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Promising plant growth promoting rhizobacteria of *Azospirillum* spp. isolated from iron sand soils, Purworejo coast, central Java, Indonesia

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ABSTRACT

Iron sand soil of Purworejo Coast, Central Java, Indonesia isdominated by sand materials having low organic matters and contains 12.51% of iron. The objectives of the study were toobserve the occurence of Azospirillum spp.on iron sand and rhizosphere soils and to assess theirabilityin fixing atmospheric nitrogen, producing indole acetic acid, and solubilizing inorganic phosphates in vitro. The results showed that a hundred and eighteen strains of Azospirillum spp. were successfully isolated from iron sand and rhizosphere soils. The bacterial population on iron sand and rhizosphere soils were ranged from 0.01-6.0 x 10^5 CFU $g^{-1}$and 0.04-8.0 x 10^7CFU g^{-1}, respectivelly. Most of bacterial isolates were capable of fixing nitrogen ranging from 5.73-99.539 ppm, and higher abilities were shown by six isolates of HR11, HP51, KP11, KR13, KP35, and KR66. These six selected isolatesalso produced IAA and solubilized phosphates. Strain of HR11 showed the highest IAA production which was about58.84μg mL^{-1};and higher phosphate solubilization of $Ca_3(PO_4)_2$ was shown by HR11 and KP35 isolates with E values of 140.74 and 133.13, while higher solubilization to $FePO_4$ showed byKP11 and KR66 strains with E values of 140.60 and 127.22. Among all phosphorous substances, $AlPO_4$ appeared to be the most difficult substance to be solubilized by the six bacterial tested.

Keywords: *Azospirillum*, nitrogen fixation, IAA, phosphate solubilization, iron sand soil.

INTRODUCTION

Indonesia is known to have iron sand mines which are mostly located all along the coasts of south Java, west Sumatera, Kalimantan, Nusa Tenggara, Sulawesi, Papua, and Maluku islands [1]. The iron sand contains 14.6 to 56.75% of iron (Fe)[2]; moreover its physical and chemical properties are dominated by sand texture, having low cathion exchange capacity and organic matters [3]. Iron sand region is a marginal habitat occupied by a little number of plant species only. The habitatmight also limit the growth and development of soil microorganisms, as these organisms are very important for plant growth. Bacteria colonize the rhizosphere and the rhizoplane are known as rhizobacteria.

Rhizobacteria which exert beneficial effects on plant development are denominated as plant growth-promoting rhizobacteria (PGPR) [4]. They are aggressively colonize the rhizospheres[5]. Possible mechanisms of direct plant growth promotion by bacteria arebased on capability of fixing dinitrogen, producing plant hormones (indoleacetic acid, gibberellic acid, cytokinins), solubilizing inorganic phosphates, lowering ethylene level, antagonizing plant pathogens, producing siderophore, and producing β-1,3, glucanase[6-7]. Several well-known bacterial genera of PGPR are *Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella,* and *Pseudomonas* [8].*Azospirillum*is considered the most important rhizobacterial genus for improvement of plant growth and crop yields [9]. Species of *Azospirillum* are free-living nitrogen-fixing bacteria commonly found in soils and in association with roots of plants [10]. The *Azospirillum*-plant association leads to increase the development and yield of various host plants including cereal crops [11], vegetable crops [12], and mangroves [13].

Azospirillum commonly promotes the growth of plantsafter being established in the rhizosphere. Although it posses nitrogen fixing capability, the increase in yields are mainly attributed to improvement of root development due to production of plant growth promoting substances and consequently increases rates of water and mineral uptake [7, 9, 11].

Phosphorus (P) is one of the essential nutrients limiting plant growth which is mostly remains insoluble form in soil [14]. Solubilization of inorganic insoluble phosphates by microorganisms was performed by production of organic acids [15-16]. Phosphate solubilizing bacteria are potential to increase P availability for plant, especially in soils with large amounts of precipitated phosphate [17]. Seed or soil inoculated with phosphate-solubilizing bacteria has beenreported to improve solubilization of fixed soil phosphate and the use of phosphates resulted in higher crops yields [7].

