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XYLANASE GENE FROM A LOCALLY ISOLATED BACTERIUM

HUSSAIN, M.H.*, CHONG, N.F.M., CHAN, C.S.W., SAFARINA, A. and HUSAINI, A.

Proteomics Laboratory, Department of Molecular Biology,
Faculty of Resource Science and Technology, University Malaysia Sarawak,
94300 Kota Samarahan, Sarawak, Malaysia
**E-mail: hhasnain@frst.unimas.my*

ABSTRACT

A xylanolytic bacterium was isolated from sago plantation humus. Results from the morphological observation, biochemical tests and 16s rRNA sequencing suggested the bacterium to be *Klebsiella pneumoniae*. Due to the xylanolytic activity of this bacterium, isolation and characterization of the xylanase gene were attempted. A distinct fragment of about 650 bp was successfully amplified using PCR and cloned into *Escherichia coli* XL-1 Blue. A BLAST search confirmed that the DNA sequence from the amplified fragment was endo-1,4-beta-xylanase gene from the family 11 glycoside hydrolase. It showed 98% homology with *Bacillus subtilis* xylanase gene. *In silico* characterisation showed an open reading frame encoding a 213 amino acid sequence with a molecular weight of 23.3 kDa and theoretical isolectric point (pI) at pH 9.42.

ABSTRAK

Bakteria pengurai xilan telah dipencilkan daripada sampel humus yang diambil di ladang sagu. Keputusan pemerhatian morfologi, ujian biokimia dan jujukan rRNA 16s mencadangkan bahawa bakteria ini ialah *Klebsiella pneumoniae*. Kajian juga dijalankan untuk memencil dan mencirikan gen xylanase daripada bakteria pengurai xilan ini. Produk bersaiz 650 pasangan bes telah berjaya diperolehi melalui proses PCR dan diklonkan ke dalam *Escherichia coli* XL-1 blue.. Analisis BLAST telah menunjukkan bahawa jujukan DNA produk tersebut merupakan gen endo-1,4-beta-xilanase daripada keluarga 11 glikosida hidrolase. Ia menunjukkan 98% kepadanan dengan gen xylanase *Bacillus subtilis*. Pencirian secara *in silico* menunjukkan ia mempuyai rangka bacaan terbuka (ORF) yang mengekodkan rantai peptida bersaiz 213 asid amino dengan berat molekul 23.3 kDa dan ramalan titik isoelektrik (pI) pada pH 9.42.

Key words: xylanase; sago; *Klebsiella pneumonia*; humus

INTRODUCTION

Xylan is the second most abundant biopolymer in the world after cellulose. It is the major component of hemicellulose which is found abundantly in plant cell walls (Khanderparkar & Bhosle, 2006) and can consist up to 35 % dry weight of higher plants (Silva *et al*., 1999). Xylan consists of a β-1,4-linked D-xylose backbone substituted to varying degrees with *O*-acetyl, α-*L*-arabinofuranosyl, 4-*O*-methylglucuronic acid groups or α-1,2-linked glucuronic acids (Singh *et al.,* 2003). Xylan is abundantly found in agro-industrial waste and in Malaysia, it can be found in sago pith waste, oil palm waste, paddy husks, and sugarcane bagasse. Degradation of xylan can be achieved by xylanolytic enzymes such as xylanase.

Using xylanases, xylan can be degraded to xylose, which can be fermented by bacteria and yeasts into ethanol or organic acids. Therefore, bioconversion of agricultural waste not only reduces pollution but also offers a renewable source of energy. Other potential uses of xylanases are in biobleaching (Morris *et al*., 1998; Viikari *et al*., 1994), production of oligosaccharides (Khandeparker & Numan, 2008), bakery industry (Romanowska *et al*., 2006), and in animal feed and fruit juice production (Beg *et al*., 2001). Thus, the aim of this work was to isolate and characterize the xylanase gene from locally isolated bacteria.

MATERIALS AND METHODS

Isolation of Xylanolytic Bacteria

Humus samples from a sago plantation in Mukah, Sarawak were inoculated into enriched broth

* To whom correspondence should be addressed.