ABSTRACT

Proposed study was designed to purify and characterize pectin lyase (PL) by *Trichoderma harziamun*. Four different *T. harzianum* isolates (T140, T194, T296, and T 325) were screened quantitatively and qualitatively by plate method and enzyme assay for the production of PL, induced by citrus peels pectin. Screening revealed that two isolates i.e. T140 and T325 exhibited PL activity. Different physical and chemical fermentation parameters like fermentation rate, inoculum size, concentration of peptone, concentration of yeast extract, pH and tween -80 were optimized by different experiments for production of PL by *T. harzianum* isolates (T140 and T325). Maximal fermentation rate on citrus peels medium was attained *T. harzianum* isolates at pH 7.0 and 28 ^{o}C after 72 hours. Addition of yeast extract and Tween -80 into fermentation medium enhanced PL production by *T. harzianum* isolates, where as peptone showed negative effect. T140 (10.95 U/mL/min) and T325 (10.99 U/mL/min) showed maximum pectin lyase activity at pH 5.5 in the presence of 0.4% yeast extract, 4ml inoculum and 0.2 % Tween -80 after 72 hours fermentation of basal growth medium of citrus peel.

Pectin lyase was partially purified by ammonium sulphate precipitation by using 70 % ammonium sulphate. Precipitate of PL was dialyased against 0.2 M phosphate buffer at pH 5.5 for 12 hours. Dialyased fraction was used for SDS-PAGE and characterization of pectin lyase. Molecular weight of partially purified PL from T140 and T 325 isolates was 22 kDa. Purified PL expressed enzyme activity in the presence of 1.1 % pectin. Temperature and pH optima for the PL were determined as 40°C and 7.0, respectively. PL activity was significantly increased in presence of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in both isolates.

Key words: Pectin lyase, *Trichoderma harzianum*, enzyme production, Liquid state fermentation, biochemical characterization.