http://dx.doi.org/10.21172/ijiet.141.04

Effect of P-K solubilizing bacteria and nitrogen chemical fertilizer on Growth, Yield and Nitrate leaf of Brassica juncea L. cultivated on acid sulphate soils

Nguyen Thi Don[1], Cao Ngoc Diep[2]
[1]*Autthor Corresponing, Department of Biology, College of Natural Science, Can Tho University, VIET NAM*
[2]*Department of Microbiology Biotechnology, Can Tho University, VIET NAM*

Abstract- A field study was conducted to determine the effect of P-K solubilizing bacteria and nitrogen chemical fertilizer on growth, yield and nitrate in leaf of Brassica juncea L cultivated on acid sulphate soil together with soil fertility as pH soil, N total and organic matter. Twelve P-K solubilizing bacterial strains plus nitrogen chemical fertilizer (0N, 25N and 50N), bacterial liquid were directly watered into plant at 3 stages [6, 12 and 21 days after planting] during vegetable cultivation, chemical fertilizer (100 N – 80 P2O5 – 40 K2O) and control (no-inoculation). The study revealed that twelve P-K solubilizing bacterial strains are PGPR which they have characteristics as nitrogen fixation, phosphate and potassium solubilization, application of more nitrogen chemical concentration, increasing nitrate concentration of leaf and concentration of N total and organic matter in acid sulphate soil. Application of Acinetobacter calcoaceticus NT30 strain plus 25 kgN/ha chemical fertilizer in Brassica juncea cultivation was the best cultural practice because the this model not only supported the highest biomass, low nitrate in leaf of Brassica juncea but also improved soil fertility as high pH soil, N total soil and organic matter.
Index Terms- acid sulphate soil, biomass yield, Brassica juncea, nitrogen chemical fertilizer, nitrate in leaf, P-K solubilizing bacteria

I. INTRODUCTION

Vegetables are an important component of the human diet because vegetables are rich source of vitamins, proteins, carbohydrates and minerals, which constitute an important component in human nutrition. Besides the nutritional value of vegetables, increased interest is being bestowed on the functional and therapeutic benefits of vegetables in human health. Agriculture is highly dependent on the use of chemical fertilizers, growth regulators, fungicides and pesticides for obtaining increased yield. This dependence is associated with problems such as environmental pollution, health hazards, interruption of natural ecology, nutrient recycling and destruction of biological communities that otherwise support crop production. Vegetables considered to be a high source of nitrate accumulation [1], accounting for 72%–94% of the total nitrate intake of humans [2]. Nitrate by itself is relatively non-toxic; however, it may be endogenously transformed to nitrite, which can react with amines and amides to produce N-nitroso compounds [3]. Analysis of pollution sources of nitrate shows that the heavily polluted regions are usually associated with larger uses of nitrogen fertilizer and household livestock or poultry [4]. The use of bioresources to replace these chemicals is gaining importance. In this context, plant growth promoting rhizobacteria (PGPR) are often considered as novel and potential tool to provide substantial benefits to agriculture. [5]. PGPR are a heterogeneous group of bacteria that can be found in the rhizosphere, which can improve the quality of the plant growth directly and or indirectly [6] as (i) their ability to produce plant growth regulators like indoleacetic acid, gibberellic acid and cytokinins [7], (ii) asymbiotic nitrogen fixation [8], (iii) antagonism against phytopathogenic microorganisms by production of siderophores [9], antibiotics [10] and cyanide [11], (iv) solubilization of mineral phosphates and other nutrients [12] and (v) active removal and bioaccumulation of heavy metals and their capacity to assist the root growth [13].

In addition, PGPR isolates must be rhizospheric competent, able to survive and colonize in the rhizospheric soil [14]. The variability in the performance of PGPR may be due to climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil that may affect their growth and exert their effect on the plant [15].

