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Study of Root Exudate Organic Acids and Microbial Population in the Rhizosphere of Oil Palm Seedling

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ABSTRACT

Mutual interaction between plants and microbes occured in the rhizosphere is expected to increase productivity of crops or soil fertility for agriculture. Plants excrete root exudates to attract microbes, and then microbes obtain habitat and food supply from plants and can fulfill the nutrient requirements through assisted enzymatic activity. The objective of the research was to study the types and amounts of root exudate organic acids, microbial population, and the relationship between root exudate organic acids and microbial population in the rhizosphere of oil palm seedlings. The study was conducted in a greenhouse using a planting medium of sterilized quartz sand. The study was conducted using two factorials completely randomized design with three replications. The first factor was oil palm seedling age (control / no oil palm seed, 1, 3, 6, 9 and 12 months-old of oil palm seedlings) and the second factor was the periods of seedling growth (45, 90, 135 and 180 days), so in total there were 72 experimental units. The result of High Pressure Liquid Chromatography (HPLC) analysis revealed that four kinds of organic acids with the highest concentrations were identified in the rhizosphere of oil palm seedlings, *i.e.* acetic acid (1.66 ppm), citric acid (0.157 ppm), malic acid (2.061 ppm) and oxalic acid (0.675) ppm. The highest total population of microbes, fungi, Azotobacter, phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) were 19.38×10^6 cfu g^{-1} soil, 3.28×10^4 cfu g^{-1} soil, 12.09×10^5 cfu g^{-1} soil, 8.39×10^4 cfu g^{-1} soil and 1.15×10^4 cfu g^{-1} soil, respectively. There are positive correlations between concentrations of root exudate organic acids and total microbes, fungi, Azotobacter, PSB and PSF.

Keywords: microbes, organic acids, rhizosphere, root exudates

ABSTRAK

Interaksi timbal balik antara tanaman dan mikroba di rizosfer diharapkan dapat meningkatkan produktivitas tanaman atau kesuburan tanah untuk pertanian. Eksudat akar tanaman dapat menarik mikroba, dan kemudian mikroba memperoleh habitat dan suplai makanan dari akar atanaman sehingga dapat memenuhi kebutuhan nutrisi melalui aktivitas enzimatik**.** Tujuan penelitian ini adalah untuk mempelajari tipe dan jenis asam-asam organik dari eksudat akar, populasi mikroba dan hubungan antara asam-asam organik dari eksudat akar dengan populasi mikroba di rizosfer bibit kelapa sawit. Penelitian dilakukan dengan media tanam menggunakan pasir kuarsa steril dengan dua faktor yang dirancang secara acak dengan tiga ulangan. Faktor pertama adalah umur bibit kelapa sawit (kontrol/ tidak ada bibit kelapa sawit; 1; 3; 6; 9; dan 12 bulan) dan faktor kedua adalah panjang musim tanam (45; 90; 135; dan 180 hari) dengan 72 unit percobaan. Analisis HPLC menunjukkan bahwa empat jenis asam organik ditemukan dengan konsentrasi tertinggi: asam asetat (1,66 ppm), asam sitrat (0,1757 ppm), asam malat (2,061 ppm) dan asam oksalat (0,675) ppm. Jumlah populasi bakteri, jamur, Azotobacter, bakteri pelarut fosfat dan jamur pelarut fosfat ditemukan dengan jumlah maksimum sebagai berikut: 19.38×10^6 cfu g^{-1} tanah, $3,28 \times 10^4$ cfu g^{-1} tanah, $12,09 \times 10^5$ cfu g^{-1} tanah, $8,39 \times 10^4$ cfu g^{-1} tanah dan $1,15 \times 10^4$ cfu g^{-1} tanah. Terdapat korelasi positif antara akar eksudat asam organik dengan total mikroba, jamur, Azotobacter, bakteri pelarut fosfat dan jamur pelarut fosfat.

