http://dx.doi.org/10.21172/ijiet.101.01

Isolation and Characterization of Plant Growth Promoting Rhizobacteria in Black Pepper (Piper nigrum L.) Cultivated in Chon Thanh and LocNinh Districts of BinhPhuoc Province, Vietnam

Dang Thi Ngoc Thanh[1], Do Thi Thuy Tram[2]
[1,2]*Dept. Natural Science Teacher Training, SaiGon University, HCM City, Vietnam*

Abstract- Rhizospheric bacterial diversity in black pepper plant cultivated in BinhPhuoc province, Vietnam was studied. Rhizosphere samples was collected from 43 pepper farms of this region. Rhizobacteria were isolated in two media Burk's N free and NBRIP. A total of 118 isolates were isolated and all of them have ability of nitrogen fixation and phosphate solubilization. The qualitative test by dropping directly 100 µL the Salkowski reagent on the colonies showed 107 of the 118 isolates have the ability of IAA production. There were 43 isolates had expressed superiority in capabilities of nitrogen fixation, phosphatesolubilization and IAA production in quantitative tests but there were only 17 isolates have the ability of siderophore production in CAS liquid assay. These 17 isolates were identified by using matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) analyzing. Five genera belong to Bacillus (11 isolates), Brevibacillus (1 isolate), Staphylococcus (2 isolates), Acinetobater(2 isolates) and Proteus (1 isolate) were explored. Except Acinetobactergerneri having no information on the possibility of promoting of plant growth, the remaining 10 species are PGPR that have been reported in black pepper and other crops. Especially, diversity of aerobic endospore forming bacteria, viz., species of Bacillus promises the possibility of application for crops under extreme conditions. Hence, these strains should be recommended to test their effectiveness on the growth of black pepper in in vivo experiments.

Keywords – PGPR, MALDI-TOF, black pepper, nitrogen fixation, phosphate solubilization, IAA production, siderophore production

I. INTRODUCTION

Black pepper (Piper nigrum L.) is a major tropical spice and it is the most important spice traded. Black pepper is native to South Asia and Southeast Asia but Vietnam has just gradually increased its influence on pepper international market over the last ten years and becomes the world's leading exporter of this spice. At present, Vietnam exports pepper to more than 100 different countries and provides an important source of income for many farmers in the country. Among six key pepper areas, BinhPhuoc province has the largest pepper area with 13,843 ha and its annual production is about 26,956 tons (Department of Agriculture and Rural Development of BinhPhuoc province, 2016, http://www.binhphuoc.gov.vn). With the advantage of ferrasol soils, LocNinh district is considered as the capital of pepper in BinhPhuoc with over 30% of the pepper area and nearly 40% of the pepper yield of the province. Whereas Chon Thanh is smaller pepper region but the acrisol having poor nutrient accounts over 80% of natural area of this district. To supply pepper according to the demand as well as to sustain the soil fertility and the yield of pepper, farmers are insistently using chemical fertilizers. In contrast, organic and biological approaches are recently favourable in enhancing the growth and productivity of crops including black pepper.

Nowadays, the concept of Plant Growth-Promoting Rhizobacteria (PGPR) were no longer strange. PGPR often carried out as inoculants to promote sustainability and health of pepper plant. These bacteria exhibit plant growth-promoting properties via some direct mechanisms such as biological nitrogen fixation, phosphate solubilisation, and phytohormone production, or indirectly via antagonist. Pseudomonas sp. and Azospirillum sp. isolated from the rhizospheric soil and root cuttings of Piper nigrum L. resulted in significant phosphate solubilization [1]. Greenhouse trials using these PSB showed very clearly the growth promoting activity and field studies also had some promising results. Two strains of rhizobacteriumBacillus cereus UPMLH1 and UPMLH24 were tested on its ability to induce and elongate roots of pepper stem cuttings [2]. Strain UPMLH24 was the most promising inoculant for root induction in pepper stem cuttings. UPMLH1 had been characterised as a N_2-fixing and an IAA producing strain while UPMLH24 had displayed only the former trait [3]. Rhizobacteria of pepper such as Bacillus spp., Pseudomonas spp. were also tested their antifungal activities [4 – 6]. However, little is known aboutrhizospheric bacteria of black pepper cultivated inBinhPhuoc, Vietnam. Furthermore, reports on the plant growth-promoting

characteristics of these bacteria are also scarce. So, the present study was conducted to (i) isolate nitrogen-fixing bacteria and phosphate-solubilizing bacteria, (ii) collect isolates having both of abilities, (iii) screen isolates for production of indole-3-acetic acid (IAA), (iv) select of outstanding isolates and (v) identify of these isolates. These bacteria can be considered promising candidates for sustainable pepper production in this region.

II. MATERIALS AND METHODS

A. Samples collection and preparation

The location of BinhPhuoc province is at 11°45' N latitude and 106°55' E longitude with elevations of between 50m and 200m throughout most of natural area. The pepper rhizosphere samples of 6 months old plants were collected in 43 pepper farms of 2 wards of LocNinh district and 5 wards of Chon Thanh district from August to December of 2016 (Figure 1). The samples of LocNinh were red brown basaltic soils (rhodicferrasols) while the samples of Chon Thanh were gley soils (haplicacrisols). Sample preparation of rhizospheric soil was according to "shaking method" (ID: 13_Turpault) [7]. This method did not allow to collect the rhizoplane.

