CHAPTER 5

Microbial community in anaerobic co-digestion of pig manure with food waste in channel digester-upflow anaerobic sludge blanket (CD-UASB) and completely stirred tank reactor (CSTR)

5.1 Introduction

Anaerobic digestion is an efficient method to treat wastewater containing highly concentrated organic compounds. This process not only removes organic pollutants in wastewater, but also produces renewable energy in the form of biogas. Anaerobic digestion is a biological process comprised of four major steps – hydrolysis, acidogenesis, acetogenesis and methanogenesis – performed by various groups of microorganisms. The complex organic compounds are reduced to organic acids, such as acetic acid, hydrogen (H_2) and carbon dioxide (CO_2), by bacteria during the first three steps. Finally, these products are converted to methane and CO_2 by methanogenic bacteria in the last step.

Recently, the energy demand of pig farms in Thailand has been increased due to development of automatic feeding system within the farms. One of the approaches for improving the biogas production is co-digesting the manure with degradable wastes as well as wastes that available in the vicinity of pig farms and farm land. Co-digestion with other wastes could provide more suitable physicochemical property of feedstock and more balanced nutrients for efficient digestion. Thus, it could be possible to achieve higher digestion efficiency and biogas production (Esposito *et al*., 2012). In addition, co-digestion could also reduce inhibition by ammonia when pig manure is digested individually (Yenigün and Demirel, 2013). There are a number of wastes, which could be used to co-digest with pig manure. Food waste is one appropriate substrate for co-digestion due to the availability and its suitable physicochemical characteristics with high moisture (70-90%) and volatile solid (VS) content, and

excellent biodegradability (Li *et al*., 2010; Gou, *et al*., 2014). Moreover, co-digestion with food waste also reduces serious environmental problems such as odor and leachate production from improper food treatment (Li *et al*., 2010).

Previous studies showed that many factors such as, wastewater composition, reactor design and operation conditions have impacts on the microbial community (Akarsubasi *et al*., 2005; Pholchan *et al*., 2010). Therefore, successful biogas production is based on stable, adaptation and activity of these microbial communities which depends on environmental conditions in the reactor (Gerardi, 2003). For this reason, the knowledge and understanding about microbial behavior involved in the system are required in order to control and improve the reactor performance (Fernández *et al*., 1999). Co-digestion of animal manure with organic wastes has been extensively studied (El-Mashad and Zhang, 2010; Zhang *et al*., 2013). However, little works have been done on the microbial community so the information of microbial community structure in anaerobic co-digestion is still insufficient.

Denaturing gradient gel electrophoresis (DGGE) is a technique used for comparative study of microorganisms in a variety of environments, including soil, water (Muyzer and Smalla, 1998) and activated sludge in wastewater treatment (Lee *et al*., 2008). This method is a powerful tool for analysis structure and identifying the dominant species in environmental samples. DGGE also presents the overall picture of the microbial community composition (Muyzer and Smalla, 1998). In this study, microbial community (archaea and bacteria) of anaerobic co-digestion of food waste with pig manure in CD-UASB and CSTR under different hydraulic retention time (HRT) of 10, 20 and 30 days were investigated during operation by DGGE method.

5.2 Materials and Methods

5.2.1 Sludge sampling and DNA extraction

Sludge samples were collected at different time (see Chapter 3) from laboratory scale CD-UASB and CSTR with working volume of 1000 L and 500 L, respectively. Pig manure mixed with food waste in the ratio of 60:40 (base on VS) were fed into 3 reactors with different HRT of 10, 20 and 30 days (Rerkkriangkrai *et al*., 2009; Muenjee, 2010). The sludge samples were stored at -20 °C until use. Total genomic DNA was extracted as described in Chapter 3.

5.2.2 PCR amplification

The variable V3-V5 region of bacterial 16S rDNA was amplified by PCR using the universal bacterial primers 357f-GC and 907rM (Muyzer *et al*., 2004) which attached a GC-clamp at 5' end of the forward primer. The primer details, reaction mixtures and cycle conditions were described in Chapter 3.

For analysis of archaeal population, the variable V3 region of 16S rDNA was amplified using 2 sets of universal archaeal primer. The primers PRE46f and PREA1100r (Øvreås *et al*., 1997) were used in first round and these PCR products were then used as template in a second round of PCR amplification using the primers PARCH340f-GC and PARCH519r (Øvreås *et al*., 1997) which attached a GC-clamp at 5' end of forward primer. The primer details, reaction mixtures and cycle conditions were described in Chapter 3. The size and concentration of PCR products were estimated by electrophoresis in a 1.4% (w/v) agarose gel followed by ethidium bromide (EtBr) staining and visualized under UV light.

5.2.3 DGGE analysis

DGGE analysis of the PCR products was performed using Dcode^{TM} Universal Mutation Detection System (Bio-Rad, USA) according to Muyzer *et al*. (1993). 8% and 6% (w/v) polyacrylamide gel (40% acrylamide/bis solution, 37:1) with a denaturing gradient of 20-45% and 30-55% (100% denaturant contain 7M urea and 40% (v/v) formamide) were used for archaea and bacteria, respectively. Electrophoresis was performed in 1X TAE buffer at 130 Volts for 5 hours at 60 ºC. After electrophoresis, the gels were stained in EtBr for 20 min and visualized under UV light.

Prominent DGGE bands were excised from the DGGE gel with sterile blade and suspended in 30 µl of sterile deionized water at 4 ºC overnight to diffuse DNA. The 2 µl of the eluted DNA was re-amplified by PCR with the same primers without GC-clamp and the same condition as described in Chapter 3. The PCR products were then purified with GF-1 Ambiclean Kit (Vivantis, Malaysia) and ligated into the pGEM-T Easy vector (Promega, USA) according to the manufacturer's instructions. Plasmid was purified with PureYield™ Plasmid Miniprep System (Promega, USA) according to the manufacturer's instructions and sent for sequencing to 1st BASE, Malaysia.

5.2.4 Statistical analysis of DGGE profile

To analyses microbial community structure in each gel, the banding pattern was converted as a binary matrix base on the presence (1) or absence (0) of each band. Jaccard coefficients of similarity were calculated for the similarities of a binary banding matrix, which were clustered by unweighted pair group method with arithmetic mean (UPGMA) algorithm with 1000 bootstrap replicates to obtain confidence estimates. These calculations were performed using FreeTree (Hampl *et al*., 2001) and the resultant tree displayed using TreeView (Page, 1996).

5.2.5 Establishment of archaeal 16S rDNA library

Archaeal clone library was generated from PCR amplified 16S rDNA using archaeal primer set PRE46f and PREA1100r under condition as described above. The PCR products were then purified with illustra™ GFX™ PCR DNA and Gel Band Purification Kits (GE Healthcare, UK). The PCR product was cloned into pGEM-T Easy vector (Promega, USA) and checked insert using the same method as describe in Chapter 3.

5.2.6 DNA sequencing and phylogenetic analysis

The 16S rDNA inserts were sequenced using the M13 primer by 1st BASE, Malaysia. All sequences were checked for chimeric molecules using Bellerophon program (http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl) and sequences determined to be chimera were removed from further analysis. Sequences were compared with related available sequences in the GenBank (http://blast.ncbi.nlm. nih.gov/Blast.cgi) and EzTaxon (http://www.ezbiocloud.net/eztaxon) databases. The sequences were then aligned using Clustral X and neighbor-joining tree was constructed from the aligned

sequences using the molecular evolutionary genetics analysis program version 4.0 (MEGA 4.0).

5.3 Results and discussion

5.3.1 Microbial community

Microbial communities (bacteria and archaea) were monitored during operation of CD-UASB and CSTR fed with 60% pig manure and 40% food waste under different HRT of 10, 20 and 30 days by DGGE technique. Moreover, similarity coefficient (Jaccard's index) was used to compare the bacterial DGGE profiles during operation of the system.

