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DYNAMIC CHANGES OF BACTERIAL COMMUNITY DURING THE COMPOSTING OF OIL PALM EMPTY FRUIT BUNCHES (OPEFB) AS ANALYZED BY DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) ANALYSIS

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ABSTRACT

In this study cultivation-independent approach was performed to characterize and understand the bacterial community dynamics during the oil palm empty fruit bunches (OPEFB) composting process. OPEFB was composted in a compost heap of 1m × 1m × 1m. During the process, bacterial community structure in specific composting time were characterized by denaturing gradient gel electrophoresis (DGGE) analysis based on the hypervariable V3 region of 16S rRNA gene. The DGGE banding patterns revealed significant change in the bacterial community structure throughout the composting process. A total of 38 bands were selected for sequencing analysis. Majority of the DGGE bands' sequences were related to known counterparts with low percentage of identity indicating novel composting bacterial populations. These DNA sequences were related to culturable relatives of *Firmicutes, Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Fibrobacteres* and *Chloroflexi*. In general, distinctive predomination by *Firmicutes* followed by succession by *Proteobacteria* were revealed during the OPEFB composting process. Information attained in this study may be useful for improving the efficiency of OPEFB composting by indigenous bacterial population. The diverse sequences from known bacteria indicated novelty in the composting bacterial populations and potentially their functions.

Key words: Oil palm empty fruit bunches (OPEFB), compost, bacterial communities, denaturing gradient gel electrophoresis (DGGE), 16S rDNA

INTRODUCTION

Oil palm (scientific name, *Elaies guineensis Jacq.*) is an important commodity crop in tropical countries, including Malaysia and Indonesia. In Malaysia, 97.56 million tons of fresh fruit bunches were processed in year 2015 to extract crude palm oil (Anonymous, 2016). During the process, oil palm empty fruit bunches (OPEFB) that accounted for 23-25% of total fresh fruit bunches (H-Kittikun *et al.*, 2000), is rapidly being generated by the palm oil processing mills at increasing volume by year due to huge increasing demand for crude palm oil.

OPEFB consists of 77.7% of holocellulose, 44.2% of alpha-cellulose, 33.5% of hemicellulose and 20.4% of lignin (Khalid *et al.,* 2008) which is highly resistant to microbial degradation. Successive groups of microbes in each composting stages are believed to play different physico-chemical role(s) in degradation of the OPEFB. The understanding of microbial community at each successive OPEFB composting stage would therefore provide in-depth information useful towards enhancement of composting efficiency and compost quality by revealing the novel microbial structures. Although studies have been carried out extensively to optimize the composting process, there are limited reports on the microbial dynamics during composting of OPEFB (Thambirajah *et al.,* 1995; Baharuddin *et al*., 2009; Yahya *et al*., 2010). While most studies reported on culturable microbial communities, Zainudin *et al* (2014) reported on

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the bacterial diversity of OPEFB compost based on DGGE. However, the research group analysed 7 DGGE bands which did not represent the richness of the community.

The recent advancement of molecular techniques in culture-independent methods including PCR-DGGE enabled more thorough investigation on microbial ecology. In this regard, these advance techniques help to overcome the limitation of culture-dependent approaches which were known to reveal less than 1% of the bacterial communities from the environmental samples (Amann *et al.,* 1995; Davis *et al.,* 2005). PCR-denaturing gradient gel electrophoresis (PCR-DGGE) is one of the cultivation-independent techniques that can provide a higher resolution in determination of predominant microbial community from environments (Muyzer, 1999) and provide a promising method to monitor the microbial community shift during a process. Microbial diversity studies from various sources using DGGE have been reported. These include samples from animal manure compost (Song *et al*., 2014), crude oil (Liew & Jong, 2008), microbial fuel cell (Jong *et al*., 2006), rhizosphere soil (Yang *et al.,* 2016), ryegrass silage (Li & Nishino, 2011), agricultural vegetable compost (Adams & Frostick, 2009; Wang *et al*., 2015), biogas digester (Liu *et al.,* 2009), kitchen waste compost (Wang *et al*., 2015) and many more.

