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Insights into microbial community structure and diversity in oil palm waste compost

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Abstract

Empty fruit bunch (EFB) and palm oil mill effluent (POME) are the major wastes generated by the oil palm industry in Malaysia. The practice of EFB and POME digester sludge co-composting has shown positive results, both in mitigating otherwise environmentally damaging waste streams and in producing a useful product (compost) from these streams. In this study, the bacterial ecosystems of 12 week-old EFB-POME co-compost and POME biogas sludge from Felda Maokil, Johor were analysed using 16S metagenome sequencing. Over 10 phyla were detected with Chloroflexi being the predominant phylum, representing approximately 53% of compost and 23% of the POME microbiome reads. The main bacterial lineage found in compost and POME was Anaerolinaceae (Chloroflexi) with 30% and 18% of the total gene fragments, respectively. The significant differences between compost and POME communities were abundances of *Syntrophobacter, Sulfuricurvum,* and *Coprococcus*. No methanogens were identified due to the bias of general 16S primers to eubacteria. The preponderance of anaerobic species in the compost, and high abundance of secondary metabolite fermenting bacteria is due to an extended composting time, with anaerobic collapse of the pile in the tropical heat. Predictive functional profiles of the metagenomes using 16S rRNA marker genes suggest the presence of enzymes involved in polysaccharide degradation such as glucoamylase, endoglucanase, arabinofuranosidase, all of which were strongly active in POME. Eubacterial species associated with cellulytic methanogenesis were present in both samples.

Keyword Oil palm empty fruit bunch. Palm oil mill effluent. Compost. Metagenomics. Microbial diversity.

1.0 Introduction

The Malaysian oil palm industry is growing rapidly and Malaysia has become the second largest producer of palm oil after Indonesia (MPOB 2017). Approximately 5.74 Mha of Malaysia’s land area was covered with oil palm plantations in 2016 (MPOB 2017) which produced 17,320,000 tonnes of palm oil. This in turn generated a large amount of oil palm derived waste. The oil palm industry produces millions of tonnes of oil palm biomass, especially empty fruit bunch (EFB). There is 1 kg of biomass, such as empty fruit bunch (EFB), palm kernel shell (PKS) and mesocarp fibre (MF), generated for each kg of oil palm extracted (Sulaiman et al. 2011). Conversion of organic waste such as EFB into usable horticultural by-products has been found to be the most efficient way to reuse this raw waste material (Siddiquee et al. 2017).

EFB and palm oil mill effluent (POME) are the most abundant waste produced in oil palm mills. At present, EFB and POME have been used as raw materials for co-composting and the resulting co-compost has been used in the oil palm plantations at Felda Maokil. In this case, POME was used to provide moisture to the compost. Composting is one of the most efficient solutions for sustainable management of organic waste, it is an aerobic process that effectively converts cellulosic organic waste into a nutrient-rich organic amendment for agricultural application (Neher et al. 2013). The conversion of organic waste to compost is carried out by a successive microbial community combining both mesophilic and thermophilic activities (Krishnan et al. 2017). However, the microbial community of end product compost in the tropics has not been well characterized.

Understanding the microbial diversity of compost systems is important in order to produce high quality compost and determine its effectiveness (Krishnan et al. 2017). Most studies that have explored this rich ecosystem have utilized culture-based methods (Ryckeboer et al. 2003; Ahmad et al. 2007; Vishan et al. 2017). But, culture-based methods are only useful for identifying less than 1% of the total microbial diversity, as the majority of microorganisms are unculturable under standard media and aerobic growth conditions (Handelsman 2004; Ito et al. 2018). The advent of Next-Generation Sequencing (NGS) and metagenomics has opened an avenue to perform comprehensive studies to characterize the total microbial diversity using a culture-independent method. Metagenomics is an alternative that has been widely applied over the last few years (Fernández-Arrojo et al. 2010).

Metagenomics refers to the direct isolation of DNA from an environmental sample (Handelsman 2004). There are two different approaches, amplicon sequencing and shotgun metagenomics (Escobar-Zepeda et al. 2015). Amplicon sequencing targets specific regions of DNA from communities by amplifying specific regions using taxonomical informative primer targets such as intergenic transcribed spacers (ITS) and the large ribosomal subunit (LSU) for eukaryotes and the 16S rRNA gene for prokaryotes (Sharpton 2014). Shotgun metagenomics randomly sequences all DNA from a community, which produces a less biased assessment of species abundance but at greater cost. Metagenomics has been commonly used in large and complex samples containing organisms from different life domains or where less bias is required. 16S amplicon sequencing, or metaprofiling (Escobar-Zepeda et al. 2015), is currently the most cost-effective method for DNA library preparation in conjunction with sequencing by platforms such as the Illumina MiSeq. This approach has been widely utilized, not only for studying resident microbiota in wastewater and compost (Krishnan et al. 2017; Wang et al. 2016), but also for studying soil samples (Yan et al. 2016), hot springs (Chan et al. 2015), termite gut (Chew et al. 2018), faecal samples (Costea et al. 2017) and many others.

In the present study, the microbial community of the EFB-POME co-compost and POME biogas sludge has been studied using the culture independent 16S amplicon sequencing approach. Metagenomes from the EFB-POME co-compost and POME were directly isolated from the samples without any microorganism cultivation. The V3-V4 regions of prokaryotic 16S rRNA genes were amplified from the metagenome and directly sequenced using Illumina's MiSeq platform. The detailed information on the microbial residents will support further research to improve the duration of the composting process and the quality of final compost by addition of specified microbial species.

2.0 Materials and Methods

2.1 Collection of samples

The 12 weeks-old EFB-POME co-compost and POME were obtained from Felda Maokil, Labis, Johor (2°17'09.6"N 102°59'37.7"E). A 1 m height compost pile was made with the ratio of 40 ton EFB: 120 ton POME, turned over every 3 days to provide aeration. The composting was done by first laying down the EFB on the ground, followed by the addition of POME to the EFB pile at three day intervals to maintain a final moisture content of 65-75% as it is a solid state aerobic fermentation process. A total of 1kg of 12 weeks-old compost was randomly sampled at a depth of 0.5 m inside the compost pile. For POME, 2 litres was collected directly from the anaerobic digester (AD) effluent. The POME and compost samples were collected in sterile containers and stored at 4 °C for further studies.

