BIODIVERSITAS
Volume 19, Number 2, March 2018
Pages: 701-705

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190243

Short Communication: The role of microbial rhizosphere in enhancing plant growth of *Jatropha curcas* in soil contaminated mercury

WIWIK EKYASTUTI♥, HANNA ARTUTI EKAMAWANTI
[1]Faculty of Forestry, Universitas Tanjungpura. Jl. Prof. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia. Tel.: +62-561-765342, 583865, 732500, Fax.: +62-561-765342, ♥email: wieky_serdam@yahoo.co.id

Manuscript received: 12 June 2017. Revision accepted: 27 March 2018.

Abstract. *Ekyastuti W, Ekamawanti HA. 2018. Short Communication: The role of microbial rhizosphere in enhancing plant growth of* Jatropha curcas *in soil contaminated mercury. Biodiversitas 19: 701-705.* Soil in the area of ex-gold mining, has the chemical-physical constraints to the growth of plants. These chemical-physical constraints are low organic matter, poor of nutrient, acid pH, very low CEC, soil texture dominated by sand, and mercury contamination. This area needs to be rehabilitated. Previous research has found that *Jatropha curcas* as a plant tolerant to mercury. On the other hand, some types of microbial rhizosphere such as arbuscular mycorrhizal fungi (AMF) and mercury reducing bacteria (MRB) also have an ability to reduce mercury. The purpose of this study was to determine the role of microbial components of AMF and MRB in enhancing the growth of *J. curcas* in tailings contaminated mercury. The study was conducted in two places, in the greenhouse and in the tailing area of ex-gold mining, using factorial completely randomized design. Results showed that interactions between AMF and MRB were simultaneously able to enhance the growth of *J. curcas* not only in the greenhouse, but also in the field (tailing area). In the greenhouse (nursery), several isolates of *Bacillus* sp, *Bacillus* sp + *Glomus* SS11 and *Bacillus* sp + *Glomus* SS18 in the forms of inoculum were very effective in enhancing the seedling growth of *J. curcas*. However, results were apparently changed after those seedlings were planted in the field (tailings of ex-gold mining). The combination of *Bacillus* sp. + *Glomus* SS18 was the best treatment to enhance the growth of *J. curcas* of all used treatments. This result proves that the role of microbial rhizosphere, especially AMF and MRB, could effectively enhance the growth of *J. curcas* in tailings contaminated with mercury.

Keywords: Gold mining, *Jatropha curcas*, microbial rhizosphere, tailings

INTRODUCTION

The tailing area previously precedes as forest environment. Ecologically, forests have a function as a source of biodiversity, oxygen source, water management and biomass source (Astiani and Ripin 2016; Astiani et al. 2017). After mining activities, these ecological functions plummet and even disappear. Soil in the area of the ex-gold mining consisting of chemical and physical agents constraining the growth of plants. These chemical-physical constraints content with low organic matter, poor of nutrient, acid pH, very low CEC, and soil texture dominated by sand. In addition, the soil is also contaminated with mercury (Ekyastuti et al. 2016) such as tailings ex-communities gold-mining (illegal mining) in Mandor Nature Reserve, West Kalimantan, Indonesia containing mercury 6-18 times higher than that of the safe threshold. Meanwhile, that was also found in the water-sediment with > 2000 times higher than that of the safe threshold (Ekamawanti et al. 2005). Therefore, the growth of plants in tailings ex-gold mining is restricted not only because of marginal soil condition, but also because of mercury contamination in tailings (Figure 1).