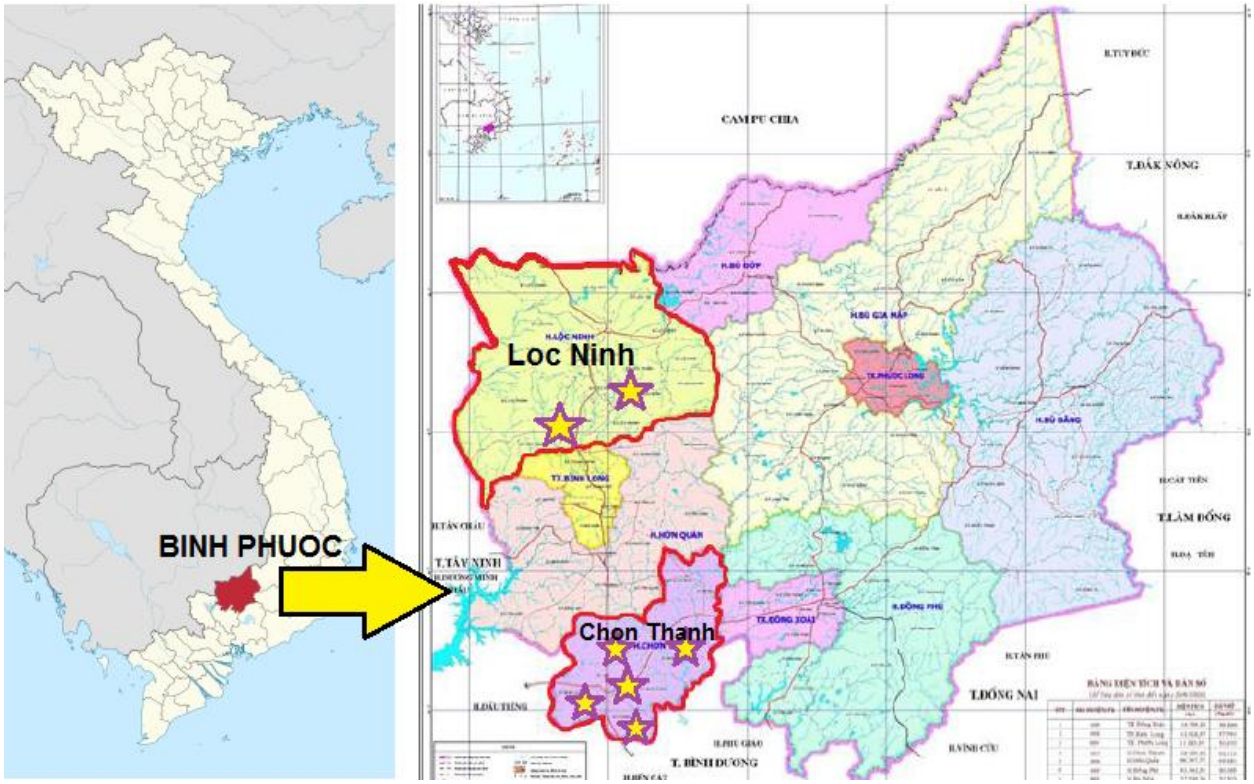

Figure 1. Map of BinhPhuoc province with LocNinh and Chon Thanh districts and places of sampling sites Star shapes used to illustrate wards LocDien and LocThinh of LocNinh district, and wards Chon Thanh, Minh Long, Minh Hung, NhaBich and Thanh Tam of Chon Thanh district.

B. Isolation and collection of nitrogen-fixing and phosphate-solubilizing bacteria

The rhizospheric soil samples were homogenized with dilution factor of 100 in sterilized distilled water containing 0.85% NaCl (w/w). After shaking 120 rpm for 12 hours and depositing for 3 hours, the supernatant was collected and spread on Burk's N free [8] or NBRIP [9] agar plates. Morphologically distinct bacterial colonies were selected for further purifications. Subsequently, the pure isolates would be streaked reciprocally between those two media to collect isolates having two abilities of nitrogen fixation and phosphate solubilization [10]. These isolates were preserved temporarily in 10% glycerol solution at -20°C.

C. Morphological characterization of bacterial isolates

Colony morphology including form, elevation, margin, surface and size were recorded after 24 hours of growth on LB agar plates at 28 ± 2°C. Cellular size, shape and mobile were observed by light microscopy. The Gram reaction

was performed [11]. "KOH String Test" [12] was also used as a complementary method to distinguish between Gram-groups.

D. PGP funtional characterization of bacterial isolates

Quantification of nitrogen fixation and phosphate solubilization

Before quantitative survey, precultuerd bacterial suspension had been prepared with two types of culture media: Burk's N-freeand NBRIP broth for testing phosphate-solubilizing. After 48 hours, 1 mL growing bacterial suspension had been adjusted to the turbidity of a 0.5 McFarland Standard [13] was transferred to 50 mL of Burk's N free or NBRIP solution and incubated at 120 rpm, 28±2^{o}C. Suspension had been periodically collected [2, 4, 6, 8 DAI (days after inoculation) to test nitrogen, and 5, 10, 15, 20 DAI to test phosphorus] then conducted centrifugation process [12,000 rpm (rounds per minute), 5 minutes] to obtain supernatant for next colorimetric.

Colorimetric method was used to estimate the nitrogen fixation or phosphate solubilization of bacteria. For nitrogen fixation, reagent formulas described by Solarzano (1969) were applied [14]. Sample and reagent ratio was 5:1 (v/v). The absorbance of the sample was measured by spectrophotometer with 640 nm wavelength after 30 minutes of reaction stability. Similarly, quantitative survey of phosphate-solubilizing was conducted with the reagent formulas described by Murphy and Riley (1962) [15] and the sample and reagent ratio was also 5:1 (v/v). The OD_{880} was measured after 15 minutes of reaction stability.

Detection and quantification of IAA production

The bacterial isolates were grown in Burk's agar plates after 8 days following inoculation were visually detected their abilities of IAA production by dropping directly 100 µL the Salkowski reagent (formulas described by [16])on their colonies and incubated in dark for 15 min. The appearance of pink to red colors on colonies mean positive results [17]. These isolates so sequently were carried out IAA quantitative survey.

For quantification, periodic sampling occurred each of 2 days within 8 DAI. Colorimetric method was employed with recipe of reagent Fe-H_2SO_4 follow [16], ratio of sample and reagents was also 5:1 (v/v), wavelength was 530 nm, and condition of the reaction stability was 15 mins in darkness.

Detection of siderophore production

This experiment was limited among outstanding isolates (having the best quantitative results of N_2-fixation, phosphate-solubilization, and IAA production). Qualitification of siderophores using CAS (Chrome AzurolSulphonate) solution asssay (formulas according to [18]) was conducted by inoculating one colony of the bacterial isolate in 5 mL of an LB medium and incubated at 28±2^{o}C for 48 hours with agitation (120 rpm). After centrifugating at 12,000 rpm, 5 minutes, 1 mL of supernatant was transferred to fresh tubes, then an equal volume of the CAS reagent was added to each tube, and the mixture incubated in the dark for 30 minutes at 28±2^{o}C. The change in color of the blue dye solution indicating the presence of siderophore.

