

Title: SELECTION OF MICROORGANISMS PRODUCING PECTINASES BY FT-IR AND CHEMOMETRICS

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Abstract:

Pectinolytic enzymes or pectinases are produced by several species of microorganisms and comprise a heterogeneous group of enzymes that hydrolyze the pectic substances. They are widely used in the food industry, vegetable waste treatment, textile and paper industry, animal nutrition, protein enrichment of children's food and oil extraction. In contrast to traditional methods of selection of fungi and bacteria producing such enzymes, the development of fast techniques to reduce environmental impacts is relevant to promote the microbial screening for future applications in bioprocesses. The aim of this study was to verify the applicability of Fourier transform infrared (FT-IR) as a tool in the selection of microorganisms potentially producers of pectinases. Seventeen fungal strains and 15 bacterial strains were selected from the Collection of Microorganisms of Industrial Microbiology at the University of Santa Cruz do Sul, and maintained on potato dextrose agar and nutrient agar, respectively. The pectinase production in submerged fermentation was performed for 72 h at 30°C in culture medium containing 5 g L⁻¹ of yeast extract and 2.5 g L⁻¹ of commercial pectin. The determination of the enzymatic activity of the supernatant extracts was performed by determination of reducing sugars by the method dinitrosalicylic acid. Triplicate samples of each extract (30 µL) were applied to stainless probes which were dried at 37 °C for 3 hours and then analyzed by FT-IR in the range of 4000-450 cm⁻¹, 8 scan pulses and 4 cm⁻¹ of resolution. The spectra were normalized, corrected for light scattering, pre-processed and analyzed via regression by partial least squares (PLS) with means of reducing sugar values, with cross-validation by mutual exclusion one at a time in Pirouette 4.0 software. The model was optimized by systematic exclusion of 50 cm⁻¹ bands. The optimized model PLS / FT-IR for reducing sugars utilized the spectral bands of 4000-3201, 3150-3051, 2300-551 cm⁻¹, with a cross-validation correlation coefficient (R²) of 0.999 and a standard cross-validation error 2,2x10⁻² mmol L⁻¹. The method showed detection limits and sensitivity adequate to prospect microorganisms with pectinolytic potential.

Key-words: chemometrics, FT-IR, pectinolytic activity

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