# Preliminary studies on the isolation of xylanase producing bacteria and kinetic studies of the enzyme

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This study was aimed at isolating a thermostable xylanase producing bacterial strain. From cowdung (3 samples), hot rice broth (one sample), water used in autoclave (3 samples), opened agar plate (3 samples), and beetroot peel (9 samples), a total of 19 bacterial strains were isolated. *Bacillus licheniformis M27* (CFTRI, Mysore) and *Bacillus licheniformis* (ATCC, 6346) were also used. Single colonies of the bacteria were obtained by cultivating the organisms in xylan-agar medium containing ($gl^{-1}$) nutrient broth, 25.0; agar, 10.0; and xylan, 2.0. To select the potential xylanase producer, single colonies from the samples mentioned above (21 samples) were selected, activated in xylan-nutrient broth medium (containing ($gl^{-1}$) xylan, 2.0; and nutrient broth, 25.0) at pH 7.0 and 42°C for 18h and used as inoculum. The inoculum was transferred into the fermentation medium containing ($gl^{-1}$) xylan, 2.0; 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. Enzyme assay was carried out at pH 6.9 and 60°C by incubating the enzyme extract with $10gl^{-1}$ xylan in 0.01M sodium phosphate buffer (pH 6.9) for 5 min. Among the strains, the strains from cow dung, hot rice broth and opened agar plate; *Bacillus licheniformis M27 & Bacillus licheniformis* (6346, ATCC) have not produced xylanase. One of the strains isolated from beetroot peel named as $BR_3$ produced $4040U^{-1}ml^{-1}$ xylanase activity ($U=nmolmin^{-1}$). Out of the 3 strains isolated from the water used in autoclave ($AC_1$, $AC_2$, $AC_3$), $AC_2$ and $AC_3$ showed 3340 and $6.16U^{-1}ml^{-1}$ xylanase activity respectively. The other strains $BR_1$, $BR_2$, $BR_3$, $BR_4$, $BR_5$, $BR_6$, $BR_7$, $BR_8$ and $BR_9$ from beetroot, produced 156, 450, 4040, 30, 1000, 0, 234, 500 and $560U^{-1}ml^{-1}$ enzyme. Therefore the strain $BR_3$ was selected for further studies. The strain $BR_3$ produced the maximum xylanase activity at 48h ($4040U^{-1}ml^{-1}$) in fermentation medium. The reaction time for the enzyme assay was fixed, as 05 min. Kinetic properties of xylanase obtained from $BR_3$ were determined. The optimum pH for the enzyme activity was 6.9 in 0.01M sodium phosphate buffer at 60°C. The enzyme showed the highest activity at 60°C and pH 6.9. An investigation of the temperature stability showed that 65% of the original activity ($3250\ U^{-1}ml^{-1}$) present when incubated at 60°C for 4h. However the enzyme was more stable at temperature and showed 72.7% of the original activity ($3564.08\ U^{-1}ml^{-1}$). The $K_m$ value for the xylanase for xylan at pH 6.9 and 60°C was $0.125gl^{-1}$. The highest stability of xylanase was observed in 0.01M sodium phosphate buffer at pH 6.9 and 60°C. Further studies are underway to improve the organism and to increase the xylanase enzyme production.