Isolation and Selection of Beneficial Microorganism Used for Peanut Growing in Sandy Soil of Binhdin Province, Vietnam

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Abstract: With the aim to apply the beneficial microorganisms to peanut (groundnut (*Arachis hypogaea*)) growing in sandy soil of Binhdin province in Vietnam, the paper was conducted to study the isolation, selection of N-fixing, phosphate, potassium solubilizing and slime producing microorganism from sandy soil and peanut root samples, and evaluate the capability of selected isolates to adapt to the sandy soil condition. The isolation and selection of beneficial microbes were based on the biological activity of isolates, which was determined by acetylene reduction assay and nodulation on the peanut for *Rhizobium* spp., by testing the capability to solubilize $\text{Ca}_3(\text{PO}_4)_2$ or fenspat in the growth medium for phosphate, potassium solubilizing microbes, and by measurement of the viscosity of Hansen medium for slime producing microorganism. All isolates were evaluated for the effectiveness on the nutrition uptake and growth of peanut by carrying out at the greenhouse and field experiments. To evaluate the adaptation of selected isolates to the sandy soil condition, the experiments were carried out in the laboratory to determine the microbial density in suitable medium, containing different NaCl concentrations or with different pH or at different temperatures. The results showed that rhizobial strain RA18 had an N-fixing activity value of $3,458 \pm 10.95$ nmol C_2H_2 /plant and can supply 30% of N required for peanut. The bacterial strain P1107 is able to solubilize phosphate and can increase phosphorus uptake by 30% in peanut. Further, inoculation of peanut with the bacterial strain S3.1 can save 30% required mineral potassium, and the *Lipomyces* strain PT5.1 can produce the slime, which can provide the sandy soil to keep moisture for 15 d longer than the control without the inoculation. All selected isolates are able to grow at temperatures from 20 °C to 35 °C, pH from 4.5 to 6.0 and the salinity of 0.2‰-0.6‰ NaCl, and can well adapt to the sandy soil conditions.

Key words: Sandy soil, peanut, N-fixing *Rhizobium*, phosphate and potassium solubilizing bacteria, slime yeast *Lipomyces*.

1. Introduction

Biofertilizers are products containing cell of different types of beneficial microorganisms, such as N-fixing, phosphate solubilizing or cellulolytic microorganisms used for application of seed, soil or composting areas to increase the numbers of beneficial microorganisms and extend the availability of nutrients in a form which can be assimilated by plant. Common biofertilizers are N fixers, K solubilizer and P solubilizer or with the combination of them. Most of the bacteria contained in biofertilizer

have a close relationship with plant roots.

Symbiotic N-fixing and phosphate solubilizing microorganisms play an important role in supplementing N and P, respectively, to the plant [1]. N inputs through biological N fixation support sustainable and environment friendly agricultural production. The value of N-fixing legumes in improving yield of legumes and other crops can be achieved by the application of biofertilizers [2]. Phosphate solubilizing bacteria have the capacity to convert inorganic unavailable P form to soluble forms $(\text{HPO}_4)_2^-$ and H_2PO_4 through the process of organic acid production, chelating and ion exchange reactions, and make them available to plants. Phosphate

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solubilizing bacteria can also mobilize insoluble P from fertilizers and soils to soluble P [3, 4]. In addition, phosphate solubilizing microorganisms can improve the plant growth by enhancing the availability of other trace element, such as iron (Fe), zinc (Zn), etc. [5], and synthesize enzymes that can modulate plant hormone level, may limit the available iron via siderophore production and can also kill the pathogen with antibiotic [6].

The inoculation with bacterial mixtures provided more nutrition for the plants and the improvement in root uptake of N and P. Co-inoculation of some phosphate solubilizing bacteria, like *Pseudomonas* and *Bacillus*, with effective *Rhizobium* spp., is shown to stimulate chickpea growth, nodulation, N fixation and have significant effects on nutrient uptake of chickpea [7]. From results of field experiments and field demonstration, Toan [8] concluded that N-fixing inoculants on peanut can reduce N application by 30-60 kg N/ha/year, and increase the crops yield by 5% to 25%. On peanuts, phosphate solubilizing inoculants and rock phosphate can replace 30%-50% mineral P fertilizer without significant change of crops yield, and mixing of symbiotic N-fixing and phosphate solubilizing microorganisms have the same yield, like reducing 20% of basal mineral N and P doses.