Plants have different sensitivity levels to mercury in the environment. In general, plant is susceptible to mercury in the environment. This is because mercury in plant tissue may interfere with the process of photosynthesis and metabolism of plants, so that the growth of plant is stunted (Rugh et al. 2000). However, some plants have a high tolerance level for mercury in the environment, and potentially used as mercury phytoremediator. Phytoremediation is defined as the leaching of pollutants or contaminants by involving the use of plants to remove, move, stabilizing and/or degrade contaminants in soil, sediment and water (Prasad 2003; Mudgal et al. 2010; Favas et al. 2014; Laghlimi et al. 2015). Previous studies have been conducted by Ekamawanti and Ekyastuti (2010) showed that jatropha (*Jatropha curcas*) was a plant that tolerant to mercury, so it could be potentially used as mercury phytoremediator. On the other hand, some types of microbial rhizosphere such as arbuscular mycorrhizal fungi (AMF) and mercury reducing bacteria (MRB) also has an ability to reduce mercury from soil. Previous research has reported the success results in collecting three isolates of AMF i.e. *Glomus* SS11, *Glomus* SS15, and *Glomus* SS18 (Ekamawanti and Ekyastuti 2011) and two isolates of MRB i.e. *Pseudomonas* sp. and *Bacillus* sp. (Ekyastuti and Setyawati 2014), which had an ability to reduce mercury. However, the symbiosis correlation between the microbial rhizosphere (AMF and MRB) with *J. curcas* in enhancing plant growth is still clearly unknown.

Therefore, the purpose of this study was to determine the role of microbial rhizosphere especially AMF and MRB in enhancing the growth of *J. curcas* in tailings ex-gold mining contaminated with mercury. The benefit of this research is to utilize *Jatropha* inoculated with AMF and MRB to rehabilitate the ex-gold mining area.

MATERIALS AND METHODS

The study was conducted in two different places, in the greenhouse and in the field (tailings ex-illegal gold mining of Mandor, West Kalimantan, Indonesia; Figure 1). Research was conducted with a factorial completely randomized design with two-type treatments of microbial rhizosphere AMF and MRB. AMF consisted of three isolates (*Glomus* SS11, *Glomus* SS15, and *Glomus* SS18) and MRB consisted of two isolates (*Pseudomonas* sp. and *Bacillus* sp.). The total number of treatment either single or combination was 12, namely: Control, *Glomus* SS11, *Glomus* SS15, *Glomus* SS18, *Pseudomonas* sp, *Bacillus* sp, *Glomus* SS11 + *Bacillus* sp., *Glomus* SS11 + *Pseudomonas* sp., *Glomus* SS15 + *Bacillus* sp., *Glomus* SS15 + *Pseudomonas* sp, *Glomus* SS18 + *Bacillus* sp and *Glomus* SS18 + *Pseudomonas* sp. Each of treatment combinations was repeated five times both in the greenhouse and in the field. In the greenhouse, concentration of mercury in the tailings was 10 ppm. The addition of mercury in the tailings was in accordance with Rabie (2005). The plant growth responses were observed and collected in the greenhouse as well as in the field for 4 months (Figure 2). Plant growth parameters i.e the increase of plant height, diameter, number of leaves, total dry weight, and the ratio of root shoots were measured. In the end of this study, values of translocation factor and tolerance index using techniques of Rabie (2005), AMF infections using modified of slide methods (McGonigle et al. 1990) and re-isolation of MRB using Canstein's selective media (Canstein et al. 2002) were carried out and analyzed.

Figure 1. Tailings ex-gold mining in Mandor Nature Reserve, West Kalimantan, Indonesia

Figure 2: *Jatropha curcas* in polybags in the greenhouse (*left*) and the field (tailings ex-illegal gold mining) (*right*)

Furthermore, to determine the microbial rhizosphere role in enhancing plant growth, we approached with the correspondence class using results of the analysis of variance. The value of control plants (without inoculation of microbial rhizosphere) compared with the median trial (mean of all trials included control) was used as standard for each variable. Suitability classes were determined as follows: (i) ineffective class, if the tested variables did not differ from controls, (ii) low effectiveness if the tested variables were in higher difference than that in control, but they were lower than that of the average of the experiment, (iii) moderate effectiveness if the tested variables were higher difference than that in control, but they were equal to the average of the experiment, and (iv) high effectiveness (very effective) if the tested variables were higher difference both than that of control or experiment.

RESULTS AND DISCUSSION

The growth response of *Jatropha curcas* as a result of microbial rhizosphere inoculation

Results showed that interactions between AMF with MRB were able to enhance the growth of *J. curcas* not only in the greenhouse, but also in the field (tailings area ex-gold mining). However, the sole inoculation of AMF in the plants had a less effect on the plant growth with low-effective response (Tables 1 and 2). This indicates that three isolates of AMF (*Glomus* SS11, *Glomus* SS15, and *Glomus* SS18) and two isolates of MRB (*Pseudomonas* sp and *Bacillus* sp) could be symbiosis not only among their communities but also with plants in this case with *J. curcas*. This condition occurred because each type of microbes including AMF has a specific response to the suitability of plant species (Turjaman 1998 as quoted by Agustian et al. 2011).

