Abstract

The accumulation of plastic waste, especially polyethylene terephthalate (PET), causes environmental problems and poses a threat to the well-being of surrounding organisms. Several methods such as mechanical and chemical method that have been carried out to overcome the accumulation of plastic waste require expensive materials and complicated methods. The latest approach utilizes natural plastic degrading enzymes derived from Ideonella sakaiensis to degrade PET plastic. Ideonella sakaiensis produces PET hydrolase enzymes that are able to degrade PET into bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate(MHET), and a small amount of terephthalic acid (TPA) and MHET hydrolase (MHETase) enzymes that degrade MHET into TPA dan ethylene glycol (EG). Advances in molecular technology serves as the foundation for the development of MHET enzymes, especially through metagenomic data mining that enables the discovery of potential putative MHETase genes. This study aims to observe the expression of the putative MHETase gene and asses the ability of the MHETase enzyme expressed by the putative MHEtase gene in degrading MHET into TPA and EG. This study was conducted through several stages including transformation, cloning, and plasmid verification in Escherichia coli (E. coli) DH5α which is followed by protein target expression in E. coli BL21 (DE3) using IPTG inducer. The expressed protein was extracted to obtain whole cell, lysate cell (soluble), and cell debris (insoluble) fractions which were then characterized and analyzed for their ability to degrade MHET. The results showed that the putative MHETase gene successfully expressed MHETase enzyme which was observed in the whole cell, lysate cell (soluble), and cell debris (insoluble) fractions. Unfortunately, the activity of MHET degradation by MHETase putative has not been observed. However, the putative MHETase enzyme appears to be able to degrade BHET with low activity which open up new possibilities for further exploration into MHETase's potential role as a BHET degrader.

Keyword: PET, Recombinant protein expression, Escherichia coli BL21 (DE3), MHETase