CHARACTERIZATION AND POLYPHASIC IDENTIFICATION OF NOVEL RHIZOBACTERIA STRAIN ISOLATED FROM SAND DUNES ECOSYSTEM

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ABSTRACT

The coastal sand dune ecosystem at the Parangtritis Coast of Yogyakarta, Indonesia has unique characteristics such as low moisture sandy soil, high salinity and low nutrient content. *Fimbristylis cymosa* is one of the plant species having the capability to survive in that unique ecosystem. In this study, rhizobacteria isolated from the rhizosphere of *F. cymosa* were isolated to be further analyzed on their phosphate solubilizing and antagonistic properties against *Fusarium oxysporum* which cause the Wilt disease. The isolates of Phosphate Solubilizing Rhizobacteria (PSR) having the most potential capabilities were then polyphasically identified based on phenotypic and genotypic characters followed by 16S rDNA sequencing. The results showed that four PSR isolates (I8, I11, I12 and I24) have high phosphate dissolution indices. The highest indices were observed in isolates I11 (3.08) and I12 (3.44), respectively. Analysis of the dual plate experiments for PSR I11 and PSR I12 isolates against the growth of *F. oxysporum* also showed quite high inhibitory activities, i.e., isolate PSR I11 was 42.40%, while isolate PSR I12 was 42.08%. The two isolates were polyphasically identified as *Burkholderia dolosa*. This study clearly showed that PSR I11 and PSR I12 isolates are very potential and prospective to be used as marginal land inoculants and as providers of phosphorus. This study also showed that the isolates are useful as biocontrol agents against *F. oxysporum* in plants.

Keywords: inhibitory activity, phosphate dissolution index, phosphorus, polyphasic identification, sandy soil

INTRODUCTION

Coastal sand dunes are Aeolian landforms commonly found in coastal areas at all latitudes in the world. Indonesia has coastal sand dunes located in the southern part of Java Island, extending from the southern coast of West Java Province to Yogyakarta Province. The most significant sand dunes formation occurs in the Parangtritis Coastal Area, located on the southern coast of Yogyakarta Province. The sand dunes ecosystem has a high temperature, relatively little vegetation, strong winds, high salt content and very low groundwater content, making it a dry and infertile area (Mahdavi & Bergmeier 2016; Campos *et al.* 2020). In general, plants are difficult to grow in dry and less fertile areas. However, there are several groups of plants that can survive in the sand dunes habitat. Among plants that survived the sand dunes habitat on Yogyakarta's southern coast is *Fimbristylis cymosa*, a family of Cyperaceae, which grows well and is widespread along with the coastal sand dunes habitat.

The growth and development of *F. cymosa* in the coastal sand dunes habitat are strongly influenced by the availability of essential soil macronutrients. One of the main macronutrients supporting the growth and development of *F. cymosa* is phosphorus. Phosphorus is among minerals needed at 0.2% dry weight of *F. cymosa*, as a component of nucleic acids (DNA and RNA) for conserving energy. Plants absorb phosphorus in the form of phosphate anions (PO_4^- or PO_4^{2-}) which are dissolved in groundwater. However, phosphate anions have

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a high tendency to bind with calcium, aluminum, and iron in the soil, which makes them insoluble and easily settled so that they cannot be absorbed by plants, including *F. cymosa* (Yang *et al.* 2021). Undissolved phosphorus in the soil can be dissolved by the Phosphate Solubilizing Bacteria (PSB) group.

A study conducted by Liu *et al.* (2015) in calcareous soil found PSB genera *Bacillus, Pseudomonas, Rhizobium* and *Acinetobacter*. Pastore *et al.* (2020) reported PSB genera *Burkholderia, Bacillus*, and *Pseudomonas* in forest soils. In the mangrove forest rhizosphere, Teymouri *et al.* (2016) observed the existence of PSB genera *Bacillus, Pseudomonas* and *Acinetobacter*. Rasul *et al.* (2019) obtained PSB genera *Pseudomonas* and *Bacillus* from rhizospheric paddy field soil, while the PSB genus *Paenibacillus* was found from wheat rhizosphere (Cherchali *et al.* 2019).

