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SHORT COMMUNICATION

HOT PHENOL EXTRACTION OF TOTAL RNA FROM *Thermoascus aurantiacus* AND CHARACTERIZATION OF ITS THERMOSTABLE XYLANASE GENE

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ABSTRACT

Total RNA was successfully isolated using hot phenol extraction method. Three bands representing the 18S, 5.8S and 28S rRNA was observed. No heavy smearing was observed in the RNA band patterns, indicating low levels of polysaccharide contamination, when subjected to 1% agarose gel electrophoresis. Genomic DNA was eliminated using DNase I digestion and lithium chloride (LiCl) precipitation. Two-steps reverse transcriptase polymerase chain reaction (RT-PCR) using M-MuLV Reverse Transcriptase and sequence specific primers for *xylanase* gene, XynA(F) and XynA(R), successfully generated the target amplicon of 500 base pairs (bp). Sequence analysis of the PCR product indicated as partial sequence of *Thermoascus aurantiacus xylanase* gene (*XynA*) deposited in the NCBI GenBank with accession number: AF127529.1 and AJ132635.1. Hot phenol extraction is useful for extracting large quantities of total RNA sufficient for complementary DNA (cDNA) synthesis in shorter period of time.

Keywords: Hot phenol extraction, thermophilic fungus, *Thermoascus aurantiacus*, DNase I digestion, cDNA synthesis, thermostable xylanase

Xylanases of thermophilic fungi with high thermostability has received considerable attentions due to their potential applications in pulps bleaching that occurred at high temperature (Gupta *et al*. 2000; Maheswari *et al*. 2000). Genetic engineering of thermostable *xylanase* gene isolated from thermophilic fungus has been carried out to obtain high levels of thermostable recombinant xylanase that can be used for industrial applications (Emami & Hack 2000; Shibuya *et al*. 2000; Damaso *et al*. 2003).

In this study, a thermophilic fungus *T. aurantiacus* was successfully isolated from water sediment samples of Gadek Hot Spring in Melaka. The fungus produced high levels of thermostable xylanase when cultured in basal media. Xylanase of *T. aurantiacus* that was isolated in this study appeared to be active over a board range of temperature ranging from 40ºC to 70ºC (Husaini, unpublished). The finding of the thermostable xylanase of this local *T. aurantiacus* is very useful in the bio-bleaching of pulps in the paper industry. Isolation of total RNA is essential for amplification of functional gene from xylanolytic fungus. Like other eukaryotic organisms, introns presence in genomic DNA of *T. aurantiacus* interferes with the expression of the recombinant xylanase in expression vector. Therefore, cDNA was constructed in order to obtain functional thermostable *xylanase* gene using reverse transcription-polymerase chain reaction (RT-PCR).

Thus, the objective of this study is to use the hot phenol method in order to obtain a large quantity of total RNA sufficient to be used for synthesis of *T. aurantiacus* thermostable *xylanase* gene. This in turn will determine whether the hot phenol method used is an effective and simplified method in extracting total RNA from any fungal species.

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