Exploring of promising bacteria from the rhizosphere of maize, cocoa and lamtoro

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Abstract. *Sukmawati, Ala A, Patandjengi B, Gusli S. 2020. Exploring of promising bacteria from the rhizosphere of maize, cocoa and lamtoro. Biodiversitas 21: 5665-5673.* Alginate-producing bacteria are important for improving the quality of dry land, as they can both dissolve phosphate and fix nitrogen. Until now, the alginate-producing bacteria are largely isolated from seaweed. These bacteria were from the root ecosystem of cultivated plants. This study was conducted to explore bacteria that were capable of producing alginates, dissolving phosphates, and fixing nitrogen from the rhizosphere of corn (*Zea mays*), cocoa (*Theobroma cacao*), and lamtoro (*Leucaena leucocephala*). The characterization was carried out both morphologically and physiologically. A total of 17 isolates were successfully grown on alginate media, of which six isolates were from maize rhizosphere, five isolates from cocoa, and six isolates from the lamtoro rhizosphere. Bacterial isolates from the rhizosphere of maize and cocoa varied in terms of colony colors. In contrast, isolates from the lamtoro rhizosphere varied in colony forms. The KK1-40 isolates showed the highest cell biomass and dry weight which were 0.082 g mL-1 and 0.068 g respectively. The KK3-32 isolate showed the highest phosphate dissolution concentration of 10.85 mg L-1 with phosphate solubility efficiency value (PSE) and phosphate solubility index (PSI) which were 166.7 and a phosphate solubility index (PSI) 2.67 with a phosphate dissolution concentration of 10.85 mg L-1. LR1-25 isolates were able to fix the highest amount of nitrogen with a total N content of 0.36%. Isolates KK1-40 and LR1-25 were identified as Gram-negative bacteria and isolate KK-32 were identified as Gram-positive bacteria. The bacterial isolates KK1-40, KK3-32, and LR1-25 were superior bacteria that can be formulated to increase the productivity of dry land.

Keywords: Alginates, biofilm, rhizosphere, dry land, exopolysaccharide, soil nutrition

INTRODUCTION

Drought is a large-scale constraint affecting the productivity of dry land (Lesk et al. 2016). Whereas the dry land is the main source of food production (FAO 2017). Understanding the roles of bacterial communities associated with the plant roots may offer an environmental friendly solution to drought problems (Naylor and Coleman-derr 2018). Plant Growth Promoting Rhizobacteria (PGPR) plays important role in maintaining both soil fertility and plant growth (Naylor and Coleman-derr 2018), including the ability to dissolve phosphate and fixing nitrogen. Isobe and Ohte (2014) stated that nitrogen-fixing microbes are safe and sustainable nitrogen source for agriculture practices. The use of PGPR is a possible alternative to reduce chemical inputs in agriculture (Etesami and Maheshwari 2018). Hence, exploration on the use of microorganisms to enhance soil fertility and plant nutrition continues to be interesting research topics reported by (Yan et al. 2015; Pacheco-leyva et al. 2016; Ahmad and Husain 2017; Patel et al. 2017; Arfarita et al.

2019; Deng et al. 2019; Sheirdil et al. 2019).

Alginate bacteria is a family of polysaccharides that have structures and properties related to water and nutrient retention. These structures belong to biofilms (Wingender et al. 1999), a polymer matrix known as bacterial aggregate (Ahmad and Husain 2017). Alginate is secreted as a biofilm with a thick structure and resistant to drought (Hay et al. 2013). It contributes to osmotolerance, and adaptive to water stress (Freeman et al. 2013; Sá et al. 2019). Also, alginate plays a key role in nitrogen fixation (Sabra et al. 2000; Goh et al. 2012; Nosrati et al. 2012). The ability of soil microorganisms to reproduce is determined by the osmotic forces of the environment (Patel et al. (2017).

The use of alginate as a natural polymer is related to its stabilization properties, viscosity, gel formation and ability to hold water. Its gel is able to forms complex ions with divalent cations (Davis et al. 2003). In this way, alginate has a surface area that can absorb water, bind micronutrients (Cu, Mn, Mg, Fe and Ca), and strengthen inorganic materials such as clay (Barreca et al. 2014).

Alginate oligosaccharides can be used as growth regulator in agriculture (Zhang et al. 2013) which can increase crop yields especially on rice (Xu et al. 2003), wheat (Zhang et al. 2013), lettuce (Iwasaki and Matsubara 2000), and plant growth, especially on maize cultivated in clay soils (Sukmawati et al. 2020). Unfortunately, most of the bacterial isolates that are capable of producing alginate are isolated from seaweed (Chang et al. 2007; Tang et al. 2009; Lee and Mooney 2012; Zhang et al. 2014; Subaryono et al. 2015; Mori et al. 2016). These bacteria also capable of producing alginates (Sá et al. 2019), fixing nitrogen, dissolving phosphate (Etesami and Maheshwari 2018) and mineralizing organic matter into nutrients that are available to plants (Yan et al. 2015). However, these are indigenous in sub-tropical areas, not in tropical regions. Isolation of alginate-producing bacteria from the rhizosphere of the tropical dry land is important as it relates to diversity, efficiency, virulence, adaptability and sustainability in improving soil fertility, as it is part of the root ecosystem. Therefore, the aim of this research is to obtain superior alginate-producing bacterial isolates from maize, cocoa, and lamtoro rhizosphere with phosphate dissolving and nitrogen-fixing capability to increase dry land productivity.

MATERIALS AND METHODS

Study area

Rhizosphere of bacteria was isolated from dry land at an elevation of 150 m above sea level in Parenring Village, Lilirilau Subdistrict, Soppeng District, South Sulawesi Province, Indonesia (Figure 1). Soil samples were taken from rhizosphere of lamtoro, cocoa, and maize at the coordinates of 120º2'45.43'E-4º20'48.24"S, 120º3'2.87'E-4º20'37.35"S, 120º2'43.24'E-4º20'45.01"S, respectively at a depth of 0-20 cm according to the guidelines provided by FAO (2008).