In soil environments, there are also soilborne pathogens causing plants infection. One species of a soil-borne pathogen is *Fusarium oxysporum* causing the *Fusarium* Wilt disease in various types of agricultural crops within the Solanaceae family, such as tomatoes, potatoes, and chilies (Ávila *et al.* 2019; Srinivas *et al.* 2019; Chowdhury *et al.* 2020). The wilting phenomenon that occurred in those crops due to *Fusarium* Wilt disease resulted in a significant reduction in crop yields and has been a major problem for agriculture worldwide. The use of chemical compounds for treating the Wilt disease has adverse effects on non-target organisms. Therefore, it is pertinent to find biocontrol agents which is considered safer for the environment.

In soil environments, Phosphate Solubilizing Bacteria (PSB) are reported to be more dominantly existing in the rhizosphere. In addition, PSB from the rhizosphere is known to produce active metabolites compared to other sources and is able to increase plant growth and development. PSB originating from extreme environments such as sand dunes, might have specific characteristics as a biocontrol agent against *F. oxysporum* (Ahluwalia *et al.* 2021; Rasool *et al.* 2021) Therefore, this study aimed to: 1. isolate and characterize Phosphate Solubilizing Bacteria (PSB) from the rhizosphere of *F. cymosa* living in sand dunes habitat; and 2. screen the capability of isolated PSB as a biocontrol agent against *F. oxysporum*.

MATERIALS AND METHODS

Rhizosphere Soil Samples and Isolate of *F. oxysporum*

Rhizosphere soil samples from the planting area of *F. cymosa* were taken and collected from 8 different sampling points along with the coastal sand dune habitat in the Parangtritis Coastal Area, Yogyakarta. The roots of the *F. cymosa* were carefully extracted with a shovel. Soil attached to the roots was collected in sterile plastic containers as rhizosphere soil samples. The samples were then stored at 4 °C for further isolation of Phosphate Solubilizing Bacteria (PSB).

The isolate of *Fusarium oxysporum* was obtained from the culture collection of the Microbiology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia and was grown in Potato Dextrose Agar (PDA) medium at 30 °C for 10 days.

PSB Isolation and Phosphate Dissolution Index Analysis

Ten (10) g of rhizosphere soil samples were poured into a 50 mL Erlenmeyer and suspended in 90 mL of sterile distilled water, then shaken using a vortex at a speed of 200 rpm for 30 min. The sample suspension was diluted in a series of up to 10^{-4} dilutions. A total of 0.1 mL of the respective 10^{-3} and 10^{-4} diluted sample suspensions was inoculated on the National Botanical Research Institute's Phosphate (NBRIP) agar medium by means of the spread plate method, then incubated at 30 °C for 48 - 72 hours. The NBRIP medium consists of 1% glucose, 0.5% $Ca_3(PO_4)_2$, 0.5% $MgCl_2$, 0.01% $(NH_4)_2SO_4$, 0.0.5% $MgSO_4.7H_2O$, and 0.02% KCl (Nautiyal 1999). PSB isolates having a clear zone around the colony were then re-isolated as PSR, and purified on an NBRIP medium.

In order to measure the phosphate dissolution index, the PSR isolates were re-cultured in NBRIP liquid medium and incubated at 30 °C for 48 h. Forty (40) µL of the incubated culture was diluted until reaching a colony having cell number 10^8 CFU/mL. The liquid culture was then poured into a Petri dish containing NBRIP agar medium by means of the pour plate method and incubated at 30 °C for 48 h.

