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Isolation and Identification of Phosphate-solubilizing Bacteria and Their Effects on the Growth of Vanilla (*Vanilla planifolia*) and Absorption of Phosphorus

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Abstract Vanilla (*Vanilla planifolia*) is a heavily-phosphorus absorption crop. A phosphate-solubilizing bacterium, *Burkholderia stabilis* (V-29), was isolated from the rhizosphere soil of vanilla field. The National Botanical Research Institute's phosphate (NBRIP) liquid medium culture showed strain V-29 lowered medium pH and released water soluble P up to 475.3 μg/mL with five days of incubation. A field experiment was carried out to investigate the effects of green fluorescent protein-tagged V-29 (V-29-gfp) and its bio-organic fertilizers (BIOs) on plant growth and phosphorus uptake. The results showed that the inoculation of V-29-gfp or application of BIOs significantly increased the vanilla plant dry weights and soil available phosphorus content; the rhizosphere soil population of V-29-gfp was approximately 10^6 cfu/g soil at 120 days after transplantation, suggesting that V-29 had potential to be exploited as biofertilizers to decrease chemical fertilizers application.

Keywords bio-organic fertilizer; *Burkholderia* sp.; phosphate-solubilizing bacteria; *Vanilla planifolia*

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解磷细菌 *Burkholderia* 的分离鉴定及对香草兰生长和 P 吸收的影响

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摘　要 香草兰为喜磷作物，从香草兰种植园中分离筛选到一株解磷微生物——伯克霍尔德氏菌 V-29。在 NBRIP 液体培养基中摇床振荡培养 5 d 后可溶性磷含量达 475.3 μg/mL，培养基 pH 下降。通过大田试验研究了接种绿色荧光蛋白标记后的解磷菌株 V-29 及其与有机肥发酵制得的微生物有机肥对香草兰生长和磷素吸收的影响。结果表明：单独接种 V-29 或施用微生物有机肥可显著增加香草兰植株干重、土壤有效磷含量；移栽 4 个月后，标记菌株 V-29 在香草兰根际土壤中的含量可达 10^6 cfu/g 土壤。由此可见，伯克霍尔德氏菌 V-29 可单独作为生物菌剂或与有机肥发酵制得微生物有机肥后用于农业生产中，以减少化肥施用量。

关键词 微生物有机肥；伯克霍尔德氏菌；解磷细菌；香草兰

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1 Introduction

Vanilla (*Vanilla planifolia* Andrews.) is a rare tropical spice crops with high economic value. Cured vanilla beans are widely used in the food, beverage and cosmetic industries[1-2]. Phosphorus (P) is one of the most important limiting mineral nutrients, plays critical roles in plant growth, while most crops can not use it efficiently as the p element in insoluble form in soil[3]. Vanilla is a heavily-phosphorus absorption crops, however, intensive soil evaluations in tropical seasonal rain forest laterite zone leads to soil deficiency of phosphorus[4]. So, a large amount of chemical fertilizers were applied to vanilla plantation, resulting in serious environmental problems. Thus, developing other ecologically compatible and environmentally friendly techniques is urgent to replace chemical inputs[5]. Phosphate solubilizing bacteria (PSB) can help plant efficiently use the P element through dissolving soil insoluble P into soluble form[3].

Many research documented that application of PSB could increase available P concentrations in soils and promote plant growth[6-9]. Thus, the use of PSB could reduce the environmental problems caused by chemical fertilizer application. However, strains must be combined with a suitable substrate to improve their efficacy because they can survive for a long time after applied to soil[5]. To the best of our knowledge, the use of PSB on promoting vanilla plant growth has not yet been studied. The aims of this research were: (1) to isolate an efficacy PSB and develop a bio-organic fertilizer for promote vanilla plant growth; (2) to investigate the effects of the bio-organic fertilizer under field conditions.

2 Materials and methods

2.1 Isolation of PSB

A rhizosphere soil was sampled from vanilla plants, 10-fold serially diluted, and spread onto National Botanical Research Institute's phosphate growth medium (NBRIP)[10] containing insoluble aluminium phosphate (3 g/L) for selective screening of PSB. The plates were incubated at 28 ℃ for 5 days. A strain that produces a wide halo zones was purified and named as V-29.

