The *ureC* Gene Diversity of Soil Bacteria in Tropical Rain Forest and Oil Palm Plantations

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Abstract: Analysis of microbial community composition and *ureC* genes was studied by using Polymerase Chain Reaction-Denatured Gradient Gel Electrophoresis (PCR-DGGE) approach. The aim of this study was to describe the abundance and diversity of *ureC* gene bacteria from tropical rain forest and oil palm plantations in Jambi Province, Sumatera, Indonesia. The abundance of bacteria was calculated through the method of Most Probable Number (MPN). Shannon-wiener analysis and DGGE profile showed that the diversity of microbial *ureC* gene in oil palm plantation was higher than that of the tropical rain forest. The abundance profile of microbes towards the depth was increased. The microbial abundance in oil palm plantations was higher than tropical rain forest.

Keywords: Tropical rain forest; Oil Palm Plantation; ureC; abundance; diversity; DGGE.

1. Introduction

Microorganisms are the most abundant organisms on earth and play key roles in natural ecosystem, including the biogeochemical cycling of carbon, nitrogen, sulfur, phosphorus, and metals, and biodegradation or stabilization of environmental contaminants [1–3]. Because of their important roles, changes in soil microbial community may directly affect soil ecosystem function, particularly carbon and nitrogen cycling [4]. Nitrogen can be a limiting nutrient and nitrogen availability in the soil environment may therefore be a major factor in controlling biomass production. Most nitrogen cycling-related studies focus on nitrate and ammonium as the primary sources of nitrogen available, because they are generally the preferred forms for assimilation [5]. But tropical forest ecosystems rely heavily on regenerated nitrogen sources [4]. In low nitrate systems, regenerated nitrogen such as urea or ammonium can provide up to 75% of the requirements. Urea represents the single dominant component of the diverse group of organic nitrogenous compounds. [6]. However, in total, the role of regenerated utilization of urea in the nitrogen cycle of forest ecosystem is poorly understood.

A deeply analysis of microbial community structure and their roles in ecological processes would improve our understanding of the biogeochemical elemental cycles affected by microbial communities in natural or man-made environments [7]. However, our understanding of soil microbial communities in terms of structure, composition, and functional activity are still limited, especially for soil microbial metabolic activity and ecosystem function have received little attention to date.

Urease is one of the important enzymes in nitrogen cycle [8]. Most organisms that use urea as a source of nitrogen rely on a urease such as urea amidohydrolase (EC 3.5.1.5), that can catalyze the hydrolysis of urea to yield ammonia and carbamate [9]− [10]. Bacterial urease is a trimer of three subunits (encoded by *ure*A, *ure*B, and *ure*C) and requires up to four accessory proteins for activation and Ni^+ incorporation (most commonly encoded by *ure*D, *ure*E, *ure*F and *ure*G) [11]. The urease peptides have highly conserved active sites and Ni^+ binding residues [10]. However, we don't know whether urease genes are active in tropical rain forest and palm oil soil and what is the phylogenetic diversity of urease genes of microbial community in tropical rain forest and palm oil soil.

The *ure*C gene was chosen as the target gene for urease analysis because it is the largest of the genes encoding urease functional subunits and contains several highly conserved regions that are suitable as PCR priming sites [12]. In this study, using *ure*C gene as marker, the phylogenetically diverse *ure*C genes were investigated for the first time.

Thus far, many studies have been conducted on *ureC* genetic diversity in different habitats, but the impact of the tropical forest conversion into oil palm plantations has been less reported. An understanding diversity of microbes that play a role in nitrogen cycle changes would allow a policy to be make regarding on land use and agricultural land development in the future.

This paper studied the effects of land conversion on the abundance and diversity of *ureC* genes in Jambi, because Jambi is one area that experiencing a quick change in land use. The purpose of this study were (1) to obtain information diversity and abundance structure of ammonification microbial communities, (2) identify the structure of microbial communities that contribute to the N_2 fixation in tropical rain forests and oil palm plantations, and (3) compare community analysis microbes of tropical rain forests with oil palm plantations in Jambi Province, Sumatera, Indonesia.

2. Material & Methodology

2.1. Site description and sampling.

Samples were taken from tropical rain forest Bukit Dua Belas National Park (TC, TD) and oil palm plantation (SA, SB) in Sarolangun Distric, Jambi Province, Sumatera, Indonesia. All spatial geographical coordinates and altitudes were recorded by GPS (eTrex Venture, Garmin, Lenexa, KS, USA). Detailed site information is listed in Table 1. Sample were collected in October 2015. At each site, we collected a total of 24 soil samples at a 0–15-cm depth during the peak plant growth stage. The samples were transported to the laboratory immediately after collection and stored at 4 °C for soil physicochemical measurements and at −20 °C for DNA extraction.

