Isolation and Characterization of Bacterial Strains Producing Xylanase

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This study was aimed at isolating a moderately thermophilic xylanase producing bacterial strain. Therefore strains from hot environment were selected. From cow dung (3 samples CD_1, CD_2 and CD_3), hot rice broth (one sample HB), water used in autoclave (3 samples AC_1, AC_2 and AC_3), opened agar plate (3 samples SM_1, SM_2 and SM_3), beetroot peel (9 samples BR_1 to BR_9) and opened xylan liquid medium (26 samples GS_1 to GS_{26}), 45 bacterial strains expected to produce xylanase were isolated. Single colonies of the bacterial strains were obtained by cultivating the organisms in xylan-agar medium containing (gl^{-1}) nutrient agar, 25.0; and xylan, 20.0. To select the potential xylanase producer, single colonies from the samples mentioned above (45 samples) were selected, activated in xylan-nutrient broth medium [containing (gl^{-1}) xylan, 20.0; and nutrient broth, 25.0] at pH 7.0 and 42°C while shaking at 100rpm for 16h and used as inoculum. The inoculum was transferred into the fermentation medium containing (gl^{-1}) xylan, 20.0; peptone, 2.0; yeast extract, 2.5; $CaCl_2.2H_2O$, 0.005; $MgCl_2 6H_2O$, 0.005; $FeCl_3$, 0.005; K_2HPO_4, 2.5; KH_2PO_4, 1.0; NaCl, 0.1 and $(NH_4)_2SO_4$, 2.0. The fermentation was carried out at 42°C and pH 7.0, while shaking at 100 rpm. The enzyme activity was measured at optimised conditions of pH 6.9 and 60°C by incubating the enzyme with $20gl^{-1}$ xylan in 0.01M sodium phosphate buffer (pH 6.9) for 4 min. Among the 45 strains, 2 strains did not show xylanase activity under the experimental conditions. However among the strains, 32, 4, 4 and 3 strains have respectively produced the xylanase activity in the range less than 10, 10-20, 20-40 and 40-46Uml^{-1} ($U=mgmin^{-1}$). Hence the three strains (GS_{19}, GS_{22} and GS_{24}), which produced the xylanase activity in the range of 40-46Uml^{-1} were selected for characterization. Single colonies of GS_{19}, GS_{22} and GS_{24} were isolated and their microscopic, cultural and biochemical studies were carried out. Since the strains GS_{19}, GS_{22} and GS_{24} were gram-positive, sporulating, motile, catalase positive, aerobic, -haemolytic rods these three strains were confirmed to belong to the Genus *Bacillus*. Among the three strains, the best xylanase producer GS_{22} was selected and further studies of microscopic, cultural and biochemical studies were studied to identify its species. In Genus *Bacillus,* 48 species are described. Based on the characteristics such as shape and arrangement of spores, ability to produce acid and inability to produce gas from glucose fermentation, growth temperature, ability to hydrolyse starch and to utilise $(NH_4)_2SO_4$ for growth, strain GS_{22} was identified as *Bacillus pasteuri.*