E. Identifying selected isolates by their molecular fingerprint

This experiment was limited among selected isolates which having the best quantitative results of N_2-fixation, phosphate-solubilization, IAA production, and the positive result of siderophores production. The isolates were cultured on LB for 24 hours then were carried out the BrukerDaltonikMaldiBiotyper Classification (MALDIBiotyper System) according to the protocol recommended by BrukerDaltonik GmbH, Bremen, Germany. This method using MALDI-TOF (Matrix-Assisted Laser Desorption Ionization/Time of Flight) Mass Spectrometry 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.

The result overview includes names of organisms that are the best match and the second best match with their score values. The score indicated is a logarithmic value resulting from the alignment of the peak list of the unknown raw spectrum and the best matching database spectrum. According to the specifications of the manufacturer, a highly probable species identification has a score value lying between 3.000 and 2.300; a secure genus and probable species identification has a score value lying between 2.299 and 2.000; a probable gnus identification has a score value lying between 1.999 and 1.700. A low score of <1.700 was considered unreliable for identification.

F. Experimental design and Data analyses

Quantitative experiments were random assignment (Completely Randomized Design) with three replicates. Negativecontrols were conducted similarly to treatments, did not use bacterial suspension but put on sterilized correspondingmedium instead.

Statistics methods were ANOVA (Analysis of Variance) One Factor and Duncan test at α=0.05 by using SPSS Statistics versions 16.0.

III. RESULTS AND DISCUSSION

A. Bacterial isolation

A total of 118 purified baterial isolates having nitrogen-fixation and phosphate-solubilization abilities was collected from 43 samples of pepper rhizospheric soil. Table 1 below would present the origins and the growth media of those isolates.

Table - 1 Origins and isolation media of 118 isolates

Site (District)	Burk's medium	NBRIP medium	Total
Chon Thanh	31	42	73
LocNinh	19	26	45
Total	50	68	118

This indicated that NBRIP medium seeming to appropriate for the isolation of bacteria that having nitrogen-fixing ability besides the capability of phosphate-solubilizing from rhizospheric soil of pepper. Besides Pikovskaya medium, NBRIP was commonly used in the isolation of phosphate solubilizing bateria from soil or rhizosphere [19] [20]. Using this medium, Thanh and Diep (2012) [21] have also isolated many strains that have good nitrogen fixation potential besides their ability to dissolve inorganic phosphate.

B. Morphological characterization

On isolation media, bacterial isolates are often milky on Burk's medium or yellow and some isolates had haloes around colonies on NBRIP medium (Figure 2) as descriptions of Thanh and Diep (2014) or Tam and Diep (2017) [10] [22].

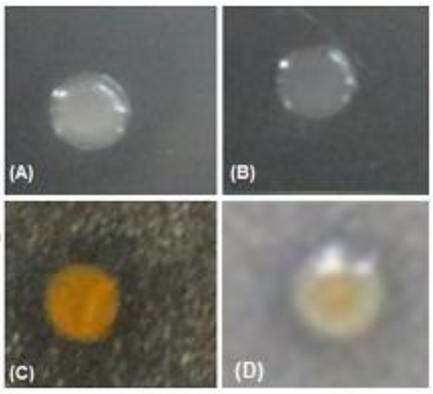

Figure 2. Colony morphology of bacteria on Burk's (A, B) and NBRIP (C, D) media

Whereas on the LB medium, they are often distinguished. Moreover, profile of morphological characteristics of each isolate would support the identification later (Figure 3). After streaking on LB agar plates, 118 isolates of nitrogen-fixing and phosphate-solubilizing rhizobacteria primarily showed some morphological characteristics of colonies including circular (89.8%) and irregular or rhizoid (10.2%), entire (66.1%) and undulate or filamentous (33.9%), convex or pulvinate (79.7%) and raised or flat (20.3%), white color with the transparent or opaque opacity (54.2%) or different colors in the pigmentation such as orange, yellow, or beige. Diameters of colonies were measured ranging from 1 to 4 mm. Through microscopic observation, there were about 68.6% of the total rhizospheric bacteria exhibited their motility and 31.2% of them were non-motile. Rod-shaped (bacillus) was the main shape of 55.1% of the total isolates whilecoccus accounted for 32.2% and coccobacillusaccounted for 12.7% of the total isolates. The results of Gram-staining and "KOH string test" showed that up to 66.1% of the total isolates were Gram-positive and 33.9% of them were Gram-negative. Many Gram-positive bacteria have the ability of endospores forming to survive in harsh conditions of environment. This helps explain the abundance of these bacteria in soil and plant roots. Multiple species of them are known to promote plant growth [23].

