ABSTRACT

Widespread utilization and inherent persistent nature of polyethylene (PE) enlist it as a major environmental threat. Since current methods to manage PE waste are not environmental-friendly, the focus has been shifted towards bioremediation. However, to date, the underlying mechanism of PE biodegradation has not been studied comprehensively. Hence, this study focused on obtaining insights into PE biodegradation mechanism via transcriptome sequencing of a PE-degrading bacterium (*Pseudomonas aeruginosa* AIMST H2), previously isolated by our team. The present study reports an optimized RNA extraction method for RNA-Seq, evaluation of *de novo* transcriptome assemblers and aligners as well as differential gene expression analysis towards understanding the PE biodegradation mechanism. RNA was extracted from mid-logarithmic cultures of *P. aeruginosa* AIMST H2 grown in glucose (control) and PE-supplemented medium using 3 methods. Based on the RIN value, purity and total RNA yield, Amresco's phenol-free kit yielded the best quality RNA. Using this kit, RNA was extracted in duplicates from each growth condition prior to sequencing via Illumina HiSeq 2000 platform. The resulting sequencing raw reads were subjected to an in-house analysis pipeline for *de novo* transcriptome assembly and reference-based alignment evaluation. The analyses revealed that SOAPdenovo-Trans and Bowtie 2 performed the best for their respective tasks. The resulting transcriptome assembly was 6,527,763 bases with a GC content of 66.4% and it consisted of 5,434 protein-coding genes and 527 non-coding RNAs. In addition, several potential novel intergenic sRNAs and alternate splicing events were observed. DESeq analysis revealed that 3,824 genes were differentially expressed; 1,780 were up-regulated and 2,044 genes were down-regulated during PE biodegradation. The PE biodegradation pathway was deduced and it was observed to mimic the alkane degradation pathway. Genes involved in stress response, biofilm generation and chemotaxis were co-regulated to aid the biodegradation process. In conclusion, completion

of this study marked the successful identification of the key enzymes involved in PE biodegradation pathway and development of a bioinformatics analysis pipeline for bacterial transcriptome dataset generated via RNA-Seq. Insights obtained from this study can serve as a stepping stone to an efficient PE bioremediation in the near future.