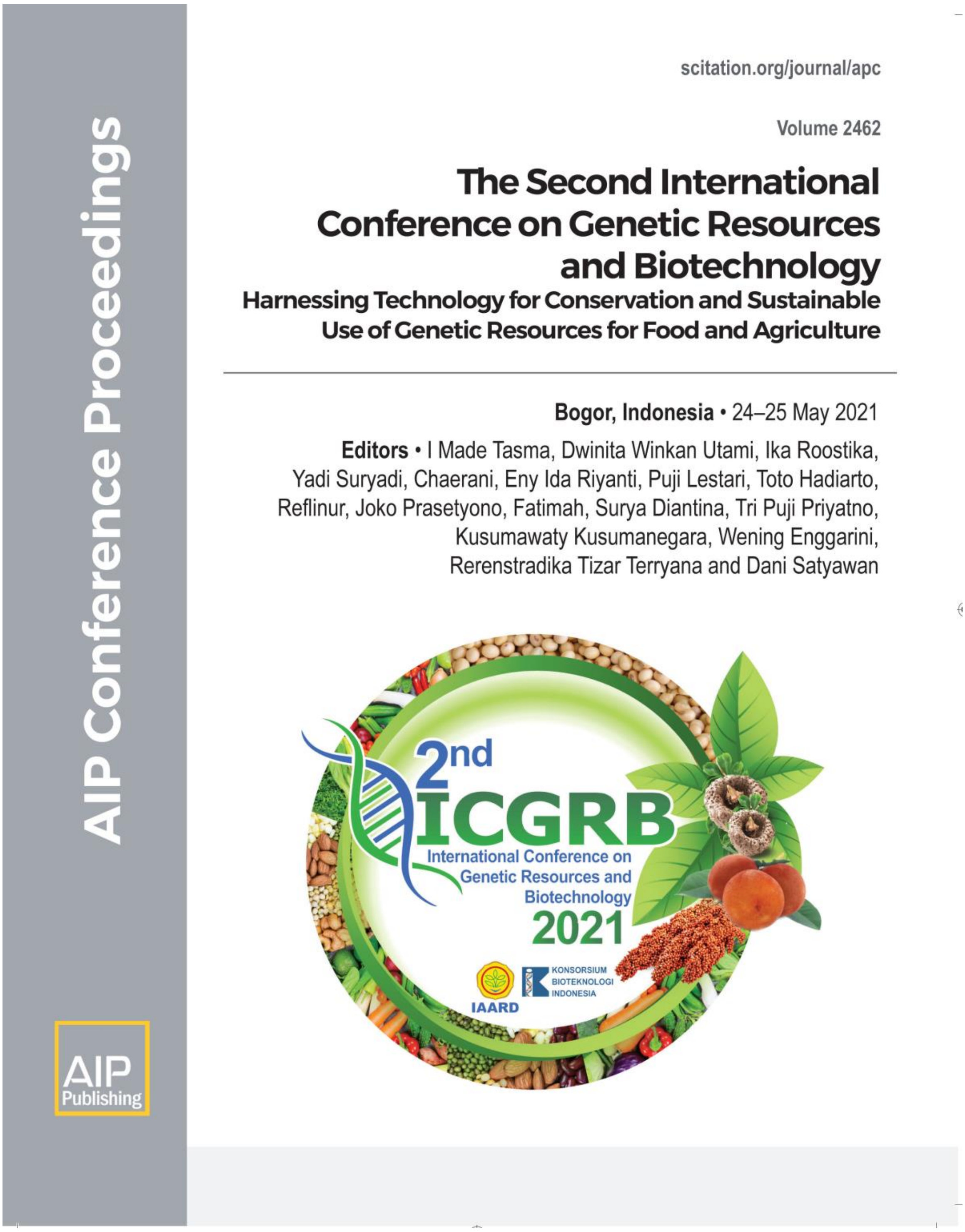

January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Cite as: AIP Conference Proceedings **2462**, 010001 (2022); https://doi.org/10.1063/12.0006897
Published Online: 19 January 2022

Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24^{th}-25^{th} of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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AIP Conf. Proc. 2462, 010001-1–010001-1; https://doi.org/10.1063/12.0006897
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Location: Bogor, Indonesia
ISBN: 978-0-7354-4172-9
Editors:
I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tru Puji Priyanto, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan
Volume number: 2462
Published: Jan 19, 2022

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Location: Bogor, Indonesia
ISBN: 978-0-7354-4172-9
Editors:

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Published Online: 19 January 2022

Edy Listanto, Ahmad Warsun, Ahmad Dadang, et al.

