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Novel biosurfactants are effective in inhibiting fungal and bacterial biofilm formation on medical grade silicone in vitro

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Background: Biosurfactants (BS), produced by bacteria, have gained importance thanks to their ability to affect the adhesion properties of cells/microorganisms, thus emerging as a potential new generation of anti-biofilm agents. Coating of medical grade materials with these compounds can be a preventive strategy to limit pathogenic biofilm growth on the surface of medical devices entering sterile body districts. This study aimed at assessing *in-vitro* fungal and bacterial antibiofilm activity of two novel BS deposited on medical grade silicone.

Material/methods: AC7BS and R89BS were extracted from cultures of *Bacillus subtilis* AC7 and *Pseudomonas aeruginosa* 89 respectively, as reported by Rivardo et al. 2009. Sterilized medical-grade silicone elastomeric disks were BS coated by physical absorption (dipping in 2 mg/ml AC7BS or R89BS solutions for 24 h and drying). Control disks were sterilized, but uncoated. Fungal biofilm on treated and control disks was formed as described by Ceresa et al. 2016. Bacterial biofilm was formed by incubating at 37°C each disk in 1 ml of a bacterial suspension (1×10^7 CFU/ml)

in TSB (*S. epidermidis*) or TSB+1% glucose (*S. aureus*). The anti-biofilm activity was evaluated after 1.5, 24, 48 and 72h for *Candida albicans* IHEM 2894, and after 24 and 48h for *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 35984. Biofilm biomass and cell viability were quantified by crystal violet staining and MTT assay. The surface coated by biofilm was quantified by scanning electron microscopy (SEM).

Antifungal activity of BS was evaluated according to EUCAST. Anti-bacterial activity of BSs was detected according to Wiegand *et al.* 2008.

Inhibition in biofilm biomass and cell viability was calculated for disks coated by AC7BS and R89BS in respect to uncoated control samples.

Student's t-test was used to compare treated to control disks at different incubation times. Results were considered significant when $p < 0.05$

Results: Both AC7BS and R89BS were able to significantly counteract fungal biofilm (Figure 1a,d) and biomass was more inhibited than cells viability. A significant inhibition was also observed on *S. aureus* biofilms (Figure 1b,e), with similar values for biomass and cells viability. *S. epidermidis* biofilm formation was mostly reduced in terms of cells viability than of biofilm biomass (Figure 1c,f). SEM data were qualitatively in accordance. AC7BS did not show biocidal activity against any test strain. R89BS had antimicrobial activity against planktonic *S. aureus*, but no biocidal effect on the other strains.

Conclusions: AC7BS and R89BS are able to significantly inhibit both fungal and bacterial biofilm formation on medial grade silicone up to 72h for *C. albicans* IHEM 2894 and 48h for *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 35984. This relatively long-term effect on both fungal and bacterial strains make these BS promising compounds for limiting biofilm formation on medical device.

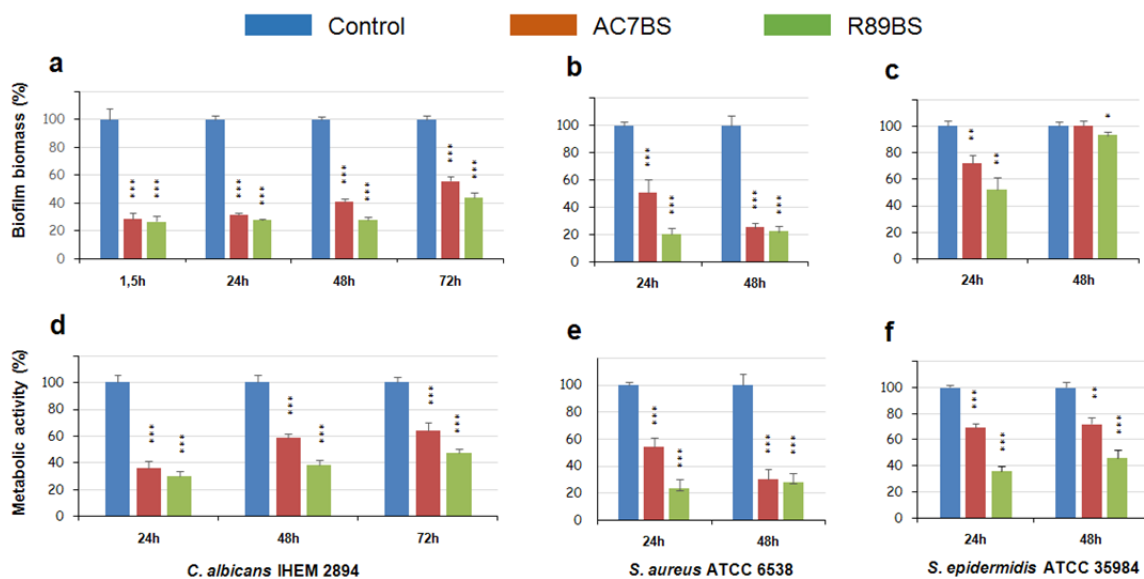


Figure 1: The antibiofilm activity of AC7BS and R89BS coated silicone disks. Inhibition of biofilm biomass and metabolic activity is reported as percentage of controls (uncoated disks). Timepoints refer to biofilm maturation age. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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