The Potency of Bacterial Associating Endemic Plants of The Java Coastal Area in Inducing Salt Tolerant in Agricultural Crops

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Abstract. Recent studies indicate that many plant species have ability to adapt to environmental stress because of their association with microbes. The aim of the research is to explore and to characterize bacterial endophyte and rhizoplane from endemic plants of the Java coastal area that induced salt tolerance in agricultural crops. Among the 1358 isolates of salt tolerant bacteria that have been isolated from 218 of different coastal plants showed that 108 isolates of bacterial rhizoplane, 87 isolates of bacterial root endophytes and 35 isolates of bacterial leaf endophyte have the ability to promote rice seedling growth. An amount of 33 isolates with vigor index (VI) value more than 1200 were tested at different levels of NaCl concentration (0, 50, 100, 150 and 200 mM). Fifteen bacterial isolates significantly increased VI value of rice seedling by up to 50% over uninoculated control at NaCl concentration more than 100 mM. Characterization of these 33 isolates revealed 32 isolates degraded pectin, 26 isolates produced indole acetic acid (IAA), 18 isolates solubilized phosphate, 15 isolates showed 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, 11 isolates produced hydrogen cyanide (HCN).

Keyword: salt tolerant, Java coastal area, rhizoplane bacteria, root endophyte bacteria, leaf endophyte bacteria

1. Introduction
Today's, soil salinity is a growing problem worldwide. As also in Indonesia, soil salinity has become considered as a problem that limiting plant productivity. Soil salinity is the salt content in the soil. The process of increasing the salt content is known as salinization. Salts occur naturally within soils and water. Salinization can be caused by natural processes such as mineral weathering or by the gradual withdrawals of the ocean. It can also come about through artificial processes such as irrigation [1]. Sea water intrusion may also contribute to a loss of arable land through inundation and increased...
soil salinity. Sea water intrusion along the coastal line has affecting crop growth and yield of agriculture land in Indonesia. Sea level in Indonesia has increased by 1–8 mm per year [2]. Indonesia has approximately 81,000 km² of coastline [3]. Agricultural land covers 26.4% of the country, and agriculture plays a major role along the coast. Nicholls and Mimura [4] estimate that approximately 1,600,000 ha of rice harvest area could be in danger due to a 1 m sea level rise. Suroso et al [5] also estimated that by 2050 the area of paddy rice fields in Indonesia could be reduced by 182,556 ha in Java and Bali, 78,701 ha in Sulawesi, 25,372 ha in Kalimantan, 3,170 ha in Sumatra, and 2,123 ha in Lombok. Therefore, it is becoming increasingly important to utilize sustainable techniques for inducing salinity tolerance in plants for better adaptation to environmental stress.

Recent studies indicate that many plant species have ability to adapt to environmental stress because of their association with certain microbes [6]. The major benefits for host plants partnering with endophytic microbes can include enhanced nutrition and improved tolerance to biotic and abiotic stress [6-8]. As an example, bacterial endophytes which are defined as those bacteria that can be isolated from surface-disinfected plant tissue or extracted from inside the plant, and do not cause a visible harm to the plant [7,8]. Bacterial endophyte can promote plant growth directly by the production of plant growth hormones and enhanced nutrition. They can also promote plant growth indirectly by alleviating the effect of environmental stress and the prevention of pathogenic organisms [9]. The term of Induced Systemic Tolerance (IST) has been projected for plant growth promoting rhizobacteria that induced physical and chemical changes that result in enhanced tolerance to abiotic stress [10,11]. Some bacterial endophytes provide an advantage to the host they colonize over non-infected plants. Therefore, this mechanism of ability for stress tolerance and survival can be induced to other plants by inoculating the plants with this bacterial endophyte. The development of stress tolerant crop through genetic engineering and plant breeding is essential, but it is a long drawn process, while using microbial inoculation to alleviate stresses could be a more cost effective, simple to use, have no adverse effect and an environmentally friendly option which could be available in a shorter time frame [12].