Different bacteria that have been reported as PGPR belong to the following genera: Pseudomonas, Bacillus, Azospirillum, Agrobacterium, Azotobacter, Arthrobacter, Alcaligenes, Serratia, Rhizobium, Enterobacter,

Burkholderia, Beijerinckia, Klebsiella, Clostridium, Vario vovax, Xanthomonas, and Phyllobacterium (16-17). Plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB), were suggested as a sustainable solution to improve plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors [18-19]. In previous our results [20-21] showed that 3 strains including CA09 (Agrobacterium tumefaciens), CA29 (Rhizobium tropici) and K16B (Azotobacter tropicalis) proposed as potential microbial inoculants or biofertilizers for sustainable crop production (peanut, rice and white-radish) in sandy acid soil in Vietnam because of their benefits and biosafety.

The aim of this study was to evaluate the effect of nitrogen fixation of P-K solubilizing bacteria and nitrogen chemical fertilizer on growth, yield, concentration of nitrate in leaf of Brassica juncea L. (leaf-eating vegetable) together with soil pH, N total and organic matter (after harvesting) in order to select a good modal for vegetable cultivation safety and soil fertility.

II. MATERIALS AND METHODS

2.1. Materials

2.1.1. Soil experiment

Soil experiment is acid sulphate soils (22) with pH and physical and chemical characteristics of arenosols (Table 1). Soil experiment has low pH but high N total, available P and exchangeable K, low organic matter.

Table 1. pH and physical & chemical characteristics of acid sulphate soils (soil experiment)

Characteristics	
pH	4.013
CEC (meq/100g)	10.50
Organic matter (%)	1.265
Available P (mg/kg)	950.01
Exchangeable K (mg/kg)	1951,77
N total (%)	4.217
P total (%)	0.29

Origin: Analysed at Advanced Lad., Can Tho University, Vietnam, 2019

2.1.2. P-K solubilizing bacteria

Twelve P-K bacterial strains were used in this study which they were isolates and selected from weathered materials of Granite Rock Mountain, That Son, An Giang Province, Vietnam, was presented in table 2.

Table 2. Twelve P-K solubilizing strains had ability of solubilization of phosphate and solubilization of potassium in Aleksandrov medium [20]

No	Bacterial name	(mg P2O5 l-1)	(mg K2O l-1)
01	Agrobacterium tumefaciens CA09	23.16 a	42.12 e
02	Bacillus subtilis CA18	12.31 cd	47.61 b
03	Azotobacter tropicalis CA21	14.98 c	43.08 e
04	Rhizobium tropici CA29	15.33 bc	48.32 b
05	Rhizobium tropici D9	14.17 cd	34.09 gh
06	Rhi. leguminosarum DG1	17.59 bc	08.70 q
07	Acinetobacter calcoaceticus NT1	18.02 bc	43.07 e
08	Acinetobacter calcoaceticus NT4	17.15 bc	31.61 k
09	Acinetobacter calcoaceticus NT30	22.67 ab	36.78 g
10	Rhizobium sp Tu09	18.85 bc	30.20 l
11	Rhi. tropici N18	12.45 cd	40.17 f
12	Rhi. leguminosarum K35	11.30 cd	31.06 k
	Control	00.63 e	00.00 r
	C.V (%)	8.50	1.18

Data were recorded at 10 days after incubation, the means of 3 replications Numbers following the same word not difference at 1% level

2.1.3. P-K solubilizing bacteria production

Twelve P-K solubilizing bacteria strains was used in this study which isolated and selected by Don and Diep [20], which proliferated by incubation in flasks containing 1litre in 5 days in broth Aleksandrov medium [23]. P-K solubilizing bacteria liquid reached to108 cells/ml and they already used to experiment.

