GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Influence of two phosphate-potassium solubilizing bacterial species on biomass and nitrate concentration on mustard greens (*Brassica juncea* (L.) Czernjaew) cultivated on acid sulfate soils

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GSC Biological and Pharmaceutical Sciences, 2021, 16(03), 155–163

Publication history: Received on 17 August 2021; revised on 19 September 2021; accepted on 21 September 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.16.3.0278

Abstract

Two field trials were conducted to determine the effect of P-K solubilizing bacteria and nitrogen chemical fertilizer on growth, yield and nitrate in leaf of mustard greens *Brassica juncea* L.) cultivated on acid sulfate soils. Eight P-K solubilizing bacterial strains composed of 3 strains of *Acinetobacter calcoaceticus* and 5 strains of *Rhizobium* sp., bacterial liquid were directly watered into plant at 3 stages [6, 12 and 21 days after planting] during vegetable cultivation, chemical fertilizer (60N – 40 P_2O_5 – 20 K_2O) and control (no-inoculation). The study revealed that eight P-K solubilizing bacterial strains have good characteristics as nitrogen fixation, phosphate and potassium solubilisation. Application of nitrogen chemical concentration, increasing nitrate concentration of leaf of mustard greens. Application of bacterial liquid with *Acinetobacter calcoaceticus* strain NT4 and *Acinetobacter calcoaceticus* strain NT30 strain, *Rhizobium tropici* strain N18 and *Rhizobium leguminosarum* strain K35 on mustard greens cultivated on acid sulfate soils (basal fertilizer: 40 P_2O_5 – 20 K_2O) was the best cultural practice because the this model not only supported the highest biomass, but also decreased nitrate concentration in leaf of mustard greens.

Keywords: Acid sulfate soil; Biomass yield; Brassica juncea; Nitrogen chemical fertilizer; Nitrate in leaf; P-K solubilizing bacteria

1. Introduction

Vegetables are rich source of vitamins, proteins, carbohydrates and minerals, which constitute an important component in human nutrition. Besides the nutritional value, it is more interested in the therapeutic benefits on human's health. To have high yield of vegetables, the farmers often use high dose of chemical fertilizers, growth stimulants, and pesticides. It will lead to environmental pollution, health hazards, interruption of natural ecology, destruction of nutrient recycling and activities of biological communities. Hence, plant growth promoting rhizobacteria (PGPR) are considered as novel and potential tool to provide substantial benefits to sustainable agriculture [1].

PGPR are a heterogeneous group of bacteria that can be found in the soil rhizosphere, which can improve the quality of the plant growth directly or indirectly [2] and the experiment of Lai Chi Quoc et al. [3] showed that the effectiveness of the phosphate-potassium solubilizing bacterial strains on the growth of vegetables cultivated in the greenhouse and they discovered three strains having the good ability of nitrogen fixation, contributed to yield-biomass of *Allium fistulosum* sp. and *Basella alba* L. Recently, Nguyen Thi Don and Cao Ngoc Diep [4] applied the 12 phosphate and potassium solubilizing bacterial strains into safety vegetable cultivation successfully and limitation of nitrate stimulated in the leaf-eating vegetables in order to prevent chemical pollution and clean foods production.

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The aims of this study (i) study on three phosphate and potassium solubilizing bacteria strains belonged to *Acinetobacte*r *calcoaceticus* and five strains of *Rhizobium* sp…..on mustard greens (*Brassica juncea* (L.) Czernjaew) cultivated on acid sulfate soils and (ii) stimulation of nitrate concentration in leaf of mustard greens.

2. Material and methods

2.1. Materials

2.1.1. Soil

Chemical characteristics of soil used in this study were presented in Table 1. These results were analyzed in Advanced Lab., Can Tho University, Vietnam.

Table 1 Chemical characteristics of acid sulfate soils used in this study

Characteristics	value
pH	4.30
Available P (mg/kg)	157.36
Exchangeable K (mg/kg)	176.30
N total (%)	0.21
P total (%)	0.08
Cation Exchange Capacity (meq/100g)	13.20
Organic matter (%)	1.13

Origin: Soil samples were analyzed at Advanced Lab, Can Tho University

2.1.2. Bacterial strains

Eight strains were chosen to study (from the result of Nguyen Thi Don and Cao Ngoc Diep' experiment)[4] composing of three *Acinetobacer calcoaceticus* strains (NT1, NT4, NT30) and five *Rhizobium* strains (*Rhizobium tropici* CA29, *Rhizobium. leguminosarum* K35, *Rhizobium. leguminosarum* DG1, *Rhizobium* sp. Tu09, *Rhizobium. tropici* N18). All eight strains were isolated from weathering-rock samples from Cam Mountain, An Giang province and selected many times and they are the high potential strains in bio-fertilizer production.

