
Disruption of *Candida albicans* Biofilms by Rhamnolipid Obtained from *Pseudomonas aeruginosa* RT

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Abstract: *Candida albicans* is an opportunistic human pathogen that causes candidiasis. With a worldwide improvement in healthcare, the number of immunocompromised patients has increased to a greater extent and they are highly susceptible to various pathogenic microbes especially *C. albicans* which has been prominent among the fungal pathogens. Earlier work has shown that rhamnolipids are efficient dispersants of bacterial biofilms. However, their effectiveness studies against fungal pathogens are limited. The aim of this study was to determine the effect of rhamnolipid on a biofilm forming strain of *C. albicans*. Two chemical surfactants, sodium dodecyl sulphate (SDS) and cetyl-trimethyl ammonium bromide (CTAB) were used as controls. Microtiter plate assay showed that the surfactant coating decreased *C. albicans* biofilm formation by 55%. Rhamnolipid treatment disrupted pre-formed biofilms in a more effective manner than the other two surfactants. The disruption of biofilms after rhamnolipid treatment was significant when compared to SDS and CTAB. The results indicate a potential application of the rhamnolipid to disrupt *C. albicans* biofilms

Keywords: Biofilm, CTAB, Rhamnolipid, SDS, *C. albicans*

1. INTRODUCTION

Candida albicans is an opportunistic fungal pathogen that is present as a harmless commensal in the gastrointestinal and genitourinary tracts in about 70% of human population. Approximately 75% of women suffer from *Candida* infection at least once in their lifetime [1–4]. For some immunologically weak individuals or even for healthy persons it becomes opportunistic pathogen. *Candida spp.* are responsible for causing mucosal infections known as thrush which is characterized by white spots in the infected membranes. However, it can also cause life-threatening, systemic infections in severely ill patients with a mortality rate about 30% [5–8]. It is well known that microorganism's growth in the biofilm mode often resist a variety of antimicrobial agents. There is thus a need to explore other means of disrupting biofilms. Biocides and surfactants have already been used to control biofilms [9]. Chemical surfactants have various applications in areas of medical care settings. For example, cetyl trimethyl ammonium bromide (CTAB) is used in various medical settings as disinfectant agent [8]. Sodium dodecyl sulfate (SDS) leads to leakage of cellular contents from microorganisms [9]. Widespread use of chemical surfactants is not favored due to their inherent toxicity. Biosurfactants are being favored in this context [10]. Biosurfactants offer several advantages in being relatively non-toxic, effective under various environmental conditions and being bio-compatible [11, 12]. Biosurfactants have been used to disrupt bacterial biofilms [13, 14]. However the reports on the efficacy of biosurfactants on fungal biofilms are very limited [15]. *Candida albicans* forms biofilms on a variety of substrates. The objective of this work was therefore to test the effectiveness of rhamnolipids in (i) preventing biofilm formation and (ii) in disrupting pre-established biofilms of *C. albicans*, the results have been compared with two chemical surfactants.

2. MATERIALS AND METHODS

2.1. Chemical Agents

Purified Rhamnolipid biosurfactant, previously isolated in our laboratory was used. CTAB and SDS were obtained from HiMedia, India. Stock solutions of the surfactants were prepared in sterile distilled water.

2.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration values (MFC)

MIC of surfactants against *C. albicans* was determined by broth microdilution assay in sterile 96 well microtiter plates (Tarsons, India) [15]. 36 hr. old cells of *C. albicans* were added to the microtiter plate wells containing Yeast Extract Peptone Dextrose (YEPD) medium to achieve the final cell numbers of 1×10^7 cells ml^{-1} . Surfactants (Rhamnolipid, SDS and CTAB) were added to these wells at different concentrations (0.05% w/v to 10% w/v). The final volume in the microtiter plate wells was maintained to 200 μl . The plates were incubated for 48 hr. at 30°C and after the incubation period, growth in presence of the surfactants was estimated as O.D. 600 using a microtiter plate reader (Multiskan, Thermo Lab systems). Control used were wells without surfactants and those lacking the cells. The MFC was determined by streaking the culture grown in presence of various concentrations of the surfactant on YEPD agar plates. The plates were incubated for 48 hr. and growth was recorded. MIC was determined as the lowest concentration without visible growth and the MFC as the lowest concentration showing no growth on the agar surface. All the experiments were performed in triplicate and the mean values were determined.

2.3. Effect of Surfactant Pre-Coating on Biofilm Formation

Rhamnolipid, SDS and CTAB (100 μl containing 0.3% w/v -10% w/v concentration) were added to wells of the polystyrene microtiter plates and incubated at 4°C for 12 hr. to facilitate effective coating [16]. After the incubation period, the wells were emptied of surfactants, rinsed with sterile distilled water and air dried in a laminar air flow chamber for 5 min. Cells (100 μl containing 1×10^7 cells ml^{-1}) of *C. albicans* were added to the microtiter plate wells and incubated for 24 hr. at 30°C . After the incubation period, the microtiter plate wells were emptied of the non-adherent cells and the plates were rinsed with sterile distilled water. Quantification of adherent cells was done using crystal violet assay [14]. All experiments were carried out in triplicates and average values were obtained.