1) Bacterial population

1.1) Bacterial population profile

The DGGE profiles of bacteria in CD-UASB reactor under different HRT were shown in Figure 5.1 (a, c and e). The results of HRT 10 days showed that bands 1, 2 and 3 were found in the beginning of operation. Bands 4, 5, 6 and 7 were appeared during the middle and end of operation. Bands S2, S3, S4 and S5 were detected throughout the operation of process and also found in seed. Considering these bands, bands S2 and S4 were the most dominant band as shown in Figure 5.1a. The resulting bacterial profile in CD-UASB reactor at HRT 20 days showed that bands 9, 10, 11, 12, 13, S1, S2, S3, S4 and S5 appeared throughout the process although, some bands were faint in some periods of operation. However, band 13 was found from day 6. Bands S1, S2, S3, S4 and S5 were originated from seed, with bands S2 and S4 as the most dominant band (Figure 5.1c). The resulting bacterial profile in CD-UASB reactor at HRT 30 days was shown in Figured 5.1e. This profile showed bands 15, 16, 17, 18, S2, S3 and S4 throughout the operation of process. Bands 16, 17 and 18 were more intense during the first 10 days of operation while band 15, S2 and S3 were more intense during days 15-45. Band 19 was also found during this period. Band 20 was found in beginning and had more intensity at the end of operation. Considering these bands, S2 and S4, which found in seed were the most dominant band.

The generated dendrograms from DGGE profiles of CD-UASB reactor at HRT 10, 20 and 30 days exhibited two different clusters as shown in Figure 5.1(b, d and f). The first cluster contained DGGE profile of seed and day 3 for HRT 10 and 30 days,

and seed and days 3-6 days for HRT 20 days. The second cluster could be classified into two sub clusters; days 6-15 and 22-62 for HRT 10 days, days 10-22 and 36-62 for HRT 20 days, and days 6-10 and 15-22 for HRT 30 days. The results showed that bacterial population structure in the first week of operation was similar to seed. Then, bacterial population was periodically changed. However, structure of bacterial population after the first week of operation was similar.

The resulting profiles of bacteria in CSTR at different HRT were shown in Figure 5.2 (a, c and e). The result of HRT 10 days showed that bands 1, 2, 3, 4, S2, S4 and S5 were detected throughout the process, whereas bands S2, S4 and S5 originated from seed. Bands 1 and 3 were the most dominant band (Figure 5.2a). The resulting profile of bacteria in CSTR at HRT 20 days showed bands 5, 6 and 7 throughout the process. Moreover, bands S2, S4 and S5 from seed were also found and bands 5 and 6 were the most dominant band as shown in Figure 5.2c. The DGGE profile of bacteria in CSTR at HRT 30 days revealed bands 8, 9, 10, S1, S2, S4 and S5 throughout the process, whereas bands S2, S4 and S5 were also found in seed. Considering these bands, bands 8 and 9 were the most dominant bands as shown in Figure 5.2e.

The generated dendrograms from DGGE profiles of CSTR at HRT 10 and 30 days exhibited two different clusters as shown in Figure 5.2(b, d and f). The first cluster contained DGGE profile of seed. The second cluster of HRT 10 and 30 days could be classified into two sub clusters; days 3-12 and 17-64 for HRT 10 days and days 3-17 and 22-64 for HRT 30 days, while HRT 20 days contained profile of days 3-64. Bacterial population structure in the first 2 weeks of operation was similar. After that, they were change and stable. Nevertheless, bacterial population structure from begin to end of operation were similar. Seed was different from the others, probably due to the low number of some bacterial species in seed and they became more abundant in the reactor due to more favorable conditions during operation. Thus, bacterial pattern in the first stage of operation seemed to differ from seed.

The DGGE profiles and generated dendrograms from the profiles during operation of both reactor types showed different in bacterial pattern (Figure 5.1 and 5.2). Bacterial population structure in CD-UASB reactor at the beginning of operation was similar to seed while those of CSTR were differed. This is because the seed was

collected from CD-UASB reactor which could result in the bacterial population in CD-UASB adapted to the system better than CSTR. Moreover, it is evident that the bacterial population structures during operation were similar. They showed little change during operation and could adapt to the variation of influent VS throughout the operation of system (Rerkkriangkrai *et al.*, 2009; Muenjee, 2010). Because food waste contained easily degradable compounds so bacteria were easy to adapt and utilize these organic materials. Moreover, the results of CD-UASB reactor also showed that bacteria in seed, especially bands S2 and S4 were the most dominant in all HRT. This indicated that bacteria in the seed can adapt to live under new condition and play crucial role in the digestion. In addition, the bacterial profiles of each reactor also contained many small DNA bands, which are difficult to detect. These bands indicated that the bacterial population in anaerobic digestion is relatively complex and high diversity.

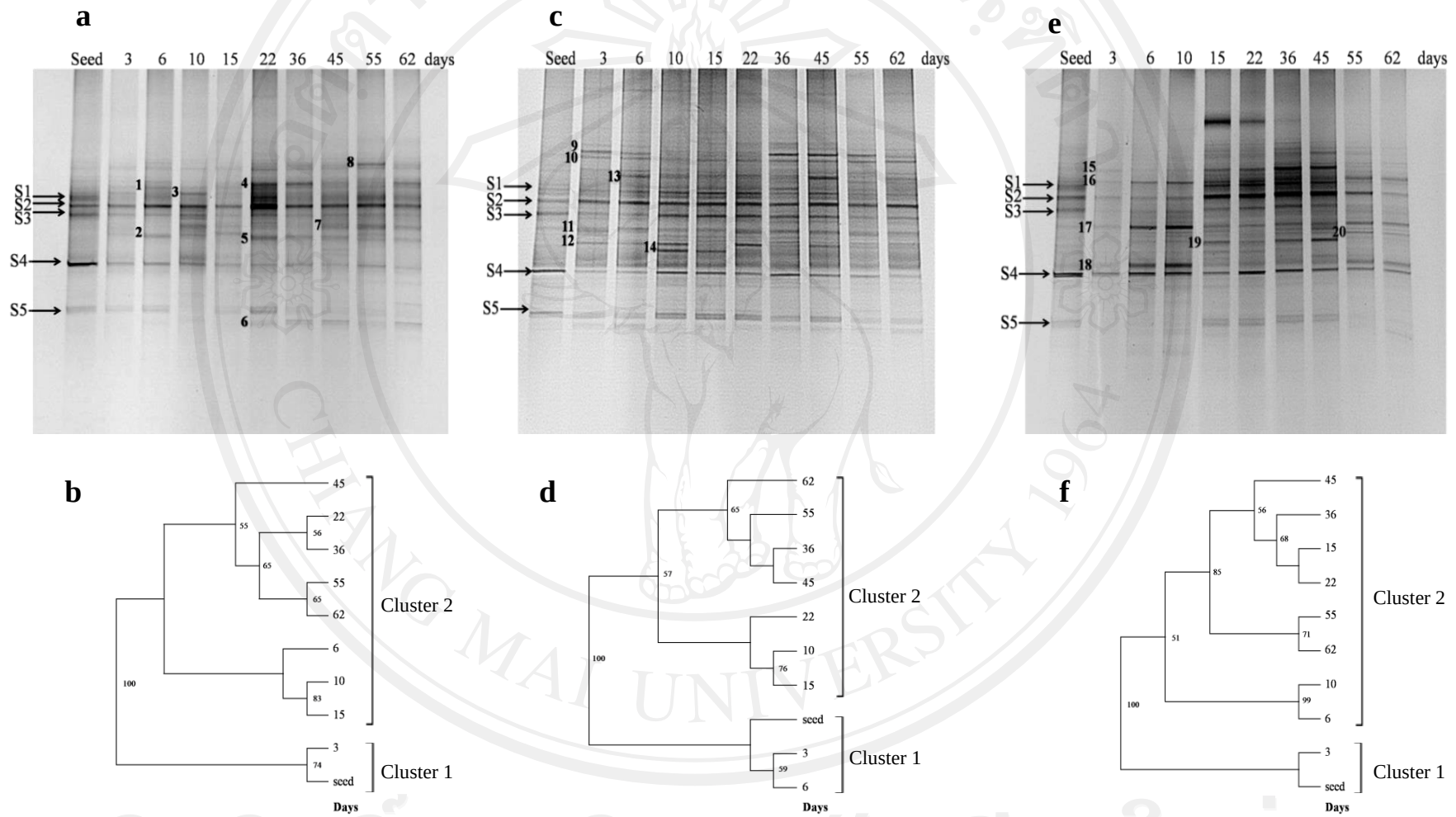

Figure 5.1 DGGE profiles and dendrograms (UPGMA clustering) of bacterial 16S rDNA fragments amplified from sludge in CD-UASB reactor co-digested with food waste under different HRT; HRT 10 days (a, b), HRT 20 days (c, d) and HRT 30 days (e, f).

