

Title: Characterization of metabolome of bioreduction products of 6-nitrocoumarin by *Candida* spp: an analysis by FT-IR and chemometrics

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Abstract:

Assays with fluorogenic substrates (FS) are commonly employed in the microbial identification for being fast, specific and efficient. The great challenge of this technique is the development of specific probes for every type of microorganism to be analyzed since they can react with different substrates. In this context, the coumarin derivatives are between the most commons FS. In this work, 6-nitrocoumarin (6NC) was employed as FS in association with Fourier transform infrared spectroscopy (FT-IR) and chemometrics for characterization and discrimination of *Candida* spp. 6NC was synthesized by nitration of coumarin and characterized by spectroscopic and physical chemistry methods. The fluorogenic assay was carried out using 17 strains of *Candida* spp (*C. albicans*, *C. krusei*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*, *C. parapsilosis*, *C. stellacoidea*, *C. tropicalis*) precultured in Sabouraud agar plates by 48h at 37°C. One loop of each microorganism was inoculated in assay tube with a glucose (1%) solution containing 6NC 1 mmolL⁻¹ and incubated by 48 h at 37°C. Then, the cultures was extracted by equal volume of NaCl saturated solution and ethyl acetate (0,4 mL). The fluorescence of organic layer was evaluated by exposition to 365 nm uv light. In the sequence, the organic layers were analyzed by FT-IR and exploratory and classification algorithms. The fluorogenic assays revealed 14 positive samples with blue or green fluorescence. The FT-IR analysis in association with principal Component Analysis of organic layers separated the positive samples in two groups (G1: *C. famata*, *C. guilhermondii*, *C. lusitaniae*, *C. parapsilosis*; GII: *C. albicans*, *C. krusei*, *C. glabrata*, *C. guilliermondii*, *C. stellacoidea*, *C. tropicalis*) according to PC2 from negative (one group according to PC1). Some species presented two spectral patterns associated with 6NC-based bioreduction metabolome. The use of Soft Independent Modeling of Class Analogy (SIMCA) allowing the discrimination of the species investigated. In conclusion, the fluorogenic assay with 6NC associated with FT-IR and chemometrics can be employed as a tool for discrimination of *Candida* spp.

Keywords: *Candida* spp, fluorogenic substrates, 6-nitrocoumarin,

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