The objectives of this study were to observe*Azospirillum*spp. from iron sand and rhizosphere soils and to assess their ability of fixing atmospheric nitrogen, producing indole acetic acid, and solubilizing inorganic phosphates *in vitro.*

MATERIALS AND METHODS

The bacterial reference strains are*Azospirillumbrasilense* DSM 1690^T, *A. lipoferum*DSM 1840^T (ATCC 29708^T) and *A. halopraeferens*DSM 3675^{T}obtained from Leibniz-Institut DSMZ Germany.

Soil sampling site

The coast of Munggangsari is one of the iron sand mining areas located in Purworejo regency, Central Java province, Indonesia (7^{o}50'37" S longitude; 109^{o}52'34" E latitude). Several plantsgrowing in this area are *Digitaria ischaemum*, *Spinifex littorius* Merr., *Calotropis gigantean* (L.) R.Br., *Calopogonium mucunoides* Desv., *Premna serratifolia* L. (Malbau), *Sebastianachamaelea*, *Pandanus* sp., *Crotalaria pumila* Ortega, *Tilia cordifolia*, *Heliotropium ovalifolium*, *Microstachy schamaelea*, *Richardia scabra* L., *Althernanther amaritima* (Mart.) A.St.-Hill, *Alysicarpus monilifer*(L.) DC., and *Ipomoea pres-caprae*(L.) R.Br..

Isolation and identification of *Azospirillum* spp.

Ten gramsof iron sand soil or rhizospheric soil was suspended in 90 mL sterile distilled water in Erlenmeyer flask and mixed thoroughly on a magnetic stirrer. One millilitre of aliquot was then transferred to 9 mL of sterile distilled water in a test tube and series of dilutions were madeup to 10^{-5}. Serial dilutions were made by spreading 0.1 mL aliquots onto Congo red (RC) medium [18]. After two days of incubation at 30^{o}C, colonies appearing pink or scarlet colour were transferred onto the fresh mediums. The medium for isolation of *Azospirillum* was a semiselective medium, which basically nitrogen-free bromothymol blue (NFb) medium supplemented with Congo red [19].

Azospirillum population was estimated by total plate count method. The total number of bacterial isolates was expressed as colony forming unit (CFU) per gram of iron sand soil or rhizosphericsoil. Isolates grown separately on RCmedium were identified as members of *Azospirillum.* Identification based on morphological (cell shape, colony colour, Gram stain, motility, pellicle formation, pleomorphism), biochemical (catalase, oxidase, nitrate reduction), and nutritional (carbon sources as a sole energy)characteristics was referred to Bergey's Manual of Determinative Bacteriology 9th Edition [20].

Efficiency of N_2 fixation by Kjeldahl analysis [21]

Dinitrogen fixation efficiency analysis of *Azospirillum*isolates were done in semisolid NfB medium. The tubes were incubated for 10 days at 33^{o}C. The amount of N_2 fixation was determined by a Kjeldahl analysis. After incubation, the medium growing isolates was poured into Kjeldahltubes with the salt mixture (40:2.5:1.5 ratio of K_2SO_4, $CuSO_4$ and metallic selenium) and 3mL of concentrated sulphuric acid were added into the tube. The tubes were digested in *Digester DK6/48* (VELP Scientifica) at 420^{o}C for 20 minutes. After digestion and the tubes were cooled, distilled water was added until the final volume of 50 mL.Twentymililitres of digested sample were poured into distillation tube and put under distillation apparatus. In a 250 mL erlenmeyer flask, 20 mL of 4% boric acid and 6 drops of Conway reagent (1000mg methyl red, 150mg bromcresol green, 200ml ethanol 96%) were added. The flask was placed under the condenser of the distillation apparatus and the tip of the condenser outlet was beneath of the solution. Distillation was carried out using *UDK 132 SemiAutomatic distillation unit* (VELP Scientifica) and delivery of 30 mL of 40% NaOH and 100 mL of distilled water was automatically poured through the distillation apparatus. The solution containing distilled NH_3, boric acid and mixed indicator was titrated against 0,05N HCL using Autotitrator (BOECO DCB5000). Calculation of N_2 concentration in the sample was based on the relation:

$$N_2 \text{ in the sample (ppm)} = \frac{\text{Sample titer} - \text{Blank titer x Normality of HClx 14 x 1000000}}{\text{Sample weight (g) x 1000}}$$