2.2. Determination of physicochemical factors of the soil sample

Physicochemical factors of the soil sample were determined according to the instructions, ISRIC standards. Total Organic Carbon (TOC) analyzer was used to determine the TOC in the soil. Potential method was used to determine soil pH value (soil water ratio: 1:2.5); Kjeldahl method was used for total nitrogen contents; for nitrate nitrogen content; $HCl–H_2SO_4$ method, for total phosphorus content; and $NaHCO_3$ extraction – molybdenum antimony anti – colorimetric method, for available phosphorus content.

2.3. Abundance of N_2 Fixing Bacteria

An abundance of bacteria is calculated through the method of Most Probable Number (MPN) [13]. A total of 1 g of a soil sample is diluted with physiological saline solution (0.85%) with some serial dilution. Further dilution of 1 ml of 3 final dilution inoculated in 9 ml of N-free liquid medium with the major components (/L): 1 g K_2HPO_4, 3 g KH_2PO_4, 0.065 g $MgSO_4$, 0.01 g $FeCl_3{\cdot}6H_2O$, 0.07 g $CaCl_2{\cdot}2H_2O$, 5 g peptone and minor components namely 240 µg $Na_2MoO_4{\cdot}2H_2O$, 3 µg H_3BO_4, 1,83 µg $MnSO_4{\cdot}H_2O$, 290 µg $ZnSO_4 7H_2O$, 130 µg $CuSO_4{\cdot}5H_2O$ and120 µg $CoCl_2{\cdot}6H_2O$ (Phillips *et al.* 2000). Incubation was performed at room temperature for 7 days. Test positive ammonification bacteria declared blue when the bacterial culture after being given a phenol reagent alcohol 10%, nitroprusside 0.5%, and a mixture of technical hypochlorite and 20% citric acid (1: 4) [14].

Table 1. Site Description: Location, altitude, Soil thermo, Soil pH, and RH for the tropical rain forest and oil palm plantation sites sampled in Jambi.

Sample	Location	Altitude (m above sea level)	T ^{0}C (Soil Thermo)	pH (Soil pH Tester)	RH (Hygrometer)
TRANSFORMATION FOREST : OIL PALM PLANTATION					
1 (SA)	01^o 56' 491'' LS 103^o 15' 140" BT	64 mdpl	**29 ^{o}C**	**6.2**	**73%**
A2 (SA)	01^o 56' 477'' LS 103^o 15' 142" BT	53 mdpl	**29 ^{o}C**	**5.4**	**62%**
A3 (SA)	01^o 56' 472'' LS 103^o 15' 134" BT	55 mdpl	**29.5 ^{o}C**	**6.1**	**62%**
B1 (SB)	01^o 56' 592'' LS 103^o 15' 104" BT	48 mdpl	**29 ^{o}C**	**5.2**	**59%**
B2 (SB)	01^o 56' 591'' LS 103^o 15' 119" BT	42 mdpl	**28.5 ^{o}C**	**5.8**	**62%**
B3 (SB)	01^o 56' 958'' LS 103^o 15' 122" BT	42 mdpl	**28 ^{o}C**	**6.2**	**64%**
TROPICAL FOREST : TAMAN NASIONAL BUKIT DUA BELAS (TNBD)					
C1 (TC)	01^o 56' 576'' LS 102^o 34' 879" BT	87 mdpl	**27 ^{o}C**	**5.6**	**55%**
C2 (TC)	01^o 56' 571'' LS 102^o 34' 874" BT	95 mdpl	**28 ^{o}C**	**5.6**	**35%**
C3 (TC)	01^o 56' 566'' LS 102^o 34' 865" BT	95 mdpl	**28 ^{o}C**	**4.3**	**75%**
D1 (TD)	01^o 56' 487'' LS 102^o 34' 852" BT	111 mdpl	**27 ^{o}C**	**5.7**	**60%**
D2 (TD)	01^o 56' 481'' LS 102^o 34' 860" BT	114 mdpl	**27 ^{o}C**	**5.6**	**55%**
D3 (TD)	01^o 56' 502'' LS 102^o 34' 836" BT	116 mdpl	**27.5 ^{o}C**	**4.9**	**70%**

