Article

Evaluation laboratory produced biosurfactant by *Rhodotorula* species and its antifungal activity

Maral Gharaghani[1,2], Marzieh Halvaeezadeh[1] and Ali Zarei Mahmoudabadi[1,2*]

[1] Infectious and Tropical Diseases Research Centre, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

* Correspondence: zarei40@hotmail.com; Tel: +9861 33330074

Abstract: Background: Biosurfactants are amphiphilic surface active compounds that produced by several microorganisms, including, bacteria and fungi. Biodegradability, low toxicity, applications diversity and functionality under extreme conditions are characterized them from chemically biosurfactants. It is found that, *Rhodotorula* species, read yeasts, have high potency for biosurfactant producing. Recently, antimicrobial activities of biosurfactants have been subjected for new antibiotic therapy. The aim of the present study was to evaluate biosurfactant production by the different strains of *Rhodotorula* species in laboratory conditions. In addition, antifungal activity of produced biosurfactant was assessed against several saprophytic fungi. In the present study 54 strains of *Rhodotorula* including, *R. glutinis* (48 strains), *R. minuta* (2 strains), *R. mucilaginosa* (2 strains) and *Rhodotorula* species (2 strains) were screened for biosurfactant production. The biosurfactant was produced using the Sabouraud dextrose broth medium and confirmed by specific tests. Antifungal assay was also evaluated by disk diffusion method and the serial dilutions of biosurfactant. In the present study, although all tested strains were capable to produce biosurfactant *in vitro*, the degree of biosurfactant production was varied among stains. 7.4% strains had the highest (+5) biosurfactant activity followed by 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 biosurfactant. *Rhodotorula* species are appropriate organisms for the production of biosurfactants and *R. glutinis* strains have the greatest ability to producing biosurfactant than another species. Furthermore, our results were demonstrated that the produced biosurfactant by *R. glutinis* presented a valuable potential for biopharmaceutical applications.

Keywords: Biosurfactant; *Rhodotorula glutinis*; Antifungal activity; Saprophytic fungi

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1. Introduction

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

Several reports were indicated that bacterial species such as *Acinetobacter* [9], *Pseudomonas* [10, 11], *Bacillus* [12], *Lactococcus* [13] and *Nocardia mediterranie* [14] are applicable biosurfactants sources. Several researches have shown that bacterial biosurfactants possess antimicrobial properties [9, 10, 15, 16]. Mostafapour et al. have shown that a biosurfactant produced by *Acinetobacter* species has

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anti-Gram positive and negative bacteria effect *in vitro* [9]. In addition, Gomaa was examined the biological properties of biosurfactant produced by *B. licheniformis* and found that it has a great potential for the biotechnological and biopharmaceutical applications [17]. Moreover, it was found that there is a synergistic effect against plant pathogenic fungi and pathogenic bacteria when silver nanoparticles used with biosurfactant [18]. Furthermore, Basit et al., were recommended lipopeptide biosurfactants produced by *B. cereus* as safe antimicrobial and antioxidant agent [19].

Producing biosurfactants by fungi are 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 mainly composed biosurfactants, hence they have new possibilities for industrial application. The antibacterial, antifungal and antiviral activities of several biosurfactants have been reported by researchers and subjected 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 were distinguished [27], it seems that lipopeptides, represent remarkable biological activities, such as antibacterial, antifungal, antitumor, antiviral and antiadhesive activities [15, 17]. Environmental applications of biosurfactant were focused by many of researchers and during the last decades, related applications to the biomedical field have been carried out. New more active antifungal agents with fewer side effects are usually demanded by researchers, clinicians and patients.

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

2. Materials and Methods

2.1. Ethics statement

The present study protocol was reviewed and approved by the Ethical and Research Committee of Ahvaz Jundishapur University of Medical Sciences (ethic number: 92151).

2.2. Organisms

In the present study 54 strains of *Rhodotorula* species including, *R. glutinis* (48 strains), *R. mucilaginosa* (2), *R. minuta* (2) and *Rhodotorula* species (2) were examined for biosurfactant production. All strains were previously collected from different sources, identified and kept at distilled water in the Department of Medical Mycology affiliated to Ahvaz Jundishapur University of Medical Sciences, Iran [28]. All tested strains were cultured on Sabouraud dextrose agar (SDA, Merck, Germany) slants, incubated at room temperature for 4-5 days for strains recovery.