2.2. Experimental procedures

A field experiment was done for Brassica juncea L. (leaf-eating vegetable), the land for the field experiment was prepared manually. The experiment had three blocks, with each block consisting of five beds, making a total number of fifteen beds, with each bed measuring 1 x 3 m and 0.5 m in between beds, and block size of 17.5 x 4.5 m. The total land area used for each experiment was 78.75 m2. (Figure 1). The seedlings were prepared in the plastic glass (Figure 2) which were planted with one glass per hole at a spacing of 0.60 x 0.60 m. The experimental design was a randomized complete block design. There were 38 treatments: NT1 (control, without fertilizer, P-K solubilizing bacteria), NT2 (100 N - 80 P2O5 – 40 K2O/ha), NT3,4,5 (Agrobacterium tumefaciens CA09 + 0N, 25N and 50N), NT6,7,8 (Bacillus subtilis CA18 + 0N, 25N and 50N), NT9,10,11 (Azotobacter tropicalis CA21 0N, 25N and 50N), NT12,13,14 (Rhizobium tropici CA29 + 0N, 25N and 50N), NT15,16,17 (Rhizobium tropici D9 + 0N, 25N and 50N), NT18,19,20 (Rhizobium leguminosarum DG1 + 0N, 25N and 50N), NT21,22,23 (Acinetobacter calcoaceticus NT1 + 0N, 25N and 50N), NT24, 25, 26 (Acinetobacter calcoaceticus NT4 + 0N, 25N and 50N), NT27,28,29 (Acinetobacter calcoaceticus NT30 + 0N, 25N and 50N), NT30,31,32 (Rhizobium sp. Tu09 + 0N, 25N and 50N), NT33,34,35 (Rhizobium tropici N18 + 0N, 25N and 50N), NT36,37,38 (Rhizobium leguminosarum K35 + 0N, 25N and 50N); from NT3 to NT 38 applied basal fertilizer [80 P2O5 – 40 K2O/ha]. However. the treatments without N fertilizer were supplemented into watering every day, the treatments applied with N chemical fertilizer [urea 46%N] at 3 stages: time 1 (0 day after planting [DAP] [60%], time 2 (6 DAP) [20%] and time 3 (12 DAP) [20%] by N chemical fertilizer dissolved with 1L water/m2, P fertilizer (super phosphate 15% P2O5) was applied at 0 DAP, K fertilizer (KCl 60% K2O) was broadcasted at 0, 6 and 12 DAP with 70%, 15% and 15%, respectively. P-K solubilizing bacteria liquid were applied at 6, 12, and 18 DAP with 0.5 L/m2 (>108 cells/ml) in all the inoculated treatments.

Figure 1. Experimental plot as a bed, land were prepared by manually

Figure 2. Seedlings were prepared to put in a hole

Insecticides did not used in the experiment, weed control by hand and eating-leaf plants were harvested at 24 days-old to measure plant height, leaf number/plant, weight of a plant, biomass yield, nitrate concentration in leaf Plant Determination of nitrate colorimetric method [24], pH soil by pH meter, N total in soil (micro-Kjeldahl method [25] and organic matter content (Walkley - Black method) (after harvesting).

III. RESULTS AND DISCUSSION

3.1. Effect of N chemical fertilizer on plant height, yield component of Brassica junca and soil characteristics

Application of chemical fertilizer increased plant height of three vegetables and plant height was the lowest in the control treatment, using compost also increased plant height, leaf of number/plant, weigh plant and biomass (green yield) of Brassica juncea cultivated on acid sulphate soil together soil N total and organic matter but it reduced soil pH significantly (Table 3).

Table 3**.** Effect of P-K solubilizing bacterial strains and N chemical fertilizer on Plant height, Leaf number/plant, weight plant of *Brassica junca* L. of cultivated on acid sulphate soils together with pH soil, N total and organic matter of soil after harvesting

Treatment	Plant height (cm)	Leaf number/ plant	Weight Plant (gr)	pH soil	N total soil (%)	Organic matter (%)
Initial				4.013 c	4.217 d	1.265 c
Control	6.608 e	6.162 e	5.75 e	4.182 b	4.321 cd	1.296 bc
100 N - 80 P_2O_5 – 40 K_2O/ha	10.314 d	7.105 d	13.54 d	4.280 a	8.096 a	2.428 a
0 N - 80 P_2O_5 – 40 K_2O/ha	10.979 c	7.747 c	20.26 c	4.212 ab	4.288 cd	1.289 c
25 N - 80 P_2O_5 – 40 K_2O/ha	12.006 b	8.082 b	22.96 b	4.143 bc	4.388 bc	1.314 b
50 N - 80 P_2O_5 – 40 K_2O/ha	12.778 b	8.341 a	28.13 a	3.996 bc	4.433 b	1.334 b
Calculated F	**	**	**	**	**	**
C.V (%)	**2.59**	**3.14**	**1.51**	**1.57**	**0.82**	**0.72**