Plants identified as harbouring endophytic bacteria that span a great range of diversity and include both monocotyledonous and dicotyledonous species, including both herbaceous and woody plants, including many of agronomic importance [13]. The endemic plants of the Java coastal area could be a huge source for the beneficial microbe in inducing salt tolerant plants that not yet been clearly revealed. This region is characterized by long, heavily populated, and rapidly urbanizing coastlines along which many economic activities are located [2]. The Java coastal area is unique due to the presence of natural sea water that intruded into the area which harbor diverse microorganisms due to extreme conditions of soil salinity, swamped and ultraviolet irradiation. The extreme conditions present at the sites might naturally habitat for unique microbial communities. The bacterial endophytes may increase plant fitness in such extreme environments. There is also potential to find bacterial endophytes as well as rhizoplane bacteria that have the ability to alleviate salt stress.

The aim of the research was to explore and to characterize bacterial endophyte and rhizoplane from endemic plants of the Java coastal area that induced salt tolerant in agricultural crops. The characterization of such bacteria can be used to develop inoculants for alleviating salt stress in agricultural applications and could lead to new ways towards a salt tolerant plant for sustainable farming. The main hypothesis of this study was that plants grown in saline soils of the Java coastal area are host of bacteria that can ameliorate salinity stress. We studied the rhizoplane as well as endophytic bacteria (root and leaf) from endemic plants of the Java coastal area for their ability in enhancing salt tolerant in rice seedling at different levels of Sodium Chloride (NaCl).

2. Materials and methods

2.1. Plants sampling and isolation of bacterial endophyte and rhizoplane
Root and leaf of healthy plants growing in coastal soils of varying salinity in the Java coastal area were collected and placed in ice box for transfer to the laboratory. Electrical conductivity (EC) and pH
of soil where the plant samples collected were recorded. Leaves and roots were washed with tap water to remove attached clay and other materials. Plant roots for isolating bacterial rhizoplane were washed with sterile water three times. Rhizoplane bacteria were isolated by vortexes 1g of washed plant root for 10 min in 10 ml phosphate buffer saline (PBS) at maximum speed. Suspensions were diluted up to $10^{-5}$ dilution and spread in 10% of tryptic soy agar (TSA) medium containing 300 mM NaCl. Subsequently for isolation bacterial endophyte, roots and leaves were immersed in 70% ethanol for 3 min, washed with fresh sodium hypochlorite solution (5% NaOCl) for 5 min, and finally washed five times with sterile distilled water. To confirm that the sterilization process was successful, the aliquots of the sterile distilled water used in the final rinse were set on TSA medium plates. Surface-sterilized plant root and leaf samples were used for further analysis. To isolate the endophytic bacteria, leaf and root slices were placed in a sterilized blender and added with the PBS solution in appropriate volume before macerating for 3 min. The macerate was made to serial dilution up to $10^{-5}$ dilution and spread in 10% TSA medium containing 300 mM NaCl. The cultures were then incubated for 3 to 5 days at $\pm 30^\circ C$ to observe the bacterial colonies. A similar individual bacterial colony structure was selected based on their morphological characteristics, picked and re-cultured in fresh media for purification to generate pure cultures. Cultures were stored in a refrigerator at 4°C for further studies. For long-term storage, bacterial cultures were maintained in -80°C in TSA broth that contained 20% glycerol.

2.2. Screening for salt tolerance bacteria
For determining salt tolerance of the isolated bacteria, they were cultured on 10% tryptic soy broth (TSB) supplemented with 500, 600, 700, 800, 900 and 1000 mM NaCl gradually from the lowest NaCl concentration which act as a selective medium. After incubated for 48 hour, isolates were marked positive or negative for their ability to grow in different concentration of NaCl by measuring the optical density at 600nm.

2.3. Seedling vigor assay
Seedling vigor assay was used to screen bacterial isolates for their ability to promote plant growth that performed by the vigor of inoculated rice seedlings. Bacterial isolates growth at 1000 mM NaCl or more and showed no pathogen potential were screened for plant growth promotion ability. Rice seeds of Ciherang were surface sterilized by treatment with 70% ethanol for 1 min followed by 5% sodium hypochlorite solution for 5 min and then washed for 5 times with sterile water. Surface-sterilized rice seeds were treated with pure cultures of these isolates with the density of $10^6$-$10^9$ colony forming unit (CFU) mL$^{-1}$ in 0.85% sterilized saline solution for 24 h. Control seeds were incubated in 0.85% sterilized saline solution for 24 h. Germination tests were carried out by the filter paper which was put at the bottom of a jam jar. The filter paper was saturated with sterilized distilled water, and 10 bacterially treated seeds and untreated seeds were placed in those germination jars, and incubated at $\pm 25^\circ C$ for seven days. Treatment was conducted in triplicates. Seedling vigor was analyzed using the method of Abdul Baki and Anderson [14]. The lengths of the roots and hypocotyls of all the individual seedlings were measured. The vigor index (VI) was calculated using the formula VI = (mean root length + mean hypocotyl length)*% germination. The strains which gave high germination and vigor were selected for further experiments.

