

## ABSTRACT

Leeches are one of nature's bloodsucking wonders, whose use in clinical settings as a medical device has been recognised by FDA. The approval has led to widespread usage of leeches, *Hirudo medicinalis* and *Hirudo verbana* for Hirudotherapy in many western countries. However in Malaysia, leech farmers and traditional medical practitioners have been widely marketing their products and treating various ailments without knowing the identity of the leeches used. Therefore, this study was focused on the characterization of two commercially farmed local leech varieties based on morphological features and molecular markers (18S, 28S, 12S rDNA and COI genes). The study confirmed that the two local leech varieties belonged to the species, *H. manillensis* and *H. javanica*, respectively. A pictorial key was developed and substantiated with the molecular findings to distinguish both these leech species. Phylogenetic analysis using the molecular markers revealed that both the leech species forms a clade under Hirudinidae family. Aside from this, the present study also focused on both the leeches feeding and *H. manillensis* breeding preference (brood stock). The feeding studies demonstrated that both leech species fed with live African catfishes had significantly higher percentage weight gain and specific growth rate (SGR) ( $p < 0.05$ ) compared to leeches fed with cattle blood and formulated feed. The breeding studies, on the other hand, revealed that only 12 *H. manillensis* juveniles were obtained in triplicate experiments of 3 brood stock densities (30, 60, 90) combined, with no cocoon formation. In addition, this study also managed to isolate and characterize 6 phages, spbP, etecP, eaP, epP, yeP and apP phage. The first 5 aforementioned phages reduced bacteria titer between 3.67-6.23  $\log_{10}$  CFU/ml, indicating their great potential as water sanitization agents. Phage eaP also showed great potential as leech sterilization agent by eradicating *P. aeruginosa*, a leech microflora that was isolated in this study. Aside from *P. aeruginosa*, 9 other bacterial strains were isolated from leech surface out of which 3 were also found in the leech gut. Since, total eradication of all

microflora was not achieved, genome sequencing of phages were performed to obtain insights into phages. This led to characterization of lysin and holin genes encoded by 5 phages (eaP, epP, yeP, etecP and apP) which in future can be modified to eradicate a broader range of bacteria. Apart from that, the sequencing, assembly and annotation of *H. javanica* genome resulted in the establishment of a genome assembly evaluation method and an annotation pipeline. Using this pipeline, 62,645 protein coding genes were predicted. In conclusion, this study established an efficient leech identification method based on pictorial key, evaluated the potential of phages in leech sterilization and water sanitization as well as devised a user friendly pipeline for leech genome annotation.