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Article

Inhibition of Some Virulence Factors in *Candida albicans* by Bioemulsifier Purified from *Saccharomyces cerevisiae*

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Abstract: As viable alternatives to some synthetic surfactants, biosurfactants, which are amphiphilic compounds, are gaining more and more interest from the scientific community. Therefore, *Saccharomyces cerevisiae* was used to create biosurfactant as a bioemulsifier at different levels. The best producer isolate was selected for biosurfactant extraction by boiling, acetone precipitation, and chromatographic techniques on sephadex G-75, resulting in a final recovery of 55%. *Candida albicans* was identified as the primary pathogen responsible for vulvovaginitis. Certain virulence factors, including the synthesis of phospholipase, haemolysin, and the hyphal transition, were inhibited by the pure biosurfactant for control of the increasing infections caused by *Candida albicans*.

Keywords: Bioemulsifier, *Saccharomyces cerevisiae*, *Candida albicans*

1. Introduction

An infection known as vulvovaginal candidiasis is caused by abnormal development of various Candida species in a female's genital system. Candida infections most frequently manifest as vulvovaginitis [1]. After bacterial vaginosis, vulvovaginitis is thought to be the second most common vaginal infection. It is an inflammatory disease of the vulva and vagina [2], [3]. Vulvar pruritus, vaginal itching, atypical curd-like vaginal discharge, irritation, burning feeling, pain during intercourse, and vaginal erythema are among the indications and symptoms of vulvovaginitis [4]. Numerous investigations revealed that *Candida albicans* is the primary cause of vulvovaginitis, with *Candida glabrata* and *Candida tropicalis* following closely after [5].

Candida is an opportunistic infection that can appear in the places where the urine, gastrointestinal system, vagina, and mouth meet [6]. Infections including thrush, denture stomatitis, and urinary tract infections are frequently caused by *Candida albicans*, but it can also result in more serious systemic diseases. Because of its emulsification, de-emulsification, solubilization, mobilization, low toxicity, and biodegradability, biosurfactants have been applied in agriculture, bioremediation, biodegradation, pharmaceutical, cosmetic, and medical fields [7], [8].

Through the reduction of surface and interfacial tensions, surfactants, amphipathic molecules with hydrophobic and hydrophilic moieties, can produce microemulsions [9]. A variety of yeasts, filamentous fungus, and some bacteria create microbial surfactants, often known as biosurfactants [10]. Because they are biodegradable, low toxicity, highly selective, foamy, and more environmentally friendly than chemical surfactants, biosurfac-

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tants are also more effective and stable under harsh temperature, pH, and salinity conditions [9], [11]. This work aimed to extract and purify the biosurfactant from *S. cerevisiae* using heat treatment and column chromatography, as well as assess its efficacy against several virulence factors generated by *Candida albicans*.

2. Materials and Methods

2.1. Collection of S. cerevisiae

In this work procured six varieties of commercial dry yeast (*S. cerevisiae*) from the Baghdad local market.

2.2. Production of biosurfactant

In Yeast Mold Agar medium, which contains 0.3% yeast extract, 0.3% malt extract, 0.5% tryptone, 1.0%glucose, and 5.0% agar, the isolates were activated. then moved to YEPG broth that contained 10 g/l yeast extract, 20 g/l peptones, and 20 g/l glucose to produce biosurfactant, then incubated for 24 hours at 2 ^{o}C [9].

2.3. Screening for biosurfactant

The Emulsification index (E24) was calculated in the Emulsification activity by mixing 2 ml of sample with 2 ml of cooking oil, vortexing for 3 minutes, and then letting the mixture remain for 24 hours. The formula (10) was utilized to ascertain the E24. E24 is equal to (emulsion layer height / total liquid layer height) x 100%.

2.4. Extraction of biosurfactant

The chosen isolate was suspended in phosphate buffer (pH 7.5) and cooked for 15 minutes in order to extract the biosurfactant. The cell wall mannoproteins were dissolved by boiling the cells in acetate buffer (0.1 M, pH 5), which allowed for the extraction of biosurfactant. After centrifuging the yeast cells for 10 minutes at 3,000 rpm, the crude biofurfactant's emulsification activity was ascertained [12].