2.3. Screening for biosurfactant production

Each of 54 strains of four species of *Rhodotorula* was separately inoculated into 5 mL of Sabouraud dextrose broth (SDB, Merck, Germany) test tube and incubated at ambient temperature at the shaker incubator for 4-6 days. Then, cultures were centrifuged at 3000g for 10 minutes to obtain the cell free broth supernatant containing biosurfactant. Biosurfactant production was confirmed with various standard methods i.e. oil displacement [21], drop collapse [29], and haemolysin tests [30].

2.4. Biosurfactant production on a laboratory scale

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

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2.5. Antifungal assay

The antifungal activity of biosurfactant was examined against different strains of *Candida albicans* (7 isolates), *R. glutinis* (2 isolates), *Aspergillus niger* (1 isolate), *Alternaria* sp. (1 isolate), *Rhizopus* sp. (1 isolate), and *Syncephalastrum* sp. (1 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 10µL spread on surface of SDA plates, duplicatly. Subsequently, 50mg of crude biosurfactant was dissolved in 1ml of DMSO/ethanol completely. Finally, 3 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. Plates were incubated at 29 and 35ºC for moulds and yeasts, respectively. The hyaline haloes (without fungal grow) around the disks were measured and minimum inhibitory concentration (MIC) for each strain calculated (Figure 1).

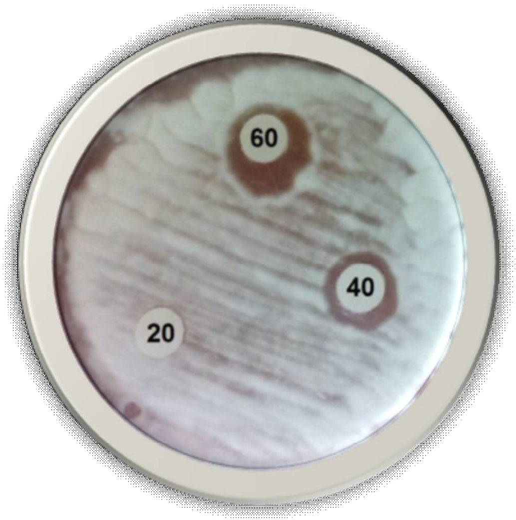

Figure 1: The antifungal activity of biosurfactant against *Aspergillus niger* after 48 h

3. Results and Discussion

In the present study, although all tested strains capable to produce biosurfactant *in vitro*, the degree of biosurfactant was different among stains. Out of 54 *Rhodotorula* strains, only 4 strains of *R. glutinis* comparatively showed higher zones in oil displacement test (2.1-2.5 cm) that was confirmed by drop collapse and haemolysin tests (Table 1). These results are indicated that they have a highly potential for biosurfactant production. Mahalingam and Sampath have been believed that the oil displacement technique is very sensitive for detecting biosurfactant even in low level amounts [29]. In this test, a larger diameter represents a higher surface activity of biosurfactant [32]. *Rhodotorula* species are new sources for producing different biosurfactant. Extracellular glycoprotein biosurfactant from *R. glutinis* [24] and astaxanthin from *R. mucilaginosa* [25] are two types of biosurfactants that were recently detected. Previous studies have shown that the components of biosurfactants produced by *R. glutinis* composed of lipids [5].

Table 1. Biosurfactant production by *Rhodotorula* species using oil displacement test.

	Rhodotorula	***R. glutinis***	***R. minuta***	***R. mucilaginosa***	***Rhodotorula* species**	**Total**
Halo diameter (cm)	>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 to the search of new natural compounds with antifungal properties has been increased. In the present study, we found that the growth of all tested fungi including, yeasts and moulds strains completely inhibited by 40µl of biosurfactant. Despite their potential for biomedical fields, only a few studies have been carried out on 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 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 were shown that the produced biosurfactant by *R. paludigena* in combination with caspofungin have synergistic effect against *C. albicans* strains [26].

Anti-adhesive activity, permeabilizing ability, and cellular damaging ability were reported as possible effective mechanism of biosurfactants [13, 16, 20]. Furthermore, biosurfactant could be inhibit the adhesion *Candida* to silicone surface [33].

4. Conclusions

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

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