2.2 P-solubilization in liquid culture

Strain V-29 was inoculated into NBRIP medium, shaken at a speed of 170 rpm at 28 ℃ and sampled at days 1, 2, 3, 4 and 5 for measuring soluble phosphate and pH. The samples were centrifuged at 12 000×*g* for 10 min. The soluble phosphorus content in filtrates was determined by the method of Bao[11]. In brief, 2 mL of ammonium molybdate reagent and 6 mL of distilled water were added to 2 mL of cell free supernatant, mixed thoroughly and then measured at 700 nm using UV-Vis spectrophotometer (U-3900H). Amount of soluble phosphate was estimated using calibration curve of standard dipotassium hydrogen phosphate.

2.3 Identification of the strain (V-29) by the 16S rDNA sequence

The isolated strain (V-29) was identified based on its 16S rDNA gene sequence. The strain V-29 was cultured in nutrient broth medium at 28 ℃ for 24 h. Cell suspensions were centrifuged at 3 000×*g* at 4 ℃ for 5 min. Genomic DNA was extracted, and the 16S ribosomal RNA gene was amplified[12] using the B27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and U1492 R (5′-GGTTACCTTGTTACGACTT-3′) primers. The PCR product was purified and sequenced. A comparison of nucleotide sequences was performed against the BLAST database (http://blast.ncbi.nlm.nih.gov) of the National Center for Biotechnology Information. Clustal-X version 1.81 program was used for construction of phylogenetic tree based on neighbour-joining phylogenetic analysis.

2.4 Construction of green fluorescent protein (gfp) -tagged V-29

The gfp plasmid HapII (GenBank accession number HM151400) that was used to tag V-29 was kindly provided by Dr. Zhang of Life Sciences College of Nanjing Agricultural University, China. The plasmid was introduced into V-29 by electroporation as described by McDonald et al.[13] and Turgeon et al.[14]. In brief, a cell suspension was washed three times with a cold electroporation buffer (contained 272 mmol/L sucrose, 1 mmol/L $MgCl_2$, and 7 mmol/L KH_2PO_4) and was then concentrated. The HapII plasmid was added to this mixture, transferred to an electroporation cuvette, and electroporated (2.5 kV, 400 Ω, 25 μF). After electroporation, cell suspensions were immediately added to Luria-Bertani (LB) medium and were shaken at 120 rpm at 37 ℃ for 1 h. Aliquots were spread onto LB agar plates supplemented with

kanamycin (300 μg/mL). Fluorescence was visualized by epifluorescence stereo microscopy (Eclipse 80i; Nikon Corporation, Japan). Gfp-tagged V-29 (V-29-gfp) was stored at –80 ℃ before use.

2.5 Preparation of bio-organic fertilizer

The cow manure compost contained 19.4% organic fertilizer, 0.8% total N, 0.6% P, and 0.4% K. The amino acid fertilizer applied in the present study has been described previously by Huang *et al*.[15] and Zhao et al.[16]. Cell suspension of V-29-gfp was produced by inoculating into LB (supplemented with 300 μg/mL kanamycin) and shaken at 180 rpm at 30 ℃ for 48 h. The suspension was separately sprayed onto cow manure compost and amino acid fertilizer at a concentration of 200 mL suspension per 1 kg organic fertilizer. These mixtures were fermented at 30-45 ℃ and 40%-50% relative humidity for five days to increase bacterial numbers. After incubation, the number of V-29-gfp was measured by the plate-count method using LB agar plates supplemented with 300 μg/mL kanamycin. The final densities of V-29-gfp were about 1.0×10^{10} cfu/g cow manure compost and 1.2×10^{10} cfu/g amino acid fertilizer. The V-29-gfp fortified cow manure compost is termed bio-organic fertilizer I (BIO1), and fortified amino acid fertilizer is termed BIOII.

