Production of Pectinases from Bacillus sp. SMIA-2 for Hydrolyzing Pectin from Passion Fruit Flour to Improve the Colonic In Vitro Fermentation

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Pectin is a structural heteropolysaccharide found in the cell walls of terrestrial plants. Pectin can be hydrolyzed into smaller molecules through chemical, thermal, enzymatic, or microbial processes. The hydrolysis breaks down the complex polysaccharides into simpler pectic oligosaccharides. Enzymatic hydrolysis, using pectinases, is a method for producing pectic oligosaccharides with specific properties and functionalities. This work aimed to produce satisfactory levels of pectinases by Bacillus sp. SMIA-2 in submerged cultures containing agro-industrial residues as substrates to hydrolyze pectin from passion fruit peel flour, evaluating the resulting product (partially hydrolyzed pectin) regarding its physicochemical properties and improving the colonic in vitro fermentation. Bacillus sp. SMIA-2 secreted pectinases (18.43 U/mL), avicelase (0.0487 U/mL), carboxymethylcellulase (0.0405 U/mL), amylase (0.1365 U/mL), xylanase (0.0220 U/mL) and protease (6.3000 U/mL) in submerged cultures containing passion fruit peel flour and corn steeping water. Pectinases were the enzymes that showed the greatest enzymatic activity. In this way, it was possible to create, unprecedentedly, a series of methodologies for producing hydrolytic enzymes – especially proteolytic and glycoside enzymes – that are highly sustainable and low-cost by using waste from the food industry and very resistant and propagating bacteria. Different enzymes are required for the degradation of complex substrates present in the plant cell wall. The bacterial strain used in this study was a thermophilic Bacillus sp. SMIA-2 isolated from a soil sample collected in Campos dos Goytacazes city, Rio de Janeiro, Brazil. The method for in vitro fermentation was based on studies by Jonathan et al. (2012) and Williams et al. (2005), in which the inoculum was immediately prepared after receiving the fecal material. The feces were pooled and diluted in a sterile anaerobic solution of 0.9% NaCl and 20% glycerol, in a 1:6 (w/v) ratio. The material was homogenized and filtered (Miracloth, EMD Millipore), and the inoculum was placed in a CO2 saturation container to maintain the anaerobic condition. The prepared fermentation medium was the basal solution (76%), vitamin/phosphate buffer solution (1%), bicarbonate buffer (1%), and reducing agent (4%). All materials were sterilized, and the medium and inoculum flasks were continuously bubbled with CO2. The carbohydrates used as substrate were modified passion fruit flour, unmodified passion fruit flour, FOS, and polydextrose. In each fermentation bottle, 200 mg of a specific carbohydrate, 24 mL of fermentation medium, and 1.5 mL of the inoculum were added. The environment was saturated with CO2 in bottles with screw caps, and the samples were incubated in a water bath at 37°C. The test for each treatment was done in triplicate for analysis at each fermentation time (0 h, 8 h, 16 h, and 24 h). At each point in the fermentation time, pH and pressure were evaluated to indicate that fermentation had occurred. During fermentation, the production of organic acids caused a

decrease in the pH of the culture medium. Regular pH monitoring indicated that fermentation had occurred. Other analyses such as the fecal microbiota composition via 16S rDNA sequencing will be carried out to understand the ecosystem and the role of our substrate.

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