Kata kunci: asam organik, eksudat akar, mikroba, rizosfer

INTRODUCTION

Mutual interaction between microbes and plants occured in the rhizosphere could be expected to increase crop productivity or soil fertility for agriculture. Rhizosphere is part of soil within few millimeters thickness from the root surface, which gets a direct influence from the root system of plants. The nature of the soil is influenced by root exudates that vary depending on the species, variety, and growth stage of crops and soil types (Manoharachary and Mukerji 2006).

Plants have the ability to optimize their own growth in poor soils. In general, there are two mechanisms of plants which can increase absorption of nutrients: morphological and physiological traits. Morphological traits include growth, distribution and root hair formation, while physiological traits include sorption kinetics and nutrient mobilization (Rao *et al*. 1999). Physiological responses of plant roots to improve nutrient uptake in soil with low nutrient availability is through the root kinetics and nutrient mobilization by increasing exudation of organic acids.

Root exudates are substantially the chemical compounds released by root into soil (Walker *et al.* 2003). Besides act as mechanical support for plants in the absorption process of water and nutrients, plant roots also show a special role, including the ability to synthesize, accumulate and excrete a series of chemical compounds (root exudates). However, the process mediated by the roots in the rhizosphere is unknown, although the process has an important role for the plant. The presence of root exudates in the rhizosphere plays a role in influencing chemical reactions and microbial activity in the neighborhood. Root exudates in organic acids form and other carbon compounds are not only directly affect the nutrient availability for the plants, but also have an indirect effect through microbial activity because the compounds are an energy source for soil microbes.

The existence of microbes in soil has an important role, especially in the decomposition of organic matter via enzymatic processes. Microbes are able to produce enzymes that can degrade organic matters outside the cell. Enzymes produced by microbes have an important role such as involved in nutrient cycling, affect fertility efficiently, stimulate decomposition of organic matter and act as an indicator of soil changes. Enzymes in soil consist of several groups, i.e. oxidoreductase, transferase, hydrolase, lyase, isomerase and ligase (Dick *et al.* 2000; Balota *et al.* 2004; Claassens *et al.* 2005). Exudates released by the plants are utilized by microbes as food source while microbes help plants to provide nutrients enzymatically. However, the complex and mutual interaction between plants and microbes process remains unknown. Furthermore, study of root exudate organic acids, microbial population, and enzymatic activities in the oil palm rhizosphere should be conducted to improve the nutrient use efficiency.

Oil palm seedling is selected as an object of current study due to oil palm in Indonesia is the leading commodity that continues to develop up to now. The development of oil palm plantations is carried out through extensification of planting area in order to increase production, but it faces the problem of the limited arable land. This study is expected to provide information for the oil palm development in Indonesia. This study was conducted on oil palm seedling stage because it was easier and more possible to do the experiment in a green house than in plantation field.

The purposes of this study were: (1) to study the types and amounts of root exudate organic acids released by oil palm seedlings, (2) to study the population of microbes in the rhizosphere of oil palm seedlings, and (3) to study the correlation between the concentrations of root exudate organic acids and the population of microbes in the rhizosphere of oil palm seedling.

MATERIALS AND METHODS

Study Location and Seedling Collection

The experiment was conducted in (1) a greenhouse of Cikabayan Experimental Station, (2) Laboratory of Soil Chemistry and Soil Fertility, Laboratory of Soil and Biotechnology, Department of Soil Science and Land Resources, Faculty of Agriculture, Bogor Agricultural University, (3) Laboratory of Residue of Agrochemical Materials, Agricultural Environment Research Institute. The research was conducted in February 2015 until February 2016.

The materials used in the current study were 18-32 mesh quartz sand and oil palm seedlings with various ages (1, 3, 6, 9 and 12 months). Quartz sand was collected from Bangka Belitung and oil palm seedlings were obtained from the Indonesian Oil Palm Research Center.

