doi: 10.17221/289/2016-PSE

Effects of Sb16 bacterial strain and herbicides on endophytic bacterial populations and growth of aerobic rice

A. Nahi, R. Othman, D. Omar

Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia

ABSTRACT

Pot experiment was conducted under glasshouse conditions to investigate the effects of the inoculation of N_2 fixing *Stenotrophomonas maltophilia* strain Sb16 and application of three herbicides (paraquat, pretilachlor and 2,4-D) at 0, 1/2X, X and 2X their recommended field application rates (X) on endophytic bacterial populations and physio-morphological parameters of aerobic rice. The physio-morphological traits such as plant height, leaf area, chlorophyll content, nitrogen (N) content, root dry mass, root length, root volume and root average diameter were assessed at 60th day after the treatment. Data on endophytic bacterial counts were collected at 15, 30, 45 and 60 days after the treatment. Results obtained from the study revealed that the number of endophytic bacteria and physio-morphological characters of aerobic rice significantly decreased with increasing herbicides dose. Sb16 inoculation significantly ($P \leq 0.0001$) increased all the parameters measured. N contents were the highest (2.53 %) in the inoculated samples treated with half dose of 2, 4-D; but the lowest contents (1.89 %) were obtained in the non-inoculated samples treated with double dose of paraquat. The results suggest that Sb16 strain can improve productivity of aerobic rice under herbicide-stressed soil.

Keywords: *Oryza sativa*; weed control; nutrient uptake; microbial communities; rhizosphere

Rice (*Oryza sativa* L.) is one of the essential food crops all over the world. Over half of the world's population, mainly in Asia, consume rice as their staple food. In Malaysia, more than 100 000 farmers survive their subsistence through rice cultivation. Therefore, Malaysia has to improve the rice production to fulfil the demand of the growing population. Aerobic rice has emerged as a bright water-saving system for rice plantation (Anwar et al. 2010). However, the alteration of water management from flooded to aerobic conditions has an impact on the availability and form of nitrogen (N) present in the soil, thus an efficient N management approach is required (Savant and De Datta 1982). Nutrient uptake efficiency and N_2 fixation is improved by free-living diazotrophic bacteria via associative and endophytic relationships with graminaceous plants (Bashan and Holguin 1997). As endophytic bacteria colonise roots and establish a desirable environment for development and function, they are considered beneficial inoculant options.

Stenotrophomonas maltophilia, formerly *Xanthomonas maltophilia* (Palleroni and Bradbury 1993) is often a dominant member of the microbial communities that exist widely on or in plants and is distributed globally (Denton and Kerr 1998). *Stenotrophomonas maltophilia* is an endophytic microorganism which has exhibited some plant growth promoting characteristics such as fungal biocontrol (Wang et al. 2007), indole acetic acid (IAA) production (Park et al. 2005), and siderophores production (Idris et al. 2007). *Stenotrophomonas maltophilia* is seen predominantly in the rhizosphere of cereal crops (Lambert and Joos 1989). The bacterial strain *S. maltophilia* Sb16 (accession number, JQ820255) was previously isolated from Tanjong Karang Rice growing area in Malaysia (Naher et al. 2008).

Aerobic rice suffers from a huge weed pressure which endangers the crop productivity. An efficient weed management approach is required to cope up this issue. Herbicide might be regarded as a feasible option to hand weeding according to lots

doi: 10.17221/289/2016-PSE

of researcher doing research on weed management in direct-seeded rice (Chauhan and Johnson 2011, Anwar et al. 2012). However, due to the probable influence of these compounds on the environment, the amounts that do not achieve the target organisms have become an issue (Kucharski et al. 2009). Furthermore, herbicides applied to soil or plant might interfere with the microbial bio-fertiliser inoculated to crop plants, in case they come in contact. Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride), a member of bipyridinium which is a non-selective cationic contact herbicide, is quickly adsorbed by soil particles and becomes inactive. Pretilachlor (2-chloro-2',6'-diethyl-N-(2-propoxyethyl) acetanilide), a member of the chloroacetamide chemical family, is a synthetic selective herbicide; however, 2,4-D (2,4-dichlorophenoxyacetic acid), a plant growth regulator is a selective phenoxyacetic acid herbicide.