Assay for indoleacetic acid (IAA) production

Production of IAA was detected by the modified method of Brick *et al.* [22] and Ahmad *et al.* [23] andquantitative analysis of IAA was performed using the method of Loper and Schroth[24]. Bacterial cultures were grown in NB medium containing 200 µg/mL *L*-Tryptophan (sterilized separately using membrane filter 0.2µm of Millipore) and incubated in shaker incubator (150 rpm) at 33^{o}C for 72h. Fully grown bacterial cultures were centrifugated at 6000 rpm for 20 min. The supernatan (2mL) was mixed with 2 mL Salkowski reagent (1 mL of 135 mg/mL $FeCl_3$, 50 mL of 35% perchloricacid). Development of pink colour indicates production of IAA. Optical density was measured on spectrophotometer (Shimadzu UV mini 1240) at 530 nm. Concentration of IAA produced by the bacterial isolates was conducted by comparing the value of optical density of the sample and IAA standard at concentration ranging from 1–20 µg/mL.

Solubilization of inorganic phosphate

Phosphate solubilization test was conducted in Pikovskaya medium [5]. A loopfull 24-hour bacterial cultures on nutrient agar medium was inoculated onto the Pikovskaya medium ($Ca_3(PO_4)_2$ 5 g,glucose 10 g, $(NH_4)_2SO_4$ 0.5 g,KCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g,yeast extract 0.5 g, $MnSO_4.7H_2O$ 0.025 g, $FeSO_4.7H_2O$ 0.025 g,agar 15 g,distilled water 1000 mL, pH 7.0), and the plates were incubated at 33^{o}Cfor nine-days. The formation of clear zone around the colony was measured. Analysis of the phosphorous solubilization was made by measuring the solubilization efficiency (E) based on the formula of Nguyen *et al.*[25].

$$E = \frac{\text{Solubilization diameter (S)}}{\text{Growth diameter (G)}} \text{ x } 100$$

RESULTS AND DISCUSSION

Isolation and identification of *Azospirillum* spp.

The physical properties of the iron sand soil of Munggangsari coast showed that air and soil temperature were 31-33^{o}C and 29–39^{o}C, and watercontent was from 2.20% to 6.14%. The chemical properties indicated that soil pH ranged from 5.75 to 6.37, carbon and nitrogen contents were 0.39% and 0.07%, respectively. Iron sand soil contained 12.51% Fe. Based on the C/N ratio indicated the fertility of sand soil of Munggangsari coast was very low (5.57). Most of coastal area was characterized by low organic matters and fertility [26], and low cathion exchange capacity causing the content of micro and macro nutrients to decrease [27].

*Azospirillum*is known to be capable of growing in marginal environments due to its efficient physiological mechanisms through formation of cyst or floc, production of melanin, synthezis of poly-β-hydroxybutyrate and polysaccharides [28]. Hundred and eighteen isolates of *Azospirillum*were successfully isolated. They consisted of 31 isolates from iron sand soils and 87 isolates from rhizospheric soils of various plants (Table1).The bacterial colony colours on RC medium showed that 9 isolates were scarlet and109 isolates were pink. Their population densities ranged from 1.0 x 10^3 to 6.0 x 10^5 CFU g^{-1} sand soil and 4.0 x 10^5 to 8.0 x 10^7 CFU g^{-1}rhizosphericsoil. This result indicated the same number with other research. The occurrence of plant growth promoting rhizobacteriaof sand dune in Chennai coast, India ranged from 4.4 x 10^6-7.5 x 10^7 CFU g^{-1} soilwas reported [29]. Higher numbers of *Azospirillum* spp. in rhizospheric soils than that found in iron sand soils also similar to the results reported[28], who stated that *Azospirillum* spp. isolated from bulk soil are usually in proportionally lower numbers than from rhizospheric soil. In bulk soil, the bacteria may survive in vegetative or cyst forms until a host plant is available. The rhizosphere is known rich in nutrients due to the accumulation of a variety of organic compounds released from roots by exudation, secretion and deposition [30].