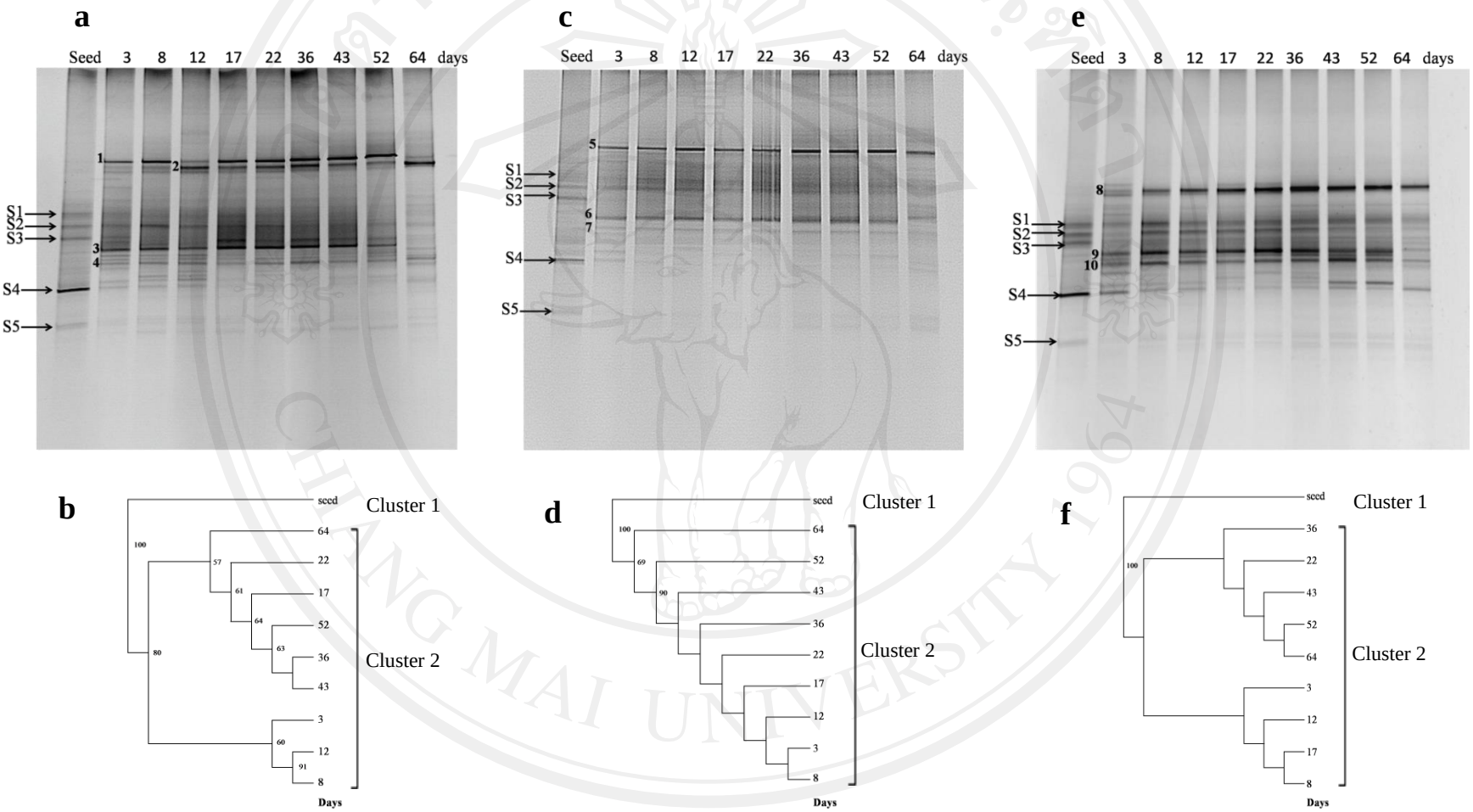

Figure 5.2 DGGE profiles and dendrograms (UPGMA clustering) of bacterial 16S rDNA fragments amplified from sludge in CSTR co-digested with food waste under different HRT; HRT 10 days (a, b), HRT 20 days (c, d) and HRT 30 days (e, f).

1.2) Comparison of bacterial population structure between reactors

Similarity coefficient (Jaccard's index) was used to compare the bacterial DGGE profiles at steady state of system. The UPGMA dendrogram of bacterial population is shown in Figure 5.3. The generated dendrogram divided bacterial population into two distinct clusters. The first cluster contained DGGE profiles of bacteria in CSTR and the second cluster contained DGGE profiles of bacteria in CD-UASB reactor. This result indicated that bacterial population in anaerobic digestion of pig manure with food waste was affected by type of reactor more than HRT. Because food waste and pig manure contained easily biodegradable compounds so bacterial population were easy to utilize these organic materials and slightly affected from the decrease or increase in HRT.

Comparison between bacterial population and average methane yields at steady state of CD-UASB reactor showed that average methane yield at steady state of CD-UASB reactor were 0.370, 0.533 and 0.430 m^3/kg VS_{add}, and those of CSTR were 0.486, 0.484 and 0.329 m^3/kg VS_{add}. These values were not significantly different (Rerkkriangkrai *et al.*, 2009; Muenjee, 2010) which was related to similar bacterial population structure obsearved in all HRT. This result is consistent with those of Dearman *et al*. (2006), who reported that methane generation rate was significantly correlated to the community structure of bacteria and different community structure could result in similar methane yield.

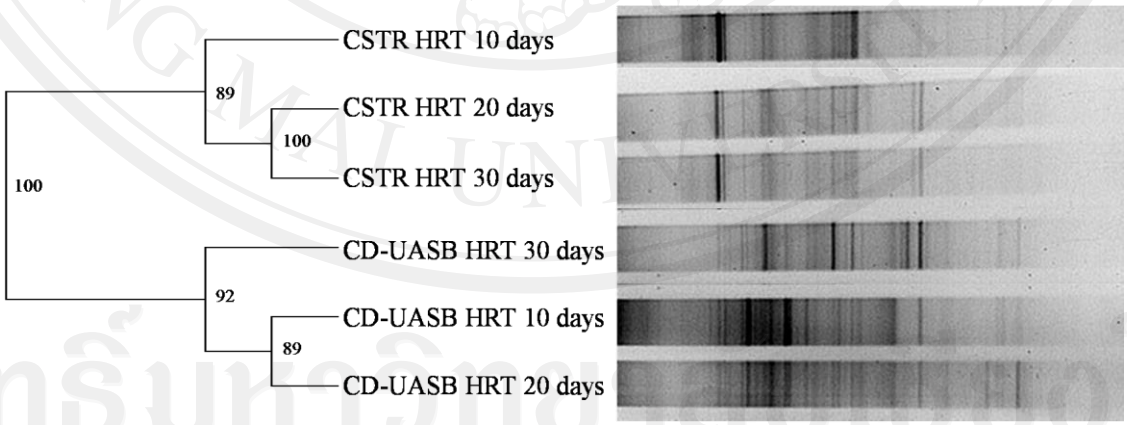

Figure 5.3 Cluster analysis of bacterial banding patterns at steady state from reactors co-digested with food waste under different HRT. The UPGMA algorithm was used to cluster pattern based on Jaccard similarity coefficient. Bootstrap values (>50%) based on 1000 replicates are given at each node.