It is general known that bacterial communities in each composting process greatly differ from one to the other depending on the initial composting material & the composting method (Ishii & Takii, 2003; Haruta *et al.,* 2005; Wang *et al*., 2015). Although Liew and co-workers (2009) reported the bacterial community in mature OPEFB compost, to the authors' knowledge, there is no study focusing on microbial succession during OPEFB composting based on the culture-independent methods. In our study, we aim to investigate the bacterial community shift during pile composting on OPEFB using PCR-DGGE analysis. The dominant populations will be identified based on the hypervariable V3 region of 16S ribosomal RNA gene sequences.

MATERIALS AND METHODS

Experimental design

The traditional composting process was carried out outdoor in a pyramid-like compost heap of 1m × 1m × 1m (length × width × height). The main composting materials included mixture of raw OPEFB and chicken manure. A total of 1.5 tonnes of raw unshredded raw OPEFB was obtained from the local palm oil processing mills. Topsoil and urea were added as additives. The compost heap consisted of repeated layers of feedstock (OPEFB, chicken litter, topsoil and urea). During the composting, water was added when needed to maintain the moisture content of compost heap. The compost heap was turned using a track loader during the process to achieve homogeneity and provide enough aeration. Sampling was performed at day 0, 2, 4, 7, 10, 14, 18, 23, 30, 37, 44, 51, 59, 66, 81 and 137. Approximately 50 g of samples from the core (0.5 m depth) of the compost heap were collected in three replicates. All samples were processed immediately after sampling.

Physico-chemical analyses

The temperature of the sampling point was measured using a thermometer with type R thermocouple probe (OAKTON Instruments, USA). The pH value and moisture content were performed according to Takaku and co-workers (2006) with some modifications. To measure the pH value, 5 g of sample was suspended into 50 ml of pure water and mixed by shaking at 200 rpm for 3 hours under room temperature. The pH value of the suspension was determined using a pH meter (Eutech instruments, Singapore). Moisture content was determined by drying 10 g of fresh compost sample in an oven at 105°C until constant weight value is obtained.

Denaturing gradient gel electrophoresis (DGGE) analysis

Total genomic DNA of all compost samples was extracted using PowerMax™ soil DNA isolation kit (MO BIO laboratories, USA). Amplifications of 16S rDNA fragments were carried out as described previously (Liew *et al.,* 2009) using the universal bacterial primer pair, 27f (5'-AGAGTTTGATCCTG GCTCAG-3') and 1492r (5'-GGTTACCTTGTTA CGACTT-3') (Lane, 1991). The PCR products were then visualised on a 1% SeaKem® LE agarose gel (Lonza, USA) using a Syngene gel documentation system (Syngene, UK) after staining with 1 µg/mL ethidium bromide solution. Subsequently, nested PCR was performed to amplify the hypervariable region V3 using the primer 341fGC (5'-CGCCCG CCGCGCCCCGCGCCCGGCCCGCCGCCCCC GCCCCCCTACGGGAGGCGACAG-3') and 534r (5'-ATTACCGCGGCTGCTGG-3') to generate PCR product (~200bp) containing 40 bp of GC-clamp as described previously (Liew & Jong, 2008).

The GC-clamp PCR products were separated using a DCode universal mutation detection system (Bio-Rad laboratories, USA). PCR products with GC-clamp were examined on a 10% (wt/vol) polyacrylamide (37.5:1 acrylamide/bis-acrylamide) gel with a denaturant ranging from 30% to 60%, where a 100% denaturant is defined as 7 M urea

and 40% (v/v) formamide. Electrophoresis was carried out in 1 × TAE (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA) for 5 hours at 130 volts, 60°C. After electrophoresis, the gels were stained with ethidium bromide and documented using a Syngene gel documentation system (Syngene, UK).

Sequencing analysis

Selected DGGE bands were excised from the gel and re-amplified using primer pair 341f/534r (without GC-clamp). The reamplified PCR products were cloned and sequencing reactions were carried out using DYEnamic ET terminator cycle sequencing kit as suggested by manufacturer (GE Healthcare, UK). Sequencing of the sample was carried out commercially. Partial 16S rDNA sequences were aligned to the non-redundant sequence database at the National Center for Biotechnology Information (NCBI) using the nucleotide-nucleotide Basic Local Alignment Search Tool (BLASTn) program. GenBank accession numbers of these bands' sequences are indicated in Table 1. The sequence data for representative DGGE bands (34) were deposited into GenBank database under the accession numbers of EU725866, EU725869- EU725874, EU725876- EU725878, EU725880- EU725881, EU921820, EU921823, GU586692, GU586694, GU586699- GU586701, GU586709- GU586710, GU586715- GU586717, GU586721- GU586723, GU586725, GU586727- GU586728 and GU586731- GU586734**.**

