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Effects of Plant Associated Bacteria on Growth of Maize and Rice in Leonard Jar Experiments

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Abstract- The research was conducted to identify the strains of plant associated bacteria possessing positive impact on growth of one month old maize and rice. The sterile seedlings were inoculated with bacteria suspension, then they were cultivated in Leonard jars with half-strength Hoagland's nutrient solution, or half-strength Hoagland's nutrient solution without nitrogen. Sterile distilled water was used as a control. Three out of the 7 bacteria strains were evaluated including BD2, BD3 and MT1 showed the best effect on both rice and maize because of increasing the leaf area, the dry root mass and dry shoot mass about from 39 to 166%, 62 - 171%, and 50 - 172%, respectively. These three strains were identified by using the MALDI Biotyper System (Germany) and investigated for their abilities of producing siderophores, and biological antagonism. The identification result indicated that the bacterial strain BD2 was similar to Bacillus pumilus, the other two (MT1 and BD3) were homologous to Bacillus subtilis. All three strains were capable of producing siderophores and inhibiting Gram-negative and Gram-positive indicator bacteria. Specially, the strain BD2 had the best capability of producing siderophores and the strain MT1 had the best capability of inhibiting the growth of indicator bacteria. The results also suggested the possibility of using all three strains coordinately in an inoculant as they did not resist each other.
Keywords- Antagonism, Bacillus pumilus, Bacillus subtilis, Leonard jar, Plant Associated Bacteria, Plant Growth Promoting Bacteria, siderophores.

I. INTRODUCTION

Rice (Oryza sativa L.) and maize (Zea mays L.) are two of the grains used as a main food for some of the world's peoples. Cultivating food crops as well as other crops needs to be based on the principle of sustainable "ecological" agriculture and minimizing negative impacts on the environment and human health. Aiming at this goal, researchers often focus on exploiting the benefits of plant growth-promoting bacteria (including plant-associated bacteria) as inoculants for plants. The bacteria help promote plant growth through good properties such as nitrogen fixation, insoluble inorganic phosphate solubilization, synthesis of phytohormones such as indole-3-acetic acid (IAA), and increase disease resistance [1].

In the last 10 years, there have been many publications on the effects of plant growth promotion of plant-associated bacteria on maize and rice. Test tube and pot experiments have been widely used. Some field-on trials have also been reported. In Thailand, Piromyou et al. (2011) studied the impact of Pseudomonas sp. SUT19 and Brevibacillus sp. SUT 47 on the germination and growth of maize plants grown in Leonard jars. The bacteria inoculation helped increase maize biomass, especially in the period of 5 to 8 weeks of age [2]. In Brazil, 136 isolates of sugarcane were isolated. There were 83 isolates showed abilities of phosphate solubilization, nitrogen fixation, and production of IAA, HCN, ammonia, chitinase, cellulase and pectinase. The seven best trains belonged to genera Klebsiella, Enterobacter and Pantoea have been tested for their abilities to promote plant growth on potted maize plants, the period of 0 - 40 days old. Thereby, 5 trains have been proposed as potential candidates for bio-fertilizer production [3]. The cited documents show that the trend of inoculation plant-associated bacteria right into the host plant or expanding on another plant is quite common. In those studies, Leonard jars and modified Leonard jars were used to grow the plant to help harvest the intact roots.

For experiments on rice, in Japan, two strains of bacteria including Pantoea sp. 18-2 isolated from sweet potato and Enterobacter sp. 35-1 isolated from sugarcane were inoculated into cultivated rice (Oryza sativa cv. Nipponbare) and wild rice (Oryza officinalis) to investigate on the colonization of endophytic bacteria. The results indicated that bacterial population densities in the roots were higher than in the aerial parts of plants, and Pantoea sp. strain 18-2 gave the best association with cultivated rice in terms of colonization and nitrogen fixation [4]. In another research, 576 bacterial strains were isolated from 10 rice cultivars in Korea. There were 12 strains identified as nitrogen fixing bacteria. Among them, 10 were auxin producing strains, 6 were siderophores producing strains, and 4 were phosphate solubilizing trains. Five strains were selected for testing on potted rice, in which strain CB-R05 identified as Bacillus subtilis showed more significant growth effect than the type strain B. subtilis (KACC 17047) [5]. Bacillus species are known as aerobic endospore-forming bacteria (AEFB) belong to the phylum Firmicutes. The characteristics of multi-layer cell wall, capable of forming endosperm, secretion of antibiotics, signaling molecules, and extracellular enzymes help these bacteria survive in adverse conditions of the environment. In addition, Bacillus and relative genera also show the capable of plant growth promoting and antagonism activities (Kumar et al., 2011).

Dini-Andreote et al. (2010) found that 10.2% of the total sequences were obtained in Brazilian sugarcane acres belonging to Firmicutes, of which Bacillus prevailed at 19.7% [7]. Studies also found Bacillus species such as Bacillus megaterium, B. subtilis were common bateria in the root and endogenous tissues of crops as sugarcane, maize grown in the Southeast region of Vietnam, in which there were some strains exhibiting abilities of in vitro plant growth promoting [8], [9].