2.2 Total DNA extraction

Extraction of total DNA from EFB-POME co-compost was done with a modified Griffiths protocol using NucleoSpin® Soil kit (Griffiths et al. 2000; Alessi et al. 2017), while the total DNA from

POME was extracted using NucleoSpin® Soil kit (Macherey-Nagel, Germany) based on the manufacturer's protocol (Verma and Satyanarayana 2011). The detailed methods are outlined below.

2.2.1 *Method 1: Modified Griffiths protocol*

Half a gram of EFB-POME co-compost was transferred into a microcentrifuge tube containing 1 g of garnet beads (OMNI International, USA), 500 µL SL2 lysis buffer (Macherey-Nagel, Germany), 500 µL 10% CTAB, 100 µL 1mg/mL lysozyme and 214.3 µL enhancer SX (Macherey-Nagel, Germany). After mixing, the samples were disrupted using Bead Ruptor 4 (OMNI International, USA) for 3 minutes at level 3. The aqueous phase was mixed with inhibitor removal solution SL3 buffer (Macherey-Nagel, Germany) and incubated at 4 °C for 5 min before centrifuging using a NucleoSpin® Inhibitor Removal column (Macherey-Nagel, Germany) to remove any impurities like humic acid and other PCR inhibitors. Following this, equal volumes of phenol: chloroform (1:1) was added to the eluted aqueous phase and separated by centrifugation for 5 minutes at 13,300 × g. One-tenth volume of ice-cold sodium acetate and 3 volumes of ice-cold absolute ethanol was added to the aqueous layer before incubating at -80°C for 2 hours to precipitate the DNA. The resulting pellet was washed twice with ice-cold 75% ethanol (Alessi et al. 2017) and the pellet was resuspended in 50 µL TE buffer. The DNA was stored at -20 °C for further use.

2.2.2 *Method 2: NucleoSpin® Soil kit*

Two mL of POME was centrifuged at 4,500 × g for 10 minutes and the resulting pellets were transferred into a microcentrifuge containing 1 g of garnet beads (OMNI International). 300 µL SL2 buffer (Macherey-Nagel, Germany), 150 µL enhancer SX (Macherey-Nagel, Germany) and 100 µL 1 mg/mL lysozyme was added to the pellet. The pellet was homogenized for 3 minutes at level 3 using Bead Ruptor 4 (OMNI International). 100 µL 1 mg/mL lysozyme was added to the homogenate and incubated at 37 °C for 30 minutes. The inhibitor removal solution and column were used to remove any impurities like humic acids or other PCR inhibitors. The binding solution was added to the supernatant before loading onto the spin column. The column was centrifuged to bind the DNA to the column and the column was washed twice with wash buffer provided in the kit. The DNA was finally eluted with the elution buffer and stored at -20 °C.

2.3 DNA yield and purity determination

DNA concentration and quality of the total DNA extracts were determined using NanoDrop™ Lite spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and 1% w/v agarose gel electrophoresis, respectively.

2.4 16S metagenomics library preparation, sequencing and data analysis

The 16S rRNA metagenome libraries were generated using purified total DNA as the template in the polymerase chain reactions (PCR). The V3–V4 region of the 16S rRNA genes were amplified using S-D-Bact-0341-b-S-17, 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3’ and S-D-Bact-0785-a-A-21, 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3’). The underlined oligonucleotide are the Illumina adapter overhang sequences, while the non-underlined sequences are locus-specific sequences which were designed according to a reported primer pair (Klindworth et al. 2013) targeting conserved regions within the V3 and V4 domains of prokaryotic 16S rRNA genes. The metagenome library was then pair-end sequenced on the Illumina MiSeq platform (San Diego, CA, USA) using MiSeq Reagent Kit (v3) for the longest length set to 2 x 300 base pairs (bp).

2.5 Bioinformatics analysis

The resulting sequencing data were analysed using the Mothur software package version 1.41.1 (Schloss et al. 2009). Firstly, the read pairs were merged to assemble them into contigs and contigs exhibiting any ambiguous positions were subsequently removed. The sequences were then aligned to the SILVA reference database (Release 132). Upon removal of unaligned sequences, the remaining sequences were further filtered, dereplicated and de-noised before removal of chimeras. Sequences were then classified based on the Greengenes database using naïve Bayesian classifier with bootstrap cut-off of 80% before removal of sequences classified as unrelated lineages. Finally, the sequences were split into bins based on taxonomy and clustered into OTUs using the *cluster.split* command.

The functional composition of EFB-POME compost and POME digester sludge was analysed using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) bioinformatics software package (Langille et al. 2013). Firstly, PICRUSt analysis was done by picking OTUs against the August 2013 Greengenes database release of gg_13_8_99 that contained

202,421 bacterial and archaeal sequences (McDonald et al. 2012). The OTU counts were normalized and used for metagenome functional predictions with KEGG orthologs (KO). The output was further analysed using the Statistical Analysis of Metagenome Profiles (STAMP) software package (Parks et al. 2014).

2.6 Data accessibility

The raw sequencing data was deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) database under accession numbers SRR8181848 and SRR8186815 for EFB-POME co-compost and POME, respectively.

3.0 Results and Discussion

3.1 Microbial diversity analysis of EFB-POME co-compost and POME

EFB-POME co-compost and POME were analysed for their microbial diversity using Illumina MiSeq sequencing of V3-V4 region of the 16S rRNA genes. A total of 72,657 and 92,677 sequence reads were generated from total DNA extracts of EFB-POME co-compost and POME, respectively. After quality filtering and read merging, EFB-POME compost and POME generated 1,272 and 10,705 contigs, respectively. POME showed a higher number of OTUs, which indicates that POME has a higher bacterial diversity compared to EFB-POME co-compost. Diversity coverage for each sample was analysed using rarefaction analysis. The rarefaction curve illustrated in Fig.1 was calculated at 3% dissimilarity. Rarefaction analysis shows the samples had reached saturation for genus level and higher taxonomic level. The coverage for mature EFB-POME co-compost was 100%, while for POME was 99.4% with Simpson indices of 113.77 and 69, respectively.

