Exploring Fluorescent Benzimidazo[1,2-α]quinolines for Candida spp. Biofilm Detection and Biocidal Activity

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The Problem 1

The Biofilm plays a major role in enhancing microbiological resistance and is a widely recognized problem in medical areas, because of persistent, recurrent and device related infections, principally. Moreover, in industries biofouling in the drink water distribution system and food processing is an additional risk to human health. This protective strategy used by bacterial and fungal species combines microbial colonies and a self-produced extracellular polymeric matrix. This matrix consists of lipids, nucleoids acids, proteins and saccharides (COSTA, 2013). With this structure (Figure 1) is possible for microorganisms to protect their cells against physical and chemical agents, preventing the penetration of various antimicrobial agents (STORTI, 2007; CERQUEIRA, 2013).

In this context, biofilms promote drug resistance being another obstacle for patient treatment and environment sanitation. Consequently, it is important for health facilities to control and eradicate biofilms existing on surfaces of medical and surgical instruments (FLEMMING, 2002).

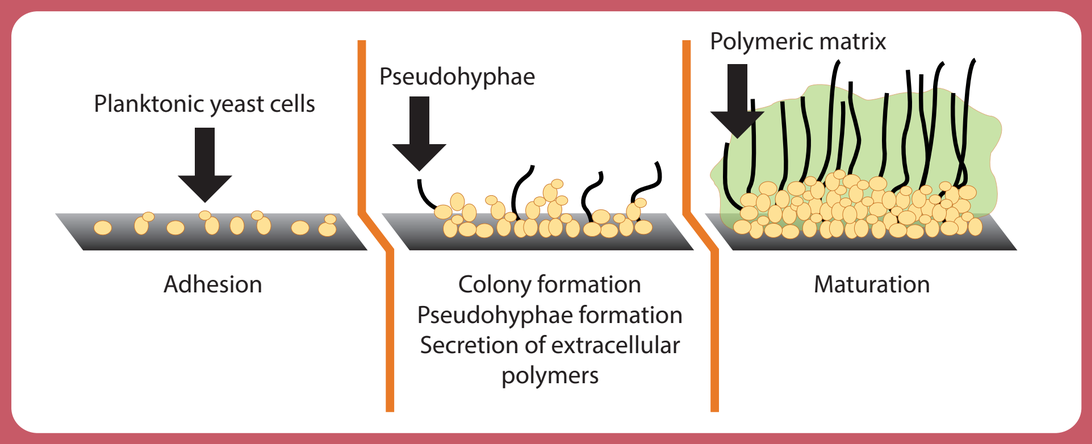

Figure 1: Biofilm growing on a surface

The Aim 2

In this study, the main goal was to find a substance that both detect and eradicate biofilms over stainless steel surfaces. We used different fluorescent benzimidazoles (Figure 2) that were tested over fungi cells following the methology of figure 3.

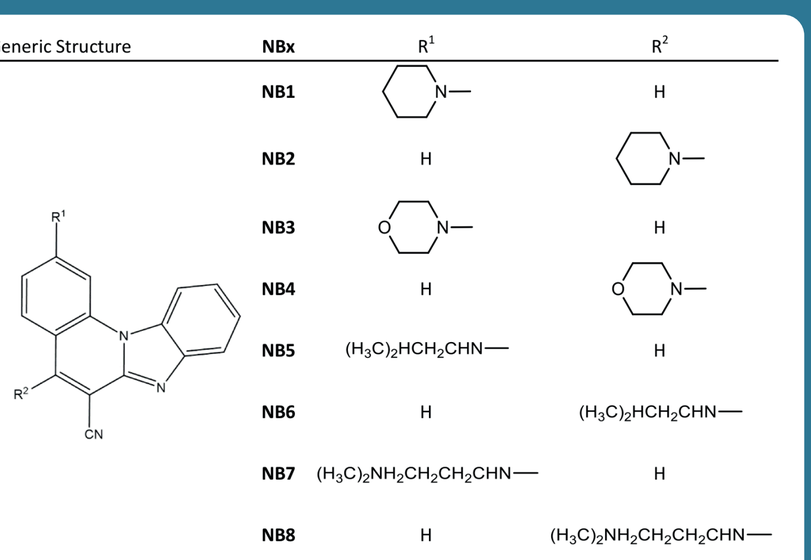

Figure 2: Chemical structures of the fluorescent benzimidazo[1,2-a]quinolines NB1-9 evaluated in this work.