Morphological characterization of soil bacteria

Bacterial isolation was carried out using a dilution method according to Rahman et al. (2017). Inoculation was performed by the spread plate method using agar medium with 2% sodium alginate (Yonemoto et al. 1991). Each single colony that grew was purified gradually using the streak plate method (Eyler 2013). Single colonies that have been purified were characterized by macroscopically (Cappucino and Sherman 2014).
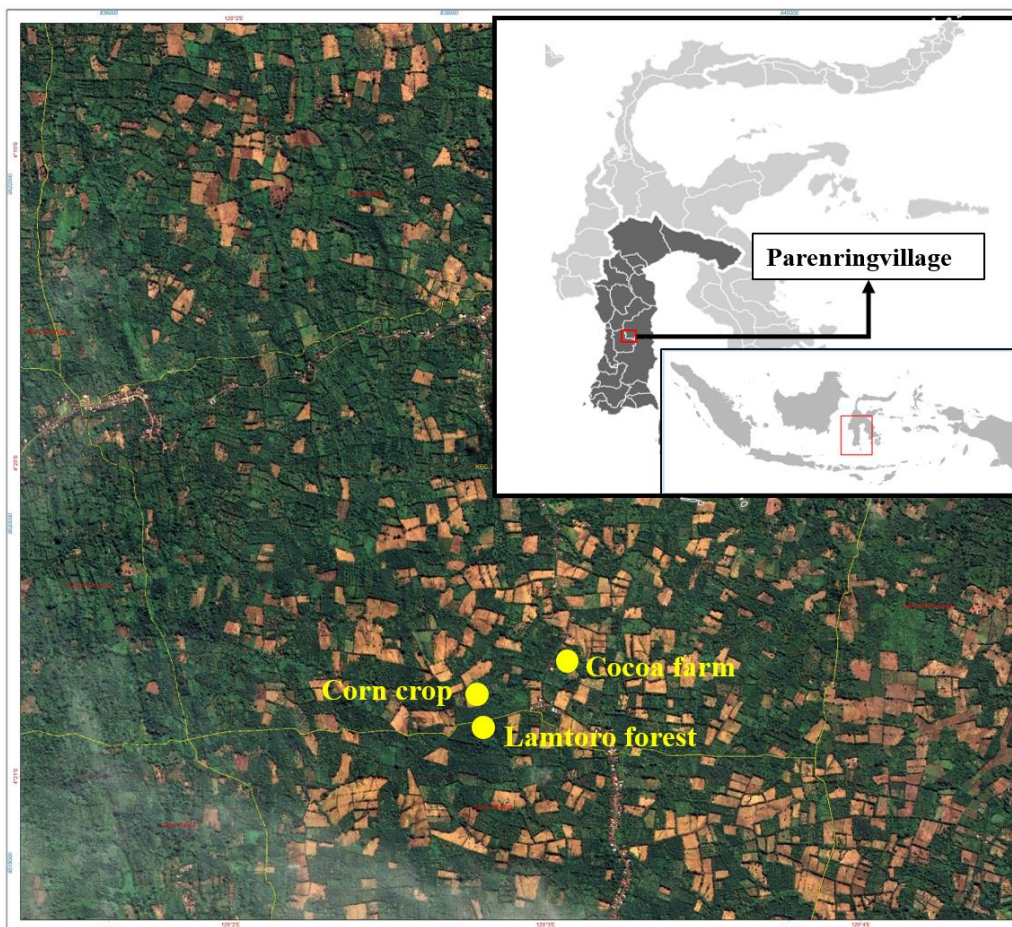
Figure 1. Sampling sites (indicated by the yellow circles) in Parenring Village, Lilirilau Subdistrict, Soppeng District, South Sulawesi Province, Indonesia based on SPOT 6 satellite imagery (source: BAPPEDA Soppeng District 2019). Light brown areas are dryland farming around the sampling area

Gram test

The Gram test was done using the Gram staining method (Hiremath and Bannigidad 2011). The bacteria culture was placed on the slide glass, then allowed to dry. The culture was stained by immersing in solution of crystal violet and iodine solution for one minute, then washed with ethanol for 10 seconds, and counterstain with safranin for 1 minute. The culture was observed under a microscope (Dialux 20), equipped with a Sony 3 CCD color camera connected to the PC.

Hypersensitivity reaction test

Hypersensitivity test determined the response of potentially pathogenic bacterial isolates to plants, which was adopted from Abdallah and Mejdoub-Trabelsi (2016). For this, 1 mL of bacterial isolate suspension with a concentration of 1×10^8 CFU was inoculated into the tobacco leaf tissue. Leaves that showed hypersensitivity symptoms such as discoloration, browning, and dry necrotic spots were not selected for physiological analysis.

Analysis of the ability of bacterial isolates to produce alginate

Alginate production

Alginate production was carried out using the gravimetric method following the procedure outlined by Nosrati, et al.(2012), but 2% sodium alginate was added to it (Yonemoto et al. 1991). The separation of cell biomass was carried out according to the method of Emtiazi, et al (2004) to obtain cell biomass and alginate dry weight.

Determination of the alginate functional group: Fourier Transform Infrared (FTIR)

Alginate functional groups in cell biomass were characterized using an IRPrestige-21 FT-IR spectrometer (Shimadzu Corp). FT-IR / ATR analysis was used for biofilms (Wingender et al. 1999).

Analysis of the ability of alginate-producing bacteria to dissolve phosphate

The ability of alginate-producing bacterial isolates to dissolve phosphate was analyzed both quantitatively and qualitatively following the method by Pande et al. (2017). The clear zone formed around the bacterial colony indicates phosphate dissolving activity. Phosphate solubility index (PSI) and phosphate solubility efficiency (PSE) were observed based on colony diameter and clear zone diameter (Paul and Sinha 2017)

$$PSI = \frac{\text{clear zone diameter} + \text{colony diameter}}{\text{colony diameter}}$$

$$PSE = \frac{\text{clear zone diameter}}{\text{colony diameter}} \times 100$$

Dissolved phosphate concentration was measured by growing bacterial isolates in Piskofkaya liquid media, followed by optical density measurements was performed using a UV-VIS spectrophotometer (Genesys 10S UV 840208100). Phosphate concentrations were measured using a standard Titrisol Curve (PO₄) prepared from 0 to 2.5 mg L^{-1} dilution.

Analysis of the ability of alginate-producing bacterial isolates to fix nitrogen

The ability of bacteria to fix nitrogen was measured using Burk's N-free solid and liquid media (Stella et al. 2010). The ability of bacteria to fix nitrogen is shown by their ability to grow on N Burk free medium, followed by measuring the total nitrogen content of bacterial cultures in liquid media using a spectrophotometer (wavelength of 636 nm).

