Title: BACTERIAL PECTINASES PRODUCTION USING WASTE GREEN ORGANIC MANDARIN (*Citrus* sp.) IN SUBMERGED FERMENTATION

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Abstract:

Microbial enzymes with biotechnological applications has been the focus of extensive studies. The industrialization of citrus products produces thousands of tonnes of solid wastes rich in soluble carbohydrates and polysaccharides insoluble with high potential of biological conversion to produce pectinolytic enzymes used in the food, pharmaceutical and textile industries. The aim of this study was to evaluate the utilization of waste green organic mandarins (*Citrus* sp.), derived from the essential oil extraction, in order to isolate bacteria with potential for the production of pectinolytic enzymes in submerged fermentation. The solid residue, composed of shells, seeds and pulp, was sown in nutrient broth, incubated at 30 ° C for 24 h, and spread on nutrient agar for growth of microorganisms. The different bacterial colonies obtained were striated on nutrient agar to obtain pure strains. The isolates were screened for their pectinolytic potential based on their ability to produce a halo on the pectin solid media. A total of 15 strains were isolated from the solid wastes, 7 of which showed pectinase activity. The overall diameter of degradation halos (colony halo more halo pectinase) varied from 0.9 to 1.7 cm. Among the isolates one strain showed higher activity index and was then submitted to identification by biochemical methods and the 16S RNA and was identified as *Arthrobacter* sp. The bacteria were so selected for production of the pectinase enzyme by submerged fermentation on three different substrates, the first containing the solid waste of mandarins, the second containing flour elaborated from the skin and pulp of green organic mandarins and the third commercial pectin, all plus yeast extract. The fermentation was carried out in 250 mL Erlenmeyer flasks containing the substrates and incubated at 30 °C with shaking on a rotary incubator shaker at 130 rpm for 96 h. The enzimatic activity was determined by the dinitrosalicylate (DNS) method. The maximum production of pectinase by *Arthrobacter* sp. in submerged fermentation occurred with the solid waste when compared with to the other substrates. Therefore, the solid residue of mandarins is the selected substrate for our next study using the methodology of the factorial experimental design and response surface analysis to verify the effects of temperature, pH and fermentation time on the yield in the production of pectinases by *Arthrobacter* sp.

Key-words: bacterial pectinases, green organic mandarin, submerged fermentation, wastes

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