1.3) Identification of dominant bands in bacterial DGGE profiles

Identification of dominant bands in DGGE profiles from CD-UASB (Figure 5.4) and CSTR (Figure 5.5) was performed with HRT 20 days due to this HRT gave high methane yield with better consistency in removal organic compounds (Rerkkriangkrai *et al*., 2009; Muenjee, 2010). The dominant bands from each profile were visually detected and excised from the gel for subsequent sequencing analyses in order to determine the composition of dominant bacterial population. However, small bands with low intensity were difficult to excise and therefore excluded from our study. The dominant 16S rDNA sequences were compared with available sequences in the GenBank database and compared with cultured species in the EzTaxon database. The phylogenetic affiliations of bacterial sequences were presented in Table 5.1. Their phylogenetic relationships were presented in Figure 5.6 and 5.7.

The bacterial DGGE profile of CD-UASB reactors was shown in Figure 5.4. Band B1 was detected in the beginning of operation and disappeared. Then, this band re-appeared again during steady state from day 36 onward. The sequence of band B1 was closely related to uncultured bacterium clone Comp1-69 with 95% similarity and closely related with the cultured species of *Lishizhenia tianjinensis* (89%) belonged to class *Flavobacteri*a in phylum *Bacteroidetes*. The member of class *Flavobacteri*a were found in many anaerobic reactors such as the reactor treated agro-industrial energetic crops and food industry byproducts (Merlino *et al*., 2012), and biowaste (different organic materials from food crop residues to waste from industrial processing) (Ritari *et al*., 2012). Quiñones *et al*. (2012) reported that they involved in degradation, hydrolysis and acidogenesis stage of anaerobic digestion. Bands B2, B3, B4, B5, B7 and B8 existed throughout the operation although, some bands were faint in some periods of operation whereas band B8 was found from day 6. B8 was related to uncultured bacterium clone FS55 with 89% similarity and related to cultured species of *Pseudomonas tremae* with 85% similarity. This genus has ability to degrade amino acids and sugars to produce acetate (Anderson *et al*., 2003; Insam *et al*., 2010). Bands B2 and B5 were similar (99%) with uncultured Bacteroidetes bacterium clone 207 and distantly related with sugar fermenting *Prolixibacter bellariivorans* (Holmes *et al*., 2007) with 84% and 85% similarity, respectively. Band B3 is also found in seed. It was similar to uncultured Syntrophaceae bacterium clone 7G-B with 99% similarity and

closely related with propionate-oxidizing bacterium *Smithella propionica* (Ariesyady *et al*., 2007). Band B4 was similar (98%) to uncultured bacterium clone MBF16_30 and closely related with cultured species of *Cloacamonas acidaminovorans* with 82% similarity. It is a member of a new bacterial division which found in an anaerobic digester of a municipal wastewater treatment plant and has ability to ferment amino acids and sugars. It is probably a syntrophic bacterium that presented in many anaerobic digesters (Pelletire *et al*., 2008). Band B7 was closely related to uncultured bacterium clone PB 94 with 99% similarity and closely related with cultured species of *Thermovenabulum ferriorganovorum* (class *Clostridia*) with 84% similarity. Bands B2, B3 and B7 were originated from seed. Bands B2 and B7 were the most dominant band indicated that they may be important in anaerobic digestion process. Bands B2 and B7 belonged to class *Bacteroidia* and *Clostridia*, respectively. In addition, band S1 found in seed were also detected throughout the operation process.

For the bacterial DGGE profile of CSTR (Figure 5.5), two dominant bands, B9 and B10 were found throughout the process. Band B9 was related (95%) to uncultured bacterium clone BLE38C and closely related with cultured species of *Clostridium aminobutyricum* with 90% similarity. Band 10 was similar (99%) to uncultured bacterium clone POME_T37_B04 and cultured species of *Smithella propionica* with 92% similarity. Moreover, the faint bands of S1, S2, S4 and S5 which found in seed were also found throughout the process.

Our results revealed that most 16S rDNA sequences of dominant bacteria in anaerobic reactor fed with food waste and pig manure obtained from DGGE method belonged to uncultured bacteria in the phyla *Proteobacteria* class *Deltaproteobacteria* (S1, S3, B3 and B10) and *Gammaproteobacteria* (B8), *Firmicutes* class *Clostridia* (B7, B9 and S4), *Bacteroidetes* class *Bacteroidia* (B2, B5 and S2), *Flavobacteria* (B1), *Chloroflexi* (S5), *Cloacamonas* (B4) and *Acidobacteria* (B6). This result demonstrated the coexistence and ability of these bacterial groups for degradation of complex organic matter in anaerobic digestion system. Bacterial members in phyla *Proteobacteria*, *Firmicutes*, *Bacteroidetes* are commonly detected in anaerobic reactors, while other phyla are rarely found (Insam *et al*., 2010). They are known as hydrolytic and fermentative degrader, which are able to degrade a wide variety of complex organic molecules, including protein, carbohydrates and lipid to produce VFAs, alcohol, CO_2

and H_2 (Panichnumsin *et al*., 2012). These results are in agreement with many previous studies which found members of these phyla in abundant and play important role in the degradation of complex organic matters in anaerobic reactors. Narihiro *et al*. (2009) surveyed the microbial community structure within granular sludge taken from full scale UASB reactor treating food processing wastewater by clone library method. They demonstrated that the bacterial phyla *Proteobacteria* (the class *Deltaproteobacteria* in particular), *Firmicutes* and *Bacteroidetes* were observed in abundance within the bacterial clone libraries examined and indicated that phyla were common bacteria in treatment system. Moreover, Wan *et al*. (2013) also found that bacteria in the phyla *Firmicutes* and *Bacteroidetes* were dominant during the anaerobic digestion of food waste with Chinese silver grass. Our results also showed important of seed such as band S2 and S4 were the most dominant in CD-UASB reactor, indicated that bacteria in seed can adapt to new condition and play crucial role in the digestion

Most of identified sequences were related to their closet sequences with less than 97% similarity indicating that they are potentially new bacterial taxa that have never been reported. Knowledge regarding bacterial species in anaerobic co-digestion is still lacking. The cultivation and determination of microbiological characteristics of these bacteria are important in order to understand their role during anaerobic digestion.

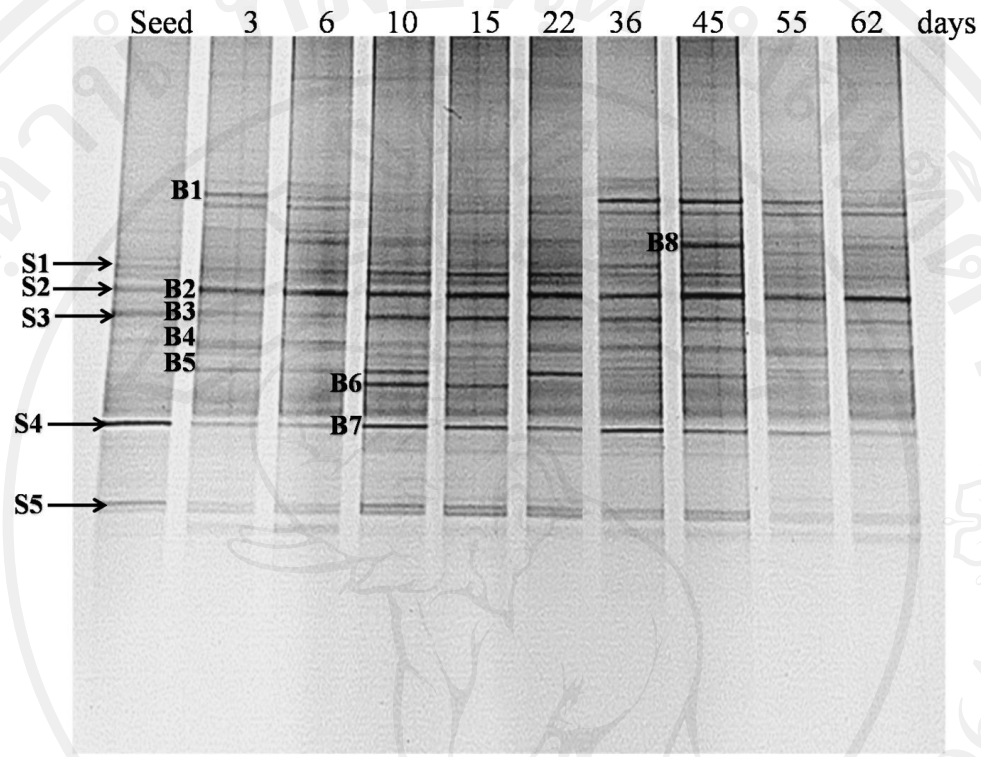

Figure 5.4 DGGE profile of bacterial 16S rDNA fragments amplified from sludge in CD-UASB reactor co-digested with food waste at HRT 20 days.