In previous studies, a collection of bacteria associated with sugarcane and maize which the abilities of nitrogen fixation, inorganic phosphate solubilization, and IAA production were selected and stored in the microbiology laboratory of Saigon University, Ho Chi Minh city, Vietnam. In this sequent study, seven strains of well-characterized bacteria selected from the collection continue to be evaluated for their abilities of plant growth promoting on the host plant as maize, or another plant as rice. This is a significant research step in making a microbial fertilizer with broad host spectrum for food crops. In addition to the function of fertilizer, the determination of the ability of siderophores production and the antibacterial properties of the inoculant also helps to exploit the biological control aspect. The identification for tested strains has also allowed consideration of the biosecurity of the inoculant when implementing field trials later.

II. MATERIALS AND METHODS

2.1 Materials

Seven strains of plant-associated-bacteria have been assessed for their abilities of nitrogen fixation, inorganic phosphate solubilization, and IAA production. Four out of 7 strains were isolated from maize: BD1, BD2, BD3, BT2; and 3 strains were isolated from sugarcane: MT1, MR1, MR2.

Seeds of rice cultivar OM5451 (Cuu Long Delta Rice Research Institute) and maize cultivar (Trang Nong Limited Liability Company) were used in gnotobiotic inoculation experiments. Plant growth cycles of two cultivars OM5451 and TN177 are 90 - 95 days and 60 - 62 days, respectively.

2.2 Activation of bacteria and quantification of nitrogen fixation, phosphate solubilization, and IAA production

The purpose of this experiment was to confirm the in vitro Plant-Growth-Promoting abilities of bacterial strains after a storage period.

Each of seven selected strains was transplanted on LB agar plates [10] by streak method to check again for purity. Subsequently, a pure colony from a streaked agar plate was inoculated into 10 mL of Burk’s broth (for testing nitrogen fixation or IAA production) or NBRIP broth (for testing phosphate-solubilizing), then incubated at 28±2oC and 120 rpm (rounds per minute) to prepare precultured suspension. After 48 hours, 1 mL precultured suspension which had the turbidity adjusted to McFarland Standard 0.5 [11] was transferred to 50 mL of Burk’s N free or NBRIP broth, continuously incubated at 28±2oC and 120 rpm within 8 days for the quantification of nitrogen fixation or IAA production and 20 days for the quantification of phosphate solubilization.

Periodically, 10 mL suspension was collected at 2, 4, 6, 8 DAI (days after inoculation) or at 5, 10, 15, 20 DAI, then centrifuged at 12,000 rpm in 5 minutes to obtain the supernatant for the next colorimetric analysis. Colorimetric procedures were based on the description of Thanh and Tram (2018) [12].

The experiments were completely randomized designs with three replicates. Statistics methods were ANOVA (Analysis of Variance) One Factor and Duncan test at $\alpha=0.05$ by using IBM SPSS Statistics 20.0.

2.3 Evaluation of plant growth promoting of maize and rice treated with plant-associated-bacteria

2.3.1 Seeds sterilization and producing gnotobiotic seedlings

Rice seeds and maize seeds were washed with sterile distilled water for 3 minutes (4 times), shaken in alcohol 70o for 5 minutes and then soaked seeds in NaClO or Ca(ClO)2 10% in the period of 5, or 10, or 15 minutes. After each stage using sanitizing chemical, the seeds were rinsed with distilled water for 2 minutes (4 times).

The sterilized seeds were placed in Petri dishes containing solid LB medium with 0.07% agar. After 2 days of incubation at 25±2oC in dark, the seeds showing infection were removed. The uninfected seeds were cultured in glass jars containing 0.07% agar and 1% sucrose, incubated at 25 ± 2oC, under a 14 hours light/10 hours dark, with the light level approach 1500 lux.

A total of 6 treatments were conducted and repeated 3 times, forming 18 experimental units. Statistical processing was used to select the best sterilization process.

2.3.2 Bacterial suspension preparation and inoculation into seedlings

The bacteria were cultured for 2 days in LB broth. Subsequently, precultured bacterial suspension were transferred into LB broth (20% v/v) and incubated at 28±2oC for 3 days at 120 rpm. The cultured bacteria were then harvested by centrifugation at 5000 rpm for 20 min. Collecting bacterial biomass and adjusting cell density in LB broth

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corresponding to McFarland Equivalence Turbidity Standard 0.5 with a photometer at a wavelength of 600 nm [13]. Approximate cell density of this suspension is about 1x10^8 CFU/mL.
The uninfected seedlings having 2 - 3 roots harvested after 2 - 3 DAS (days after sowing) were soaked into the prepared bacterial suspension about 2 hours and then transplanted into modified Leonard jars [4]. The plants were also incubated in sterile distilled water under the same conditions as the untreated control.

