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Bacterial community and their functional characteristics in leachate from Jeram and Jabor landfills

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ABSTRACT

Aims: A landfill is one of the environments that nurtures a diverse microorganism community, which has the potential to carry various dioxygenase enzymes that can degrade possible contaminants in the landfill leachate. However, the information on the microbial community that is responsible for the degradation process is limited due to the culture-dependent approach. The aim of this study was to assess the distribution of the bacteria that inhabited the landfills using 16S rRNA gene amplicon sequencing approach.

Methodology and results: The leachate sample collected from Jeram Sanitary Landfill, Selangor and Jabor-Jerangau Integrated Landfill, Pahang, was extracted and purified before being sent for 16S rRNA gene sequencing to proceed with the bioinformatics analyses. The findings show that 265 and 273 bacterial amplicon sequence variant (ASV) were sequenced for Jeram and Jabor landfills, respectively, with the dominant phyla Firmicutes for both leachate samples. On average, diverse genera associated with significant functions, such as cellulolytic bacteria (e.g., *Taibaiella*) with a percentage abundance of 0.3% and hydrogen-reducing bacteria (e.g., Anaerobic digester metagenome) with a percentage abundance of 0.375% were analyzed in the Jabor and Jeram landfill leachate, respectively. In addition, the Shannon index, which accounts for the species diversity of Jabor leachate, was lower than that of the Jeram leachate, and the Simpson index, which refers to random selection of the sample, was similar for both samples with no significant difference.

Conclusion, significance and impact of study: Research findings indicate that both leachate samples contained diverse bacterial species. Using PICRUSt2, 10 metabolic pathways were predicted in both landfill leachates. Therefore, this approach can serve as a platform to analyze the distribution of bacterial communities and their functional characteristics in landfill leachate.

Keywords: 16S rRNA, landfill leachate, metabolism pathway, microbial community, PICRUSt2 prediction

INTRODUCTION

Nationwide, the most common option to dispose of municipal solid waste (MSW) is landfilling. (Aslam *et al.*, 2022). In Malaysia, 1.1 kg/day of waste per capita is generated, and more than 26,500 tons of waste are dumped into 166 active landfills (Kamaruddin *et al.*, 2017). Although this is the most suitable option, the effect of the contamination produced by the leachate during landfilling should be taken into consideration (Naveen *et al.*, 2017). Leachate can be described as the byproducts discharged from the precipitation of rainwater onto waste, resulting from aerobic and aerobic biochemical reactions during the decomposition process (Kjeldsen *et al.*, 2002),

comprised of various chemical pollutants that might be harmful to humans and the environment (Costa et al., 2019). A diverse microbial community has been identified to metabolize these complex constituents (Sekhohola-Dlamini and Tekere, 2020). Thus, a culture-dependent and culture-independent approach can be conducted to evaluate this microbial community (Chien et al., 2015; Imron et al., 2019). From a culture-independent approach, diversity and functionality of the microbial the communities could be identified (Kochling et al., 2015). significant However these elements are not comprehensively characterized due to the differences in internal and external factors (Stamps et al., 2016). Accordingly, characterizing the microbial communities

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from different regions may give a significant result based on the survival of the microorganisms towards different factors. Recently, the advancement of a cultureindependent approach, next-generation sequencing (NGS) has tackled the limitation (Fuhrman, 2009) by providing the microbial composition with a comprehensive characterization (Tan *et al.*, 2019). Thus, in this context, the comprehensive 16S amplicon sequencing was adopted to analyze the bacterial communities and PICRUSt2 analysis was conducted to predict the microbial functions.

MATERIALS AND METHODS

Sample collection and processing

Leachate sources from Jeram and Jabor landfill sites, located in the state of Selangor and Pahang, Malaysia, respectively, were collected. Sampling devices were utilized to collect samples. The collected leachate samples were kept in a dry ice box before being chilled at 4 °C in the laboratory for future analysis.

Environmental DNA extraction

Environmental DNA isolation was performed using CTAB based extraction method with simple modification (Healey et al., 2014). Briefly, 10 mL extraction buffer with a composition of 100 mM Tris-HCI (pH 7.5), 1.5 M NaCI, 25 mM EDTA, 0.3% (v/v) β-mercaptoethanol and 2% (w/v) CTAB were heated in 65 °C water bath before added with the filtered leachate sample and incubated in 65 °C for 60 min (inverse every 15 min). The solution was spined at 5000 rpm for 5 min to get the supernatant. The supernatant was transferred to a new falcon tube before being added with Chloroform (24): Isoamyl alcohol (1) to 1 volume and inversed for 5 min. The solution was spined at 5000 rpm for 5 min. The clear layer (upper) was pipetted into a new falcon tube before adding 5 µL RNAse (10 mg/mL) and incubated at 37 °C for 15 min with gentle mixing. Chloroform (24): Isoamyl alcohol (1) with 1 volume was added before inversed for 5 min, and spined at 5000 rpm for 5 min. The clear layer (upper) was again pipetted and added with 1/2 volume of 5M NaCl and inverted. Then, chilled 95% Ethanol to 3 volumes was added and inversed before incubating at -20 °C for 1 h. The incubated sample was spined at 5000 rpm for 10 min to pellet DNA before being washed with 3 mL of 70% ethanol and swirled. The solution was spined at 5000 rpm for 10 min to get the DNA pellet and air dried at room temperature for 15 min. The targeted eDNA was redissolved in 50 µL TE buffer before being sent for Next Generation Sequencing (NGS).