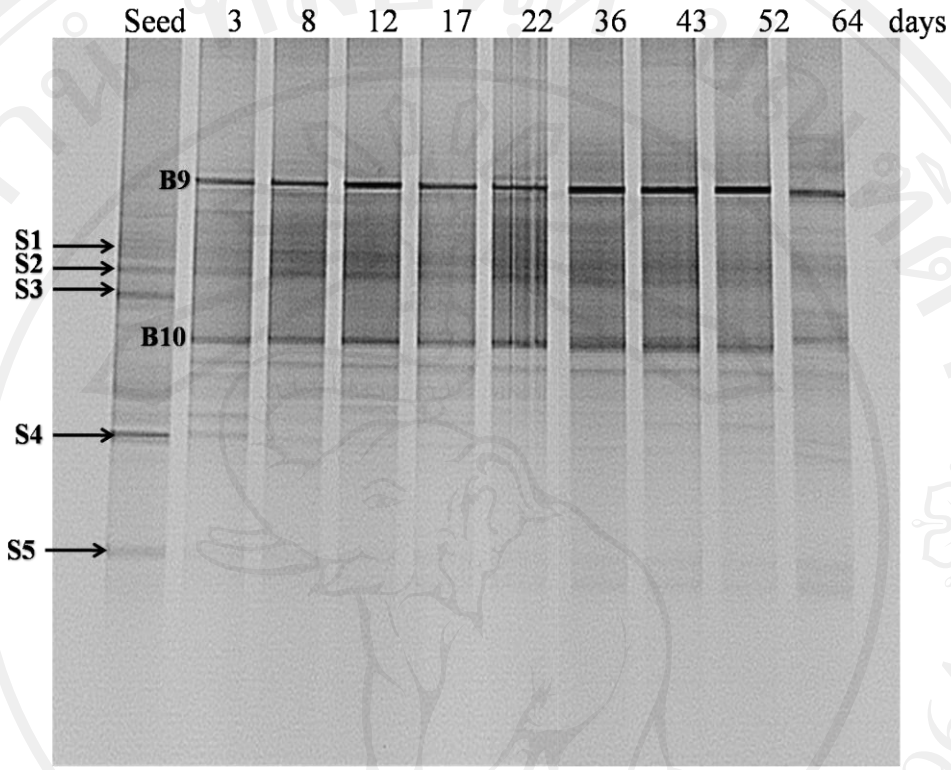

Figure 5.5 DGGE profile of bacterial 16S rDNA fragments amplified from sludge in CSTR co-digested with food waste at HRT 20 days.

Table 5.1 Phylogenetic affiliation of the bacterial 16S rDNA sequences from DGGE bands using BLAST search in GenBank and EzTaxon database from sludge in CD-UASB and CSTR co-digested with food waste at HRT 20 days.

DGGE band	Accession number	Closest sequence match* with BLASTN (accession number)	Similarity (%)	Closest type strain** (accession number)	Similarity (%)	Taxonomic assignment
S1	KJ630802	Uncultured bacterium clone QEDV3AD12, Mesophilic anaerobic digester which treats municipal wastewater sludge (CU919758)	94	*Smithella propionica* R4b16, Granular sludge (AF482441)	94	*Proteobacteria*/ *Deltaproteobacteria*
S2	KJ630803	Uncultured bacterium clone QEDR1AH11, Mesophilic anaerobic digester which treats municipal wastewater sludge (CU922702)	95	*Prolixibacter bellariivorans* F2, Sediment (AY918928)	85	*Bacteroidetes*/ *Bacteroidia*
S3	KJ630804	Uncultured bacterium clone KID28_P3_G08, DHS reactor treating sweage after UASB reactor (AB902603)	99	*Smithella propionica* R4b16, Granular sludge (AF482441)	97	*Proteobacteria*/ *Deltaproteobacteria*
S4	KJ630805	Uncultured bacterium clone PB 94, Sediment (HQ330563)	99	*Thermovenabulum ferriorganovorum* Z-9801, Terrestrial hydrothermal source (AY033493)	84	*Firmicutes/ Clostridia*

Table 5.1 (Continued)

DGGE band	Accession number	Closest sequence match* with BLASTN (accession number)	Similarity (%)	Closest type strain** (accession number)	Similarity (%)	Taxonomic assignment
S5	KJ630789	Uncultured bacterium clone BUT1_OTUB16, Anaerobic UASB bioreactor fed with butyrate (JN995355)	98	*Dehalogenimonas lykanthroporepellens* BL-DC-9, Chlorinated solvent contaminated groundwater (CP002084)	86	*Chloroflexi*/ *Dehalococcoidetes*
B1	KJ630798	Uncultured bacterium clone Comp1-69, Composting process (KF911150)	95	*Lishizhenia tianjinensis* H6, Coastal seawater (EU183317)	89	*Bacteroidetes*/ *Flavobacteria*
B2	KJ630800	Uncultured Bacteroidetes bacterium clone 207, biogas slurry from anaerobic fermentation of pig manure (GQ468584)	99	*Prolixibacter bellariivorans* F2, Sediment (AY918928)	84	*Bacteroidetes*/ *Bacteroidia*
B3	KJ630794	Uncultured Syntrophaceae bacterium clone 7G-B, domestic sewage treatment plant (JX843902)	99	*Smithella propionica* R4b16, Granular sludge (AF482441)	98	*Proteobacteria*/ *Deltaproteobacteria*

Table 5.1 (Continued)

DGGE band	Accession number	Closest sequence match* with BLASTN (accession number)	Similarity (%)	Closest type strain** (accession number)	Similarity (%)	Taxonomic assignment
B4	KJ630801	Uncultured bacterium clone: MBF16_30, Mesophilic anaerobic sludge treating palm oil mill effluent (AB290395)	98	*Cloacamonas acidaminovorans* Evry, Anaerobic digester (CU466930)	82	*Cloacamonas/ Cloacamonas*
B5	KJ630795	Uncultured Bacteroidetes bacterium clone 207, biogas slurry from anaerobic fermentation of pig manure (GQ468584)	99	*Prolixibacter bellariivorans* F2, Sediment (AY918928)	85	*Bacteroidetes/ Bacteroidia*
B6	KJ630796	Uncultured Acidobacteria bacterium clone QEDP1AB03, Mesophilic anaerobic digester which treats municipal wastewater sludge (CU924312)	94	*Solibacter usitatus* Ellin6076 (CP000473)	88	*Acidobacteria/ Solibacteres*

Table 5.1 (Continued)