*The numbers followed by the same letter do not differ at 1% level significantly

Table 4. Effect of 12 P-K solubilizing bacterial strains and 100 kg/N on plant height and plant component of *Brassica junca* L cultivated on acid sulphate soils

Treatment	Plant height (cm)	Leaf number /plant	Weight Plant (gr)	pH soil	N total soil (%)	Organic matter (%)
Initial				4.013 d	4.217 ef	1.265 f
No-Inoculated – 0NPK	6.608 g	6.162 e	5.75 l	4.182 c	4.321 d	1.296 d
100 N – no-inoculated	10.314 d	7.105 d	13.54 g	4.280 b	8.096 a	2.428 a
CA09 strain – 0N	10.121 d	7.036 d	15.79 f	4.420 a	4.313 d	1.296 d
CA18 strain – 0N	9.928 de	6.944 d	15.79 f	4.164 c	4.434 c	1.329 c
CA21 strain – 0N	9.364 e	6.884 d	8.96 i	4.310 ab	4.548 b	1.364 b
CA29 strain – 0N	10.097 de	6.761 de	15.62 f	4.387 a	4.461 c	1.344 bc
D9 strain – 0N	8.736 f	6.435 e	10.12 h	4.065 cd	4.228 e	1.285 d
DG1 strain – 0N	8.424 f	6.210 e	6.20 l	4.126 c	4.541 b	1.349 bc
NT1 strain – 0N	11.215 c	8.550 b	24.15 c	4.087 c	4.182 f	1.262 f
NT4 strain – 0N	13.335 a	9.351 a	32.86 b	4.093 c	4.114 f	1.242 f
NT30 strain – 0N	13.455 a	9.815 a	38.07 a	4.266 b	4.428 c	1.327 c
u09 strain – 0N	11.219 c	7.867 c	19.34 e	4.427 a	4.103 f	1.228 fg
N18 strain – 0N	13.542 a	8.631 b	32.72 b	4.077 cd	4.114 f	1.234 fg
K35 strain – 0N	12.306 b	8.482 bc	23.42 d	4.126 c	3.982 f	1.207 g
Calculated F	**	**	**	**	**	**
C.V (%)	**2.59**	**3.14**	**1.51**	**1.57**	**0.82**	**0.72**

Basal fertilizer: 80 P_2O_5 – 40 K_2O/ha

*The numbers followed by the same letter do not differ at 1% level significantly

3.2. Effects of P-K solubilizing bacteria on plant height, yield component of Brassica junca and soil characteristics

Application of 12 P-K solubilizing bacteria strains for vegetable cultivation supported plant height and plant component clearly especially strains as follows: NT4, NT30, NT1, Tu09, N18 and K35. Applied 100 kg N/ha increased plant height and plant component of Brassica junca slightly but N chemical fertilizer induced pH soil, N total and organic matter after harvesting (Table 4). However, several P-K solubilizing bacterial strains not only enhanced plant height, plant component of Brassica junca but also improved soil fertility slightly in comparison to initial as CA29, DG1 and NT30 strains. This showed that the effectiveness of these strains as nitrogen-fixing bacteria strains provided nitrogen as nutrient for plant growth and soil fertility, they can be the potiential strains for biofertilizer production.

Table I: The Arrangement of Channels

Channels	Group 1	Group 2	…	Group c
Main channel	Channel 1	Channel 2	…	Channel c
Assistant channel	Channel 2	Channel 3	…	Channel 1

Control and inoculated strains treatments without nitrogen chemical fertilizer did not increase nitrate concentration in leaf of Brassica juncea while application of 100 kg N/ha without inoculation enhanced nitrate concentration in leaf of Brassica juncea and nitrate concentration exceeded the allowed threshold (>500 mg/kg TCVN7373/2004) (Fig. 3). Inoculation of NT4, NT30 and N18 strains supported the highest biomass of Brassica juncea significantly but nitrate concentration in leaf of Brassica juncea L of these treatments were low, this results demonstrated the these potential