RESULTS AND DISCUSSION

The diversity of alginate-producing bacteria based on morphological characters and pathogenic potential

A total of 17 bacterial isolates were obtained from three types of rhizospheres, i.e. corn, cacao, and lamtoro, which produced alginates. All isolates different colony characteristics based on color, edge, elevation, and shape (Table 1). The isolates from the maize rhizosphere (JG) showed differences in colony colors and shapes, but dominated the wavy edges and flat elevations. All isolates were identified as Gram-negative bacteria, which were potentially pathogenic to the plants (JG1-16 and JG3-1). Likewise, bacterial isolates from the cocoa rhizosphere (KK) showed differences on their colony colors, with the round and irregular form, and corrugated edges with a flat elevation and elevated flat. Of the five isolates from the cocoa rhizosphere, two were identified as Gram-negative bacteria (KK1-28 and KK1-40). All isolates showed necrotic symptoms on the tobacco leaves, so there was no potential pathogen to the plants. While bacterial isolates from the rhizosphere of the lamtoro (LR), which was dominated by cream and pale cream colony color, with various shapes, edges, and elevations. Gram staining showed that five of them were Gram-negative (LR1-1, LR1-25, LR2-18, LR3-21, LR3-33). Two out of six isolates from lamtoro were potentially pathogenic for the plants, which were LR2-18 and LR3-21. All isolates with positive hypersensitivity reactions were not selected for further physiological characterization.
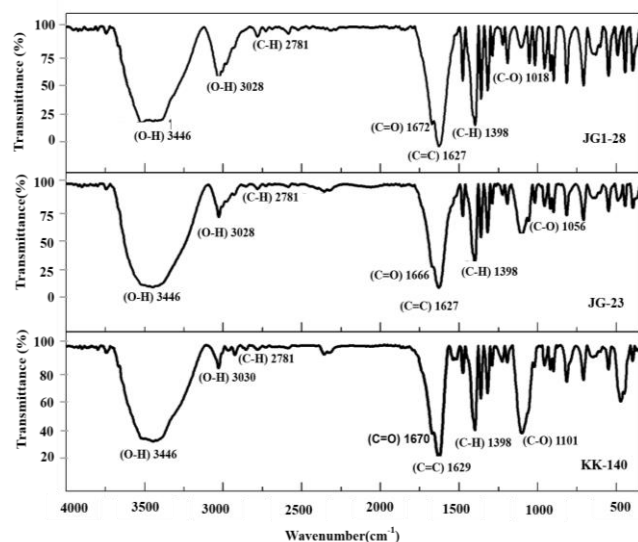
Alginate production capability

Alginate production

Isolate KK1-40 showed the highest results both in cell biomass and dry weight which were 0.082 g mL^{-1} and 0.0681 g, respectively. While lowest result was recorded in KK3-32 isolate (Table 2).

Cell biomass surface functional groups

The functional groups of the cell biomass of bacterial isolates KK1 40, JG2-3 and JG1-28, were characterized by six types of bands at different wave numbers (Figure 2). The characteristic of hydroxyl (OH) bond was detected in three bands, i.e. 3446 cm^{-1} (found in the three bacterial isolates), band 3028 cm^{-1} (isolates JG1 28 and JG2 3), and band 3030 cm^{-1} (KK1-40). Characteristics of alkanes (C-H) in bands 2781 cm^{-1} and 1398 cm^{-1} (fingerprint area) with

strong intensity for all isolates. Characteristics of carboxyl acid (C = O) were detected in three different bands of KK1-40, JG1-28, and JG2-3 which were 1670 cm-1 1672 cm-1, and 1666 cm-1 respectively. The aromatic ring absorption characteristics (C = C) were detected in the 1627 cm^{-1} band (JG128 and JG2 3) and the 1629 cm^{-1} band (KK1 40). The alcohol bond characteristic of the ether group was detected with a strong intensity between the 1000-1300 cm^{-1} bands, where the three isolates had different bands

Figure 2. Surface functional groups of cellulosic biomass of bacterial isolates (KK1-40, JG2-3, and JG1-28) based on Fourier-transform infrared spectroscopy (FTIR)

The ability of alginate-producing bacteria to dissolve phosphate

Results exhibited that three were 12 bacterial isolates capable of forming clear zones on Piskovkaya solid media (Figure 3) with different clear zones and colony diameters (Table 3). The phosphate dissolution index of alginate-producing bacteria ranged from 2.3 to 3.8. LR1-25 isolates showed the highest result on phosphate dissolution index and dissolve phosphate which was 3.8 and 275 respectively. In terms of quantity, the highest (10.85 mg L^{-1}) concentration was phosphate dissolution produced by KK3-32 l isolate (Figure 4).

Tabel 1. Cell biomass and alginate dry weight of each alginate-producing bacterial isolate from the rhizosphere of JG - maize (*Zea mays*), KK - cocoa (*Theobroma cacao*), LR - lamtoro (*Leucaena leucocephala*).
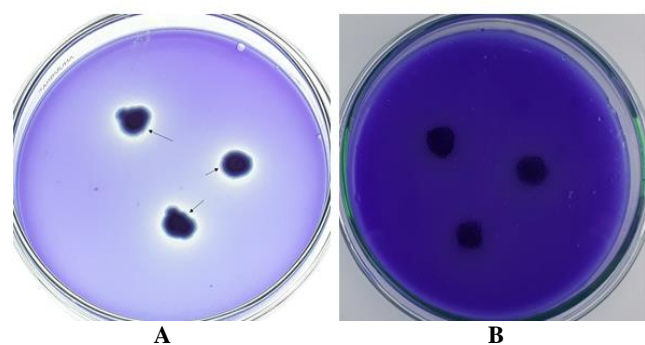
Bacterial isolate	Biomass cellulose (g mL^{-1})	Dry weight (g)
JG1-28	0.0440	0.0391
JG1-45	0.0401	0.0357
JG2-3	0.0658	0.0579
JG3-53	0.0226	0.0218
KK1-28	0.0247	0.0232
KK1-40	0.0817	0.0683
KK3-6	0.0172	0.0164
KK3-23	0.0203	0.0197
KK3-32	0.0096	0.0084
LR1-1	0.0419	0.0382
LR1-25	0.0234	0.0224
LR1-37	0.0232	0.0231
LR3-33	0.0258	0.0231

Tabel 2. Diversity of alginate-producing bacterial isolates from the rhizosphere of JG - maize (*Zea mays*), KK - cocoa (*Theobroma cacao*), LR - lamtoro (*Leucaena leucocephala*) based on colony morphology, gram staining, and hypersensitivity reactions.

Isolate code	Colony color	Colony edges	Elevation	Colony form	Gram staining reaction (+/-)	Reaction hypersensitive (+/-)
JG1-16	Yellow	Undulate	Flat	Circular	-	+
JG1-28	Creamy pale	Undulate	Flat	Circular	-	-
JG1-45	Yellow	Undulate	Flat	Filamentous	-	-
JG 2-3	Creamy pale	Undulate	Flat	Irregular	-	-
JG3-1	White	Undulate	Flat	Circular	-	+
JG3-53	Cream	Entire	Raised	Circular	-	-
KK1-28	Cream	Undulate	Raised	Circular	-	-
KK1-40	Yellow	Undulate	Raised	Circular	-	-
KK3-32	Creamy pale	Undulate	Flat	Irregular	+	-
KK3-23	Cream	Undulate	Raised	Irregular	+	-
KK3-6	Yellow	Undulate	Raised	Circular	+	-
LR1-1	Creamy pale	Curled	Flat	Filamentous	-	-
LR1-25	Cream	Entire	Flat	Circular	-	-
LR1-37	Creamy pale	Undulate	Raised	Circular	+	-
LR2-18	Cream	Undulate	Raised	Irregular	-	+
LR3-21	Cream	Undulate	Flat	Irregular	-	+
LR3-33	Cream	Curled	Flat	Filamentous	-	-