The most abundant phylum present in both samples was Chloroflexi, which constituted 23% of the total gene fragment abundance in POME and 53% in EFB-POME co-compost (Fig.2A). The remaining phyla present in POME were Firmicutes (19%), Bacteroidetes (16%), Proteobacteria (16%) and Synergistetes (3%), while in EFB-POME co-compost the phyla were Bacteroidetes (15%), Firmicutes (12%), Proteobacteria (7%) and Actinobacteria (3%). According to Chandna et al. (2013), the number of microbial species in early stages of composting depends on the initial substrate used and the prevailing environmental conditions during the composting process, especially the temperature. Composting can be divided into several phases based on the temperature. These include mesophilic, thermophilic and maturing phases, during which different bacterial populations thrive. Chandna et al. (2013) found that Firmicutes are abundant during mesophilic phase and decrease in the maturation phase, while Actinobacteria are stable during mesophilic and thermophilic phases. Neher et al. (2013) found Bacteroidetes dominated at the end of the thermophilic phase, whereas Proteobacteria was dominant after the thermophilic phase. The predominance of anaerobes in the compost sample are strongly suggestive of a secondary fermentation of the pile after collapse of all air spaces and exhaustion of oxygen throughout the mass. The compost method was similar to that developed by Raabe at Berkeley, which takes 18 days in a Meditteranean climate (Raabe 1981). After 84 days in the tropics, the compost has further fermented. The 16S data for the EFB-POME compost is a glimpse of the microbial structure of this mature compost post aerobic composting.

The major family that was observed in both samples was Anaerolinaceae, which represents 18% abundance in POME and 30% abundance in EFB-POME co-compost as shown in Fig.2B. These OTUs represent a diversity of species, rather than a single dominant species. The other five major families observed in POME were Syntrophaceae (9%), Syntrophomonadaceae (5%), Porphyromonadaceae (5%), Tissierellaceae (3%), and Synergistacaea (3%), while in EFB-POME co-compost, the other major families were Porphyromonadaceae (7%), Lachnospiraceae (3%), Helicobacteraceae (2%), Ruminococcacea (2%), and Tissierellaceae (2%).

The most abundant genus observed in POME was *Syntrophus*, which accounted for 9% of the total gene fragments (Fig.2C). The remaining genus observed in POME include *Syntrophomonas* (5%), *Sedimentibacter* (3%), *Gracilibacter* (3%), *Solibacillus* (3%). Most of the bacteria found in POME digester sludge were anaerobic as methanogensis is an anaerobic process. In a previous study by Krishnan et al. (2017), *Parabacteroides, Levilinea, Smithella, Prolixibacter* and *Bellilinea* were identified as the common genera found in POME. *Bellilinea* was also found in DNA extracts from POME in the present study. However, this genus represented a small majority, which only accounted for 1% of the community. In the EFB-POME co-compost, on the other hand, *Coprococcus* was identified as the most common genera accounting for 3% of the gene fragments, followed by *Sulfuricuvum* (2%), *Sedimenterbacter* (2%) and *Proteiniphilium* (2%). *Coprococcus* are anaerobic bacteria and a major bacterial taxa in the rumen microbiota of some ruminants (Jia et al. 2016). The bacteria from the *Coprococcus* family are commonly involved in the degradation of cellulosic materials (Moore et al. 2011) and can be found enriched in xylan based cultures (Jia et al. 2016) which are often found in anaerobic cellulose digestion. Apart from these genera, *Steroidobacter, Nitriliruptor, Anaeomyxobacter, Filomicrobium* and *Truepera* were also found inside lignocellulose biomass compost by Krishnan et al. (2017).

The overall population in compost is illustrated in Fig.3A. The most common phyla found in the EFB-POME co-compost was Chloroflexi (53% of the total gene fragments) and this phyla was represented 100% of the total Chloroflexi by Anaerolinaceae family. The second most abundant phyla was Bacteroidetes, which accounted for 15% of the total 16S rRNA gene fragments. The major family in Bacteroidetes was Porphyromonadaceae comprising 47.5% of the total Bacteroidetes. *Petrimonas* and *Proteiniphilum* were identified which represent 9.2% and 32.2% of the total Porphyromonadaceae gene fragments. The remaining family in the Bacteroidetes was unclassified with 52.5% of the total Bacteroidetes gene fragments. The third major phyla in compost was Firmicutes accounting for 12% of the total gene fragments. Fig.3B shows that the major order was Clostridiales, which amounts to 89% of the total Firmicutes gene fragments. The remaining were Bacillales and unclassified Firmicutes with 2.7% and 8.3%, respectively. Clostridiales were represented commonly by the family of Lachnospiraceae (24.6% of the total Clostridiales gene fragments) followed by Tissierellaceae (23.8%), Ruminoccoccaceae (23%), Syntrophomonadaceae (7.7%), Gracilibacteraceae (3.8%), Christenellaceae (2.3%) and Clostridiaceae (1.5%) as shown in Fig.3B.