Population of the bacteria was dominated by pink colonies,howevernine isolates had scarlet colonies grown on RC medium after two days of incubation. The pink colonies became red or scarlet after 3-5 days incubation (Figure 1), and the isolates were assumed as *Azospirillum.*Species of*A. lipoferum*, *A. brasilense*, *A. largimobile*, and *A. doebereinerae* are scarlet on Congo red medium [31]. According to Caceres [18], *Azospirillum*has light-pinkand colourless colonies after 48 h incubation and they become scarlet following 72 h.Further confirmative tests resulted that 44 strains have rod shape cells, and 56 isolates were vibroid cells. All isolates revealedas Gram negative,motilityusing single polar flagellum, forming pellicle in NfB semisolid, and showing pleomorphic cells. Biochemical tests showed that all the strains were positive in oxidase, catalase, and nitrate reduction. Similarly, nutritional tests showed that all isolates could utilize malate, succinate, pyruvate, or lactate as sole carbon sources.

According to Bergey's Manual of Determinative Bacteriology 9th edition [20], these isolates belonged to *Azospirillum.*The dominant forms of *Azospirillum* on solid malate medium are curved rods with various sizes and it revealed polymorphism [31].

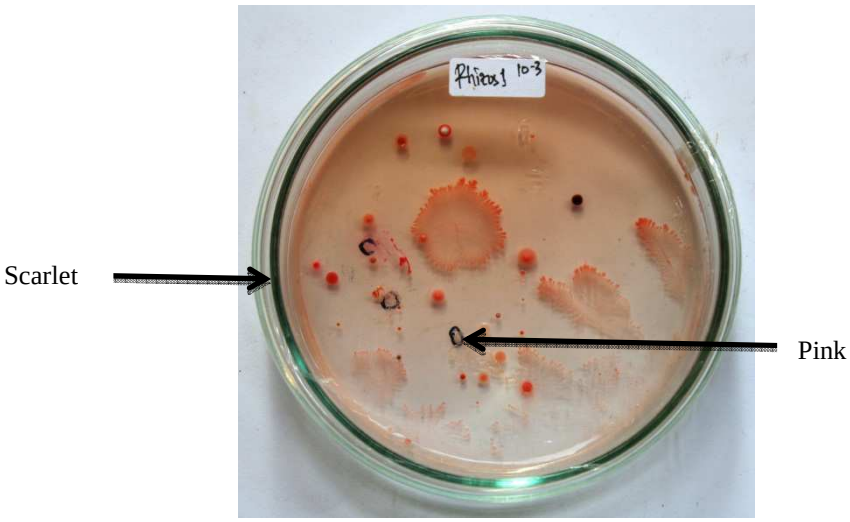

Figure 1. Colony appearence of *Azospirillum* on Congo red medium

Table 1. Sources of isolation of bacterial isolates

Code of isolates	Source of isolation
KP11, KP12, KP13, KP14, HP11, HP12, HP13	Iron sand soil sample 1
KP21, KP22, KP23, KP24, KP25, KP26, KP27, HP21, HP22	Iron sand soil sample 2
KP31, KP32, KP33, KP34, KP35, KP36, KP37, HP31, HP32, HP33	Iron sand soil sample 3
HP41, HP42	Iron sand soil sample 4
HP51, HP52, HP53	Iron sand soil sample 5
KR11, KR12, KR13, KR14, KR15, KR16, KR17, KR18, KR19, KR110, HR11	*D. ischaemum*rhizosphere
KR21, KR22, KR23, KR24, KR25, KR26, KR27, KR28, HR21	*S. littorius*rhizosphere
KR31, KR32, KR33, KR34, KR35, KR36, KR37, KR38, KR39, HR31, HR32, HR33	*C. gigantea*rhizosphere
HR41, HR42, HR43	*C. mucunoides*rhizosphere
HR51, HR52, HR53	*P. serratifolia*rhizosphere
KR61, KR62, KR63, KR64, KR65, KR66, KR67, HR61, HR62, HR63	*S. chamaelea*rhizosphere
KR71, KR72, KR73, KR74, HR71, HR72, HR73, HR74	*Pandanus* sp. rhizosphere
KR81, KR82, KR83, HR81, HR82, HR83	*C. pumila*rhizosphere
HR91, HR92	*T. cordifolia*rhizosphere
HR101, HR102, HR103	*H. ovalifolium*rhizosphere
HR111, HR112	*M. chamaelea*rhizosphere
HR121, HR122, HR123, HR124	*R. scabra*rhizosphere
HR13A1, HR13A2, HR13A3, HR13A4, HR13B1, HR13B2, HR13B3	*A. maritima*rhizosphere
HR141, HR142, HR143	*A. moniliferr*hizosphere
HR151, HR152, HR153, HR154	*I. pres-caprae*rhizosphere