DGGE band	Accession number	Closest sequence match* with BLASTN (accession number)	Similarity (%)	Closest type strain** (accession number)	Similarity (%)	Taxonomic assignment
B7	KJ630797	Uncultured bacterium clone PB 94, Sediment (HQ330563)	99	*Thermovenabulum ferriorganovorum* Z-9801, Terrestrial hydrothermal source (AY033493)	84	*Firmicutes/ Clostridia*
B8	KJ630799	Uncultured bacterium clone FS55, Sludge (EU593854)	89	*Pseudomonas tremae*, CFBP6111T, Phyllosphere(AJ492826)	85	*Proteobacteria*/ *Gammaproteobacteria*
B9	KJ630791	Uncultured bacterium clone BLE38C, Anaerobic digester treating feedstock (GU389560)	95	*Clostridium aminobutyricum* DSM 2634 (X76161)	90	*Firmicutes/ Clostridia*
B10	KJ630792	Uncultured bacterium clone POME_T37_B04, Continuous anaerobic digestion (HM440282)	99	*Smithella propionica* R4b16, Granular sludge (AF482441)	92	*Proteobacteria*/ *Deltaproteobacteria*

* Data taken from GenBank database

** Data taken from EzTaxon database

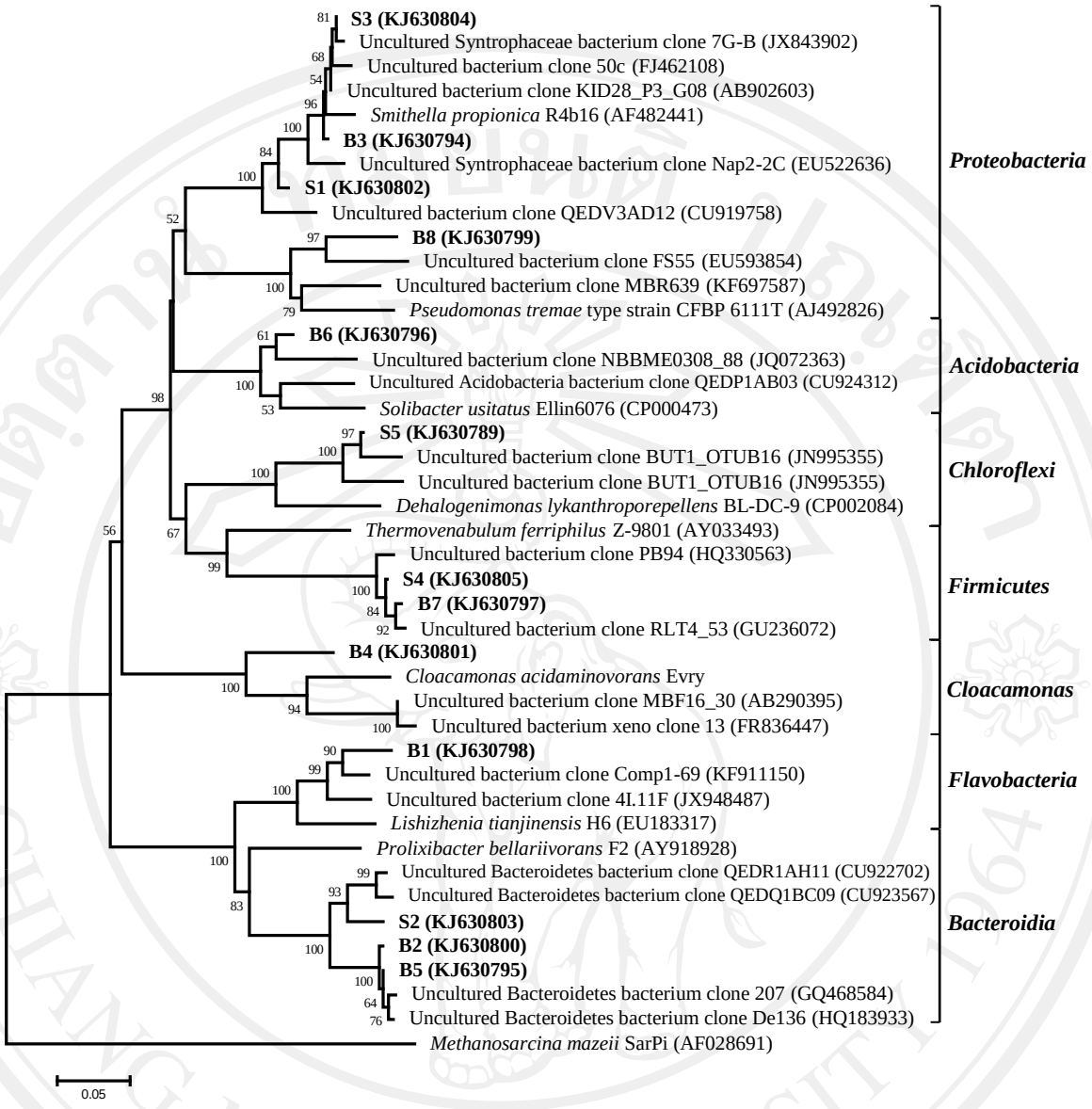

Figure 5.6 Phylogenetic tree of 16S rDNA bacterial sequences from sludge DNA sludge in CD-UASB reactor co-digested with food waste at HRT 20 days as determined by the neighbor-joining method. *Methanosarcina mazeii* was used as an out group. The percentage of 1000 bootstrap samplings that supported a cluster is indicated. The scale bar indicates 0.05 nucleotide substitution per site. Number in round brackets indicates contributing GenBank accession number of reference nucleotide sequence.