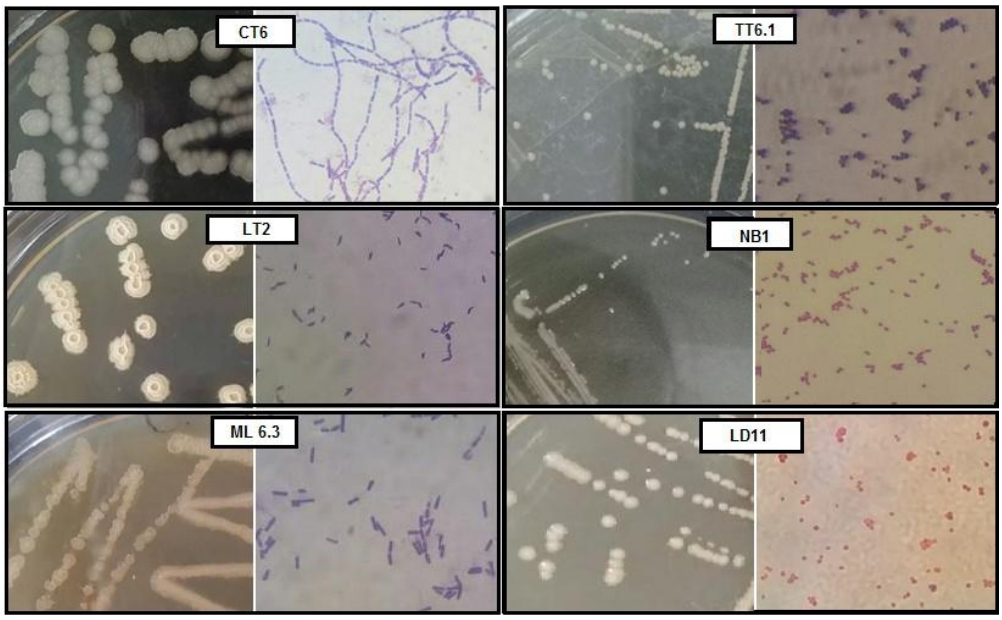

Figure 3. Colony morphology and Gram staining results of some isolates

C. PGP funtional characterization

Abilities of nitrogen-fixing and phosphate-solubilizing

All of 118 isolates were examined the abilities of nitrogen-fixing and phosphate-solubilizing by colorimetric methods and the results were listed in the appendix part of the thesis. The average values of 4 times of measurement at 2-day distance were in range from 0.15 to 5.20 mg NH_4^+/L while the average values of dissolved phosphate contents of 4 times of measurement at 5-day distance were in range from 2.75 to 61.88 mg P_2O_5/L. Particularly, NH_4^+ or dissolved phosphate content generated by each isolate in each measurement exhibiting the variation and there were no general model for all isolates that reflecting the different adaptation of them with growth media. However, "2 DAI" appeared to be the optimal time-point for nitrogen fixation of the majority of bacterial isolates while "5 DAI" seemed to be the suitable time-point for phosphate solubilizationasTaiwoandOgundiya (2008) had reported [24].

Ability of IAA production

The qualitative test showed 107/118 of isolates (90.7%) have the ability of IAA production through the appearance of pink to red colors on colonies appearance of pink to red colors on colonies (Figure 4). However, it is difficult to observe the pink color of the reaction in a pigmented colony (Figure 4C). In quantitative experiment, the average values of IAA contents of 4 times of measurement at 2-day distance in the absence of tryptophan in culture medium (Burk's N free) of 107 isolates was in range of 0.51 to 10.41 mg/L. This was similar to the results of the research of some authors [25] [26]. Phytohormones is often produced by plant associated bacteria. According to Zakharovaet al. (1999), there were 80% of bacteria isolated from rhizosphere were capable to produce auxin such as IAA. Particularly, this quantitative survey results also revealed the different adaptation of the bacteria to the culture media. "2 DAI" appeared to be the optimal time-point for IAA production of most of isolates but the best isolates had the highest quantitative values at 4 DAI. Perhaps so some authors did not consistently survey but stop at "2 DAI" for IAA synthesis test [28]similar to"7 DAI" for quantitative survey of phosphate solubilization[29].

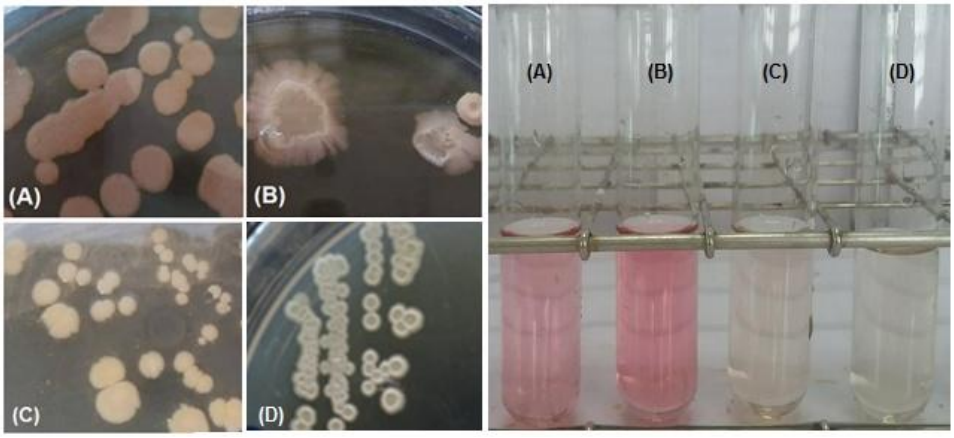

Figure 4. The qualitative (left) and quantitative (right) results of IAA production testsof some isolates using Salkowski reagent at 8 DAI

A- Isolate TT6.1, B- Isolate NB5, C- Isolate LT5: Positive results; D- Isolate LT7: Negative results

Detection of siderophore production

Base on the results of quantitative surveys mentioned above, there were 43 isolates had expressed superiority in capabilities of nitrogen fixation, phophatesolubilization and IAA production. Hence they had been tested siderophore production by using CAS liquid assay. This experiment showed only 17 isolates (39.5%) have the ability (Figure 5). Consequently, identification based on MALDI-TOF technique would be performed on these isolates.