RESULTS

Physico-chemical properties' changes during composting

The changes of the measured mean temperature, pH and moisture contents for the compost are shown in Figure 1. The compost temperature drastically increased from 39.3°C at Day 0 to 65.8°C at Day 2, which is the highest temperature recorded. The temperature dropped gradually and maintained at approximately 60°C during the thermophilic phase (Day 2-10). During the cooling phase (Day 14-59), the temperature declined slowly and maintained around 45°C. The compost core slowly attained ambient temperature during maturation phase (Day 59-137). The pH increased within the first 2 days from an initial value of 7.7 at Day 0 to approximately 9 at Day 2. The alkaline pH maintained between pH 8.4 to pH 9.0 onwards. On the other hand, the compost's moisture content fluctuated between 38.6 – 61.7% throughout the composting process. The initial moisture content was about 61% after setting up the compost heap. At the end of composting, the moisture content was around 45%.

DGGE analysis

In this study, DGGE bands adopting the same migration rate during gel electrophoresis were decided to represent the same bacterial species. Bacterial community structure dynamic over composting time is observed based on changes in DGGE banding patterns. The decreasing number of bands indicates reduction in the bacterial diversity over the period of composting (Figure 2). In total, 38 DNA bands were excised aseptically from the DGGE gels. DNA sequencing analysis (Table 1) revealed that these bands were distributed among 6 bacterial phyla, specifically *Firmicutes* (18 bands), *Proteobacteria* (sub-division *alpha*- (3 bands), *beta*- (6 bands), *delta*- (1 band), *gamma*- (3 bands)), *Acidobacteria* (2 bands) *Bacteroidetes* (3 bands), *Fibrobacteres* (1 band) and *Chloroflexi* (1 band). Seven bands were matched to cultivated bacterial sequences at species level with an identity index of ≥97% and eight bands matched to cultivated bacterial sequences at genus level with index of ≥95% but < 97%. The remaining 19 bands exhibited percentage of identity less than 95% to their cultivated counterparts. However, all 16 bands show an identity index of ≥97% with their uncultivable relatives in GenBank database, 8 with an identity index of ≥95% but < 97% and 10 bands with percentage of identity less than 95%.

The bacterial diversity at Day 0 and 2 were the most diverse among other sampling days. At Day 0, the initial phase, bands related to *Acidobacteria, Bacteriodetes, Fibrobacteres, Firmicutes, alpha-Proteobacteria* and *beta-Proteobacteria* were detected. During the thermophilic phase, maximum banding patterns was observed on Day 2 when the temperature of the compost heap peaked (65.8°C) but the number of bands reduced thereafter. DNA sequences of these bands were distributed among five bacterial lineages, including *Bacteroidetes, Chloroflexi*, *Fibrobacteres, Firmicutes* and *Proteobacteria*. The most intense band appeared on Day 2 was distantly related to Bacterium Ellin 5299 (accession number EU029132, originating from soil, 95% sequence similarity).

During Day 10-23 of the cooling phase, most of the dominant/intense bands detected were related to *Firmicutes*. The *Firmicutes* group consisted of Bacillales bacterium (P4), *Bacillus* (P14, P29), *Clostrdium* (P5, P33), *Eubacterium* (P28), *Natranaerobiaceae* bacterium (P16), *Oceanobacillus* (P7, P19) and *Paenebacillus* (P20). Successive predominance by *Proteobacteria* was observed from Day 37 onwards until Day 66 of maturation phase, including *Burkhlolderia pseudo-mallei,* (P27), and *Hydrogenophaga* sp. (P15, P17, P17a and P17b) of the *beta-Proteobacteria*. The bands recovered during the maturing phase were