Efficiency of N_2 fixation

The ability of nitrogen fixation of 118 *Azospirillum*isolates was measured by Kjeldahl method in nitrogen-free bromothymol blue semisolid medium. Among the 118 isolates tested, 110 isolates were able to fix nitrogen(Table 2).The amount of nitrogen fixed by the isolates ranged from 5.73–94.54 ppm and strain KR66 isolated from rhizosphere of *S. chamaelea*showed the highest result. The capability of fixing nitrogen of several isolates (KR66, KP11, KR13, HP51) was higher than reference strains of *A. brasilense*DSM 1690^T, *A. lipoferum*DSM 1840^T and *A. halopraeferens*DSM 3675^T. Most of the isolates originated from rhizospheric soil appeared to be higher in fixing nitrogen compared to the iron sand soil isolates. High capability of fixing nitrogen was initial selection of the isolates as PGPR candidates and furthermore they were assayed for IAA production and phosphate solubilization.The selected isolates were KR66, KP11, KR13, HP51, KP35, and HR11.

The ability of *Azospirillum* in fixing nitrogen is also mentioned by many researcher. Nitrogen fixation was the first mechanism proposed to explain the improvement of plant growth following *Azospirillum*inoculations [32]. *Azospirillum* could converts atmospheric nitrogen into ammonium under microaerophilic conditions at low nitrogen levels through the action of nitrogenase[31]. A number of 10 strains of *Azospirillum*spp. isolated from paddy rhizosphere soil were able to fix nitrogen ranging from 11.0–15.06 mg 'N" kg^{-1} measured by micro Kjeldahl method [21]. The nitrogen fixing ability of *Azospirillum*spp. isolated from rhizosphere of Taro (*Colocasiaesculenta* L.

Schott) was between 2.0–6.16 mg N g^{-1} substrate [33].Variability of the nitrogenase activity of *Azospirillum*has also been observed *in vitro* with ARA method varying from 5.70–14 nmol C_2H_4 $hour^{-1}$[34]. Amounts of fixed nitrogen by *A. zeae*and *A. brasilense*were 6–7.6 and 7.1–44.3 nmol C_2H_4 $hour^{-1}mg^{-1}$ protein [35].

Table 2.Nitrogen fixation of *Azospirillum*isolates and reference strains

Isolates	Concentration of N_2 (ppm)
KR66	94.54
KP11	90.24
KR13	87.38
HP51	84.51
KP35	58.73
HR11	55.86
HR141	54.43
KR67	50.14
HR154	48.70
KR110	45.84
HR124	44.41
HR42	42.97
KR33	41.54
HR152	40.11
KR16, HR92, HR121, HR13B2, KR36, KR39	35.81
KR61, HP12, HP53, HR53, HR13A3	34.38
KP25, KR22, KR26, HP42, HR122	32.95
KR15, KR35, HR13A4,	31.51
KR17	30.08
KP14, KP21, KR82	28.65
KR18, KR24, HR21, HR52, HR61, HR13B1	27.22
HP22, KR32, HR33, HR13A2	25.78
KP26, KR25, HR43, HR153, HR41, HR103	24.35
KR74, HP32, HP41, HR73, HR81, HR112	22.92
KR21, KR63, KR72, HR51, HR13B3, HR143	21.49
KR11, HR32, KP33, HR31	20.05
HP21, HR72, HR151, KP37	18.62
KP13, HP33, HR102	17.19
KP23, KP34, KR12, KR73, HP13, HR74, HR82	15.76
KP24, KR27, KR28, KR62, KR83, HP52	14.32
KP36, KR14, KR81, HP31, HR62, HR111	12.89
KP31, HP11, HR83	11.46
KR23, KR64, KR71	10.03
KR65, KP32, KR19, HR63, HR123, HR13A1	8.59
KR34	7.16
HR71	5.73
KP27, KR37, KP12, HR91, KR31, HR101, HR142, KR38	ND
DSM 3675^T	71.62
DSM 1840^T	31.51
DSM 1690^T	28.65