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Cite as: AIP Conference Proceedings **2462**, 060011 (2022); https://doi.org/10.1063/5.0075843
Published Online: 19 January 2022

Kurnia Dewi Sasmita, Iswandi Anas, Syaiful Anwar, et al.

Application of Phosphate Solubilizing Microbes to Promote the Effectiveness of Rock Phosphate on Cacao Seedling Growth in Acid Soil

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Abstract. The effectiveness of rock phosphates (RP) on plant growth is often lower than that of chemically synthesized fertilizer SP-36. Phosphate solubilizing microbes (PSM) are potentially used to enhance RP dissolution and agronomic effectiveness for cacao plant. This study was carried out to assess the ability of several PSM to dissolve insoluble phosphate, produce phytohormones, and increase the effectiveness of RP in cacao seedling at acid soil. Five phosphate solubilizing bacteria (PSB) and one phosphate solubilizing fungi (PSF) were evaluated for their ability to solubilize insoluble phosphate in liquid medium (added $Ca_3[PO_4]_2$, RP, and $AlPO_4$) and to produce phytohormones. Six isolates were also evaluated in cacao nursery consisting of nine treatments (without P fertilizer; SP-36 fertilizer; only RP; RP with inoculation of five PSB and one PSF, respectively). The result showed that the highest P solubilization in liquid medium with $Ca_3(PO_4)_2$, RP, and $AlPO_4$ was obtained by *Aspergillus niger* F-E1, *Burkholderia ambifaria* B-SS1.2, and *Pseudomonas fluorescens* B-SS13.5, respectively. All isolates could produce the IAA, GA3, trans-zeatin, and kinetin. Whereas *B. ambifaria* B-SS1.2, *B. cepacia* B-SS19.7, and *A. niger* F-E1 increased shoot dry weight and relative agronomic effectiveness (RAE) of RP. Notably, the *B. ambifaria* B-SS1.2 increased N, P, and K uptake in shoot of cacao seedlings. The utilization of these microbes potentially promote the effectiveness of RP for cacao plant growth in acid soil.

INTRODUCTION

Phosphorus (P) is one of the essential nutrients for plant growth, which is involved in energy metabolism, biosynthesis of nucleic acids and cell membranes, respiration, and photosynthesis [1]. Phosphor uptake in the plant is relatively low compared to other macro elements, in which, P limitations may cause low plant growth. Visually, P-deficient plants showed symptoms of inhibited leaves development, i.e. smaller and less number of leaves [1]. Phosphorus deficiency in plant often occurs in tropical soils especially in acid soils. The low P availability in the acid soil is caused by P fixation in the soils by soluble Al and Fe ions, oxides/hydroxides of Al and Fe, and clay minerals [2]. This low P availability in soil may decrease the growth and yield of the perennial crop including cacao (*Theobroma cacao L.*).

An adequate P supply is necessary to support optimal cacao growth and productivity in acid tropical soils. Cacao plant gives a positive response to P fertilizer application on soil with low P availability [3]. Rock phosphate (RP) is suitable as source of P and potential as liming material in acid soil [4], but the effectiveness of RP on plant growth are often lower than that of the SP-36 fertilizer, and its efficiency may be low because the soluble P would be fixed

The Second International Conference on Genetic Resources and Biotechnology
AIP Conf. Proc. 2462, 060011-1–060011-9; https://doi.org/10.1063/5.0075843
Published by AIP Publishing. 978-0-7354-4172-9/$30.00

by Al and Fe in acid soils. Therefore, an alternative method using phosphate solubilizing microbes (PSM) for improving the effectiveness of RP for crops needs to be considered.

Many researchers have isolated and characterized various species of PSM from various sources including non-rhizosphere soils, rhizoplane, phyllosphere, rhizosphere, soil with low P availability, and RP [5, 6]. Evaluation of the ability of microbes in solubilizing phosphate quantitatively at liquid culture medium commonly uses some type of inorganic P, such as $CaHPO_4$, $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and RP [7–9]. Various kinds of phosphate solubilizing bacteria (PSB) which were characterized belong to the genera *Agrobacterium*, *Burkholderia*, *Mesorhizobium*, *Rhizobium*, *Pseudomonas*, *Streptomyces*, *Bacillus*, *Enterobacter*, *Achromobacter*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, *Azotobacter*, *Azospirillum*, and *Erwinia* [9, 10], and fungi that belong to genera *Aspergillus*, *Penicillium*, and *Trichoderma* [11].

