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Isolation and in vitro Screening of Plant Growth Promoting Rhizospheric Bacteria from Corn (*Zea mays* var. *indentata*)

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Abstract—Many farmers use organic fertilizers to preserve the environment, but unfortunately, it's not as effective as chemical fertilizers. In recent years, the discovery of plant growth-promoting rhizobacteria (PGPR) has gained some attraction. PGPR have abilities to promote plant growth efficiently, including corn plants. Corn (*Zea mays* L.) is a staple food needed in large quantities. Our results show that some bacteria may have the potential to become PGPR for corn. Corn plant samples were obtained from maize plantations in Bunulrejo district, Blimbing, Malang (Republic of Indonesia). The potential of bacterial isolates obtained from the rhizosphere and endophyte area of corn as PGPR was determined based on several *in vitro* screening results, including the siderophore production test, indole-3-acetic acid (IAA) phytohormone production test, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production test, and phosphate dissolution test. Five isolates contained the best potential to become PGPR, namely E6.2, E3.1, E4.2, R4.2, and R2.1. Later, E6.2 and E3.1 were known to be able to fix nitrogen and produce siderophores, IAA and ACC deaminase. Based on the sequencing results, the R2.1 isolate was considered *Bordetella muralis*, the E3.1 isolate was identified as *Cellulosimicrobium cellulans*, while the R4.2, E4.2, and E6.2 isolates belonged to *Serratia nematodiphila*. These isolates could be used as potential inoculants for biofertilizers for better agricultural practice.

Keywords: *rhizobacteria*, *Bordetella muralis*, *Cellulosimicrobium cellulans*, *Serratia nematodiphila*

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Corn (*Zea mays* L.) is a plant that plays an important role, both as a food source and for other purposes such as a substitute for gas fuel, craft materials, and so on. In many countries, corn is widely used for human food as a staple food. It was also used in the animal feed industry. Today, corn is the most important cereal grain in terms of production, surpassing wheat and grain. Corn is the most extensively cultivated and consumed cereal globally, and in 2016, corn produced more than 1 billion metric tons [1]. In Indonesia, corn was produced up to 18.5 million tons in 2013, and about 13.9 million tons were used for feed [2], the need for corn continues to increase from year to year, and in 2020, corn was produced up to 24.95 tons. The world population is expected to grow to 9.8 billion in 2050, and it will lead to increased demand for foods, for example, corn.

Corn requires nutrients such as nitrogen (N), dissolved potassium (K), and phosphorus (P) for its growth [3]. Elements of $N : P_2O_5 : K_2O$ are required at least 75 : 50 : 30 kg/ha to increase the growth of corn plants. Because these elements are needed in large quantities, and the availability in the soil is limited, until now [4], many farmers have added chemical compounds to increase the growth of corn plants, such as urea, SP-36, ZK, and ZA fertilizers. The overuse of chemical fertilizers hardens the soil, reduces soil fertility, pollutes air, water, and soil, and lessens important nutrients of soil and minerals. Constant use of chemical fertilizer can alter the pH of soil, increase pests, acidify it, and stunting plant growth [5]. World population growth will lead to increased demand for foods and excessive use of chemical fertilizers. Excessive use of chemical fertilizers brings serious challenges to present and future generations like polluted air, soil, and water. Chemical fertilizers are not only hazardous to the environment but also to humans, animals, and microbial life forms [6]. To preserve the environment, organic fertilizers are now widely used. Organic fertilizers have several advantages, such as increasing soil fertility, improving the chemical, physical and biological conditions of soil, being safe for the environment, etc. However, using organic fertilizers also has disadvantages, such as nutrient content that is difficult to predict, inconsistent quality, and low nutrient content. It can't help promote stable plant growth [7].