Preparation and Sterilization of Growing Media

Before use, the quartz sand growing media were sterilized from organic materials. Quartz sand was washed 3-5 times by water to remove coarse organic materials and then burnt (roasted) for 30

minutes to remove more subtle organic materials. Roasted quartz sand was then washed back. After that, the quartz sand was air-dried and sterilized using fumigants of Dazomet 98%.

Planting and Nurturing of Oil Palm Seedlings

Planting of oil palm seedlings of tenera type was conducted in a greenhouse using a completely randomized design (CRD) in a factorial arrangement, with three replications. The first factor was oil palm seedlings age (without oil palm seed, 1, 3, 6, 9 and 12 months of oil palm seedlings) and the second factor was growing period (45, 90, 135 and 180 days). In total, there were 72 experimental units.

Oil palm seedling roots that would be transplanted were cleaned from the previous planting medium and sterilized using clorox 10%. After that, the oil palm seedlings were planted in the quartz sand media with the amount of 32 kg polybag^{-1}. Watering of oil palm seedlings was conducted using distilled water through drip irrigation. Fertilization of oil palm seedlings was conducted using Hoagland solution that was sprayed carefully on the leaves so the solution did not fall into the planting media.

Analysis of Root Exudate Organic Acids

Analysis of root exudate organic acids was performed on each growing period for each plant age. Planting medium near the rhizosphere was sampled carefully by removing the quartz sand outside the root volume and taking quartz sand that was in the root volume. Root exudate organic acids were analyzed using the centrifugation method (Angeles *et al*. 2006) using sterilized distilled extractor. The organic acids concentrations were measured less than 24 hours after sand sampling using HPLC (Waters Associates model of 440) in the mobile phase of 12.05 N H_2SO_4 with 1 ml min^{-1} speed flow, 60 psi pressure and a 254 nm wavelength.

Microbial Analysis

Microbial counting in diluted culture solution was conducted using the pour plate counting method. The methods and media used in the microbial analysis are presented in Table 1.

Statistical Analysis

The data were analysed using *Analysis of Variance* (ANOVA) with F-test at 5% significance level. The differences of treatment effects were further analyzed using *Duncan Multiple Range Test* (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

Root Exudate Organic Acids

The predominant compounds of root exudates measured in the rhizosphere were organic acids, sugars, amino acids, fats, coumarin, flavonoids, proteins, enzymes, aliphatic and aromatic compounds. Organic acids get more attention because of its role in providing a substrate for microbial metabolism and mediating biochemical reactions in soil (Angle *et al.* 1996; Koo *et al.* 2005). The predominant organic acid compound in soil-root interface is an organic acid with low molecular weight, *i.e*. below 250 g mol^{-1} (Tan 2000).

The low molecular weight organic acids play an important role in many processes in the soil, such as increasing nutrient availability. However, a fundamental understanding of the important roles of organic acids in the soil is unclear (Wang and Qiu 2006). Organic acid components of root exudates consist of tartaric, oxalic, citric, malic, acetic, propionic, butyric, succinic, fumaric, glycolic, valerate and malonate. Organic acids are released into the soil by the roots to actively improve the plant's ability to live and develop normally under severe nutrient deficiency (Shen *et al.* 1996).

In this study (Table 2), the root exudate organic acids found in the rhizosphere of oil palm seedlings planted in various ages were acetic acid, citric acid, malic acid and oxalic acid. Analysis of variance showed that the single factor of oil palm seedling age significantly affects the concentration of acetic acid, citric acid, malic acid and oxalic acid excreted by the roots. The single factor of growing period also showed a significant effect on the concentration of each organic acid. Analysis of variance also showed that the interaction between plant age and growing period significantly affects the concentration of citric acid and malic acid excreted by oil palm seedlings, but does not significantly affect the

Table 1. Methods and media used in the microbial analysis.

Parameter	Method	Media
Total Microbes	Plate count	Nutrient Agar (Hastuti and Ginting 2007)
Azotobacter	Plate count	NFM (Hastuti 2007)
Phosphate Solubilizing Microbes	Plate count	Pikovskaya (Santosa 2007)

Table 2. The concentrations of acetic acid, citric acid, malic acid, oxalic acid in the rhizosphere of oil palm seedlings with various ages and growing periods.