The effects of inoculation with N_2-fixing *Bradyrhizobium* sp. (vigna), along with herbicides on greengram [*Vigna radiata* (L.) Wilczek], have been studied (Zaidi et al. 2005, Ahemad and Khan 2011). However, the effect of N_2 fixing endophytic bacteria on the growth of aerobic rice in soil applied with herbicides has not been studied to date. Therefore, this study was carried out to determine the effects of selected herbicides commonly used for rice production (paraquat, pretilachlor and 2,4-D) on the endophytic bacterial populations and growth of aerobic rice inoculated with *S. maltophilia* (Sb16) under glasshouse condition.

MATERIAL AND METHODS

Aerobic rice line MR 219–9 mutant (M-9) was obtained from Malaysian Agricultural Research and Development Institute (MARDI). Seeds of aerobic rice were surface sterilised according to the method of Somasegaran and Hoben (1994) and allowed to germinate on Petri dishes.

A pot experiment was conducted under glasshouse conditions at 25 ± 2°C and 60% relative humidity (RH) at Ladang 2 of University Putra Malaysia in Serdang, Selangor, Malaysia. The study was laid out as factorial in a randomised complete block design (RCBD) with three replications.

Soil samples collected from Ladang 2 were alluvial sandy clay loam with pH 5.3, organic carbon 1.28%, cation exchange capacity (CEC) 12.1 $cmol_+$/kg and total nitrogen (N) 0.29%. The soils were air-dried in laboratory conditions (25°C; 50% RH) for 48 h, crushed and passed through a 2 mm sieve, and 2 kg of sieved soil was poured into plastic pots (17 cm diameter × 23 cm height).

The herbicides used in this study were formulations of paraquat dichloride (13% w/w) Syngenta Capayam, pretilachlor (28.7% w/w) Syngenta Sofit N300 EC and 2,4-D isopropylamine (35.5% w/w) (28% equivalent) Kompressor Ancom Cropcare. The concentrations (rates) of each herbicide treatment applied in this study are as follows: paraquat at 0.78, 1.56 and 3.12 mg a.i./g soil; pretilachlor at 0.72, 1.44 and 2.88 mg a.i./g soil); and 2,4-D at 1.42, 2.84 and 5.68 mg a.i./g soil). These treatment rates represented 1/2, 1, and 2 times the recommended field rate of the products (paraquat: 700 g a.i./ha; pretilachlor: 430 g a.i./ha; and 2,4-D: 710 g a.i./ha). The herbicides solutions were applied pre-plant to the pot and incorporated to the soil to disperse the herbicides uniformly. The untreated soil samples (control) received the same amount of distilled water. The soil moisture content was adjusted to 60% of water holding capacity and checked regularly.

Stenotrophomonas maltophilia (Sb16) was obtained from the Laboratory of Microbiology, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. The Sb16 strain was cultured in Erlenmeyer flasks containing 100 mL Jensen's N-free medium and shaken on an orbital shaker at 150 rpm at 37°C for 3–4 days. The drop plate method was employed to confirm the population of inoculum (10^7 colony forming unit (CFU)/mL). Seven day-old aerobic rice seedlings were soaked in cultured inoculum for 30 min and 5 seedlings were transplanted into each pot at a depth of 5 cm. After emergence, plants were thinned to 2 per pot. The pots were hand-weeded at regular intervals and watered on a daily basis using distilled water.

Data collection to determine the population size of endophytes was performed every 15 days starting from 15–60 days after the treatment (DAT). Fresh roots were surface sterilised by immersion in 70% ethanol for 5 min, followed by 2% sodium hypochlorite (NaOCl) solution for 2 min, and three sequential washing with sterilised distilled water. Root pieces were cut into smaller sections (5 cm) and surface sterilised by submersion into 95% ethanol. Surface-sterilised roots were then

doi: 10.17221/289/2016-PSE

homogenized in sterile 0.85% phosphate buffer solution using sterilised mortar and pestle in order to release the endophytic bacterial cells. Ten-fold serial dilutions ranging from 10^{-3} through 10^{-8} were prepared and 100 μL of each dilution was spread plated on the nitrogen-free (Nfb) semi-solid malate medium (Prasad et al. 2001) per L consisting of 5 g malic acid, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7\ H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2$, 0.5% bromothymol blue in 0.2 mol/L KOH (2 mL), 1.64% Fe-EDTA solution (4 mL) and 2 g agar, after which plates were incubated for one week at 28°C.