The further investigation showed that isolates of *Bacillus* sp, *Bacillus* sp + *Glomus* SS11 and *Bacillus* sp + *Glomus* SS18 were the most effective microbes to enhance the growth of *J. curcas* in nurseries (greenhouse), which could be observed from all improved data of growth parameters especially in increasing plant height, diameter, number of leaves and plant dry weight (Table 1 and 2). This result was in accordance with other published papers reporting some types of microbial rhizosphere could help plants in acquiring nutrients from the soil (Harman 2011; Pii et al. 2015; Kumar et al. 2015). In addition, some plant growth-promoting bacteria could decrease the heavy metal toxicity in plants (Burd et al. 2000). However, after planting in the field (tailing areas) the different response occurred. In the field, the treatment combination of *Bacillus* sp + *Glomus* SS18 still had the most effective, response to the improvement of plant growth, while another two treatments showed the decreased response (ineffective). This suggested that isolate of *Bacillus* sp. inoculated either solely or in combination with Glomus SS11 might have the good ability to compete with other microbes in the tailings ex-gold mining, so that the symbiosis system with plant might also work effectively. Göhre and Paszkowski (2006) reported that several symbiosis mechanisms between plants and microbial rhizosphere (AMF and MRB) related to the removability of heavy metals including mercury. Furthermore, microbes might involve in the removing heavy metals as a chelating agent by binding metals to the cell wall. It also has a function as a selection barrier of chelating agent to actively and passively eliminate the metal-specific and non-metal specific in the cytosol and the cell vacuole, as well as transport metal both in the hyphae and fungal arbuscles. It shows that after being transferred to the field, this mechanism seems to be limited because of unknown factors, so that the effectiveness of the symbiosis between plants with microbial rhizosphere (*Bacillus* sp and *Glomus* SS11) also decreased.

Translocation Factor (TF), Tolerant Index (TI) and % AMF Infection

Translocation factor (TF) is used to determine the location of mercury for being accumulated in plant tissues, whether in roots or shoots (Rabie 2005). Furthermore, if the value of TF $\geq$ 1, it means that mercury is mostly accumulated in shoots, whereas if the value of TF <1, it means that mercury is accumulated in roots. AMF and MRB inoculations had caused the varying value of TF in the *J. curcas* (Table 3). Besides, TF is also used to determine the level of plant tolerance to mercury accumulation with the value of TF < 30% is considered as low tolerance, moderate tolerance (TF 31%-70%) and high tolerance (TF > 71%, very tolerant).

Results showed that TF value of all inoculation treatment combinations except for *Glomus* SS11 + *Pseudomonas* sp treatment in *J. curcas* plants grown in the greenhouse showed less than 1. It means that most mercury was accumulated in the root. However, after planting in the field (tailings area), there is little change. TF value of all inoculation treatment combinations except for *Glomus* SS18 + *Pseudomonas* sp treatment showed more than 1. It means that mercury was accumulated in plant shoots. Remediation mechanism accumulating mercury in shoots is phytoextraction (Ghosh and Singh 2005; Malik et al. 2010). Through the mechanism of phytoextraction, usually, the level of plant tolerance will decrease. However, in this study plant tolerance index (TI) remained high with a mean of > 75% (Table 3), both in the nursery (greenhouse) and in the field. It means that microbial rhizosphere has an important role in eliminating mercury accumulation. According to Suharno and Sancayaningsih (2013), the presence of heavy metals including mercury can disrupt plant growth and changes in microbial communities, which are more resistant to metals. This condition is also supported by the obtained results of AMF infection in this study. AMF infection was found in plants either in the greenhouse or the field.

As a conclusion, the application both types of rhizosphere microbial namely AMF *Glomus* SS18 and MRB *Bacillus* sp simultaneously inoculated in *J. curcas* is highly recommended to remedy tailings (soil) contaminated with mercury. Furthermore, the results of this study could be used for reclamation program, especially in the tailings of ex-gold mining.