2.4. Effect of bacterial isolates on the growth of rice seedling under saline
Selected isolates from previous experiment were used for evaluating their ability to enhanced salinity tolerance in rice seeds. Bacterial isolates were grown in TSA medium and incubated for 24 h at 30°C. After incubation, the cell suspension was centrifuged at 5,000g for 10 min, and the pellet was resuspended in 0.85% sterilized saline solution. The bacterial inoculum was standardized to $10^6$-$10^9$ CFU mL$^{-1}$. Ten surface sterilized rice seeds of Ciherang were immersed in the bacterial inoculum for 24h in the dark under sterile conditions. Filter paper was used and put in the bottom of the jam jar as growth chamber. Sterilized Hoagland nutrient solution [15] of half strength was applied to the filter paper in appropriate volume for seedling nutrition. Jars were arranged using completely randomized
design with three replications for each treatment. Plants were incubated in a growth room with temperatures maintained at ±25°C, with a cycle of 12 h dark/light. After 14 days, germinated seeds were counted and percent of germination was calculated. Root and shoot length of rice seedlings were also recorded. Seedling vigor index (VI) was calculated using the formula as described above.

2.5. Characterization of plant growth promoting (PGP) traits

An amount of 33 isolates were tested for production of indole acetic acid (IAA). IAA production was detected as described by Brick et al. [16]. Bacterial cultures were grown for 72 h in nutrient broth media at 36-38°C. Fully grown cultures were centrifuged at 5000 rpm for 10 min. The supernatant (2 ml) was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Tube then incubated for 20 min at room temperature before the absorbance was measured at 527 nm (Shimadzu UV Probe). The concentration of each sample was calculated from a standard plot ranging from 0.5-30 µg ml⁻¹ pure IAA (Sigma). The ability of the isolates to produce ACC deaminase was screened on minimal media containing ACC as their sole nitrogen source as described by Penrose and Glick [17]. Optical density (OD) was measured after 48 h at 540 nm by spectrophotometer and considered as an index for evaluating ACC deaminase producing isolates. Isolates with OD more than 0.6 indicated positive for ACC deaminase production. Phosphate solubilization of isolates was evaluated for the ability to solubilize inorganic phosphate. Pikovskaya’s agar medium containing calcium phosphate as the inorganic form of phosphate was used in the assay. A loopful of bacterial culture were streaked on the plates and kept for incubation at 28°C for 4-5 days. The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria [18]. Production of HCN was detected according to the method of Lorck [19]. The nutrient agar medium was amended with 4.4 g glycine / L and bacteria were streaked on this agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate and 0.5% picric acid and placed in the lid of a petri plate inoculated with bacterial isolates. The plates were incubated at 28-30°C for 5 days. HCN production was assessed by the color change of yellow filter paper to reddish brown. Ammonia production was detected using method describe by Cappucino and Sherman [20]. Bacterial isolates were screened for the production of ammonia in peptone water. Freshly grown culture were inoculated in 10 ml peptone water in each tube and incubated for 48-72 h at 28-30°C. Each tube was then added with 0.5 ml of Nessler’s reagent. Development of brown to yellow color was a positive for ammonia production. For exopolysaccharide (EPS) activity (qualitative), strains were grown on Weaver mineral media enriched with glucose and production of EPS was assessed visually [21]. The EPS production was monitored as the formation of fluffy material on the plates after 48 h of incubation at 28-30°C.