2.5. Purification of biosurfactant

With a small modification, the purification was carried out in accordance with [12] as following 4:1 vol/vol of cold 95% acetone was used to precipitate the crude biosurfactant. Centrifugation was used to gather the precipitate, which was then resuspended in acetate buffer (0.1 M, pH 5) and dialyzed against the same buffer. A Sephadex G-75 column was treated with the biosurfactant that had been partially purified. The same buffer was used to elute and equilibrate the column. 5 mL of fractions were collected. Each collected fraction's protein content was calculated at 280 nm of absorbance. Protein and carbohydrate contents, as well as the emulsification index (E24), were assessed when fractions exhibiting emulsification activity were combined.

2.6. Protein and Carbohydrate content

Using a standard of bovine serum albumin, the protein content was calculated using procedure [13]. The phenol-sulfuric acid assay [14] was utilized to ascertain the carbohydrate content, with glucose serving as the standard.

2.7. Isolation of Candida albicans

Twenty females with vaginal candidiosis were the subjects of vaginal specimen collection. The Vitek 2 system, along with cultural, microscopical, and biochemical testing, were applied to all developing isolates.

2.8. Detection virulence of Candida albicans

1) Phospholipase activity

The selected isolates were cultured on the surface of an egg yolk agar plate, then incubated at 37°C for 48 hours to measure the phospholipase activity. After measuring the opacity zone diameters surrounding the colonies, the following activity was identified as: phospholipase index value= Diameter of colony (mm) / Diameter of opacity zone (mm) + Dimeter of colony (mm), with a value of 1 denoting a negative value. The activity of phospholipase is classified as weak at 0.80-0.89, moderate at 0.70-0.79, and strong at <0.70 [15].

2) Haemolysin production

The chosen isolates were cultivated for 48 hours at 37 degrees on Sabouraud dextrose blood agar medium. The colony's bright halo indicates that hemolysin production is positive. The formula for calculating the hemolytic index was: hemolytic index = colony diameter (mm)/opacity zone diameter (mm) + colony diameter (mm). A value of 1 indicates negative hemolysis, a value of 064-0.99 indicates moderate activity, and a value of <0.63 indicates strong activity [16].

3) Yeast–hyphal transition

The chosen isolates were put to a broth made of yeast extract and peptone. The amount of hyphae was then calculated by counting the yeast on a hemocytometer slide and counting them under a microscope [17].

2.9. Effect of purified biosurfactant on some virulence factors

1) Reduction of Phospholipase productivity

To find out if purified biosurfactant could inhibit phospholipase activity, 20 µl of yeast isolate, 30 µl of sabouraud dextrose broth, and 50 µl of purified biosurfactant were mixed together. The isolate was then placed on an egg yolk agar plate and after that incubated at 37 °C for 24 hours, while the control group did not receive any purified biosurfactant. Then, as previously mentioned, phospholipase activity was determined.

2) Reduction of haemolysin productivity

To find out if purified biosurfactant could stop the production of haemolysin, 20 µl of yeast isolate, 30 µl of sabouraud dextrose broth, and 50 µl of purified biosurfactant were mixed together. A control group did not have any purified biosurfactant. The isolate was then put to sabouraud dextrose blood agar and incubated at 37°C for 24 hours. Following that, hemolytic activity was assessed using the previously mentioned method.

3) Reduction of Hyphal transition

By combining 20 µl of yeast isolate, 30 µl of yeast extract peptone broth, and 50 µl of purified biosurfactant, while keeping the control without purified biosurfactant, it was possible to detect the ability of purified biosurfactant to inhibit the yeast-hyphal transition. The number of hyphae was then counted using a hemocytometer slide and observed under a microscope.

3. Results and Discussion

3.1. Screening for biosurfactant production

When six *Saccharomyces cerevisiae* isolates were tested for their ability to produce biosurfactant, the findings showed that each isolate produced biosurfactant to varying degrees, as indicated by the emulsification indexes (E24%), which ranged from 27.8 to 46.2%, as illustrated in Figure 1.