2.4. DNA extraction, purification, and quantitation.

Soil DNA was extracted using a Fast DNA SPIN Kit for soil (Bio 101, Inc. Vista, CA, USA) following the manufacturer's instructions. In this procedure, cell lyses was performed by vigorous shaking in a mini-beadbeater (Biospec product, Wakenyaku, Co., Tokyo, Japan) with intense speed of 4.8 for 30 sec. DNA extracts were stored at -20 °C before used as a template for subsequent PCR reaction. DNA quality was assessed using 260 nm/280 nm and 260 nm/230 nm ratios, and final DNA concentrations were quantified by Nano drop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.5. PCR amplification of ure*C* gene

The DNA amplification was performed using Polymerase Chain Reaction (PCR) by T1-thermocycler (Biometra, Goettingen, Germany). To amplify the genes coding for the *ureC* subunit of the urease enzyme, PCR was performed with primers UreC-F (5_-TGGGCCTTAAAATHCA YGARGAYTGGG-3_) and UreC-R (5_-GGTGGTGGCACACCATNANCATRTC-3_) as previously described by Reed [15].The length of the expected amplified fragment was 382 bp. To examine the diversity of the partial *ureC* DNA fragments by DGGE, a 40-bp GC clamp (5_-CGCCCGCCG CGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3_) [15]was attached to the 5_ end of the UreC-F primer. The PCR conditions were as follows: 94°C for 5 min, followed by 35 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 2 min. A final extension was carried out at 72°C for 10 min. PCR products were analyzed by 1.5% agarose gel electrophoresis followed by staining with ethidium bromide to confirm their sizes.

2.6. Analysis of DGGE

DGGE was performed using Bio-Rad DCode system (Bio-Rad, Hercules, CA, USA) in 8% (w/v) polyacrylamide gel (acrylamide-bisacrylamide, 37.5:1) with 40% to 60% denaturing concentrations for ureC gene DGGE analysis (100% denaturant corresponding to 7M urea and 40% deionized formamide). Electrophoresis was performed at 150 V and 60°C for 6 hr in 1X Tris-acetateEDTA (TAE). The gel was stained for 15 min with EtBr gel stain (Molecular Probes, Invitrogen, Cergy Pontoise, France). Gel was rinsed with 500 mL of TAE buffer and scanned by G:BOX gel documentation (Syngene, Frederick, MD, USA). Band profile image was analyzed using Phoretix 1D software (Total Lab) to estimate the total bands that appeared on polyacrylamide gel. Single appeared band was excised using sterile scalpel and put into micro tube containing 100 µL ddH_2O. The micro tube was incubated at 4^{o}C overnight and 60^{o}C for 2 h [15]. Ten µL (~50 ng µL_1) of template was used for re-PCR using primer without GC-clamp. The condition of re-PCR was the same with the previous PCR condition.

Statistical analysis of DGGE profile was conducted by using alpha diversity (Shannon-wiener/He) to estimate the diversity within each sample and beta diversity (Dice similarity coefficient/SD) to estimate the similarity of band pattern between the samples. The quantification of statistical analysis was conducted using PAST3 Software based on the estimation analysis of band intensity using CLIQS 1D software. The index was calculated by following equation:

$$SD = 2Nc/(Na + Nb)$$

$$He = \sum_{i-1}^{S} Pi \ln pi$$

Where, Na represented the number of bands detected in sample a; Nb represented the number of bands detected in sample b; Nc represented the number of bands detected in both samples; s represented the number of species in the sample; Pi represented the proportion of species i in the sample [16]

3. Results and Discussion

3.1. Soil characteristics

The results of analysis of the characteristics and physical chemistry indicate that the oil palm plantations soil contains 38.37% sand, 61.34% dust and clay 31.00%. Tropical rain forest soils containing sand 74.19%, 10.76% of dust and clay 5.00%. Total organic carbon in the soil of oil palm plantation, and Tropical rain forest is 2.61%, and 3.94%. The total content of organic nitrogen in the soil of oil palm plantation, and Tropical rain forest is 0.24% and 12.08%.

3.2. The Abundance of ammonification Bacteria

The analysis results of ammonification microbe population abundance at Tropical rain forest and oil palm plantations are not much different. Oil palm plantation has the lowest microbes abundance value at 7.18 Log cells/g (TC) in strata 0-5 cm, meanwhile the highest is in oil palm plantations in strata 5-10 cm (8.56 Log cells /g). The abundance profile of ammonification microbes towards the depth increases tend to increasing (Figure 1). The microbial abundance in oil palm plantations are higher than Tropical rain forest.