PSM potentially enhance RP dissolution and reduce phosphate fixation in soil and and thus improve the agronomic effectiveness of RP as fertilizer. Phosphate solubilizing activity of PSM was associated with the release of organic acids and lowering of pH in the medium by proton release [8]. The role of organic acids produced by PSM in solubilizing insoluble phosphate may be due to the lowering of pH, chelation of cations both iron and aluminum ions associated with phosphate and by competing with phosphate for adsorption sites in soil [12]. PSM also could promote plant growth by producing phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA3), production of siderophores, N_2 fixation, ACC deaminase activity, secrete biocontrol agent, and enhance drought tolerance [8, 13–16].

The PSM including bacteria, fungi, and actinomycetes in soil has been shown to improve the effectiveness of RP to various crops including cacao [17–19]. Studies on the use of P solubilizers from cacao rhizosphere to increase cacao growth in acid soils are still limited. This study aimed to assess the ability of several PSM to dissolve insoluble phosphate, produce phytohormones and increase the effectiveness of RP in cacao seedling at acid soil.

MATERIALS AND METHODS

Characterization of Potential Phosphate Solubilizing Microbes

Bacteria isolates were isolated from the rhizosphere of cacao plant in Bulukumba, South Sulawesi Province, which characterized being as acid soil. Bacteria isolates were isolated by the dilution plate methods and grown on Pikovskaya's agar medium. Halozone (zone of solubilization)-forming isolates were purified in the nutrient agar. The fungi used in this study was F-E1 which is the collection of Soil and Environmental Biotechnology Laboratory, IPB University.

Five bacteria isolates (B-SS1.2, B-SS13.5, B-SS19.2, B-SS19.7, and B-SS20.4) and one fungi isolate (F-E1) were grown at Pikovskaya's agar, and incubated at 30°C for 7 days. Solubilization index was measured using that formula [20]:

$$SI = \frac{\text{colony diameter} + \text{halozone diameter}}{\text{colony diameter}}$$

Furthermore, the PSM were grown at liquid Pikovskaya's medium with insoluble tricalcium phosphate (TCP), RP, and aluminum phosphate ($AlPO_4$) 5 g/l. One ose culture of PSM was transferred into 250 ml Erlenmeyer flask containing 50 ml of each three insoluble phosphate type, previously sterilized in an autoclave (120°C, 15 min). The PSM isolates were incubated at 30°C with shaking (120 rpm) for 7 days. The solution was determined for soluble P using the phospho-molybdate blue color method [21] and pH of solution using a pH meter.

Identification of all isolates was carried out using molecular techniques. Bacteria B-SS2.1 and fungi F-E1 were previously published by Sasmita *et al*. [22] as *B. ambifaria* B-SS1.2 and *A. niger* F-E1. Bacteria isolates were grown in Nutrient Broth medium at 25°C on a rotary shaker (150 rpm) for 3 days and its biomass was harvested with centrifugation (13,000 rpm) for 5 min. Bacterial biomass were then extracted with GF-1 Nucleic Acid Extraction Kits (Vivantis Technologies, Malaysia) according to the manufacture protocol. Bacterial isolates were identified by 16S rDNA region with primer PSL/16S2 (5'-AGGATTAGATACCCTGGTAG-3') and XB4/16S2 (5'-GTGTGTACAAGGCCCGGGAA-3'). PCR was performed in a total volume of 40 µl with the composition: 2 µl genomic DNA 20–40 ng/µl, 25 µl KAPA2G Fast ReadyMix (Kapa Biosystems, USA), 2 µl PSL/16S2 and XB4/16S2 primers, and 14 µl sterile ddH_2O. The PCR reaction was performed with initial denaturation at 95°C for 5 min, 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and elongation at 72°C for 1 min. The final extension was carried out at 60°C for 15 min. The amplified PCR product exhibiting a single band on agarose gel electrophoresis was purified and used as a template for sequencing using a BigDye Terminator Cycle Sequencing Kit V.3.1 (Applied Biosystems, USA). Sequence analysis was performed with an automatic sequencer, ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA) and sequence results were analyzed using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). For homology analysis, sequences were compared with non-redundant sequence data using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The analysis of plant growth promoting hormones (IAA, GA3, trans-zeatin, and kinetin) from isolates was carried out using the high performance liquid chromatography (HPLC) [23–25]. Bacterial isolates were grown on nutrient broth and fungi was grown on potatoes dextrose broth media for 5 days, then 5 ml of liquid sample were taken and 20 ml of 65% MeOH solvent was added. The solution was then centrifuged at 4,000 rpm for 30 min, and filtered to remove the supernatant. The filtrates was then injected 10 µl into the HPLC for measurement of IAA, GA3, zeatin, and kinetin.