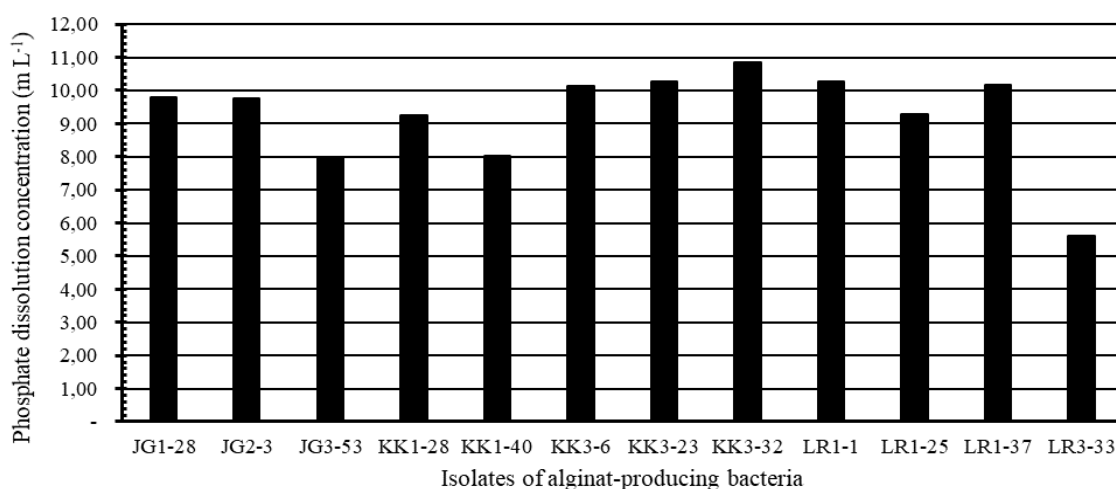**The ability of alginate-producing bacteria to fix nitrogen**

A total of 10 alginate-producing bacterial isolates were grown on Burk's N-free media. This indicated that were bacteria capable of fixing nitrogen (Figure 5). These alginate-producing bacterial isolates produced nitrogen content ranging from 0.27%-0.39%, the highest produced by LR1-25 (0.39%), followed by bacterial isolates JG3-53 (0.36%) and KK1-40 (0.36%) bacterial isolates (Figure 6).

Table 3. The phosphate dissolving ability of alginate-producing bacteria isolates based on phosphate solubility index (PSI) and phosphate solubility efficiency (PSE).

Isolate code	Diameter of clear zone (cm)	Colony diameter (cm)	PSE	PSI
JG1-28	1.5000	0.7667	195.65	3.0
JG2-3	0.9667	0.5333	181.25	2.8
JG3-53	0.6667	0.5333	125.00	2.3
KK1-28	1.1667	0.9333	125.00	2.3
KK1-40	0.9667	0.5333	181.25	2.8
KK3-6	1,2667	0.5000	253.33	3.5
KK3-23	0.8333	0.4000	208.33	3.1
KK3-32	1.4667	0.7000	209.52	3.1
LR1-1	1.1667	0.5333	218.75	3.2
LR1-25	1.4667	0.5333	275.00	3.8
LR1-37	0.4333	0.2667	162.50	2.6
LR3-33	1.1000	0.5000	220.00	3.2

Figure 3. Growth of bacterial isolates on Pikovskaya media: A. Clear zone was formed (arrows), B. No clear zone was formed

Figure 4. The ability to dissolve the phosphate of the alginate-producing bacterial isolate based on the dissolution concentration of the phosphate
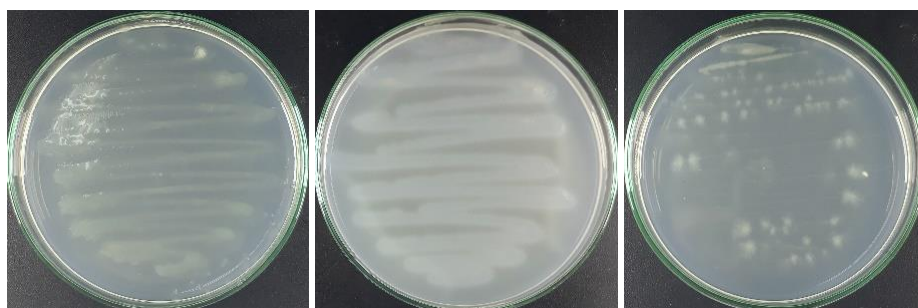
Figure 5. Culture of alginate-producing bacterial isolates on Burkes's N-free media
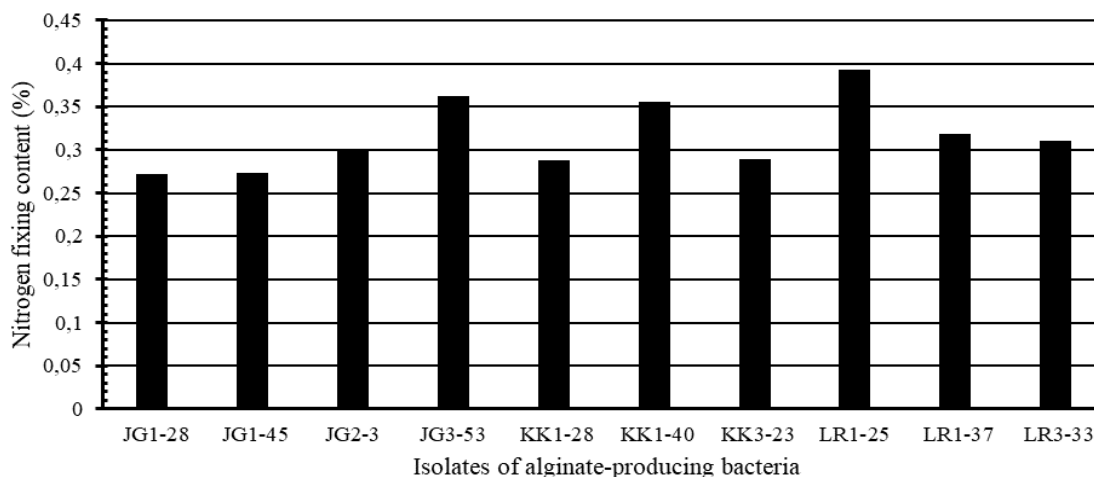
Figure 6. The ability of nitrogen fixation of alginate-producing bacterial isolates

