Effect of antifungal agents on Non-Candida albicans Candida species enzymes secretion

M. Negri, T. Lorenço, S. Silva¹, M. Henriques¹, J. Azeredo¹ and R. Oliveira¹
¹Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

The infective ability of Candida species depends on specific virulence mechanisms that confer the ability to colonize host surfaces, to invade deeper host tissue or to evade host defences. During the pathogenic process many virulence attributes may be involved including, production of extracellular proteases and haemolytic activity. Nevertheless, in vitro studies have indicated that antifungal agents could be able to influence the enzymatic activity of Candida species. Therefore, the purpose of this work was to investigate the action of antifungals on protease and haemolytic activity of Candida species.

This study was conducted with C. albicans (1), C. glabrata (4), C. parapsilosis (5) and C. tropicalis (6) recovered from different body sites (blood, oral, vaginal and urinary tract). Four reference strains of C. albicans ATCC 90028, C. glabrata ATCC 2001, C. parapsilosis ATCC 22019 and C. tropicalis ATCC 750 were also examined. The susceptibility to fluconazole and amphotericin B was determined by the microdilution test in order to allow the determination of the minimal inhibitory concentrations (MIC) and the maximum antifungal concentration (MAC). Then, the proteinase and hemolytic activity was determined for yeasts grown at MIC and MAC.

It was observed that all Candida species assayed were sensible to both antifungal agents. Concerning the antifungal effect on enzymatic activity of Candida species, C. parapsilosis from candiduria presented a decreased proteinase and haemolysin activity for both MIC and MAC of both antifungal agents. Moreover, the other species presented differences in terms of production of proteinase and haemolysin at MIC and MAC. Candida albicans reference strain presented lower protease activity at MIC of fluconazole (46.7%) but presented higher activity for MAC (61.9%) in comparison to the control (60%). Furthermore, regarding haemolysin activity there were isolates that expressed high levels of enzymes in the presence of both antifungals such as: C. glabrata from urine and from vaginal tract; and C. tropicalis from urine. Conversely, some clinical isolates, presented low levels of enzymatic activity after contact with the antifungal agents, such as: C. albicans (oral isolate); C. glabrata (oral isolate and vaginal isolate); C. parapsilosis (from urine) and also all C. tropicalis except one urinary isolate.

It was possible to conclude that the proteinase and haemolysin activities were strain and species dependent and no correlation was found among activity profile and the site of isolation. Moreover, fluconazole and amphotericin B were able to influence the tested Candida species enzymatic activity.

Keywords: Candida; Fluconazole; Amphotericin B; Proteinase; Haemolytic activity

Effect of chitosan, nisin and storage time on the growth of Listeria innocua and Shewanella putrefaciens in fish homogenates

L. I. Schelegueda¹, M. F. Gliemmo¹, ² and C. A. Campos¹, ²
¹Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Universitaria, Capital Federal, 1428, Argentina.
²CONICET

Fish and other fishery products are highly perishable food. The process of degradation starts immediately after the fish’s slaughter and it often develops more quickly than in other meats. Therefore, the use of a preservation method is essential when it comes to the mentioned products. The use of natural antimicrobials together with other preservation measures has proved to be a good option to extend the shelf life of this kind of food.

The aim of this study was to evaluate the effect of chitosan, nisin and storage time on the inhibition of Listeria innocua and Shewanella putrefaciens growth in fish homogenates. For such purpose a factorial design in three blocks was performed, on which the concentration of chitosan and nisin, and the storage time were the factors studied. The selected levels were: chitosan (0, 2000 ppm), nisin (0, 5000 ppm) and storage time (0, 48 hours). A central point was included (chitosan 1000 ppm, nisin 2500 ppm, storage time 24 hours).

L. innocua was selected as a representative microorganism of Gram-positive flora and as an alternative to the pathogen Listeria monocytogenes. S. putrefaciens, which is one of the typical spoilage microorganism of fishery products when they are stored in cold and aerobic conditions, was selected as the Gram-negative bacterium representative.

Fish homogenate was prepared processing Argentine hake fillets (Merluccius hubbsi) and distilled water in a ratio of 1:1; pH was adjusted to 5.5 using citric acid 10% w/v and homogenate aliquots were put into screw-cap flasks. They were sterilized for 15 minutes at 100ºC. Preservatives were added and then microorganisms were inoculated reaching a level of 105 CFU g⁻¹. The microorganisms had been previously incubated in Mueller-Hinton broth, overnight at 30ºC. The inoculated homogenates were stored at 30ºC for 48 h. S. putrefaciens and L. innocua populations were enumerated by pour-plating in TSA agar and Mueller-Hinton agar, respectively. The plates were incubated for 48 hours at 30ºC.

As it was expected, in all cases, time exerted a significant effect on the growth of both microorganisms. When antimicrobials were added, counts were significantly reduced (P < 0.05). A significant interaction (P < 0.05) between antimicrobials and time was noted; however, this effect depended on the microorganism being tested. In the case of S. putrefaciens, both preservatives reduced growth during storage; on the other hand, the development of L. innocua was reduced by chitosan during storage, but it increased in the presence of nisin. There was no significant interaction between the two preservatives. However, different trends in respect of time are noted. At time zero, nisin alone or together with chitosan reduced the counts of both microorganisms between 2 and 3 log cycles. After 48 hours of storage, both antimicrobials showed an additive effect reducing the development of both microorganisms between 4 and 5 log cycles.

To sum up, nisin and chitosan were able to inhibit the development of S. putrefaciens and L. innocua in fish homogenate at pH 5.5. It is clear that nisin controlled the growth of S. putrefaciens, Gram-negative bacterium. Although the interaction was not significant, the joint use of the studied antimicrobials could be promising, since the nisin’s effects on counts are shown immediately after its application, while chitosan’s action can be noticed throughout the storage.

Keywords: nisin, chitosan