Biofertilizer is a type of fertilizer that contains living beneficial microorganisms for plants. A healthy

plant usually has a healthy rhizosphere dominated by beneficial microbes. Biofertilizers differ from chemical and organic fertilizers; these compounds do not directly supply nutrients to plants. Biofertilizers belong to environmentally friendly fertilizers and can be used to replace chemical and organic ones [6]. Plant growth-promoting rhizobacteria (PGPR) are soil microorganisms that inhabit the areas around, on and in plant roots [7]. They can directly or indirectly promote plant growth and development through the production and secretion of various compounds around the rhizosphere [8]. PGPR can also have the ability to fix nitrogen, dissolve potassium and phosphorus, and produce siderophores, indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and several other elements that are important for plant growth. That means it can facilitate the availability of nutrients for plants [9]. A study revealed some types of bacteria, such as *Azotobacter*, *Azospirillum,* and phosphate solubilizing microorganisms having some promoting effects on the growth of corn plants, such as increasing plant height, fresh weight and dry weight of plants, and so on [10]. Although there are many studies regarding the effect of giving PGPR on corn plants in Indonesia, there are only a few studies about the isolation of PGPR from the corn, and there has been no study investigated completely the isolation of PGPR from corn plants used in *in vitro* screening and identification of bacteria obtained. PGPR isolated from corn plants will be able to form a relationship with these plants and give some beneficial effects. Although there are some studies about the effect of PGPR on a non-host plant, not all bacteria can form a relationship with a non-host plant. Several studies revealed that PGPR are only able to promote the growth of plants that are very closely related to their natural host [11]. Therefore, the present study aimed to isolate rhizospheric and endophytic bacteria from corn plants that have some potential to become PGPR for these plants. Several PGPR abilities become the focus of this research, which can produce siderophores, IAA, ACC deaminase, and dissolve phosphate. Isolates obtained from this research can be used to develop a bacteria consortium or as a single isolate that can be used for biofertilizer development for corn plants. The use of these isolates is expected to help preserve the environment by reducing the use of chemical fertilizers.

MATERIALS AND METHODS

Sample collection. Corn plant (*Zea mays* var. *indentata*) samples that can grow without fertilizers were collected from corn plantation, Blimbing, Malang (Republic of Indonesia).

Preparation of rhizosphere and endophyte samples. At the tip of the plant roots, soil samples attached to the root surface were taken aseptically using a toothbrush. Approximately 1 g of rhizosphere soil was put into 9 mL of sterile 0.9% NaCl solution and carried out in serial dilution from 10^{-2} to 10^{-6}. Endophytic sample preparation was done by cleaning the plant roots using running water and sterilizing the root surface with the following details: (1) soaking the roots in 95% ethanol for 90 s and rinsing with distilled water, (2) soaking the roots in 3.1% NaOCl for 6 min and rinsing with sterile distilled water, (3) soaking the roots in 95% ethanol for 60 s and rinsing with sterile distilled water [12]. After sterilizing the surface, the roots were then mashed aseptically, and ± 1 g of the biomass was mixed in 9 mL 0,9% NaCl solution, which was then carried out in serial dilutions from 10^{-2} to 10^{-3}.

Bacteria isolation and purification. Ten μL of the diluted samples were inoculated into Lowenstein-Jensen (HiMedia, India), SEA (HiMedia, India), and TSA (HiMedia, India) media separately and incubated at 25 and 37°C. Tryptic Soy Agar (TSA) is a general-purpose medium. Soil Extract Agar (SEA) is a selective medium for soil microorganisms. Lowenstein-Jensen medium is a selective medium for nitrogen-fixing bacteria [13]. Observations were performed every day, and when colonies were found growing, the colonies were streaked on the new portion of growth medium until some single colonies were obtained and the colony morphology was revealed. Gram staining was done to determine the chemical makeup of bacteria's cell walls. Single colonies obtained were then subcultured on nutrient-rich medium such as Trypticase Soy Broth (TSB, HiMedia, India). The obtained isolates were stored by adding 20–30% glycerol or further analyzed [13].