The formed clear zone and the size of the bacterial colony were calculated. The phosphate dissolution index was determined with the following formula developed by Haile *et al.* (2016):

$$PSI = \frac{(HZD + CD)}{CD}$$

where:
PSI = Phosphate Solubilization Index
HZD = Halo Zone Diameter (mm)
CD = Colony Diameter (mm)

Antagonistic Activity of PSR Against *F. oxysporum*

In vitro test of PSR antagonist activity against *F. oxysporum* was carried out by using the dual plate experiments method according to Zhang *et al.* (2017) with minor modifications. Potato Dextrose Agar (PDA) medium was prepared on a 9 cm diameter Petri dish, followed by making a 5-mm diameter well in the PDA medium at the center of the Petri dish by using a cork borer. The other four 5-mm diameter wells were made at a distance of 2 cm from the well located at the center of the Petri dish and from each other. *F. oxysporum* mycelium with a diameter of 5 mm was taken from the outermost part of the 10-day-old fungal colony and was inoculated into the well at the center of the Petri dish containing PDA medium by using an inoculation needle. A total of 8 µL of PSR isolate suspension with a cell number of 10^9 CFU/mL was inoculated into the other four wells in the same Petri dish and then incubated at 30 °C for 10 days. The control agar plates were then prepared with the same agar medium without inoculating the PSR isolates suspension. At the end of the incubation time, the diameter of *F. oxysporum* colonies was measured diagonally, vertically and horizontally. The inhibition percentage of PSR isolates against *F. oxysporum* was determined using the following formula of Royse and Ries (1978):

$$IP = \frac{(R1 - R2)}{R1} \times 100\%$$

where:
IP = Inhibition Percentage
R1 = Diameter of *F. oxysporum* colonies on the control plate
R2 = Diameter of *F. oxysporum* colonies on the treated plate

Polyphasic Identification of PSR Isolates

Polyphasic identification of PSR isolates was determined based on a combination of phenotypic and genotypic characters. Phenotypic characters of the observed isolates, including the morphological and biochemical characters, were determined by referring to Bergey's Manual of Systematic Bacteriology (Brenner *et al.* 2005). The morphological characters were observed based on cell and colony morphologies. The cell morphology of PSB isolates was observed based on the shape and characteristics resulting from the gram staining procedure. Meanwhile, colony morphology of PSB isolates was observed based on the shape, color, margins, elevation, internal structure and optical features of the colony. The biochemical characters were determined based on several tests, such as the motility, catalase, oxidase tests, glucose, sucrose, lactose fermentation tests, urease and Voges-Proskauer (VP) tests.

The isolates were then characterized genotypically based on the 16S rDNA nucleotide sequence. Total genomic DNA of PSB isolates was extracted using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA). PCR amplification of the 16S rDNA gene of the PSB isolates was analyzed by using 27F primers (5'-AGAGTTTGATCCT GGCTCAG-3') and 1492R (5'- GGTTACCTT GTTACGACTT-3') (Hossain *et al.* 2020). The PCR amplification was performed using a DNA thermal cycler (Bio-Rad, USA) with the following program: pre-denaturation at 95 °C for 30 min, followed by 30 cycles consisting of denaturation at 95 °C for 30 sec, annealing at 57 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR products were purified and sequenced. The nucleotide sequences were then compared with DNA sequence databases (GenBank) through the BLAST program (BLASTn) at the National Biotechnology Information Center (NCBI). A phylogenetic tree was constructed through the maximum likelihood method using Molecular Evolutionary Genetics Analysis X (MEGA version X).

Statistical Analysis

Data were statistically analyzed using the one-way variance analysis (ANOVA) followed by the Duncan Multiple Range Test (DMRT) approach for testing the mean differences. At $P < 0.05$,

the differences were considered significant. Results are expressed as means of three replicates ± standard deviation. The software used was IBM SPSS Statistics version 21.

RESULTS AND DISCUSSION

PSR Isolate and Phosphate Solubilization Index

A total of 26 bacteria were isolated from *F. cymosa* rhizosphere soil samples in the sand dunes habitat of Parangtritis Coastal Area, Yogyakarta, Indonesia. Of the 26 rhizobacterial isolates, 4 rhizobacterial isolates were selected as PSR based on the clear halo formation around the colony in the NBRIP medium containing tricalcium phosphate. The clear halo was formed due to the production of organic acids or polysaccharides as a result of the phosphatase enzyme activity of the PSR isolate (Paul & Sinha 2013; Behera *et al.* 2017). The phosphate solubilization indices of each PSR isolate after 10 days of incubation are presented in Table 1 (Widane *et al.* 2018).