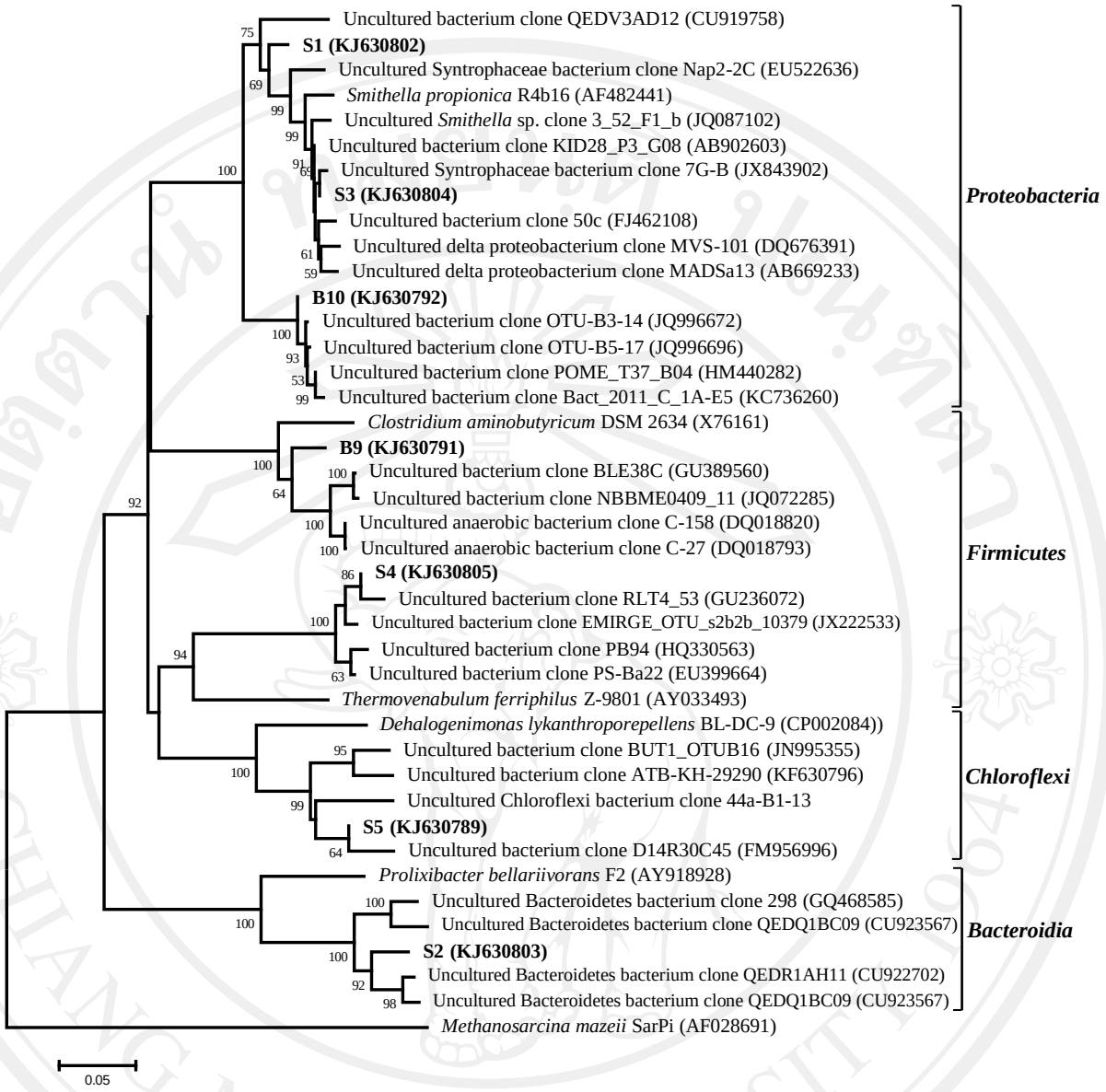

Figure 5.7 Phylogenetic tree of 16S rDNA bacterial sequences from DNA of sludge in CSTR co-digested with food waste at HRT 20 days as determined by the neighbor-joining method. *Methanosarcina mazeii* was used as an out group. The percentage of 1000 bootstrap samplings that supported a cluster is indicated. The scale bar indicates 0.05 nucleotide substitution per site. Number in round brackets indicates contributing GenBank accession number of reference nucleotide sequence.

2) Archaeal population

2.1) Archaeal population profile

The result of monitoring change in archaeal population in CD-UASB reactors under HRT 10, 20 and 30 days showed similar pattern as shown in Figure 5.8. Archaeal pattern in each reactor was stable throughout the process, with five bands designated as bands 1, 2, 3, 4 and 5.

The archaeal population in CSTRs under HRT 10, 20 and 30 were different pattern from CD-UASB reactor as shown in Figure 5.9. In each profile, five major DNA bands, 1, 2, 3, 4 and 5 were presented. However, band 3 decreased its intensity around days 17 and 43-52 in all HRT. Band 5 in DGGE profile of HRT 20 and 30 days had lower intensity than that of HRT 10 days. This may be caused by the effect of low influent pH of around 5.8 and 6.5 during these periods (Muenjee, 2010). The optimum pH for methanogen in anaerobic digestion was in a range of 6.8-7.2, so improper pH can result in methanogen growth inhibition (Gerardi, 2003). Moreover, new DGGE bands were also found in some days of operation.

The DGGE profiles of archaeal in all reactors displayed a similar banding pattern indicated that the archaeal population remained stable during operation. Similary, Gómez *et al*. (2011) who found the archaeal community was stable during operation of anaerobic digester treating municipal sewage sludge with increasing the organic loading rate. This observation suggested that HRT and type of reactor had less effect on archaeal population. Each profile displayed five bands throughout the operation compared to 10-19 bands in DGGE profile of bacterial population, implied that archaeal population in the reactors was less diverse than bacteria. Because archaea can only utilize substrates like acetate, H_2 and CO_2 or methyl compound for energy (Gerardi, 2003). The low diversity of archaeal population is in accordance with Bengelsdorf *et al*. (2013) who found low diversity of archaeal community in mesophilic continuously operating biogas treating food residue.

Because each profile displayed five major DNA bands in the operational process, therefore, the PCR products of 16S rDNA of archaea from the sludge of each reactor and seed were re-run on DGGE to confirm that they migrated to the same position. As shown in Figure 5.10, the five dominant DNA bands (A1, A2, A3, A4 and

A5) from the sludge in each reactor and seed migrated to the same position on the DGGE gel. This demonstrated that the dominant archaea found in each reactor were the same taxa and might have originated from the seed.

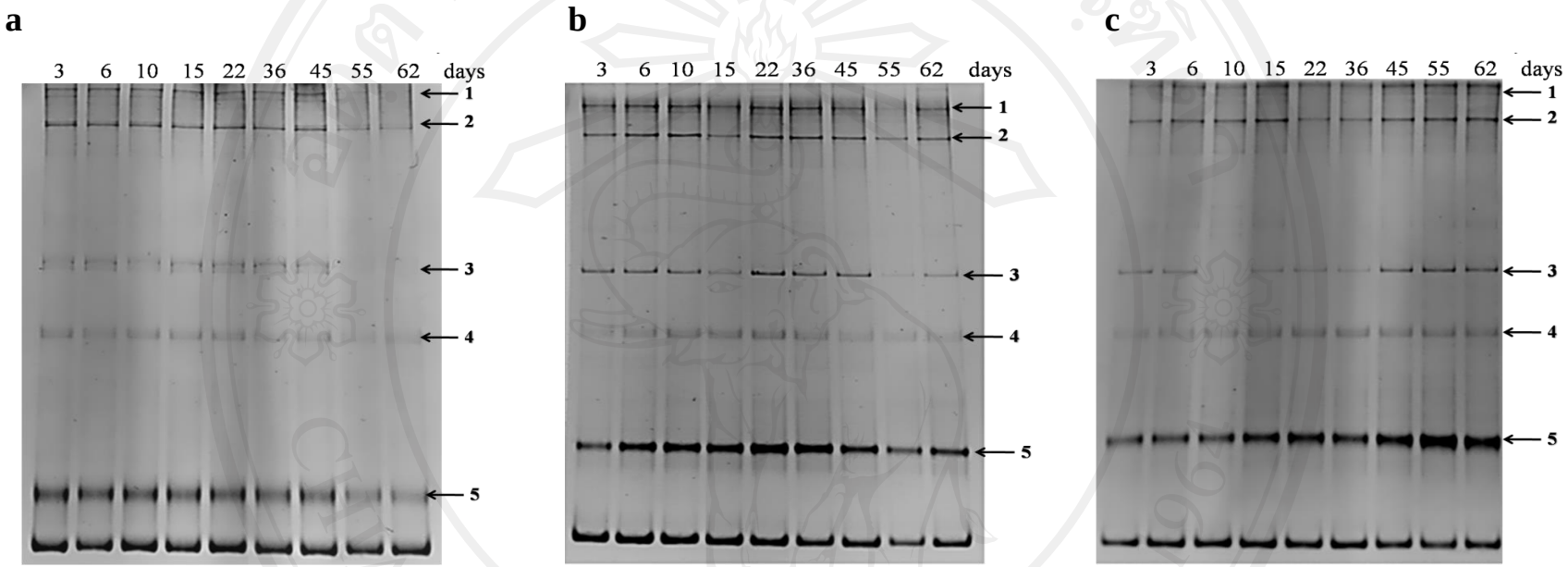

Figure 5.8 DGGE profiles of archaeal 16S rDNA fragments amplified from sludge in CD-UASB reactor co-digested with food waste under different HRT; HRT 10 days (a), HRT 20 days (b) and HRT 30 day (c).

