1.0 ABSTRACT

The objective of this study was to select and identify an alkaline xylanase producing thermophilic bacterial strain, to maximize the production of xylanase by optimizing the conditions for solid state fermentation and to purify and characterize the enzyme. Among the 45 bacterial strains isolated from different sources and available at the laboratory, five strains, which were expected to produce alkaline xylanase, at temperatures above 40°C, isolated from opened media containing xylan as carbon source (GS_7, GS_{15}, GS_{17}, GS_{20} & GS*) were selected for this studies. Xylanase production by strains GS_{17} and GS* was less affected than the strains GS_7, GS_{15} and GS_{20} when the pH of the medium was maintained between 8.0 and 9.5. Strains GS_{17} and GS* produced xylanase at pH 10.0. Strain GS_{17} was selected because it produced xylanase 1.2, 1.3, 1.2 and 3.2 times higher at 40, 45, 50 and 55°C than by the strain GS* at 39 hours. Based on the morphological and biochemical tests, the strain GS_{17} was identified as *Bacillus pumilus*. When *Bacillus pumilus* was subjected to 3 cycles of UV-irradiation, xylanase production was increased by 1.22 times in the first two cycles while no increase after the third cycle, heat shock treatment and chemical treatment with Ethyl Methane Sulphonate.

The culture conditions were optimized to increase the xylanase production by Solid State Fermentation using paddy husk as the support. Solid media contained 200g of paddy husk and 800mL of liquid fermentation medium (containing (gL^{-1}) xylan, 20.0; peptone,2.0; yeast extract, 2.5; K_2HPO_4, 2.5; KH_2PO_4, 1.0; NaCl, 0.1; $(NH_4)_2SO_4$, 2.0 and $CaCl_2.2H_2O$, 0.005; $MgCl_2.6H_2O$, 0.005; and $FeCl_3$, 0.005 at pH 9.0). Production of xylanase activity has started on the 2nd day and the highest activity (142.0 $Ug^{-1}DM$) was obtained on the 6th day at room temperature and pH 9.0. When paddy husk to liquid fermentation medium ratio was optimized as 2:9, xylanase activity obtained was 1.0 times than that of the control when the ratio was 2:8. When the cultivation temperature was optimized as 40°C, xylanase activity was increased by 1.1 times, than that obtained at room temperature. When commercial birch wood xylan was replaced with different concentrations of corn cob, xylanase production was highest (224.2 $Ug^{-1}DM$) in the medium containing 150gL^{-1} corn cob. Xylanase production was supported by sucrose (248.2 $Ug^{-1}DM$), fructose (235.5 $Ug^{-1}DM$) and arabinose (286.4 $Ug^{-1}DM$), while the enzyme production was reduced by glucose (212.4$Ug^{-1}DM$), galactose (196.0 $Ug^{-1}DM$), lactose (207.3 $Ug^{-1}DM$) and amylose (214.0 $Ug^{-1}DM$) than in the control which contained no sugar (222.6 $Ug^{-1}DM$). When

organic nitrogen sources were replaced with locally available nitrogen sources such as groundnut powder or sesamum seed cake powder or coconut seed cake powder or soy meal powder, highest xylanase production (290.7 $Ug^{-1}DM$) was obtained in the medium containing soy meal powder. Among the different concentrations of soya meal powder, $16gL^{-1}$ gave highest xylanase production ($326.5Ug^{-1}DM$). By optimizing the culture condition and media composition, xylanase production was increased by 2.3 times and the production cost was decreased by 14.4 times than under the initial non-optimized conditions.

When the crude enzyme was subjected to fractional precipitation with solid $(NH_4)_2SO_4$, highest specific activity (33.7 Umg^{-1}protein) was precipitated at 50% (w/v) saturation. When the dialyzed enzyme was purified in a DEAE-Sepharose column under optimized conditions, the specific activity of xylanase was increased from 33.2 to 222.6 Umg^{-1} protein, with 84.2% yield. When the purified xylanase was subjected to poly acrylamide gel electrophoresis, the sample gave a single band. The molecular weight of the purified xylanase was approximately 55.3 KDa. When the crude xylanase was purified with Eudragit S100, by three phase partitioning method (TPP), 14.6 UmL^{-1} of xylanase activity was obtained with a specific activity of $31.4Umg^{-1}$ protein. The recovery of the enzyme was 52.2% with 1.8 fold purification. In the precipitation method with Eudragit S100, $15UmL^{-1}$ of xylanase activity was obtained with the specific activity of 33.4 Umg^{-1}. The recovery of the enzyme was 52.2% with 1.7 fold purification. When the xylanase was purified by precipitation method with Eudragit $40gL^{-1}$ and the Eudragit bound enzyme was eluted with 0.3M NaCl, $19.0UmL^{-1}$ of xylanase activity with the specific activity of 43.2 Umg^{-1} was obtained. Xylanase enzyme yield was 69.7% and the purification fold was 2.3. When the optimized conditions of precipitation method were used for TTP method with $40gL^{-1}$ Eudragit, 50% (w/v) ammonium sulphate and the Eudragit bound xylanase was eluted with 0.3 M NaCl, 2.0 fold of purification was obtained.

Crude, purified and commercial xylanases showed zero order kinetics for 10 minutes and highest activity was obtained at 60°C at pH 9.0. The optimum pH for crude, purified and commercial xylanases were 9.0, 9.0 and 7.0 respectively at 60°C and the Michaelis constant by Lineweaver-Burk Plot method for xylan were 3.1, 2.3 and $0.03gL^{-1}$ under optimized conditions. The half-life of the crude and purified xylanase was highest at pH 9.0 while it was highest at 50°C when incubated at pH 9.0.