2.6. Characterization of hydrolyzing enzyme activity

Amylase activity was determined on agar plates following the protocol of Männistö and Häggblom [22]. Formation of an opaque halo around colonies indicated amylase activity. Cellulase activity was assayed on plates containing (per liter) 5 g of carboxymethyl cellulose, 1 g of peptone and 1 g of yeast extract. After incubating for 48 h at 28-30°C the plates were overlayed with congo red (1 mg ml⁻¹) solution for 30 min. Congo red solution was then poured off followed by washing the surface of the plate with 1 M NaCl solution [23]. Chitinase activity of the isolates was determined as zones of clearing around colonies following the method of Chernin et al. [24]. Protease activity was determined using 1% skimmed milk agar plates, formation of halo zone around colonies was used as indication of activity [25]. Bacterial isolates were spotted on nutrient agar supplemented with 0.5% pectin for the assay of pectinase. After incubating the plates at 30 °C for 5 days the surface of the medium was overlayed with 2% hexadecyl trimethyl ammonium bromide (CTAB) solution for 30 min. CTAB solution was then poured off and the surface of the plate was washed with 1 M NaCl to visualize the halo zone around the bacterial growth [26].
3. Results

3.1. Plants sampling and isolation of bacteria

The plant samples were taken from 16 different coastal area that included in 4 different Provinces 10 Districts, 11 Sub-Districts, 13 Villages (figure 1). The Electrical Conductivity that is a measure of salinity of the soil samples were varied and ranged from 4 to 22 dS m\(^{-1}\) and the pH ranged were 6 to 8.5. A Total of 218 plant samples has been collected from 16 different points of sampling site (table 1). Plant species that were mostly found at every sampling site are Ipomea pescaprae, Rhisopora sp, Pandanus tectorius, Portulaca oleracea, Nypa fruticans, Spinifex sp., Cromolaena odorata, Calatropis gigantea, Thespesia populnea, Passiflora foetida, Sphagneticola trilobata, Hyptis capitata, Euphorbia hirta, Physalis peruviana, Terminalia catappa.

![Sampling site as marked with color and dot. Included in 4 Provinces, 10 Districts, 11 Sub-Districts, 13 Villages](image)

The total amount of bacteria that have been isolated were 553 of bacterial rhizoplane, 637 of root bacterial endophytes and 168 of leaf bacterial endophytes. A number of 1025 isolates from those isolates i.e. 477 rhizoplane isolates, 431 root endophyte isolates and 117 leaf endophyte isolates were able to grow in TSB medium containing 1000 mM NaCl. These isolates then were tested for hypersensitive response (HR) test and hemolysis test to determine their pathogen for plant, human and animals respectively. The result has indicated that only a few numbers of isolate that indicated as pathogen potential. This isolate then discarded according to the safety laboratory procedures. The isolates that were not indicated as pathogen potential were further screened for their ability to promote plant growth. The result was summarized in table 2.
Table 1. Soil electrical conductivity (EC) and pH of sampling sites.

| Province/District | Village, Subdistrict | Soil EC (dS/m) | Soils pH |
|-------------------|----------------------|----------------|----------|
| West Java:        |                      |                |          |
| Ciamis            | Bagolo, Kalipucang   | 11-17          | 6.5-7    |
| Indramayu         | Junti, Juntinyuat    | 10-18          | 6.5-7.5  |
| Central Java:     |                      |                |          |
| Cilacap           | Sodong, Adipala      | 15-20          | 6-7.5    |
|                   | Welahan, Adipala     | 10-18          | 6-7.5    |
|                   | Segara anakan        | 16-22          | 7-7.5    |
|                   | Karangkandri, Kesugihan | 5-8        | 6.5-7    |
| Demak             | Tambak bulusan, Karang | 18-21      | 6-7.5    |
|                   | Tengah               |                |          |
| Tegal             | Demangharjo, Warureja| 10-17          | 6.5-7.5  |
| Daerah Istimewa   |                      |                |          |
| Yogyakarta:       |                      |                |          |
| Kulonprogo        | Jangkaran, Temon     | 7-11           | 6-7.5    |
| Bantul            | Poncosari, Srandakan | 4-7           | 6-7.5    |
| Gunung Kidul      | Kemadang, Tanjungsari| 5-12         | 6.5-7.5  |
| East Java:        |                      |                |          |
| Lamongan          | Sendang Agung, Paciran| 18-20     | 7-8.5    |
|                   | Tunggul, Paciran     | 18-22          | 7-8.5    |
| Tuban             | Gesikharjo, Palang   |                | 7-8      |
|                   | Jenu, Jenu           | 13-15          | 7-8      |

Table 2. Total of saline tolerant bacteria isolated from 218 endemic plants of Java coastal soils.

| Numbers of bacteria | Rhizoplane | Root endophyte | Leaf endophyte |
|---------------------|------------|----------------|----------------|
|                     | 553        | 637            | 168            |
| Bacterial growth at NaCl>1000mM | 477        | 431            | 117            |
| Hemolysis test positive | 71         | 91             | 47             |
| Hypersensitivity Respond (HR) test positive | 70         | 36             | 10             |
| Bacterial growth promoting test positive | 108        | 87             | 35             |

