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Evaluation of Laboratory-Produced Biosurfactant by *Rhodotorula* Species and Its Antifungal Activity

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Abstract

Background: Biosurfactants are amphiphilic surface-active compounds that are produced by several microorganisms, including bacteria and fungi. Biodegradability, low toxicity, application diversity, and functionality under extreme conditions characterize them from chemical biosurfactants. It is found that *Rhodotorula* species, read yeasts, have high potency for biosurfactant production. Recently, antimicrobial activities of biosurfactants have been subjected to new antibiotic therapy.
Objectives: The aim of the present study was to evaluate the biosurfactant production by the different strains of *Rhodotorula* species in laboratory conditions. In addition, the antifungal activity of produced biosurfactant was assessed against several saprophytic fungi.
Methods: In the present study, 54 strains of *Rhodotorula* including *R. glutinis* (48 strains), *R. minuta* (two strains), *R. mucilaginosa* (two strains), and *Rhodotorula* species (two strains) were screened for biosurfactant production. The biosurfactant was produced in Sabouraud dextrose broth medium and confirmed by specific tests. The antifungal assay was carried out by a disk diffusion method using serial dilutions of biosurfactant.
Results: In the present study, although all tested strains were capable of producing biosurfactant *in vitro*, the degree of biosurfactant production varied among the strains. 7.4% of the strains had the highest (+5) biosurfactant activity while 16.7%, 29.5%, 25.8%, and 20.4% had +4, +3, +2, and +1, respectively. In the present study, all tested fungi were inhibited at 40 μL of the biosurfactant.
Conclusions: *Rhodotorula* species are appropriate organisms for the production of biosurfactants and *R. glutinis* strains have the greatest ability to produce biosurfactant among other species. Furthermore, our results demonstrated that the produced biosurfactant by *R. glutinis* presents a valuable potential for biopharmaceutical applications.

Keywords: Biosurfactant, *Rhodotorula glutinis*, Antifungal Activity, Saprophytic Fungi

1. Background

Biosurfactants are surface-active compounds with extracellular (secondary metabolites) or cell wall-associated sources that are produced by several microorganisms such as bacteria and fungi (1-4). In contrast to synthetic surfactants that are not environmentally friendly, biosurfactants have several advantages including biodegradability, low toxicity, diversity of application, and functionality under extreme conditions (5, 6). In addition, biosurfactants are emerging as potential nanoparticle stabilizing agents and used for the treatment of wastewaters containing heavy metals (Cd^{++} ions) and biodegradation of model hydrocarbons and crude oil in soil (7, 8). Moreover, production of biosurfactants by microorganisms from renewable and cheaper substrates is another reason for its increased use as a green alternative to synthetic surfactants.

Several reports indicated that bacterial species such as *Acinetobacter* (9), *Pseudomonas* (10, 11), *Bacillus* (12), *Lactococcus* (13), and *Nocardia mediterranei* (14) are applicable biosurfactants sources. Much research has shown that bacterial biosurfactants possess antimicrobial properties (9, 10, 15, 16). Mostafapour et al. have shown that a biosurfactant produced by *Acinetobacter* species has anti-Gram positive and negative bacteria effects *in vitro* (9). In addition, Gomaa examined the biological properties of biosurfactant produced by *B. licheniformis* and found that it has great potential for the biotechnological and biopharmaceutical applications (17). Moreover, a synergistic effect was found against plant pathogenic fungi and pathogenic bacteria when silver nanoparticles were used with biosurfactants (18). Furthermore, Basit et al. recommended lipopep-

tide biosurfactant produced by *B. cereus* as a safe antimicrobial and antioxidant agent (19).

Producing biosurfactants by fungi is limited to some species of *Candida, Pseudozyma, Yarrowia, Penicillium* and *Aspergillus* species (5, 20-23). Recently, the production of biosurfactants from *Rhodotorula glutinis* (24), *R. mucilaginosa* (25), and *R. paludigena* (26) has been investigated by researchers. It seems that *Rhodotorula* species are the main producers of biosurfactants; hence, they have new possibilities for industrial application. The antibacterial, antifungal, and antiviral activities of several biosurfactants have been reported by researchers and used for new antibiotic therapy (10, 12, 17, 20).