Seeds Age	Growing Period				Average
	45 days	90 days	135 days	180 days	
			Acetic Acid (ppm)		
Control	0.000 f	0.000 f	0.000 f	0.000 f	0.000 d
1 month	0.092 ef	0.197 ef	0.224 def	0.526 bcdef	0.260 cd
3 months	0.257 def	0.204 ef	0.357 cdef	0.663 bcdef	0.370 c
6 months	0.427 bcdef	0.276 def	0.419 bcdef	0.908 abcd	0.507 bc
9 months	0.533 bcdef	0.478 bcdef	0.724 bcde	1.088 ab	0.706 b
12 months	0.714 bcde	1.022 abc	1.497 a	1.662 a	1.224 a
Average	0.337 b	0.363 b	0.537 ab	0.808 a	
			Citric Acid (ppm)		
Control	0.000 f	0.000 f	0.000 f	0.000 f	0.000 d
1 month	0.005 f	0.016 f	0.035 ef	0.081 cd	0.034 c
3 months	0.003 f	0.015 f	0.055 de	0.073 cd	0.037 c
6 months	0.004 f	0.016 f	0.085 cd	0.130 ab	0.059 b
9 months	0.008 f	0.024 ef	0.078 cd	0.151 a	0.065 ab
12 months	0.008 f	0.035 ef	0.107 bc	0.157 a	0.077 a
Average	0.005 c	0.018 c	0.060 b	0.099 a	
			Malic Acid (ppm)		
Control	0.000 f	0.000 f	0.000 f	0.000 f	0.000 d
1 month	0.035 ef	0.070 ef	0.106 ef	0.686 cde	0.224 cd
3 months	0.028 ef	0.058 ef	0.314 def	1.203 bc	0.401 bc
6 months	0.084 ef	0.092 ef	0.440 def	1.552 ab	0.542 b
9 months	0.112 ef	0.100 ef	0.876 cd	1.618 ab	0.677 b
12 months	0.131 ef	0.171 ef	2.061 a	1.796 ab	1.040 a
Average	0.065 c	0.082 c	0.633 b	1.143 a	
			Oxalic Acid (ppm)		
Control	0.000 g	0.000 g	0.000 g	0.000 g	0.000 e
1 month	0.107 fg	0.120 fg	0.140 efg	0.305 bcdef	0.168 d
3 months	0.129 efg	0.101 fg	0.232 defg	0.333 bcdef	0.199 cd
6 months	0.231 defg	0.250 cdef	0.273 bcdef	0.372 bcde	0.282 bc
9 months	0.252 cdefg	0.275 bcdef	0.314 bcdef	0.478 abc	0.330 b
12 months	0.331 bcdef	0.435 bcd	0.512 ab	0.675 a	0.488 a
Average	0.175 b	0.197 b	0.245 b	0.361 a	
			Total Organic Acids (ppm)		
Control	0.000 h	0.000 h	0.000 h	0.000 h	0.000 e
1 month	0.239 gh	0.404 gh	0.504 fgh	1.598 cde	0.686 d
3 months	0.417 gh	0.378 gh	0.958 defg	2.272 c	1.006 d
6 months	0.746 fgh	0.635 fgh	1.218 def	2.962 b	1.390 c
9 months	0.905 efg	0.877 efg	1.992 c	3.336 b	1.778 b
12 months	1.184 def	1.663 cd	4.177 a	3.182 b	2.552 a
Average	0.582 c	0.660 c	1.475 b	2.225 a	

The same letters in the same row and column indicate no significant difference at 5% significance level according to DMRT

concentrations of acetic acid and oxalic acid. The concentration of each type of organic acids increased with plant age. The highest concentration of acetic acid was 1.66 ppm, citric acid was 0.157 ppm, malic acid and oxalic acid were 2.061 ppm and 0.675 ppm, respectively.