The data on physio-morphological attributes such as plant height (cm), leaf area per plant (cm^2), leaf chlorophyll content (SPAD value), plant N content (%), root dry mass, length, volume and average diameter were collected at 60th DAT. Plant height was measured from the base of plant to the tip of the uppermost part of the plant. A portable chlorophyll meter (Minolta^{TM} SPAD-502, Tokyo, Japan) was used to determine the leaf chlorophyll content. The plant samples were transferred directly to the laboratory after collection, washed with distilled water and leaf, root and shoot portions were separated. The leaves from each plant were randomly taken and total surface area of each leaf was measured using a leaf area meter (Licor, Model LI-3100 Area Meter, LI-COR Inc. Lincoln, USA). The Kjeldahl method (Bremner 1996) was employed to determine the nitrogen concentration (%) in the plant tissue. The root dry mass was estimated by forced air oven drying at 65°C until a constant weight was obtained. Fresh roots were washed thoroughly with distilled water and placed on the Root Scanner Image Analyser Win Rhizo STD1600 WIA-Epson Expression 1680 (Nagano, Japan) to determine the length, volume and surface area of the root.

Data were analysed using the Statistical analysis system (SAS) version 9.3 (Cary, USA), and treatment means were compared using the Tukey's test ($P < 0.05$). Analysis of variance (ANOVA) was conducted for each herbicide to test the effects of inoculation and concentrations on physio-morphological parameters of aerobic rice and inoculation (–Sb16, +Sb16), concentrations (0 – control; 1/2, 1, 2 times their recommended field application rates (X) and sampling dates (15, 30, 45, 60 days after the treatment) on endophytic bacterial populations. Data on endophytic population were log-transformed and expressed as $\log_{10}$ CFU/g root fresh weight.

RESULTS AND DISCUSSION

The results of the three-way ANOVA demonstrated that inoculation and inoculation × sampling time interaction had significant effects on populations of endophytic bacteria in all samples. There were no significant differences in endophytic bacterial communities between the 15th and 30th day of sampling of non-inoculated samples treated with herbicides and between the 30th and 45th day in inoculated samples, except the control (Figure 1). The populations of endophytic bacteria in the inoculated samples treated with herbicides were not significantly different between the 45th and 60th sampling days, with the exception of the sample treated with double dose of paraquat ($P = 0.0035$). An enhancement in the bacterial endophytes of aerobic rice, following inoculation with Sb16, indicates the beneficial establishment of introduced bacterium on and within the root of aerobic rice and consequently increase in N uptake, leading to the overall improvement in plant growth.

The main effects (inoculation, concentrations ($P < 0.0001$)) and their interaction significantly influenced plant height. An increase in plant height following inoculation can be due to the atmospheric N fixation by Sb16 strain in plant roots, leading to overall plant growth. Previous studies exhibited the ability of free-living N_2-fixing bacteria to improve rice plant heights (Islam et al. 2009). Inoculation showed a significant effect ($P = 0.0004$) on N content in sample treated with half dose of 2,4-D (Table 1). In non-inoculated samples, half and double doses of paraquat significantly reduced N content compared to inoculated samples ($P = 0.0006$ and $P = 0.0007$), respectively. The better response of greengram plant in terms of its whole biomass and N in root and shoot after herbicides application and inoculation of *Bradyrhizobium* sp. (vigna) strain, compared to non-inoculated soil treated with the same concentration of herbicides quizalafop-p-ethyl and clodinafop in the study by Ahemad and Khan (2011), agrees with the findings of the present study.

In terms of leaf area, inoculated sample treated with pretilachlor at double dose was significantly different ($P < 0.0001$) from those with half and full doses (Table 1). There were significant main effect of inoculation ($P < 0.0001$) and interaction effects of inoculation and concentrations on leaf area. Inoculation and concentrations of herbicides did not show any significant effects on chlorophyll