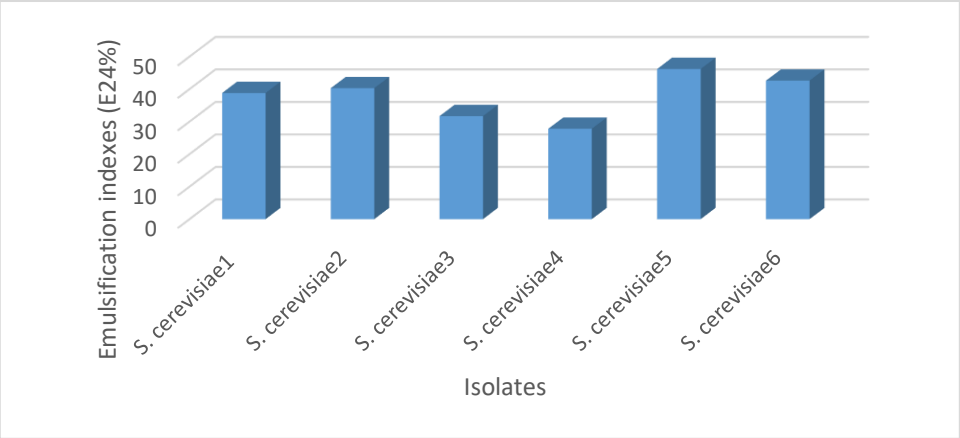

Figure 1. Levels of Production of biosurfactant from *S. cerevisiae* isolates

An outstanding emulsifier was the biosurfactant from *S. cerevisiae* that was discovered to be a mannoprotein [11]. The biosurfactant produced by utilizing Cooper and Paddock's medium to produce biosurfactant from S. cerevisiae was not able to lower surface tension, indicating that its primary function was that of an emulsifier rather than a surfactant [9], [18].

3.2. Extraction and purification of biosurfactant

It's possible that the biosurfactant from *S. cerevisiae* was released when a significant amount of cell wall glucan was broken down and boiled for 15 minutes. Subsequently, employing acetone as a solvent enhanced the biosurfactant's emulsification activity to 52.5%. To get rid of extraneous protein and other insoluble impurities that can cause a biosurfactant to lose its activity or become unstable, chilled acetone was added to the mixture. Any methodology for the purification of biosurfactants must include this step. With three protein peaks and a final emulsification activity of 59.4% as shown in Table 1, the white precipitate from acetone precipitation was further purified using Sephadex G-75 gel filtration chromatography. The second peak in the peak depicted in Figure 2 included biosurfactant activity.

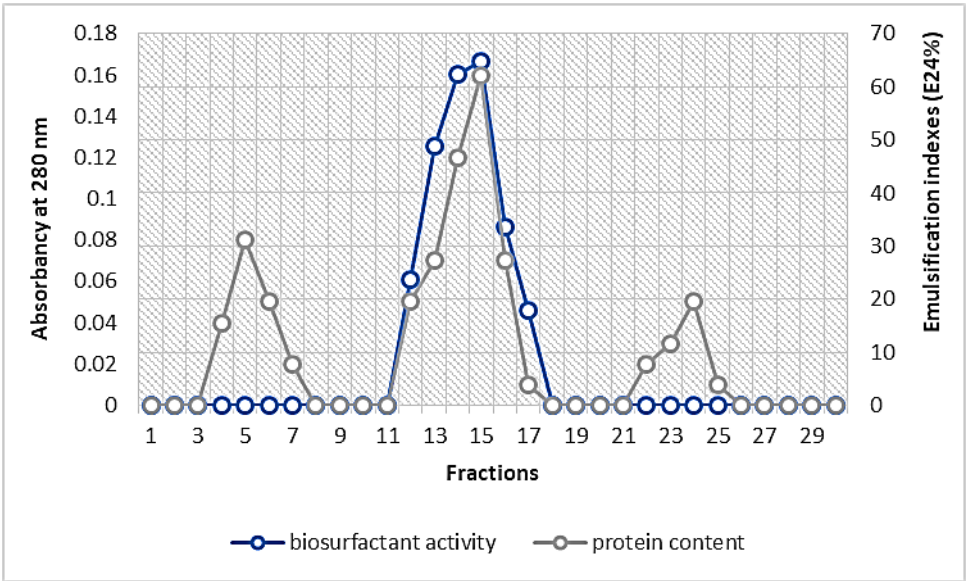

Figure 2. Purification of biosurfactant from *S. cerevisiae* in Gel filtration on sephadex G-75 column

Table 1. Serial stages in biosurfactant purification from *S. cerevisiae*

Purification step	Size (ml)	Carbohydrate (mg/mL)	Emulsification activity (%)	Protein conc. (mg/ml)	Specific activity (%/ mg)	Total activity	Purification fold	Yield (%)
Crude extract	35	1.7	46.2	2.4	19.25	1617	1	100
Acetone precipitation	18	1.3	52,5	1.3	40.38	945	2	58.4
Sephadex G-75	15	0.8	59.4	1	59.4	891	3	55.1

Extracellular biosurfactant was successfully dissolved during the 5-minute heat treatment of S. cerevisiae to isolate the biosurfactant [19]. The precipitation with solvents such as ethanol and acetone created an emulsion that was sticky, viscous, compact, and stable, in contrast to the ammonium sulfate precipitate's dispersed and unstable emulsion [12]. Numerous investigations have demonstrated that emulsion activity is significantly influenced by the protein moiety of biosurfactants and bioemulsifiers [20]. It is in charge of emulsification, and the carbohydrate component is in charge of enhancing the emulsion's stability [12].

