Isolation and screening test of indigenous endophytic bacteria from areca nut rhizosphere as plant growth promoting bacteria

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Abstract. The study aimed was to find out the endophytic bacteria isolated from areca nut rhizosphere in Southeast Sulawesi. The study was conducted at the Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University. It using a completely randomized design (CRD) consist of four serial treatment of isolates isolated from 4 regencies. The isolates have tested their ability to improve viability and vigour of rice seed. The rice seeds before application, treated with endophytic bacteria isolates and then germinated. The germination rate was recorded daily by seven days. Each treatment using three replications, and data result were analysed using analysis of variance (ANOVA) and followed with DMRT (Duncan's Multiple Range Test). The result showed that the treatment of endophytic bacteria on rice seed has significantly increased the viability and vigour of rice seed. It also found four selected isolates which have potential to increase of rice seed growth, namely ME4, WSE4, WSE15, LE7, and LAE2. The future research is needed to find out the isolate that has effectivity on increase of areca nut seedling growth.

1. Introduction
Increasing public awareness of the negative impacts of chemical pesticides using in the production process of agricultural crops and the importance of consuming healthy and safe food from chemical pesticide residues, must be accompanied by a variety of technological innovations in plant cultivation based environmentally friendly technology. Currently, microbial-based plant cultivation technology products receive special attention and are widely developed because it is proven to be quite effective and efficient in replacing the role of fertilizers and even chemical pesticides. One type of microbe which has recently been studied is endophytic microbes (bacteria). These types of microbes are generally saprophytic and are in plant tissue, so they can directly interact and provide benefits for plant growth. It was stated that interactions between plants and endophytic bacteria could help plants adapt to various environmental conditions which are less conducive to plant growth [1].
Different plant species may be hosts of different endophytic bacterial species. Therefore, it is necessary to study continuously related to exploration and isolation of the endophytic bacteria, in different plants type and environmental conditions to obtain potential isolates that could be used as growth promoters and agent for biological control. Very limited information about the utilization of endophytic bacteria originating from areca nut plants. In general, the mechanism of action of endophytic bacteria is almost the same as rhizosphere bacteria, among others through its ability to fix nitrogen, dissolve phosphate and synthesize growth hormones such as IAA [2] [3-4]. Endophytic bacteria inoculation from group Klebsiella sp. and Enterobacter sp. in sugarcane can increase its growth through nitrogen fixation [5]. The role of endophytic bacteria and their interactions with plants have also related to their ability in improving plant health, growth and yields, and also improve soil quality [6-10]. This natural association of endophytic bacteria-plant provides positive benefits not only for endophytic bacterial species and their hosts (plants), but also more broadly on the sustainability of agroecosystems, increasing farmers' income through significantly increasing crop production [2, 11]. It was further mentioned that in addition to increasing plant growth, the utilization of endophytic bacteria can also increase plant tolerance to environmental stress [12].

2. Materials and methods

2.1. Place and time
The study was conducted at the agronomy Laboratory, Faculty of Agriculture, University of Halu Oleo from March to June 2019.

2.2. Research design
The study arranged in a completely randomized design, carried out serially with a number of different test isolates based on sampling locations namely Lainea (7 isolates), Benua (13 isolates), Mowila (7 isolates) and Landono (15 isolates). There were 42 test isolates, then each added 1 control, so that in total there were 129 treatment units.

2.3. Seed treatment with endophytic bacteria
Endophytic bacterial isolates were grown in solid TSA media, after that incubated for 48 hours. The growing bacterial colony was suspended in liquid medium until a population density reached of $10^9$ cfu ml$^{-1}$ [13]. The upland rice seeds were sanitized for five minutes using 2% sodium hypochlorite, and then washed times with sterile water, and dried for 60 minutes in a laminar air flow cabinet. A ten grams of dried seeds doused in the suspension of each endophytic bacterial isolate (50 ml) at 28 °C for 24 hours. The seeds are re-dried after the treatment, in a laminar wind current bureau until they arrive at the underlying weight and the seeds are prepared to use for testing.

2.4. Viability and vigour test
The upland rice seed that have been treated using endophytic bacterial isolates, are germinated in a plastic tub estimating 20 cm x 15 cm x 10 cm (length x width x stature), in media of sterile husk charcoal as a germination pad. Every treatment were planted 25 seeds and using three replications. The count of these observational factors is completed utilizing an equation:
1. Maximum growth potential (MGP), observed by calculated the number of seed germinated in the latest observation (7 DAP).
2. Germination rate (GP), delineating seed potential viability, was estimated dependent on the level of ordinary seedlings (NS) in the late perception (7 DAP).
3. Relative growth rate (RG-r), is the proportion of growth rate (RG) to maximum growth rate (RG-m). The maximum growth rate was obtained from the supposition that in the first observation, that normal seedlings had arrived 100%. RG was determined dependent on the collection of every day development rate:
4. Vigour index (VI), delineating the growth rate of vigour. It was measured based on the percentage of normal seedlings at the first time of observation (i.e. 5 DAP).

