1. ABSTRACT

Micro organisms are rich sources of cyanoses, which are used mainly in paper industries. To produce xylanase, 45 strains from different sources such as cow dung, boiled rice broth, water used in autoclave, opened agar plate, beetroot peel and opened xylan liquid medium were isolated. From single colonies of the bacterial strains potential xylanase producer was selected. Among the 45 strains, strain GS_{22} from contaminated xylan-nutrient broth medium produced the highest xylanase activity ($45.7UmL^{-1}$) was selected for further characterization. Since Strain GS_{22} was gram-positive, sporulating, motile, catalase positive, aerobic, β-haemolytic rod with ability to produce acid from glucose, it was confirmed as Genus *Bacillus*. Further studies of microscopic, cultural and biochemical studies indicated that isolated strain has the ability to produce acid from xylose and mannose; shown positive results to Vogues Proscaeur test; ability to grow at 45 and 50°C, inability to grow at 55°C; inability to hydrolyse starch and tyrosine; halophobic; inability to produce indole, oxidase and urease, inability to utilise citrate and reduce nitrate. From these experiments, GS_{22} was classified as belonging to the Kingdom: Procaryotae; Division: Bacteria; Order: Bacillales; Family: Bacillaceae; Genus: *Bacillus*; Species: *laterosporus*.

Bacillus laterosporus grew optimally at 42°C and produced xylanase. Xylanase production was high in the medium containing $20gL^{-1}$ xylan [$49.25UmL^{-1}$ ($U=mgmin^{-1}$)]. The optimum amount of $(NH_4)_2SO_4$ concentration in the medium for highest xylanase ($45.7UmL^{-1}$) production at 48h was $2gL^{-1}$. Peptone inhibited the production of xylanase.

For xylanase production locally available corn cob was selected. Water soluble and fermentable sugars were removed from corn cob and from the residue xylan was extracted by alkali treatment, precipitated with ethanol and dried with ether. From 1kg of dried corn cob, 63.3g of xylan was extracted. The yield of xylan was 6.33%. The presence of xylan in the extract was confirmed by subjecting it to acid hydrolysis and separating in paper chromatography. This was compared with commercial xylan. The activity of commercial xylanase on the extracted and commercial xylan was compared. Further the activity of xylanase produced by *Bacillus laterosporus* compared with the commercial and extracted xylan. The growth and xylanase production of *Bacillus laterosporus* was observed in extracted xylan containing medium.

Purification of xylanase from *Bacillus laterosporus* was optimised and the continuous purification was carried out under optimised conditions. Xylanase produced by *Bacillus laterosporus* was purified using the anion exchanger DEAE Sepharose fast flow under batch mode. The suitable pH for coupling the enzyme with DEAE Sepharose fast flow, the optimum amount of enzyme to be applied and the concentration of NaCl for the elution of xylanase were optimised. Optimum pH for coupling of the enzyme to DEAE-Sepharose fast flow was 6.9. The volume of the crude enzyme for coupling with 5mL of DEAE Sepharose fast flow was 1mL (with 4.15mg of protein and 40.6UmL^{-1} xylanase activity). The concentration of NaCl for the elution of xylanase was 0.75M. When the enzyme was batch purified under optimised conditions in continuous mode the total activities of crude and purified xylanases were 24.76Umg^{-1} and 58.3Umg^{-1}. The purification fold was 2.4 and 143.96% yield was obtained.

Kinetic properties of crude and purified xylanase obtained from *Bacillus laterosporus* were determined and compared with commercial xylanase. The time for incubation was optimised as 10min for crude, purified and commercial xylanase. The optimum pH was 6.9, 7.5 and 7.0 for crude, purified and commercial xylanase respectively. Crude and purified enzymes showed the highest activity at 60°C (26.0 and 17.5UmL^{-1}) while commercial xylanase showed highest activity at 45°C. Michaelis constant of crude, purified and commercial xylanases for xylan were 9, 8.5 and 5.

At pH 7.0 the crude enzyme incubated at 37°C retained higher activity (98%) than at 50°C (72.4%) and 60°C (10%) for 30min. At pH 7.0 purified enzyme also retained higher activity at 37°C (98%) than at 50°C (80%) and 60°C (80%). Purified enzyme was more stable than the crude enzyme at all temperatures. Crude and purified xylanase were pre-incubated at different pH values (ranging from 5.0 to 9.0) at 60°C and the activities of xylanase at different intervals were measured. The activities of crude and purified xylanase were optimum at pH 7.0 and 7.5 in phosphate buffer. The pH stability of the xylanase was studied. When the crude enzyme was allowed to react at pH 5 and 60°C no activity was observed even at zero time. When the crude enzyme was pre-incubated at pH 6 for 30min the activity was totally lost. It retained 10% of the original activity when it was pre-incubated at pH 7 and 60°C for 30min. At pH values 8 and 9, 31.6 and 9% of the activities were retained respectively indicating xylanase is more stable in pH 8 even though the activity at alkaline pH was lower than in the neutral pH.