The potential of indigenous rhizobacteria from areca nut rhizosphere in South Konawe regency as a plant growth promoter

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Abstract. Sustainable agriculture focuses on the applied of biological resources in agricultural cultivation process, therefore exploration and isolation of potential microbes as biological fertilizers need to be continuously developed. The research aim was to get the potential indigenous rhizobacteria isolates from the rhizosphere of areca nut plants as plant growth promoter. The experiment consists of 4 series based on the location of isolate collection, namely Angata 7 isolates, Landono 7 isolates, Palangga 6 isolates, and 4 isolates Wolasi, using a complete randomized design with three replications. The potential of these various isolates on a laboratory scale was evaluated via seed viability and vigour tests using upland rice as indicator plant. Inoculation of seeds using rhizobacteria isolates was able to improve the viability and vigour of rice seeds as shown by increasing potential development, germination percentage, vigour index, and relative growth rate as compared to control. The research found that 19 isolates which have the potential to promote plant growth were selected, namely AG1, AG2, AG3, AG4 and AG7 (Angata), LD1, LD2, LD3, LD4, LD5 and LD7 (Landono), PL1, PL2, PL5, PL6 (Palangga) and WL1, WL2, WL3 and WL4 (Wolasi). Field-scale experiments is required to evaluate these potential isolates as biofertilizers.

1. Introduction
The development of organic agriculture as a solution for healthy and environmentally friendly living requires the support of cultivation technology free from synthetic chemicals. Various research studies related to the use of environmentally friendly technology are currently more focused on using indigenous microbes as fertilizer and biological control in plant cultivation. The increasing public awareness in consuming healthy food products and free of chemical pesticides also encourages the expanding exploration of beneficial microbes from various plant rhizosphere. Indonesia, as a mega-biodiversity country, has enormous potential in producing microbial-based bio-fertilizer products. Several studies have shown that the rhizobacteria have a role as plant growth promoter (PGPR), and members group microbes are effective in stimulating the growth of walnuts [1], pears, date palm and tomatoes [2,3], carob tree [4], and medicinal plant [5]. It also has potential to increase plant growth in onion [6], cocoa [7] and rice [8-10]. The exploration and isolation of rhizobacteria from the plant rhizosphere consider
several things. It is including vigorous plants' performance, healthy or not contaminated by disease, being able to produce even though they do not get fertilization applications. One of them is the areca nut—the areca nut condition grows wild but is fertile and has beneficial microbes that work in the plant's root area. The utilization of rhizobacteria is still intensively carried out in all parts of the world. The products produced as an alternative to chemical fertilizers play a significant role in supporting sustainable organic agriculture. Previous studies showed that rhizobacteria generally act as biofertilizers \[1-13\], bioprotectant \[14,15\] or play a dual role as a biofertilizer and bioprotectant \[16,17\]. These rhizobacteria generally come from Serratia, Pseudomonas, Azotobacter, Micrococcus, Burkholderia, Bacillus, and Azospirillum \[18,19\]. The ability of PGPR to fix nitrogen is related to its function as a biofertilizer, such as the groups of Rhizobia, Azotobacter, and Azospirillum \[20-23\]. It is also as phosphate solubilization \[24\], antagonistic agents such as Bacillus, Pseudomonas dan Serratia \[25\]. The research on the rhizobacteria isolated from the rhizosphere of areca nuts is still limited. Evaluation of the potential of rhizobacterial isolates as plant growth promoters on a laboratory test scale was carried out through seed viability testing, using upland rice seeds as indicator crops.

2. Materials and methods

2.1. Place and time
Evaluation of the potential for indigenous rhizobacteria explored from Konawe Selatan District was carried out in Agronomy unit of Agrotechnology Laboratory of Agriculture Faculty, Halu Oleo University from January to June 2020.