Table 1. BLAST results of DGGE band sequences from OPEFB compost

Band	Accession no	Culturable				Unculturable		
		Closest relative (accession no)	Id (%)	Source	Affiliation	Closest relative (accession no)	Id (%)	Source
P1	EU725866	Chitinophagaceae bacterium HU1-GB11 (FJ263934)	97	Freshwater sediment	*Bacteroidetes*	Uncultured *Flavobacteria* bacterium clone GASP-MB1S3_G10 (EF664640)	97	Forest soil
P2	GU586699	*Parapedobacter koreensis* Jip14 (NR043933)	91	Dried rice straw	*Bacteroidetes*	Uncultured bacterium DGGE band A10-2 (DQ640331)	91	SBR reactor
P3	GU586700	Bacterium Ellin6075 (AY234727)	96	Soil	*Acidobacteria*	Uncultured bacterium clone ZG7023_SP6.ab1 (EU007648)	97	Soil
P4	GU586709	Bacillales bacterium YT0311 (AB362830)	97	Alkaline high CO_2 soil	*Firmicutes*	Uncultured bacterium clone A089 (AB269486)	97	Solid waste compost
P5	GU586692	*Clostridium* sp. 6-31 (FJ808611)	95	Biocompost	*Firmicutes*	Uncultured bacterium clone TR4 (DQ661712)	98	Methanogenic bioreactor
P6	GU586701	*Lysinibacillus* sp. CB22 (JN247744)	97	Rhizosphere	*Firmicutes*	Uncultured bacterium clone AFEL2_aao29g12 (EU466815)	98	African elephant feces
P7	GU586725	Oceanobacillus sp. A21 (AB623011)	95	Unknown	*Firmicutes*	Uncultured bacterium clone A195 (AB269539)	98	Sewage sludge compost
P8	EU725869	Bacterium Ellin6067 (AY234719)	93	Activated sludge system	*Beta-proteobacteria*	Uncultured bacterium clone CS2_153 (EF222008)	95	Oil palm empty fruit bunches compost heap
P9	EU725873	*Chromatium* sp. EP2204 (Y12299)	93	Unknown	*Gamma-proteobacteria*	Uncultured bacterium clone H49-814 (EF174279)	95	Compost maturation stage
P10	EU725870	*Paenibacillus pinihumi* S23 (GQ423057)	96	Unknown	*Firmicutes*	Uncultured bacterium clone P_2C02 (HE589879)	97	rice phyllosphere
P11	EU725871	*Sphingomonas* sp. S-2 (AY081166)	96	Tropical soil	*Alpha-proteobacteria*	Uncultured bacterium clone F1Q32TO04EDBNQ (GU755491)	96	Drinking water treatment plant
P12	EU725872	Bacterium Ellin6075 (AY234727)	94	Soil	*Acidobacteria*	Uncultured Acidobacteria bacterium clone RP16.0059 (EF457481)	96	Uranium contaminated subsurface
P13	GU586716	*Steroidobacter denitrificans* FS (NR044309)	90	Anoxic digested sludge	*Gamma-proteobacteria*	Uncultured bacterium clone Niitsu18-45 (AB187888)	96	Compost
P14	EU725874	*Bacillus* sp. PML14 (EF165014)	97	Waste water	*Firmicutes*	Uncultured bacterium clone SMG36 (AM930289)	97	Composting sample
P15	GU586732	*Hydrogenophaga intermedia* 0310ARD8H_6 (FR848406)	90	Air from a cave	*Beta-proteobacteria*	Uncultured bacterium clone RO118 (EF219024)	90	Osmosis membrane
P16	GU586727	Natranaerobiaceae bacterium Z1001 (HQ322120)	91	Sediment of soda lake	*Firmicutes*	Uncultured compost bacterium clone 2B06 (DQ346478)	97	Compost
P17 P17a P17b	EU725876 EU921822 GU586696	*Hydrogenophaga intermedia* 0310ARD8H_6 (FR848406)	98	Air from a cave	*Beta-proteobacteria*	Uncultured bacterium clone RO118 (EF219024)	97	Osmosis membrane

Table 1 continued...