The overall bacterial population of POME are shown in Fig.4A. Similar to EFB-POME co-compost, Cloroflexi was also identified as the dominant phyla (23% of the total gene fragments) which was 100% represented (of the total Cloroflexi gene fragments) by the Anearolinaceae family. Anearolinaceae dominates the population of POME and EFB-POME co-compost. Anearolinaceae is anaerobic and involved in methanogenesis. This family of bacteria is indigenous in many environments rich in oil and hydrocarbon (Liang et al. 2015) and associated with the anaerobic degradation of oil-related compounds (Sutton et al. 2013). Anaerolinaceae has been reported as the predominant species isolated from anaerobic digester systems and has a fermentative metabolism, utilizing carbohydrates and proteinaceous carbon sources under anaerobic conditions (McIlroy et al. 2017; Sun et al. 2016;

Yamada et al. 2006). The absence of the archaean methanogens in the data is due to the known poor ability of the standard 16S primers to amplify these organisms (Klindworth et al. 2013). As all known methanogens are archaea, methanogenic archaea ecosystems are only served by specific 16S primers such as S-D-Arch-0349-a-S-17 and S-D-Arch-0786-a-A-20 primer pair (Fischer et al. 2016). The second common phyla identified in the POME population was Firmicutes with 12% of the total gene fragments (Fig.4B). The major order observed from this phylum was Clostridiales with 77.3% of the total Firmicutes gene fragments. The family of Syntrophomonadaceae was the most dominant, accounting for 31.7% of the total Clostridiales gene fragments. The remaining families observed were Gracilibacteraceae (22.5%), Tissierellaceae (21.7%), Ruminococcaceae (5.7%), Clostridiaceae (5.7%) and Lachnospiraceae (3.1%) as shown in Fig.4B. The family of bacteria found in Clostridiales were similar to the community found in co-compost, with slight variations in abundances as many bacteria in this family are thermotolerant and are to survive the composting process.

3.2 Comparative analysis of EFB-POME co-compost and POME bacterial communities

Sequence data sets retrieved from EFB-POME co-compost and POME digester sludge were compared. Fig.5 compared the relative abundance of the 12 major genus represented in both EFB-POME compost and POME. There were fewer bacterial genera in POME than during the composting process. The dominant bacteria in the compost were *Syntrophobacter, Sulfuricuvum* and *Coprococcus.* There is limited evidence that these bacteria are able to produce compost, and in fact are anaerobes. These organisms likely represent the secondary fermentation of the compost, once the pile had collapsed and oxygen and easily metabolisable carbon had been exhausted. Their fermentative abilities are directed to metabolites likely present after thermophilic composting of woody biomass. Bacteria that did not survive aerobic composting but were found in the POME sludge were *Petrimonas, Syntrophus, Treponema, Bellilinea, Solibacillus, Clostridium, Gracilibacter, Syntrophomonas,* and *Acholeplasma.* Most of these bacteria are anaerobes and facultative anaerobes as POME is an anaerobic digester effluent.

In this study, *Sulfuricurvum* was identified as the predominant bacteria in the mature compost. *Sulfuricurvum* is chemolithoautotrophic and a sulphur-oxidizing bacterium, capable of thriving under microaerobic and anaerobic conditions (Kodama and Watanabe 2004). The condition inside the EFB-

POME compost is facultative anaerobic which is therefore, favourable for the growth of *Sulfuricurvum*. *Sulfuricurvum* has previously been identified in contaminated soil (Liu et al. 2015), river sediments (Liu et al. 2018), underground crude-oil storage (Kodama and Watanabe 2004) and wastewater sludge (Hatamoto et al. 2011). The presence of a chemolithoautotroph demonstrates how limited the nutrients were and how mature the compost was. Liu et al. (2015) reported that the abundance of *Sulfuricurvum* increases with higher moisture, since high moisture content is associated with low redox potential and anaerobic environments (Brockett et al. 2012).

Syntrophobacter was also found in a higher relative abundance in EFB-POME compost compared to POME. *Syntrophobacter* has the ability to degrade propionate, which is usually isolated from methanogenic ecosystems (Boone and Bryant 1980), characteristic of the POME sludge. In anaerobic digestion, the acetogenesis stage is predominantly acetogenic bacteria such as *Syntrophobacter*, which converts fermentation products with more than two carbon atoms, alcohols and aromatics fatty acids into acetate and hydrogen (Kangle et al. 2012). In this stage, the bacteria convert products from the first phase (hydrolysis) to butyric acid, propionic acid, ethanol, acetic acid, carbon dioxide and hydrogen (Nalo et al. 2014).

3.3 Predicted functional metagenome profiles

Metagenome functional prediction was carried out using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis based on the Greengenes 16S rRNA database and KO. A bar graph was plotted to compare the abundance of the metabolic features between the two samples as shown in Fig.6. From the result of this study, energy metabolism was found to be highly represented in POME and compost community. The subfunctions in energy metabolism included carbon fixation pathways in prokaryote, oxidative phosphorylation, nitrogen metabolism, sulphur metabolism and methane metabolism. The proportion of sequences for the energy metabolism is higher in POME sample compared to compost, as the secondary fermentation of the compost in anaerobic conditions is relatively energy limited. For the xenobiotics biodegradation and metabolism, the subfunctions benzoates degradation, bisphenol degradation, drug metabolism by cytochrome P450, naphthalene degradation and polycyclic aromatic hydrocarbon degradation were observed to have higher sequence proportion in the compost community compared to the POME community. These functional classes are explained by secondary fermentation of the remaining recalcitrant substrates in the now anoxic pile.

Carbohydrate metabolism such as nucleotide sugar, fructose and mannose, starch and sucrose and butanoate were observed to be slightly higher in proportion in the POME community compared to compost due to active anaerobic processing of the oil palm products in POME. The degradation of cellulose and hemicellulose during the composting process can produce carbohydrates (Toledo et al. 2017). Those compounds are easily degradable substances, which are preferentially degraded by aerobic eubacteria. Carbohydrate metabolism plays an important role in degradation of hemicellulose and cellulose during the composting process (Wei et al. 2018). Furthermore, amino acids are sources of energy and carbon for bacterial metabolism produced throughout the composting process (López-González et al. 2015). Wu et al. (2017) suggest that a higher abundancy of bacteria with active amino acid metabolism increases humic substance synthesis.

In order to determine the potential roles of microbial communities in the decomposition of plant polymers, carbon degradation enzymes were identified and their presence is illustrated in Fig.7. The enzymes include genes encoding alpha-amylase, glucoamylase and neopullulanase for starch degradation; beta-glucanase, endoglucanase, and beta-glucosidase for cellulose degradation; arabinofuranosidase and xylanase for hemicelluloase degradation; and lastly, beta-hexosaminidase, chitinase, and peptidoglycan hydrolase involved in degradation of chitins derived from fungal decomposition of the plant mass. Apart from that, genes related to chemotaxis was also more abundant in the POME sample as this was a liquid culture.