Nursery Assay of PSM on Cacao Seedlings Growth

Five bacteria and one fungi strains were prepared for cacao nursery assay until 20 weeks. Acid soil for planting media was collected at a depth of 0–20 cm from Typic Hapludults in Jasinga, Bogor. The properties of the studied soil are shown in Table 1.

TABLE 1. Characterization of the studied soil [26].

Soil properties	Value	Criteria
pH H_2O (1:5 w/w)	3.90	Highly acidic
Organic C (%)	1.60	Low
Total N (%)	0.26	Medium
Available P (mg/kg)	1.77	Very low
Exchangeable K (cmol [+]/kg)	0.27	Low
Exchangeable Ca (cmol [+]/kg)	1.62	Very low
Exchangeable Mg (cmol [+]/kg)	1.68	Medium
Exchangeable Na (cmol [+]/kg)	0.20	Low
CEC (cmol/kg)	31.90	High
P HCl 25% (P_2O_5 mg/100 g)	18.10	Low
K HCl 25% (K_2O mg/100 g)	10.00	Low
Exchangeable Al (cmol/kg)	19.82	High

RP used from Ciamis has characteristic 21.89% total P_2O_5, 16.67% P_2O_5, soluble citric acid 2%, 45.96% CaO, and 0.11% MgO [22]. Medium of seedlings was 2.2 kg dried soil passed through 2 mm mesh sieve filled in polybag sized 20 cm × 30 cm. F1 hybrid seed from Sulawesi 1 x TSH 858 crosses were firstly planted for 10 days in sand medium that has been sterilized with autoclave at 121°C for 15 min, then transplanted to polybag. This study used completely randomized design (CRD) with four replications. The treatments consisted of 9 treatments: P0 = without P fertilizer, P1 = SP-36 fertilizer, P2 = only RP, P3 = RP + B-SS1.2, P4 = RP + B-SS13.5, P5 = RP + B-SS19.2, P6 = RP + B-SS19.7, P7 = RP + B-SS20.4, and P8 = RP + F-E1.

Treatments of RP and SP-36 with dose of 400 mg/kg P were homogenized in the soil media, with basic fertilizers of 2 g of N and 2 g of K_2O. The media were incubated for one week at field capacity before the cacao seed grown in each polybag. An isolate of PSB was grown in nutrient broth for 3 days and applied 5 ml culture concentrate (10^9 CFU/ml) per polybag. An isolate of phosphate solubilizing fungi (PSF) was grown in potato dextrose broth (PDB) for 5 days and was applied 5 ml culture concentrate (1×10^8 CFU/ml) per polybag. Inoculation of PSM was applied in soil around the roots of cacao seedlings when the seedlings transplanted in the polybag. The plant were maintained in field capacity. Plant growth parameters were observed at 20 weeks after planting including plant height, stem diameter, number of leaves, plant dry weight (shoot and root dry weight), and nitrogen (N), phosphor (P), and potassium (K) uptake on shoot of cacao seedlings.

Analysis of total N, P, and K content in shoot was carried out by using wet digestion method with H_2SO_4-H_2O_2. The extractant was then measured for N content by distillation, P content with a spectrophotometer, and K content with atomic absorption spectrophotometry (AAS). The N, P, and K content in the shoot was then multiplied by the dry weight of the shoot to obtain the amount of nutrient uptake in shoot for each plant.

Data Analysis

Relative agronomic effectiveness (RAE) of RP was calculated based on total plant dry weight at 400 mg/kg P with the calculation formula as follows [27]:

$$\text{RAE (\%)} = \frac{TdRP - TdC}{TdS - TdC} x100$$

where TdRP = total dry weight of plant at RP treatment, TdS = total dry weight of plant at SP-36 treatment, and TdC = total dry weight of plant at control (without P). Classification of effectiveness consisted of four groups, i.e. high (RAE>90), medium (90–70 RAE), low (70–30 RAE), and very low (RAE<30).

Data were statistically analyzed the ANOVA to test all means and LSD test at 5% significance level using Statistical Analysis System (SAS).

RESULTS AND DISCUSSIONS

Characterization of Potential Phosphate Solubilizing Microbes

Phosphate Solubilization Assays

The bacterial and fungal isolates used in this study were isolated using Pikovskaya's agar medium. The halo zone formed around the colony on Pikovskaya's agar medium indicates that these isolates have the potential to solubilize tricalcium phosphate. Phosphate solubilization index (SI) in Pikovskaya's agar medium was resulted by 5 bacterial isolates and 1 fungal strains ranged from 1.19 to 6.20 at 7 days of incubation period (Table 2). The highest SI value was produced by B-SS19.7 and the lowest was produced by F-E1. The bacteria strains from *Burkholderia* reported in the previous study [28] had an SI ranging between 3.23±0.48 and 4.88±0.84 at 5 days of incubation period. While Elias *et al*. [29] reported SI in several strains of fungal (*Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.) ranged from 1.10 to 3.05 at 7 days of incubation period.

