RESEARCH ARTICLE

The Efficiency of Purified Pyocyanin from *Pseudomonas aeruginosa* in Disruption of Biofilm Formation by *Candida albicans* Causing Oral Candidiasis

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ABSTRACT

Candida albicans is the most usually isolated species in invasive oral candidiasis, which is a major health concern in underdeveloped nations. So pyocyanin was screened in *Pseudomonas aeruginosa* and found that the highest level of productivity in King A medium appeared compared with cetrimide agar, also, the productivity was increased in presence of sweet potato in comparison with soya bean. The pyocyanin was purified with silica gel chromatography as a single peak with high purity. *C. albicans* causing oral candidiasis is considered a stronger producer of biofilms. The effect of pyocyanin on *Candida* biofilm formation was inhibited with increasing incubation concentration and with increasing incubation period and the percentage of biofilm inhibition reached 73–87% after 72 hours.

Keywords: Oral candidiasis, *Pseudomonas aeruginosa*, Pyocyanin.

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INTRODUCTION

Pseudomonas aeruginosa is a bacterium that is frequently isolated from natural environments or contaminated human body systems. Humans, animals, plants, and nematodes can all be infected by this bacterium.[1] The host flexibility stems from *P. aeruginosa* natural distribution in soil and water and its lengthy survival on dry surfaces, which enhances the spread of infections, particularly nosocomial infections. *P. aeruginosa* is commonly found in cystic fibrosis patients, wounds, burns, and urinary tract infections.[2] *P. aeruginosa* pathogenicity is attributed to several virulence factors, including enzymes, toxins, and pigments.[3]

Pseudomonas produces a range of extracellular pigments, the most important of which are phenazines. The formation of soluble pyocyanin pigment, a water-soluble blue-green phenazine molecule, is the most distinguishing trait of *P. aeruginosa*. Pyocyanin has been utilized as a reversible dye with a redox potential similar to menaquinone since its inception.[4]

Pyocyanin's propensity to form reactive oxygen species (ROS) and its ability to transfer electrons make it useful in various disciplines, including health, agriculture, and industry, all of which positively impact the environment.[5-7] Because of the multiple beneficial applications of pyocyanin, this research aims to improve its synthesis through a low-cost fermentation technique rather than a high-cost synthetic pathway and detect its antibiofilm action against *Candida albicans*.

MATERIALS AND METHODS

Detection of Pyocyanin Productivity

Burn and wound swabs 16 were taken from people who had wound infections. The samples were cultured for 24 hours at 37°C in cetrimide agar and King A agar. The diagnostic was then completed with cultural characteristics, microscopic characteristics, and a biochemical API test to diagnostic *P. aeruginosa* isolates that could produce blue-green pigment (pyocyanin).

Production and Extraction of Pyocyanin

After adding 5% sweet potato and soya bean to the medium separately, the chosen isolates were cultured in King A broth and incubated at 37°C for 24 hours. After that, the bacterial

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cell pellet was removed using 15 minutes centrifugation at 4000 rpm. The supernatant was filter-sterilized for pyocyanin extraction using the chloroform-acid extraction technique as follows: In a 1:2 ratio, chloroform was added to the recovered supernatant. The top layer was removed after a brief vortex and centrifugation at 3000 rpm for 1-minute, and the volume of the blue bottom layer (chloroform + pyocyanin) was determined. The acidified top pink-red phase was collected in a separate tube and neutralized with 0.5 mol.l^{-1} NaOH after adding 20% (v/v) 0.1 N HCl to the blue mixture and centrifuging at 3000 rpm for 1-minute. The above stages were repeated three times to obtain a great purity pigment. The solution was filtered and dried after the final pH was adjusted to 7.5 to generate pyocyanin blue needle-like crystals.[8] After that, it was weighted.

Purification of Pyocyanin Pigment

According to the[9] that described a method for purifying crude pyocyanin pigment. To summarize the procedure, the crude pigment was suspended in chloroform and absorbed into silica gel. The absorbed pigment samples were then loaded onto a column that had been equilibrated with methanol and chloroform, and the pure pigment was eluted with a mixture of methanol and chloroform.

Isolation of *C. albicans* from Oral Candidiasis

Fourteen oral candidiasis swabs were cultivated on sabouraud dextrose agar and chromogenic Candida agar before being incubated aerobically for 48 hours at 37ºC. The Vitek 2 system was used to confirm the diagnosis.

