Screening of Yeast Strains For Pectinolytic Activity: Effects Of Different Carbon And Nitrogen Sources In Submerged Fermentations

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Objectives: In the industrial production of fruit juices it is necessary to eliminate the pectin released during fruit processing in order to reduce the time of filtration and enhance the production at the end of the process. The present investigation was to select yeast strains with the best pectinase production and characterize some of the properties of the enzymes produced by submerged fermentation (SmF) processes.

Materials and Methods: For plate assay, yeast strains were inoculated in the solid medium including citrus or apple pectin (1.0%) and incubated for 72h at 30°C. After, iodine-potassium iodide solution or 6M HCl was added to detect clearance zones around the colonies. Strains presenting large clearing zones were used for enzyme production assays on liquid medium. SmF was carried out using 250 ml Erlenmeyer flasks with 40 ml of medium in a rotary shaker (120 rpm) at 30°C. After 96h the biomass was separated by centrifugation at 11000 rpm for 10 min and the supernatant was used to evaluate polygalacturonase (PGase) activity. Assay of PGase activity was determined by measuring the release of reducing groups using the 3,5-dinitrosalicylic acid (DNS) reagent. The reaction mixture containing 250 µl of 1% citric pectin in 250 µl citrate-phosphate, pH 6.0 buffer and 100 µl of culture supernatant, was incubated at 30°C for 5 min.

Results: Yeast strains were tested for pectin hydrolysis by plate assay, and were classified as very good producers of pectin depolymerizing enzymes when presented clear halos around colonies of at least 1.5 cm (1), good producers when the halos were of at least 1 cm (4), weak producers when halos were at least 0.5 cm (4) and poor producers when no pectinolytic activity and no clear lysis zones were observed (11). Maximal PGase production activity was obtained for strains Kluyveromyces marxianus NRRL-Y-1195 and Pichia pastoris. Pectinolytic activity was the highest at pH 5.0 and 5.5 for these strains respectively, except for Candida rugosa NRRL-Y-95, which presented optimal pH between 6.0. Assay for determination of the optimal temperature for PGase activity indicated maximal activities between 40-50°C for high pectinolytic yeast strains. PGase secreted by these yeasts showed an activity between pH 3.5 and 6.0, which is typical of PGase secreted by yeasts.

Conclusion: The pectinolytic activity of yeasts has specific properties which may offer advantages over currently available pectinase preparations. The enzyme solution can be applied directly to fruit juice industries without the need for pH modification. Furthermore, because of the temperature stability of the enzyme, it can be possibly used at processing temperature of 40-50°C, which is sufficient for industrial process.

Key words: Food processing, Kluyveromyces marxianus, pectinase production, yeasts.