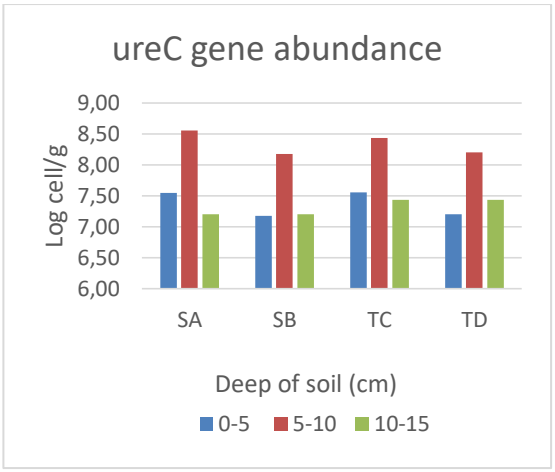
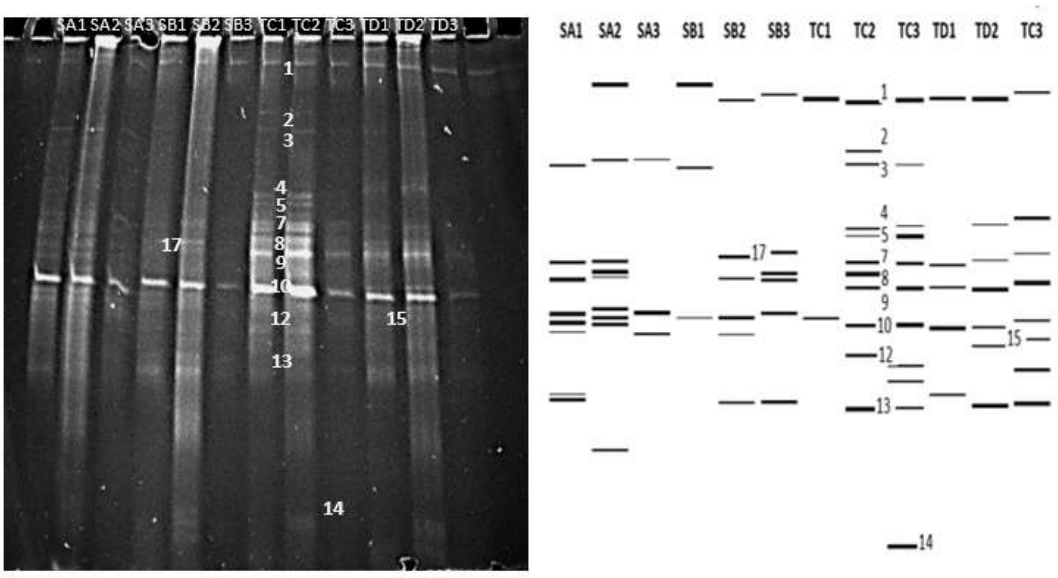
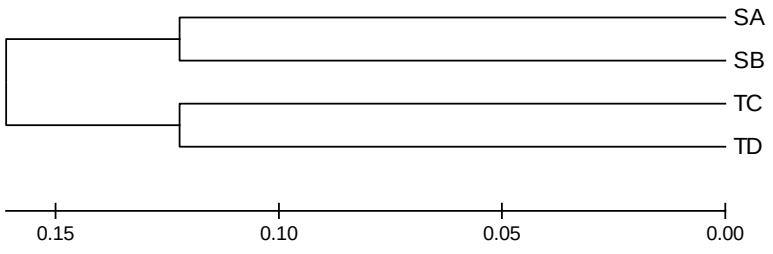


Figure 1. Profile of microbe abundance in role of ammonification from oil palm plantation (SA, SB) and Tropical rain forest (TC, TD).



(A)



(B)

Figure. 2. (A) DGGE band profile of the PCR products of nif*H* gene from Tropical rain forest (TC;TD) and oil palm plantations (SA:SB) (left). Illustration of DGGE band using CLIQS 1D software (right). (B) Hierarchical cluster analysis results of ureC gene DGGE profiles demonstrated graphically as an UPGMA dendrogram (p-distance).

3.3. PCR amplification, DGGE and phylogenetic profile of ure*C* gene

DNA extracted from tropical rain forest and oil palm plantation samples were successfully amplified using the PCR protocol. The resulting ≈300-bp-long ure*C* gene fragments were then resolved by DGGE, whose profiles showed various banding patterns in the different forest samples (Fig. 2). Based on DGGE result, it showed that community structure of N_2 fixing bacteria in soil samples were more varied than that of the oil palm plantation (Fig. 2A). Cluster analysis of community showed that the community of amonification bacteria in soil samples were different forest (Fig. 2B) ammonification community in tropical forests were more diversed than of oil palm plantation based on the distribution pattern of bands seen on polyacrylamide gel (Fig. 2A). Similarity pattern analysis using binary data (Fig. 2B) showed that ammonification community in soil of SA had similarities with soil of SB. Meanwhile, soil of SA and SB had similar community pattern but their cluster separated with soil sample of TC and TD which indicates that they have different community pattern with soil sample of SA and TC.