Antifungal Activity of Purified Pyocyanin

To investigate pyocyanin activity against biofilm formation, different concentrations of pyocyanin (50, 100, and 200 µg/mL) were made and 125 µL of each concentration was coupled with 125 µL of chosen cells suspension in a microtiter plate method. The biofilm experiment was carried out as mentioned above after a 24 and 48 hours incubation period at 37°C. The biofilm activity was replicated, as previously indicated. The proportion of biofilm inhibition was calculated using[10]: Biofilm inhibition percentage (%) = [O.D control-O.D treatment]/O.D control x100.

RESULTS AND DISCUSSION

Detection of Pyocyanin Productivity

After culturing of burn and wound samples on cetrimide agar and King A agar, there were eight *P. aeruginosa* that showed their capacity to produce pyocyanin pigment with of blue-green color. While two isolates don't produce any pigment. The producer isolates a higher level of productivity in King A medium compared with cetrimide agar since the diameter of this pigment expended from 24 to 36 mm but in cetrimide agar, the diameter ranged between 18–26 mm (Figure 1).

An inoculation of King's A fluid medium, previously adjusted to pH 7, and incubation at 37°C for three to four days with shaking at 200 rpm are suitable production conditions for pyocyanin.[11] The presence or absence of specific gene regulators that may have a favorable or negative impact on the production of bacterial secondary metabolites can explain the variance in pyocyanin production among studied strains. When QteE is overexpressed in *P. aeruginosa*, it reduces homoserine lactone signals, which impairs pyocyanin synthesis.[12]

Production and Extraction of Pyocyanin

The quantity of productivity for pyocyanin pigment in King A broth after the addition of 5% of sweet potato and soya bean, separately, ranged between 1.2–3.7 mg/mL after growing *P. aeruginosa* isolates in the presence of soya bean while in presence of sweet potato, the productivity level ranged between 2.3–4.9 mg/mL as shown in Figure 2.

As secondary metabolites, *P. aeruginosa* strains produce many redox-active chemicals. Pyocyanin, the most well-studied, is a blue-green pigment that is soluble in chloroform and plays a significant role in *P. aeruginosa* pathogenicity.[13,14]

Purification of Pyocyanin Pigment

The extracted pyocyanin was loaded on column chromatography using a silica gel as the stationary phase, and after elution, only one peak formed which contained pyocyanin pigment, as shown in Figure 3, with a final concentration of 4.8 mg/mL.

Screening of Biofilm Production

In 14 cotton swab samples taken from children with oral candidiasis, six *C. albicans* isolates were detected. They were gram-positive, oval to spherical, and had light greenish

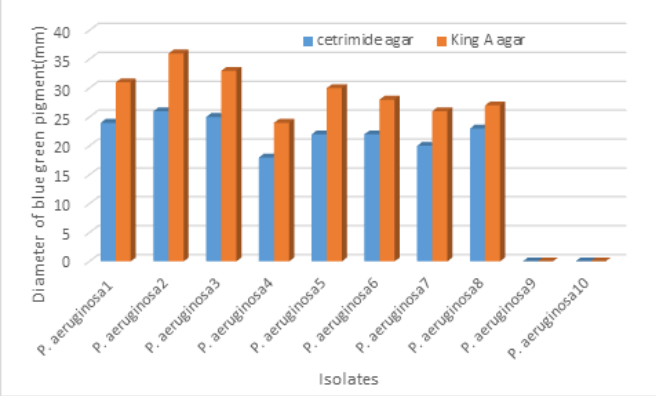

Figure 1: Pyocyanin production by *P. aeruginosa* in different culture media

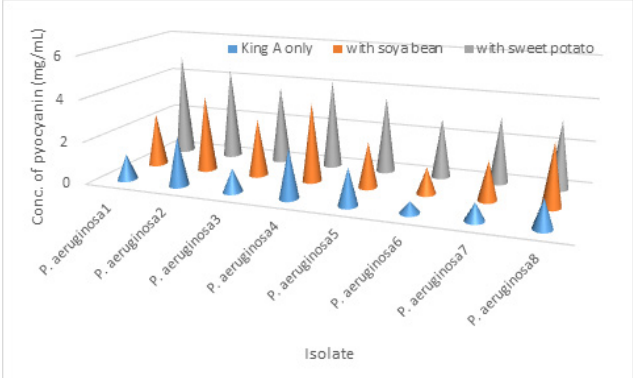

Figure 2: Pyocyanin production by *P. aeruginosa* in King A broth supplemented with natural feedings.