P18	GU586715	*Natranaerobius* sp. JW/NM-KB 411 (GU570705)	91	Kenyan-Tanzanian Rift	*Firmicutes*	Uncultured compost bacterium clone 2B06 (DQ346478)	94	Compost
P19	EU725877	*Oceanobacillus profundus* (FR750971)	96	Marine Sponge	*Firmicutes*	Uncultured bacterium clone A160 (AB269526)	97	Solid waste compost
P20	GU586731	*Paenibacillus sp. SF3-28* (AM268317)	88	Bryophyte of peat bog	*Firmicutes*	Uncultured bacterium clone HAW-RM37-2-B-650d-A5 (FN563170)	88	Mesophilic biogas digester
P21	GU586717	*Fibrobacter intestinalis* DR7 (M62687)	85	Porcine cecal contents	*Fibrobacteres*	Uncultured bacterium clone Niitsu24_25 (AB187930)	95	Compost
P22	EU921823	*Flavobacterium* sp. JJ011 (JN712176)	90	Lake	*Bacteroidetes*	Uncultured Eubacterium clone U79-1 (DQ137959)	97	Wetland
P23	GU586721	*Sphaerobacter thermophilus* (NR042118)	91	Sewage sludge	*Chloroflexi*	Uncultured *Sphaerobacter* sp. clone DGGE band A-1 (DQ082870)	92	Mushroom compost
P24	EU725878	*Steroidobacter* sp. ZUMI 37 (AB548216)	95	Soil	*Gamma-proteobacteria*	Uncultured bacterium clone Niitsu31-45 (AB188010)	98	Compost
P25	GU586722	*Planomicrobium okeanokoites* SJ111 (GQ140340)	77	Deteriorated stone	*Firmicutes*	Uncultured bacterium clone A128 (AB269505)	79	Solid waste compost
P26	EU725880	Bacterium Ellin 5299 (AY234650)	95	Soil	*Alpha-Proteobacteria*	Uncultured bacterium LR A2-25 (DQ988306)	98	A2 reactor
P27	GU586728	*Burkholderia pseudomallei* DRDEBPS1004 (JN001989)	91	Soil	*Beta-proteobacteria*	Uncultured bacterium clone OUT_3 (EU083481)	92	Hexadecane-degrading contsortium
P28	GU586734	*Eubacterium* sp. WAL 18692 (GQ461730)	88	Stool sample	*Firmicutes*	Uncultured bacterium clone M55_D21_H_B_A07 (EF586054)	89	Solid waste
P29	EU725881	*Bacillus* sp. M71_S11 (FM992820)	98	Sediment	*Firmicutes*	Uncultured compost bacterium 5-33 (AB034720)	98	Compost
P30	GU586723	*Paracoccus* sp. SK198 (HQ836455)	97	Carbon source enrichment	*Alpha-Proteobacteria*	Uncultured bacterium clone NGA7 (EF613992)	80	Paddy soil
P31 P31a P31b	EU921820 EU921821 EU725882	*Natronovirga wadinatrunensis* JW/NM-WN-LH1 (EU338489)	92	Soda lake	*Firmicutes*	Uncultured compost bacterium clone 2B06 (DQ346478)	98	Compost
P32	GU586694	*Alkalibaculum bacchi* CP11 (FJ438469)	91	Livestock-impacted soil	*Firmicutes*	Uncultured bacterium clone CK8 (GU320655)	97	Sludge from thermophilic site
P33	GU586710	*Clostridium scatologenes* K29(AB610570)	91	Silage	*Firmicutes*	Uncultured bacterium clone M55_D21_H_B_A07 (EF586054)	97	Solid waste digester
P34	GU586733	*Desulfuromonas acetoxidans* (AY187305)	87	Chicken cecum	*Delta-Proteobacteria*	Uncultured bacterium clone HM64 (AM909864)	79	Soil

Id (%): % Identity.