2.6 Field experimental design

Field experiments were carried out in Wanning city, Hainan province, China. Plots, each 15 m× 1.6 m in size, planted with 12 plants, were randomly distributed. The field soil, sandy loam in texture, contained 12.67 g/kg of organic matter, 87.74 mg/kg of available N, 21.02 mg/kg of available P (Olsen), and 64.32 mg/kg of available K (NH_4OAc). Treatments included: (1) CK: soil was untreated; (2) V-29: soil was amended with V-29-gfp suspension at the concentration of 10^8 cfu/g soil; (3) CM: soil was amended with cow manure compost; (4) BIOI: soil was amended with BIOI; (5) AA: soil was amended with amino acid fertilizer; (6) BIOII: soil was amended with BIOII. The application rate of organic fertilizer or BIO was 7 500 kg/ha in field plots. Two blocks were randomly laid out and each treatment with three plots was randomly arranged within each block.

Six plants per treatment were sampled to assess available P content in rhizosphere soil, total P content in plant and V-29-gfp number in rhizosphere soil after 120 days of transplantation. The sampled plants were carefully shaken by hand to obtain rhizosphere soil, which was tightly adhered to roots[17]. One portion soil samples were air dried for analyzing available P content, the other portion were stored at 4 ℃ for analyzing V-29-gfp population within five days. The V-29-gfp population was measured using beef extract peptone agar plate supplemented with 300 μg/mL of kanamycin. Other three plants per treatment were used to assess plant dry weight.

2.7 Data analysis

The data were statistically analyzed using the statistical program SPSS for Windows, version 19 (SPSS, Inc., Chicago, IL, USA). Data were subjected to Duncan's analysis of variance (ANOVA), and the means were compared by Duncan's tests at $p<0.05$.

3 Results

3.1 Isolation of PSB

Six strains showing P-solubilizing zone were isolated from the rhizosphere soil of vanilla plants. One P-solubilizing strain, V-29, was selected for its consistent production of a wide clear zone on NBRIP plates (Fig. 1). The soluble phosphate content increased with culture time. We also observed an increase in phosphate solubilization and a decline in pH during the time course (Fig. 2). The maximum P-solubilizing ability was 475.3 μg/mL at incubation of 3 days in liquid NBRIP medium. The culture pH was decreased from 6.3 to 5.1 after incubation of 5 days (Fig. 2).

3.2 Identification of strain V-29

The 16S rDNA gene fragment of strain V-29

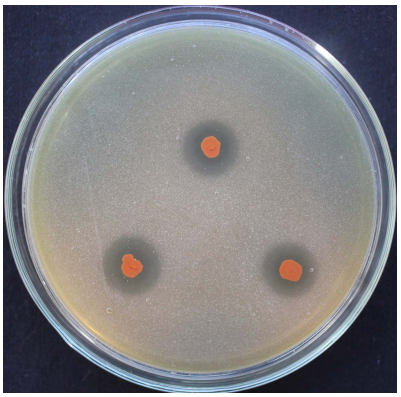


Fig. 1　Phosphate solubilizing zone of strain V-29 on NBRIP plates after three days of incubation

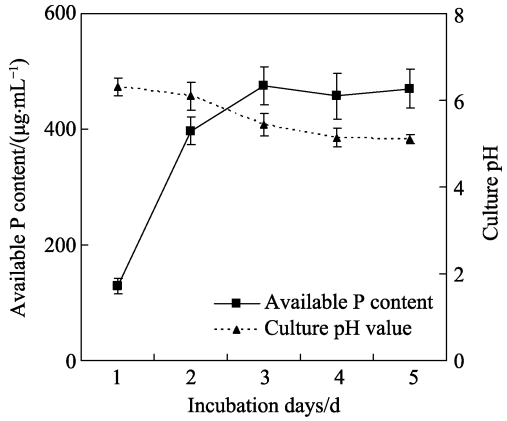


Fig. 2 Phosphate solubilizing ability of strain V-29 in liquid NBRIP culture

(1 453 bp) produced a 100% similarity to *Burkholderia stabilis* strain S000428831 in the BLAST search against all of the nucleotide sequences in the NCBI database (Fig. 3). Consequently, the strain V-29 was identified as *Burkholderia stabilis*. The V-29 16S rDNA gene sequence was deposited into GenBank under accession number KF137574. The strain V-29 was deposited at the China General Microbiological Culture Collection Center under accession no. 7980.