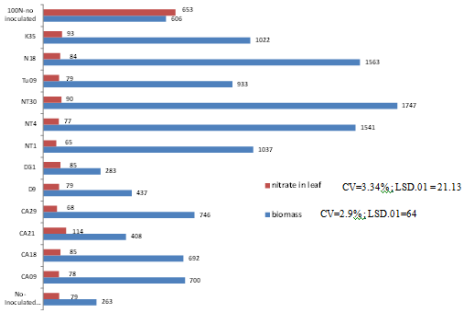

Figure 3. Effect of 12 P-K solubilizing bacterial strains and 100 kg/N on nitrate concentration in leaf of Brassica juncea L. cultivated on acid sulphate soil (biomass: g/m2; nitrate in leaf: mg/kg)

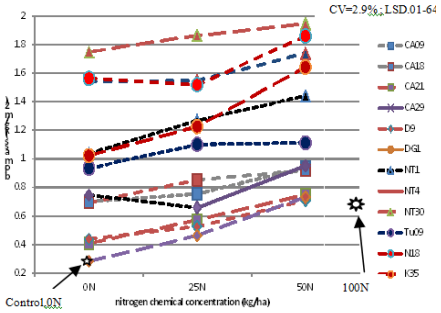

Figure 4. Effect of 12 P-K solubilizing bacterial strains and 100 kg N/ha on biomass of Brassica juncea L. cultivated on acid sulphate soil

In Figure 4 presented the correlation of 12 P-K solubilizing bacterial strains and nitrogen chemical concentration showed that strains as N18, NT30, NT1 had the high biomass at 0N level, increasing 25N and 50N enhanced biomass slightly while control (no-inoculation, O-NPK) treament had 0.263 kg/m2 and 100 kgN/ha treament had 0.606 kg/m2). Based on figure 4, at 25N level, 10 P-K solubilizing bacterial strains had higher biomass than 100 kgN/ha treatment unless 2 strains (D9 and DG1) and at 50N level, all 12 strains had higher biomass than 100 kgN/ha treatment. However increasing nitrogen chemical concentration from 25N up to 50N, enhanced nitrate in leaf on Brassica juncea, especially 3 strains (NT30, N18, K35) had nitrate in leaf exceeded over 500 mg/kg (500 mg nitrate in leaf from QĐ 99/2008/QD-BNN 15/October, 2008 of Ministry of Agriculture and Rural Development, Vietnam) while 100 kgN/ha without inoculation increased the highest nitrate in leaf of Brassica juncea (653 mg/kg) (Figure 5).

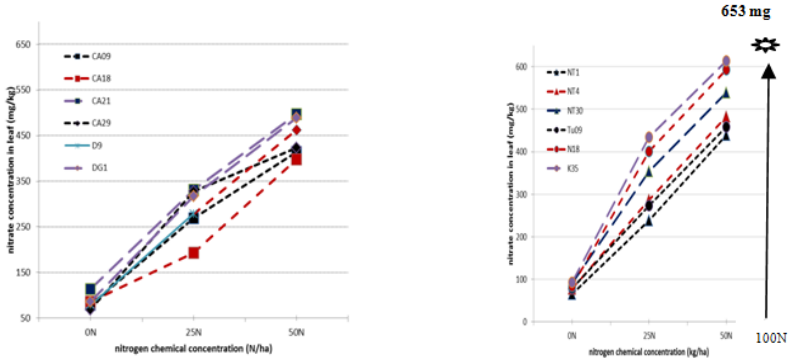

Figure 5. Effect of 12 P-K solubilizing bacterial strains and nitrogen chemical concentration in nitrate in leaf of Brassica junca L. cultivated on acid sulphated soil

Brassica juncea cultivation on acid sulphate soil with and without inoculation and application of nitrogen chemical fertilizer with many different levels showed the variation of soil fertility (at after harvesting) pH soil, N total soil and OM of acid sulphate soil after harvested vegetable increased slightly, this showed that acid soil was improved physical soil as soil structure, aeration…before planting, this work supported to plant growth. Application of 100 kg N/ha to acid sulphate soil increased pH soil, N total and OM, nitrogen concentration as nutrient was moved to vegetable for plant growth and most nitrogen fertilizer fixed in soil and this enhanced N total soil (nearly two folds). Unless two strains (D9 and DG1), 10 P-K solubilizing bacterial strains without nitrogen fertilizer had higher biomass than 100 kg N/ha treatment but the NT30 strain not only had high biomass but also had stable soil fertility through high pH soil, N total soil and OM in comparison to initial (Table 4, Table 5).