Due to the poor amplification of archaean 16S sequences however, methanogenesis is only observed in a limited way in both samples. Limitations of 16S primers targeting the V3-V4 domains are clear in this study, where the primary fermentation was not observed. To overcome this limitation and obtain an unbiased view of the archaean diversity, shotgun metagenomics sequencing could be employed.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Fig. 1 Rarefaction curve for a dissimilarity of 3% from sample POME and compost

Fig. 2 Relative abundance of prokaryotes 16S rRNA sequences in **(A)** phyla **(B)** family level and **(C)** genera level for POME and compost samples

Fig. 3 Taxonomic affiliation of 16S rRNA metagenomics fragment. The phylogenetic distribution for **(A)** the overall population and **(B)** Firmicutes in compost

Fig. 4 Taxonomic affiliation of 16S rRNA metagenomics fragment. The phylogenetic distribution for **(A)** the overall population and **(B)** Firmicutes in POME

Fig. 5 Heatmap visualizing the z-score distribution of the major genera among POME and compost samples

Fig. 6 Proportion of sequences for the predicted metabolic profile in POME and mature compost

Fig. 7 Number of sequences for the predicted enzymes involved in carbon (polysaccharides) degradation

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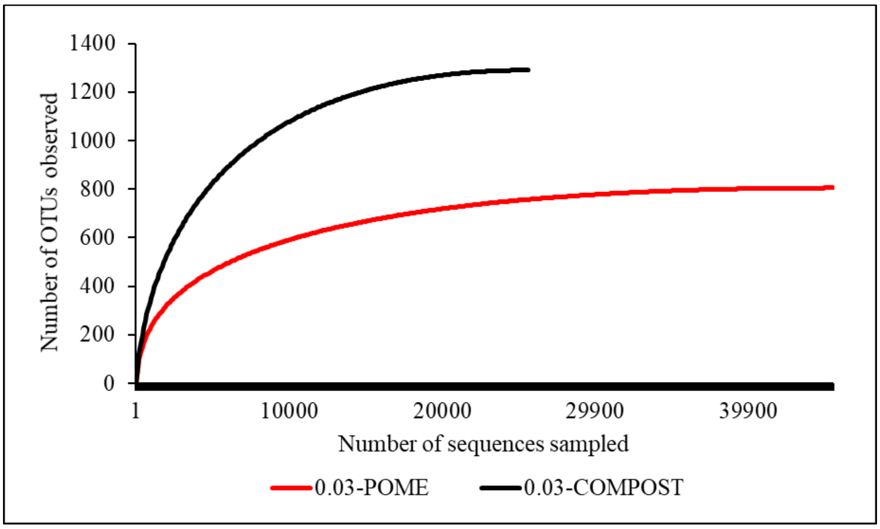