2.4. Disruption of Preformed Biofilms

C. albicans biofilms were allowed to form in sterile polystyrene 96 well microtiter plate wells for 36 hr. [17]. Planktonic cells were removed after the incubation period, and varying concentrations (0.3 w/v -10% w/v) of Rhamnolipid, SDS and CTAB were individually added to the wells. The plates were further incubated at 30°C for 1, 2 and 3 hr. The microtiter plate wells were emptied of the non-adherent cells and rinsed with sterile distilled water. Quantification of residual biofilms was performed using crystal violet assay [15]. All experiments were performed in triplicates and average values were obtained.

3. RESULTS AND DISCUSSION

3.1. MIC and MFC Values of Surfactants

Rhamnolipid, SDS and CTAB displayed antifungal activity against the cells of *C. albicans* MTCC 227. Rhamnolipid displayed a minimum inhibitory concentration (MIC) of $5.0\% \pm 0.01$ w/v and minimum fungicidal concentration (MFC) value $>10\% \pm 0.05$ w/v, CTAB showed MIC and MFC values of $7.5\% \pm 0.03$ w/v and $>10\% \pm 0.1$ w/v while SDS showed MIC and MFC values of $1.25\% \pm 0.02$ w/v. SDS was more effective as an antifungal agent compared to rhamnolipid and CTAB. SDS is an anionic surfactant and is known to possess antimicrobial properties [18]. The surfactant permeabilizes cells by targeting the cell membranes and by affecting membrane bound enzymes [18]. Rhamnolipids are anionic in nature and they disrupt cells by interacting with the phospholipid components of the biological membranes [18, 19]. Rhamnolipids derived from *Pseudomonas aeruginosa* are known to possess antifungal activity against some plant pathogenic fungi [20]. A cationic surfactant, CTAB displayed lower antifungal activity towards *C. albicans* as compared to SDS or rhamnolipids. Lower antifungal activity of CTAB could be a result of reversal of fungal cell surface charge and not due to cell lysis, as observed with SDS [10].

3.2. Effect of Surfactant Pre-Coating on Biofilm Growth

Microbial surfactants are known to exhibit anti-adhesive properties [12, 13, and 21]. Pre-coating of microtiter plate wells with the surfactants effectively reduced the development of *C. albicans*

biofilms. Adhesion of *C. albicans* cells to the microtiter plate wells was inhibited to 50% with rhamnolipids at MIC concentration (5%). Rhamnolipids showed significant anti-adhesive ability as compared to SDS and CTAB suggesting the potential of rhamnolipids as anti-adhesive agents in the treatment of fungal biofilms.

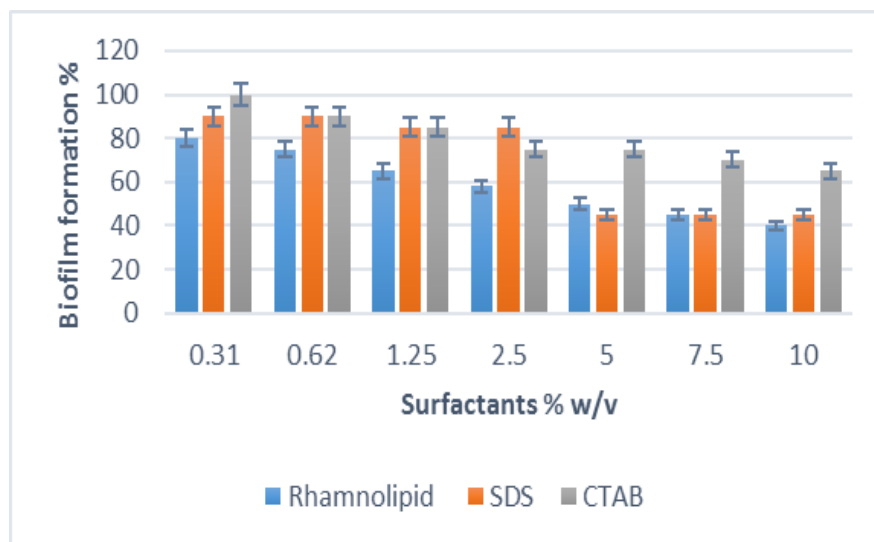


Fig A. Inhibition of *C. albicans* biofilms by surfactants. Observations in microtiter plate wells after pre-coating with different concentrations of Rhamnolipid, SDS or CTAB. The O.D. values are normalized with reference to control (considered as 100%).

3.3. Disruption of Preformed Biofilms of *C. Albicans*

The pre-formed biofilms of *C. albicans* were treated with the surfactants for 1, 2 or 3 hr. Biofilms of *C. albicans* formed for 36 hr. in microtiter plate wells were disrupted effectively (52% with rhamnolipid, 37% with CTAB and 45% with SDS at respective MIC values) within 1 hr. of treatment with the surfactants. At higher concentrations (>2.5%), the effect of SDS was slightly better than that of the rhamnolipid. However, over a period of time, the efficacy of rhamnolipids was found to be similar to that of SDS (Figure 3b and 3c).