3.2. Seedling vigor assay

Seedling vigor assay was used to screen the endophytic bacterial isolates for their plant growth promoting ability by examining the effect of isolates on seed germination, root growth and hypocotyl growth of rice seedling. Bacterial isolates that grow in saline medium containing NaCl up to 1000 mM e.i. 336 rhizoplane isolates, 304 root endophyte isolates, 60 leaf endophyte isolates were evaluated their ability to promote rice seedling growth. The result shows that total amount of 108 bacterial rizoplane, 87 isolates of root endophitic bacteria and 35 isolates of leaf endophitic bacteria were increased rice seedling vigor compare to uninoculated control. These isolates were further screened for their ability to promote growth of rice seedling by measuring the VI of rice seedling after 7 days. The VI data of rice seedling showed that amount of 33 isolates (12 rhizoplane isolates, 11 root endophytic isolates and 10 leaf endophytic isolates) have VI value more than 1200 (figure 2). The intermediate VI value (1001-1200) was shown in 40 isolates of rhizoplane, 38 isolates of root endophyte and 10 isolates of leaf endophyte. The highest value of VI indicates that this isolates have a better positive effect on the growth of rice seedling and used for further study.
3.3. **Effect of bacterial isolates on the growth of rice seedling under saline**

Isolates with a VI value up to 1200 from previous experiment have been evaluated by their effect on rice seedling growth at different salinity levels (0; 50; 100; 150 and 200 mM NaCl) (figure 3). Each isolates performed different respon at different level of salinity (figure 4). Isolates E194-3 and R146-6 have significantly increased VI 55% and 52% respectively compare to control at no salinity condition. Isolate D150 has significantly increased VI 78% over control, isolate E194-3 and isolate R146-6 significantly increased VI by 74% compare to uninoculated control at 50 mM NaCl. At NaCl 100 mM, isolatesE181-4, R146-6 and R55-11 significantly increased VI of rice seedling by 171%, 157% and 153% compare to uninoculated control. Isolate D102-1 and E196-1 showed the highest VI value at 150 mM. At NaCl 200 mM, isolate D205-1, E203-1and isolate E194-3 has an VI value of 300 for each while control has VI value 50. Hence we concluded that the most promising isolates among the 33 tested isolates were rhizoplane bacteria: R146-6; R188-2; R55-3; R55-11; R50-2; R17-9 root endophyte: E194-3; E196-1; E109-2; E203-1; E181-4, and leaf endophyte: D102-1; D150; D205-1; D183-4. These isolates significantly increased growth of rice seedling up to 50% (as measured by VI value) at NaCl more than 100 mM. However, five isolates i.e. D151-1; D151-3; E79-3; R110-1 and R191-2have no positive effect at salinity levels 150 mM and 200 mM as compared to control.

**Figure 3.** Growth of rice seedling inoculated with endophytic isolates (a) E203-1 (b) E109-2 (c) D217-2; rhizoplane isolates (d) R146-6 (e) R55-11 and (f) uninoculated control at the concentration of NaCl 100 mM.
Figure 4. Effect inoculation of bacterial isolates on the growth of rice seedling at the concentration of NaCl 0, 50, 100, 150 and 200 mM. Values are the means of three replicates.
3.4. Characterization of PGP traits

Characterization of these 33 isolates has indicated that 26 isolates produced IAA for more than 10 µg/ml, 18 isolates has the ability to solubilize phosphate, 15 isolates showed ACC deaminase activity and 3 isolates produced EPS (table 3). The result also obtained that 5 isolates have the ability to fixed nitrogen and 11 isolates have the ability to produced HCN. Several isolates showed the ability to produce multiple PGP traits. Except isolate R191-2, all isolates demonstrated pectinase activity. Characterization of hydrolyzing enzyme activity have resulted that only 4 isolates have no ability to produced ammonia, 15 showed ability to produced chitinase, 8 isolates produced cellulase, 6 isolates have no protease activity and 9 isolates have no amylase activity.