Table 1: An inhibition of biofilm formation by *C. albicans* using pyocyanin

Isolate	*Biofilm inhibition (%) after 24 hour*		
	Conc. 50 µg/mL	*Conc. 100 µg/mL*	*Conc. 200 µg/mL*
C. albicans 1	35	47	54
C. albicans 2	42	49	63
C. albicans 3	48	55	69
C. albicans 4	23	46	52
C. albicans 5	35	57	59
C. albicans 6	41	61	67

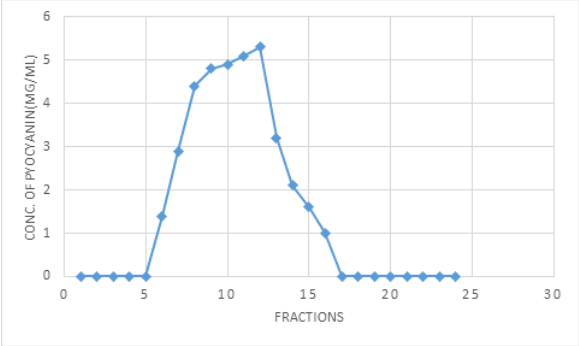

Figure 3: Purification of pyocyanin by a silica gel chromatography

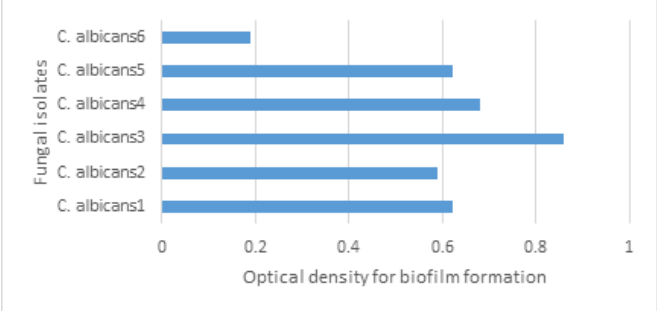

Figure 4: Detection of biofilm formation by C. albicans isolates.

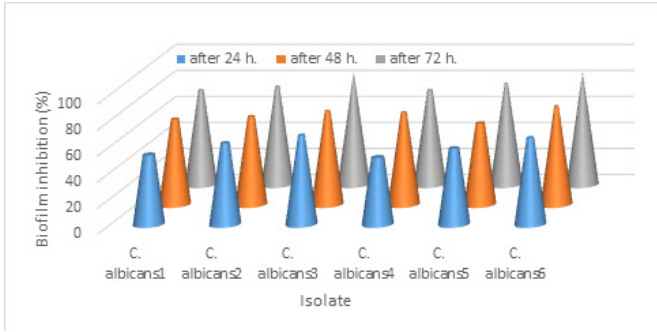

Figure 5: An inhibition of biofilm formation by *C. albicans* using pyocyanin at different incubation times

colonies on chromogenic media. As shown in the table, all *C. albicans* isolates were capable of producing biofilm (Figure 4).

C. albicans can infect numerous host niches due to a wide range of virulence and fitness features. Virulence factors include the ability to morphologically transition between yeast and hyphae, the generation of adhesions and invasions on the cell surface, the development of biofilms, phenotypic exchange, and the secretion of hydrolytic enzymes.[15]

Antifungal Activity of Purified Pyocyanin

All *C. albicans* isolates were used to investigate pyocyanin activity against biofilm development. The purified pyocyanin from *P. aeruginosa* was found to have antibiofilm action against various *C. albicans* isolates. Compared to the control group, biofilm inhibition increased with increasing the concentrations of purified pyocyanin as in Table 1 after 24 hours and the percentage of inhibition ranged between 52–69%. In Figure 5 the percentage of inhibition for biofilm increased with time and reached a range of 73–87% after 72 hours for *C. albicans* isolates.

C. albicans and *C. tropicalis* biofilm production were greatly reduced by culture filtrate, both plain and heated, and pyocyanin.[16] Pyocyanin, a phenazine molecule released by *P. aeruginosa*, is thought to be responsible for the inhibitory impact. Farnesol, a quorum-sensing chemical generated by *C. albicans*, has been demonstrated to suppress pyocyanin production by *P. aeruginosa* in a mixed environment by dramatically inhibiting pqs A gene transcription. This could be a way for the yeast pathogen to shield itself from the toxicity of pyocyanin, an iron scavenger and potential *P. aeruginosa* virulence factor.[17] Pyocyanin is a powerful bacterial pigment produced by *P. aeruginosa* that can stop fungi's electron transport chain and so has antifungal properties.[18]

CONCLUSION

Pyocyanin can inhibit *C.* biofilm formation (time dependant) which helps in efficient oral candidiasis treatment.

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