Three *Acinetobacter calcoaceticus* strains NT1, NT4, NT30, and five R*hizobium* sp. strains CA29, DG1, TU9, N18, K35) were each proliferated by incubation in container 120-L with 100 liters water of 10% sugar in 10 days. In this stage, bacterial density reached 10^7 cells/ml and then they already used for inoculating (by mixed bacterial liquid with water and watering for mustard greens) at 6, 12 and 21 days after planting.

2.1.3. Composting procedure

Compost was prepared from rice straw (*Oryza sativa*). The compost added with 0.02% *Trichoderma* spore (Dept. of Plant Protection, College of Agriculture, Can Tho University), incubated by covering plastic membrane; the compost was inverted and watering fortnightly. After 6 weeks, the volume of compost was reduced 50%, compost to keep moisture at 50 – 60%, compost was incubated 4 weeks and compost from the bucket, air dried and later sieved to remove the shaft, shredded and bagged. Compost used in this study with pH and physical and chemical characteristics presented as follows: pH 6.68, Neutral Available N (mg/kg) 134.17, Available P (mg/kg) 950.01, Exchangeable K (mg/kg) 5951.77, N total (%) 2.37, P total (%) 0.29 (Compost sample was Analyzed at Advanced Lab. Can Tho University, Vietnam, 2016) and compost used to plant mustard greens seedlings in the plastic cups before planting the holes of beds.

2.1.4. Chemical fertilizers

- Urea (46% N) from Ca Mau fertilizer factory
- Superphosphate (15% P_2O_5) from Lam Thao fertilizer factory
- KCl (60% K_2O) from Singapore Ltd.

Superphosphate and KCl was mixed and spread evenly 1 day before seedlings put into the holes in every plot unless control treatment; urea (divided 2 times to apply mustard greens) was mixed into water to watering to mustard greens at 4 DAP (50%) and 12 DAP (50%).

2.2. Experimental procedures

Experiments were conducted in greenhouse conditions with four blocks, consisting of nine beds per plot. Each beds was size of 1 x 1 m and the block was size of 13 x 5.5 m. The total land area was 71.5 m^2 (Figure 1). The seedlings were prepared in the plastic glass with one seed per glass (Figure 2). The experimental design was a randomized complete block design with three replications.

Figure 1 Two experiments were conducted in greenhouse

Figure 2 Seedlings in plastic cup

There were five treatments with three strains of *Acinetobacter calcoaceticus* including: NT1 treatment (control, without fertilizer and bacteria), NT2 treatment (recommended fertilizer dose) (60 N- 40 P_2O_5 – 20 K_2O/ha), NT3 treatment (*Acinetobacter calcoaceticusstrain* strain NT1 + 40 P_2O_5 – 20 K_2O/ha), NT4 treatment (*Acinetobacter calcoaceticus* strain NT4 + 40 P_2O_5 – 20 K_2O/ha), NT5 treatment (*Acinetobacter calcoaceticus* strain NT30 + 40 P_2O_5 – 20 K_2O/ha). Besides, the treatments: NT3, NT4 and NT5 were supplemented bacterial liquid into watering at time 1 (6 days after planting [DAP] with 500 ml/m^2, time 2 (12 DAP with 300 ml/m^2, and time 3 (21 DAP) with 200 ml/m^2.

There were seven treatments with five strains of R*hizobium* sp. including R*hizobium* strain CA29, DG1, TU9, N18 and K35. These treatments were similar as above described with the NT1 treatment (control), the recommended fertilizer dose (NT2 treatment) and treatment of each R*hizobium* sp. strain as NT3 treatment (*Rhizobium tropici* strain CA29 + 40 P_2O_5 – 20 K_2O/ha), NT4 treatment (*Rhizobium. leguminosarum* strain K35 + 40 P_2O_5 – 20 K_2O/ha), NT5 treatment (*Rhizobium leguminosarum* strain DG1 + 40 P_2O_5 – 20 K_2O/ha), NT6 treatment (*Rhizobium* sp. strain Tu09 + 40 P_2O_5 – 20 K_2O/ha), NT7 treatment (*Rhizobium tropici* strain N18 + 40 P_2O_5 – 20 K_2O/ha)).