Table 1: Summary of effectiveness of microbial rhizosphere on growth of *Jatropha curcas*

Locations	Microbial rhizosphere isolates	Plant height (cm)		Plant diameter (mm)		Number of leaves (pieces)		Plant dry weight (g)	
		Mean	Effectiveness	Mean	Effectiveness	Mean	Effectiveness	Mean	Effectiveness
Greenhouse	Control	10.50	-	3.75	-	2.25	-	5.46	-
	Glomus SS11	9.40	-	3.50	-	2.00	-	4.96	-
	Glomus SS15	9.24	-	3.37	-	3.00	+++	5.07	-
	Glomus SS18	7.87	-	3.75	+	2.12	-	5.48	+
	Bacillus sp	14.56	+++	4.87	+++	2.62	++	6.68	+++
	Pseudomonas sp	12.15	+++	3.37	-	2.25	+	6.18	+++
	Glomus SS 11 + *Bacillus* sp	11.25	+++	4.94	+++	2.25	+	5.57	+
	Glomus SS 11 + *Pseudomonas* sp	11.31	+++	3.87	+	2.62	++	5.30	-
	Glomus SS 15 + *Bacillus* sp	11.06	+++	4.00	+++	1.50	-	6.08	+++
	Glomus SS 15 + *Pseudomonas* sp	11.31	+++	4.25	++	1.50	-	6.50	+++
	Glomus SS 18 + *Bacillus* sp	11.37	+++	3.87	+	2.62	++	6.21	+++
	Glomus SS 18 + *Pseudomonas* sp	11.72	+++	3.12	-	3.25	+++	5.10	-
Tailings area	Control	11.3	-	8.24	-	2.20	-	19.8	-
	Glomus SS11	12.8	+	8.12	-	1.52	-	7.9	-
	Glomus SS15	1.0.8	-	7.57	-	1.69	-	15.0	-
	Glomus SS18	16.6	+++	7.56	-	1.41	-	22.8	++
	Bacillus sp	14.6	+++	6.32	-	1.41	-	21.7	++
	Pseudomonas sp	13.3	++	7.46	-	1.52	-	19.3	-
	Glomus SS 11 + *Bacillus* sp	13.7	++	6.71	-	1.41	-	21.7	+
	Glomus SS 11 + *Pseudomonas* sp	14.6	+++	7.48	-	1.52	-	19.3	-
	Glomus SS 15 + *Bacillus* sp	15.5	+++	8.12	-	2.33	-	17.1	-
	Glomus SS 15 + *Pseudomonas* sp	15.2	+++	9.35	+++	2.15	-	23.5	++
	Glomus SS 18 + *Bacillus* sp	14.0	++	9.41	+++	3.77	+++	20.9	++
	Glomus SS 18 + *Pseudomonas* sp	15.6	+++	7.29	-	2.66	+++	17.9	-

Note: - : ineffective, (++): moderate effectiveness, (+): low effectiveness, (+++): high effectiveness

Table 2: Value of TF, TI and AMF infections in *Jatropha curcas*

Microbial rhizosphere isolates	In the greenhouse			In the field (tailings ex-illegal gold mining)		
	TF (%)	TI (%)	FMA	TF (%)	TI (%)	FMA
Control	0.546	-	Moderate	3.14	-	Very low
Glomus SS11	0.291	98.13	Moderate	0.12	39.9	Low
Glomus SS15	0.407	115.8	Moderate	3.99	75.8	Moderate
Glomus SS18	0.142	102.3	Moderate	0.22	115.2	Low
Bacillus sp	0.052	113.0	Moderate	0.06	109.6	Very low
Pseudomonas sp	0.099	128.8	Moderate	3.79	97.5	Very low
Glomus SS 11 + *Bacillus* sp	0.816	93.5	Moderate	1.14	109.6	Low
Glomus SS 11 + *Pseudomonas* sp	1.066	106.5	Moderate	3.69	97.5	Low
Glomus SS 15 + *Bacillus* sp	0.002	98.1	Moderate	1.52	86.4	Moderate
Glomus SS 15 + *Pseudomonas* sp	0.144	106.9	High	4.82	118.7	Moderate
Glomus SS 18 + *Bacillus* sp	0.044	114.0	Moderate	1.41	105.6	Low
Glomus SS 18 + *Pseudomonas* sp	0.129	77.7	Moderate	0.08	90.4	Low

ACKNOWLEDGEMENTS

We thank the Directorate General of Higher Education (DIKTI), the Republic of Indonesia on the funding for this research through research scheme National Strategy.