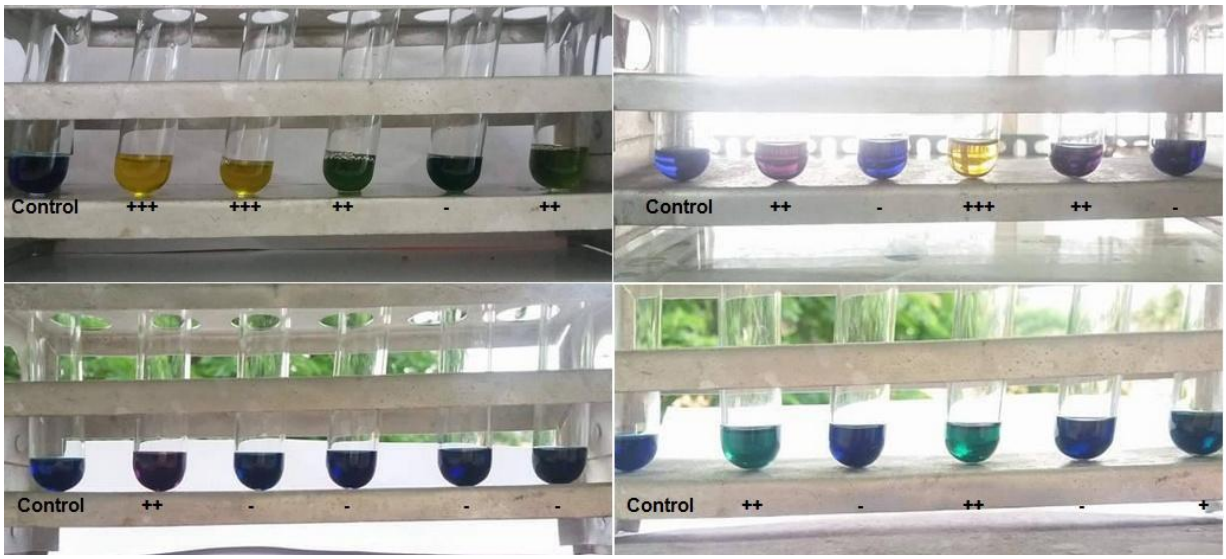

Figure 5. The qualitative results of siderophore production tests of some isolates at 2 DAI using CAS liquid

(-): negative result; (+): positive result;

(++)/(+++): stronger positive results based on the color arrangement illustrated by Nielsen et al. (2012) [30]

D. Identification of selected isolates

There were 17 selected isolates had been identified by MALDI-TOF technique. This approach was used by many authors to identify PGP bacteria associate with plants [31 – 33]. Profiles of morphology of 17 selected isolates had been checked. It showed the suitability between species name and some tested cellular characteristics such as Gram staining, cell shape and motility. Species names and PGP characteristics in vitro of them were presented in Table 2 in which isolate LD8 was identified as Bacillus cereus (the second best match) instead of Acinetobacterbaylyi(the best match) because of Gram-positive that it belongs to.

Table 2- Identification and PGP funtionalcharacteration of 17 selected isolates

Isolate	Daltonik MALDI-TOF Biotyper classification		N_2-fixation	P-solubilization	IAA-production	Siderophore-production [a]
	Best match	Score value	mg NH_4^+/L (at 2 DAI)	mg P_2O_5/L (at 5 DAI)	mg IAA/L (at 2 DAI)	(Qualitative test)
Low GC Firmicutes						
CT1	Bacillus cereus	2.170	7,68 ab	16,78st	6,75 b	+
CT6	Bacillus weihenstephanensis	2.060	8,35 a	19,71 rs	4,86 fghi	++
ML42	Bacillus megaterium	2.113	2,82 ijklmn	48,90 ef	4,94 defghi	++
ML63	Bacillus megaterium	1.977	1,55 jklmnop	59,27 bc	4,77 ghij	+++
NB11	Staphylococcus saprophyticus	2.219	0,97 lmnop	36,95 klm	1,17 p	+++
TT6.1	Staphylococcus warneri	1.817	3,99 ghi	14,31 tu	5,45 cdef	++
TT6.2	Bacillus subtilis	2.007	6,98 abcd	9,16 wx	3,94 l	+
TT6.3	Brevibacillusbrevis	1.902	3,53 hij	67,40 a	4,83 fghi	+
TT7	Bacillus pumilus	1.849	3,28 hijkl	38,73 ijk	5,59 cd	+
LD4	Bacillus megaterium	2.095	4,41 efghi	46,14 fg	4,76 ghij	++
LD5	Bacillus subtilis	2.019	6,11 abcdefg	44,22 gh	4,62 hijk	+
LD8	Bacillus cereus*	2.005*	6,59 abcdef	58,53 bc	5,22 cdefgh	+++
LT4	Bacillus megaterium	2.315	3,03 ijklm	52,01 de	5,61 c	++
LT2	Bacillus subtilis	2.125	4,39 efghi	11,71 uvw	6,67 b	+++
Gammaproteobacteria						
NB1	Acinetobacterbaylyi	2.170	0,99 lmnop	66,45 a	2,16 n	++
TT6.4	Acinetobactergerneri	2.209	0,46 nop	52,86 d	3,33 m	+
LD11	Proteus mirabilis	2.342	8,16 a	14,44 tu	5,06 cdefgh	+

Means within a column followed by the same letters are not significantly different at α =0.05 using Duncan test.
a: (+) positive result; (++)/(+++) stronger positive results based on the illustration of Nielsen et al.(2012) [30].
*: the result presented is the second best match.
CT, ML, TT, NB: Symbols of strains originating from Chon Thanh.
LD, LT: Symbols of strains originating from LocNinh.

Table 2 showed that the abilities of nitrogen-fixing and phosphate-solubilizing of strains isolated from pepper grown in Chon Thanh seem to be better than the abilities of strains isolated from pepper rhizosphere of LocNinh. As mentioned above, soils for pepper cultivation in Chon Thanh are acrisols while soils for pepper grown in LocNinh are ferrasols. The texture of two types of soil are sandy clay with clay of the surface accounting 50 – 60%. Both of soil types are slightly acidic in nature but chemical fertility of ferrasols is higher than acrisols [34]. Tam and Diep (2015) also conducted the isolation of nitrogen-fixing and phosphate-solubilizing bacteria from the rhizosphere of sugarcane cultivated on Dong Nai province, Vietnam. The best selected strain P14 was also isolated from gray soil [35]. These results support the theory of using different environments with stringent conditions to find out potential PGPB strains [36].