Both experiments were conducted from May to August of 2019 at the College of Rural Development, Hoa An, Hau Giang province.

Insecticides did not used in the experiment. Weed was controlled by hand. Leaf plants were harvested at 28 days to measure plant height, leaf length, leaf number/plant, weight of a plant, biomass yield, available ratio and nitrate content in mustard greens leaf.

3. Results and discussion

In general, mustard greens seedlings developed very well on acid sulfate soil in the first day because they were planted in compost (in plastic cup) (Figure 2). Furthermore, their roots only developed from 5 -7 cm depth and roots did not grow into the acid sulfate soil.

3.1. Effects of NPK and bacterial liquid on plant height and yield component of mustard greens

Application of chemical fertilizer (NPK) and two species (phosphate and potassium solubilizing bacteria) increased plant height of two vegetables and plant height was the lowest in the control treatment, especially application of *Acinetobacter calcoaceticus* strain NT4 (NT4 treatment) and *Acinetobacter calcoaceticus* strain NT30 (NT5 treatment) enhanced 4 parameters of yield components of mustard greens while only treatment of *Rhizobium tropici* strain N18 + 40 P_2O_5-20 K_2O (NT6 treatment) increased 4 parameters of yield components of mustard greens therefore *Rhizobium tropici* strain N18 had the good ability of nitrogen fixation (replaced or saved of 60 kg N/ha). Thus this strain provided enough of nutrients for the growth of mustard greens in the short time (4 weeks).

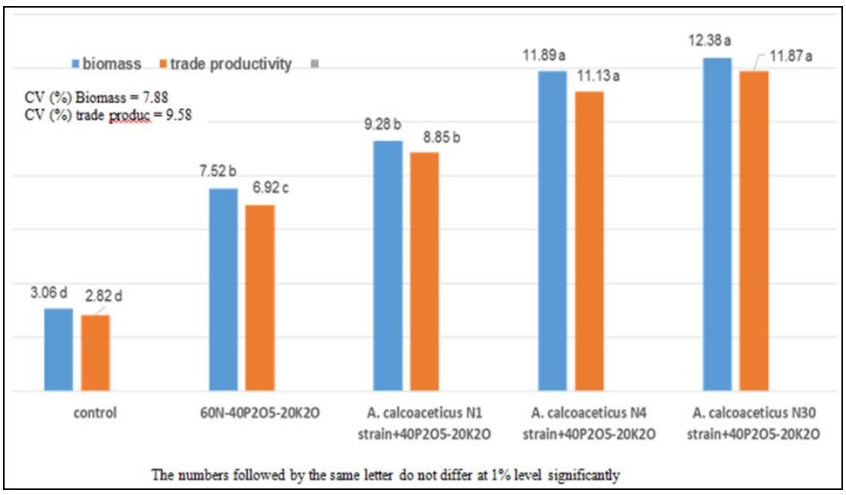
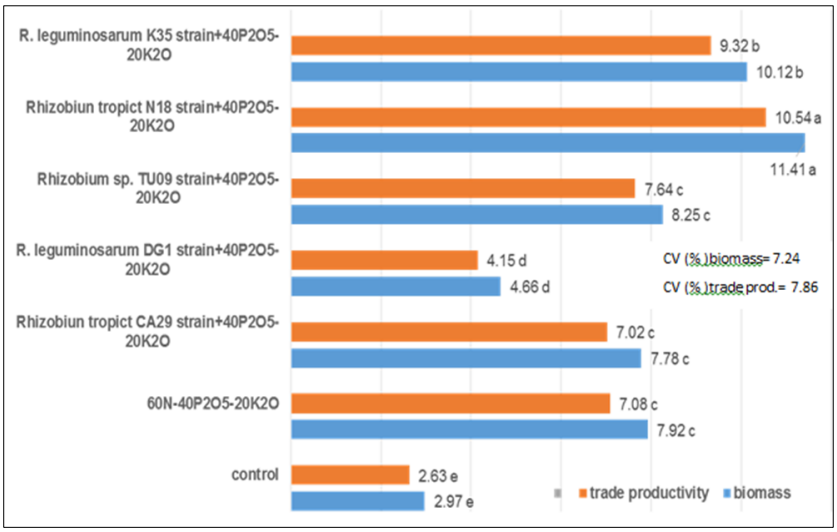