REFERENCES

Agustian, Faiza M, Maira F. 2011. The growth response of *Tithonia diversifolia* to the inoculation of arbuscular mycorrhizal fungi (AMF). J Solum 8 (2): 70-77.

Astiani D, Ripin. 2016. The roles of community fruit garden tembawang on maintaining forest structure, diversity and standing biomass allocation: an alternative effort on reducing carbon emission. Biodiversitas 17 (1): 359-365.

Astiani D, Mujiman, Curran LM. 2017. Trees of tropical peatland forest influence on variability of water and carbon input through stem flow. Biodiversitas 18 (1): 383-388.

Burd GI, Dixon DG, Glick BR. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Canadian J Microbiol 46 (3): 237-245.

Canstein HV, Leonhauser Y, Li J, Haase E, Felske A, Deckwer WD, Dobler IW. 2002. Spatially oscillating activity and microbial succession of mercury-reducing biofilms in a technician-scale bioremediation system. Appl Environ Microbiol 68: 1938-1946.

Ekamawanti HA, Utomo RS, Liwono. 2005. Restoration of terrestrial, riparian and aquatic area of gold mining area with bioremediation technology in Mandor sub-district, Landak District West Kalimantan; Final report of Research Incentive Mathematics and Natural Sciences. Forestry Faculty Tanjungpura University, Pontianak.

Ekamawanti HA, Ekyastuti W. 2010. Effectiveness test of rhizosphere microbe isolates on growth of some species of plants in mercury tailed media. Faculty of Forestry, Tanjungpura University, Pontianak.

Ekamawanti HA, Ekyastuti W. 2011. The effectiveness of rhizosphere microbial isolates on growth of *Paraserianthes falcataria* and *Enterolobium cyclocarpum* seedlings in mercury-contaminated tailings. Agrotropika 16 (2): 62-69.

Ekyastuti W, Setyawati TR. 2014. Identification and in vitro effectiveness test of four isolates of mercury-resistant bacteria as bioaccumulation agents of mercury. Procedia Environ Sci 28 (2015): 258-264.

Ekyastuti W, Faridah E, Sumardi, Setiadi Y. 2016. Mitigation of mercury contamination through the acceleration of vegetation succession. Biodiversitas 17 (1): 84-89.

Ghosh M, Singh SP. 2005. A review on phytoremediation of heavy metals and utilization of its by-products. Appl Ecol Environ Res 3: 1-18.

Göhre V, Paszkowski U. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223 (6): 1115-1122.

Harman GE. 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. New Phytologist 189 (3): 647-649.

Kumar A, Bahadur I, Maurya BR, Raghuwanshi R, Meena VS, Singh DK, Dixit J. 2015. Does a plant growth promoting rhizobacteria enhance agricultural sustainability?. J Pure Appl Microbiol 9 (1): 715-724.

Malik RN, Husain SZ, Nazir I. 2010. Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pak J Bot 42 (1): 291-301.

McGonigle TP, Miller MH, Evans DG, Fairchild GL and Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol (115): 495-501.

Mudgal, Varsha, Madaan N, Mudgal A. 2010. Heavy metals in plants: phytoremediation: Plants used to remediate heavy metal pollution. Agric Biol J North Amer 1 (1): 40-46.

Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C. 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process (a review). Biol Fertil Soils (51)4: 403-415.

Rugh CL, Bizily SP, Meagher RB. 2000. Phytoreduction of environmental mercury pollution. In: Raskin I, Ensley BD (eds.). Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. Wiley-Interscience Publication. John Wiley & Sons, Inc. New York.

Rabie GH. 2005. Contribution of Arbuscular Mycorrhizal Fungus to Red Kidney and Wheat Plants Tolerance Grown In Heavy Metal Polluted Soil. African J Biotechnol 4 (4): 332-345.

Suharno, Sancayaningsih RP. 2013. Arbuscular Mycorrhizal Fungi: The potential use of heavy metal mycorrhizal-remediation technology in mined field rehabilitation. Bioteknologi 10: 31-42.