Microbial community analysis

For metagenomic, microbial sequencing was generated by MiSeq using 16S rRNA gene sequencing at V3-V4 region with two universal primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'- GGTTACCTTGTTACGACTT-3'). By using BBTools, an initial 200,000 reads were screened out to eliminate unnecessary reads. After that, QIIME2 was used to merge forward and reverse reads. Then, DADA2 (*https://benijneb.github.io/dada2/*) was applied to trim the error and low-quality reads. The amplicon sequence variant (ASV) was produced and proceeded to the next stage. To complete the taxonomic analysis, Scikit-learn (*https://scikit-learn.org/stable/*) and Naïve Bayes' classifiers from SILVA were applied. Finally, diversity analysis proceeded to analyze the microbial community of the landfill leachate.

Microbial function prediction using PICRUSt2

Generally, 16S rRNA marker gene profiles in the PICRUSt2 were selected to predict microbial function (Douglas *et al.*, 2020). Firstly, the amplicon sequence variant (ASV) was aligned to reference sequences (HMMER). Then, ASV was placed into a reference tree (EPA-NG and GAPPA), and the gene family copy number of ASV was inferred. After that, the gene content per ASV was predicted to generate the gene family profiles. Finally, the map of genes was used to predict the sample pathway abundance for a stringent prediction.

RESULTS

Microbial diversity in landfill leachate

As shown in Figure 1, a total of 538 ASVs were generated using the primer pair (27F-1492R), resulting in 240 and 232 unique ASVs from Jabor and Jeram leachate, respectively. Meanwhile, 33 of the remaining ASVs were found in both leachate samples.



Figure 1: Venn diagram of ASVs in Jabor and Jeram landfill.

The α -diversity indices for both leachate samples are illustrated in Figure 2. The Chao-1 index indicated the community richness in Jabor and Jeram were slightly similar, with a value of 273 and 265, respectively. The Shannon index, with a value of 4.663 for Jabor leachate and 4.822 for Jeram leachate, indicated the diversity of species in the leachate samples. Other than that, both Jabor leachate and Jeram leachate have high Simpson values, 0.968 and 0.973, respectively, indicating all the samples were randomly selected.



Figure 2: The α-diversity indices.



Figure 3: Rarefaction curve for Jeram and Jabor leachate.

In addition, the rarefaction curves for Jeram and Jabor leachate have reached the saturation plateau (Figure 3), indicating that the sampling was sufficiently high to fully represent the saturation of the species richness, which means that most of the species within the system have been detected. The curves also illustrated that their plateau was consistent, indicating that no further sequencing was needed.

Microbial community in landfill leachate

From the phylum level, 17 phyla were classified for both leachate samples (Figure 4). From this composition, the percentage of phyla for both leachate samples denoted a difference in value. As such, Firmicutes (40%), Bacteroidetes (25%) and Proteobacteria (22.5%) were among the most abundant phyla in the Jabor leachate. Jeram leachate was dominated by Firmicutes (60%), Proteobacteria (20%) and Synergistetes (15%). Meanwhile, from the genera level, 60 bacterial genera were identified in both leachate samples.

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Figure 4: Taxonomy profile at phylum level for Jeram and Jabor leachate.



Figure 5: Taxonomy profile at the genus level for Jeram and Jabor leachate.

As shown in Figure 5, Anaerobic digester metagenome, *Taibaiella, Pusillimonas,* uncultured bacterium, *Caldicoprobacter, Rubellimicrobium, Syntrophaceticus,* and uncultured bacteria were the 8 dominant genera for both leachate samples, with a different relative abundance. Specifically, the genus *Taibaiella*, belonging to the Bacteroidetes phylum, was dominant in Jabor leachate (30%), while the Anaerobic digester metagenome (35%), belonging to Firmicutes phylum was dominated by Jeram leachate sample.



Metabolism Pathway

Figure 6: Metabolism pathways predicted by PICRUSt2 based on KEGG database.