TABLE 2. Solubilization index (SI), pH, and phosphate solubilized by PSM in liquid medium with tricalcium phosphate ($Ca_3[PO_4]_2$), rock phosphate, and $AlPO_4$.

Isolates	Solubilization index	$Ca_3(PO_4)_2$		Rock phosphate		$AlPO_4$	
		pH	Soluble P (µg/ml)	pH	Soluble P (µg/ml)	pH	Soluble P (µg/ml)
B-SS1.2	3.43	3.43	185.19	3.14	360.70	3.38	105.39
B-SS13.5	2.06	3.99	189.86	3.71	98.64	2.99	174.42
B-SS19.2	3.87	3.32	203.92	3.47	171.89	3.02	88.74
B-SS19.7	6.20	3.37	110.18	3.93	219.50	3.04	125.46
B-SS20.4	3.92	3.64	518.65	3.32	93.14	3.61	166.29
F-E1	1.19	3.71	530.56	3.29	171.82	2.92	151.00
Control	-	6.14	58.29	7.37	25.08	5.05	19.36

The ability of microbes to solubilize insoluble phosphates in liquid medium which added with tricalcium phosphate, RP, and $AlPO_4$ was measured quantitatively (Table 2). This study showed that the effectiveness of microbes in phosphate solubilization was different in some types of P inorganic source. Phosphate solubilization by bacterial and fungal on medium with tricalcium phosphate ranged from 110.18 to 530.56 µg/ml P. The highest soluble P on tricalcium phosphate medium was produced by F-E1. Meanwhile, phosphate solubilization in the medium with RP ranged from 93.14 to 360.70 µg/ml P, with the highest solubility from B-SS1.2 strain. The highest soluble P from $AlPO_4$ medium was resulted by B-SS13.5, with solubilization by all strains ranged from 88.74 to 174.42 µg/ml P.

The solubilization of phosphate by microbes inoculation was accompanied with reduction of pH in liquid medium. The final pH of the liquid medium with tricalcium phosphate inoculated ranged from 3.32 to 3.99, lower than the control (6.14). The medium containing RP resulted pH in the ranged from 3.14 to 3.93, lower than the control (7.37), and in medium with $AlPO_4$ produced pH in the ranged from 2.92–3.61, with control (5.05). The decrease in pH in the liquid medium is related to the phosphate solubilizing process. The main mechanism of phosphate solubilization was acid organic production leading to decrease in pH [30]. This acid is a product of microbial metabolism, from oxidative respiration or from fermentation of organic carbon sources [11].

Acidification does not to be the only mechanism of solubilization, organic acids also will release P minerals by enhancing chelation of the cations bound to P and by forming soluble complexes with metal ions associated with insoluble P (Ca, Al, and Fe) [11]. The phosphate solubilizing activity of microbes is determined by the biochemical ability to produce and release organic acids [31]. Organic acids which can be released by the PSM were gluconic acid, oxalic acid, citric acid, acetic acid, lactic acid, malic acid, succinic acid, propionic acid, tartaric acid, aspartic, glyoxalate, fumarate, glutamate, pyruvate, and oxaloacetate [8, 11, 12, 32, 33]. Other mechanisms for P dissolving besides the release of organic acids are the production of siderophores, the release of protons through cellular respiration and absorption of NH_4, and the production of exopolysaccharides [11, 34].

Identification of Microbes

The results of the identification of bacterial isolates are presented in Table 3. Bacteria B-SS2.1 as *B. ambifaria* have been reported by Sasmita *et al*. [22].

TABLE 3. Molecular identification of phosphate solubilizing microbes.

Isolate	E-value	Homology	Species
B-SS1.2	0	99%	*Burkholderia ambifaria*
B-SS13.5	0	99%	*Pseudomonas fluorescens*
B-SS19.2	6e-174	86%	*Staphylococcus sp.*
B-SS19.7	0	99%	*B. cepacia*
B-SS20.4	0	99%	*B. cepacia*

Five isolates of PSM belonged to the genera *Burkholderia*, *Pseudomonas*, and S*taphylococcus* with close to 100% homology, except B-SS19.2 (Table 3). Three isolates belonged to *Burkholderia* species, especially *B. ambifaria* (B-SS1.2), and *B. cepacia* (B-SS19.7 and B-SS20.4). B-SS13.5 was identical to *P. fluorescens*, while B-19.2 belong to *Staphylococcus* sp.