Discussion

The morphological characters of bacteria showed diversity of the bacterial species isolated from the rhizosphere of maize, cocoa, and lamtoro. Total of 17 bacterial isolates was recorded with different morphological characters. Isolates from the maize rhizosphere showed various morphological characteristics, while isolates from the cocoa rhizosphere tend to be more uniform, both in color and shape. Plants are the main determinant of soil microbial diversity (Philippot et al. 2013), as plant roots secrete a substrate that affects microbial activity (Bowen and Rovira 1999; Berg and Smalla 2009). The bacterial morphology of the maize rhizosphere was influenced by environmental extremes (9% soil moisture content and high synthetic fertilizer input). This can be seen from the various colors and forms of colonies. Bacteria produce pigments in response to environmental stresses (Ivanov et al. 2007) and virulence (Muhlestein et al. 2011). In addition, the form of colonies determines the interaction between bacterial cells and their environment. This is a consequence of adaptation to pressure to optimize bacterial survival (Teeseling, Pedro, and Cava 2017). The lamtoro stands to provide highamount of C-organic (Valpassos et al. 2007) where is the main factor affecting the number, composition and activity of microbes (Wardle 1992), including various morphologies, due to differences in nutrient uptake, motility and predation (Young 2007). Thus, microbes have a different structure and composition in each plant species, because plant interactions with microbes are diverse (Mendes 2013).

Morphological characterization with Gram staining differentiates bacteria based on the cell wall structure. Characterization with Gram stain can provide an indication of the use of bacteria as an inoculum to be applied to agricultural land. Gram-negative bacteria produce pure osmolites in response to dryness, whereas Gram-positive bacteria produce osmolites for substance exchange (Schimel et al. 2007). This is consistent with our findings, where six isolates from the maize rhizosphere with only 9% soil moisture content are identified with Gram-negative

bacteria. While, gram-positive bacteria are identified in three bacterial isolates from the cocoa rhizosphere (KK3-6, KK3-23, KK3-32) and one isolate from the rhizosphere lamtoro (LR1-37).

The pathogenic potential of alginate-producing bacterial isolates is an important feature in its utilization as a land improvement agent. In the hypersensitivity test, 4 bacterial isolates showed a response while 13 bacterial isolates showed a negative response. All isolates that showed positive correction were identified as Gram-negative bacteria. Most Gram-negative bacteria are more reactive to tobacco leaves than Gram-positive bacteria (Umesha et al. 2008). This is caused by the EPS produced by gram-negative bacteria (Vanneste et al. 1990).

A total of 13 bacterial isolates had alginate production capacity, including four bacterial isolates from the maize rhizosphere, five bacterial isolates from cocoa, and four bacterial isolates from the rhizosphere lamtoro. Therefore, dryland agriculture may be a potential source of alginate-producing bacterial isolates. Alginate is an important component of exopolysaccharide (Chang et al. 2007). It is produced rapidly by bacteria in the soil (Kaur et al. 2014), so that it can interact directly with clay particles as a binding agent or adhesive (Costa et al. 2018). In addition, alginate prevents high oxygen pressure against nitrogen fixation (Gaurav et al. 2009). On the other hand, bacteria secrete alginate to keep moisture from drying out (Ngumbi and Kloepper 2016), thereby increasing water retention in the soil (Nasser et al. 2008). This is consistent with present study, where the bacterial isolates from the rhizosphere of maize are able to produce average alginate biomass compared to bacterial isolates from the rhizosphere of lamtoro and cocoa.

The functional groups formed on the surface of the cellulose biomass of the alginate-producing bacterial isolates are detected through FTIR spectra. The changes in the metabolic activity of surface-related bacteria during biofilm development are evaluated from the surface groups formed (Bremer and Geesey 1991). FTIR spectra show functional groups (OH, CH and C=C) that lead to the

presence of alginate polymers in cell biomass produced by bacterial isolates KK1-40, JG2-3 and JG1-28. The characteristics of the functional groups O-H, C-H, and C = O are polar or hydrophilic. This is useful in the bonding mechanism between the primary particles of materials formed through the negative charge of functional groups with clay particles (Chenu and Stotzky 2002).

According to Edathil et al. (2018), alginate polysaccharides are detected in the 3310 cm^{-1} bands, and Sujana et al. (2013) detected alginate in the 3542 cm^{-1} bands. These bands are assigned to a hydroxyl bond. Furthermore, the carbon matrix of the biofilm is shown by the alkane (C-H) group in the 2781 cm^{-1} band. Alkane functional groups exist in all organic compounds, as they are the carbon skeleton (Jindo et al. 2014). The ability of cation exchange capacity on the biomass surface is determined by the presence of carboxyl acid (C = O). The structure of the carboxylic groups in the acidic form allows electrostatic interactions with positively charged species (Furuyama Lima et al. 2018). These are detected in a different band among the three bacterial isolates. The carbonyl vibrations of the carboxylate groups occur at very different wave numbers (Lawrie et al. 2007), while the alginate matrix is shown by the C = C bond (aromatic ring) in the 1629 cm-1, 1627 cm^{-1} bands. The exopolysaccharide matrix is an important multipurpose element for the microbial community related to adhesion, structure, protection, and physiology (Wingender et al. 1999). The characteristic alcohol (C-O) bond is detected in different bands which are 1056, 1018 and 1101 cm^{-1}. Edathil et al. (2018) reported that alcohol was detected in the 1018 cm^{-1} band. This is a pyranose subunit stretch of bacterial polysaccharide (Gardella et al. 1984). The carbon element bound to oxygen indicates that the cell biomass of the bacteria leads to the formation of the alginate polymer. The alginate polymer is formed by carbon linked by oxygen from the ether group (Penman and Sanderson 1972). Based on this, isolate KK1-40 with its ability to produce alginate is interesting for further study related to its use as a dry land improvement agent, especially in relation to water retention.

One of the physiological characteristics of alginate-producing bacterial isolates was their ability to dissolve phosphate that was considered important for the study. Alginate had a potential effect on the dissolution potential of phosphate. This involves the production of exopolysaccharides in phosphate dissolution process (Yi et al. 2008), by utilizing alkyl phosphonate esters in polysaccharides to obtain P (Repeta et al. 2016). This potential was assessed both qualitatively (based on the solubility efficiency of phosphate and the solubility index of phosphate), and quantitatively (based on the dissolution concentration of phosphate). The results showed that there were differences in the efficiency of the phosphate solvent and the phosphate solubility index by alginate-producing bacteria, where the LR1-25 bacterial isolate dissolved higher the phosphate than the other 11 isolates.

Results showed that alginate producing bacterial isolates has the potential to dissolve phosphate. Bacteria can contribute to plant nutrition by releasing P from

organic compounds (Unno et al. 2005). This group of bacteria is capable of hydrolyzing organic and inorganic phosphorus compounds form insoluble compounds (Kalayu 2019) through dissolving and absorption (Chen and Liu 2019). It is caused by bacterial biofilms that contain carboxyl, sulfate, and phosphate groups that produce a negative charge on their surface (Ahmad and Husain 2017).