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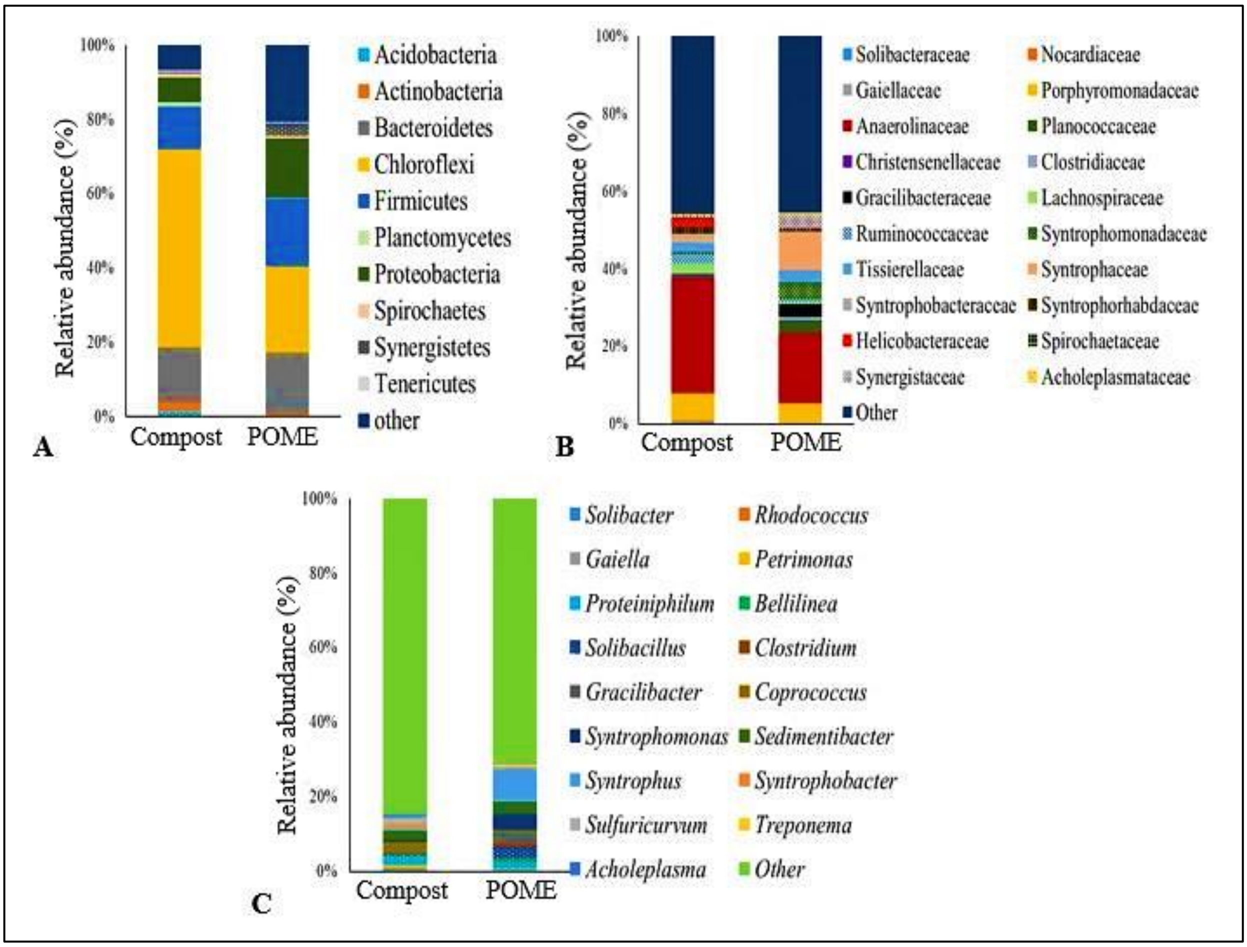
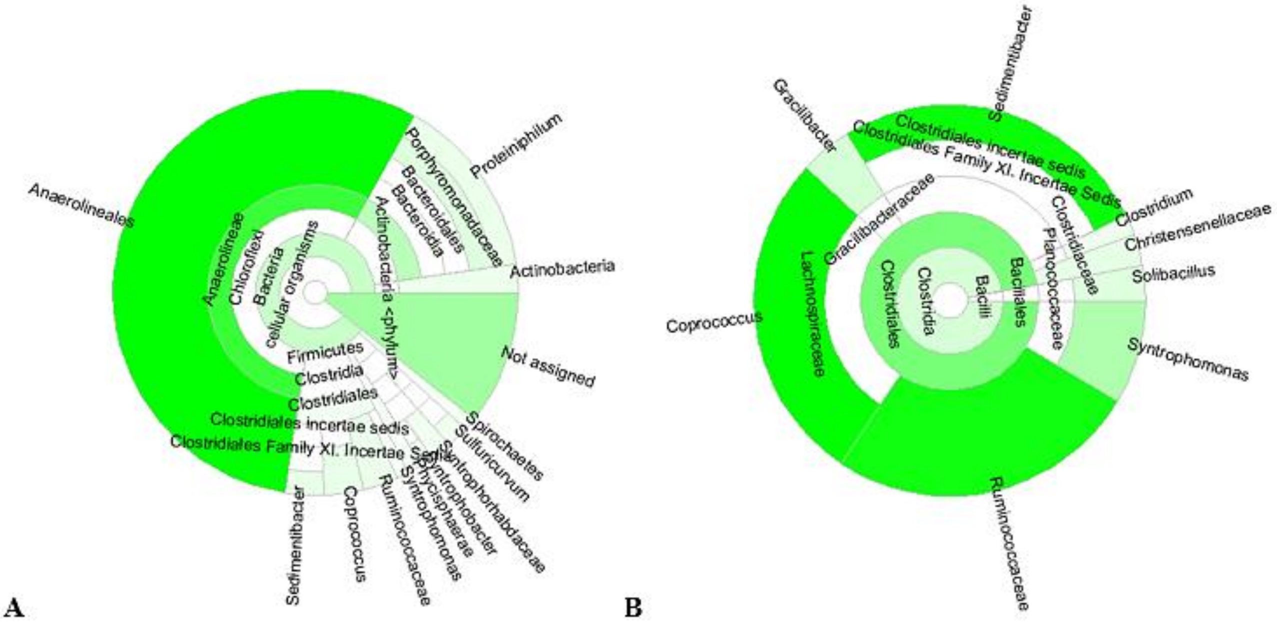


Fig. 2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Anaerolineae
Chloroflexi
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Bacteroidia
Bacteroidales
Porphyromonadaceae
Proteiniphilum
Actinobacteria
Not assigned
Firmicutes
Clostridia
Clostridiales
Clostridiales incertae sedis
Clostridiales Family XI. Incertae Sedis
Spirochaetes
Sulfuricurvum
Syntrophorhabdaceae
Syntrophobacter
Phycisphaerae
Syntrophomonas
Ruminococcaceae
Coprococcus
Sedimentibacter
A
Gracilibacter
Sedimentibacter
Clostridiales incertae sedis
Clostridiales Family XI. Incertae Sedis
Clostridium
Christensenellaceae
Solibacillus
Gracilibacteraceae
Clostridiaceae
Planococcaceae
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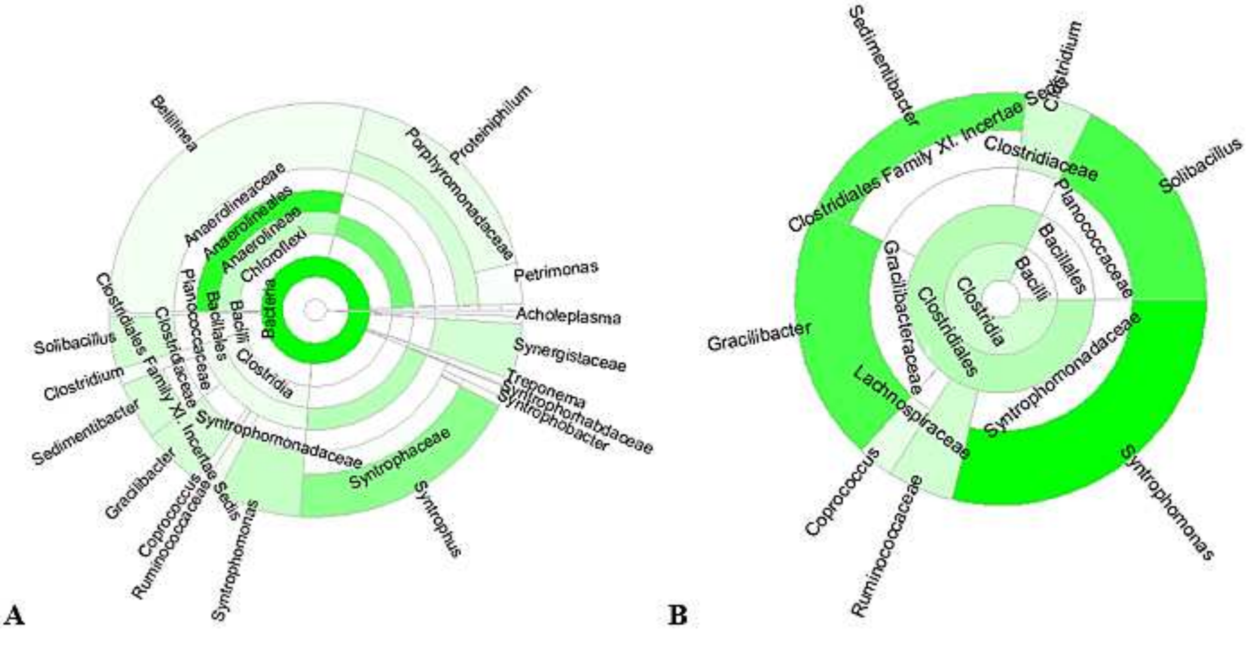
Syntrophomonas
Ruminococcaceae
B