2.3.3 Experimental design
In the bottom, each Leonard jars containing 500 mL one-half strength Hoagland solution [14], or one-half strength Hoagland lacking nitrogen (N-free) solution [15]. Sterile distilled water was used as a control. A mixture of sand and perlite (1:1 v/v) was filled into the upper part and covered with a layer of paraffin sand to limit the infection from outside. The experimental design was completely randomized with four replications. Factor 1 was the individual inoculation of seven bacterial strains, plus one treatment without inoculation. Factor 2 was two forms of nutrient supply: one-half strength Hoagland solution, and one-half strength Hoagland N-free solution, plus one treatment using sterile distilled water alternatively. For each crop, a total of 24 treatments were conducted with 4 replications forming 96 experimental units.
Potted plants are placed in a greenhouse with a natural light and temperature regime within 30 days (about 14 hours of lighting/day, 28±2oC).

2.3.4 Plants harvesting and Data analysis
Data were collected at 30 DAS (Days After Sowing). All maize and rice plants and roots were harvested. The evaluated criteria were included shoot height (height of aerial parts), root length (corresponding to the longest roots), leaf area (surface area of leaves), weight of fresh mass and weight of dry mass of shoot and root.
Statistics methods were ANOVA (Analysis of Variance) Two Factors and Duncan test at α=0.05 by using IBM SPSS Statistics 20.0.

2.4 Identification of the best plant growth promoting bacteria
This experiment was limited among selected bacteria which having the best effects on both maize and rice's growth. These selected strains were cultured on LB for 24 hours, then were identified by Bruker Daltonik Biotyper Classification System (Germany). This method was used to determine the unique proteomic fingerprint of an organism. The characteristic spectrum pattern of this proteomic fingerprint is used to reliably and accurately identify a particular microorganism by matching thousands of reference spectra from microorganism strains.

2.5 Preliminary survey of biological control ability of selected strains
Qualitative test of siderophores production using Chrome Azurol Sulphonate (CAS) solution was based on procedure described by Thanh and Tram (2018) [12].
Testing of antibacterial activities of selected strains against 2 indicator bacteria: Escherichia coli and Staphylococcus aureus (provided by Biotechnology Development and Research Institute, Can Tho University). The procedure was based on "agar disk-diffusion" method, then measured the diameter of the zone of inhibition [16], [17].

2.6 Investigation for antagonism among selected bacteria
The investigation aims to evaluate possible uses of a combination of selected strains in the same composition at a later time.
Detecting antagonism among selected bacteria was carried out by using cross-streak method on LB agar plates. After the incubation at 28 ± 2oC for 48 hours, occurrence of bacteria on the cross-cutting lines helped to assess mutual resistance.

III. RESULTS AND DISCUSSION

3.1 Abilities of nitrogen fixation, phosphate solubilization, and IAA production of bacterial strains after a storage period
All of 7 strains were examined the abilities of nitrogen fixation, inorganic phosphate solubilization, and IAA production by colorimetric method (Figure 1). The average values of 4 times of measurement from 2nd day up to 8th day with 2 days interval were in range from 0.42 to 2.22 mg NH4+/L, and 3.33 to 8.54 mg/L IAA; while the average values of dissolved phosphate contents of 4 times of measurement from 5th day up to 20th day with 5 days interval were in range from 37.60 to 60.47 mg P2O5/L. Particularly, the optimal time-point for nitrogen fixation and IAA production of the majority of bacterial strains was "4 DAI" (Days After Inoculation) while "10 DAI" seemed to

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be the suitable time-point for phosphate solubilization as Lwin et al. (2012) and Taiwo and Ogundiya (2008) had observed [18], [19] (Table 1).