In general, some bacterial isolates in this study had a high level of homology with PSB (B-SS19.7 and B-SS20.4), plant-growth promoting bacteria (B-SS1.2), and animal development (B-SS13.5). Thus, the PSB in this study enriched the microbes of the previous research, which is not only as PSB, but also having other functions, such as plant growth promoters and biological agents [9, 35, 36].

The isolate of *A. niger* F-E1 have been reported by Sasmita *et al*. [22]. Fungus isolate F-E1 was homologous with *A. niger* strain H1, a fungi reference of PSF as reported by Gong and Tang [37]. In addition, the strain of the *Aspergillus* species were also reported by other studies related to its capability of dissolving phosphates from various phosphate sources [38, 39].

Phytohormones Production Assays

The results of the phytohormone assays showed that all six strains in this study could produce IAA, GA3, trans-zeatin, and kinetin (Table 4). The production of IAA was evaluated without supplementation of L-tryptophan within the range of 1.70–4.93 µg/ml. The highest IAA quantities was produced by *A. niger* F-E1, followed by *B. ambifaria* B-SS1.2 (2.98 µg/ml). The other study reported that strain *A. niger* CSR3 produce IAA in the absence of L-tryptophan approximately 0.185±0.234 µg/ml [40]. Tang *et al*. [41] also reported that *B. cepacia* strains PC8 and PB3 without the addition of tryptophan were found produced IAA approximately 8.45 and 7.81 µg/ml, respectively.

The production of GA3 in this study ranged from 2.49 to7.54 µg/ml, with the highest content was produced by *Staphylococcus* sp. B-SS19.2, followed by *B. ambifaria* B-SS1.2 (5.21 µg/ml). Other study reported that many of strains produce GA3 within range 1.96–4.32 mg/l [42]. The optimal gibberellin content which produced by microbes was affected by several factors such as culture conditions (nitrogen source and carbon source) [43].

Trans-zeatin and kinetin belong to the group of cytokinins phytohormones. The trans-zeatin and kinetin were produced by all strains ranged from 1.29 to 3.59 µg/ml and from 0.09 to 0.76 µg/ml, respectively. The highest trans-zeatin and kinetin content were respectively obtained by *B. cepacia* B-SS19.7 and *A. niger* F-E1 strains. Another study stated that strains of *B. licheniformis* (Am2train) produce maximum trans-zeatin and zeatin riboside was 1,09 µg/ml (TZ) and 0.521 µg/ml (ZR) after 120 and 96 h incubation, respectively [44].

The ability of *Burkholderia*, *Staphylococcus*, and *Aspergillus* strains to solubilize phosphate and to produce plant growth promoting hormones, such as IAA, GA3, and cytokinins, were also studied by others [6, 40, 41, 45, 46].

TABLE 4. Production of IAA, GA3, trans-zeatin, and kinetin.

Isolates	Phytohormones production (μg/ml)			
	IAA	GA3	Trans-zeatin	Kinetin
Burkholderia ambifaria B-SS1.2	2.98	5.21	2.41	0.19
Pseudomonas fluorescens B-SS13.5	2.10	2.98	2.05	0.18
*Staphylococcus sp.*B-SS19.2	1.70	7.54	2.60	0.15
B. cepacia B-SS19.7	2.19	2.49	3.59	0.10
B. cepacia B-SS20.4	2.27	3.32	2.01	0.09
Aspergillus niger F-E1	4.93	2.71	1.29	0.76

The phytohormones produced by microbial can play a role in regulating several aspects of plant growth and development [47]. IAA plays a role in stimulating the elongation of shoots and/or root structures so that nutrient uptake is more efficient [41]. While gibberellin is a phytohormone that regulates growth and development, including seed germination, seed development, stem elongation, root growth, flowering, regulating vegetative and reproductive, delaying senescence of many plant organs, and increasing resistance to various stresses (drought and salinity stress) [40, 46, 48]. Cytokinins, which are phytohormones that play an important role in cell division or cytokinesis in the roots and shoots, affect apical dominance, are associated with axillary bud growth and leaf senescence, increase plant defense and are involved in numerous plant-biotic interactions [48, 49].