The increase of organic acid concentrations derived from root exudates in each plant age and growing period was thought to be triggered by a lack of nutrient supply to the plants. Oil palm seedlings were planted in sterilized quartz sand media without addition of nutrient, and this condition was intended to trigger the production of root exudates. The addition of nutrients through the leaves was probably still insufficient, which was indicated by the plant growth that was not optimal. Marschner (1997) suggested that tolerance mechanisms of plants to nutrient deficiency is via excreting exudate organic acids around the plant roots. These organic acids can then dissolve the nutrients that are previously unavailable to become available to plants.

The increase of organic acids was also due to the aging of the seedlings and root weight gain. The increase in root weight was indicated with many emerging young roots. The study of Guckert *et al.* (1991) indicated that production of plant root exudates will vary depending on either plant age or plant growth phase. Plant excretes more exudates when plant roots are still young or in vegetative phase. Exudate excreted in the vegetative phase is rich in organic acids and protein. According to Islami and Utomo (1995), young roots are the root part which actively excrete exudates so that the area with many young roots will excrete more exudates.

Total Population of Microbes and Soil Functional Microbes

Analysis of variance showed that the plant age significantly affects population of microbes, fungi, Azotobacter, phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungus (PSF). The growing period also showed a similar significant effect. Analysis of variance also showed that the interaction between plant age and growing period significantly affects the total population of microbes, fungi, Azotobacter, PSB, but does not significantly affect the population of PSF. The highest population of microbes was 19.38×10^7 cfu g^{-1} soil, fungi was 3.28×10^4 cfu g^{-1} soil, Azotobacter was 12.09×10^5cfu g^{-1} soil, PSB was 8.39×10^4 cfu g^{-1} soil and PSF was 1.15×10^4 cfu g^{-1} soil. The microbial populations measured in the current study are relatively lower than the microbial populations that are commonly found in the rhizosphere of plants. This phenomenon is because of the sterilization process of the growing media and root seedlings at the beginning of oil palm planting.

The microbial population is influenced by the process of sterilization, temperature and humidity of environment. The temperature in the greenhouse was relatively higher than that in the field. The highest temperature was about 44°C with an average temperature of 39°C. Soil moisture was also low due to the nature of the media in the form of sand that could not hold water. Soepardi (1983) indicated that the increasing amount of microbes in the soil depends on soil conditions such as nutrition, temperature, humidity, aeration, oxygen supply and the nature of the organic material.

Microbial population increased with the increase of age of oil palm seedlings, although the microbial population was low. The increase of the microbial population was probably caused by the emergence of new roots. As a result, the rhizosphere extends as microhabitat that supports the increase of microbial population. The growth of new roots caused the wet weight of roots increased followed by an increase of the amount of organic compounds excreted by the roots. Plant roots affect the rhizosphere in various ways. When the root cells die and exfoliate, then microbes rapidly degrade the root cell components. However, root exudates as well as various organic compounds are more important and will affect the amount and diversity of microbes in the rhizosphere (Angle *et al.* 1996).

The population of the fungi increased on day 90 and then decreased on day 135 and 180. A decline in population of fungi on day 135 and 180 was probably due to the decrease of pH (although it was not significantly decreased), and low water and oxygen levels in the rhizosphere. The pH of medium is not classified as dynamic conditions for fungal growth, although the pH of the rhizosphere of oil palm seedlings was not significantly decreased.

According to Sutedjo (1991), an optimum pH for fungi to grow is between 4.5 - 5.5. Population of fungi decreases along with soil acidity, while the population of bacteria and actinomycetes increase with soil pH. Fungi are heterotrophic organisms that depend on the availability of organic matter, carbon and oxygen. Because fungi need carbon and oxygen, so they are usually more common in the upper soil layer than in the vicinity of the rhizosphere (Handayanto and Hairiah 2007).

Azotobacter population increased along with the plant age and growing period. This increase is due to the limited amount of nitrogen in the rhizosphere of oil palm seedlings and no addition of extra nutrients from fertilizer. This limitation led to an increase of root weight of oil palm seedlings,

Table 3. The population of microbes at different plant ages and growing periods.