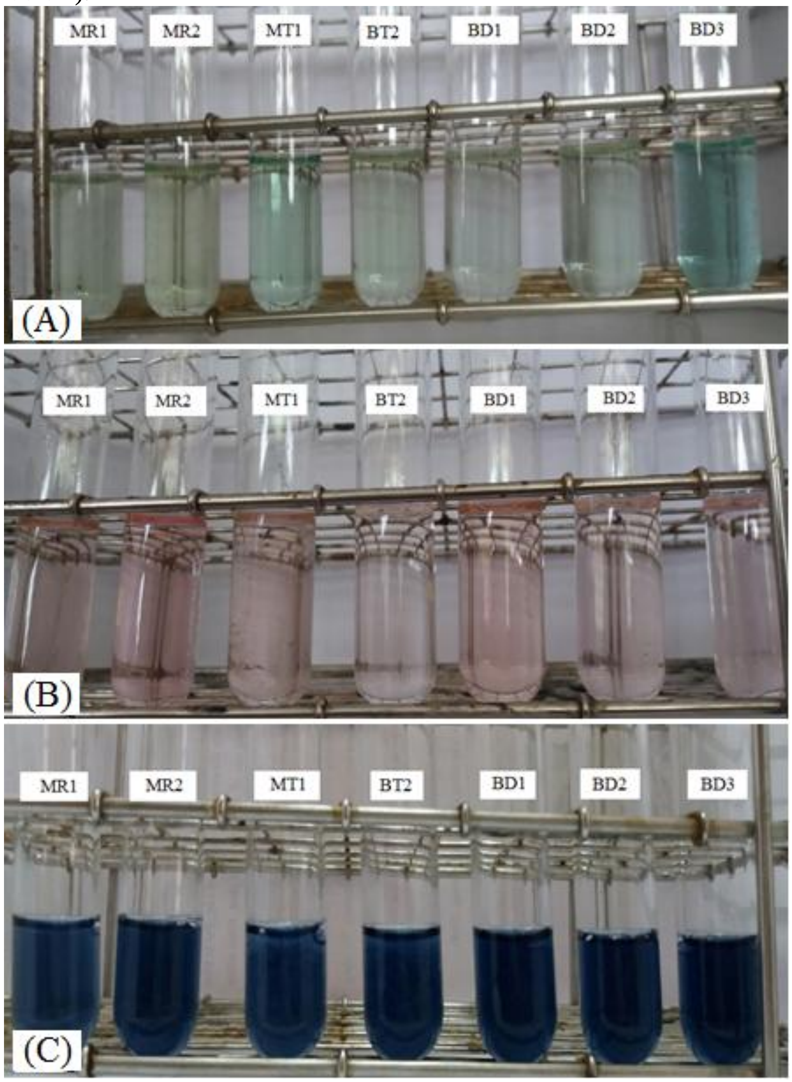

Figure 1. Forming colored complex with the aid of a reagent in colorimetric analysis

to detect ammonia at 4 DAI (A), IAA at 4 DAI (B), and solubilized phosphate at 10 DAI (C)

IAA is the main auxin which controls many important physiological processes of plant. Some soil microorganisms, especially PAB have the ability of producing IAA that allows them to interfere plant's physiology towards their own advantages. Root is the most sensitive organ in term of response to exogenous IAA. Under the impact of IAA, plants elongate the primary root, form lateral roots to increase nutrient absorbance for their growth; in return, they provide root exudates as a source of carbon and energy of rhizospheric microorganisms [20]. So some bacteria strains such as MR1 and BD1 that were capable of producing a lot of IAA would have the advantage in interacting with host plants and have the potential to stimulate growth of plants, especially the roots.

In addition, among of the three indicators that have just been investigated, the bacterial nitrogen fixation is the only indicator that can be evaluated based on growth of plants which were grown in media without nitrogen. In contrast, the phosphate-dissolving ability of bacteria could only be effective on the growth of the plant once the plant is grown in a medium containing a large amount of condensed phosphate. In this study, the nutrient medium for plants containing dissolved minerals so the indicator of "phosphate-dissolving" of 7 bacteria strains only for reference. However, an inoculant containing phosphate-solubilizing bacteria such as these strains will be very useful for plants once fertilized to agricultural soil which has a lot of fixed phosphorus.

Table 1- In vitro Plant Growth Promoting functional characterization of seven strains

Strain	Source	In vitro Plant Growth Promoting Functional Characterization		
		N2-fixation (mg NH4+/L, at 4 DAI)	IAA-production* (mg IAA/L, at 4 DAI)	P-solubilization (mg P2O5/L, at 10 DAI)
MR1	Root of sugarcane	1.10e	8.97c	36.04d
MR2	Root of sugarcane	2.56d	10.67a	64.58a
MT1	Stem of sugarcane	3.44b	9.33c	48.87c
BT2	Stem of maize	1.12e	5.17e	52.86c
BD1	Rhizosphere of maize	1.11e	10.17b	65.79a
BD2	Rhizosphere of maize	3.22c	6.17d	58.50b
BD3	Rhizosphere of maize	4.56a	6.50d	62.91a
CV (%)		0.79	3.22	4.98

Means within a column followed by the same letters are not significantly different at α =0.05 using Duncan test.
* Do not add tryptophan to the culture medium.

3.2 Results of seed sterilization and sterile seedling creation in vitro

This experiment showed that using Ca(OCl)2 helps shorten the sterilizing time more than using NaClO. Ruiza et al. (2011) also proposed the treatment with a mixture of alkali salts including commercial detergent (25% sodium hypochlorite), Na2CO3 (1 g/L), NaCl (30 g/L), NaOH (1.5 g/L) but it takes up to 40 minutes [21]. Some other authors had proposed sterilizing seeds with HgCl2 but this chemical is toxic, needing careful treatment [22].

The best general procedure of sterilization for both of maize seed and rice seed was using Ca(OCl)2 10% for 10 minutes. Rate of aseptic seeds of maize and rice was 96.7% and 94.4% respectively. After transferring the aseptic seed to semi-solid agar medium, rate of germination and forming aseptic seedlings was 96.7% for maize and 97.2% for rice. Thus, the overall effectiveness of seed sterilization and in vitro aseptic seedlings creation was 93.5% for maize, and 91.8% for rice. Besides, sowing the sterilized seeds into a cultural jar with 2 - 3 days helped to select the uniform aseptic seedlings for the experiment of inoculation target bacteria into plants (Figure 2). This experimental step has the effect of enhancing gnotobiotic conditions in in vitro planting experiments as mentioned by Mehnaz (2011) [23].