Figure 3 Effects of NPK and 3 strains of *Acinetobacter* sp. on biomass (ton/ha) and trade productivity (ton/ha) of mustard greens cultivated on acid sulfate soils



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

Figure 4 Effects of NPK and 5 strains of *Rhizobi*um sp. on biomass (ton/ha) and trade productivity (ton/ha) on mustard greens cultivated on acid sulfate soils

This result led to biomass and trade productivity of treatment of *Acinetobacter calcoaceticus* strain NT4 + 40P_2O_5 - 20K_2O (treatment NT4) and *Acinetobacter calcoaceticus* strain NT30 + 40P_2O_5 - 20K_2O (treatment NT5) had the highest and differed from other treatments (Figure 3). In experiment with *Rhizobium* sp. as *Rhizobium tropici* strain N18 + 40P_2O_5 - 20K_2O (treatment NT6) had the highest biomass and trade productivity and it differed from others (Figure 4). Therefore, in genus *Acinetobacter* and *Rhizobium* had the strains the famous strains as they can fix nitrogen from the air to provide for the growth of mustard greens beside they dissolve insoluble phosphate and potassium.

Table 2 Effects of NPK and two phosphate and potassium solubilizing bacteria species on the growth components of mustard greens cultivated on acid sulfate soils

Treatment	Plant height (cm)	Number of leafs	Length of leaf (cm)	Width of leaf (cm)
Acinetobacter sp.				
0% NPK	8.86 d	8.80 c	8.51 d	4.03 d
100% NPK (60N-40P_2O_5-20K_2O)	13.59 c	11.22 b	13.17 c	6.21 c
Acinetobacter sp. NT1 + 40P_2O_5-20K_2O	16.61 b	11.23 b	16.22 b	7.42 b
Acinetobacter sp. NT4 + 40P_2O_5-20K_2O	19.68 a	12.06 ab	19.30 a	8.84 a
Acinetobacter sp. NT30 + 40P_2O_5-20K_2O	20.74 a	12.83 a	20.38 a	9.49 a
F	66.64**	21.99**	65.35**	64.65**
CV %	6.55	4.97	6.66	6.52
***Rhizobium* sp.**				
0% NPK	8.29 e	7.13 e	7.81 e	3.45 e
100% NPK (60N-40P_2O_5-20K_2O)	14.43 c	9.38 c	14.00 c	6.37 c
Rhizobium sp. CA29 + 40P_2O_5-20K_2O	15.98 bc	9.23 c	15.30 bc	6.51 c
Rhizobium sp. DG1 + 40P_2O_5-20K_2O	12.37 d	8.30 d	11.84 d	5.50 d
Rhizobium sp. TU9 + 40P_2O_5-20K_2O	16.84 b	9.56 c	16.11 b	6.98 bc
Rhizobium sp. N18 + 40P_2O_5-20K_2O	20.38 a	11.03 a	19.67 a	8.73 a
Rhizobium sp. K35 + 40P_2O_5-20K_2O	17.08 b	10.25 b	16.50 b	7.26 b
F	37.80**	41.22**	35.10**	50.01**
CV %	7.24	4.54	7.65	6.25

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

Yield component of 2 strains (*Acinetobacter calcoaaceticus* and *Rhizobium* sp.) correlated with biomass very closely (**) (Table 2). In both experiments, application of *Acinetobacter calcoaceticus* strain NT1 + 40P_2O_5 - 20K_2O (NT3 treatment) and 60N - 40P_2O_5 - 20K_2O treatment (NT2 treatment) did not differ from biomass but two treatments: NT4 treatment (*Acinetobacter calcoaceticus* strain NT4 + 40P_2O_5 - 20K_2O) and NT5 treatment (*Acinetobacter calcoaceticu* strain NT30 + 40P_2O_5 - 20K_2O) had the highest biomass and differed from others significantly. Two strains of genus *Acinetobacter* (*Acinetobacter calcoaceticus* NT4 and *Acinetobacter calcoaceticus* NT30), therefore strain NT4 and strain NT30 saved 60 kg N/ha or 60 kg N/ha from nitrogen fixation and this amount of nitrogen provided directly to mustard greens through biological nitrogen fixation.