3.3 *Burkholderia stabilis* V-29-gfp

The plasmid was successfully transformed into strain V-29 by electroporation. Green-colored colonies and the emission of green fluorescence were visualized under UV light (Fig. 4). The gfp-tagged

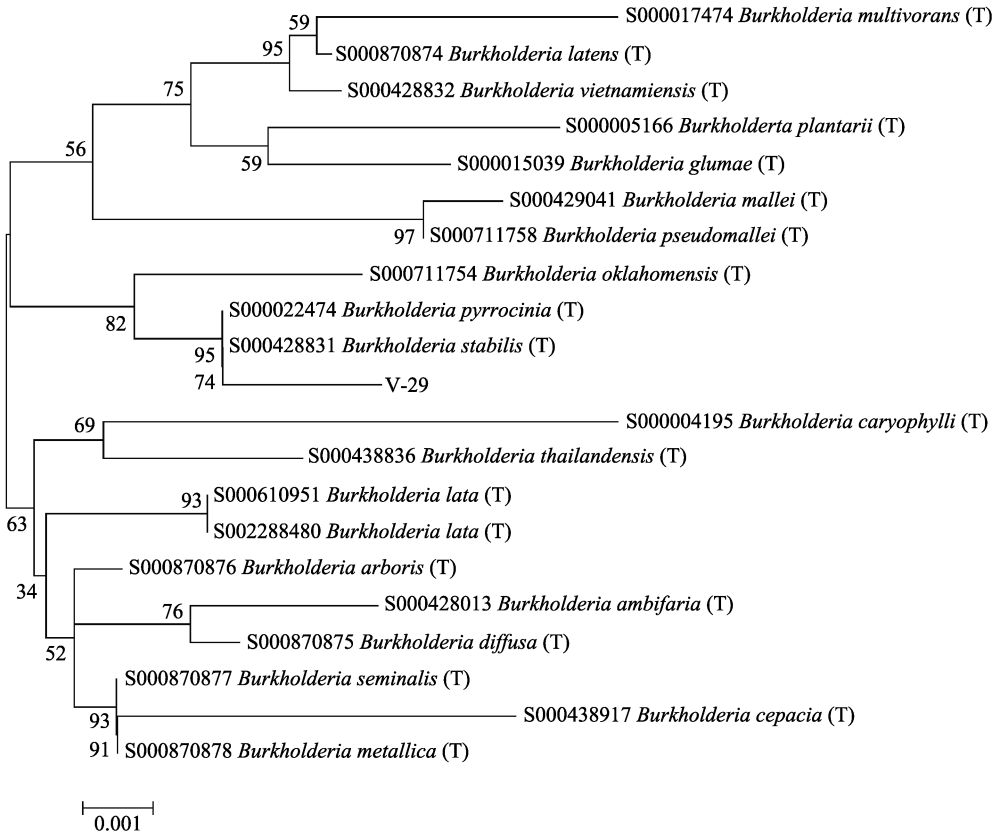


Fig. 3 Phylogenetic tree of strain V-29.

