

Titre: Activity of metabolites produced by *Saccharomyces boulardii* against multidrug-resistant bacteria

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Abstract

Probiotics are defined as living microorganisms that in adequate quantity, confer benefit to the health of the host. Most probiotics are prepared with bacteria, however yeasts, especially those of the genus *Saccharomyces*, are used in various preparations. *Saccharomyces boulardii* is a probiotic whose efficacy has been demonstrated in several clinical studies. Therefore, the aim of this work was to investigate the antimicrobial properties of two cell free supernatants (CFSs) from *Saccharomyces boulardii* strains isolated from probiotic products against Gram-positive and Gram-negative pathogenic bacteria. CFSs were obtained from these *Sacharomyces boulardii* cultures after centrifugation at 7.500 rpm, 4°C for 10 min. The supernatants were filtered through a 0.22 µm cellulose membrane and with them, organic acids (lactic acid and acetic acid) and ethanol were quantified by gas chromatography with Flame Ionization Detector (GC-FID). The determination of antibacterial activities was performed in 96-well microplates in which 180 µL of CFSs or neutralized CFSs were deposited. Then, 20 µL of each indicator bacteria were inoculated. Microdilution plates containing only culture of that bacteria were used as control. Inhibition of indicator bacteria growth was assessed by reading the optical density at 630 nm using a microplate reader (Thermo plate-TP Reader ®). GC-FID analysis of CFS showed concentrations equal to 114.5 and 9.6 mM for etanol and acetic acid. Lactic acid was not detected. CFSs were able to inhibit all pathogenic bacteria evaluated. The percentage of inhibition ranged from 69.3 to 94.2% depending on bacteria evaluated. Neutralization of CFSs with

NaOH 1N drastically reduced antibacterial activity. After the neutralization, the CFSs totally lost activity on *Klebsiella pneumoniae* and *Escherichia coli* O157:H7. The antibacterial activity is probably due to the presence of ethanol that was detected in higher concentrations.

Keywords: CFS, *Saccharomyces boulardii*, GC-FID, ethanol, acetic acid

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