In term of classification, among 17 identified isolates, there were 14 isolates including 3 genera belong to low GC Firmicutes, 3 isolates including 2 genera belong to Gammaproteobacteria (Figure 6). A diversity of Bacillus spp. were discovered including 11 strains identified as Bacillus cereus, B. weihenstephanensis, B.megaterium, B.subtilis, and B. pumilus. In the research of Aravind et al. (2009), among 74 strains of endophytic bacteria were isolated from root and stem tissues of black pepper, there were 22 strains were identified as Bacillus spp. in which B. megaterium IISRBP 17 were found promising for suppression of P. capsici [37]. B. cereus, B. megaterium were isolated from

rhizosphere of pepper also exposed plant growth-promoting properties such as nitrogen fixation, phosphate solubilisation, phytohormone production and the inhibition of antagonisticactivity of harmful biological agents in the soil [3] [6] [2]. Meanwhile, Bacillus weihenstephanensis were reported as metal-resistant plant-growth promoting bacteria [38] and B. pumilus were considered as plant growth-promoting bacteria to enhance establishment of plants in mine tailings [39] or in acclimatization stage of Grande naine banana (Musa AAA) [40]. Three species of Low GC Firmicutes including Brevibacillusbrevis, Staphylococcus warneri,Staphylococcus saprophyticuswere potential plant growth promoting rhizobacteria of cotton, maize, rice, swainsonpea, cashew, and had abilities of nitrogen-fixation, salt-tolerance or antagonism [41– 47].

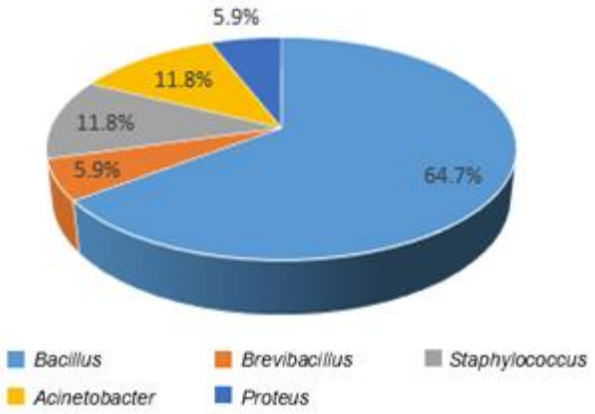

Figure 6. The proportions of groups (genus level) that 17 identified isolates belong to

Three Gram-negative isolates of this study identified as Acinetobacterbaylyi, Acinetobactergerneri, and Proteus mirabilisall belong to Gammaproteobacteria. While Acinetobactergerneri was reported as a novel species isolated from activated sludge plants in Victoria, Australia [48], Acinetobacterbaylyi was considered as plant growth-promoting bacteria with many traits such as nitrogen fixation, siderophore production and mineral solubilization [49] or producing aroma compound 2-acetyl-1-pyrroline (2AP) in rice [50]. Proteus mirabilis was reported about the production of auxin (indole-3-acetic acid), gibberellin, cytokinin (zeatin) and abscisic acid with other bacteria such as Proteus vulgaris, Klebsiellapneumoniae, Bacillus megaterium, and B. cereus [51] or reducing oxidative stress caused under Zn toxicity in maize [52]. Thus, except Acinetobactergernerihaving no information on the possibility of promoting of plant growth, the remaining 10 species are PGPRs that have been reported in black pepper and other crops. Especially, diversity of aerobic endospore forming bacteria, viz., species of Bacillus with physiological traits such as multilayered cell wall, stress resistant endospore formation promises the possibility of application for crops under extreme conditions [23].

IV.CONCLUSION

A total of 118 isolates having two abilities of nitrogen fixation and phosphate solubilizationwere obtained from 43rhizospheresamples of pepper plants cultivated in seven wards of BinhPhuoc province,the Southeast of Vietnam. The qualitative test showed 107 of the 118 isolates (90.7%) have the ability of IAA production. There were 43 isolates had expressed superiority in capabilities of nitrogen fixation, phosphatesolubilization and IAA production in quantitative tests but there were only 17 isolates (39.5%) have the ability of siderophore production in CAS liquid assay. These 17isolates were identified as PGPB including genera Bacillus(64.7%), Brevibacillus(5.9%), Staphylococcus (11.8%), Acinetobater(11.8%) and Proteus (5.9%). These strains should be recommended to test their effectiveness on the growth of black pepper in in vivo experiments.

V. ACKNOWLEDGEMENT

The authors wish to thank SaiGon University for financing this research. We would like to especially thank Assist. Prof. Dr.PhanThi Phuong Trang from University of Science whoprovided expertise assisted the identification research, and our colleagues Chau Kim Xuyen, and Ha Bao Son for assistances with laboratory expriments.

VI. REFERENCE

[1] K. Ramachandran, V. Srinivasan, S. Hamza, M. Anandaraj, "Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (Piper nigrum L.) cuttings", in: E. Velázquezand C. Rodríguez-Barrueco (eds.), First International Meeting on Microbial Phosphate Solubilization, Vol. 102 of the series Developments in Plant and Soil Sciences, Springer Netherlands, pp. 325-333, 2007.

[2] Z.F.A.Aziz, M.S.Halimi, F.R.Kundat, M.Jiwan, S.K.Wong, "Rhizobacterium Bacillus cereus induces root formationof pepper (Piper nigrum L.) stem cuttings",Research in Biotechnology, Vol. 6(2), pp. 23-30, 2015.

[3] F.A.A. Zakry, M.S. Halimi, K.B. Abdul Rahim, H.A. Osumanu, S.K. Wong, R.K. Franklin, L.C.T. Stephen,andJ. Make, "Isolation and plantgrowth-promoting properties ofrhizobacterial diazotrophs from peppervine (Piper nigrum L.)",Malays. Appl. Biol., Vol. 39, pp. 41-45, 2010.

[4] K.N. Anith, N.V. Radhakrishnan, and T.P. Manomohandas, "Screening of antagonistic bacteria for biological controlof nursery wilt of black pepper (Piper nigrum)", Microbiol. Res., Vol. 158, pp. 91–97, 2003.

[5] H. Tran, M. Kruijt,J.M. Raaijmakers, "Diversity and activity of biosurfactant-producing Pseudomonas in the rhizosphere of black pepper in Vietnam", Journal of Applied Microbiology, Vol. 104(3), pp. 839-851, 2008.

[6] Y.C. Ann,"Rhizobacteria of pepper (Piper nigrum) and their antifungal activities,African Journal of Microbiology Research, Vol. 6(19), pp. 4185-4193, 2012.

[7] J. Luster, and R. Finlay, Handbook of Methods Used in Rhizosphere Research. Birmensdorf, Swiss Federal Research Institute WSL, 536 pages, 2006.