Antibiotic test. To test the bacterial resistance to several antibiotics, bacteria colonies were inoculated to some media containing antibiotics (kanamycin, gentamicin, and rifampicin). This test was carried out by inoculating the bacterial cultures into TSA medium containing antibiotics [14] (50 g/mL for kanamycin; 10 g/mL for gentamicin; 5 g/mL for rifampicin), incubated for ±2–3 days at 25°C, then the growth of bacteria on the media was observed.

In vitro screening. Bacterial isolates were tested for their potential to become PGPR through a siderophore production test, IAA production test, ACC deaminase production test, and phosphate dissolution test. These tests were first performed by inoculating some isolates on a TSB medium, incubating at 25°C for 1–2 days and measuring OD_{600} [15]. If the OD_{600} ranged from 0.3–0.9, then the culture was concentrated 10×, but if the OD_{600} was above 1, the culture was concentrated 5×. The culture concentrate was prepared by centrifugation at 1000 *g* for 10 min, taking out the supernatant (removing 90% supernatant volume for making 10× concentrated culture or 80% supernatant volume for making it 5× concentrated), resuspending the cells pellet before using it as a sample for other tests.

Siderophore production test. A siderophore production test was carried out by inoculating 10 μL of the concentrated culture on a modified King's B medium (BD Difco™, USA). The culture was incubated for 2–3 days at 25°C, and a medium color change indicated a positive result from blue to orange [16].

IAA production test. IAA production test was carried out by inoculating 1 full loop of bacterial culture into TSB and TSB + Trp (1 mg/mL; L-Trp, Sigma-Aldrich, USA) media. Incubation was performed at 25°C, and sampling was carried out on days 1, 3, 5, and 7. Samples were centrifuged at 2500 *g* for 10 min, then 0.5 mL of the supernatant was taken and mixed with 1 mL of Salkowski's reagent containing 0.5 M ferric chloride (Sigma-Aldrich, USA) and 35% perchloric acid (Sigma-Aldrich, USA). The sample mixture with reagent was incubated in the dark for 30 min, and then OD_{530} was measured. The quantity of IAA was determined by comparing the results obtained with standard curves made using IAA (Sigma-Aldrich, USA) at various concentrations (1–10 ppm and 10–100 ppm) [17].

ACC deaminase production test. ACC deaminase production test was carried out by inoculating 10 μL of concentrated culture on modified Dworkin and Foster (DF) medium containing (g/L: KH_2PO_4—4.0, Na_2HPO_4—6.0, 0.2 g $MgSO_4$·7H2O—0.2, glucose—2.0, gluconic acid—2.0 and citric acid—2.0 with trace elements (1 mg $FeSO_4 \cdot 7H_2O$, 10 mg H_3BO_3, 11.19 mg $MnSO_4 \cdot H_2O$, 124.6 mg $ZnSO_4 \cdot 7H_2O$, 78.22 mg $CuSO_4 \cdot 5H_2O$, 10 mg MoO_3, pH 7.2) amended with 3 mM 1-aminocyclopropane-1-carboxylic acid (Sigma-Aldrich, USA). The culture was incubated for 2–3 days at 25°C, and a positive result was indicated by colony growth on the medium [18].

Phosphate dissolution test. The phosphate dissolution test was carried out by inoculating 10 μL of concentrated culture on 1/2 Pikovskaya medium(Himedia, India). The culture was incubated for 2–3 days at 25°C, and a positive result was indicated by the formation of a clear zone around the colony [19].