The highest phosphate solubilization index of 3.44 was obtained from PSB I12. Meanwhile, the lowest phosphate solubilization index of 3.00 was obtained from PSB I24. A previous study by Teymouri *et al.* (2016) reported the phosphate solubilization index of 3.5 which was obtained from *Bacillus* sp. found in the rhizosphere of mangrove forest. Mardad *et al.* (2013) reported the phosphate solubilization index of 3.5 which was obtained from *Enterobacter hormaechei* found in the phosphate rock deposits. In contrast, several other researchers reported a lower-than-three phosphate solubilization index, such as those obtained from *Pseudomonas* (2.6) and *Acinetobacter* (2.0) found in the rhizosphere of forest (Teymouri *et al.* 2016). The *Paenibacillus* genus from the wheat rhizosphere was reported to have a very low phosphate solubilization index of 1.32 (Cherchali *et al.* 2019). *Pseudomonas* and *Serratia* obtained from the rhizosphere of *Allium cepa* L. were also reported to have low phosphate solubilization indices of 2.0 and 2.1, respectively (Blanco-Vargas *et al.* 2020).

Table 1 Phosphate solubilization index of PSR isolates obtained from the *F. cymosa* rhizosphere after 10 days of incubation

No	PSR Isolate	Phosphate solubilization index
1	I8	3.04 ± 0.51^{bcd}
2	I11	3.08 ± 0.38^{cd}
3	I12	3.44 ± 0.19^{d}
4	I24	3.00 ± 0.43^{abcd}

Notes: The index is presented as mean ± SD; Numbers followed by the same letter in a column do not show a significant difference according to the Duncan test (DMRT) at $P < 0.05$.

Different values of phosphate solubilization index among PSR isolates are closely related to organic acids production, causing a pH decrease in bacterial cells and their environment, leading to proton substitution in the phosphate mineral and P release (Ludueña *et al.* 2018; Rasul *et al.* 2021). Organic acids produced by several PSRs include gluconic, glycolic, oxalic, malonic and succinic acids. Gluconic acid is an organic acid having a role as a phosphate solvent (Joe *et al.* 2018; Santos-Torres *et al.* 2021), which is useful in providing P minerals needed for plant growth (Valetti *et al.* 2018; Sahandi *et al.* 2019). PSR I11 and PSR I12 isolates have a high ability to dissolve phosphate *in vitro*. Therefore, these two isolates are the potential to be developed as agents for providing P for plants.

Antagonistic Activity of PSR Isolates Against *F. oxysporum*

PSR I8, PSR I11, PSR I12, and PSR I24 isolates were tested *in-vitro* for their antagonistic activity against *F. oxysporum* by using the dual plate technique. The results showed a significant inhibitory effect on the growth of *F. oxysporum* mycelium (Fig. 1), which was clearly seen by comparing the inhibitory effect of the four isolates with the control against the growth of *F. oxysporum*. The four PSR isolates clearly caused a smaller colony size of *F. oxysporum* compared to that in the control treatment. The colony diameter of *F. oxysporum*, however, was different among the four PSR isolates, i.e., 44.75 mm was observed in PSR I11 isolate, 45.00 mm in PSR I12, 53.00 mm in PSR I24 and 62.50 mm in PSR I8 (Widane *et al.* 2018).