Figure 2. Sowing the sterilized seeds into a cultural jar help to select uniform aseptic seedlings

3.3 Influence of growing media and plant-associated bacteria on growth of one month year old rice and maize

The results of ANOVA showed the interaction of the two factors “bacteria” and “nutrient supplying solution” on the growth of one month old rice plants as well as maize plants. The one-half strength Hoagland solution had the best impact on most measurement criteria on plants because it provides all of micro and macro elements, including nitrogen (Table 2).

Table 2- Impact of nutrient supply on the growth of one-month-old crops

Crop	Nutrient Supply	Root length (cm)	Shoot height (cm)	Leaf area (cm2)	Root fresh weight (g)	Shoot fresh weight (g)	Dry root weight (mg)	Dry shoot weight (mg)
Rice	Control	5.49a	13.13c	2.26b	0.057a	0.050c	8.50c	10.60c
	Hoagland ½	4.66b	16.63a	2.76a	0.054b	0.077a	10.40a	13.80a
	Hoagland ½ 0N	5.63a	14.78b	1.81c	0.048c	0.060b	9.90b	11.60b
Maize	Control	8.55a	25.44b	11.43c	0.680a	0.710b	96.30c	85.50c
	Hoagland ½	7.33b	32.41a	25.87a	0.496b	0.891a	106.60a	147.20a
	Hoagland ½ 0N	8.75a	21.59c	15.20b	0.415c	0.417c	98.80b	98.20b

For each crop, means within a column followed by the same letters are not significantly different at α =0.05 using Duncan test.
Control: sterile distilled water
Hoagland ½: one-half strength Hoagland solution
Hoagland ½ 0N: one-half strength Hoagland lacking nitrogen solution
Meanwhile, the increase of root length once the plants were grown in a nutrient lacking media such as distilled water could be explained through some author's reports [24]. Similarly, the factor “bacteria” also had a statistically significant effect on the plant growth. The inoculation of bacteria into plants helped them grow better than the control treatments that had not supplemented with bacteria. For the one-month-old rice plants, three strains of BD2, BD1 and MT1 had the best impact on many of examined indicators. Meanwhile, for the 1-month-old maize plants, two strains of BD3, BT2 and BD2 showed better impacts on the growth of this crop (Table 3).

Table 3- Impact of bacterial inoculation on the growth of one-month-old crops (*)

Crop	Nutrient Supply	Root length (cm)	Shoot height (cm)	Leaf area (cm2)	Root fresh weight (g)	Shoot fresh weight (g)	Dry root weight (mg)	Dry shoot weight (mg)
Rice	Control	3.61c	13.08de	2.08e	0.03g	0.04g	4.60h	8.00f
	BD2	6.17a	17.33a	2.90c	0.07a	0.09a	12.50b	15.30a
	BD1	6.29a	17.00ab	2.79c	0.05f	0.07c	13.80a	15.20a
	MT1	3.96c	15.67bc	3.53 a	0.04e	0.08b	7.80g	12.00c
Maize	Control	4.21f	20.83d	9.08h	0.296h	0.38f	56.30g	54.40e
	BT2	10.82a	27.17b	22.68b	0.49e	0.67c	133.80a	153.80a
	BD2	7.17d	27.79b	24.23a	0.67b	0.66cd	133.10a	148.50a
	BD3	9.67b	29.38a	20.54c	0.85a	0.94a	115.50b	147.20a

For each crop, means within a column followed by the same letters are not significantly different at α =0.05 using Duncan test.
(*) The table lists only the best 3 strain for each crop.
Control: sterile distilled water
Because of the interaction of two factors had a statistically significant, the significance of difference in means was tested. The results helped to find the best treatment for each crop as presented in detail as follows.
For root length, fresh and dry root weight
For one month old rice plants, in the growing medium only containing distilled water, the BD2 strain had the best impact on the root length when increasing the length of 79% more than the non-bacteria control treatment. Meanwhile, for maize, MT1 strain had an increase of 112% compared to non-bacteria control treatment. Root length can increase once plants were grown under nutrient lacking conditions. Meanwhile, plants can enhance the growth of the roots to effectively access to nutrients through regulation of hormones, including IAA from plant-associated bacteria. Thus, mass of the root, especially the dry root weight is a better indicator of root growth than root length.
Fresh weight of rice root under the influence of the strain MR1 and Hoagland 1/2 solution had increased by 160% compared to the non-bacteria control treatment; while for maize plants, the two strains BD3 and MR2 could increase 102% and 113%, respectively, in the same of nutrient supply condition. For the dry rice root weight, when this crop

were inoculated with BD1 and grown Hoagland 1/2 solution, this indicator had increased 5 times compared to the non-bacteria control treatment. Especially, the treatment including strain BD2 and Hoagland 1/2 without N also helped the dry weight of rice and maize roots increased 4 - 5 times compared to the control.

For shoot height, fresh and dry shoot height

For plant height, all treatments with bacterial inoculation showed better results than non-bacteria control treatments. In particular, for rice, treatments with BD1 and BD2 helped increase height of 47 - 56% compared to the corresponding control. For maize, four strains BD1, BD2, MT1, and BD3 had increased plant height by 11 - 21% when were grown in the Hoagland 1/2 solution. In particular, the BD3 also helped to increase the height of plant by 58% compared to non-bacteria treatment in condition of planting in distilled water. However, in the fourth week, leaves of plants all turned yellow as nutrition deficiency.