[8] M. Park, C. Kim, J. Yang, H. Lee, W. Shin, S. Kim, and T. Sa, "Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea",Microbiological Research, Vol. 160, pp. 127-133, 2005.

[9] C.S.Nautiyal, "An efficient microbiological growth medium for screening phosphate solubilizing microorganisms",FEMS Microbiology Letters, Vol. 170, pp. 256-270, 1999.

[10] D.T.N. Thanh, C.N. Diep,"Isolation and Identification of Rhizospheric Bacteria in Acrisols of Maize (Zea mays L.) in theEastern of South Vietnam",American Journal of Life Sciences,Vol. 2, No. 2, pp. 82-89, 2014. doi: 10.11648/j.ajls.20140202.18.

[11] N.Garg, K. L. Garg, and K. G. Mukerji, Laboratory Manual of Food Microbiology, I.K. International Publishing House Pvt. Ltd., New Delhi, India, 199 pages, 2010.

[12] A. von Graevenitz, and C. Bucher, "Accuracy of the KOH and Vancomycin Tests in Determining the Gram Reaction of Non-Enterobacterial Rods", J. Clin. Microbiol., Vol. 16(4), pp. 983-985, 1983.

[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., Vol. 20(4), pp. 255-266, 2014.

[14] L. Solarzano, "Determination of ammonia in natural waters by the phenolhypochlorite methods",Limnol.Oceanogr., Vol.14(5), pp. 799-801., 1969.

[15] J. Murphy, and J.P.Riley, "A modified single solution method for the determrnation of phosphate in natural waters",Anal. Chim. Acta., Vol. 27, pp. 31-36, 1962.

[16] S.A. Gordon, and R.P. Weber, "Colorimetric estimation of indoleacetic acid",Plant physiol., Vol. 26(1), pp. 192-195, 1951.

[17] R.S.D.P. Raj, and G.R. Rex, "Modified medium for isolation and preliminary screening of indole acetic acid (IAA) producing bacteria from soil samples",Int. J. Modn. Res. Revs., Vol. 2(9), pp. 275-276, 2014.

[18] B.C. Louden, D. Haarmann, and A.M. Lynne, "Use of Blue Agar CAS Assay for Siderophore Detection",Journal of Microbiology & Biology Education, pp. 51-53, 2011.

[19] D.D. Kadiri, N. Gorle, K.V.R. Peetala, and S. Peela, "Isolation, screening and identification of phosphate solubilizing bacteria from different regions of Visakhapatnam and Araku valley",International Journal of Advanced Biotechnology and Research,Vol. 4(4), pp. 518-526,2013.

[20] L.D.P. Salcedo, C. Prieto, and M.F. Correa, "Screening phosphate solubilizing actinobacteria isolated from the rhizosphere of wild plants from the Eastern Cordillera of the Colombian Andes",African Journal of Microbiology Research, Vol. 8(8), pp. 734-742,2014.

[21] Dang Thi Ngoc Thanh and Cao Ngoc Diep, "Isolation and identification of beneficial bacteria (nitrogen fixation, phosphorus and potassium solubilization) in rhizospheric soil of maize cultivated in Trang Bom district, Dong Nai province", Journal of Agriculture and Rural Development, Vol. 14, pp. 49-56, 2012. (Vietnamese)

[22] Hoang Minh Tam and Cao Ngoc Diep, "Isolation and Identification of Rhizospheric Bacteria in Sugarcane (Saccharum spp. L.) Cultivated on Acrisols of Tay Ninh Province, Vietnam", International Journal of Innovations in Engineering and Technology, Vol. 8(2), pp. 323-335, 2017.

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

[24] L.B. Taiwo, and M. Ogundiya, "Microbial solubilization of Ogun rock phosphate in the laboratory and in soil",African Journal of Microbiology Research, Vol. 2, pp. 308-312, 2008.

[25] F. Ahmad, I.Ahmad, and M.S.Khan, "Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absence of Tryptophan",Turkish Journal of Biology, Vol. 29(1), pp. 29-34, 2005.

[26] Dang Thi Ngoc Thanh, Nguyen Thi Xuan My, Cao Ngoc Diep, "Indole acetic acid andsiderophore production byselected isolates of plantassociated bacteria andtheir effects on growth ofmaize (Zea mays L.) in potexperiments",Science Journal of Can Tho University, Vol. 47a, pp. 59-67, 2016.(Vietnamese)

[27] E. Zakharova, A. Shcherbakov, V. Brudnik, N. Skripko, N. Bulkhin and V. Ignatov, "Biosynthesis of indole-3-acetic acid in Azospirillum brasilense Insights from quantum chemistry",Eur. J. Biochem., Vol. 259, pp. 572-576, 1999.

[28] O.A. Almaghrabi, T.S. Abdelmoneim, H.M. Albishri, and T.A.A. Moussa,"Enhancement of Maize Growth Using Some Plant Growth Promoting Rhizobacteria (PGPR) Under LaboratoryConditions",Life Science Journal, Vol. 11(11), pp.764-772, 2014.

[29] N.U. Maheswar, and G. Sathiyavani,"Solubilization of phosphate by Bacillus Sps, from groundnut rhizosphere(Arachishypogaea L), Journal of Chemical and Pharmaceutical Research, Vol. 4(8), pp. 4007-4011, 2012.