Sequencing, editing, and sequence identification. Based on *in vitro* screening results, 5 isolates with the best results were chosen for sequencing using 785F primer 5'(GGATTAGATACCCTGGTA)3' and 907R primer 5'(CCGTCAATTCMTT TRAGTTT)3'. Sequences obtained from the sequencing were edited using Seqman Ultra software. Sequencing results from both primers were merged and ordered to create a sequence-contig scaffold. If there was a discrepancy between the reading of the nucleotide signal and the justification of the nucleotide sequence among the sequencing results obtained, then correction was made by changing the nucleotide sequence in the sequence. Sequence identification was performed using NCBI BLAST (web address: https://blast.ncbi.nlm.nih.gov/Blast.cgi) and EzBioCloud (web address: https://www.ezbiocloud.net/). The results obtained are the results of the sequence that are most similar to the sample sequence.

RESULTS AND DISCUSSION

Bacteria isolation, Gram staining, and antibiotic test. There were 2 kinds of samples used for this experiment: rhizosphere samples and endophyte samples collected from the root area of the corn plant. From the rhizosphere samples, a total of 11 different types of colonies were successfully isolated, where 8 isolates were obtained from the TSA medium, and 3 isolates were obtained from the SEA medium. From the endophyte samples, a total of 9 different types of colonies were successfully isolated, where 4 isolates were obtained from TSA medium, 2 isolates were obtained from SEA medium, and 3 isolates were obtained from Jensen medium.

Colony morphology observation, Gram staining, and antibiotic tests were done for every bacterial isolate. The results of observations of colony morphology, Gram staining, and antibiotic tests for the 5 best isolates are shown in Table 1. Based on the results shown in Table 1, almost all bacteria are Gram-negative, coccobacillus bacteria except for E3.1, a Gram-negative bacillus type bacterium. It was observed that all bacteria were circular with an entire margin, and almost all bacteria had a glistening appearance, except for E3.1, which had a dull appearance. Isolates that showed resistance to antibiotics (able to grow on TSA medium containing antibiotics) were marked with R, and non-resistant isolates (didn't grow on this medium) were marked with S. Based on the results in Table 1, it can be observed that: (1) isolates E4.2, E6.2, R2.1 and E3.1 were not resistant to kanamycin, while isolate R4.2 was resistant to it; (2) isolates R4.2, E4.2, E6.2 and R2.1 were resistant to gentamicin, while isolate E3.1 was not resistant to it; (3) isolates R4.2, E4.2, E6.2 and E3.1 were resistant to rifampicin, while isolate E3.1 was not resistant to it.

In vitro screening and sequence identification. Molecular identification was carried out to ensure the appearance of the colony/morphology of the bacteria obtained. There were 5 isolates selected for sequencing based on the in vitro screening results, namely isolates R4.2, E4.2, E6.2, R2.1, and E3.1 (Table 2). Sequence identification was performed using 2 different sources, NCBI and EzBioCloud. In Table 2, % ident or similarity represents the similarity between sequences obtained with sequences from the database. For the siderophore production test, a positive result is indicated with a media color change from blue to orange [16] and symbolized with a "+" sign in the table, and the greater the color change is, the more "+" signs ("2+" or "3+") present in the table. Based on siderophore production test results obtained, it can be seen that isolate E4.2 had the highest ability to produce siderophores when compared to the other 4 isolates. IAA production tests were done using 2 types of media,

Table 1. Colony morphology, Gram staining, and antibiotic test results

Temperature	Medium	Isolate	Colony morphology	Gram staining	Antibiotic test		
					Kanamycin	Gentamicin	Rifampicin
25°C	1/2 TSA	*R4.2	Circular, bright yellow, medium-large, entire, glistening	Coccobacillus, negative	**R	R	R
		E4.2	Circular, yellow, medium-large, entire, glistening	Coccobacillus, negative	S	R	R
	Jensen	E6.2	Circular, yellow, small-medium, entire, glistening	Coccobacillus, negative	S	R	R
37°C	SEA	R2.1	Circular, milky white, medium-large, entire, glistening	Coccobacillus, negative	S	R	R
	Jensen	E3.1	Circular, yellowish-white, small-medium, entire, dull	Bacillus, negative	S	S	S

* R = rhizosphere sample, E = endophyte sample; ** R = resistance, S = susceptible.

namely TSB and TSB + TRP. The addition of Trp to TSB media was carried out to improve conditions for IAA production (sufficient Trp as a precursor was available). The level of IAA produced by bacteria inoculated in TSB + Trp media was higher than that for bacteria inoculated in TSB medium; this is because the higher the concentration of Trp in the medium, the higher the production of IAA by bacteria [20]. Based on IAA production test results in Table 2, isolate R4.2 showed the highest IAA production level.