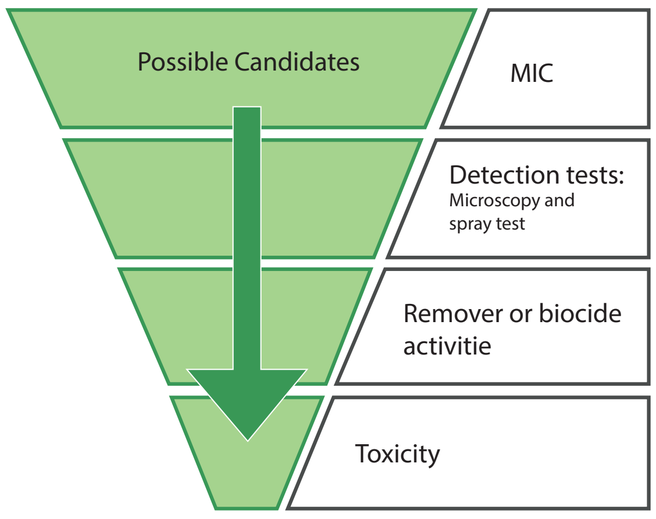

Figure 3: Methodology for testing substances.

The Methods 3

Minimum Inhibitory concentration (MIC): this test was made following the CLSI protocol (M27-S3) and shows the concentration of substance that inhibits the yeast proliferation.

Microscopy: We inoculated 20uL of a 10^6 *Candida* sp. suspension over the surface of glass plate and incubated for 24h. On growing cells is inoculated the substance, then this glass plate is observed with microscopy showing if substance is capable of mark cells, when using fluorescent substances was used a florescent microscope.

Spray test: We sprayed the substance over the surface of the stainless steel with biofilm grown observing if the substance was capable of showing the contamination. When the substance was fluorescent is used a fluorescent 254nm filtered lamp.

Determination of the capacity to remove the biofilm and/or to eradicate it: on the first part, the stainless steel plate with biofilm was immerged in the substance for a determined time, then an aliquot was removed and inoculated on sabouraud agar medium, then incubated for 24h and 32ºC. Thus, on the second part the plate with the remaining biofilm, strongly attached on the plate, was immerged in peptone water and with sonication was possible to remove the remaining biofilm. An aliquot was removed and was inoculated on sabouraud agar, then incubated for 24h and 32ºC. A positive control without the substance was necessary to validate the test. If the substance eradicates biofilms, neither the first part of the experiment nor the second shows microorganism proliferation, and when there is a proliferation on agar on first part, the substance can be capable of remove biofilm but not eradicates it, we can also compare with the control results (Figure 4).

Toxicity: cytotoxic tests were performed by Federal University of Pampa, they are based on the capacity of the substance to destroy leucocyte cells and fragmenting of deoxyribonucleic acid (ADN).

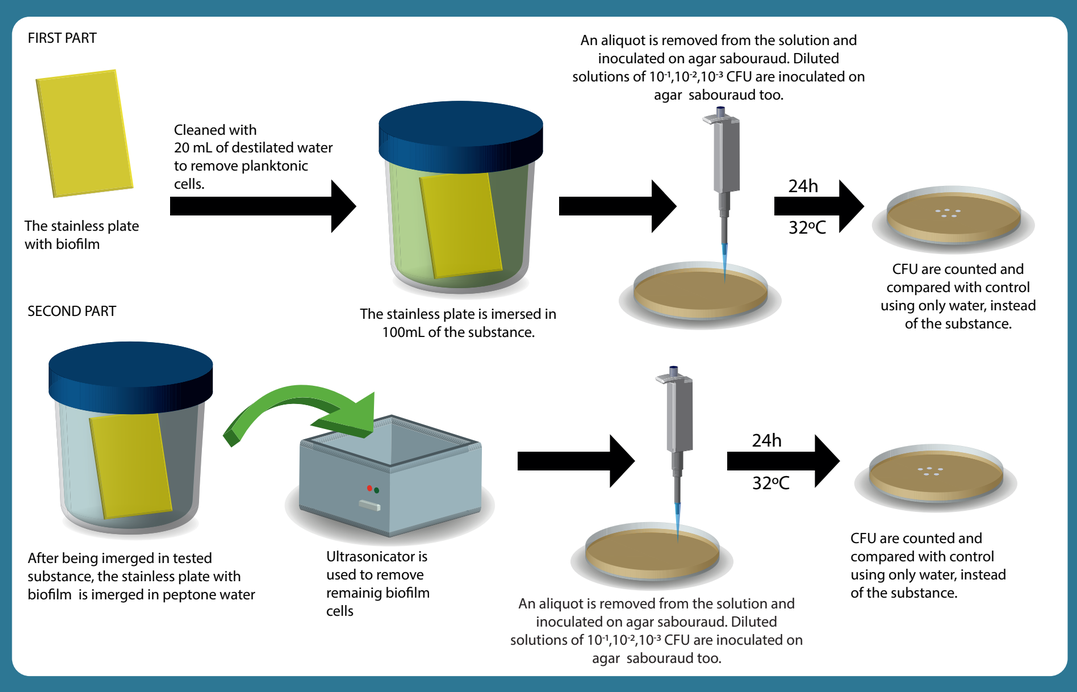

Figure 4: Scheme of eradication and/or remover test.

The Results 4

Of all the nine substances, only one (NB7) was able to both detect biofilms and have a biocidal activity against it. In collaboration with the University of Zagreb we synthetized a fluorescent substance that marks and eradicate biofilms from three species of Candida tested, including ATCC and clinical strains (Figure 5 and 6).