The potential for nitrogen fixation by alginate-producing bacteria was one of the characteristics studied. This is due to the ability of nitrogen-fixing bacteria to convert free nitrogen from the atmosphere into ammonia using the nitrogenase enzyme system (Ahmad and Husain 2017). While alginate protects the nitrogenase enzyme from oxygen and plays a role in nitrogen fixation (Sabra et al. 2000; Goh et al. 2012; Nosrati et al. 2012). Results show that bacterial isolates were able to fix nitrogen and also had the ability to produce alginates. These results are consistent with Nosrati et al. (2012), who stated that the increase in nitrogen fixation is depended on the amount of alginate produced.

Both morphological and physiological characterization revealed that three types of superior bacterial isolates (KK1-40, KK3-32, and LR1-25) exhibit the ability to produce alginate, phosphate dissolving, and nitrogen-fixing respectively. The bacterial isolate KK1-40 was identified as Gram-negative bacteria which was capable of producing more alginate with hydrophilic surface functional groups, so it was a potential being an agent for increasing water retention in dry land. Meanwhile, the KK3-32 isolate was detected as Gram-positive bacteria which was capable of dissolving phosphate. In addition, Gram-positive bacteria also be used as potential biological control agents (Suyono and Baharuddin 2019).

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REFERENCES

Ahmad I, Husain FM. 2017. Biofilms in Plant and Soil Health. In Biofilms in Plant and Soil Health. John Wiley & Sons Ltd., New York. DOI: 10.1002/9781119246329.

Arfarita N, Muhibuddin A, Imai T. 2019. Azotobacter population, soil nitrogen and groundnut growth in mercury-contaminated tailing inoculated with Azotobacter. J Degrade Min Land Manage 6: 1617-1623. DOI: 10.15243/jdmlm. 2019.062.1617.

Barreca S, Orecchio S, Pace A. 2014. The effect of montmorillonite clay in alginate gel beads for polychlorinated biphenyl adsorption:

Isothermal and kinetic studies. Appl Clay Sci 99: 220-228. DOI: 10.1016/j.clay.2014.06.037.

Ben Abdallah RA, Mejdoub-Trabelsi BM. 2016. Isolation of Endophytic Bacteria from Withania Somnifera and Assessment of their Ability to Suppress Fusarium Wilt Disease in Tomato and to Promote Plant Growth. J Plant Pathol Microbiol 7 (5). DOI: 10.4172/2157-7471.1000352.

Berg G, Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68 (1): 1-13. DOI: 10.1111/j.1574-6941.2009.00654.x.

Bowen G, Rovira A. 1999. The Rhizosphere and its management to improve plant growth. Adv Agron 66: 1-102. DOI: 10.1016/S0065-2113(08)60425-3.

Bremer PJ, Geese GG. 1991. Biofouling: An evaluation of biofilm development utilizing non-destructive attenuated total reflectance Fourier transform infrared spectroscopy. Biofouling 3: 89-100. DOI: 10.1080/08927019109378165.

Cappucino JG, Sherman N. 2014. New features make the micro lab more clinical application gram staining: the first. In: Cappuccino JG. (ed.), Clinical Application (XI). Pearson Education, UK.

Chang WS, Van De Mortel M, Nielsen L, De Guzman GN, Li X, Halverson LJ. 2007. Alginate production by Pseudomonas putida creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. J Bacteriol 189 (22): 8290-8299. DOI: 10.1128/JB.00727-07.

Chen Q, Liu S. 2019. Identification and characterization of the phosphate-solubilizing bacterium *Pantoea* sp. S32 in reclamation soil in Shanxi, China. Front Microbiol 10: 1-12. DOI: 10.3389/fmicb.2019.02171.

Chenu C, Stotzky G. 2002. Interactions between soil particles and microorganisms-impact on the terrestrial ecosystem: an overview. In: Huang PM, Bollag JM, Senesi N (eds.). Interactions between Soil Particles and Microorganisms. John Wiley & Sons, Ltd., New York.

Costa OYA, Raaijmaker JM, Kuramae EE. 2018. Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. Front Microbiol 9: 1-14. DOI: 10.3389/fmicb.2018.01636.

Davis TA, Volesky B, Mucci A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37: 4311-4330. DOI: 10.1016/S0043-1354(03)00293-8.

Deng S, Wipf HML, Pierroz G, Raab TK, Khanna R, Coleman-Derr D. 2019. A plant growth-promoting microbial soil amendment dynamically alters the strawberry root bacterial microbiome. Sci Rep 9 (1): 1-15. DOI: 10.1038/s41598-019-53623-2.

Edathil AA, Pal P, Banat F. 2018. Alginate clay hybrid composite adsorbents for the reclamation of industrial lean methyldiethanolamine solutions. Appl Clay Sci 156: 213-223. DOI: 10.1016/j.clay.2018.02.015.

Emtiazi G, Ethemadifar Z, Habibi MH. 2004. Production of extra-cellular polymer in Azotobacter and biosorption of metal by exopolymer. J Biotechnol 3: 330-333.

Etesami H, Maheshwar DK. 2018. Use of plant growth-promoting rhizobacteria (PGPRs) with multiple plant growth-promoting traits in stress agriculture: Action mechanisms and future prospects. Ecotoxicol Environ Saf 156: 225-246. DOI: 10.1016/j.ecoenv.2018.03.013.

Eyler E. 2013. Pouring agar plates and streaking or spreading to isolate individual colonies. In: Methods in Enzymology 533: 3-14. DOI: 10.1016/B978-0-12-420067-8.00001-5.

FAO. 2008. Guide to laboratory establishment for plant nutrient analysis, Food and Agriculture Organization of the United Nations, Rome. 2008. In: Motsara MR (ed.), FAO Fertilizer and Plant Nutrition Bulletin 19. Electronic Publishing Policy and Support Branch Communication Division, FAO, Rome.

FAO. 2017. Water for Sustainable Food and Agriculture Water for Sustainable Food and Agriculture. A report produced for the G20 Presidency of Germany, FAO, Rome.

Freeman BC, Chen C, Yu X, Nielsen L, Peterson K, Beatti GA. 2013. Physiological and Transcriptional Responses to Osmotic Stress of Two *Pseudomonas syringae* Strains That Differ in Epiphytic Fitness and Osmotolerance. J Bacteriol 195 (20): 4742-4752. DOI: 10.1128/JB.00787-13.

Furuyama Lima AM, De Freitas Lima M, Garrido Assis OB, Raab A., Amoroso HC, De Oliveira Tiera VA, De Andrad MB, José Tiera M. 2018. Synthesis and physicochemical characterization of multiwalled

carbon nanotubes/hydroxamic alginate nanocomposite scaffolds. J Nanomater. 2018. DOI: 10.1155/2018/4218270.