doi: 10.17221/289/2016-PSE

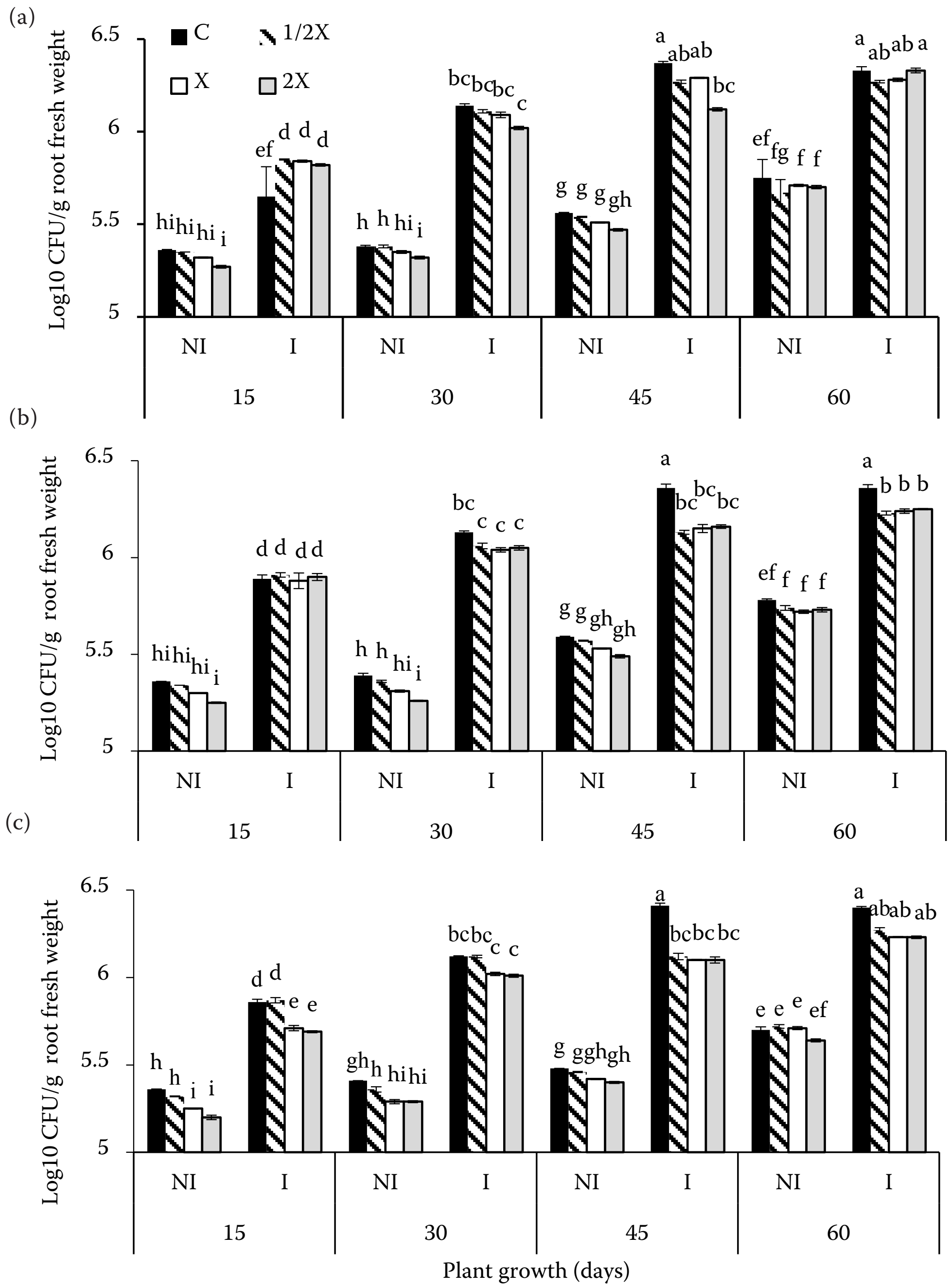

Figure 1. Effect of Sb16 bacterial inoculation and herbicides application on population of endophytic bacteria within 60 days after transplanting of aerobic rice; (a) paraquat; (b) pretilachlor; (c) 2,4-D; NI – non-inoculated; I – inoculated; C – control; 1/2X – half dose; X – full dose; 2X – double dose; Bars on top of each column represent standard error of three experimental replicates. Different letters indicating statistical differences of the Tukey's test at the 0.05 level of significance from a four-factorial ANOVA are shown above the bars

content of aerobic rice (Table 1). Increases in leaf area, chlorophyll content and N content of aerobic rice after Sb16 inoculation in the present study are similar to those in a previous study by Sheng (2005), who observed higher N content of the above ground components in cotton and ripe plants following inoculation of bacterial strain *B. edaphicus* NBT. Leaf area and photosynthesis metabolism of green plants are considerably influenced by N supply (Lloyd and Farquhar 1996).