Similarly, application of *Rhizobium tropici* strain CA29 + 40P_2O_5 - 20K_2O (treatment NT3) and *Rhizobium* sp. strain TU09 + 40P_2O_5 - 20K_2O (treatment NT5) and 60N-40P_2O_5-20K_2O (treatment NT2) did not differ from biomass but two treatments (treatment NT7) *Rhizobium tropici* strain N18 + 40P_2O_5 - 20K_2O and *Rhizobium leguminosarum* strain K35 + 40P_2O_5 - 20K_2O (treatment NT5) had the highest biomass and they differed from others significantly. However with treatment of *Rhizobium* sp. strain DG1 + 40P_2O_5 - 20K_2O (NT6 treatment) had biomass lower than 60N-40P_2O_5-20K_2O treatment (NT2 treatment) and its biomass only was higher than control (no fertilizer, no bacteria).

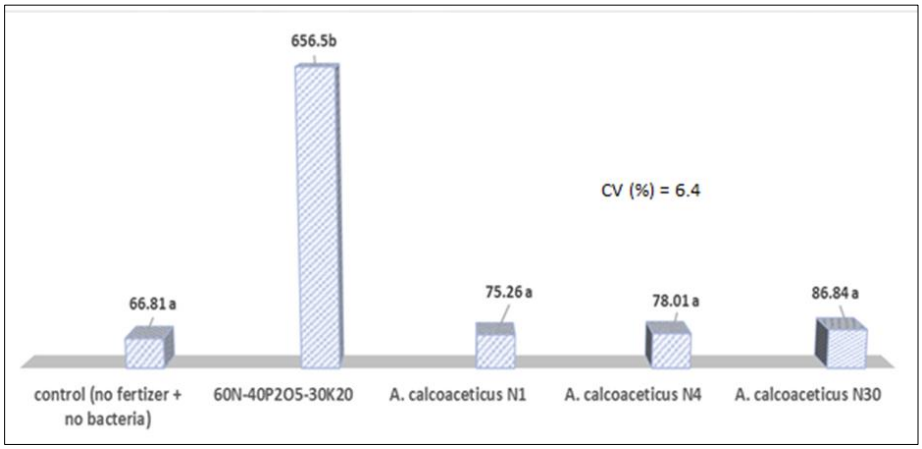
Table 3 The relationship between yield components and biomass of mustard greens cultivated on acid sulfate soils

Acinetobacter calcoaceticus			
Yield components	**Regression equation**	**regression coefficient**	
Plant height	y = 0.7717x - 3.4433	R^2 = 0.9481	r = 0.973**
Number of leaf	y = 2.2007x - 15.892	R^2 = 0.8546	r = 0.924**
Length of leaf	y = 0.7702x - 3.1286	R^2 = 0.9423	r = 0.943**
Width of leaf	y = 0.5718x + 2.1529	R^2 = 0.9578	r = 0.978**
***Rhizobium* sp.**			
Plant height	y = 0.6917x - 2.8272	R^2 = 0.8836	r = 0.940**
Number of leaf	y = 2.2355x - 13.136	R^2 = 0.9441	r = 0.971**
Length of leaf	y = 1.2503x + 4.979	R^2 = 0.8896	r = 0.943**
Width of leaf	y = 1.6413x - 2.9186	R^2 = 0.8894	r = 0.943**

Among 5 strains of genus *Rhizobium*, two strains (*Rhizobium tropici* strain N18 and *Rhizobium. leguminosarum* strain K35) were the outstanding strains; they not only saved more 60 kg N/ha or this was amount of nitrogen from nitrogen fixation which *Rhizobium* sp. (*Rhizobium. tropici* strain N18 (treatment NT7) and *Rhizobium. leguminosarum* strain K35 (treatment NT4) fixed nitrogen from the air and transferred amount of nitrogen to mustard greens as biological nitrogen fixation in the short time (more than 3 weeks).