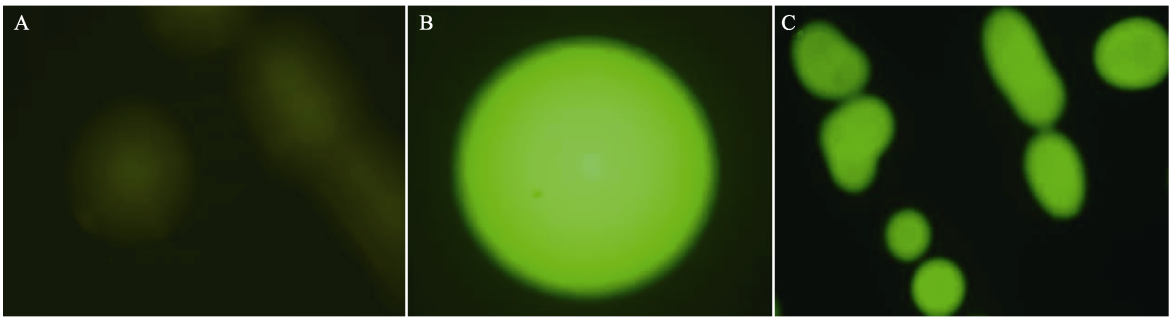
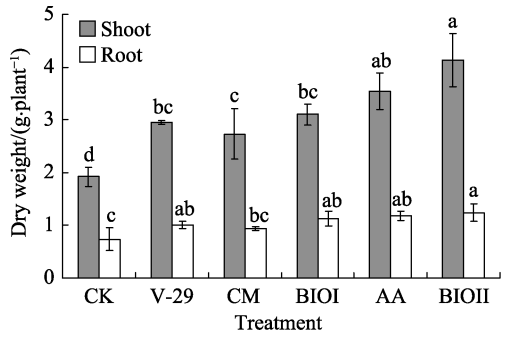


Fig. 4 Colonies of V-29 (A) and V-29-gfp (B, C) under epifluorescence microscopy.

V-29 strain was named V-29-gfp. Green fluorescence could still be visualized under UV light after multiple sub-culturing of V-29-gfp for one month, confirming that the vector was stable in terms of expressing gfp genes for the length of the present experiment.

3.4 Effects of *Burkholderia stabilis* V-29-gfp on vanilla plant growth

Plants in treatments received BIOII produced the highest shoot biomass, while those in control had the smallest (Fig. 5). The dry weights of the vanilla shoots and roots in the BIOII treatment were 18.2% and 5.1% higher compared with the AA treatment, respectively. There were no significant differences both in shoots and roots between the BIOI and V-29 treatment, but all significantly higher than those in control treatment. As for the plant roots, no significant differences were observed among the BIOII, AA, BIOI and V-29 treatments. For the shoots, plants treated with BIOII and AA significantly higher compared with CK, V-29 and CM treatments. These results suggest that inoculation of strain V-29-gfp or application of V-29-gfp fortified organic fertilizer could significantly promote vanilla plant growth.



CK: soil was untreated; V-29: soil was amended with V-29-gfp suspension at the concentration of 10^8 cfu/g soil; CM: soil was amended with cow manure compost; BIOI: soil was amended with BIOI; AA: soil was amended with amino acid fertilizer; BIOII: soil was amended with BIOII. Vertical bars are the mean ± standard deviation of three replicates. Bars with different letters indicate significant differences (p<0.05) by Duncan's test.

Fig. 5　Effects of different treatments on vanilla plant growth

3.5 Effects of *Burkholderia stabilis* V-29-gfp on P content

Inoculations of strain V-29-gfp (V-29) significantly increased the available P content in rhizosphere soil, compared with non-treated soil (Tab. 1), but significantly lower than amino acid fertilizer (AA) or BIOII treatments. In the AA or BIOII treated soil, showed obvious higher available P content than the non-treated soil, while, no obvious difference between the two treatments.

Similar tendency was found in P accumulation in plant tissues. Plants accumulated much more P in BIOII, AA, or BIOI treatments compared to those in other treatments (Tab. 1). No significant differences were observed among treatments of control, V-29 and CM.

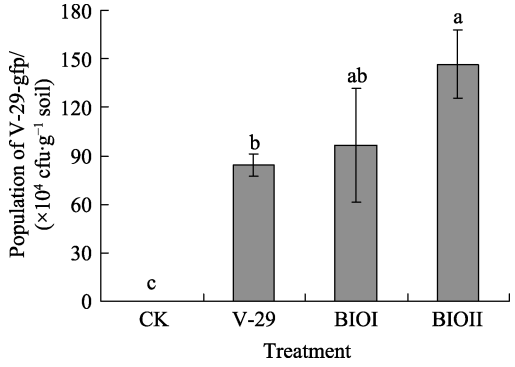
Tab. 1　Effects of different treatments on P content

