

Title: Screening of yeasts from Antarctic continent for L-asparaginase production

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Abstract:

L-Asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonium ion. This is an important chemotherapeutic agent for management of acute lymphoblastic leukaemia (ALL) and other hematopoietic malignancies. The enzyme has been extensively investigated since it was found that the enzyme from certain microorganisms has antitumor activity. Eukaryotic microorganisms such as yeast and filamentous fungi have the potential to produce less adverse effects of L-asparaginase. The production of some asparaginases by yeasts has been reported, and they present some characteristics that make them attractive for the production of biopharmaceuticals, since they grow relatively fast in relatively cheap and simple cultivation medium. Additionally, enzymes from Antarctic's yeast present special properties as high specific activity and sometimes these are located at the periplasmic space. In this study, 41 yeasts isolated from different location at Antarctic continent were employed to perform a screening of L-asparaginase production. Initially, the enzyme production was tested at 15 °C for 96 h in plates with Czapek Dox medium (pH 5.5) supplemented with 2% agar and 0.007% bromothymol blue. The plates were inoculated with yeast strains previously cultivated on potato dextrose (PDA) medium at 15 °C for 72 h. Uninoculated plates were set as control. The diameter of the blue color halo was measured and five yeasts strains with biggest diameter of halo were selected to produce L-asparaginase in liquid medium. The selected strains were cultivated in 50 mL of Czapek Dox medium (pH 5.5) at 15 °C and 150 rpm for 96 h inoculated with 1.5 (DO_{600 nm}) of cell cultivated in PDA medium at 15 °C for 72 h. Then, the asparaginase activity was measured in whole cell suspensions (periplasmic activity) by the method based on asparagine hydroxylaminolysis. The best activity of L-asparaginase (37 UI.g⁻¹ dry biomass) was produced by the yeast identified as *Leucosporidium scotti*. This yeast is a psychrotolerant or psychrophilic organism, and has been isolated from rather cold environments around the world, including terrestrial and marine Antarctic ecosystems. To date, *L. scotti* has not been reported as L-asparaginase producer. Therefore, yeast from Antarctic continent could be an important eukaryotic source of L-asparaginase.