Fig. 3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Synergistaceae
Treponema
Syntrophorhabdaceae
Syntrophobacter
Clostridiales Family XI. Incertae Sedis
Clostridiaceae
Planococcaceae
Bacillales
Bacilli
Clostridia
Solibacillus
Clostridium
Sedimentibacter
Gracilibacter
Coprococcus
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Syntrophomonadaceae
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A
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Clostridia
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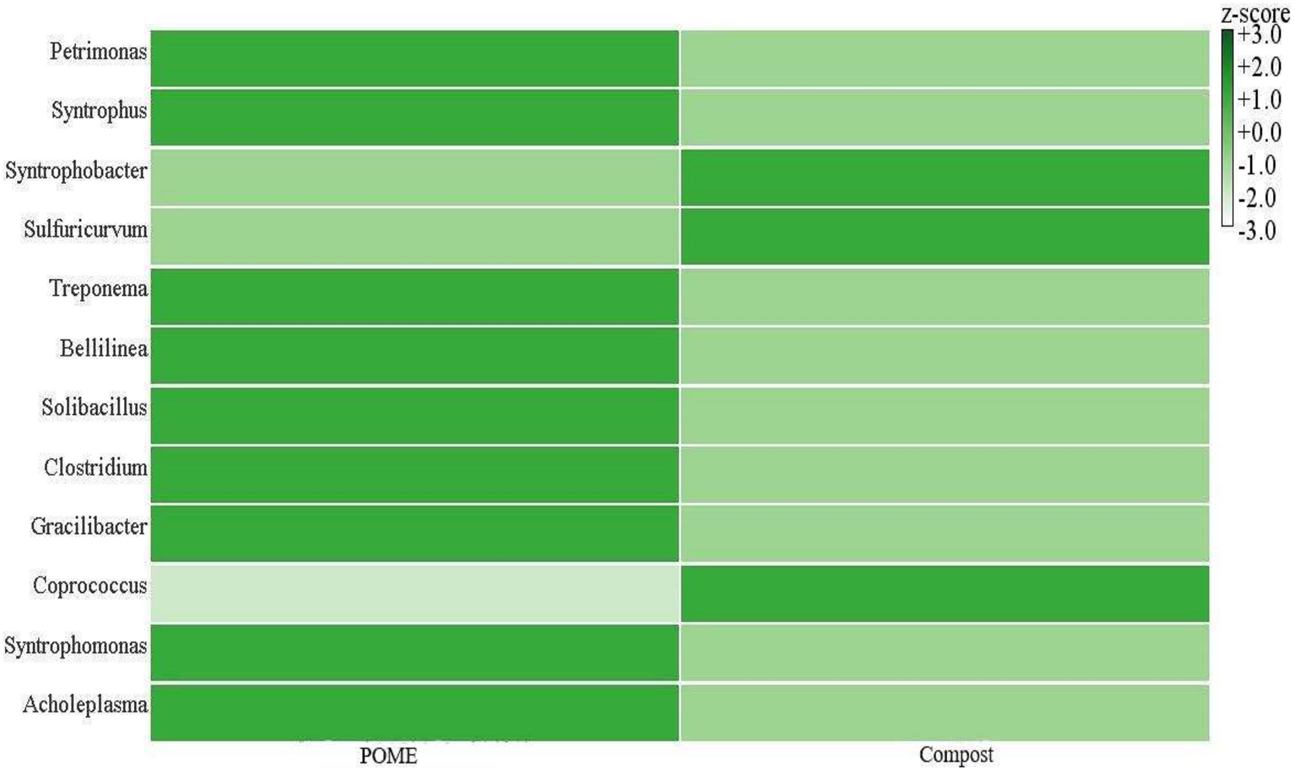
B