Treatment	Available P content in rhizosphere soil/(mg·kg^{-1})	P uptake in vanilla plant/(mg·kg^{-1})
CK	20.91±1.67^{d}	3.28±0.19^{c}
V-29	21.54±1.91^{c}	3.57±0.11^{bc}
CM	22.81±2.03^{bc}	3.76±0.17^{bc}
BIOI	23.46±2.91^{ab}	3.82±0.19^{ab}
AA	24.02±1.84^{a}	3.97±0.34^{ab}
BIOII	24.93±2.09^{a}	4.43±0.28^{a}

Note: The data in a column with a different letter differ significantly at Duncan's significance level 0.05.

3.6 *Burkholderia* Stabilis V-29-gfp colonization ability in rhizosphere soil

Strain V-29-gfp was not detected in the treatments of control, CM and AA (data not shown). In the V-29 treatment, the V-29-gfp number enumerated 120 days after transplantation was 8.4×10^5 cfu/g soil, significant difference was not observed between treatments of V-29 and BIOI (Fig. 6). The V-29-gfp population in BIOII treatment was obviously higher than that of the V-29 treatment at 120 days after transplantation.



CK: soil was untreated; V-29: soil was amended with V-29-gfp suspension at the concentration of 10^8 cfu/g soil; BIOI: soil was amended with BIOI; BIOII: soil was amended with BIOII. Bars with different letters indicate significant differences (p<0.05) by Duncan's test.

Fig. 6　Population of *Burkholderia stabilis* V-29-gfp in rhizosphere soil

4 Discussion

In our research, a novel phosphate solubilizing bacterium, designed strain V-29, was isolated from vanilla rhizosphere soil and identified as *Burkholderia stabilis* by 16S rDNA gene sequence. Our study demonstrated that inoculation of V-29-gfp or fortified with organic fertilizer (BIO), significantly promoted vanilla plant growth and increased available P content in rhizosphere soil compared with control. These suggested that *Burkholderia stabilis* V-29 could be of potential to develop as biofertilizers to promote vanilla plant growth. This is the first report of the isolation of *Burkholderia stabilis* strain from vanilla rhizosphere which has mineral phosphate solubilizing ability.

Various bacteria, such as *Acinetobacter*, *Azotobacter*, *Bacillus, Burkholderia*, *Pseudomonas*, and *Rhizobium* are reported as P solubilizer, have been popularly employed for promotion of crops growth[18-21]. Stephen et al.[22] have demonstrated that inoculation of phosphate solubilizing *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) increased P uptake and enhanced rice (*Oryza sativa*) growth under greenhouse conditions. *Rhododendron arboretum* seeds treated by phosphate solubilizing *Burkholderia* sp. gave the higher germination percentage[23]. Inoculation of *Serratia* sp. J260, *Enterobacter* sp. J33, *Acinetobacter* sp. L176, *Enterococcus* sp. L185, *Enterococcus* sp. L191 and *Bacillus* sp. L55 on peanut plants led to an increase in plant or soil P content [6]. In this study, a newly phosphate solubilizing bacterium *Burkholderia stabilis* V-29 was isolated from vanilla field (Fig 1). Application of V-29 or V-29 fortified bio-organic fertilizer obviously increased vanilla plant growth and soil P content, suggesting V-29 could be an alternative strategy to chemical fertilizer for vanilla growth.

The positive effects on vanilla plant growth after application of strain V-29 or bioorganic fertilizer may be linked to the phosphate solubilizing. The maximum content of soluble P in V-29 culture filtrate was 475.3 μg/mL, with a 1.2 drop in pH (Fig. 2). Li et al.[24] detected that the soluble P was 584.8 μg/mL after 7-day incubation of *Pseudomonas* K3 in the liquid medium NBRIP. A similar relationship between phosphate solubilization and pH was also found by Hwangbo et al.[25] and Shahid et al.[7]. The inoculation with V-29 or BIO positively increased the phosphorus content and uptake of plants (Tab. 1). Similar results on the increased P uptake in other crops attribute to inoculation of P solubilizer have been described by Kaur and Reddy[26] and Stephen et al.[22]. Application phosphate solubilizing bacteria of *Pantoea cypripedii* and *Pseudomonas plecoglossicida* as bio-inoculants significantly increased maize and wheat grain yield, phosphorus uptake and available P at three different agroclimatic regions.