Gardella JA, Grobe LI, Hopson WL, Eyring EM. 1984. Comparison of attenuated total reflectance and photoacoustic sampling for surface analysis of polymer mixtures by Fourier Transform Infrared Spectroscopy. Anal Chem 56 (7): 1169-1177. DOI: 10.1021/ac00271a026.

Gaurav SS, Sachin C, Chandra M. 2009. Exopolysaccharides and lipopolysaccharide production by Sinorhizobiobium fredi Tn5 mutans infection *Vigna radiata* L. Amer J Agric Biol Sci 4 (5): 32-38.

Goh CH, Heng PWS, Chan LW. 2012. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohydrate Polymers 88 (1): 1-12. DOI: 10.1016/j.carbpol.2011.11.012.

Hay ID, Rehman ZU, Moradali MF, Wang Y, Rehm BHA. 2013. Minireview Microbial alginate production, modification and its applications. Microb Biotechnol 6: 637-650. DOI: 10.1111/1751-7915.12076.

Hiremath PS, Bannigidad P. 2011. Automated Gram-staining characterisation of bacterial cells using colour and cell wall properties. Intl J Biomed Eng Technol 7 (3): 257-265. DOI: 10.1504/IJBET.2011.043298.

Isobe K, Ohte N. 2014. Ecological perspectives on microbes involved in N-cycling. Micro Environ 29 (1): 4-16. DOI: 10.1264/jsme2.ME13159.

Ivanov AG, Krol M, Selstam E, Sane PV, Sveshnikov D, Park Yll, Öquist G, Hune NPA. 2007. The induction of CP43′ by iron-stress in Synechococcus sp. PCC 7942 is associated with carotenoid accumulation and enhanced fatty acid unsaturation. Biochimica et Biophysica Acta-Bioenergetics 1767 (6): 807-813 DOI: 10.1016/j.bbabio.2007.02.006.

Iwasaki KI, Matsubara Y. 2000. Purification of pectate oligosaccharides showing root-growth-promoting activity in lettuce using ultrafiltration and nanofiltration membranes. J Biosci Bioeng 5: 495-497. DOI: 10.1016/S1389-1723(00)89104-5.

Jindo K, Mizumoto H, Sawada Y, Sanchez-Monedero MA, Sonoki T. 2014. Physical and chemical characterization of biochars derived from different agricultural residues. Biogeosciences 11 (23): 6613-6621. DOI: 10.5194/bg-11-6613-2014.

Kalayu G. 2019. Phosphate solubilizing microorganisms: Promising approach as biofertilizers. Intl J Agron. DOI: 10.1155/2019/4917256.

Kaur V, Ber MB, Panesar PS, Kumar H, Kennedy JF. 2014. Welan gum: Microbial production, characterization, and applications. Intl J Biol Macromol 65: 454-461. DOI: 10.1016/j.ijbiomac.2014.01.061.

Lawrie G, Keen I, Drew B, Chandler-Temple A, Rintoul L, Fredericks P, Gröndahl L. 2007. Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS. Biomacromolecules 8 (8): 2533-2541. DOI: 10.1021/bm070014y.

Lee KY, Mooney DJ. 2012. Alginate: Properties and biomedical applications. Prog Polymer Sci (Oxford) 37 (1): 106-126. DOI: 10.1016/j.progpolymsci.2011.06.003.

Lesk C, Rowhani P, Ramankutty N. 2016. Influence of extreme weather disasters on global crop production. Nature 529 (7584): 84-87. DOI: 10.1038/nature16467.

Mendes R. 2013. Deciphering the rhizosphere Microbiome. 1097 (2011). DOI: 10.1126/science.1203980.

Mori T, Takahashi M, Tanaka R, Miyake T, Shibata T, Chow S, Kuroda K, Ueda M, Takeyama H. 2016. Falsirhodobacter sp. alg1 Harbors Single Homologs of Endo and Exo-Type Alginate Lyases Efficient for Alginate Depolymerization. PLoS ONE 11 (5): e0155537. DOI: 10.1371/journal.pone.0155537.

Muhlestein JB, Johnson EG, Zhu C, Xiao J, Revelo MP, et al. 2011. Effect of the Chinese drugs Nao Xintong and Dan Hong on markers of inflammation and the lipid profile in a hypercholesterolemic rabbit model. J Clinic Experiment Cardiol 2: 168. DOI: 10.4172/2155-9880.1000168

Nasser A. Mingelgrin U, Gerstl Z. 2008. Effect of soil moisture on the release of alachlor from alginate-based controlled-release formulations. J Agric Food Chem 56 (4): 1322-1327. DOI: 10.1021/jf0718392.

Naylor D, Coleman-derr D. 2018. Drought stress and root-associated bacterial communities. Front Plant Sci 8: 1-16. DOI: 10.3389/fpls.2017.02223.

Ngumbi E, Kloepper J. 2016. Bacterial-mediated drought tolerance: Current and future prospects. Appl Soil Ecol 105: 109-125. DOI: 10.1016/j.apsoil.2016.04.009.

Nosrati R, Owlia P, Saderi H, Olamaee M, Rasooli I, AAT. 2012. Correlation between nitrogen fixation rate and alginate productivity of an indigenous *Azotobacter vinelandii* from Iran. Iran J Microbiol 4 (3): 153-159.

Pacheco-Leyva I, Pezoa FG, Díaz-Barrera A. 2016. Alginate biosynthesis in *Azotobacter vinelandii*: overview of molecular mechanisms in connection with the oxygen availability. Intl J Polymer Sci 2016: 2062360. DOI: 10.1155/2016/2062360.

Pande A, Pandey P, Mehra S, Singh M. 2017. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. J Genet Eng Biotechnol. DOI: 10.1016/j.jgeb.2017.06.005.

Patel S, Jinal HN, Amaresan N. 2017. Isolation and characterization of drought resistance bacteria for plant growth-promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress. Biocatal Agric Biotechnol 12: 85-89. DOI: 10.1016/j.bcab.2017.09.002.

Paul, D., & Sinha, S. N. 2017. Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. Ann Agrar Sci 15 (1): 130-136. DOI: 10.1016/j.aasci.2016.10.001.

Penman A, Sanderson GR. 1972. A method for the determination of uronic acid sequence in alginates. Carbohydrate Res 25 (2): 273-282. DOI: 10.1016/S0008-6215(00)81637-7.

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH. 2013. Going back to the roots: The microbial ecology of the rhizosphere. Nature Rev Microbiol 11 (11): 789-799. DOI: 10.1038/nrmicro3109.

Rahman SS, Siddique R, Tabassum N. 2017. Isolation and identification of halotolerant soil bacteria from coastal Patenga area. BMC Res Notes 10 (1): 4-9. DOI: 10.1186/s13104-017-2855-7.