Efficacy of PSM on Cacao Seedling Growth and Nutrient Uptake

The application of RP with *A. niger* F-E1 resulted in higher plant where it was significantly different from RP without inoculation, with an increase of plant height to 30.5%. The addition of RP with *B. cepacia* B-SS19.7, *B. ambifaria* B-SS1.2, and *A. niger* F-E1 could increase the number of leaves and stem diameter significantly ($p \leq 0.05$) compared with control, increased number of leaves were 47.3%, 45.6%, and 45.0%, respectively, and increased stem diameter of 28.03%, 33.0%, and 33.4%, respectively, but not significantly different ($p > 0.05$) compared with RP without inoculation (Table 5).

Shoot dry weights of cacao seedling increased significantly ($p \leq 0.05$) by the treatment of RP with *B. ambifaria* B-SS1.2, *B. cepacia* B-SS19.7, and *A. niger* F-E1 compared with RP without inoculation. These three inoculants revealed an increased in dry weights of 73.6%, 58.2%, and 60.0%, respectively, compared with RP application without inoculation. *B. ambifaria* B-SS1.2 and *A. niger* F-E1 also significantly increased root dry weight of 80.1% and 80.1% respectively in comparison with RP without inoculation (Table 5).

TABLE 5. Effect of P fertilizers and PSM inoculation on plant height, number of leaves, stem diameter, shoot and root dry weights of cacao seedling, and RAE of RP.

Treatments	Plant height (cm)	Number of leaves	Stem diameter (mm)	Shoot dry weight (g)	Root dry weight (g)	RAE (%)
Without P	19.6 c	14.3 c	7.05 c	4.46 d	1.58 d	-
SP-36	33.8 ab	20.5 ab	8.57 ab	13.99 a	3.78 ab	100
RP	29.8 b	17.8 abc	8.17 abc	8.23 c	2.56 bc	40
RP + B-SS1.2	36.4 ab	20.8 a	9.38 a	14.29 a	4.61 a	110
RP + B-SS13.5	30.5 b	17.0 bc	8.46 abc	8.97 c	2.12 c	43
RP + B- SS19.2	30.8 b	17.8 abc	7.73 bc	9.95 bc	1.89 c	50
RP + B- SS19.7	35.0 ab	21.0 a	9.02 ab	13.02 ab	2.79 bc	83
RP + B- SS20.4	33.7 ab	18.3 ab	8.03 abc	8.88 c	2.41 bc	45
RP + F- E1	38.8 a	20.7 a	9.40 a	13.17 ab	4.61 a	100

PSM = phosphate solubilizing microbes, RAE = relative agronomic effectiveness, RP = rock phosphates. Number in the same column followed by different letter are significantly different according LSD test at $P \leq 0.05$ level.

The effectiveness of RP to the growth and yield of plant often demonstrated in a value of RAE. This value in this study was calculated by comparing the total dry weight of cacao seedlings that influenced by inoculant with RP compared with SP-36 treatment as standard. RAE of RP with inoculation of *B. ambifaria* B-SS1.2, *A. niger* F-E1, and *B. cepacia* B-SS19.7 increased to 110%, 100%, and 83%, respectively.

N, P, and K uptakes in shoot were significantly affected by phosphate fertilizer and inoculation treatment (Table 6). Inoculation of *B. ambifaria* B-SS1.2 and *B. cepacia* B-SS19.7 resulted in higher P uptake of shoot than that of only RP treatment by an increase in uptake at 66.4% and 55.6% compared with RP only. A significant increase of N accumulation in shoots was observed at the RP treatments with *B. ambifaria* B-SS1.2 and *A. niger* F-E1, compared with only RP treatment, with an increase of 84.6% and 61.3%, respectively. RP with *B. ambifaria* B-SS1.2, *B. cepacia* B-SS19.7, and *A. niger* F-E1 treatments significantly increased K uptake in shoot compared to that only RP treatment.

TABLE 6. Effect of P fertilizers and PSM inoculation on nutrient uptake at shoot of cacao seedling.

Treatments	Nutrient uptake at shoot (mg/plant)		
	N	P	K
Without P	74.27 d	6.83 e	65.58 e
SP-36	271.44 ab	37.16 ab	272.19 a
RP	156.72 c	20.34 d	133.08 d
RP + B-SS1.2	289.33 a	33.84 ab	245.84 ab
RP + B-SS13.5	163.00 c	19.18 d	179.01 cd
RP + B- SS19.2	178.03 c	22.97 cd	186.84 bcd
RP + B- SS19.7	213.14 bc	31.64 abc	200.65 bc
RP + B- SS20.4	146.89 c	20.31 d	140.09 cd
RP + F- E1	252.74 ab	27.34 bcd	244.19 ab

PSM = phosphate solubilizing microbes, RP = rock phosphates. Number in the same column followed by different letter are significantly different according LSD test at $P \leq 0.05$ level.