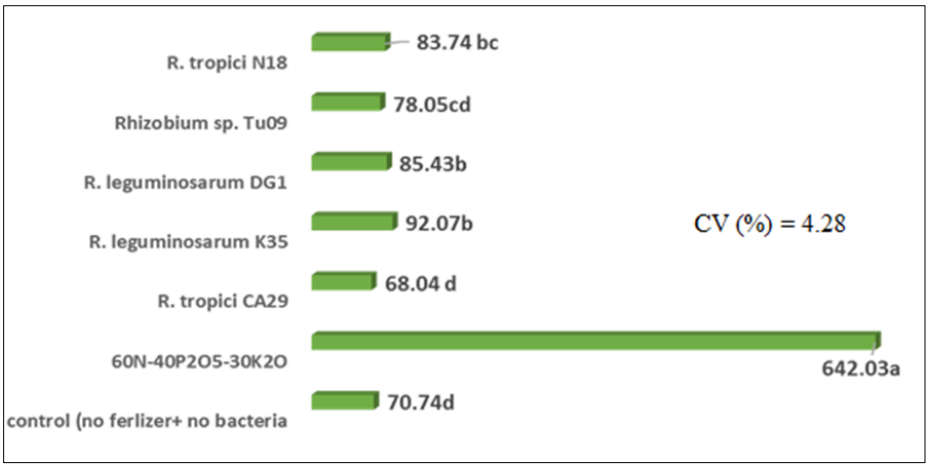
Total of 4 strains (NT4 and NT30; N18 and K35) had the highest biomass of mustard greens, 2 species of *Acintobacter calcoaceticus* demonstrated as two nitrogen-fixing species while 2 species (N18 and K35) are the nitrogen-fixing species.

Both genus (*Acinetobacter* and *Rhizobium* sp.) or these strains as *Acinetobacter calcoaceticus* NT4 and NT30 strains and *Rhizobium tropici* strain N18 and *Rhizobium leguminosarum* strain K35 were strains not only good P-K solubilisation but also strong nitrogen fixation in the short time to provided nutrients (NPK) for the growth of mustard greens cultivated on acid sulfate soils. Besides, application of inorganic nitrogen in mustard greens cultivation enhanced nitrate concentration in leaf with high levels (>650 mg/kg) while treatments of bacterial liquid in mustard greens cultivation did not increase nitrate concentration in leaf (equivalent with control) (Figure 5 and Figure 6).



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

Figure 5 Effects of NPK and 3 strains of *Acinetobacter calcoaceticus*. on nitrate concentration in leaf of mustard greens cultivated on acid sulfate soils



The numbers followed by the same letter do not differ

Figure 6 Effects of NPK and 5 strains of Rhizobium sp. on nitrate concentration in leaf of mustard greens cultivated on acid sulfate soils differ at 1% level significantly)

In 1991, Martinez-Romero and colleagues [5] identified a new species of common bean symbionts: *Rhizobium tropici*. This new rhizobial species was described as aerobic, Gram-negative, with optimal pH for growth ranging between 5 and 7, and characterized by high genetic stability of the symbiotic plasmid and tolerance to tropical environmental stresses such as high temperature and low soil pH; it can nodulate in *Phaseolus vulgaris* (common bean) and some other leguminous species [6] and it was recognized as an effective symbiont of the common bean, *Leucaena leucocephala* and some other leguminous species [7]. *Rhizobium tropici* had good competition with other rhizobia during successive bean cultures and this strain is currently recommended (authorized) for the production of commercial rhizobial inoculant for common bean production in Brazil [8].

The first '*Acinetobacter*' microorganisms were identified in 1911 by the Dutch microbiologist, Beijerinck, who assigned the bacteria the name *Micrococcus calcoaceticus* following their isolation from soil [9]. *Acinetobacter* cells are Gram-negative short rods (coccobacilli), measuring 1.0-1.5 by 1.5-2.5 microns during growth; they often become more coccoid during the stationary phase. The *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex has emerged as a high priority among hospital-acquired pathogens in intensive care units (ICUs), posing a challenge to infection management practices [9]. However, *Acinetobacter* sp and *A. calcoaceticus* play an important role in the fermentation process of the cocoa bean. For example, bacteria from the genus *Acinetobacter* oxidize ethanol, produced to form acetic acid. While acetic acid the acetic acid is formed during the cocoa fermentation, the temperature increases due to biochemical reactions, killing germ and breaking the cell-wall [10].