Seeds Age	Growing Period				Average
	45 days	90 days	135 days	180 days	
			Population of Microbes 10^7 cfu g^{-1} soil		
Control	0.28 m	0.31 m	0.45 m	0.31 m	0.34 f
1 month	3.66 l	5.68 jkl	8.17 ghij	7.39 hijk	6.22 cde
3 months	4.37 kl	5.39 jkl	8.80 fghi	9.60 efghi	7.13 cd
6 months	5.39 jkl	8.06 ghij	10.12 defgh	10.80 cdefg	8.59 c
9 months	6.80 ijk	11.45 cdef	11.91 cde	12.52 cd	10.67 b
12 months	7.62 hijk	13.27 c	16.64 b	19.38 a	14.23 a
Average	4.75 c	7.36 b	9.35 a	10.00 a	
			Population of fungi 10^4 cfu g^{-1} soil		
Control	0.01 m	0.07 m	0.08 m	0.05 m	0.05 f
1 month	0.37 lm	1.35 fg	1.06 ghi	0.73 jkl	0.88 e
3 months	0.48 l	1.91 de	1.29 fgh	0.94 ghijk	1.16 d
6 months	0.59 kl	2.35 c	1.65 ef	1.18 ghi	1.44 c
9 months	0.76 ijkl	2.81 b	2.11 cd	1.12 ghij	1.70 b
12 months	0.91 hijk	3.28 a	2.74 b	1.86 de	2.20 a
Average	0.52 d	1.96 a	1.49 b	0.98 c	
			Population of *Azotobacter* 10^5 cfu g^{-1} soil		
Control	0.03 n	0.12 n	0.00 n	0.01 n	0.04 f
1 month	1.22 m	2.59 kl	4.17 ij	3.70 j	2.92 e
3 months	2.04 lm	3.55 jk	4.85 hi	4.93 hi	3.84 d
6 months	1.84 lm	5.41 gh	6.63 ef	6.42 efg	5.08 c
9 months	2.63 kl	5.78 fgh	7.74 cd	8.22 c	6.09 b
12 months	3.41 jk	6.92 de	9.60 b	12.09 a	8.01 a
Average	1.86 c	4.06 b	5.50 a	5.90 a	
			Population of PSB 10^4 cfu g^{-1} soil		
Control	0.01 j	0.04 j	0.06 j	0.07 j	0.05 f
1 month	0.12 j	1.90 i	2.79 gh	3.11 fgh	1.98 e
3 months	0.22 j	2.37 hi	3.77 ef	4.52 cde	2.72 d
6 months	0.43 j	3.20 fg	5.12 cd	5.26 c	3.50 c
9 months	0.56 j	3.59 f	6.72 b	6.49 b	4.34 b
12 months	0.60 j	4.45 de	8.34 a	8.39 a	5.45 a
Average	0.32 c	2.59 b	4.47 a	4.64 a	
			Populationof PSF 10^4 cfu g^{-1} soil		
Control	0.02 h	0.07 gh	0.10 gh	0.07 gh	0.07 c
1 month	0.28 efgh	0.50 cdefg	0.59 bcdef	0.60 bcdef	0.49 b
3 months	0.24 fgh	0.41 defgh	0.49 defg	0.66 bcdef	0.45 b
6 months	0.37defgh	0.73 bcd	0.69 bcde	0.63 bcdef	0.61 b
9 months	0.23 fgh	0.39 defgh	0.65 bcdef	0.98 ab	0.56 b
12 months	0.48 defg	0.75 bcd	0.92 abc	1.15 a	0.83 a
Average	0.27 c	0.48 b	0.57 ab	0.68 a	

The same letters in the same row and column indicate no significant difference at 5% significance level according to DMRT.

Table 4. Correlation between concentrations of root exudate organic acids and microbial population.