[30] M.I. Stets, A.S. Pinto Jr., L.F. Huergo,E.M. de Souza, V.F. Guimaraes, et al., "Rapid identification of bacterial isolates from wheat roots by highresolution whole cell MALDI-TOF MS analysis", Journal of Biotechnology, Vol. 165, pp. 167-174, 2013.
[31] A. Tani, N. Sahin, Y. Fujitani, A. Kato, K. Sato, K. Kimbara, "Methylobacterium Species Promoting Rice and Barley Growth and Interaction Specificity Revealed with Whole-Cell Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) Analysis", PLOS ONE, Open Acess, Published on June 8, 2015.
[32] C.C. Urquiza, I.A. Hernández, J.A.C. Medina, M.A.R. López, J.P. Cruz, R.L.R. Zarazúa, et al., "Identification by MALDI-TOF Mass Spectrometry of Mercury-resistant Bacteria Associated with the Rhizosphere of an Apple Orchard", Geomicrobiology Journal, Vol.33, pp. 176-182, 2017.
[33] Le Huy Ba, Soil Resource Environmentof Vietnam, Vietnam Education Publishing House, 1,300 pages, 2009. (Vietnamese)
[34] Hoang Minh Tam, Cao Ngoc Diep. Isolation and Identification of Rhizospheric Bacteria in Sugarcane (Saccharum spp. L.) Cultivated onAcrisols and Ferrasols of Dong Nai Province, the Southeast of Vietnam. American Journal of Life Sciences. Vol. 3, No. 2, pp. 109-118, 2015. doi: 10.11648/j.ajls.20150302.18P.
[35] M. Karadayi, M. Gulluce, S. Dogan, E. Gulluce, "Isolation and Molecular Characterization of Bacteria with Plant Growth-Promoting Characteristics from Magnesite Mining Fields in Kütahya-Turkey", Journal of Applied Biological Sciences, Vol. 10(3), pp. 27-32, 2016.
[36] R. Aravind, A. Kumar, S.J. Eapen and K.V. Ramana,"Endophytic bacterial flora in root and stem tissues of blackpepper (Piper nigrum L.) genotype: isolation, identificationand evaluation against Phytophthora capsici", Letters in Applied Microbiology, Vol. 48,pp. 58–64, 2009.
[37] M. Rajkumar, Y. Ma and H. Freitas, "Characterization of metal-resistant plant-growth promotingBacillus weihenstephanensis isolated from serpentine soilin Portugal", Journal of Basic Microbiology, Vol.48,pp. 500-508, 2008.
[38] L.E. de-Bashan, J-P. Hernandez, Y. Bashan, and R. Maier,"Bacillus pumilus ES4: candidate plant growth-promoting bacterium to enhance establishment of plants in mine tailings",Environ. Exp. Bot., Vol. 69(3), pp. 343-352, 2010.
[39] M. Cruz-Martín, E. Mena, C. Sánchez-García, B. Roque, M. Acosta-Suárez, T. Pichardo, M. Leiva-Mora, Y.Alvarado-Capó, "The effects of plant growth promoting Bacillus pumilus CCIBPC5on 'Grande naine' (Musa AAA) plants in acclimatization stage", Artículo original Biotecnología Vegetal, Vol. 15, No. 3, pp. 151 - 156, 2015.
[40] V. Nehra, B.S. Saharan, and M. Choudhary, "Evaluation of Brevibacillus brevis as a potential plant growth promoting rhizobacteria for cotton (Gossypium hirsutum) crop", Springerplus,Vol. 5(1), p. 948, 2016.
[41] Q. Hou, C. Wang, X. Hou, Z. Xia, J. Ye, K. Liu, H.Liu, et al., "Draft genome sequence of Brevibacillus brevis DZQ7, a plant growth-promoting rhizobacterium with broad-spectrum antimicrobial activity", Genome Announc., Vol. 3(4), pp. e00831-15, 2015. doi:10.1128/genomeA.00831-15.
[42] S.P. Chowdhury, M. Schmid, A. Hartmann, and A.K. Tripathi, "Identification of Diazotrophs in the Culturable BacterialCommunity Associated with Roots of Lasiurus sindicus, a Perennial Grass of Thar Desert, India", Microbial Ecology, Vol. 54, pp. 82-90,2007.
[43] Z.S. Deng, L.F. Zhao, Z.Y. Kong, W.Q. Yang, K. Lindström, E.T. Wang, and G.H. Wei, "Diversity of endophytic bacteria within nodules of the Sphaerophysa salsula in different regions of Loess Plateau in China. FEMS Microbiol. Ecol., Vol. 76, pp. 463-475, 2011.
[44] M.R. da C.R. Lins, J.M. Fontes, N.M. de Vasconcelos, D.M. da S. Santos, O.E. Ferreira, J.L. de Azevedo, et al., "Plant growth promoting potential of endophytic bacteria isolated from cashew leaves", African Journal of Biotechnology, Vol. 13(33), pp. 3360-3365, 2014.
[45] A.W. Qurashi, and A.N. Sabri, "Osmoadaptation and plant growth promotion by salt tolerant bacteria under salt stress", African Journal of Microbiology Research, Vol. 5(21), pp. 3546-3554, 2011.
[46] N.A. Azman, K. Sijam, E.M. Hata, R. Othma, and H.M. Saud, "Screening of Bacteria as Antagonist againstXanthomonas oryzae pv. oryzae, the Causal Agent ofBacterial Leaf Blight of Paddy and as Plant GrowthPromoter", Journal of Experimental Agriculture International, Vol. 16(4), pp. 1-15, 2017.
[47] E.L.Carr, P.Kämpfer, B.K.Patel , V.Gürtler , R.J. Seviour,"Seven novel species of Acinetobacter isolated from activated sludge", Int. J. Syst. Evol. Microbiol., Vol. 53(Pt 4), pp. 953-63, 2003.
[48] D. Sachdev, P.Nema, P.Dhakephalkar, S. Zinjarde, and B. Chopade, "Assessment of 16S rRNA gene-based phylogenetic diversity and promising plant growth-promoting traits of Acinetobacter community from the rhizosphere of wheat", Microbiol. Res.,Vol. 165(8), pp. 627-638, 2010.
[49] Y. Deshmukh, P. Khare, D. Patra,"Rhizobacteria elevate principal basmati aroma compound accumulation in rice variety", Rhizosphere, Vol. 1, pp. 53-57, 2016.
[50] A. Karadeniz, S.F. Topeuoglu, and S. Inan, "Auxins, gibberellin, cytokinin and abscisic acid production in some bacteria",World J. Microbiol. Biotechnol., Vol.22, pp. 1061-1064, 2006.
[51] F. Islam, T. Yasmeen, M. Riaz, M.S. Arif, S. Ali, and S.H. Raza, "Proteus mirabilisalleviates zinc toxicity bypreventing oxidative stress in maize (Zea mays) plants". Ecotoxicol. Environ. Saf., Vol. 110, pp. 143-152, 2014.