From results of Table 4, Figure 5 and Table 5, P-K solubilizing bacterial NT30 strain was the best strain because it not only supported the highest biomass, low nitrate in leaf but also improved soil fertility through pH soil, N total, organic matter after harvesting vegetable for Brassica junca cultivation in acid sulphate soil.

In Table 2 showed that 8/12 strains are strains belonging to Rhizobiaceae or they have nif gene and they fix nitrogen from the air to provide for host plant and themselve. 4/8 strains are Bacillus subtilis (1) and Acinetobacter calcoaceticus (3), these strains demonstrated that they act as nitrogen-fixing bacteria or Promoting Growth Plant Rhizobacteria (PGPR) (unpublished data) and in this study all of them act as nitrogen-fixing bacteria when they fixed and provide biological nitrogen to vegetable (Brassica juncea) at 0N level (Figure 4). Therefore 12 P-K solubilizing bacterial strains are PGPRs because they have characteristics as nitrogen fixation, phosphate and potassiun solubilization and they can use as PGPRs for biofertilizer production for leaf-eating vegetable production in the future.

Table 5. Effect of 12 P-K solubilizing bacterial strains and nitrogen chemical concentration to pH soil, N total soil and organic matter in acid sulphate soil after harvesting *Brassica juncea* L.

No	Treatment	pH soil	N total (%)	OM (%)	No	Treatment	pH soil	N total (%)	OM* (%)
1	Control-0 NPK	4.182	4.321	1.296	25	NT1-0N	4.087	4.182	1.262
2	100N–No Inoculation	**4.280**	**8.096**	**2.428**	26	NT1-25N	4.139	4.434	1.335
3	CA09-0N	4.420	4.313	1.296	27	NT1-50N	**4.213**	**4.232**	**1.274**
4	CA09-25N	4.205	4.466	1.343	28	NT4-0N	4.093	4.114	1.242
5	CA09-50N	4.313	4.564	1.374	29	NT4-25N	4.130	4.444	1.335
6	CA18-0N	4.164	4.434	1.329	30	NT4-50N	4.178	4.117	1.252
7	CA18-25N	4.047	4.314	1.291	31	NT30-0N	4.266	4.428	1.327
8	CA18-50N	4.478	4.630	1.389	32	NT30-25N	4.569	4.636	1.388
9	CA21-0N	4.310	4.548	1.364	33	NT30-50N	**4.475**	**4.712**	**1.408**
10	CA21-25N	4.617	4.116	1.240	36	Tu09-0N	4.427	4.103	1.228
11	CA21-50N	4.408	4.371	1.334	37	Tu09-25N	4.163	4.067	1.222
14	CA29-0N	4.387	4.461	1.344	38	Tu09-50N	3.448	4.300	1.294
15	CA29-25N	4.170	4.979	1.493	39	N18-0N	4.077	4.114	1.234
16	CA29-50N	3.481	4.134	1.243	40	N18-25N	3.416	4.334	1.254
17	D9-0N	4.065	4.228	1.285	41	N18-50N	2.941	4.674	1.390
18	D9-25N	3.749	4.219	1.277	42	K35-0N	4.126	3.982	1.207
19	D9-50N	3.784	4.581	1.378	43	K35-25N	4.252	4.097	1.226
20	DG1-0N	4.126	4.541	1.349	44	K35-50N	4.116	4.121	1.244
21	DG1-25N	4.255	4.554	1.367		**LSD.01**	**0.138**	**0.078**	**0.021**
22	DG1-50N	4.116	4.755	1.425		**C.V (%)**	**1.57**	**0.82**	**0.72**

*OM = organic matter

Plant rhizosphere is known to be the preferred ecological niche for various types of PGPR (Rhizobium, Azotobacter and Azospirillium) due to rich nutrient availability. The three main intrinsic characteristics of PGPR must be ability to: (i) colonize roots, (ii) survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities, and (iii) promote plant growth [26-27].