ND: Not detected.

Table 3. Production of IAA by selected isolates and reference strains

Isolates	IAA Productions (µg/mL)
HR11	58.84± 13.48
HP51	38.41± 3.28
KP11	36.05± 2.35
KR13	41.83± 1.30
KP35	50.25± 9.66
KR66	32.20± 4.75
DSM 1840^T	20.02± 4.76
DSM 3675^T	14.80± 6.13
DSM 1690^T	5.73± 1.29

Production of IAA

Production of plant growth-promoting substances such as IAA is one of the principal mechanisms of PGPR candidates. The results showed that IAA produced by six selected isolates were higher compared to the three reference strains, and isolate of HR11 showed the highest IAA production of 58.84µg/mL (Table 3). The six selected *Azospirillum* spp also showed higher IAA production compared to *A. brasilense*strains Cd and Az39 which were produced IAA in amounts of 10.8 and 0.75 µgmL^{-1}[36]. In contrast, concentration of IAA produced by HR11

was lower compared to several *Azospirillum* strains reported[37] which produced IAAin the range of 29 to761 ppm in LBT medium containing *DL*-Tryptophan.

Solubilization of phosphates

Formation of clear zones around the colony on Pikovskaya medium indicates that the bacterial isolates are able to solubilize inorganic phosphates. High phosphate solubilization of $Ca_3(PO_4)_2$ was found on HR11, KP35 and DSM 1690^T isolates, while toward $FePO_4$ was by KP11, KR66, and DSM 3675^T strains (Table 4). Among all phosphorous substances, $AlPO_4$ appeared to be the most difficult to be solubilized by the bacterial tested. Phosphorous solubilization efficiency of most isolates tended to decrease based on the solubility level of inorganic phosphates tested inwhich $AlPO_4$ is known to be the most insoluble followed by $FePO_4$ and $Ca_3(PO_4)_2$. Solubilization of inorganic phosphates is due to the action of organic acids, especially gluconic acid, synthesized by the bacteria [38].

Table 4.Phosphatesolubilization of selected *Azospirillum*isolates and reference strains

Isolates	Phosphates Solubilization Efficiency (E)		
	$Ca_3(PO_4)_2$	$FePO_4$	$AlPO_4$
HR11	140.74 ± 16.18	119.69 ± 0.68	105.96 ± 2.37
HP51	123.81 ± 10.12	118.52 ± 12.81	108.59 ± 1.27
KP11	124.61 ± 8.08	140.60 ± 19.17	119.40 ± 3.85
KR13	111.84 ± 12.55	121.90 ± 1.18	100.00 ± 0.00
KP35	133.13 ± 20.84	125.79 ± 10.70	110.93 ± 7.04
KR66	100.76 ± 1.31	127.22 ± 4.34	100.00 ± 0.00
DSM 1840^T	123.25 ± 7.59	119.65 ± 9.65	100.00 ± 0.00
DSM 3675^T	110.72 ± 11.49	136.17 ± 14.64	111.69 ± 5.47
DSM 1690^T	136.68 ± 15.49	115.51 ± 1.47	109.25 ± 3.80

Phosphorous is the most limiting nutrient in tropical soil, there is only 0.1% of the total P available to the plants because of its chemical bonding and low solubility [39]. Theability of phosphate solubilization of selected *Azospirillum* isolates of HR11, HP51, KP11, KR13, KP35, and KR66 were similar to 6 bacterial strains isolated [29] from coastal sand dunes of Chennai coast, India. Strains of *Azospirillum*spp. isolated from different environments, including coastal areas were also able to solubilize phosphate with E values ranging from 100 to 160 [40].Regarding IAA production and phosphate solubilization activity, isolates from the rhizosphere appeared to be more efficient as auxin producers than the isolates from the bulk soil, and a considerably higher concentration of phosphate solubilizing bacteria are commonly isolated from the rhizosphere [29].