3.3. Isolation of Candida albicans

Six isolates of *Candida albicans* were found in 20 vaginal specimens collected using cotton swabs from patients with vaginal candidiasis. In addition to growing on chromogenic agar with light greenish colonies, the bacteria showed up as gram positive under a microscope, with an oval to spherical shape.

One of the most typical signs of a Candida infection is vulvovaginitis. Although other species, *Candida albicans*, is typically the cause [21]. A high frequency of vaginal candidiasis in pregnant women with varying genotypes of *Candida albicans*. Consequently, while diagnosing vaginal candidiasis, it is advisable to take a vaginal swab culture along with clinical signs into account [22].

3.4. Effect of purified biosurfactant on some virulence factors

3.4.1. Reduction of Phospholipase productivity

The generation of phospholipase by *C. albicans* isolates that caused vaginal candidiasis was significantly impacted by the pure biosurfactant. In comparison to the control, as shown in Figure 3, phospholipase activity declined in each of the *C. albicans* isolates, and the phospholipase index value raised to 0.8.

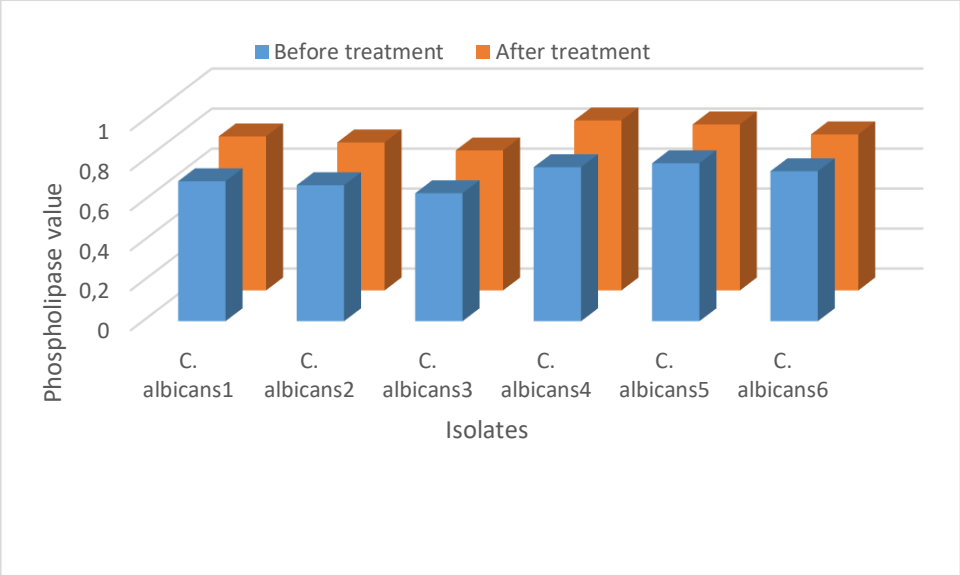

Figure 3. Detection the effect of biosurfactant on of Phospholipase productivity by *C. albicans*

3.4.2. Reduction of haemolysin productivity

Figure 4 shows the inhibitory impact of pure biosurfactant on the synthesis of haemolysin from isolates of *Candida albicans* that cause vaginal candidiasis. When the hemolytic index increased to 0.99 from *C. albicans* 6 before treatment, which was equal to 0.82, the hemolysin activity dropped in the tested *C. albicans* isolates and the hemolytic activity fell as well.