NB7 showed antifungal activity against all tested Candida strains. Spraying of NB7 over Candida sp. biofilm contaminated stainless steel 304 plates resulted in the successful detection of the biofilms under UV light. At the same time, NB7 showed the potential to eradicate the detected biofilms. Altogether, benzimidazole NB7 has the potential to guarantee the use of disinfected medical and surgical instruments in clinical and surgical procedures, consequently, contributing to an increased safety for patients.

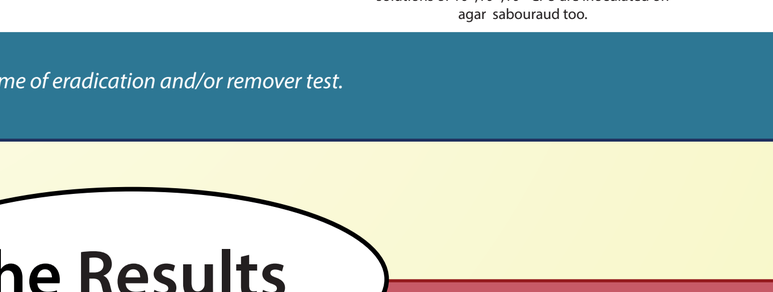

Figure 5: Fluorescent microscopy showing fungi cells (ATCC 18804). On the left using Blue filter and on the right using Violet filter.

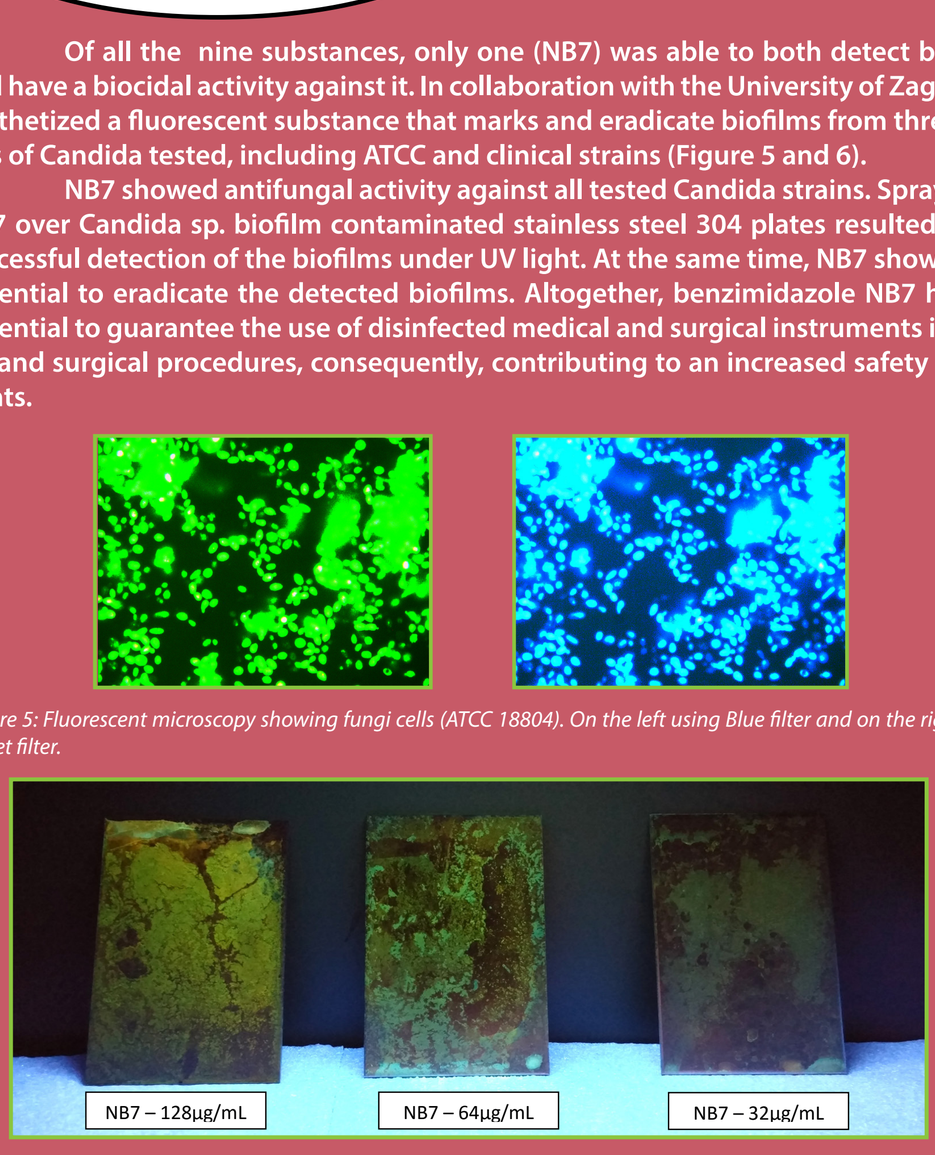

Figure 6: Different concentrations for biofilm revelation over surface with fluorescent substance (ATCC 950).

References

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