For the weight of fresh rice shoot, two strains BD2 and MT1 gave the best effect when plants were grown in the Hoagland 1/2 solution and increased weight by 105% compared to the non-bacteria treatments. Meanwhile, for maize, the strain BD2 has an average increase 126% and the MT1 line has a 139% increase compared to the non-bacteria control. For dry shoot weight of rice, 3 strains MT1, BD2 and BD1 had the better impacts once helped to increase this indicator about 77%, 100%, and 155% compared to non-bacteria treatments when plants were grown in the Hoagland 1/2 solution. While in maize, BD3 and BD2 contributed an increase 123% and 163% compared with the non-bacteria treatments, respectively.

For leaf area

The study results showed that rice and maize plants treated with bacterial strains and grown in the same type media had better leaf area than non-bacterial controls. The strains had the best impact on rice leaf area are BD1, BT2, BD3, MR2, MT1. Especially, the MT1 increased the leaf area by 69% when the rice were grown in the Hoagland 1/2 without N and increased by 70% when the rice were grown in the Hoagland 1/2 solution. The strain BD3 helped to increase the leaf area of rice by 51% when the crop was planted in the Hoagland 1/2 solution. On the one month old maize plants, meanwhile, three trains MT1, MR1, and BD2 having good impacts on leaf area indicator. Once growing in the nutrient solution Hoagland 1/2, these strains could help increasing the leaf area by 3 - 4 times compared to the non-bacterial controls.

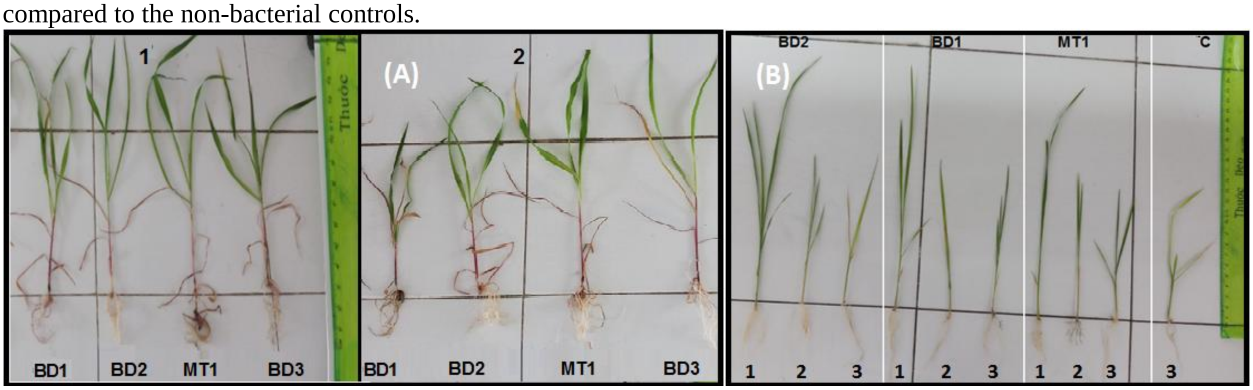

Figure 3. Effect of the best treatment on one month old maize plant (A) and on one month old rice plant (B)
(1): Hoagland ½; (2): Hoagland ½ 0N; (C): sterile distilled water

3.4 Results of selection and identification of plant-associated bacteria strains

The results of statistical analysis show that three strains MT1, BD2 and BD3 had the good impacts on the growth of both maize and rice plants. Hence, they were selected for identification by using MALDI method. In particular, strain BD2 had the best impact on 6 monitoring indicators in rice and 5 monitoring indicators in maize. Two strains BD2 and BD3 were isolated from the rhizospheric soil of maize and the strain MT1 was isolated from stem of sugarcane. This also showed the nonspecificity with the host of plant-associated bacteria, and the potential of supplying them as biofertilizer for a range of crops.

Results of identification showed that the BD2 was similar to Bacillus pumilus. MT1 and BD3 were similar to Bacillus subtilis. These are Gram-positive, endometriosis bacteria, so they are able to withstand and spread well. Bacillus subtilis has been known for its ability to promote plant growth, bioavailability and combined in many commercial preparations due to its high biosecurity. Bacillus pumilus, B. subtilis have proved to be effective alternatives for chemical fertilizer. These bacteria also have abilities to produce IAA, siderophores, control Fusarium fungi and increase the length of root and shoot on many plants including rice and maize [25], [26], [27].

3.5 Ability to produce siderophores and bacteria inhibition

The results of qualitative experiments showed that all strains BD2, BD3 and MT1 had the ability to produce siderophores and inhibited Gram (-) and Gram (+) bacteria indicators. In particular, BD2 strain produced siderophores best and MT1 strain showed the best antibacterial ability with sizes of inhibit zones were 0.2 cm of diameter for E. coli and 0.5 cm for S. aureus (Figure 4).