A high rate of colonization is crucial for achieve beneficial effects[27-29]. In the present study, we have successfully tagged V-29 with gfp technology for the detection and quantification of V-29-gfp from vanilla rhizosphere soil. The number of V-29-gfp in the rhizosphere soil of the V-29 and BIO treatments was approximately 10^6 cfu/g soil after transplantation of 120 days (Fig. 6), suggesting that V-29 can successfully colonization in vanilla rhizosphere soil to provision of beneficial effects. Shahid et al.[7] found that *Bacillus* sp. Ps-5 and *Alcaligenes faecalis* Ss-2 were good root colonizers and significantly (p<0.05) increased sunflower growth and phosphorus uptake. Both strains were recovered up to 30 days after transplantation at a population density of 10^6 cfu/g soils in the pot experiment[7]. However, Li et al.[24] reported that the total population of K3 strain was decreased to 10^3 cfu/g soil after inoculation of 40 days[24].

A basic mechanism in phosphate solubilization includes production of organic and inorganic acid by PSB[30]. Our further research should focus on the isolation and identification of organic and inorganic acids produced by V-29 to understand the mechanisms underlying these beneficial effects.

References

[1] Priefert H, Rabenhorst J, Steinbuchel A. Biotechnological production of vanillin[J]. Applied Microbiology and Biotechnology, 2001, 56: 296-314.

[2] Chen Y G, Gu F L, Li J H, *et al*. *Bacillus vanillea* sp. nov., isolated from the cured vanilla bean[J]. Current Microbiology, 2015, 70(2): 235-239.

[3] Rodriguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion[J]. Biotechnology Advances, 1999, 17: 319-339.

[4] 王 华，庄辉发，王 辉，等. 海南省香草兰种植园植株养分状况研究[J]. 热带作物学报, 2010, 31 (1): 547-550. Wang H, Zhuang H F, Yang J F, *et al*. Study of the Plant

Nutrient Status of *Vanilla planifolia* Andr in Hainan Province[J]. Chinese Journal of Tropical Crops, 2010, 31 (1): 547-550.

[5] Zhang N, Wu K, He X, *et al*. A new bioorganic fertilizer can effectively control banana wilt by strong colonization with *Bacillus subtilis* N11[J]. Plant Soil, 2011, 11: 87-97.

[6] Anzuay M S, Ludueña L M, Angelini J G, *et al*. Beneficial effects of native phosphate solubilizing bacteria on peanut (*Arachis hypogaea* L.) growth and phosphorus acquisition[J]. Symbiosis, 2015, 66: 89-97.

[7] Shahid M, Hameed S, Tariq M, *et al*. Characterization of mineral phosphate-solubilizing bacteria for enhanced sunflower growth and yield-attributing traits[J]. Annals of Microbiology, 2015, 65: 1 525-1 536.

[8] Hussein K A, Joo J H. Isolation and characterization of rhizomicrobial isolates for phosphate solubilization and indole acetic acid production[J]. Journal of the Korean Society for Applied Biological Chemistry, 2015, 58(6): 847-855.

[9] Gupta M, Kiran S, Gulati A, *et al*. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of *Aloe barbadensis* Miller[J]. Microbiological Research, 2012, 167: 358-363.

[10] Nautiyal C S. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms[J]. FEMS Microbiology Letters, 1999, 170: 265-270.

[11] 鲍士旦. 土壤农化分析[M]. 北京. 中国农业出版社, 2000: 70-86. Bao S D. Soil and agricultural chemistry analysis[M]. Beijing: China Agriculture Press, 2000: 70-86.

[12] Rong L, Guo X, Chen K, *et al*. Isolation of an isocarbophos degrading strain of *Arthrobacter* sp. scl-2 and identification of the degradation pathway[J]. Journal of Microbiology and Biotechnology, 2009, 19: 1 439-1 446.