Kudpeng et al. [11] recognized only *M. caseolyticus* and *Acinetobacter calcoaceticus* were capable of gold bioleaching and the growth supernatant of *M. caseolyticus* and *A. calcoaceticus* can be applied in gold bioleaching. Seventy-six isolates were isolated from 25 rhizosphere soil samples of 13 different leaf- eating vegetables species grown in 6 districts of Can Tho City. Among them, 48 isolates had good characteristics as nitrogen fixation, phosphorus solubility and IAA synthesis. Especially, one of good PGPR was identified as *Acetinetobacter calcoaceticus* [12]. The good P-K solubilizing bacteria isolated in granite-weathered materials from CAM mountain were *Acinetobacter calcoaceticus* [13]. Therefore, among *Aciinetobacter calcoaceticus* isolates influenced to human healthy, there were *Acinetobacter calcoaceticus* isolates as PGPR to bio-fertilizer production for crops cultivation as in the our experiment [4].

Twelve P-K solubilizing bacterial strains without nitrogen fertilizer had higher biomass than 60 kg N/ha treatment but the *Acinetobacter calcoaceticus* NT4 and *Acinetobacter calcoaceticus* NT30 strains not only had high biomass but also had stable soil fertility through high pH soil, N total soil and OM in comparison to initial [4]. Furthermore, *Acinetobacter calcoaceticus* strain NT4 and strain NT30 were the best strains because they not only supported the highest biomass, low nitrate residue in leaf but also improved soil fertility through pH soil, N total, organic matter after harvesting vegetable for mustard greens cultivation in acid sulfate soil. Besides, *Rhizobium tropici* N18 strain together with *Acinetobacter calcoaceticus* NT4 and NT30 strains were the best strains for mustard greens cultivation in acid sulfate soils [4]. In this experiment, these strains (NT4, NT30 and N18 strains) demonstrated the good characteristics as good phosphate-potassium solubilisation and N fixation provided to mustard greens in short time.

However increasing nitrogen chemical concentration from 25N up to 50N, enhanced nitrate in leaf of mustard greens, especially 3 strains (NT30, N18, K35) had nitrate in leaf exceeded over 500 mg/kg [4] but in this experiment, application of bacterial strains without nitrogen fertilizer led to nitrate concentration in leaf of mustard greens as equivalent as control treatment (Figure 5 and Figure 6),, this showed that application of inorganic nitrogen fertilizer for mustard greens cultivation stimulated and enhanced nitrate concentration in leaf of mustard greens, this result also demonstrated by Nguyen Thi Lan Huong [14] when she studied on the relationship of 8 kinds of vegetables with nitrogen fertilizer at Kim Boi market, Ha Noi city. Ministry of Agriculture and Rural Development, Vietnam promulgated with Decision: 99/2008/QĐ-BNN 15/10/2008 limitation of nitrate in leaf of vegetable are 500 mg/kg. Therefore vegetables production, especially leaf-eating vegetable cultivation not only supported high biomass and good quality but also improved soil fertility and environmental protection.

The strains of phosphate-potassium solubilizing bacteria had the characteristics as 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 solubilisation; and (v) dinitrogen fixation [15] when they studied on *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on nonlegumes: on radishes (*Raphanus sativus* L.). With these results, we will select the good strains to produce bio-fertilizer for vegetable cultivation, especially on acid sulfate soils.

4. Conclusion

Four P-K solubilizing bacterial strains (*Acinetobacter calcoaceticus* N4 and NT30) and (*Rhizobioum tropici* N18 and *Rhizobioum leguminosarum* K35) supported the growth of mustard greens (biomass) but nitrate concentration in leaf of mustard greens (*Brassica juncea*) had the lowest; application of nitrogen fertilizer enhanced nitrate concentration in leaf of mustard greens but using these bacterial strains increased not only biomass but also decreased nitrate concentrate in leaf of mustard greens; these strains will be suggested to use in bio-fertilizer production for leaf-eating vegetable nearly in the future.

Compliance with ethical standards

Acknowledgments

The authors thank the helpness of students of Department of Agricultural Technology, College of Rural Development, Can Tho University, Vietnam.

Disclosure of conflict of interest

No conflict of interest.

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