Although many types of biosurfactants including, glycolipids, rhamnolipids, sophorolipids, mannosylerythritol lipids, phospholipids, polymeric compounds, mycolic acids, and lipopolysaccharides are distinguished (27), it seems that lipopeptides represent remarkable biological activities, such as antibacterial, antifungal, antitumor, antiviral, and antiadhesive activities (15, 17). Environmental applications of biosurfactants were focused by many researchers and during the last decades, the biomedical field applications have been carried out. New more-active antifungal agents with fewer side effects are usually demanded by researchers, clinicians, and patients.

2. Objectives

In the present study, we evaluated the biosurfactant production ability of different strains of *Rhodotorula* species in laboratory conditions. Furthermore, the antifungal activity of produced biosurfactant was assessed against several fungi (molds and yeasts).

3. Methods

3.1. Organisms

In the present study, 54 strains of *Rhodotorula* species including, *R. glutinis* (48 strains), *R. mucilaginosa* (two strains), *R. minuta* (two strains), and *Rhodotorula* species (two strains) were examined for biosurfactant production. All strains had been already collected from different sources, identified, and kept in distilled water in the Department of Medical Mycology affiliated to Ahvaz Jundishapur University of Medical Sciences, Iran (28). All the tested strains were cultured on Sabouraud dextrose agar (SDA, Merck, Germany) slants and incubated at room temperature for four to five days for strains recovery.

3.2. Screening for Biosurfactant Production

Each of the 54 strains of four species of *Rhodotorula* was separately inoculated into 5 mL portions of Sabouraud dextrose broth (SDB, Merck, Germany) in test tubes and incubated at ambient temperature in a shaker incubator for four to six days. Then, the cultures were centrifuged at 3000 g for 10 minutes to obtain the cell-free broth supernatant containing biosurfactant. Biosurfactant production was confirmed by various standard methods including oil displacement (21), drop collapse (29), and haemolysin tests (30).

3.3. Biosurfactant Production on a Laboratory Scale

At this stage, only was one strain of *R. glutinis* selected with high ability to produce biosurfactant. The isolate was inoculated into several Erlenmeyer flasks (250 mL volume) containing 100 mL of SDB and incubated in the shaking incubator at 29°C for one week. The free cell supernatant was removed using centrifugation at 3000 g for 10 minutes. The same volume of supernatant (crude biosurfactant) and chloroform-methanol (2:1 v/v) were mixed, and then the biosurfactant was extracted by centrifugation (26, 31). The gather biosurfactant was dried and kept at -20°C until use.

3.4. Antifungal Assay

The antifungal activity of the biosurfactant was examined against different strains of *Candida albicans* (seven isolates), *R. glutinis* (two isolates), *Aspergillus niger* (one isolate), *Alternaria* sp. (one isolate), *Rhizopus* sp. (one isolate), and *Syncephalastrum* sp. (one isolate). The antifungal activity was evaluated by the disk diffusion method. Briefly, a standard suspension of each strain was prepared in sterile distilled water and then, 10 μL was spread on the surface of SDA plates in duplicate. Subsequently, 50 mg of the crude biosurfactant was dissolved in 1 mL of DMSO/ethanol completely. Finally, three blank disks were put on each plate and an aliquot of diluted biosurfactant (20, 40, 60, 80, 100, and 120 μL) was added into each disk. The plates were incubated at 29 and 35°C for molds and yeasts, respectively. The hyaline haloes (without fungal growth) around the disks were measured and the minimum inhibitory concentration (MIC) for each strain was calculated (Figure 1).