Fig. 4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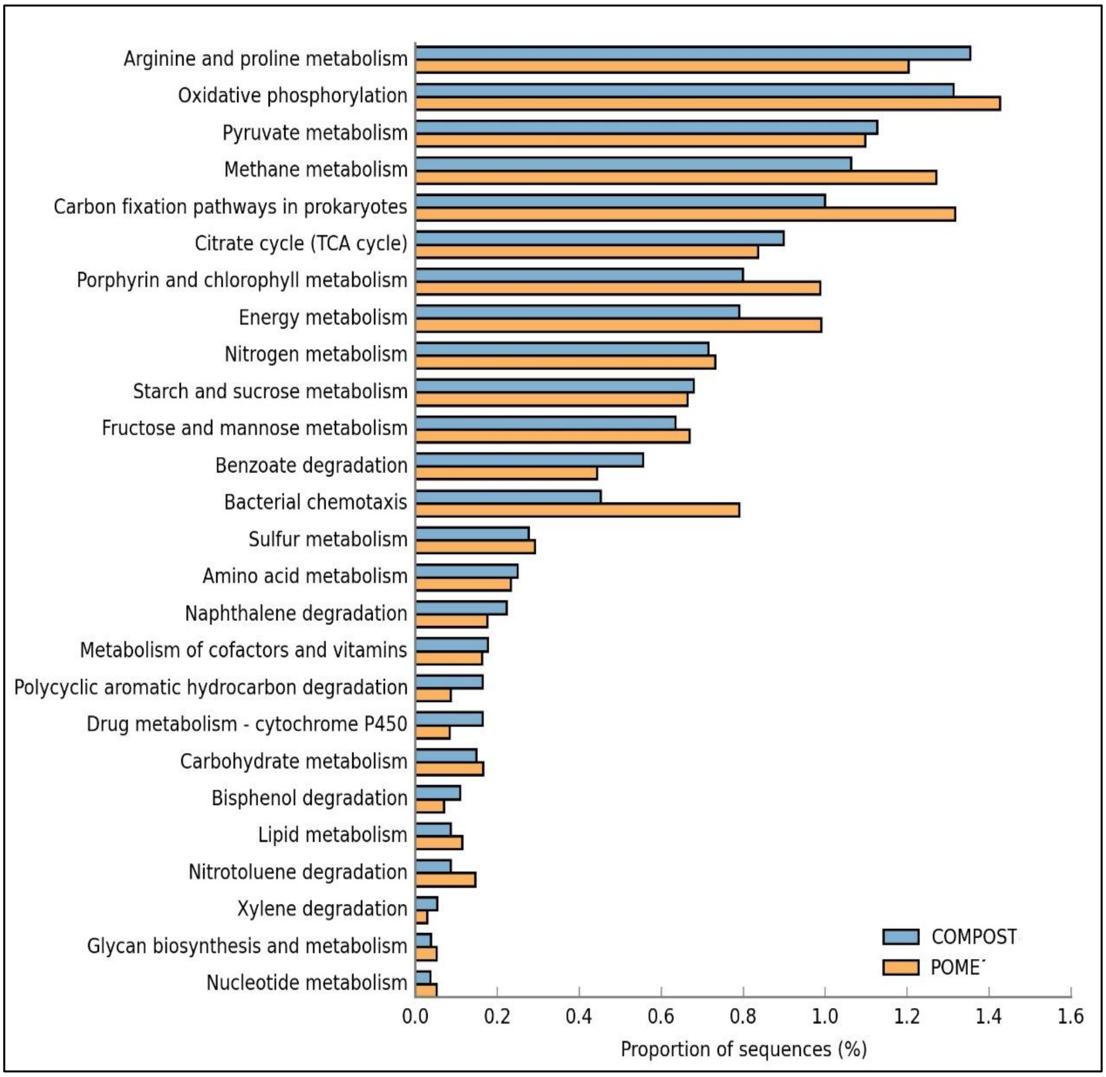
Gracilibacter
Coprococcus
Syntrophomonas
Acholeplasma
POME
Compost
z-score
+3.0
+2.0
+1.0
+0.0
-1.0
-2.0
-3.0

Fig.5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Oxidative phosphorylation
Pyruvate metabolism
Methane metabolism
Carbon fixation pathways in prokaryotes
Citrate cycle (TCA cycle)
Porphyrin and chlorophyll metabolism
Energy metabolism
Nitrogen metabolism
Starch and sucrose metabolism
Fructose and mannose metabolism
Benzoate degradation
Bacterial chemotaxis
Sulfur metabolism
Amino acid metabolism
Naphthalene degradation
Metabolism of cofactors and vitamins
Polycyclic aromatic hydrocarbon degradation
Drug metabolism - cytochrome P450
Carbohydrate metabolism
Bisphenol degradation
Lipid metabolism
Nitrotoluene degradation
Xylene degradation
Glycan biosynthesis and metabolism
Nucleotide metabolism
COMPOST
POME
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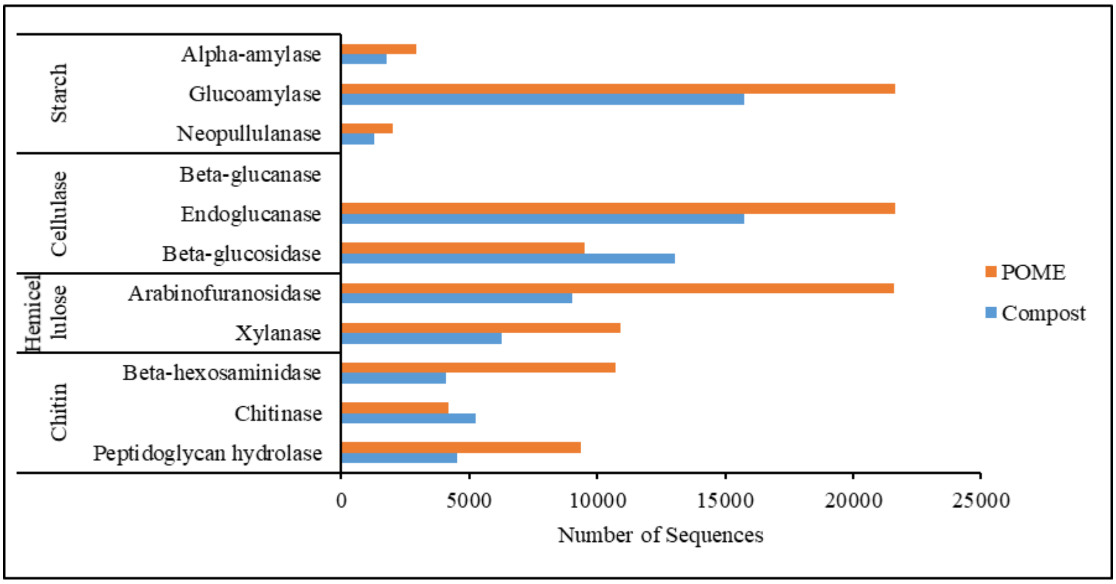
0.8
1.0
1.2
1.4
1.6
Proportion of sequences (%)

Fig. 6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Endoglucanase
Beta-glucosidase
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Arabinofuranosidase
Xylanase
Chitin
Beta-hexosaminidase
Chitinase
Peptidoglycan hydrolase
POME
Compost
0
5000
10000
15000
20000
25000
Number of Sequences

Fig. 7