CONCLUSION

It was concluded that the six selected*Azospirillum* sppof HR11, HP51, KP11, KR13, KP35, and KR66 might play a role in the growth of plants in the iron sand habitat. Based on the results of nitrogen fixation, IAA production, and phosphate solubilization, they appeared to be promising as PGPR candidates, especially for plants growing in marginal agricultural practices.Further study about characterization and identification of the selected isolates by systematic polyphasicapproach are in progress.

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REFERENCES

[1] B.N.Widi, Penyelidikanendapanpasirbesi di daerahpesisirselatanEnde-Flores Propinsi Nusa Tenggara Timur. LaporanHasilPenyelidikan PT. Ever Mining, **2004.**

[2] I.Z. Yasir,EksplorasipasirbesipantaiAdipalaCilacapJawa Tengah. http://tri-online.biz/eksplorasi-pasir-besi-pantai-adipala-cilacap-jawa-tengah/,**2011**, (Accessed on25th June 2012).

[3] G.Djajakirana, D.Tjahyandari,Supijatno,Reklamasilahanbekastambangpasirbesimelaluiteknikameliorasi in situ bahanorganik,LaporanPenelitian, IPB Bogor, **2009**.

[4] J.W. Klopper,M.N.Schroth, Plant growth-promoting rhizobacteria in radish.In: Angers (Ed.), Proceedings of the 4th International Conference on Plant Pathogenic Bacteria, Gilbert-Clarey, Tours, France,**1978**, 879-882.

[5] N.S. SubbaRao, Biofertilizer in agriculture, Oxford and IBH Publishing Co., New Delhi, Bombay, Calcuta, **1982**.

[6] S. Mahalakshmi,D. Reetha,*Recent Research in Science and Technology*, **2009**,1(1), 26-29.