Peanut, also known as groundnut (*Arachis hypogaea*) is a crop of global importance and widely cultivated in tropical and subtropical regions. World annual production is about 46 million tons per year [9]. Vietnam has peanut growing area of 208,000 ha in all ecological zones. In last 10 years, the peanut yield in Vietnam increased continuously and reached 2.18 tonnes/ha in 2014, but low compared to other countries, like Israel, America, China and Brasilia. Sandy soil is widely used for peanut production in Vietnam, but it has some constraints for agricultural production, like acid, low inorganic matter, poor in plant nutrients and suffering from serious wind and water soil erosion [10]. New management to improve

the sandy soils fertility already has been reported by Ha et al. [11], but not easy to realize. Practical peanut production in Vietnam needs new solution for sandy soils management.

The purpose of this paper was to isolate, select and evaluate the capability of beneficial microbes used as biofertilizer for peanut growing in sandy soil of Binhdin province.

2. Materials and Methods

Soil and root of peanut were collected following the Vietnam standard [12]. The analyse of the chemical properties was done according to the methods described in Vietnam standard [13-18]. The *Rhizobium* spp. are isolated and evaluated for nodulation on the peanut by methods described by Berk et al. [19]. The N-fixing activity of selected rhizobial strains was determined by acetylene reduction assay according the method described in Vietnam standard [20].

Phosphate and potassium solubilizing bacteria were isolated from soil samples by serial dilution plate count method using Pikovskaya medium for phosphate solubilizer and Aleksandrov medium for potassium solubilizing microbes, respectively. The phosphate and potassium solubilizing activities were determined through the available P and K in the supernatant of growth medium, using the flame photometric method described by Sugumaran and Janarthanam [21] for potassium solubilizer and according to the method described by Gaur [22] for phosphate solubilizer, respectively.

To isolate the slime yeast (*Lipomyces* spp.), soil samples were ground, mixed with water to make a soil ball, placed on agar disk containing Hasen medium and incubated at 28-30 °C for three weeks. The slime apperance around the soil ball was observed to isolate and purify the slime producing microorganisms. The activity of slime producing microorganisms was determined by the method described by Tam [23] as the viscosity of Hansen medium inoculated with

isolated *Lipomyces* spp. after 3 d cultivated on shaker at temperature of 28-30 °C. The viscosity was measured using the Elcometer 2300 and calculated by software Viscosity MasterTM as N·s/m².

To evaluate the effect of isolates on nutrient uptake by peanut, the pot experiments were conducted using the seed inoculation methods with the microbial density of 10⁶ CFU/seed. Mineral N, P, K were applied with a dose of 0.64 g urea, 2.5 g super P and 0.5 g KCl per 10 kg pot for the control. As experimental treatments with inoculation, the amount of mineral fertilizers was reduced 10%, 20% and 30% of required N for N fixer, 10%, 20% and 30% of required P for phosphate solubilizer or 10%, 20% and 30% of required K for potassium solubilizer, respectively.

For study on the moisture keeping of sandy soil inoculated with slime *Lipomyces* spp., pot experiment was also conducted. Sandy soil was inoculated with *Lipomyces* spp. at density of 10⁶ CFU/g soil and has the initial moisture of 40%. The moisture of soil was measured using moisture probe meter in the time of 15, 30, 45 and 60 d after inoculation according to the Vietnam standard TCVN 4048:2011 [24].

The experiments were carried out in the laboratory to study the capability of isolates to adapt the sandy soil condition. Each isolate was grown in a suitable

medium containing NaCl concentration of 0.2‰-0.6‰, with pH from 4.5 to 6.0, at different temperatures ranging from 20 °C to 35 °C. The microbial density of each isolate was determined by plate count method at the time of 48 h after cultivation.

All experiments were repeated three times and data analysis was carried out using the IRRISTAT and Excel program.