Table 3. Characterization of PGP traits and hydrolyzing enzyme activity of bacterial isolates (ACC=ACC deaminase, IAA; PO₄=phosphate solubilizing; HCN; NH₃= ammonia; Pro=protease; Amy=amylase; Chit=chitinase, Cell= cellulase; EPS= exopolysaccharide; N₂-Fix= N₂ fixing; Pec=pectinase)

| No | Isolates | ACC (µg/ml) | IAA | PO₄ | HCN | NH₃ | Pro | Amy | Chit | Cell | EPS | N₂-Fix | Pec |
|----|----------|-------------|------|-----|-----|-----|-----|-----|------|------|-----|--------|-----|
| 1  | D150     | -           | 19.442 | +   | +   | +   | +   | +   | +    | -    | -    | +      | -   |
| 2  | D151-1   | -           | 14.468 | +   | -   | +   | +   | +   | +    | -    | -    | +      | -   |
| 3  | D151-2   | -           | 12.836 | +   | -   | -   | +   | +   | +    | -    | +    | -      | +   |
| 4  | D217-1   | -           | 17.721 | +   | -   | +   | +   | -   | +    | -    | +    | -      | +   |
| 5  | D217-2   | -           | 15.825 | +   | +   | +   | +   | +   | -    | +    | -    | +      | +   |
| 6  | R110-1   | -           | 0.987  | -   | +   | +   | +   | +   | -    | +    | -    | -      | +   |
| 7  | R191-2   | -           | 13.860 | +   | -   | +   | +   | -   | +    | -    | +    | -      | -   |
| 8  | E91-3    | -           | 14.355 | +   | +   | -   | -   | -   | -    | +    | -    | +      | +   |
| 9  | R146-3   | -           | 16.773 | +   | +   | -   | +   | +   | -    | -    | -    | +      | -   |
| 10 | E79-3    | -           | 13.202 | -   | -   | -   | -   | -   | -    | -    | -    | +      | -   |
| 11 | E109-2   | -           | 17.738 | +   | -   | +   | -   | +   | +    | -    | +    | -      | +   |
| 12 | E193-2   | +           | 12.670 | +   | +   | +   | -   | +   | +    | -    | +    | -      | +   |
| 13 | E101-1   | +           | 10.288 | +   | +   | +   | +   | -   | -    | +    | -    | +      | +   |
| 14 | D205-1   | +           | 35.102 | +   | -   | -   | +   | +   | +    | -    | +    | -      | +   |
| 15 | D183-4   | +           | 0.945  | -   | +   | -   | -   | -   | -    | +    | +    | +      | -   |
| 16 | E107+    | +           | 3.814  | -   | -   | +   | -   | +   | +    | -    | +    | -      | +   |
| 17 | R55-3    | +           | 14.281 | +   | +   | +   | -   | -   | -    | +    | +    | -      | +   |
| 18 | R55-1    | +           | 10.404 | +   | -   | -   | -   | -   | -    | +    | +    | -      | +   |
| 19 | R50-2    | +           | 10.385 | +   | +   | +   | -   | -   | -    | +    | +    | -      | +   |
| 20 | D102-1   | +           | 4.077  | +   | +   | +   | +   | -   | -    | -    | -    | +      | +   |
| 21 | D99      | -           | 4.169  | -   | -   | +   | -   | -   | -    | +    | +    | -      | +   |
| 22 | E196-1   | +           | 40.692 | +   | -   | +   | +   | +   | -    | -    | -    | -      | +   |
| 23 | E181-4   | +           | 26.157 | +   | -   | -   | +   | +   | -    | -    | -    | -      | +   |
| 24 | D181-1   | +           | 24.927 | +   | +   | +   | -   | -   | +    | -    | -    | -      | +   |
| 25 | E194-3   | +           | 34.390 | +   | +   | +   | -   | -   | +    | -    | -    | -      | +   |
| 26 | R167-7   | +           | 18.906 | -   | -   | -   | +   | -   | +    | -    | -    | +      | +   |
| 27 | R146-6   | +           | 11.173 | +   | -   | +   | +   | -   | -    | +    | +    | -      | +   |
| 28 | E213-7   | +           | 6.282  | -   | +   | -   | -   | -   | -    | -    | -    | +      | +   |
| 29 | R61-2    | -           | 17.344 | -   | +   | +   | -   | +   | -    | +    | -    | -      | +   |
| 30 | R184-2   | +           | 12.689 | +   | +   | +   | -   | +   | -    | -    | -    | -      | +   |
| 31 | R188-2   | +           | 34.670 | -   | -   | -   | +   | +   | +    | +    | -    | +      | +   |
| 32 | R17-9    | +           | 20.944 | +   | +   | +   | -   | -   | -    | +    | +    | -      | +   |
| 33 | E203-1   | +           | 18.068 | -   | +   | +   | -   | -   | -    | -    | -    | +      | +   |
4. Discussion
We have obtained that bacterial isolates that have been isolated from rhizoplane, root and leaf of plants endemic of the Java coastal area were mostly tolerant to high salinity medium containing up to 500 mM NaCl. This may imply that these isolated bacteria are well adapted to high salinity. Since the coastal soils with salinity are natural habitats of halophilic or halotolerant bacteria [27], isolation of bacteria associated with plants from such natural habitat of saline environments with better adaptive strategies is the environmentally friendly option to apply as biocontrol mediators to ameliorate salt stress [28]. Gnotobiotic assay experiments using axenic rice germination assay were conducted for evaluating growth promoting activities of the isolates. The assessment of the plant growth-promoting capabilities of the isolates was based on the vigour index value that configured the germination percentage, hypocotyl length and root length. Inoculation with selected isolates had considerable positive impacts on growth parameters of rice seedling under salinity conditions as compared to uninoculated control, although not all high salinity tolerant bacteria that have been isolated could promote the growth of rice seedling under saline. Bacterial isolates that have not promoted the rice seedling growth indicated that these bacterial isolates have no effect to the host. It may be the bacterial isolates could not colonize the rice seedling or it has a neutral effect on rice seedling. As explained by Lodewyckx et al. [13] that endophytic bacteria that reside within the plant are some of which believed to impart a beneficial effect, whereas others are regarded to have a neutral or detrimental effect on the host plant. Grover et al. [6] and Bal et al. [29] have implied that different microbes have different ecological functions within a plant associated community, and have different colonization and survival dynamics under stress condition. Discovery of a novel beneficial bacterial strain in association with plant hosts from saline habitat which are specialized niches for microbe diversity are promising bioprospecting value for developing future environmentally friendly technology of salt tolerance crops [6, 29].

