Supplementary information

Fundamental of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methodologies

The antimicrobial effect of cell free supernatants (CFS) was evaluated over bacteria (this article) and fungi previously. In both studies, the reduction of the capacity of the pathogenic microorganism to replicate (microbial growing) by means of the presence of bioactive compounds in the yeasts' CFS was determined as the development of visible growth in the liquid media after incubation. The development of a visible growth in the liquid media is related to the multiplication of the pathogen, and is not limited to an inhibition zone, such as in solid/agarized media, but recognizable in the total volume of the culture.

The bacteriostatic effect refers to the capacity of the CFSs to inhibit/reduce the multiplication of bacteria during the incubation period, and can be determined using the well described technique of Minimum Inhibitory Concentration (MIC), defined as "the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation" (Andrews, J. M. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001 Jul; 48 Suppl 1: 5-16. doi: 10.1093/jac/48.suppl 1.5. Erratum in: J. Antimicrob. Chemother. 2002 Jun; 49(6): 1049. PMID: 11420333). MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials. This methodology is based on the direct observation of visible microbial growth after overnight incubation of the treatments, relative to the aspect of the negative control (not inoculated tube). If the treatment shows a visible turbidity development, the conclusion is that the CFSs, at the concentration studied, is not capable of reducing/inhibiting bacteria replication and, therefore, has no bacteriostatic properties in regard to the pathogen. If the treatment has an aspect comparable to the negative control (absence of visible growth), the conclusion is that the CFSs was capable of reducing/inhibiting bacteria replication, hence a bacteriostatic effect is ascribed to it.

Continuing, the Minimum Bactericidal Concentration (MBC) is determined in the treatments that exhibit bacteriostatic properties. The MBC is defined as "the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on an antibiotic-free media" (Andrews, 2001). This methodology explores the CFSs capability to kill bacteria through inoculation in nutritive solid media of those treatments which previously did not show turbidity development in liquid media. The growth of bacterial colonies in the nutritive agar proves no bactericidal effect of the CFSs. Conversely, if after 24 h of plate incubation at 37 °C there is no evidence of bacterial growth, the bactericidal property of the CFS at certain concentration is confirmed.