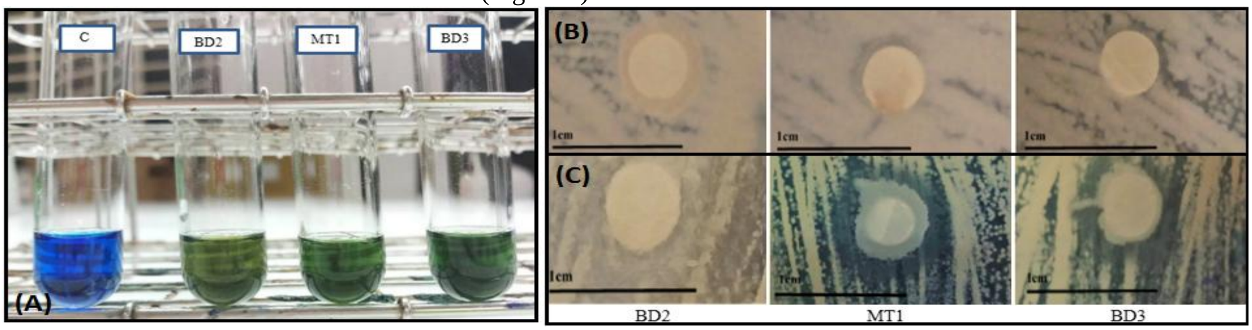

Figure 4. Possibility of siderophores production (A),
E. coli resistance (B), and S. aureus resistance (C) of three selected bacterial strains
C: sterile distilled water used as control

3.6 Antagonistic ability between selected trains

All of selected strains BD2, BD3 and MT1 can be used in combination in a microbial inoculant because they were not antagonistic to each other (Figure 5).

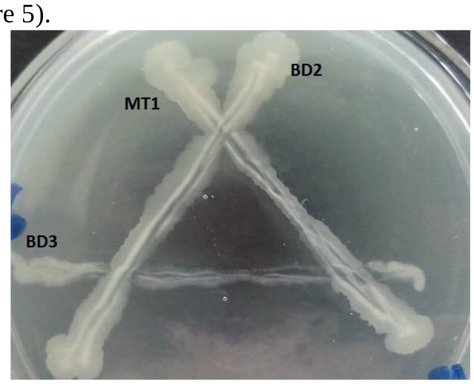

Figure 5. The result of cross-streaking of 3 selected strains

IV. CONCLUSION

Three of the seven plant-associated bacterial strains those have good effects on growth of both maize and rice were Bacillus pumilus BD2, B. subtilis MT1 and B. subtilis BD3. All of BD2, MT1, and BD3 were capable of producing siderophores and resistant to indicator bacteria. All BD2, MT1 and BD3 can be used in a combination in a biofertilizer because they are not antagonistic.

V. ACKNOWLEDGMENTS

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VI. REFERENCE

[1] R. de Souza, A. Ambrosini, L.M.P. Passaglia, “Plant growth-promoting bacteria as inoculants in agricultural soils”, Genet Mol Biol. 38(4): 401–419, 2015.
[2] P. Piromyou, B. Buranabanyat, P. Tantasawat, P. Tittabutr, N. Boonkerd, N. Teaumroong, “Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand”, Eur J Soil Biol, 47(1): 44-54, 2011.
[3] A.A. Rodrigues, M.V. Forzani, R. de S. Soares, S.T. Sibov, J.D.G. Vieira, “Isolation and selection of plant growth-promoting bacteria associated with sugarcane”, Pesq. Agropec. Trop., Goiânia, 46(2): 149-158, 2016.
[4] M. Zakria, K. Udonishi, T. Ogawa, A. Yamamoto, Y. Saeki, S. Akao, “ Influence of inoculation technique on the endophytic colonization of rice by Pantoea sp. isolated from sweet potato and by Enterobacter sp. isolated from sugarcane”, Soil Science and Plant Nutrition, 54(2):224–236, 2008.

[5] S.H. Ji, M.A. Gururani, S-C. Chun, “Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars?”, Microbiological Research, 169(1): 83-98, 2014.

[6] A. Kumar, A. Prakash, B.N. Johri, “Bacillus as PGPR in Crop Ecosystem”, in: D.K. Maheshwari (ed.), Bacteria in Agrobiology: Crop Ecosystems, Springer, Berlin, Heidelberg, pp. 37-59, 2011.

[7] F.Dini-Andreote, F.D. Andreote, R. Costa, R.G. Taketani, J.D. van Elsas, W.L. Araújo, “ Bacterial soil community in a Brazilian sugarcane field”, Plant and Soil, 336(1-2): 337–349, 2010.

[8] D.T.N. Thanh, C.N. Diep, “Isolation, characterization and identification of endophytic bacteria in maize (Zea mays L.) cultivated on acrisols of the Southeast of Vietnam”, American Journal of Life Sciences, 2(4): 224-233, 2014. Published online August 30, 2014, doi: 10.11648/j.ajls.20140204.16.