Application of different concentrations of herbicides and inoculation with Sb16 had no effect on

doi: 10.17221/289/2016-PSE

Table 1. Mean comparison of interaction effect of Sb16 bacterial inoculation, herbicides types and concentrations on physio-morphological parameters of aerobic rice

Herbicide	Dosage	Plant height (cm)		Leaf area (cm^2)		Chlorophyll content (SPAD Units)		Plant nitrogen content (%)	
		NI	I	NI	I	NI	I	NI	I
Paraquat	0	71.47^{a}	71.57^{a}	150.48^{b}	177.67^{a}	43.9^{ab}	44.57^{a}	2.24^{ab}	2.49^{a}
	1/2X	71.37^{a}	71.53^{a}	152.35^{b}	175.28^{a}	43.8^{ab}	44.33^{a}	2.14^{b}	2.47^{a}
	X	70.73^{ab}	70.97^{a}	150.15^{b}	161.04^{ab}	43.37^{ab}	43.6^{ab}	2.09^{bc}	2.32^{ab}
	2X	70.5^{ab}	70.63^{ab}	147.69^{b}	161.69^{ab}	42.03^{b}	43.3^{ab}	1.89^{c}	2.21^{ab}
Pretilachlor	0	71.47^{a}	71.57^{a}	153.37^{b}	180.02^{a}	43.6^{ab}	44.17^{a}	2.21^{ab}	2.54^{a}
	1/2X	71.13^{a}	71.4^{a}	150.19^{b}	182.55^{a}	43.63^{ab}	44^{ab}	2.16^{b}	2.41^{ab}
	X	71.07^{a}	70.93^{a}	150.08^{b}	174.02^{a}	43.17^{ab}	43.67^{ab}	2.11^{bc}	2.4^{ab}
	2X	70.87^{ab}	71.1^{a}	144.55^{b}	151.07^{b}	43.13^{ab}	43.4^{ab}	1.99^{bc}	2.13^{b}
2,4-D	0	71.57^{a}	71.67^{a}	149.63^{b}	180.73^{a}	43.97^{ab}	44.13^{a}	2.25^{ab}	2.54^{a}
	1/2X	71.3^{a}	71.5^{a}	151.79^{b}	178.67^{a}	43.8^{ab}	44.17^{a}	2.2^{b}	2.53^{a}
	X	70.47^{ab}	71.27^{a}	151.01^{b}	168.51^{ab}	43.43^{ab}	43.63^{ab}	2.02^{bc}	2.42^{ab}
	2X	68^{b}	71.17^{a}	145.56^{b}	163.74^{ab}	43.4^{ab}	43.37^{ab}	2.08^{bc}	2.28^{ab}

NI – non-inoculated; I – inoculated; 1/2X – half dose; X – full dose; 2X – double dose. No significant difference among means with the same letters in each two column

root dry mass of aerobic rice (Table 2). Inoculation resulted in a significant increase (P = 0.0083) in root length of aerobic rice in sample treated with double dose of 2,4-D (Table 2). There was a significant

Table 2. Mean comparison of interaction effect of Sb16 bacterial inoculation, herbicides types and concentrations on root morphological parameters of aerobic rice

Herbicide	Dosage	Root dry mass (g/plant)		Root length (cm)		Root volume (cm^3)		Root average diameter (mm)	
		NI	I	NI	I	NI	I	NI	I
Paraquat	0	1.153^{a}	1.158^{a}	1399.45^{ab}	1455.83^{a}	14.19^{b}	15.78^{a}	1.179^{ab}	1.224^{a}
	1/2X	1.152^{a}	1.156^{a}	1408.52^{ab}	1432.53^{a}	14.03^{b}	14.64^{ab}	1.132^{ab}	1.181^{ab}
	X	1.146^{a}	1.15^{a}	1372.26^{ab}	1372.73^{ab}	12.67^{bc}	13.76^{b}	1.117^{b}	1.156^{ab}
	2X	1.141^{a}	1.15^{a}	1334.02^{bc}	1369.51^{ab}	12.16^{c}	12.34^{c}	1.122^{ab}	1.131^{ab}
Pretilachlor	0	1.154^{a}	1.157^{a}	1391.88^{ab}	1442.2^{a}	15.18^{a}	15.37^{a}	1.178^{ab}	1.187^{ab}
	1/2X	1.154^{a}	1.153^{a}	1400.34^{ab}	1407.09^{ab}	13.52^{b}	15.11^{a}	1.161^{ab}	1.198^{a}
	X	1.147^{a}	1.152^{a}	1386.82^{ab}	1387.34^{ab}	13.17^{bc}	13.47^{b}	1.111^{b}	1.131^{ab}
	2X	1.145^{a}	1.149^{a}	1338.28^{bc}	1363.68^{b}	12.19^{c}	13.28^{bc}	1.091^{b}	1.112^{b}
2,4-D	0	1.153^{a}	1.155^{a}	1407.7^{ab}	1426.61^{a}	13.88^{b}	15.84^{a}	1.181^{ab}	1.259^{a}
	1/2X	1.153^{a}	1.157^{a}	1397.16^{ab}	1428.49^{a}	13.52^{b}	15.57^{a}	1.151^{ab}	1.252^{a}
	X	1.147^{a}	1.152^{a}	1320.98^{bc}	1357.03^{b}	12.83^{bc}	13.32^{bc}	1.116^{b}	1.169^{ab}
	2X	1.145^{a}	1.146^{a}	1287^{c}	1350.85^{b}	12.1^{c}	12.87^{bc}	1.111^{b}	1.15^{ab}