3. Results and Discussion

3.1 Chemical and Biological Properties of Sandy Soil

The analysis of chemical and biological properties of sandy soil in the research area in Table 1 showed that soil had low moisture, organic matter and pH, salinity of 0.17‰-0.26‰ NaCl, very low in total and available nutrients and low population of beneficial microbes. There are the main constraints for crops production in this area like other sandy soil in coastal area in Vietnam reported by Vinh [10] and Ha et al. [11].

3.2 Isolation and Selection of Beneficial Microbial Strains

3.2.1 N-Fixing Activity

From nodules of peanut grown in sandy soil, six rhizobial strains were isolated and tested for the N-fixing

Table 1 Chemical and biological properties of sandy soil in Phucat, Binhdin.

Soil properties	Catrinh commune	Cathiep commune
Moisture (%)	4.60 ± 0.400	4.40 ± 0.320
pH (KCl)	4.80 ± 0.180	4.60 ± 0.130
Soil salinity (‰)	0.26 ± 0.050	0.17 ± 0.040
Organic matter (%)	0.18 ± 0.020	0.18 ± 0.014
Total N (% N)	0.03 ± 0.007	0.04 ± 0.006
Total P (% P ₂ O ₅)	0.01 ± 0.005	0.02 ± 0.004
Total K (% K ₂ O)	0.01 ± 0.003	0.02 ± 0.005
Available P (mg P ₂ O ₅ /100 g soil)	6.31 ± 0.152	4.20 ± 0.158
Available K (mg K ₂ O/100 g soil)	1.34 ± 0.062	2.14 ± 0.367
Microbial density (CFU/g)		
N fixer	(2.08 ± 0.13) × 10 ⁴	(2.20 ± 0.19) × 10 ⁴
P solubilizer	(1.10 ± 0.10) × 10 ⁴	(1.90 ± 0.40) × 10 ⁴
K solubilizer	(2.52 ± 0.30) × 10 ⁴	(1.45 ± 0.41) × 10 ⁴
Slime producer	(5.80 ± 0.29) × 10 ³	(1.56 ± 0.25) × 10 ⁴

Table 2 The N-fixing capability of some rhizobial strains isolated from nodules of peanut grown in sandy soil in Phucat, Binhdin.

Rhizobial strains	Nodules number per plant	N-fixing activity (nmol C ₂ H ₂ /plant)
RA1	58.2 ± 2.86	2,355 ± 8.94
RA4	55.5 ± 2.77	1,514 ± 9.06
RA16	60.2 ± 2.28	3,100 ± 14.82
RA18	65.8 ± 2.59	3,458 ± 10.95
RA22	54.6 ± 3.58	1,600 ± 8.34
RA25	54.0 ± 2.77	1,250 ± 9.35

Table 3 Effect of rhizobial strain RA18 on yield of peanut.

Treatments	N content of green biomass (g/pot)	Nodule number (nodule/plant)	Dry green biomass (g/pot)	Pod yield (g/pot)
100% NPK	1.55	186.00	81.80	25.50
100% NPK + RA18	2.02	220.00	89.80	28.56
90% N + 100% PK + RA18	1.98	216.00	88.40	27.82
80% N + 100% PK + RA18	1.80	207.00	86.80	27.61
70% N + 100% PK + RA18	1.59	200.60	83.50	26.38
CV (%)	5.90	3.20	2.80	3.00
LSD _{0.05}	0.20	12.50	4.50	1.50

Table 4 The capability of isolates to solubilizing the Ca₃(PO₄)₂.

Microbial strains	Diameter of Ca ₃ (PO ₄) ₂ solubilizing zone (mm)	Concentration of available P in the medium (mg P ₂ O ₅ /100 mL)
P55	12.0 ± 0.35	15.2 ± 0.45
P100	16.2 ± 0.45	18.5 ± 0.50
P114	14.9 ± 0.22	16.6 ± 0.47
P1107	18.0 ± 0.35	21.2 ± 0.27
P1108	15.9 ± 0.42	16.8 ± 0.57

activity. The results in Table 2 showed that the rhizobial strain RA18 can nodulate more (65.8 ± 2.59 nodules/per plant) and had higher ARA activity (3,458 ± 10.95 nmol C₂H₂/plant) than other isolates, so can be used for further study. Similar results were obtained by Toan [8] and Hiep [25], when they evaluated the N-fixing activity of rhizobial isolated from red and black soils in Vietnam.