The increase of growth and nutrient uptake in plant by PSM is due to their ability in solubilizing insoluble P and phytohormons production. *B. ambifaria* B-SS1.2 and *B. cepacia* B-SS19.7 has the ability to solubilize RP in liquid medium higher than other strains showed that mechanism of P solubilization was important to support plant growth. PSM can also increase the release of P from AlPO4 so that it has the potential to increase the efficiency of P fertilizer in acid soils with high exchangeable Al content, such as the soil in this study. PSM potentially dissolve P by chelation of cations and competing with phosphate for adsorption sites in the soils through the production of organic and anorganic acids [50]. Microbes also could mineralize soil organic P by the production orthophosphatase and releasing inorganic phosphorus that can be absorbed by plants [50]. Phosphorus is an important component of ATP which is a high-energy compound needed in metabolic activity, thereby directly contributing to the increase of photosynthesis, stimulating root development, and vegetative growth of plants [9]. The adequacy of P nutrition promoted the better root growth and thus increased N and K uptakes and plant growth.

Plant growth promotion by microbes also occurs through the mechanism of production of phytohormones. *A. niger* F-E1 and *B. ambifaria* B-SS1.2 produced higher IAA quantities than other strains (Table 4). *B. ambifaria* B-SS1.2 also resulted the high GA3 content relatively. The genera *Burkholderia* and *Aspergillus* have been reported in other studies as PSM and as plant growth promoting microbes [9, 13, 14, 45]. In addition, there are other possible mechanisms that also play a role in increasing cacao plant growth in this study. Several studies found that the *Burkholderia* species showed activity promoting plant growth such as N_2 fixation, production of siderophores, ACC deaminase activity, and antagonistic ability against pathogenic microbes [9, 13, 14]. Other mechanisms described above were not evaluated in this paper.

The successful inoculation of PSM from the genera *Burkholderia* and *Aspergillus* in increasing the effectiveness of RP on plant growth, dry weight, and nutrient uptake in plants had also been reported by other researchers [19, 51]. Suparno [17] and Sasmita *et al*. [22] also reported that inoculation of PSM at some RP doses was able to increase plant growth, P uptake, and dry weight in cacao seedlings compared with that without inoculants.

The effectiveness of the PSM isolates on the plant growth and P uptake of cacao seedlings in this study might be determined by P solubilization ability and/or other growth-promoting abilities. The variance of PSM effectiveness on plants was also due to the viability of PSM and its colonization in the rhizosphere as well as the competition with endogenous microbes in the soil rhizosphere [12]. The success of *B. ambifaria* B-SS1.2, *B. cepacia* B-SS19.7, and *A. niger* F-E1, in increasing shoot dry weight, N, P, and K uptakes in cacao seedling, and RAE of RP for cacao seedlings indicated that the three strains are potential inoculants to be developed as biofertilizer for cacao plants on acid soils after evaluating their performance under field conditions.

CONCLUSION

Inoculation of *A. niger* F-E1, *B. ambifaria* B-SS1.2, and *P. fluorescens* B-SS13.5 resulted the highest phosphate solubilization in liquid medium containing $Ca_3(PO_4)_2$, RP, and $AlPO_4$ with P soluble of 530.56 µg/ml, 360.70 µg/ml, and 174.42 µg/ml, respectively. The highest IAA quantities was produced by *A. niger* F-E1 (4.93 µg/ml), followed by *B. ambifaria* B-SS1.2 (2.98 µg/ml). The highest GA3 content was produced by *Staphylococcus* sp. B-SS19.2 (7.54 µg/ml), followed by *B. ambifaria* B-SS1.2 (5.21 µg/ml). The highest trans-zeatin and kinetin content were resulted by *B. cepacia* B-SS19.7 and *A. niger* F-E1 strains, respectively. RP with inoculation of *B. ambifaria* B-SS1.2, *B. cepacia* B-SS19.7, and *A. niger* F-E1 significantly increased shoot dry weight and RAE of RP on cacao seedlings. RP with *B. ambifaria* B-SS1.2 resulted in of N, P, and K uptakes that were significantly higher than RP without inoculation. *B. ambifaria* B-SS1.2, *B. cepacia* B-SS19.7, and *A. niger* F-E1 have the potential to promote the effectiveness of RP for cacao plant growth.

ACKNOWLEDGMENTS

The research was supported by Ministry of Research, Technology, and Higher Education, Indonesia through a Grant of Leading Strategic Research. We thank Nika Roslina Silaen for her contribution as the technical support for the research. We also would like to thank to Dr. Puji Lestari for giving advice for the paper improvement.

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