Microbial function prediction

To predict the microbial function, the 16S rRNA marker gene was used in PICRUSt2 analysis. From this analysis, the generated NSTI value for Jabor leachate was 0.395, whereas Jeram leachate was 0.438. This low value denoted that both leachate samples were detectable for functional prediction using PICRUSt2. From the microbial composition, a total of 10 metabolism pathways belonging to KEGG database were identified in both landfill leachate. The detailed pathways (Figure 6) were associated with lipid metabolism (31.8%), carbohydrate metabolism (21.3%), nucleotide metabolism (19.7%), metabolism of terpenoids and polyketides (19.3%), amino acid metabolism (6.23%), metabolism of cofactors and vitamins (0.78%), energy metabolism (0.39%),xenobiotics biodegradation and metabolism (0.22%), metabolism of other amino acids (0.11%) and glycan biosynthesis and metabolism (0.03%). Generally, the relative abundance was similar for each pathway for both leachate samples.

DISCUSSION

Microbial community

In this study, the *Bacteroidetes*, *Proteobacteria* and *Firmicutes* have dominated the bacterial communities in both landfill leachate, regardless of the location (Wang *et al.*, 2017). The hydrolysis-acidification phyla, *Bacteroidetes* and *Firmicutes* were mostly found in anaerobic environments to metabolize macromolecules in landfills (Xu *et al.*, 2020). While the abundance of *Proteobacteria* might be responsible for fermenting the

soluble compounds (Xu et al., 2017), such as digested sugars into monosaccharides. Other than this commonly found three phyla, Synergistetes dominated the leachate samples (Song et al., 2015), probably for refuse decomposition. The differences in dominancy at the genera level also suggested the diversity of the microbial community. Previously, some genera, such as Taibaiella (Hou et al., 2021), Pusillimonas (Remmas et al., 2017), Caldicoprobacter (Che et al.. 2021). and Syntrophaceticus (Wong et al., 2019), have been classified as dominant genera in the landfill leachate.

Relatively, among the detected bacterial genera, Taibaiella genus, belonging to Bacteroidetes, was found abundantly in Jabor leachate. This genus is responsible for the elimination of aniline and other toxic landfill leachate, indicating tolerance to xenobiotics (Hou et al., 2021). Meanwhile, Jeram leachate was dominated by anaerobic digester metagenome. This genus might be helpful in metabolizing intermediates and act as a hydrogen source in the landfill (Seo et al., 2019). Other than that, Pusillimonas genus was detected, which was significant in stabilizing the aged landfill using the degradative functionality (Stolz et al., 2005). Caldicoprobacter might be involved in carbohydrate degradation (Zhou et al., 2019) by producing lactate and ethanol (Achmon et al., 2019). For Syntrophaceticus, which depended on the amount of cellulose (Dennehy et al., 2018) was useful in methanogenesis under a thermophilic environment. The presence of Rubellimicrobium is also important in the nitrogen and sulphur cycle during leachate production processes. Overall, all the identified genera were responsible for the solid waste decomposition in the landfill leachate.

Microbial function prediction

To predict the microbial function, PICRUSt2 analysis using KEGG database was performed. From the database, the pathway metabolism was found abundantly in this leachate sample. This pathway was found in MSW degradation (Yang and Song, 2019), food waste anaerobic (Wang et al., 2020) and sludge anaerobic (Zhang et al., 2015). The metabolism pathway was crucial in degrading complex molecules to simpler molecules in anaerobic conditions. As such, carbohydrate metabolism during MSW decomposition was useful in metabolizing organic compounds such as cellulose (Toledo et al., 2017). The occurrence of amino acid metabolism is also important so that enough energy and carbon supply in the landfills (Lopez-Gonzalez et al., 2015). Accordingly, to complete the process of methanogenesis, nitrogen cycle and sulphur cycle, energy metabolism was involved. Moreover, the metabolism of cofactors and vitamins has been stated to be involved in homeostasis (Petri et al., 2019). This functional diversity has proven that microbial communities were important in biodegradation during landfilling (Song et al., 2015). Importantly, the diverse bacterial composition results in similar microbial functions, even in different environments (Zhou et al., 2019). This also suggests that the microbial community undergoes survival and adaptation processes during landfilling (Yang and Song, 2019). Therefore, the distribution and functionality of the microorganisms have emphasized the various roles for possible remediation of landfill leachate.

CONCLUSION

The microbial distribution and predicted function from Jabor and Jeram leachate were examined and analyzed based on 16S rRNA gene amplicon sequencing. At the phylum level, Bacteroidetes, Firmicutes and Proteobacteria dominated the communities for both landfill leachate samples. This diversity provides insight into leachate degradation by characterizing the microbial community and function involved for microorganisms.

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