5. Dry weight of normal seedling (DWNS), depicting seed vigour and calculated in the end of the observation. All normal seedling are removed, wrapped using aluminium foil and then dried for 3 days in an oven at 60 °C. After that the seedling are put into desiccator for 30 minutes and weighed.

2.5. Data analysis

Data were analysed using ANOVA, if the treatment had a significant effect, then continued with different tests using Duncan's Multiple Range Test (DMRT) α 0.05.

3. Results and discussion

3.1. Results

3.1.1. Evaluation of endophytic bacteria from areca nut rhizosphere which explored from Mowila Sub-district. Inoculation of endophytic bacterial isolates from areca nut rhizosphere (explored from Mowila sub-district) on rice seeds was able to increase viability and vigour of seed. Among the treatments, ME4 isolates were consistently increase the viability and vigour of rice seeds compared to other isolates (Table 1). The ME4 isolates could increase the parameter of maximum seedling growth potential (MGP), percentage of germination (GP), vigour index (VI), relative growth rate (RG-r) and dry weight of normal seedlings (DWNS) respectively 72%, 131%, 70%, 118%, 169% if compared with control (Table 1).

| Isolate code | MGP (%) | GP (%) | VI (%) | RG-r (%/etmal) | DWNS (mg) |
|--------------|---------|--------|--------|----------------|-----------|
| Control     | 48.00 c | 34.67 c | 17.33 c | 33.02 c        | 36.33 c   |
| ME1         | 76.00 ab| 65.33 bc| 48.33 ab| 61.65 ab       | 56.33 bc  |
| ME2         | 76.00 ab| 71.33 ab| 49.33 a | 63.78 ab       | 70.67 bc  |
| ME3         | 64.00 b | 58.67 b | 34.00 ac| 53.87 b        | 38.00 c   |
| ME4         | 82.67 a | 80.00 a | 45.33 ab| 72.11 a        | 97.67 a   |
| ME5         | 74.67 ab| 65.33 bc| 32.00 ac| 59.3 ab        | 67.67 bc  |
| ME6         | 77.33 a | 61.33 b | 32.00 ac| 55.65 ab       | 67.33 bc  |
| ME7         | 70.67 ab| 64.00 b | 26.67 bc| 55.87 ab       | 43.00 c   |

Note: Number in the same column followed by the different suffixed are significantly different at DMRT.
3.1.2. Evaluation of endophytic bacteria from areca nut rhizosphere which explored from Landono Sub-district. The result showed that inoculation of endophytic bacterial isolates from areca nut rhizosphere (explored from Landono sub-district) on rice seeds was also able to increase seed viability and vigour in all observed variables. Among the treatments of endophytic bacterial isolates tested, two isolates (WSE4 and WSE15) were consistently and able to increase the rice seed viability and vigour (Table 2). Both of isolates could increase the parameter of MGP, GP, VI, RG-r and DWNS respectively 67%, 123%, 99%, 117%, 162% for WSE4 and 80%, 131%, 50%, 117%, 189% for WSE15 if compared with control (Table 2).

Table 2. The effect of endophytic bacterial isolates from the rhizosphere of areca nut explored from Landono sub-district on seedling rice parameters