Repeta DJ, Ferrón S, Sosa OA, Johnson CG, Repeta LD, Acker M, Delong EF, Karl DM. 2016. Marine methane paradox explained by bacterial degradation of dissolved organic matter. Nature Geosci 9 (12): 884-887. DOI: 10.1038/ngeo2837.

Sá C, Cardoso P, Figueira E. 2019. Alginate as a feature of osmotolerance differentiation among soil bacteria isolated from wild legumes growing in Portugal. Sci Total Environ 681: 312-319. DOI: 10.1016/j.scitotenv.2019.05.050.

Sabra W, Zeng A, Lünsdorf H, Deckwer W. 2000. Effect of oxygen on formation and structure of *Azotobacter vinelandii* alginate and its role in protecting nitrogenase. Appl Environ Microbiol 66 (9). DOI: 10.1128/AEM.66.9.4037-4044.2000.Updated.

Schimel J, Balser T, Wallestein M. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88 (6): 1386-1394. DOI: 10.1890/06-0219.

Sheirdil RA, Hayat R, Zhang XX, Abbasi NA, Ali S, Ahmed M, Khattak JZK, Ahmad S. 2019. Exploring potential soil bacteria for sustainable wheat (*Triticum aestivum* L.) Production. Sustainability 11 (12): 3361. DOI: 10.3390/su11123361.

Stella M, Suhaimi M, Matthews S, Masduki S. 2010. Selection of suitable growth medium for free-living diazotrophs isolated from compost (Pemilihan medium pertumbuhan yang sesuai untuk bakteria pengikat nitrogen hidup bebas yang dipencil daripada kompos). J Trop Agric Fd Sc 38 (2): 211-219. [Indonesian]

Subaryono, Peranginangin R, Suharton MT, Zakaria FR. 2015. Isolation and identification bacteria producing alginate lyase derived from seaweed *Sargassum crassifolium*. JPB Kelautan dan Perikanan 10: 1-9. DOI: http://dx.doi.org/10.15578/jpbkp.v10i1.239.

Sujana MG, Mishra A, Acharya BC. 2013. Hydrous ferric oxide doped alginate beads for fluoride removal: Adsorption kinetics and equilibrium studies. Appl Surface Sci 270: 767-776. DOI: 10.1016/j.apsusc.2013.01.157.

Sukmawati, Ala A, Baharuddin, Gusli S. 2020. Biochar interventions enriched with alginate-producing bacteria support the growth of maize in degraded soils Biochar interventions enriched with alginate-producing bacteria support the growth of maize in degraded soils. IOP Conf Ser Earth Environ Sci 486 (1): 012133. DOI: 10.1088/1755-1315/486/1/012133 0-10.

Suyono, Baharuddin. 2019. Effectiveness formulation made from PGPR Bacillus spp. in the protection of shallot plants from fusarium wilt. IOP Conf Ser Earth Environ Sci 343: 012252. DOI: 10.1088/1755-1315/343/1/012252 DOI: 10.1088/1755-1315/343/1/012252.

Tang J, Taniguchi H, Chu H, Zhou Q, Nagata S. 2009. Isolation and characterization of alginate-degrading bacteria for disposal of seaweed wastes. Lett Appl Microbiol 48: 38-43. DOI: 10.1111/j.1472-765X.2008.02481.x.

Umesha S, Richardson PA, Kong P, Hong CX. 2008. A novel indicator plant to test the hypersensitivity of phytopathogenic bacteria. J Microbiol Methods 72 (1): 95-97. DOI: 10.1016/j.mimet.2007.11.002.

Unno Y, Okubo K, Wasaki J, Shinano T, Osaki M. 2005. Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of Lupin analysed by phytate utilization ability. Environ Microbiol 7 (3): 396-404. DOI: 10.1111/j.1462-2920.2004.00701.x.

Valpassos MAR, Maltoni KL, Cassiolato AMR, Nahas E. 2007. Recovery of soil microbiological properties in a degraded area planted with *Corymbia citriodora* and *Leucaena leucocephala*. Scientia Agricola 64 (1) 68-72. DOI: 10.1590/S0103-90162007000100010.

van Teeseling MCF, de Pedro MA, Cava F. 2017. Determinants of bacterial morphology: From fundamentals to possibilities for antimicrobial targeting. Front Microbiol 8: 1-18. DOI: 10.3389/fmicb.2017.01264.

Vanneste JL, Paulin JP, Expert D. 1990. Bacteriophage Mu as a genetic tool to study *Erwinia amylovora* pathogenicity and hypersensitive reaction on tobacco. J Bacteriol 172 (2): 932-941. DOI: 10.1128/jb.172.2.932-941.1990.

Wardle DA. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol Rev Cambridge Philos Soc 67 (3): 321-358. DOI: 10.1111/j.1469-185X.1992.tb00728.x.

Wingender J. Neu TR, Flemming HC. 1999. Microbial Extracellular Polymeric Substances: Characterization, Structure, and Function. Springer, Nederland. DOI: 10.1007/BF00582584.

Xu X, Iwamoto Y, Kitamura Y, Oda T, Muramatsu T. 2003. Root growth-promoting activity of unsaturated oligomeric uronates from alginate on carrot and rice plants. Biosci Biotechnol Biochem 67 (9): 2022-2025. DOI: 10.1271/bbb.67.2022.

Yan N, Marschner P, Cao W, Zuo C, Qin W. 2015. Influence of salinity and water content on soil microorganisms. International Soil and Water Conserv Res. DOI: 10.1016/j.iswcr.2015.11.003.

Yi Y, Huang W, Ge Y. 2008. Exopolysaccharide: A novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24 (7): 1059-1065. DOI: 10.1007/s11274-007-9575-4.

Yonemoto Y, Murata K, Kimura A, Yamaguchi H. 1991. Bacterial alginate lyase: characterization of alginate lyase-producing bacteria and purification of the enzyme. J Ferment Bioeng 72 (3): 152-157. DOI: 10.1016/0922-338X(91)90208-X.

Young, KD. 2007. Bacterial morphology: why have different shapes? Curr Opin Microbiol 10 (6): 596-600. DOI: 10.1016/j.mib.2007.09.009.

Zhang Y, Liu H, Yin H, Wang W, Zhao X, Du Y. 2013. Nitric oxide mediates alginate oligosaccharides-induced root development in wheat (*Triticum aestivum* L.). Plant Physiol Biochem 71: 49-56. DOI: 10.1016/j.plaphy.2013.06.023.

Zhang Y, Yin H, Zhao X, Wang W, Du Y, He A, Sun K. 2014. The promoting effects of alginate oligosaccharides on root development in Oryza sativa L. mediated by auxin signaling. Carbohydr Polym 113: 446-454. DOI: 10.1016/j.carbpol.2014.06.079.