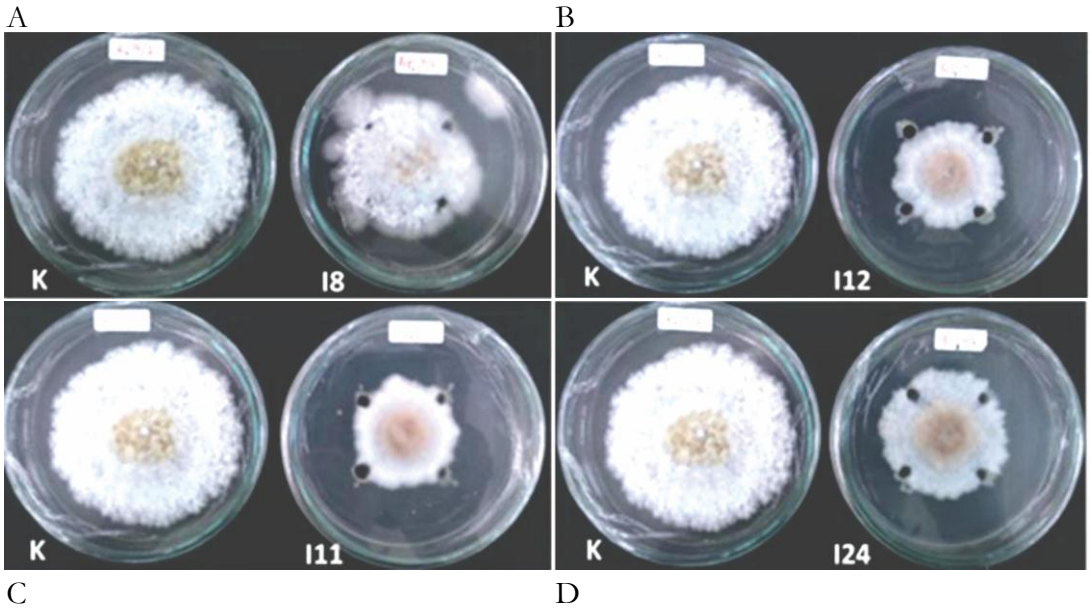

Figure 1 Antagonistic activity of PSR isolates against *F. oxysporum* observed *in-vitro* after 14 days incubation period
Notes: A = PSR I8; B = PSR I12; C = PSR I11; D = PSR I24.

In addition, our study showed that PSR isolates also caused thinner *F. oxysporum* mycelium growth compared to that in the control treatment. This explains the inability of *F. oxysporum* to grow well in the presence of PSR isolates. The graph of the inhibition percentage of PSR isolates against *F. oxysporum* is presented in Figure 2 (Widane *et al.* 2018).

The dual culture method applied in this study allows direct *in-vitro* interactions between PSR isolates and *F. oxysporum*. These interactions can be in the form of competition for space or for nutrients from the growth medium, leading to growth inhibition of *F. oxysporum*. Growth inhibition can also be caused by metabolite compounds produced by PSR isolates. Several metabolite compounds are reported to have the most effective antagonistic activity in inhibiting the growth of plant pathogens in the form of antibiotics, siderophores and bacteriocins (Khedhera *et al.* 2021). Bacteria can produce hydrolytic enzymes such as protease, lipase, chitinase and glucanase which can lyse fungal cells so that they have antagonistic activity against plant pathogenic fungi (Cui *et al.* 2019; Lau *et al.* 2020).

Our study also showed that the PSR I11 isolate was the most effective at inhibiting the growth of *F. oxysporum* with an inhibition percentage of 42.40%, while the PSR I12 isolate had an inhibition percentage of 42.08% which is not significantly different from that of PSR I11. Lower inhibition percentages were observed for PSR I24 and PSR I8, i.e., 31.82% and 19.58%, respectively (Fig. 2).

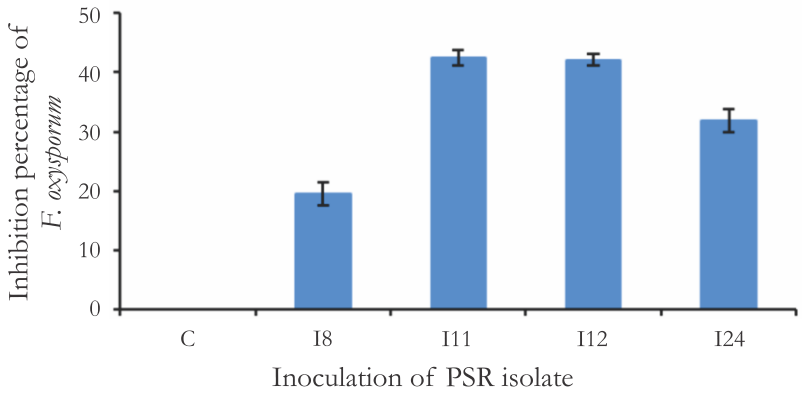

Figure 2 Inhibition percentage value of PSR isolates against *F. oxysporum* after 14 days of incubation period