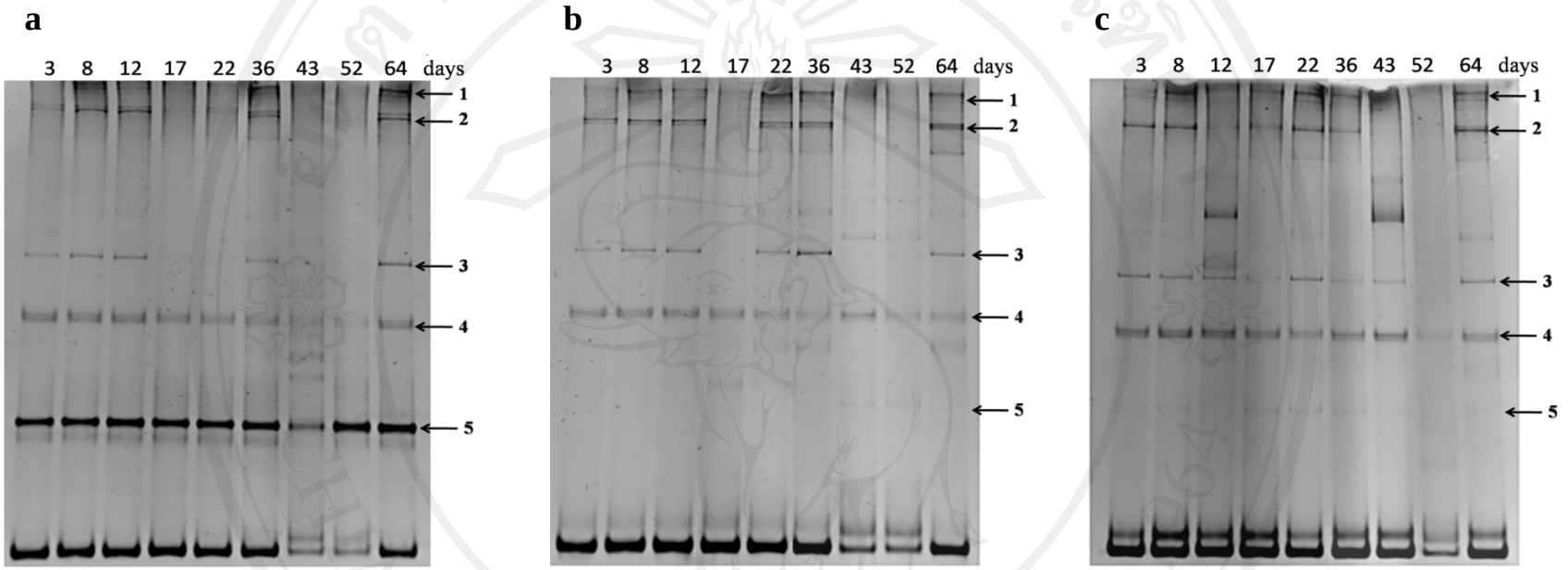

Figure 5.9 DGGE profiles of archaeal 16S rDNA fragments amplified from sludge in CSTR co-digested with food waste under different HRT; HRT 10 days (a), HRT 20 days (b) and HRT 30 day (c).

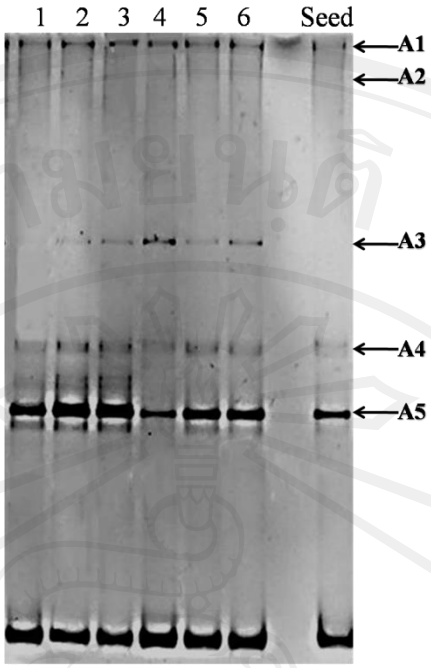

Figure 5.10 DGGE profile of archaeal 16S rDNA fragments amplified from seed and sludge samples in CD-UASB and CSTR co-digested with food waste.

Lane 1, 2 and 3: 16S rDNA fragments amplified from sludge in CD-UASB reactor under HRT 10, 20 and 30 days, respectively.

Lane 4, 5 and 6: 16S rDNA fragments amplified from sludge in CSTR under HRT 10, 20 and 30 days, respectively.

2.2) Identification of dominant bands from DGGE profiles

Like the identification of dominant bands in bacterial DGGE profile, the identification of dominant archaeal DGGE bands was performed with HRT 20 days due to this HRT gave high methane yields with better consistency in removal organic compounds than other HRT. The dominant band from each profile was visually detected and excised from the gel for subsequent sequencing analyses in order to determine the composition of dominant archaeal population. The dominant 16S rDNA sequences were compared with available sequences in the NCBI database and compared with cultured species in the EzTaxon database.

Although, the DGGE profile of archaea in CD-UASB (Figure 5.11) and CSTR (Figure 5.12) were displayed different banding pattern during operation. However, each

profile displayed five major bands of DNA as shown in Figure 5.10. The five dominant bands, A1, A2, A3, A4 and A5, were excised from the gel, sequenced and identified. The result is similar to the identification result of dominant bands in archaeal DGGE profiles of Napier grass with pig manure which found that bands A1, A2, A4 and A5 were related to uncultured clone with 83-96% similarity and related to cultured specie of *Methanocorpusculum sinense* with 78-96% similarity. Band A3 showed 87% similarity to *Methanoseata concilii* (see in Chapter 3).

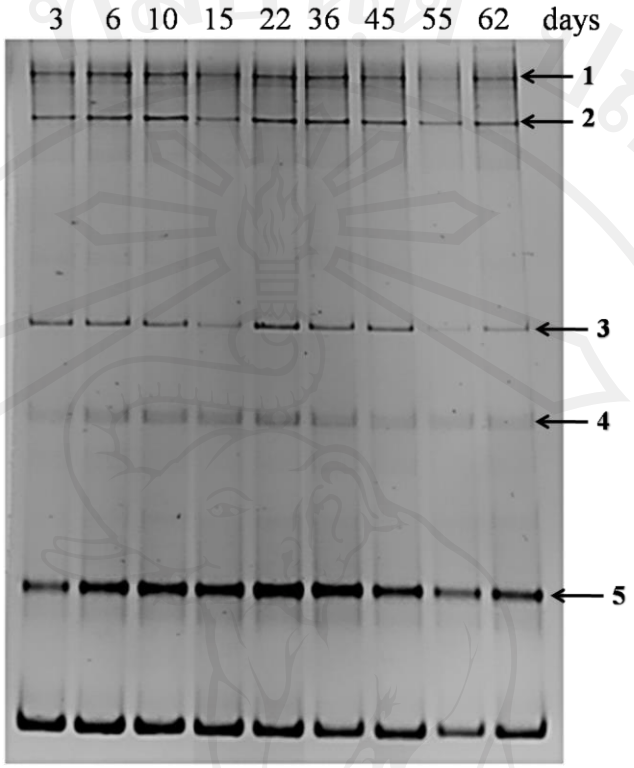

Figure 5.11 DGGE profile of archaeal 16S rDNA fragments amplified from sludge in CD-UASB reactor co-digested with food waste at HRT 20 days.

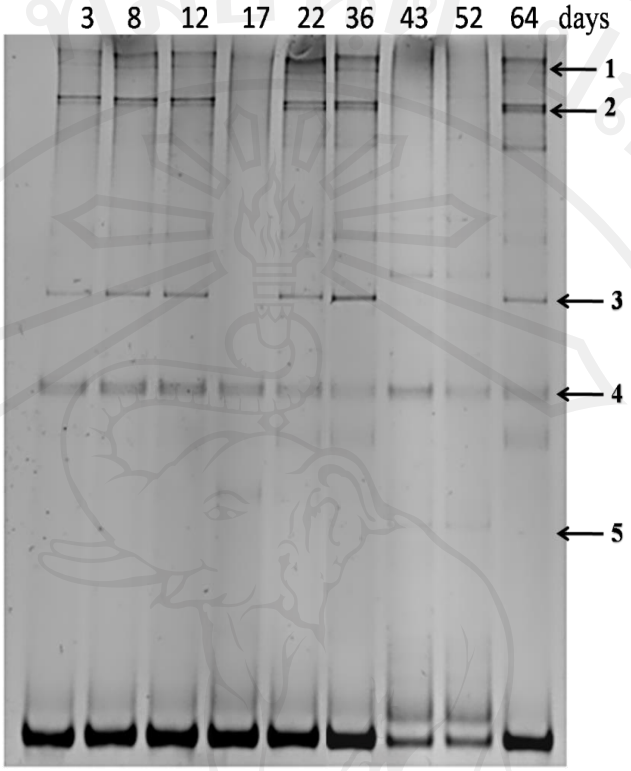

Figure 5.12 DGGE profile of archaeal 16S rDNA fragments amplified from sludge in CSTR co-digested with food waste at HRT 20 days.