For the ACC deaminase production test, isolates capable of producing ACC deaminase will be able to grow on DF media [21]. In Table 2, isolates that were able to grow on DF media were marked with "+" signs, and the more growth, the more "+" signs present in the table ("2+" or "3+"). Based on the ACC deaminase production test results in Table 2, it can be seen that isolates E4.2, E6.2, R2.1, and E3.1 were able to produce ACC deaminase with high activity.

For the phosphate dissolution test, a positive result was indicated by the formation of a clear zone around the growing colonies [19], but it was not revealed in our experiments (Table 2, a "–" sign).

Corn is widely used for human food and in the animal feed industry. The need for corn will continue to increase from year to year due to the growth of the world population (the world population's growth will lead to increased demand for foods like corn). The industrial revolution, followed by the green revolution, helped fulfil the need for food in the growing population, but the use of chemical fertilizers in agriculture will also increase [6]. Excessive use of chemical fertilizers will give some negative effects not only on the environment but also on humans and animals. To preserve the environment, eco-friendly organic fertilizers are now widely used, but organic fertilizers aren't as effective as chemical fertilizers, so they can't help to promote plant growth well.

Biofertilizer is a type of fertilizer that contains beneficial microorganisms that can help to promote plant growth. Biofertilizers are eco-friendly fertilizers that do not harm the environment and can replace chemical and organic fertilizers. PGPR can promote plant growth by producing and secretion different compounds around the rhizosphere [8]. Isolated PGPR can be used as biofertilizer development for corn plants. PGPR generally form 2 types of relationships with their host plants. The first is a rhizospheric relationship, where PGPR form colonies on the rhizosphere area of the plant. The second is an endophytic relationship, where PGPR form colonies in the host's apoplast space [22]. In this study, incubation was carried out at 2 different temperatures, where incubation at 25°C was performed to adjust to the optimal temperature in the original environment, while incubation at 37°C was carried out to test bacterial growth at higher temperatures.

There were 3 types of media used for bacteria isolation: TSA, SEA, and Lowenstein-Jensen media. TSA is a general medium used to grow microorganisms, and a total of 12 different colonies were successfully isolated from this medium. SEA was used to isolate soil microorganisms; this medium contains nutrients needed by soil microorganisms [23]. A total of 5 different colonies were successfully isolated from the SEA medium, and results indicate that these isolates are soil microorganisms that can grow in an oligotrophic environment. Jensen medium was used for initial

Table 2. In vitro screening and sequence identification results

Isolate	NCBI			EzBioCloud			Siderophore production test	IAA production test		ACC deaminase production test	Phosphate dissolution test
	best hits strain	accession number	%Ident	besthits strain	accession number	similarity		TSB	TSB + Trp		
R4.2	*S. nematodiphila* DZ0503SBS1	NR_044385.1	99.86	*Serratia nematodiphila* strain DSM 21420(T)	JPUX01000001	99.86	2+*	16.7 ppm	35.41 ppm	2+	–
E4.2	*S. nematodiphila* DZ0503SBS1	NR_044385.1	99.53	*Serratia nematodiphila* strain DSM 21420(T)	JPUX01000001	99.45	3+	4.25 ppm	29.85 ppm	3+	–
E6.2	*S. nematodiphila* DZ0503SBS1	NR_044385.1	99.80	*Serratia nematodiphila* strain DSM 21420(T)	JPUX01000001	99.66	2+	1.36 ppm	21.52 ppm	3+	–
R2.1	*B. muralis* T6220-3-2b	NR_145920.1	87.18	*Bordetella muralis* strain T6220-3-2b(T)	LC053647	85.22	2+	2.96 ppm	32.57 ppm	3+	–
E3.1	*C. cellulans* DSM 43879	NR_119095.1	99.66	*Cellulosimicrobium funkei* strain ATCC BAA-886 (T)	AY501364	99.93	2+	2.21 ppm	19.55 ppm	3+	–