[9] H.M. Tam, C.N. Diep, “Isolation and identification of rhizospheric bacteria in sugarcane (Saccharum spp. L.) cultivated on acrisols and ferrasols of Dong Nai province, the Southeast of Vietnam”, American Journal of Life Sciences, 3(2): 109-118, 2015. Published online March 25, 2015. doi: 10.11648/j.ajls.20150302.18.

[10] G. Bertani, “Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli”, J. Bact., 62: 293-300, 1951.

[11] E.Matuschek, D.F.J. Brown, and G. Kahlmeter, “Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories”, Clin. Microbiol. Infect., 20(4): 255-266, 2014.

[12] D.T.N. Thanh, DT.T. Tram, “Isolation and characterization of plant growth promoting rhizobacteria in black pepper (Piper nigrum L .) cultivated in Chon Thanh and Loc Ninh districts of Binh Phuoc province, Vietnam”, International Journal of Innovations in Engineering and Technology, Volume 10, Issue 1, Published online April, 2018. http://dx.doi.org/10.21172/ijiet.101.01

[13] E. Matuschek, D.F.J. Brown, and G. Kahlmeter, “Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories”,Clin. Microbiol. Infect., 20(4): 255-266, 2014.

[14] L.A. Spomer, W.L. Berry, T.W.Tibbitts, “Plant culture in solid media”, in R.W. Langhans, T.W.Tibbitts (eds.), Plant growth chamber handbook, Ames, IA: Iowa Agricultural and Home Economics Experiment Station, pp. 105–118, 1997

[15] J. Knoth, S. Kim, G. Ettl, S. Doty, “Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia”, New Phytol, 201: 599–609, 2014..

[16] M. Balouiri, M. Sadiki, S.K. Ibnsouda, “Methods for in vitro evaluating antimicrobial activity: A review”, J Pharm Anal., 6(2): 71–79, 2016.

[17] S. Yasmin, F.Y. Hafeez, M.S. Mirza, M. Rasul, H.M.I. Arshad, M. Zubair, M. Iqbal, “Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by rhizospheric Pseudomonas aeruginosa BRp3”, Front Microbiol., 8: 1895, 2017. Published online 26 Sep, 2017. doi: 10.3389/fmicb.2017.01895

[18] K. M. Lwin, M. M. Myint, T. Tar, W. Z. M. Aung, “Isolation of plant hormone (indole-3-acetic acid - IAA) producing rhizobacteria and study on their effects on maize seedling”, Eng. J., vol. 16, no. 5, pp. 137-144, 2012.

[19] L.B. Taiwo, and M. Ogundiya, “Microbial solubilization of Ogun rock phosphate in the laboratory and in soil”,African Journal of Microbiology Research, 2: 308-312, 2008.

[20] J.H.J. Leveau, and S.E. Lindow, “Utilization of the plant hormone Indole-3-acetic acid for growth by Pseudomonas putida strain 1290”, Applied and Environmental Microbiology, Vol. 71, No. 5, pp. 2365–2371, 2005.

[21] D. Ruiza, B. Agaras, P. de Werrab, L.G. Wall, C. Valverde, “Characterization and screening of plant probiotic traits of bacteria isolated from rice seeds cultivated in Argentina”, The Journal of Microbiology, Volume 49, Issue 6, pp 902–912, 2011.

[22] S.M. Maina, Q. Emongor, K.K. Sharma, S.T. Gichuki, S. Muthoni, M. Gathaara, S.M. de Villiers, “Surface sterilant effect on the regeneration efficiency from cotyledon explants of groundnut (Arachis hypogea L.) varieties adapted to eastern and Southern Africa”, African Journal of Biotechnology, Vol. 9(20), pp. 2866-2871, 2010.

[23] S. Mehnaz, “Plant growth promoting bacteria associated with sugarcane”, in D.K.Maheshwari (ed.), Bacteria in Agrobiology: Crop Ecosystems, Springer-Verlag, Berlin, Germany, pp 165–187, 2011.

[24] D.T.N. Thanh, N.T.X. My, C.N. Diep, “Indole acetic acid andsiderophore production byselected isolates of plant associated bacteria and their effects on growth of maize (Zea mays L.) in pot experiments”, Science Journal of Can Tho University, Vol. 47a, pp. 59-67, 2016 (Vietnamese).

[25] N. Heidarzadeh, S. Baghaee-Ravari, “Application of Bacillus pumilus as a potential biocontrol agent of Fusarium wilt of tomato”, Archives of Phytopathology and Plant Protection, 48(13): 1-9, 2015.

[26] O.A. Almaghrabi, T.S. Abdelmoneim, H.M. Albishri, T. A-M. Moussa, S. Arabialess, “Enhancement of maize growth using some plant growth promoting rhizobacteria (PGPR) under laboratory conditions”, Life Science Journal 11(11):764-772, 2014.

[27] A. Khan, Sirajuddin, X.Q. Zhao, M.T. Javed, K.S. Khan, A. Bano, R.F. Shen, S. Masood, “Bacillus pumilus enhances tolerance in rice (Oryza sativa L.) to combined stresses of NaCl and high boron due to limited uptake of Na+”, Environmental and Experimental Botany, 124: 120-129, 2016.