Several studies have also reported that bacteria isolated from the rhizosphere are able to protect plants from fungal pathogens (Babu *et al.* 2015; Panda *et al.* 2016). *Bacillus amyloliquefaciens* from the rhizosphere of apple trees were reported to have inhibition capability against *F. oxysporum* with an inhibition percentage of 35% (Guleria *et al.* 2016). Meanwhile, research conducted by Jangir *et al.* (2018) showed that *Bacillus* sp. from the rhizosphere of tomato plants had antagonistic activity against *F. oxysporum* with higher inhibition percentage (70%) and therefore, is potential as a biocontrol agent against *Fusarium* Wilt disease in tomato plants. Karthika *et al.* (2020) also isolated *Bacillus cereus* from the rhizosphere of a tomato plant, which has an inhibition percentage of 66%. Our study further showed that PSR I11 and PSR I12 isolates have the capability of phosphate solvent and have high antagonistic activity against *F. oxysporum*.

Polyphasic Identification of PSR Isolates

Observation of the morphological characters of PSR I11 and PSR I12 showed different colony morphology forms, i.e., circular and irregular, respectively (Table 2). However, the two isolates have the same colony color, elevation, internal structure, optical features and shape, such as cream, entire, convex, smooth, translucent and rod-shaped (Table 2).

Biochemical properties of the two isolates, PSR I11 and PSR I12 showed similarities in terms of motility, production of catalase and oxidase enzymes and positive reaction toward the Voges-Proskauer test. Different reactions occurred when being tested in a medium containing urea, showing that PSR I11 isolate is able to produce urease, while PSR I12 isolate is not able to produce urease (Table 3).

Comparing the results from the identification of the morphological and biochemical characters with the characters described in Bergey's Manual of Determinative Bacteriology, it is concluded that PSR I11 and PSR I12 isolates belong to the genus *Burkholderia* (Brenner *et al.* 2005).

Table 2 Morphological characters of PSR isolates from rhizosphere of *F. cymosa*

No	Morphological characters		PSR isolate	
			I11	I12
1.	Colony morphology	Shape	Circular	Irregular
		Color	Cream	Cream
		Margin	Entire	Entire
		Elevation	Convex	Convex
		Internal structure	Smooth	Smooth
		Optical feature	Translucent	Translucent
2.	Cell morphology	Shape	Rod-shaped	Rod-shaped
		Gram properties	Negative	Negative

Tabel 3 Biochemical characters of two PSR isolates from rhizosphere of *F. cymosa*

		PSR isolate	
No	Biochemical characters	I11	I12
1	Motility test	+	+
2	Catalase test	+	+
3	Oxidase test	+	+
4	Glucose fermentation	-	-
5	Sucrose fermentation	-	-
6	Lactose fermentation	-	-
7	Urease	+	-
8	Voges-proskauer (VP) test	+	+

Burkholderia spp. is reported of having the ability to dissolve phosphate and at the same time has antagonistic activity against *F. oxysporum*. Simonetti *et al.* (2018) isolated *Burkholderia ambifaria* from the rhizosphere of barley having a role as phosphate solvents and antagonistic properties against *F. oxysporum*. *Burkholderia cepacia* and *Burkholderia contaminans* with similar capabilities were isolated from the rhizosphere of maize (Zhao *et al.* 2014; Tagele *et al.* 2018). *Burkholderia* sp. was also obtained from the rhizosphere of Juçara palm (*Euterpe edulis* Mart) (de Castilho *et al.* 2020). In addition to the plant rhizosphere, *Burkholderia* spp. with the same properties is also obtained from the root nodules of fenugreek (*Trigonella foenum-graecum* L.) (Kumar *et al.* 2017). *B. contaminans* were also existed at the nodule of the common bean (*Phaseolus vulgaris*) (Tapia-García *et al.* 2020). Apart from dissolving phosphate and having antagonistic properties against *F. oxysporum*, *B. contaminans* also has nitrogenase properties (Silva *et al.* 2012).

