

COMPARATIVE MICROBIAL PROFILE OF ECO-ENZYMES PRODUCED FROM ORANGE (*Citrus Sinensis*) AND PINEAPPLE (*Ananas comosus*) PEELS USING METABARCODING OF THE 16S AND ITS REGIONS

By

Alifya Laras Ardana Pudijanto

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Supervisor: Dr. Miftahul Ilmi, S.Si., M.Si.

ABSTRACT

Eco-enzyme (EE) is an organic, antibacterial substance used as an eco-friendly cleaner. Although there are many studies on the practical use of EE, the identification of microbial diversity through 16S and ITS metabarcoding remains limited. This study aims to characterise the biodiversity of bacterial and fungal organisms in orange (*Citrus sinensis*) and pineapple (*Ananas comosus*) peel-derived EE using metabarcoding of the 16S (bacteria) and ITS (fungi) regions. The EE samples were prepared using a 1:3:10 (molasses:organic waste:water) ratio and fermented for 3 months. DNA extraction and PCR amplification using the 16S and ITS primers were performed. PCR amplicons were visualised using gel electrophoresis. DNA sequencing was conducted by the Integrated Genomic Factory (IGF) using Oxford Nanopore Technology (ONT). Data analysis was performed in RStudio, with the provided data based solely on the ONT results. The results reveal that the bacterial profile of the pineapple EE identified 10 different species, while the orange EE identified only 9 species within the top 10. The identified bacterial species in the pineapple EE are: *Lentilactobacillus buchneri* (166,579), *Komagataeibacter saccharivorans* (51,730), *Lentilactobacillus parafarraginis* (15,691), *Lentilactobacillus parabuchneri* (2550), *Lentilactobacillus fungorum* (1873), *Lentilactobacillus farraginis* (1135), *Lentilactobacillus kefir* (1223), *Lentilactobacillus parakefir* (550), and *Ligilactobacillus aviaries* (466). On the other hand, the fungal profile of the orange EE identified more species than that of the pineapple EE, which identified only 4. The fungal species found in the orange EE include: *Zygosaccharomyces bailii* (126, 832), *Wickerhamiella qilinensis* (203), *Starmerella stellata* (70,513), *Starmerella dacenportii* (235), *Pichia manshurica* (339,000), *Pichia kudriavzevii* (180), *Phaeococcomyces rothmanniae* (1,774), and *Lamelloclavaria petersenii* (351). This study was carried out with a single repetition; Therefore, further studies are recommended to include a variety of tests, such as enzyme profiling and analysis, and conducting practical use to better understand the potential applications of EEs. Furthermore, metabarcoding of the ITS regions in EEs should be further explored.

Keywords: 16S and ITS Regions, Eco-enzyme, Metabarcoding, Orange, Oxford Nanopore Technology, Pineapple