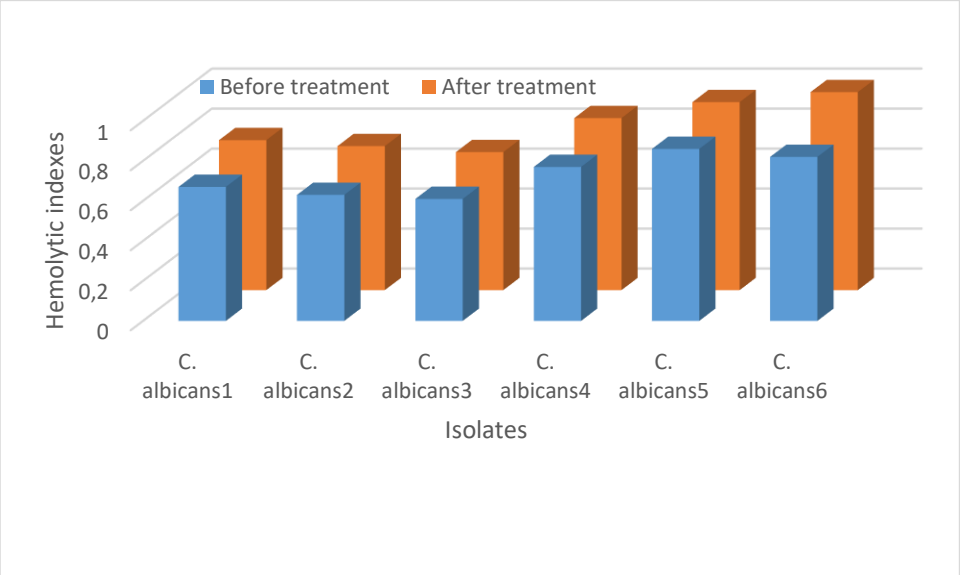

Figure 4. Detection the effect of biosurfactant on of hemolysin productivity by *C. albicans*

3.4.3. Reduction of Hyphal transition

Figure 5 shows the inhibitory impact of pure biosurfactant on the hyphal transformation of *Candida albicans* that causes vaginal candidiasis. When comparing the amount of hypha in *C. albicans* 2 to the same isolate before to treatment, the hyphal transition reduced in all of the *C. albicans* isolates, with a value of 0.48.

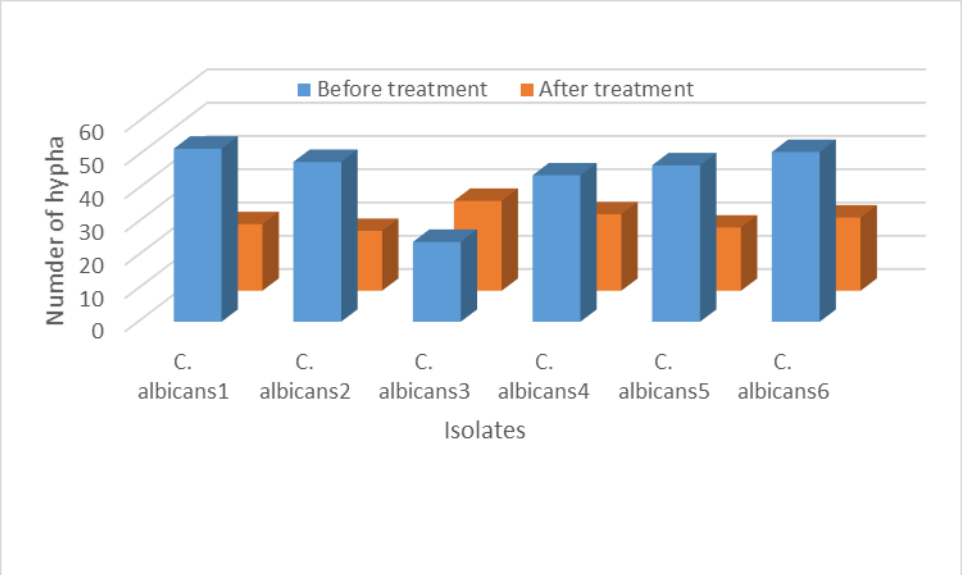

Figure 5. Detection the effect of biosurfactant on of hyphal transition productivity by *C. albicans*

Alginate oligosaccharides have been effectively used by [23] to cause noticeable changes in hyphal development, which lessen *C. albicans*' ability to invade epithelial cells.

4. Conclusion

Globally common *Candida albicans'* virulence and drug resistance have made control difficult, but advancements in potent antivirulence medications are still essential. *Saccharomyces cerevisiae's* purified invertase was found to be the most effective inhibitor of multidrug-resistant *Candida albicans'* ability to form biofilms, which is a positive sign for the development of anti-pathogenic medications that won't evolve resistance.

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