3.4. Principal component analysis and cluster analysis of DGGE fingerprints

To characterize the ammonification microbial community structure in tropical rainforest and oil palm plantation, UPGMA clustering analysis was used to analyze the ammonification microbial community similarity (Fig. 2B.). Clustering of the DGGE profiles revealed that there were low differences among the N_2-fixing microbial communities in tropical rain forest and oil palm plantation samples from Jambi.

The result for microbial community (Figure 3) showed *He* index value of SA and SB (0.592-1.611) were higher compared with TC and TD (1.164-1.546). Shannon-wiener (*He*) analysis were used in this study to estimate the microbial diversity in each sample, in which high index value referred to the high diversity of species (bands) within a sample.

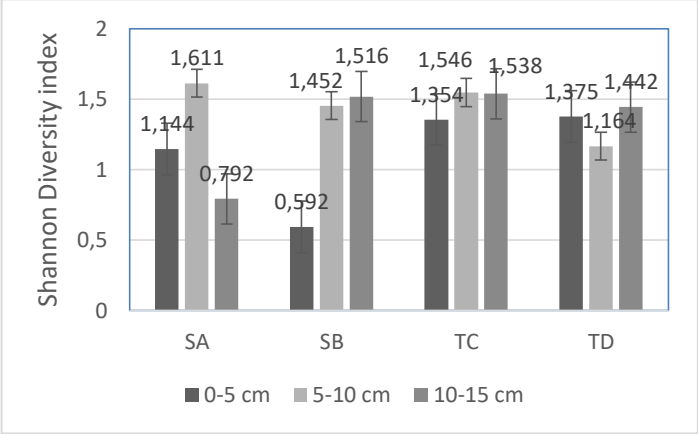


Figure 3. Shannon diversity index of Gene ureC DGGE prifiles of tropical rain forest (TC, TD) and oil palm plantation (SA, SB).

3.5. Discussion

In this study, the diversity of the ammonification microbial community in forest conversion for agriculture drove change to the soil chemistry. Soil pH was found to be the best predictor of the bacterial community across forest and oil plantation areas [17]. Soil pH in both areas were no significanlyt different (Table 1). Worlwide acid soils cultivated with oil palm represent more than 50 % of the total area. Under highly acidic soil conditions represented by Ultisols, which constitute the most important soil for oil palm growth in tropical Asia and tropical latin America (Cristancho *et al*. 2014). Oil palm plantation had been added with anorganic fertilizer for agricultural management. They changed increasing soil pH event though no significant differences between both areas.

The microbial abundance in oil palm plantations are higher than Tropical rain forest (Figure 1).

Urea is released into the environment as the result of natural degradative processes involving the turnover of proteins and nucleotides or via fertilization with urea-containing fertilizers. Rapid hydrolysis of urea into ammonia in the soil provides plants with an assimilable form of nitrogen, thus enhancing crop production. High levels of ureolytic activity in the soil are provided by either bacterial ureases (it has been estim ated th a t about 17 to 30% of soil bacteria are ureolytic) or by extracellular ureases derived from plant and microbial cells [18].

The microbial communities from tropical rain forest and oil palm plantation were analyzed using PCR-DGGE method. Based on DGGE principle, same migration distance of bands in the gel was considered to be the same microorganism, while the intensity of the band indicated its relative abundance (Meroth *et al*. 2003). The profile of bands were used to describe the shape of community structure, diversity, and the order of relatedness among the samples [16].

PCR Amplicons of ureC for each replicate sample were analyzed by using DGGE to compare profiles each time point suring the field experiment. The variability of profile patterns within replicates of a given sample was minimal, except that the relative intensity of bands occupying the same positions in the gel was not always consistens. Because of the similarity between the replicate at each time point, a representative replicate from each time point could be made simultaneously. A distance-UPMGA comparison of ureC amplicons for each time point showed that the profiles for each of sample were significantly different from each other (figure 2B). The number of ureC DGGE bands per sample varied between 3 and 11. DGGE is an effective method for evaluating mixed microbial communities in environmental sample [19].

3. Conclusion

The analysis results of ammonification microbe population abundance at Tropical rain forest and oil palm plantations are not much different. *ureC*-targeted DGGE analyses was elucidate the identities of key ureolytic bacterial species within a given community without isolating and culturing individual species. An understanding diversity of microbes that play a role in nitrogen cycle changes would allow a policy to be make regarding on land use and agricultural land development in the future.

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