[7] K.V.B.R. Tilak, K.K. Pal, R. Dey,Microbes for sustainable agriculture, I.K. International Publishing House Pvt. Ltd., New Delhi, India, **2010**.
[8] L.E. Fuentes-Ramirez, J.Caballero-Mellado, In:Z.A. Siddiqui (Ed.), PGPR: Biocontrol and biofertilization (Springer, Netherlands,**2010**) 143-172.
[9] Y.Bashan,G. Holguin, L. de-Bashan,*Can. J. Microbiol.*, **2004**, 50, 521-577.
[10] R.O. Pedraza, J. Motok, M.L. Tortora, S.M. Salazar, J.C. Diaz-Ricci, *Plant and Soil*, **2007**,295, 169-178.
[11] B.S. Saharan, V. Nehra,*Life Sciences and Medicine Research*,**2011**, 2011, 1-30.
[12] C.A.Barassi, R.J.Sueldo,C.M.Creus,L.E.Carrozzi,E.M. Casanovas, M.A.Pereyra, *Dynamic Soil, Dynamic Plant*, **2007**,1(2), 68-82.
[13] K. Sahoo, N.K. Dhal, *Indian Journal of Marine Sciences*, **2009**,38(2), 249-256.
[14] C.S. Nautiyal,*FEMS Microbiology Letters*, **1999**,170, 265-270.
[15] C.Leyval,J. Barthelin, *Plant and Soil*, **1989**, 17, 103-110.
[16] E.Husen, *Indonesian Journal of Agricultural Science*, **2003**,4(1), 27-31.
[17] A.H.Goldstein, *Am. J. Altern. Agric.* **1986**, 1, 51-57.
[18] E.A.R.Caceres, *Journal of Applied and Environmental Microbiology*,**1982**,44(4), 990-991.
[19] Y.Bashan, G. Holguin, R.Lifshitz,In:Bashan *et al.* (Eds.), Methods in Plant Molecular Biology and Biotechnology (CRC Press, Inc., **1993**) 331-345.
[20] J.D.Holt, N.R.Krieg,P.H.A.Sneath, J.T.Staley,S.T.Williams, Bergey's Manual of Determinative Bacteriology 9th Edition, Lippincott Williams & Wilkins, Philadelphia, **1994**.
[21] K. Kanimozhi,A. Panneerselvam,*Der ChemicaSinica*, **2010**,1(3), 138-145.
[22] J.M Brick, R.M.Bostock, S.E. Silverstone, *Journal ofApplied and Environmental Microbiology*, **1991**,57, 535-538.
[23] F.Ahmad, I.Ahmad, M.S.Khan, *Turk. J. Biol.*,**2005**,29, 29-34.
[24] J.E. Loper, M.N. Schroth, *Phytopathology*, **1986**,76, 386-389.
[25] C. Nguyen, W. Yan, F. Le Tacon, F.Lapeyrie,*Plant and Soil*, **1992**,143, 193-199.
[26] H.J.W. Verplancke,In:H.J.W. Verplancke, E.B.A. De Strooper, M.F.L. De Boodt (Eds.),Water saving techniques for plant growth (Kluwer Academic Publisher. Dordrecht, Netherlands, **1992**).
[27] F.I. Massoud,In:Sandy soil, Report of FAO/UNDP Seminar on Reclamation and Management of Sandy Soils in the Near East and North Africa,**1975**, FAO-UNO, Rome,47-72.
[28] Y.Bashan, *Biol. Fertil. Soils*, **1999**,29, 246-256.
[29] R. Muthezhilan, B.S.Sindhuja, A.J. Hussain, M.Jayaprakashvel,*Pakistan Journal of Biological Sciences*, **2012**,15(16), 795-799.
[30] R. Pinton, Z. Varantini, P. Nannipieri, The Rhizosphere: Biochemistry and organic substances at the soil-plant interface, Marcel Dekker Inc., New York, **2001**.
[31] J.E.Baldani, N.R.Krieg, V.L.D.Baldani,A. Hartmann, J.Dobereiner, In*:* Brenner *et al.* (Eds.),Bergey's Manual of Systematic Bacteriology, Second edition, Vol. 2, Part C(Springer, East Lansing, **2005**) 7.
[32] I.H.Attitalla, A.M.Alhasin, M.A.Nasib, A.H.Ghazali,L.Zakaria, H.M.Jais, I.A.A.Balal, B.Salleh, *American-Eurasian J. Agric. & Environ. Sci.*,**2010**,8(6), 617-625.
[33] S.N. Jolly, N.A.Shanta, Z.U.M. Khan. *International Journal of Botany*, **2010**,6(2), 117-121.
[34] I. Rusmana, D.D.Hadijaya, *Hayati*, **1994**,1(2), 51-54.
[35] A. Venieraki, M.Dimou, E. Vezyri, I. Kefalogianni, N.Argyris, G. Liara, P. Pergalis, I. Chatzipavlidis, P.Katinakis,*The Journal of Microbiology*, **2011**,49(4), 525-534.
[36] D. Perrig, M.L.Boiero, O.A. Masciarelli, C. Penna, O.A. Ruiz, F.D. Cassan, M.V. Luna,*Appl. Microbiol. Biotechnol.*,**2007**,75(5), 1143-1150.
[37] Gh.A. Akbari, S.M. Arab, H.A. Alikhani, I. Allahdadi, M.H. Arzanesh, *World Journal of Agricultural Sciences*, **2007**, 3 (4), 523-529.
[38] H. Rodriguez,R. Fraga,*Biotechnology Advances*, **1999**,17, 319-339.
[39] K.V.B.R. Tilak, N.Ranganayaki,K.K.Pal,R.Dey, A.K.Saxena, C.S.Nautiyal, S.Mittal,A.K.Triphati,B.N.Johri, *Current Science*, **2005**,89, 136-150.
[40] S. Widawati, A.Muharam,*JurnalHortikultur*, **2012**, 22(3), 258-267.