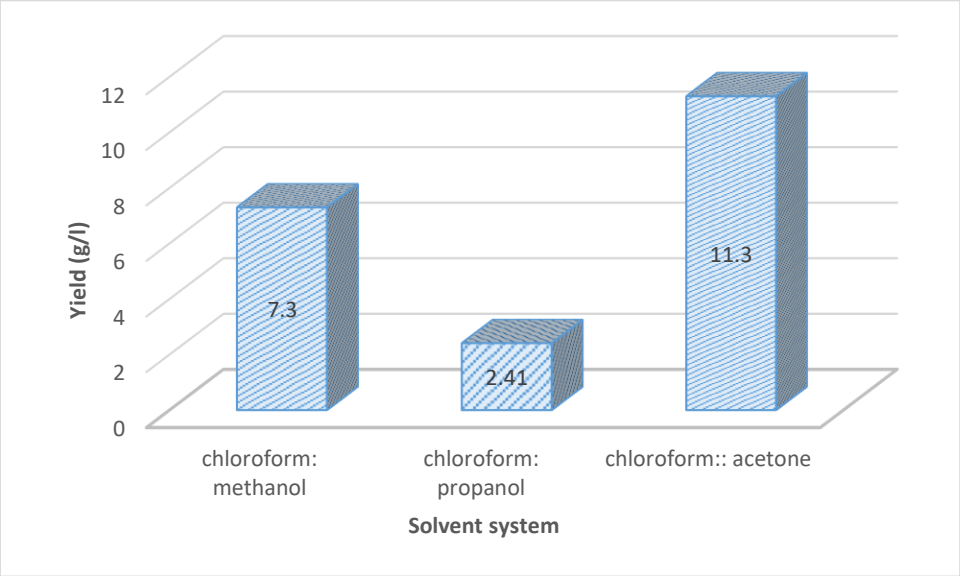

Figure 2: Extraction of biosurfactant by different mixtures of solvents

Screening of biofilm production

Candida albicans isolates were discovered in ten of the 18 cotton swab samples taken from women with vaginal candidiasis. On chromogenic media, they were gram-positive and oval to spherical, with light greenish colonies. Only seven of the ten isolates are deemed stronger producers, with the remaining isolates classified as weak biofilm formers as in table (1).

A vast spectrum of virulence characteristics and fitness traits enable the capacity of *C. albicans* to infect multiple host niches. The ability to transition morphologically between yeast and hyphae, the production of adhesions and invasions on the cell surface, the development of biofilms, phenotypic interchange, and the secretion of hydrolytic enzymes are all virulence factors(21).

Table 1: Biofilm formation by *Candida albicans*

Isolate	Absorbency at 450 nm	Density
C. albicans1	0.62	+++
C. albicans2	0.59	++
C. albicans3	0.86	+++
C. albicans4	0.68	++
C. albicans5	0.62	++
C. albicans6	0.19	+
C. albicans7	0.16	+
C. albicans8	0.24	+

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Biosurfactant as biofilm inhibitor

The strongest producers were used to investigate biosurfactant activity against biofilm development. The isolated biosurfactant from *P. aeruginosa* was found to have antibiofilm action against various *Candida albicans* isolates. In comparison to the control group, biosurfactants affected all *Candida* isolates. Figure (3) shows that the proportion of biofilm inhibition was 45-78 % after 24 hours and increased to 65-84 % after 48 hours for *Candida albicans* isolates.

An inhibit *Candida albicans* hyphal growth even at low concentrations, and biofilm cells have a punctured outer membrane with a bloated and distorted shape. Increased biosurfactant concentration resulted in full biofilm suppression, with cells remaining in yeast form (22).

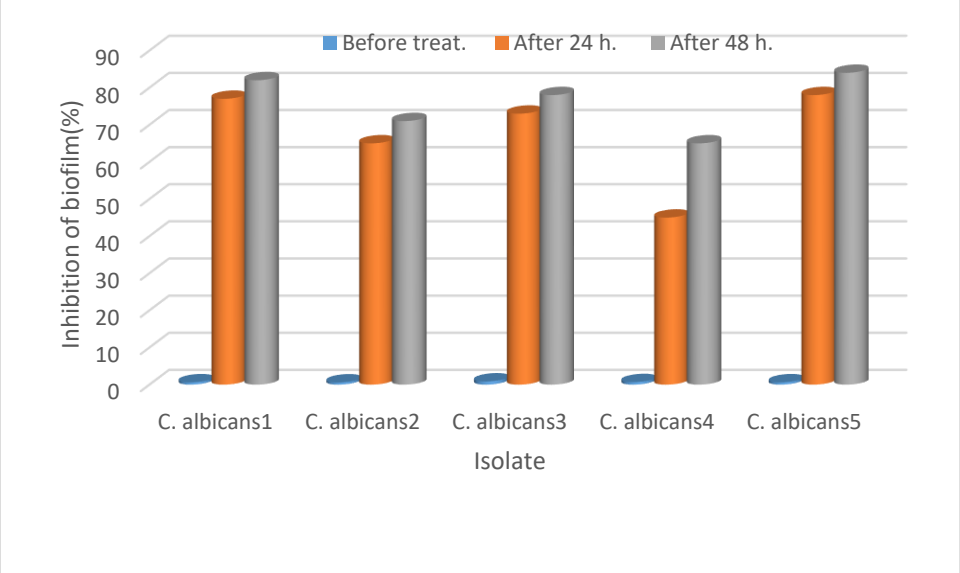

Figure 3: Inhibition of biofilm formation with surfactant at different incubation periods

CONCLUSION

The effect of biosurfactant on *Candida* biofilm formation was inhibited with increasing incubation period and the percentage of biofilm inhibition reached 45-78% after 24 hours and increased to 65-84% after 48 hours.

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