screening for nitrogen-fixing bacteria [10], and there were 3 isolates obtained under these conditions. Results showed that these 3 isolates could fix nitrogen.

Colony morphology observation, Gram staining, and antibiotic tests were done to test the colony's characteristics and differentiate between 20 isolates obtained before in vitro screening test, where each colony has its characteristics, and each bacterium has a different resistance to the antibiotic. The antibiotic test needs to be done because colony morphology observation and Gram staining results cannot be used to accurately distinguish between different types of bacteria (because several bacteria can have the same colony morphology and Gram staining results). Antibiotic tests were done using 3 types of antibiotics: kanamycin, gentamicin, and rifampicin. Kanamycin is an effective antibiotic used against Gram-negative bacteria [24]. Gentamicin—a broad-spectrum antibiotic, and rifampicin are effective against Gram-positive bacteria [25].

Bacterial isolates were tested for their potential to become PGPR through several in vitro screening tests: siderophore production test, IAA production test, ACC deaminase production test, and phosphate dissolution test. Five bacterial isolates with the highest results were isolated as R4.2, E4.2, E6.2, R2.1, and E3.1. Based on molecular identification results, R4.2, E4.2, and E6.2 isolates belonged to *Serratia nematodiphila*. This bacterium is a Gram-negative, short rod bacterium, and its colonies have several morphological characteristics, including red-colored (on Nutrient Agar), circular, entire edges, and smooth [26]. These characteristics were quite following the results obtained, where the three isolates (R4.2, E4.2, and E6.2) were Gram-negative, coccobacillus bacteria, with their colony morphology in a circular shape, yellow in color, entire edges, and glistening appearance. The color difference was due to the different media used, which led to differences in gene expression (different media can induce different gene expressions) [27]. Moreover, isolates R4.2, E4.2 and E6.2 were able to produce siderophores, IAA and ACC deaminase (Table 2). Based on the *in vitro* study conducted by Khoa et al. [28], who succeeded in isolating *S. nematodiphila* CT-78 from the rhizosphere area of rice plants, it was revealed that this bacterium could produce siderophores and inhibit the rice plant pathogen, *Xanthomonas oryzae*.

Isolate R2.1 was identified as *Bordetella muralis* based on molecular identification results. *Bordetella* is a Gram-negative, coccobacillus and can be found in soil and water [29], where the average species have the characteristics of small-medium-sized, grayish-white, smooth, and glistening colonies. When compared with the observations of colony morphology of R2.1, where the colonies were milky white, small-medium in size, with entire edges and glistening, it can be said that the results obtained were appropriate. *B. muralis* was first isolated from the surface of a mural painting wall in Takamatsuzuka tumulus [29]. Based on in vitro screening results, R2.1 was able to produce siderophores, IAA and ACC deaminase. Although there has been no specific research on the effect of *B. muralis* on plants, another *Bordetella* species, namely *Bordetella bronchiseptica,* belongs to PGPR. This bacterium isolated from the northern hills of India showed the ability to fix nitrogen under extremely low-temperature conditions and can act as a biocontrol for plant growth under cold climate conditions [30].