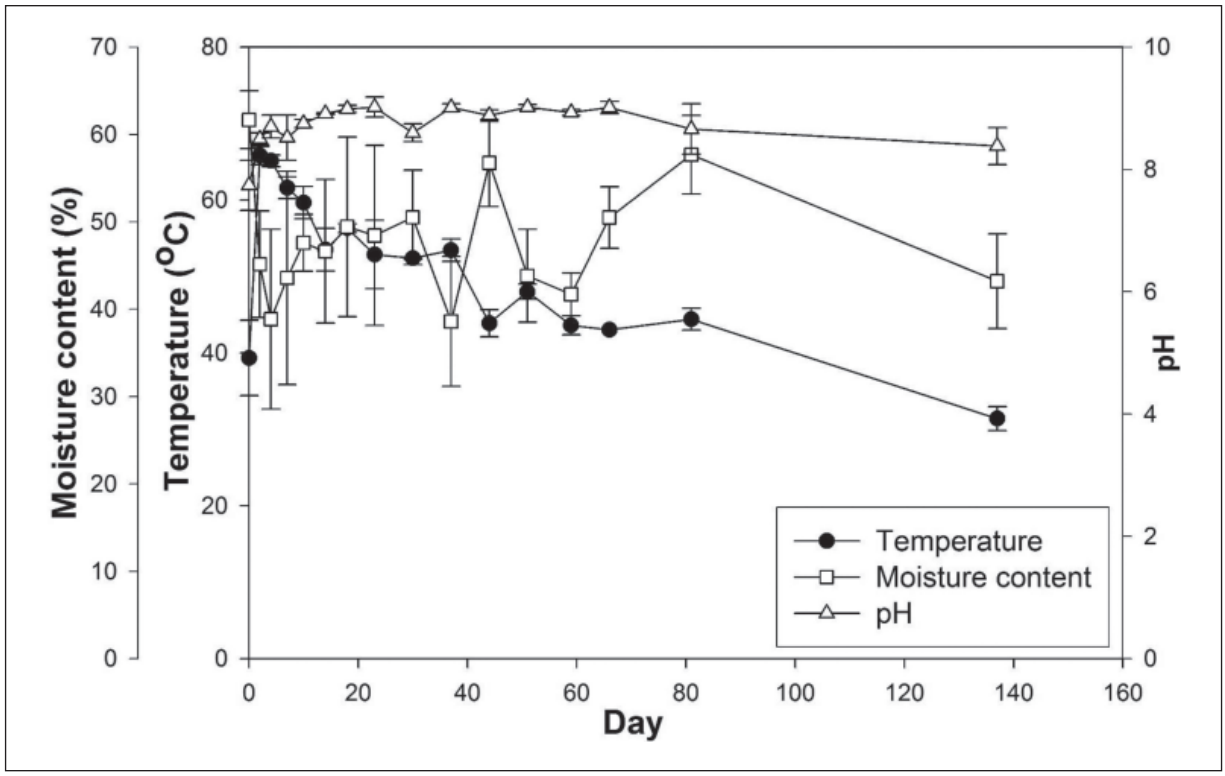

Fig. 1. Temperature, moisture content and pH value profiles of the compost heap during the composting process. Type of parameters: (•) Temperature; (□) Moisture content; (Δ) pH.