Both PSR isolates were also genotypically identified using the 16S rDNA genetic marker. Based on the 16S rDNA gene sequencing, the PSR I11 and PSR I12 isolates had similarities with *Burkholderia dolosa* strain LMG 18943B with a similarity index of 99.51% and 99.44%, respectively (Table 4). The two PSR isolates were categorized in the same species because the similarity percentage in the 16S rRNA gene sequences was both ≥ 99% (Schlaberg *et al.* 2012). Phylogenetic trees based on 16S rDNA sequences of PSR I11 and PSR I12 isolates were reconstructed by using comparative sequences with the in-group and out-group categories obtained from NCBI (Table 4).

Some of the relatively close sequences of *Burkholderia* spp. included *Burkholderia dolosa* strain LMG 18943 (Yarza *et al.* 2013), *Burkholderia latens* strain R-563 (Vanlaere *et al.* 2008), *Burkholderia multivorans* strain Struelens (Bauernfeind *et al.* 1999), *Burkholderia vietnamiensis* strain LMG 10929 (LiPuma *et al.* 1999), *Burkholderia vietnamiensis* strain TVV75 (Viallard *et al.* 1998), *Burkholderia metallica* strain R-16017 (Vanlaere *et al.* 2008), *Burkholderia territorii* strain LMG 28158 (De Smet *et al.* 2015) and *Burkholderia seminalis* strain R-2419 (Vanlaere *et al.* 2008) as the in-group. Meanwhile, *Alcaligenes faecalis* subsp. *parafaecalis* strain G 16S was used as the out-group because the species is a class-level classification group Betaproteobacteria with *Burkholderia* spp. In addition, *Alcaligenes* sp. has the ability to dissolve phosphate and antibiosis properties against *F. oxysporum* (Rasool *et al.* 2021). Reconstruction of the phylogenetic tree was carried out using the maximum likelihood (ML) method with a substitution model (Tamura-Nei 93, G: gamma-distributed) and a bootstrap of 1,000 replications (Fig. 3). This phylogenetic tree shows that both strains form clusters with *Burkholderia dolosa* strain LMG 18943 in 83% bootstrap replications; therefore, reconstruction of these phylogenic trees can be trusted (Hillis & Bull 1993). The phylogenetic tree reconstruction of the two isolates was also in accordance with the BLAST results, which indicated that the two isolates could be identified as *Burkholderia dolosa*. In addition, we already submitted the 16S rDNA sequences of both strains (*B. dolosa* I11 and *B. dolosa* I12) to the GenBank, with accession numbers of OK083732 and OK083731, respectively https://www.ncbi.nlm.nih.gov).

Tabel 4 Identify sequences of PSR isolates from the rhizosphere of *F. cymosa* based on the GenBank data by using BLAST

Isolate	Accession number	Species of PSR homolog	Identity	Query cover	Reference
PSR I11	NR_104973.1	*Burkholderia dolosa* strain LMG 18943	99.51 %	99%	Yarza *et al.* (2013)
	NR_042632.1	*Burkholderia latens* strain R-5630	99.30 %	99%	Vanlaere *et al.* (2008)
	NR_029358.1	*Burkholderia multivorans* strain Struelens	99.09 %	99%	Bauernfeind et al. (1999)
	NR_041720.1	*Burkholderia vietnamiensis* strain LMG 10929	99.09 %	99%	LiPuma *et al.* (1999)
	NR_118872.1	*Burkholderia vietnamiensis* strain TVV75	99.22%	98%	Viallard *et al.* (1998)
	NR_042636.1	*Burkholderia metallica* strain R-16017	98.95%	99%	Vanlaere *et al.* (2008)
	NR_136496.1	*Burkholderia territorii* strain LMG 28158	98.95%	99%	De Smet *et al.* (2015)
	NR_042635.1	*Burkholderia seminalis* strain R-2419	98.74%	99%	Vanlaere *et al.* (2008)
PSR I12	NR_104973.1	*Burkholderia dolosa* strain LMG 18943	99.44 %	99%	Yarza *et al.* (2013)
	NR_042632.1	*Burkholderia latens* strain R-5630	99.16 %	99%	Vanlaere *et al.* (2008)
	NR_029358.1	*Burkholderia multivorans* strain Struelens	99.09 %	99%	Bauernfeind *et al.* (1999)
	NR_041720.1	*Burkholderia vietnamiensis* strain LMG 10929	99.09 %	99%	LiPuma *et al.* (1999)
	NR_118872.1	*Burkholderia vietnamiensis* strain TVV75	99.43%	98%	Viallard *et al.* (1998)
	NR_042636.1	*Burkholderia metallica* strain R-16017	98.95%	99%	Vanlaere *et al.* (2008)
	NR_136496.1	*Burkholderia territorii* strain LMG 28158	98.88%	99%	De Smet *et al.* (2015)
	NR_042635.1	*Burkholderia seminalis* strain R-2419	98.81%	99%	Vanlaere *et al.* (2008)