| Isolate code | MGP (%)  | GP (%)  | VI (%)  | RG-r (%/etmal) | DWNS (mg) |
|--------------|----------|---------|---------|----------------|-----------|
| Control      | 48.00 b  | 34.67 c | 26.67 bc| 33.01 c        | 36.33 c   |
| WSE1         | 62.67 ab | 52.00 bc| 21.33 bc| 45.30 bc       | 28.00 cd  |
| WSE2         | 76.00 ab | 66.67 ab| 34.67 a-c| 61.17 ab       | 46.33 bd  |
| WSE3         | 76.00 ab | 70.67 ab| 28.00 bc| 61.17 ab       | 59.67 bc  |
| WSE4         | 80.00 a  | 77.33 ab| 53.33 a | 71.74 a        | 95.67 a   |
| WSE5         | 78.67 ab | 69.33 ab| 13.33 c | 58.09 ab       | 78.67 ab  |
| WSE6         | 74.67 ab | 58.67 ac| 25.33 bc| 51.52 ac       | 56.67 bc  |
| WSE7         | 74.67 ab | 68.00 ab| 40.00 ab| 62.22 ab       | 59.33 bc  |
| WSE8         | 72.00 ab | 61.33 ab| 29.33 bc| 57.68 ac       | 50.67 bd  |
| WSE9         | 70.67 ab | 62.67 ab| 20.00 bc| 53.49 ac       | 38.67 cd  |
| WSE10        | 60.00 ab | 54.67 ac| 25.33 bc| 48.19 ac       | 39.00 cd  |
| WSE11        | 69.33 ab | 66.67 ab| 16.00 bc| 57.75 ab       | 17.67 d   |
| WSE12        | 73.33 ab | 64.00 ab| 22.67 bc| 55.37 ac       | 75.00 ab  |
| WSE13        | 69.33 ab | 53.33 ac| 20.00 bc| 46.83 bc       | 46.33 bd  |
| WSE14        | 66.67 ab | 60.00 ac| 28.00 bc| 53.56 ac       | 46.33 bd  |
| WSE15        | 86.67 a  | 80.00 a | 40.00 a | 71.59 a        | 105.00 a  |

Note: Number in the same column followed by the different suffixed are significantly different at DMRT

3.1.3. Evaluation of endophytic bacteria from areca nut rhizosphere which explored from Benua Sub-district.

Table 3. The effect of endophytic bacterial isolates from the rhizosphere of areca nut from Benua sub-district on seedling rice parameters

| Isolate code | MGP (%)  | GP (%)  | VI (%)  | RG-r (%/etmal) | DWNS (mg) |
|--------------|----------|---------|---------|----------------|-----------|
| Control      | 48.00 bc | 34.67 cd| 26.67 ab| 33.02 cd       | 20.00 c   |
| LE1          | 34.67 c  | 24.00 d | 4.00 b  | 20.51 d        | 18.33 c   |
| LE2          | 50.67 bc | 42.67 ac| 21.33 ab| 38.95 bd       | 56.67 ac  |
| LE3          | 70.67 ab | 60.00 ac| 32.00 ab| 54.54 ab       | 56.00 ac  |
| LE4          | 58.67 ac | 56.00 ac| 17.33 ab| 47.81 ad       | 59.67 ac  |
| LE5          | 74.67 ab | 65.33 ac| 30.67 ab| 58.29 ac       | 45.67 ac  |
| LE6          | 82.67 ab | 72.00 ab| 44.00 a | 66.85 ab       | 73.00 ab  |
| LE7          | 88.00 a  | 77.33 a | 45.33 a | 72.89 a        | 84.00 a   |
| LE8          | 73.33 ab | 69.33 ab| 30.67 ab| 65.33 ab       | 68.67 ab  |
| PE1          | 74.67 ab | 64.00 ac| 36.00 a | 59.02 ac       | 43.00 ac  |
| PE2          | 68.00 ac | 60.00 ac| 21.33 ab| 52.13 ac       | 49.67 ac  |
| PE3          | 61.33 ac | 56.00 ac| 16.00 ab| 47.59 ad       | 59.00 ac  |
| PE4          | 69.33 ab | 66.67 ab| 38.67 a | 61.05 ac       | 43.33 ac  |
| PE5          | 70.67 ab | 61.33 ac| 26.67 ab| 54.60 ac       | 36.33 bc  |
Seed treatment with endophytic bacterial isolates isolated from areca nut rhizosphere (explorated from Benua sub-district) significantly increased of seed viability and seed vigour. Among the 13 isolates tested, LE7 isolate has better effect on increase of seed viability and seed vigour compared that others. Inoculation LE7 isolates on seeds were consistently increase all parameter tested. The average of MGP, GP, VI, RG-r and DWNS reached 83%, 123%, 70%, 121%, and 320% when compared to controls (Table 3).

**3.2. Discussion**

The results showed that endophytic bacteria isolate from areca nut rhizosphere explored from 4 subdistricts in South Konawe district, namely Mowila, Landono, Benua and Lainea have significantly effect in increasing of rice seed viability and vigour. The result is in line with the previous results reported that the application of endophytic bacteria could increase the seed viability and vigour [14]; corn [15] and onion [4].