[13] McDonald I R, Riley P W, Sharp R J, *et al*. Factors affecting the electroporation of *Bacillus subtilis*[J]. Journal of Applied Microbiology, 1995, 79: 213-218.

[14] Turgeon N, Laclamme C, Ho J, *et al*. Elaboration of an electroporation protocol for *Bacillus cereus* ATTC14579[J]. Journal of Microbiological Methods, 2006, 67: 543-548.

[15] Huang X Q, Zhang N, Yong X Y, *et al*. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43[J]. Microbiological Research, 2012, 167: 135-143.

[16] Zhao Q Y, Wang H, Zhu Z H, *et al*. Effects of *Bacillus cereus* F-6 on promoting vanilla (*Vanilla planifolia* Andrews.) plant growth and controlling stem and root rot disease[J]. Agricultural Sciences, 2015, 6: 1 068-1 078.

[17] Kinsella K, Schulthess C P, Morris T F, *et al*. Rapid quantification of *Bacillus subtilis* antibiotics in the rhizosphere[J]. Soil Biology and Biochemistry, 2009, 41: 374-379.

[18] Bahena M H R, Salazar S, Velázquez E, *et al*. Characterization of phosphate solubilizing rhizobacteria associated with pea (*Pisum sativum* L.) isolated from two agricultural soils[J]. Symbiosis, 2015, 67: 33-41.

[19] Mamta G, Kiran S, Gulati A, *et al*. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-a biosynthesis of *Aloe barbadensis* Miller[J]. Microbiological Research, 2012, 67: 358-363.

[20] Ogut M, Er F, Kandemir N. Phosphate solubilization potentials of soil *Acinetobacter* strains[J]. Biology and Fertility of Soils, 2010, 46: 707-715.

[21] Patten C L, Glick B R. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system[J]. Applied and Environmental Microbiology, 2002, 68: 3 795-3 801.

[22] Stephen J, Shabanamol S, Rishad K S, *et al*. Growth enhancement of rice (*Oryza sativa*) by phosphate solubilizing *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) under greenhouse conditions[J]. Biotechnology, 2015, 5: 831-837.

[23] Nailwal S, Anwar M S, Budhani K K, *et al*. *Burkholderia* sp. from rhizosphere of *Rhododendron arboretum*: isolation, identification and plant growth promontory (PGP) activeities[J]. Journal of Applied Natural Sciences, 2014, 6: 473-479.

[24] 李晓婷. 解磷细菌 K_3 的 GFP 标记与在土壤中定殖及对玉米生长效应研究[D]. 南京: 南京农业大学, 2009. Li X T. Research on green fluorescent protein-labelled phosphate-solubilizing bacterium K3: colonization in soil and effects on growth of corn[D]. Nanjing: Nanjing Agricultural University, 2009.

[25] Hwangbo H, Park R D, Kim Y W, *et al*. 2-Ketogluconic acid production and phosphate solubilization by *Enterobacter intermedium*[J]. Current Microbiology, 2003, 47: 87-92.

[26] Kaur G, Reddy M S. Influence of P-solubilizing bacteria on crop yield and soil fertility at multilocational sites[J]. European Journal of Soil Biology, 2014, 61: 35-40.

[27] Ramirez C A, Kloepper J W. Plant growth promotion by *Bacillus amyloliquefaciens* FZB45 depends on inoculum rate and P-related soil properties[J]. Biology and Fertility of Soils, 2010, 46: 835-844.

[28] Zhao Q Y, Shen Q R, Ran W, *et al*. Inoculation of soil by *Bacillus subtilis* Y-IVI improves plant growth and colonization of the rhizosphere and interior tissues of muskmelon (*Cucumis melo* L.)[J]. Biology and Fertility of Soils, 2011, 47: 507-514.

[29] Zhang F G, Yuan J, Yang X M, *et al*. Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indole acetic acid and plant colonization[J]. Plant Soil, 2013, 368: 433-444.

[30] Zhu F, Qu L, Hong X, *et al*. Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China[J]. Evidence-Based Complementary and Alternative Medicine, 2011: 615032.