In this study, among all PGP traits evaluated on 33 isolates, IAA production, ACC deaminase, and phosphate solubilize ability appeared to be the most promising PGP traits in evaluated isolates. IAA production by bacterial endophyte may improve the fitness of the plant host in plant bacteria-interaction. IAA is a plant hormone with that is not has significant function to bacterial cells [12]. Bacteria that produce the enzyme ACC deaminase can cleaves ACC to form oketobutyrate and ammonium and thereby lowers the level of ethylene in stressed plants [30,31]. When ACC deaminase-containing bacteria colonize the rhizosphere or plant tissues, they can act as a sink for ACC and keep ethylene levels below the point where root growth is obstructed. Lower levels of ethylene in and around roots will resulted in promoted growth and elongation of roots [30]. The majority of the isolates have demonstrated multiple plant growth promoting traits i.e.IAA production, phosphate solubilization, ACC deaminase activity, ammonia and HCN production. Volatile compounds produce by rhizosphere and endophytic bacteria, such as ammonia and HCN were reported to play an important role in the biocontrol agent for abiotic and biotic tolerant in plant [32,33]. It is better to screen the most promising bacterial strain that have ability to produced multiple PGP traits and suitable colonization to the hosts. It is one of the preferences in the selection of strains that could lead to increased plant growth [29,30,34]. Most of the evaluated isolates have demonstrated hydrolyzing enzyme activity. Plant polymer hydrolyzing activities of these isolates indicating endophytic nature of these isolates. Pectinase and cellulase enzyme which degrade pectic and cellulose substances respectively, that presents in the plant cell wall are a key enzyme for colonization [35].

5. Conclusion
In conclusion, the isolated rhizoplane, root and leaf endophyte bacteria from endemic plants of Java coastal soils are promising in developing crops tolerant to salinity. The presence of indigenous endophytic and rhizoplane bacteria from the endemic plant grown in saline soils indicates that these bacteria may contribute to salinity tolerance. Bacterial strains that are adapted to the saline conditions are of interest for agricultural applications in salinity stress environment. To determine which of the endophytic and rhizoplane bacteria have the ability to colonize and persist at high levels in agricultural
crops as well as their capacity to improve growth and yields of agricultural crops under saline conditions should be tested under field condition.

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