CTAB was less effective in controlling biofilms. Rhamnolipids have earlier shown to be effective against bacterial biofilms [15], however there are limited reports on their effect on fungal biofilms. In the present study rhamnolipids were found to be effective in disrupting biofilms of *C. albicans* compared to SDS and CTAB, suggesting their potential application as biofilm disrupting agents (Figure 4). The effectiveness of rhamnolipids against biofilms even at low concentrations makes them a good candidate for therapeutic applications.

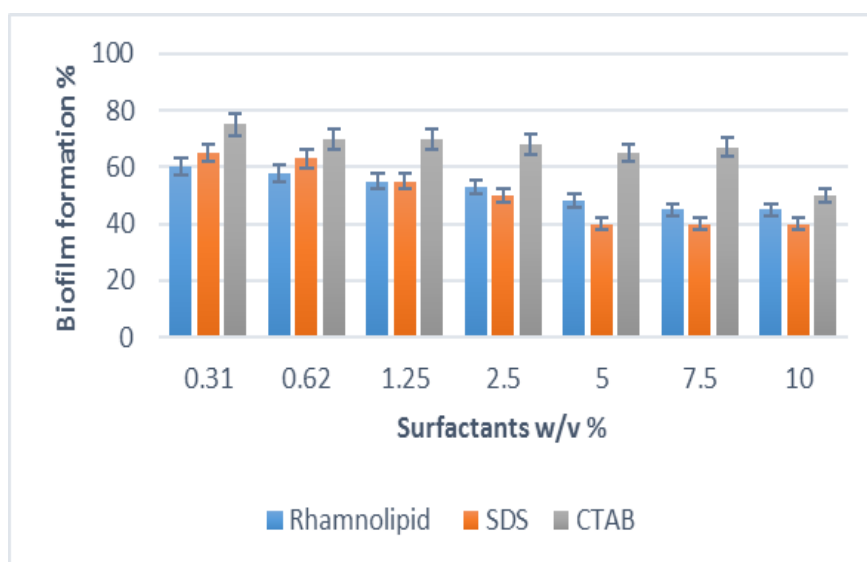


Fig B

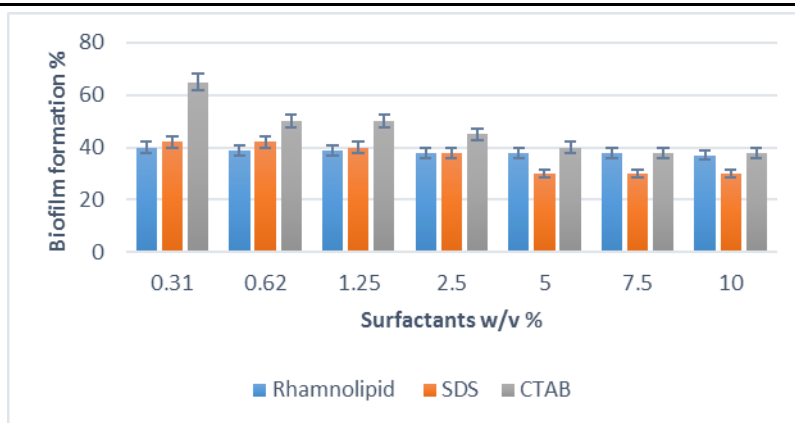


Fig C

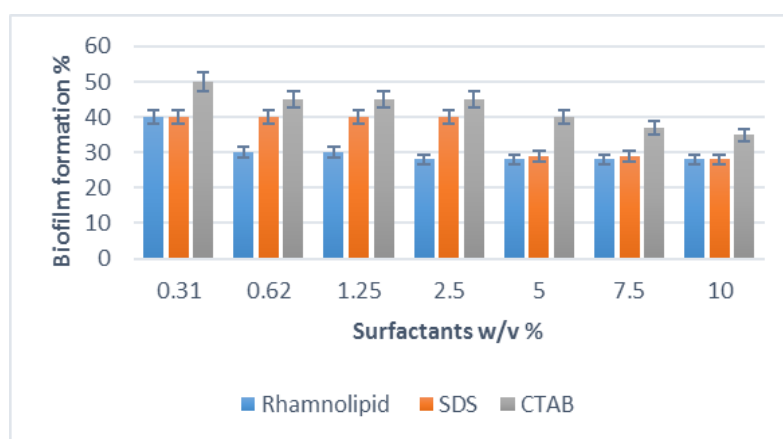


Fig D

Effect of surfactants on preformed biofilms of *C. albicans*. Observations with rhamnolipid, SDS and CTAB after (Fig. B) 1 h (Fig. C) 2 h and (Fig. D) 3 h of incubation.

4. CONCLUSIONS

Rhamnolipids have potential to disrupt *C. albicans* biofilms as compared to the tested chemical surfactants. The results suggest potential of rhamnolipid as anti-adhesive and pre-formed biofilm disrupting agents and their possible role in the treatment of fungal infections.

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