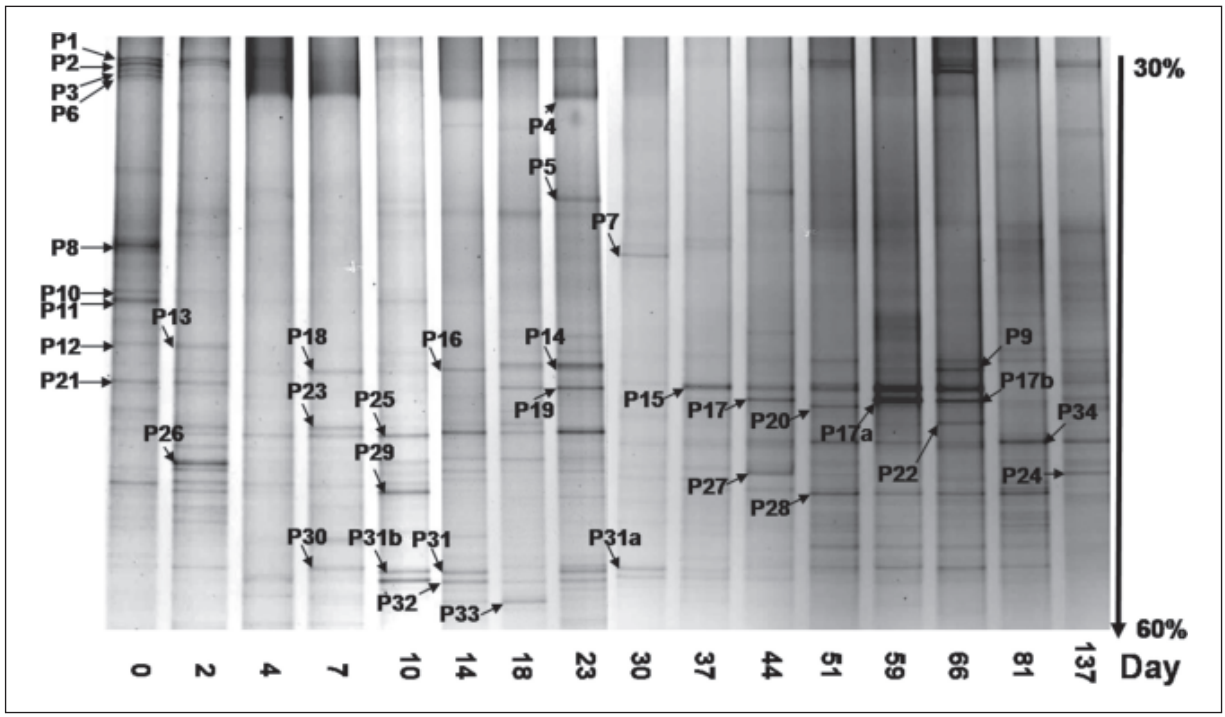

Fig. 2. DGGE profiles of bacterial communities during the composting of OPEFB. The black arrow (right) indicates the linear denaturant gradient. The numbers indicate the position of the excised DGGE bands.

related to *Firmicutes* (*Eubacterium* sp., similar migration distance with P28, *delta-Proteobacteria* (*Desulfuromonas acetoxidans,* P34); *gamma-Proteobacteria* (*Chromatium* sp., P9; *Steroidobacter* sp., P24) and *Bacteroidetes* (*Flavobacterium* sp., P22, Chitinophagaceae bacterium, P1; *Parapedobacter korensis*, P2).

DISCUSSION

Based on the DGGE bands analysis, the bands with sequences related to *Firmicutes* and *Proteobacteria* are two most abundant phyla throughout the composting process. The abundant DGGE bands observed on Day 0 of OPEFB composing suggesting a highly diverse bacterial community structure was expected (Figure 2). Most of these bands showed similarity to sequences originating from soil, rhizosphere and other environmental sources (Table 1). This is in agreement with the outdoor set up for the composting heap and the composting inputs including OPEFB and chicken manure. A DGGE band P8 was affiliated to an uncultured bacterium clone CS2_153 with 95% identity. The uncultured counterpart was previously documented in similar OPEFB compost at the same composting facility (Liew *et al*., 2009). Besides, DNA sequence of P8 was also distantly related to a previously uncultured soil bacterial isolates, *Bacterium* Ellin6067 (AY234719) from *beta-Proteobacteria* with 93% identity. The bacteria was originating from an activated sludge system.

However, most bands gradually disappeared or reduced in bands' intensities and similar findings were also reported by Song *et al* (2014) during the composting of chicken and pig manure. Based on the DGGE bands *Firmicutes* emerged as the predominance bacterial populations from Day 2 until Day 10. The disappearance or reduced predominance as described by the less complicating banding patterns and lower DGGE bands' intensities of most bacterial members suggested that the high temperature may have killed or reduced most of the initial bacterial species and subsequently contributed to the decline in microbial biomass. Similar finding was also reported by Schloss *et al* (2003).

Predomination by *Firmicutes* persisted from Day 10 onwards until Day 23 while composting started cooling phase. Similar findings were also reported by others (Zainudin *et al.*, 2014; Antunes *et al*., 2016). The predominant *Firmicutes* populations are represented by bands P4, P5, P7, P14, P16, P19, P25, P29, P31, P31a, P31b, P32 and P33. The composting temperature recorded was ~50ºC. The persistence of *Firmicutes* at the high temperature could be due to their specialized abilities to form spore which are able to tolerate higher temperatures. Most of the bands from *Firmicutes* including P18, P25, P29, P31, P31a, P31b and P32 were closely related to sequences that previously reported from environment sources agreeable to the natural presence of *Firmicutes* members (Blanc *et al.,* 1999; Ohno *et al*., 2000; Pedro *et al*., 2001; Takaku *et al.,* 2006; Watanabe *et al*., 2009). Bands distantly related to other *Firmicutes* members specifically *Alkalibacterium* (P32, 91% identity) and *Clostridium* (P33, 91% identity) were also detected in the late thermophilic phase. These bands however showed closer affiliation (≥ 97% sequence similarity) to their uncultured counterparts, uncultured bacterium clone CK8 (GU320655) and uncultured bacterium clone M55_D21_H_B_A07 (EF586054) isolated from organic waste compost and solid waste digester, respectively. Both *Alkalibacterium* (alkaliphile bacterium) and *Clostridium* were anaerobic organisms. This may indicate the anaerobic niche inside the compost heap. Similar findings were reported in other studies (Blanc *et al.*, 1999; Ryckeboer *et al.*, 2003).