NI – non-inoculated; I – inoculated; 1/2X – half dose; X – full dose; 2X – double dose. No significant difference among means with the same letters in each two column

doi: 10.17221/289/2016-PSE

inoculation × concentrations interaction on root length in 2,4-D-treated samples. Inoculation had a significantly positive effect on root volume of aerobic rice in samples treated with half dose of pretilachlor and 2,4-D (P = 0.001 and P = 0.0005, respectively) (Table 2). The interaction of inoculation × concentrations had significant effects on root volume. The root average diameter of aerobic rice varied significantly (P < 0.0001) between the inoculated samples treated with double and half dose of pretilachlor (Table 2). Improvements in the N content, plant growth and root development assert the ability of Sb16 strain to fix N_2 and produce phytohormones such as indole-3-acetic acid (IAA), which is related to improve root proliferation (Spaepen et al. 2007). Enhanced development of the root system leads to plant mineral uptake and indirectly stimulates plant growth (Lambrecht et al. 2000). The influence of the herbicides on plant growth in the present study is possibly due to disruption of certain metabolic pathways such as photosynthesis (paraquat), cell division and growth (pretilachlor and 2,4-D). Mishra et al. (2008) suggested that low dose of insecticide probably resulted in an increased cell membrane permeability, nutrient influx to the root cells and their following transition to leaf and shoot. This finding can justify the improved growth performance of plant and endophytic bacterial counts as a consequence of the application of lower dose of herbicides in the present study. Although the application of higher dose of herbicides resulted in a significant reduction in endophytic bacterial populations and growth of aerobic rice, the reductions were minimized in inoculated compared to non-inoculated samples. The detrimental effect caused by the pesticides might be neutralised by rhizobacteria via biodegradation (Yang and Lee 2008) or enzymatic hydrolysis (Herman et al. 2005).

The present study demonstrates the inhibitory effects of herbicides on the growth and endophytic bacterial populations of aerobic rice at the higher dose, but resulted in a stimulatory effect on aerobic rice at the lower dose. Meanwhile, inoculation with Sb16 strain protected the plant against detrimental effects of herbicides. Nonetheless, the study calls for finding more beneficial endophytic strains that are promising in minimising deleterious effects, as well as the costs of chemical inputs and pollutants, and providing high quality and productivity of crops.

REFERENCES

Ahemad M., Khan M.S. (2011): Response of greengram [*Vigna radiata* (L.) Wilczek] grown in herbicide-amended soil to inoculation with *Bradyrhizobium* sp. (vigna) MRM6. Journal of Agricultural Science and Technology, 13: 1209–1222.

Anwar P., Juraimi A.S., Man A., Puteh A., Selamat A., Begum M. (2010): Weed suppressive ability of rice (*Oryza sativa* L.) germplasm under aerobic soil conditions. Australian Journal of Crop Science, 4: 706–717.

Anwar M.P., Juraimi A.S., Puteh A., Man A., Rahman M.M. (2012): Efficacy, phytotoxicity and economics of different herbicides in aerobic rice. Acta Agriculturae Scandinavica, Section B – Soil and Plant Science, 62: 604–615.

Bashan Y., Holguin G. (1997): Azospirillum – Plant relationships: Environmental and physiological advances (1990–1996). Canadian Journal of Microbiology, 43: 103–121.