Plant growth-promoting rhizobacteria (PGPR) benefit plants through different mechanisms of action, including, for example, (i) the production of secondary metabolites such as antibiotics, cyanide, and hormonelike substances; (ii) the production of siderophores; (iii) antagonism to soilborne root pathogens; (iv) phosphate solubilization; and (v) dinitrogen fixation [28].

Plant growth-promoting rhizobacteria (PGPR) benefit plants through different mechanisms of action, including, for example, (i) the production of secondary metabolites such as antibiotics, cyanide, and hormonelike substances; (ii) the production of siderophores; (iii) antagonism to soilborne root pathogens; (iv) phosphate solubilization; and (v) dinitrogen fixation [28]. The establishment in the rhizosphere of organisms possessing one or more of these characteristics is interesting since it may influence plant growth. Chabot et al. [29] used phosphate-solubilizing Rhizobium leguminosarum biovar phaseoli on lettuce and Antoun et al. [28] also used Rhizobium and Bradyrhizobium species on ridishes (Raphanus sativus L.) and they noticed positively from these rhizobia species. Kalita et al. [30] showed that the mixture of PGPRs increased the shoot height, number of leaves, and total biomass content of plants as tomato, chili, cauliflower, brinja after treatment. Kumar et al. [31] recognized that bitter gourd with plant growth promoting rhizobactreia (PGPR) enhanced its growth, yield and quality attributes, especially with Bacillus subtilis.

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Nitrate levels in water and food supplies have been increased during last decades worldwide so far the nitrate pollution has become a global concern which may affect the food quality for daily use and impair the human health [32]. Nitrates are, besides being used as food additives, found in nature as part of the nitrogen cycle, and play an important role during nutrition, growth and development of plants. Because of their cumulative properties, they are an important part of vegetables [33-35]. Besides leafy vegetables that may contain a substantial proportion of nitrate, studies have shown that other types of vegetables such as oilseeds, grains, tubers and nuts also contain nitrate [36]. Absorption of nitrate occurs most often from natural sources, but vegetables accumulate a significant portion of nitrate from nitrogen-based fertilizers, which are used for fertilizing plants for faster and bigger growth [37].

For this reason, nitrate in vegetables has received increasing attention. Leafy vegetables, such as lettuce or spinach, contain the highest concentrations of nitrate [38]. Lan Huong [39] studied on the correlation between nitrogen content in soil and nitrate accumulation of 8 kinds of common vegetablesgrown in Hai Boi commune, Dong Anh district of Hanoi, she regconied the correlation coefficient of nitrate concentration in Brassicaceae Juncea and soil nitrogen is highest in comparison with the studied vegetables. After fertilizer supply, nitrate content in vegetables increased very sharply, the highest accumulation was recorded from the 5th to 11th day after fertilization depending different vegetables (over 500 mg/kg) and decreased gradually after 11th day of manuring. Ministry of Agriculture and Rural Development, Vietnam decided with QD: 99/2008/QĐ-BNN 15/10/2008 limited of nitrate in leaf of vegetable are 500 mg/kg [40]. Therefore vegetables production, especially leaf-eating vegetable cultivation not only supported high biomass and good quality but also improved soil fertility and environmental protection.

IV. CONCLUSION

Twelve P-K solubilizing bacterial strains are PGPR which they have characteristics as nitrogen fixation, phosphate and potassiun solubilization, increasing concentration of nitrogen chemical fertilizer applied to vegetable led to increase nitrate concentration in leaf of Brassica juncea and Acinetobacter calcoaceticus NT30 strain suggest to use in biofertilizer production for leaf-eating vegetable nearly in the future.

V. ACKNOWLEDGEMENTS

The authors thank the helpness of MSc. TRAN DUY PHAT and students of Department of Agricultural Technology, College of Rural Development, Can Tho University, Vietnam.

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