Sphaerobacter thermophilus of phylum *Chloroflexi,* a Gram-positive, non-spore-forming thermophile was the closest cultivable relative to band P23 which appeared only on Day 7 (Figure 2). This bacterium is previously reported in the thermophilic sludge (Hensel *et al*., 1989). However, its presence in the OPEFB composting was not known.

From the later stage of cooling phase (Day 37 - 59) until compost maturation, the decline in compost heap temperature and the alkaline condition are believed to promote the proliferation of bacterial groups belonging to *beta-Proteobacteria, gamma-Proteobacteria* and *delta-Proteobacteria*. The predominant DGGE bands of *Proteobacteria* include P15, P17, P17a, P17b and P27 which were affiliated to *beta-Proteobacteria*, P9 and P24 which were affiliated to *gamma-Proteobacteria* and P34 which was affiliated to *delta-Proteobacteria*. The presence of *gamma-Proteobacteria* (P9, P24) in cooling phase was also reported by several studies (Peters *et al*., 2000; Ishii & Takii, 2003; Takaku *et al.,* 2006). Beffa *et al.* (1996) in their study described that water-soluble depolymerised lignocellulose compounds was found in the cooling and maturation phases which promote the growth of a large variety of mesophilic bacterial species. DGGE band P34 which was affiliated to *delta-Proteobacteria* started to increase in band intensity from Day 44 towards compost maturation (Day 137).

Overall, bacterial members related to phyla *Firmicutes* predominated Day 10-23 of the cooling phase, which were then succeeded by predomination by bacterial members of *Proteobacteria* until the

end of the maturation phase. *Firmicutes* re-emerged at Day 51 (bands P20, P28) to co-dominate the compost community together with *Proteobacteria* until end of composting. *Hydrogenophaga* of *Betaproteobacteria* (bands P17, 17a P17b) was reported as a chemoorganotrophic bacterium which utilizes hydrogen and carbon dioxide as their energy and carbon sources, respectively (Willems *et al.,* 1989). It was one of the hydrogen-oxidizers previously found in maturation phase of a green waste composting (Beffa *et al.,* 1996). Hence, it was suggested that the presence of *Hydrogenophaga* in the compost most possibly play important role in the sulfur- and hydrogen- oxidization activities during the maturation phase of the OPEFB compost.

Bacteroidetes was present on Day 137 but with low band intensity. This indicated that it is a less abundant group in the maturation phase bacterial community. In the present study, *Flavobacterium* (P22) was found in the compost environment despite the fact that it is a common freshwater inhabitant (McBride *et al.,* 2003). Besides, the bacterium was also reported in the cooling phase of composting by Ryckeboer and co-workers (2003). According to McBride *et al* (2003), although *Flavobacterium* cannot degrade compost, it can decompose certain types of polysaccharides. This may explained the detection of the bacterium at the final composting stage of maturation.

The indigenous bacterial communities in a composting environment are responsible for the biodegradation of organic materials and they play an important biological factor affecting the composting process. It is generally known that the composting environment changes continuously in terms of physicochemical and microbial diversity due to the interaction between the biotic and abiotic factors within the compost as reported (Haruta *et al.*, 2005; Franke-Whittle *et al*., 2014). Several reports (Beffa *et al.,* 1996; Ryckeboer *et al.,* 2003; Haruta *et al.,* 2005; Takaku *et al.,* 2006; Kopeæ *et al*., 2015) also suggested that biotic factors are presumably the main determinant to the succession of microbial community reside in the compost. In the present study, succession/predomination by distinct microbial groups at different composting phases maybe direct or indirectly influenced by the distinctive physicochemical conditions which favored their proliferation.

CONCLUSION

In conclusion, our study provided an insight of the bacterial community shift/dynamic assisted by documentation of the physical parameters including temperature and pH during OPEFB composting. We believed that the knowledge is important for ultimate improvement of the composting efficiency and quality. Future works by pyrosequencing may provide a better understanding of the microbial diversities in the oil palm empty fruit bunches composting process.

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