Bremner J.M. (1996): Nitrogen – Total. In: Sparks D.L., Page A.L., Helmke P.A., Loeppert R.H., Soltanpour P.N., Tabatabai M.A., Johnston C.T., Sumner M.E. (eds): Methods of Soil Analysis. Part 3 – Chemical Methods. Soil Science Society of America. Madison, American Society of Agronomy, 1085–1121.

Chauhan B.S., Johnson D.E. (2011): Growth response of direct-seeded rice to oxadiazon and bispyribac-sodium in aerobic and saturated soils. Weed Science, 59: 119–122.

Denton M., Kerr K.G. (1998): Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. Clinical Microbiology Reviews, 11: 57–80.

Gyaneshwar P., James E.K., Mathan N., Reddy P.M., Reinhold-Hurek B., Ladha J.K. (2001): Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. Journal of Bacteriology, 183: 2634–2645.

Herman P.L., Behrens M., Chakraborty S., Chrastil B.M., Barycki J., Weeks D.P. (2005): A three-component dicamba O-demethylase from *Pseudomonas maltiphilia*, strain DI-6: Gene isolation, characterization, and heterologous expression. The Journal of Biological Chemistry, 280: 24759–24767.

Idris H.A., Labuschagne N., Korsten L. (2007): Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia. Biological Control, 40: 97–106.

Islam M.R., Madhaiyan M., Deka Boruah H.P., Yim W., Lee G., Saravanan V.S., Fu Q., Hu H., Sa T. (2009): Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. The Journal of Microbiology and Biotechnology, 19: 1213–1222.

Kucharski J., Baćmaga M., Wyszkowska J. (2009): Effect of herbicides on the course of ammonification in soil. Journal of Elementology, 14: 477–487.

Lambert B., Joos H. (1989): Fundamental aspects of rhizobacterial plant growth promotion research. Trends in Biotechnology, 7: 215–219.

doi: 10.17221/289/2016-PSE

Lambrecht M., Okon Y., Broek A.V., Vanderleyden J. (2000): Indole-3-acetic acid: A reciprocal signalling molecule in bacteria-plant interactions. Trends in Microbiology, 8: 298–300.

Lloyd J., Farquhar G.D. (1996): The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. Functional Ecology, 10: 4–32.

Mishra V., Srivastava G., Prasad S.M., Abraham G. (2008): Growth, photosynthetic pigments and photosynthetic activity during seedling stage of cowpea (*Vigna unguiculata*) in response to UV-B and dimethoate. Pesticide Biochemistry and Physiology, 92: 30–37.

Naher U.A., Radziah O., Halimi M.S., Shamsuddin Z.H., Mohd Razi I. (2008): Effect of inoculation on root exudates carbon sugar and amino acids production of different rice varieties. Research Journal of Microbiology, 3: 580–587.

Palleroni N.J., Bradbury J.F. (1993): *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. International Journal of Systematic and Evolutionary Microbiology, 43: 606–609.

Park M.S., Kim C.W., Yang J.C., Lee H.S., Shin W.S., Kim S.W., Sa T.M. (2005): Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiological Research, 160: 127–133.

Savant N.K., De Datta S.K. (1982): Nitrogen transformations in wetland rice soils [Includes fertilizer aspects]. Advances in Agronomy, 35: 241–302.

Sheng X.F. (2005): Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biology and Biochemistry, 37: 1918–1922.

Somasegaran P., Hoben H.J. (1994): Handbook for Rhizobia: Methods in Legume Rhizobium Technology. 1st Ed. Heidelberg, Springer-Verlag.

Spaepen S., Vanderleyden J., Remans R. (2007): Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, 31: 425–448.

Wang Y.S., Zheng Y.G., Sen Y.C. (2007): Isolation and identification of a novel valienamine-producing bacterium. Journal of Applied Microbiology, 102: 838–844.

Yang C.F., Lee C.M. (2008): Enrichment, isolation, and characterization of 4-chlorophenol-degrading bacterium *Rhizobium* sp. 4-CP-20. Biodegradation, 19: 329–336.

Zaidi A., Khan M.S., Rizvi P.Q. (2005): Effect of herbicides on growth, nodulation and nitrogen content of greengram. Agronomy for Sustainable Development, 25: 497–504.

Received on April 15, 2016
Accepted on October 3, 2016

Corresponding author:

Radziah Othman, Ph.D., Universiti Putra Malaysia, Faculty of Agriculture, Department of Land Management, Serdang, Malaysia; e-mail: radziah@agri.upm.edu.my