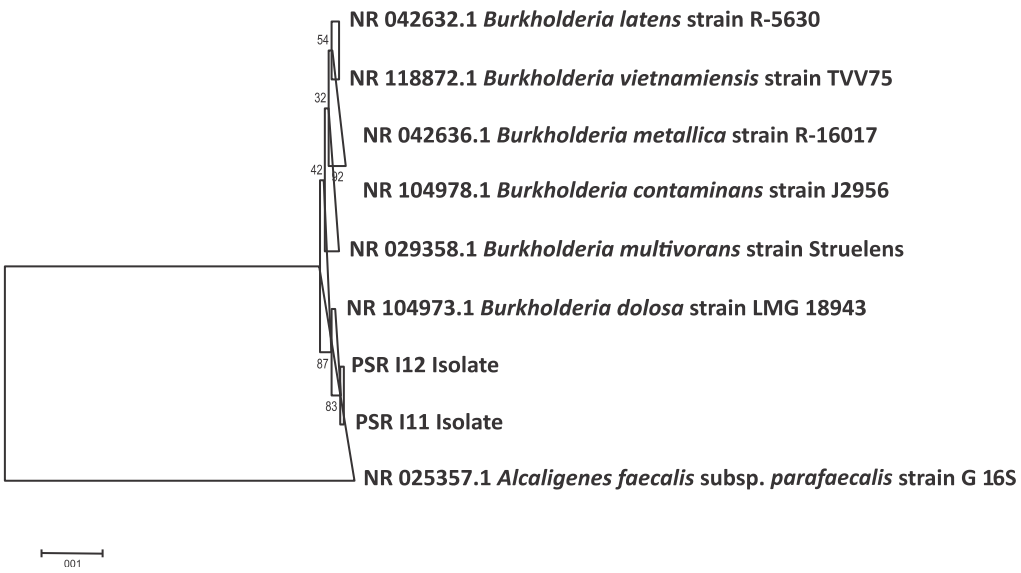

Figure 3 Phylogenetic tree based on 16S rDNA sequences constructed by the maximum likelihood method
Note: Numerals at the nodes indicate the bootstrap value (%) derived from 1,000 replication.

CONCLUSION

PSR I11 and PSR I12 bacterial isolates obtained from the rhizosphere of *F. cymosa* in the sand dunes habitat of Parangtritis, Yogyakarta, Indonesia showed the capability to dissolve phosphate. The two isolates also showed an inhibition percentage of more than 40%, and therefore, can be used as biocontrol agents against *F. oxysporum*. PSR I11 and PSR I12 isolates were polyphasically identified as *Burkholderia dolosa*. Since the two isolates were obtained from *F. cymosa* living in sand dunes habitat, which is an extreme environment, this study can be further developed into determining the possibility of obtaining these two isolates in other marginal lands, to be used as phosphate solvent and biocontrol agents against *F. oxysporum*. Further research is also suggested to determine the ability of these two isolates for procuring phosphorous mineral supply and as biocontrol agents against *F. oxysporum* in plants, especially Solanaceae family, plants both in polybags and agricultural land.

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