Characterisation of Klebsiella pneumoniae Xylanase and Increment of Its Activity in Heterologous Expression System

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ABSTRACT

A xylanase DNA sequence with a total length of 642 bp was previously isolated from a xylanolytic Klebsiella pneumoniae. Xylanase gene primers were designed with the addition of BamH1 and EcoR1 restriction enzyme sites in order get a full xylanase gene that is in-frame with pSTAG expression vector. The isolated xylanase gene was amplified using the designed primers through PCR, then cloned and expressed in E. coli BL21 (DE3). In-silico characterization showed that the recombinant xylanase has a molecular weight of 23.9 kDa and a pI of 9.32. The signal peptide cleavage site for the recombinant xylanase was predicted to be between residues 61 and 62. The activity of the crude recombinant xylanase was 2.015 U/mL, which was higher than the crude native xylanase activity, with maximum at 0.642 U/mL. Staining of the birchwood xylan agar plate with Congo red showed a clearing zone around E. coli BL21 (DE3) colonies with recombinant pSTAG plasmid even without being induced with IPTG. This implied leaky expression of the E. coli BL21 (DE3) secretion system, which recognized the signal sequence of the recombinant xylanase, and proceeded to cleave and secreted out the mature protein into the culture medium. MALDI-TOF analysis of a 20 kDa protein present in the culture medium confirmed that the recombinant xylanase had been secreted into the culture medium.

Keywords: Heterologous expression, Klebsiella pneumoniae, recombinant xylanase

INTRODUCTION

Xylan consists of β-1,4-linked xylopyranosyl residues. It is the second most abundant polysaccharide and an important component that makes up the plant cell wall. Due to the heterogeneity and complexity of the xylan structure, the complete hydrolysis of xylan is carried out by a xylanolytic enzyme system. Endo-1,4-β-D-xylanase (EC 3.2.1.8) is part of this complex system (Singh et al., 2007). Xylanase cleaves the xylan backbone at 1-4 carbon linkages to produce xylo-oligosaccharides and xylose (Kulkarni et al., 1999). From a biotechnology perspective, xylanase is an important enzyme because of its thermal stability and its ability to work alone or in combination with other enzymes, making it suitable for a number of industrial applications. Examples of xylanase applications include its use in the kraft process and biobleaching in the paper and pulp industry (Helianti et al., 2008; Kulkarni et al., 1999; Te'o et al., 2000).

Heterologous expression of a gene encoding β-1,4-endoxylanase in E. coli BL21 (DE3) was performed in this study. The xylanase gene was isolated from xylanolytic Klebsiella pneumoniae, a bacterium that had been locally isolated from soil from a sago plantation by Hussain et al. (2011). Here, we report on the expression of a xylanase gene from K. pneumoniae in E. coli BL21 (DE3) and to determine the characteristics of the recombinant xylanase expressed. To our knowledge, there are no prior publications that describe the isolation and heterologous expression of a xylanase gene from K. pneumoniae. Xylanase genes are commonly isolated from genus Bacillus which are abundantly found in soil (Gardener, 2004; Nakamura et al., 1993; Touzel et al., 2000). Thus the isolation of a xylanase gene from K. pneumoniae from agricultural soil suggests that xylanase might be naturally occurring among soil-inhabiting microbes.

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