Isolate E3.1 was identified as *Cellulosimicrobium cellulans* based on NCBI result and was thought to be *Cellulosimicrobium funkei* based on EzBioCloud result. However, *C. cellulans* was chosen because NCBI has a more complete and preferred database when compared to EzBioCloud. In addition, in NCBI, sample nucleotides were compared with 1477bp *C. cellulans* sequences from the database. The *C. cellulans* nucleotides were more than *C. funkei* sequences EzBioCloud (1443bp). Besides, the *C. cellulans* results also emerged as the second-best results on EzBioCloud. *C. cellulans* is a Gram-positive bacillus having colonies with morphological characteristics as circular and pale yellow colored [31]. It can be said that the morphology follows the colony results obtained, which are yellowish-white and circular. However, the Gram staining results can be said to be less suitable, where Gram-negative bacteria were found in the form of bacilli. Gram staining is a method with a fairly high error rate, up to 24%, where false-negative Gram staining results can be caused by poor sample preparation, staining, and observation processes [32]. In addition, the results of Gram staining that are not suitable can be caused by the destruction of lipopolysaccharides during staining. The bacteria are still too young, so the lipopolysaccharides have not been formed completely. Based on in vitro screening results, isolate E3.1 was able to fix nitrogen and produce siderophores, IAA, and ACC deaminase. *C. cellulans* isolated from the plant rhizosphere of wheat in Algeria produced IAA, dissolved phosphate, and inhibited wheat plant pathogens, such as *Verticillium dahliae*, *Botrytis cinerea* and *Fusarium oxysporum* [33].

* *

This study confirms that there are 5 isolates with a high potential to become PGPR for corn plants, which are R4.2, E.4.2, E6.2, R2.1, and E3.1. These isolates can produce IAA, ACC deaminase, and siderophores, and among them, isolate E6.2 and E3.1 can fix nitrogen. With their abilities, these isolates can be used to develop a bacteria consortium or as a single isolate that can be used as a biofertilizer, which can help promote corn plant growth and preserve the environment.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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Isolation and in vitro Screening of Plant Growth Promoting Rhizospheric Bacteria from Corn (*Zea mays* var. *indentata*)

J. Sukweenadhi, J. A. Theda, I. B. M. Artadana & S. C. Kang

Applied Biochemistry and Microbiology **58**, 806–812 (2022)

Metrics

Abstract

Many farmers use organic fertilizers to preserve the environment, but unfortunately, it's not as effective as chemical fertilizers. In recent years, the discovery of plant growth-promoting rhizobacteria (PGPR) has gained some attraction. PGPR have abilities to promote plant growth efficiently, including corn plants. Corn (*Zea mays* L.) is a staple food needed in large quantities. Our results show that some bacteria may have the potential to become PGPR for corn. Corn plant samples were obtained from maize plantations in Bunulrejo district, Blimbing, Malang (Republic of Indonesia). The potential of bacterial isolates obtained from the rhizosphere and endophyte area of corn as PGPR was determined based on several *in vitro* screening results, including the siderophore production test, indole-3-acetic acid (IAA) phytohormone production test, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production test, and phosphate dissolution test. Five isolates contained the best potential to become PGPR, namely E6.2, E3.1, E4.2, R4.2, and R2.1. Later, E6.2 and E3.1 were known to be able to fix nitrogen and produce siderophores, IAA and ACC deaminase. Based on the sequencing results, the R2.1 isolate was considered *Bordetella muralis*, the E3.1 isolate was identified as *Cellulosimicrobium cellulans*, while the R4.2, E4.2, and E6.2 isolates belonged to *Serratia nematodiphila*. These isolates could be used as potential inoculants for biofertilizers for better agricultural practice.

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Ethics declarations

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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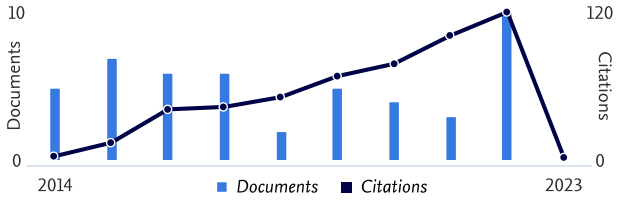

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