The increasing of viability and vigour of rice seeds that has inoculated with endophytic bacteria is caused by the role of endophytic bacteria as plant growth promotor. Endophytic bacteria could synthesize growth hormones in the form of IAA, dissolve phosphate and fix nitrogen [3,16]. Endophytic bacteria could be obligate, facultative or passive associated with plants which act as phyto stimulators of biofertilizers and biocontrol agents so that they are beneficial to host plants [17,18]. Improved of seed viability and vigour are related to the capability of endophytic bacteria to synthesize IAA [19,20,21,22,23], fix nitrogen [24,25,26] and dissolve phosphate [25,26]. The results of other studies also showed that endophytic bacteria have ability in producing of IAA hormone and dissolve phosphate [27,28]. The endophytic bacteria could increase the rice plants growth [29]. The use of endophytic bacteria as plant growth promoting is very important in order to increase crop production [30]. Application of endophytic bacteria A3 and A6 was reported to contribute N equivalent to urea [31]. It was further reported that the application of endophytic bacteria by immersion methods in seeds showed that MSJ1H and AGS1F isolates could increase tomato plant growth by up to 60% when compared to controls [19].

**Table 4.** The effect of endophytic bacterial isolates from the rhizosphere of areca nut from Lainea sub-district on seedling rice parameters

| Isolate code | MGP (%) | GP (%) | VI (%) | RG-r (%/etmal) | DWNS (mg) |
|--------------|---------|--------|--------|----------------|-----------|
| Control      | 48.00 b | 34.67 c| 26.67 ab| 33.02 c        | 36.33 b   |
| LAE1         | 80.00 a | 72.00 ab| 37.33 ab| 64.63 ab       | 51.00 ab  |
| LAE2         | 80.00 a | 85.33 a | 54.67 a | 78.95 a        | 100.33 a  |
| LAE3         | 73.33 a | 68.00 ab| 37.33 ab| 61.46 ab       | 81.00 ab  |
| LAE4         | 80.00 a | 77.33 ab| 50.67 ab| 71.78 ab       | 38.00 b   |
| LAE5         | 77.33 a | 68.00 ab| 56.00 a | 65.68 ab       | 69.67 ab  |
| LAE6         | 76.00 a | 60.00 b | 34.67 ab| 54.82 bc       | 74.33 ab  |
| LAE7         | 65.33 ab| 58.67 b | 22.67 b | 50.92 bc       | 51.67 ab  |

Note: Means in the same column suffixed with different lower case letters are different at 5% levels of significance according to DMRT

**3.1.4. Evaluation of endophytic bacteria from areca nut rhizosphere which explored from Lainea Sub-district.** The result showed that seed treatment with endophytic bacterial isolates from areca nut rhizosphere (explorated from Lainea sub-district) also has significantly effect on seed viability and vigour. Among the 7 isolates tested, LAE2 isolate has better effect on increasing the rice seed viability and vigour. Inoculation LAE7 isolates on seeds were consistently increase all parameter tested. The average increase in MGP, GP, VI, RG-r and DWNS reached 67%, 146%, 105%, 139%, and 176% when compared to controls (Table 4).
Related to the ability of endophytic bacteria to synthesize IAA growth hormone, a study on green bean plants showed that from 16 isolates that have the ability to produce IAA with different concentrations, the application of isolates that produce high IAA concentrations affected the number of lateral roots but did not affect the sprout length. The results of further analysis showed that all isolates significantly influenced the formation of lateral roots except AT and control isolates. DM and K1K1 isolates give the best results in lateral root formation [32]. The results of other studies also reported that Ke03 isolate endophytic bacteria isolated from local onion Wakatobi gave the best results in increasing germination, root length and number of roots with an average increase of 145%, 46% and 78% when compared to controls.

It was also further reported that endophytic bacteria could increase growth of lateral roots, adventitious roots and primary roots [33]. The mechanism of action of the IAA hormone is directly related to the elongation of cells in the coleoptile and vertical segment of the plant so that it directly affects the increase in plant wet weight [34].

Relevant research shows that DnAr4 isolates treated by watering the endophytic bacterial suspension one day after planting showed the best results with an increase in plant height reaching 47.69%, canopy 68.9%, root wet weight 62.9%, canopy dry weight 19% and root dry weight of 52.93% [35]. Another study also reported that EKN2.1.1 isolate was the best isolate which could increase growth reaching 22.5% with the ability to produce the highest IAA hormone reaching 22.73 ppm [36].

4. Conclusions
It concluded that the isolates of endophytic bacteria isolated from the areca nut rhizosphere that explored from South Konawe district, have showed a significant effect on the rice seed viability and vigour. Five endophytic bacterial isolates were selected as potential growth promoters, namely ME4 isolate (from Mowila sub-district), WSE4 and WSE15 (from Landono sub-district), LE7 (from Benua sub-district) and LAE2 (from Lainea sub-district).

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