Rhizobial strain RA18 was tested for N supplement to peanut. The results in Table 3 indicated that inoculation of peanut with rhizobial strain RA18 can save more than 30% of required mineral N. Sandy soil is general low in nutrients and organic matter [10, 11], which may have negative effect on the symbiotic relationship between *Rhizobium* and peanut root. This is the reason for the lower N-supplement in the study compared to the results obtained by Toan [8] and Hiep [25], who reported that the rhizobial inoculation can

save 40%-60% of required mineral N.

3.2.2 Phosphate Solubilizing Activity

From sandy soil samples, five phosphate solubilizing bacterial strains are isolated and evaluated for the phosphate solubilizing activity. The data in Table 4 showed that all isolates were able to solubilize the Ca₃(PO₄)₂. The results are in line with those obtained by Hiep [25]. The bacterial strain P1107 has higher potential to solubilize the Ca₃(PO₄)₂, i.e., solubilize more larger size (18.0 ± 0.35) of Ca₃(PO₄)₂, compared to other isolates, and thus it will be used for further experiment.

Bacterial strain P1107 was tested for improving the P uptake of peanut. The result in Table 5 showed that by using bacterial strain P1107 inoculation and reduction of 30% required mineral P, peanut green biomass and pod yield had no significant difference with the control. The analysis of P₂O₅ content of green

Table 5 Effect of bacterial strain P1107 on yield of peanut.

Treatments	P ₂ O ₅ content of green biomas (g/pot)	Dry green biomas (g/pot)	Pod yield (g/pot)
100% NPK (control)	0.18	81.80	25.50
100% NPK + P1107	0.20	89.80	27.56
90% P + 100% NK + P1107	0.20	85.90	26.77
80% P + 100% NK + P1107	0.18	82.50	26.62
70% P + 100% NK + P1107	0.17	78.30	25.70
CV (%)	3.70	2.80	2.20
LSD _{0.05}	0.01	3.35	1.09

Table 6 The capability of isolates to solubilizing the fenspat.

Microbial strains	Diameter of fenspat solubilizing zone (mm)	Concentration of available K in the medium (mg K ₂ O/1,000 mL)
S1.1	8.20 ± 0.27	18.40 ± 0.31
S3.1	12.00 ± 0.35	19.20 ± 0.21
S3.3	7.80 ± 0.45	16.00 ± 0.27
S8	6.40 ± 0.42	12.50 ± 0.35
S10	8.00 ± 0.35	16.40 ± 0.44
S11.2	7.60 ± 0.31	15.60 ± 0.31

Table 7 Effect of bacterial strain S3.1 on yield of peanut.

Treatments	K ₂ O content of green biomas (g/pot)	Dry green biomas (g/pot)	Pod yield (g/pot)
100% NPK (Contro)	1.04	81.80	25.50
100% NPK+ S3.1	1.18	87.20	28.42
90% K + 100% NP + S3.1	1.14	84.40	27.63
80% K + 100% NP + S3.1	1.12	83.80	26.83
70% K + 100% NP + S3.1	1.04	81.00	25.15
CV (%)	3.70	2.80	2.20
LSD _{0.05}	0.08	1.78	1.20

biomas showed the same result. Similar results were obtained by Hiep [25]. The data in this study indicated that bacterial strain P1107 was able to increase P uptake by 30% in peanut.

3.2.3 Potassium Solubilizing Activity

From sandy soil samples, six potassium solubilizing bacterial strains were isolated and evaluated for the potassium solubilizing activity. The data presented in Table 6 showed that all isolates were able to solubilize the fenspat and make fenspat to provide available K for plant. While the bacterial strain S3.1 has the highest potential, i.e., can solubilize the largest size (12.0 ± 0.35 mm) of fenspat, and can be used for the further study. The potassium solubilizing activity of microbes depended on the mineral resources and also

on the pH and aerobic condition. Badr [26] found that the release of K in order was illite > fenspat > muscovite.

Bacterial strain S3.1 was tested for improving the K uptake of peanut. The results in Table 7 showed that peanut had the same green biomas, pod yield and green biomas K₂O content by inoculation with bacterial strain S3.1 and reduction of 30% required mineral K compared to the control. The data in this study indicated that bacterial strain S3.1 was capable to support the peanut with an uptake of 30% K more. Similar results were obtained by Sheng [27], who by conducting the pot experiment with K-deficiency soil on cotton and rape seed, reported the K₂O content of green biomas 30% higher than the control without inoculation.