4. Results and Discussion

In the present study, although all the tested strains were capable of producing biosurfactant *in vitro*, the degree of biosurfactant was different among the strains. Out

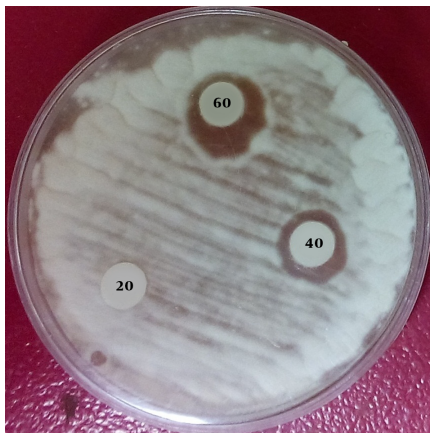

Figure 1. The antifungal activity of biosurfactant against *Aspergillus niger* after 48 h

of 54 *Rhodotorula* strains, only could four strains of *R. glutinis* comparatively show higher zones in the oil displacement test (2.1 - 2.5 cm) confirmed by drop collapse and haemolysin tests (Table 1). These results indicated that they had a high potential for biosurfactant production. Mahalingam and Sampath believe that the oil displacement technique is very sensitive for detecting biosurfactants even at low levels (29). In this test, a larger diameter represents a higher surface activity of the biosurfactant (32). *Rhodotorula* species are new sources for producing different biosurfactants. Extracellular glycoprotein biosurfactants from *R. glutinis* (24) and astaxanthin from *R. mucilaginosa* (25) are two types of biosurfactants that have been recently detected. Previous studies have shown that the biosurfactants produced by *R. glutinis* are composed of lipids (5).

Table 1. Biosurfactant Production by *Rhodotorula* Species Using Oil Displacement Test

Halo Diameter (cm)	*Rhodotorula*				
	R. glutinis	*R. minuta*	*R. mucilaginosa*	*Rhodotorula* Species	Total
> 0.5 (+1)	11 (20.4%)	0.0	0.0	0.0	11 (20.4%)
0.6 - 1 (+2)	10 (18.5%)	2 (3.7%)	1 (1.8%)	1 (1.8%)	14 (25.8%)
1.1 - 1.5 (+3)	14 (25.9%)	0.0	1 (1.8%)	1 (1.8%)	16 (29.5%)
1.6 - 2 (+4)	9 (16.7%)	0.0	0.0	0.0	9 (16.7%)
2.1 - 2.5 (+5)	4 (7.4%)	0.0	0.0	0.0	4 (7.4%)
Total	48 (88.9%)	2 (3.7%)	2 (3.6%)	2 (3.6%)	54 (100%)

Due to developing fungal resistance to antifungal agents, a lot of attention has been paid to new natural compounds with antifungal properties. In the present study, we found that the growth of all tested fungi including yeasts and molds strains completely was inhibited by 40 μL of the biosurfactant. Despite their potential for biomedical fields, only have a few studies been carried out on the antifungal activity of biosurfactants. Antifungal activities of biosurfactants produced by *Lactobacillus lactis* (13), *B. subtilis* (12), and *P. aeruginosa* (10) were investigated by several researchers. Furthermore, Ceresa et al. have shown that the biosurfactant produced by *L. brevis* has antibiofilm formation activity in *C. albicans* (33). A synergistic effect of surfactin with ketoconazole against *C. albicans* was also considered by Liu et al. (27). Furthermore, Halvaeezadeh and Zarei Mahmoudabadi showed that the produced biosurfactant by *R. paludigena* in combination with caspofungin have synergistic effects against *C. albicans* strains (26).

Anti-adhesive activity, permeabilizing ability, and cellular damaging ability are reported as the possible effective mechanisms of biosurfactants (13, 16, 20). Furthermore, biosurfactants could inhibit the adhesion of Candida to the silicon surface (33).

4.1. Conclusions

In conclusion, *Rhodotorula* species are applicable organisms for the production of biosurfactants and *R. glutinis* strains have the greatest ability to produce biosurfactants among other species. Furthermore, our results demonstrated that the biosurfactant produced by *R. glutinis* had a valuable potential for biopharmaceutical applications.

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Footnotes

Authors' Contribution: Study concept, design, and manuscript preparation: Ali Zarei Mahmoudabadi; experiment execution: Maral Gharaghani; data analysis: Marzieh Halvaeezadeh.

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