	Microbes	Fungi	Azotobacter	PSB	PSF
Total Organic Acids	0.857**	0.458*	0.858**	0.882**	0.834**
Acetic Acid	0.919**	0.580**	0.893**	0.840**	0.847**
Citric Acid	0.801**	0.328	0.856**	0.902**	0.844**
Malic Acid	0.759**	0.326	0.791**	0.857**	0.768**
Oxalic Acid	0.950**	0.623**	0.920**	0.843**	0.903**

**correlation is significant at 0.01 significance level; *correlation is significant at 0.05 significance level.

which may be due to an increase of IAA (indole acetic acid) generated by the increase of Azotobacter population. The study conducted by Razie and Anas (2005) showed that the rice inoculated with Azotobacter and not fertilized with urea produce higher IAA than inoculated and fertilized rice. In addition, an increase of root growth through the length or surface area of roots was also observed, so that the roots have the ability to absorb water and gain wet weight significantly.

The existence of Azotobacter in the rhizosphere is well known to have two roles, i.e. help to fixing $_2$ from air, and to synthesize plant growth hormones such as IAA. Root weight, plant height and leaf number are positively correlated with plant age and growing period of oil palm seedlings. El-Khawas and Adachi (1999) also found that the lateral and root hair increase root length, root surface area, dry and wet weight of rice inoculated with N_2-fixing bacteria.

The population of phosphate solubilizing microbes increased along with plant age and growing period, although it was relatively low. In general, the population of phosphate solubilizing microbes is around 10^4-10^6 per gram soil and mostly located in the root zone. The population of phosphate solubilizing microbes in the rhizosphere of oil palm seedlings was dominated by a group of bacteria due to the influence of soil pH. The soil pH, which is close to neutral pH, resulted in higher increase of bacterial growth compared to the growth of the fungi. Phosphate solubilizing fungi grow optimally at pH 5-5.5. The decline of soil pH, although it was not significant, was caused by the increase of organic acids amount excreted by oil palm seedling roots.

Correlation between Concentration of Root Exudate Organic Acids and Microbial Population

Concentrations of organic acids from root exudates are positively correlated to the total microbes, fungi, Azotobacter, PSB and PSF. Total organic acids is highly correlated to the population of PSB, acetic acid is highly correlated to the population of Azotobacter, citric acid is highly correlated to the population of PSB, malic acid is highly correlated to the population of PSB, and oxalic acid is highly correlated to the population of Azotobacter. This phenomenon is due to the planting medium did not supply sufficient amount of available nutrients to the plants, so plants excreted organic acids to fulfill their nutrients requirement.

So far there are no studies showing a correlation between the types of organic acids and type of microbes that are dominant in the rhizosphere. However, Syarif (2005) found that low phosphate level in plant can increase the exudation of organic acids, especially oxalic acid and citric acid. Organic acids are then utilized by phosphate solubilizing microbes as a substrate, so that the population of the microbes has increased along with the increasing amount of organic acids.

CONCLUSIONS

Production of acetic, citric, malic, and oxalic acids increased along with plant age and growing period. The highest production of acetic acid was 1.66 ppm, malic acid was 2.061 ppm, citric acid was 0.157 ppm, and oxalic acid was 0.657 ppm. Population of bacteria, fungi and soil functional microbes increased along with plant age and growing period. The highest population of bacteria was 19.38 $\times$ 10^7 cfu g^{-1} soil, fungi was 3.28 $\times$ 10^4 cfu g^{-1} soil, Azotobacter was 12.09 $\times$ 10^5 cfu^{-1} g^{-1} soil, PSB was 8.39 $\times$ 10^4 cfu^{-1} g^{-1} soil, and PSF was 1.15 $\times$ 10^4 cfu^{-1} g^{-1} soil. There are positive correlations between root exudate concentrations and population of soil microbes. The highest correlations were found between total organic acids and population of PSB, acetic acid and population of Azotobacter, citric acid and population of PSB, malic acid and population of PSB, and oxalic acid and population of Azotobacter.

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