Table 8 The capability of *Lipomyces* isolates to produce the polysaccharid.

<i>Lipomyces</i> strains	Viscosity (10^{-3} N·s/m ²)	Dry weight of polysaccharid in growth medium (g/L)
PT2	26.50 ± 0.79	34.50 ± 0.55
PT1	26.40 ± 0.84	32.00 ± 0.61
PT5.1	37.60 ± 0.42	36.00 ± 0.62
PT12	30.80 ± 0.57	31.80 ± 0.35
PT15	25.60 ± 0.55	30.80 ± 1.15
PT39	10.40 ± 0.42	16.20 ± 0.57

Table 9 Effect of *Lipomyces* strain PT5.1 on the improvement of sandy soil moisture.

Time after inoculation (day)	Soil moisture (%)	
	Control without inoculation	Experiment
0	40.00	40.00
15	24.20	35.40
30	22.60	28.40
45	18.30	22.50
60	13.00	16.80
CV (%)	2.30	2.60
LSD _{0.05}	0.97	1.00

3.2.4 Moisture Keeping Ability

Purposed to improve the moisture keeping for sandy soil, six slime *Lipomyces* isolated were tested for slime production. From the data in Table 8, it can be seen that all isolates were able to produce slime, and the best strain is PT5.1, which can produce 36 g dry polysaccharid in 1 L growth medium.

Lipomyces strain PT5.1 was inoculated to soil with a moisture of 40% at the beginning of experiment. The results in Table 9 showed that *Lipomyces* strain PT5.1 can help the soil to keep its moisture content 15 d longer than the control without the *Lipomyces* strain PT5.1 inoculation. Similar results were obtained by Tam [23] on research of the capability of *Lipomyces* to improve the soil moisture in Me Linh, Vinh Phuc province.

3.3 Evaluation of the Adaptation Capability of Isolates to Sandy Soil Condition

Soil physico-chemical properties can affect the growth and metabolism of microorganisms, thus influencing soil microbial activities. Salinity may restrain microbial activity and increase in soil salinity, leading to decreased soil microbial biomass and soil enzyme activities [28, 29]. Purposed to produce

inoculant for peanut growing in sandy soil, there was a need to evaluate the adaption of selected isolates

Fig. 1 Density of isolates in the growth medium at different temperature (Log₁₀).

Fig. 2 Density of isolates in the growth medium with different pH (Log₁₀).

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Fig. 3 Density of isolates in the growth medium containing different NaCl concentration (Log₁₀).

(RA18, P1107, S3.1 and PT5.1) to the sandy soil condition. From Figs. 1-3, it can be seen that all selected microbial strains were able to grow at temperature from 20 °C to 35 °C, pH from 4.5 to 6.0 and salinity of 0.2‰-0.6‰ NaCl. It can be concluded that the microbial strains RA18, P1107, S3.1 and PT5.1 can grow in the sandy soil of research area.

4. Conclusions

Sandy soil in Binhdin province has low moisture, organic matter, pH, total available nutrients, population of beneficial microbes and high salinity. There is the main limitation for agricultural production in this area. The rhizobial strain RA18 has an N-fixing activity value of $3,458 \pm 10.95$ nmol C₂H₂/plant and can supply 30% of required N for peanut, while the bacterial strain P1107 is able to solubilize phosphate and can increase P uptake by 30% in peanut. Inoculation of peanut with the bacterial strain S3.1 can save 30% required mineral K and the *Lipomyces* strain PT5.1 can produce the slime, which can provide the sandy soil to keep moisture for 15 d longer than the control without the inoculation. All selected isolates are able to grow at temperatures from 20 °C to 35 °C, pH from 4.5 to 6.0, salinity of 0.2‰-0.6‰ NaCl, and can well adapt to the sandy soil conditions.

The selected microbial strains (RA18, P1107, S3.1 and PT5.1) have positive effects on growth, yield of peanut growing in sandy soil and can be used as

biofertilizer to improve the fertility of sandy soil.

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