2.2. Research design
The experiment consists of 4 series of experiments based on the location of isolate collection, namely Angata 7 isolates, Landono 7 isolates, Palangga 6 isolates, and 4 isolates Wolasi, using a complete randomized design with three replications. Thus there were 43 test isolates (plus one as a control treatment), and overall there were 75 treatment units.

2.3. Seed inoculation using rhizobacteria isolates
The rhizobacterial isolates cultivated for 48 hours in solid TSA media. In an adequate liquid medium, the growing colony of bacteria was suspended until a population density of \(10^9\) cfu ml\(^{-1}\) was achieved \[25\]. The upland rice seed inoculation method follows the previous method has been used \[26\].

2.4. Viability and vigor test of upland rice seed
After inoculation treatment with rhizobacterial isolates, upland rice seeds were germinated in plastic box measuring 20 cm x 15 cm x 10 cm (length x width x height) and sterile rice husk charcoal as germination media. The number of seeds planted per treatment unit was 25 seeds. Observation of seed viability and vigor using variables of the growth potential maximum, germination percentage, vigor index and growth rate relative, using the formula \[27\].

2.5. Data analysis
ANOVA was used to evaluate the data and to proceed with numerous experiments using the Duncan \(\alpha\) 0.05 Multiple Range Test whether the procedure had a major effect.

3. Results and discussion

3.1. Results

3.1.1. Viability and vigour rice seed inoculated using rhizobacteria from Angata sub-district. The seeds which had inoculated with rhizobacteria isolates from Angata have significantly improved seed viability and vigor (MGP, GP, VI, RGr) compared to controls. All rhizobacterial isolates except AG5 and AG6
were more able to increase MGP, GP, VI and RGr compared to controls. The increase in MGP, GP, VI and RGr by rhizobacteria isolates reached 429-572%, 750-1076%, 733-1100%, and 853-1208% respectively compared to the control (Figure 1).

![Figure 1. Effect of rhizobacterial isolates explored from Angata sub-district on rice seed viability and vigor.](image)

3.1.2. Viability and vigor rice seed inoculated using rhizobacteria from Landono sub-district. Inoculation of seed with rhizobacteria isolates from Landono have significantly improve seed viability and vigor (MGP, GP, VI, RGr) compared to controls. In general, almost all rhizobacterial isolates except LD6 were more able to increase MGP, GP, VI and RGr compared to controls. By using this treatment, the variables of MGP, GP, VI and RGr increased 414-615%, 776-1076%, 900-1200%, and 925-1284% compared to the control (Figure 2).

![Figure 2. Effect of rhizobacterial isolates explored from Landono sub-district rice seed viability and vigor.](image)

3.1.3. Viability and vigor rice seed inoculated using rhizobacteria from Palangga sub-district. As Inoculation of rice seed with rhizobacterial isolates from Angata and Landono sub-districts, seeds inoculated with rhizobacteria isolates from Palangga sub-district were also significantly able to increase seed viability and vigor (MGP, GP, VI, RGr) compared to controls. In general, almost all rhizobacterial isolates except PL3 and PL4 were more able to increase MGP, GP, VI and RGr compared to controls. The increase in MGP, GP, VI and RGr by rhizobacteria isolates reached 500-643%, 876-1051%, 833-1267%, and 1024-1278% respectively compared to the control (Figure 3).
3.1.4. Viability and vigor of seeds inoculated using rhizobacteria from Wolasi sub-district. There was an increase in viability and vigor (MGP, GP, VI, RGr) in seeds which were inoculated using rhizobacteria from Wolasi. All tested rhizobacterial isolates showed the ability to increase MGP, GP, VI and RGr compared to controls, reaching 343-615%, 675-1038%, 467-1133%, and 765-1222%, respectively (Figure 4).

3.2. Discussion
In many parts of the world, discovery and isolation of rhizobacteria from different plant roots has been widely carried out and is still a research trend. The differences in location and type of plants are two things that should be considered when exploring microbes. As in this study, the target sampling location was in South Konawe Regency. The land conditions in this area are generally dry land, with the soil type dominated by Red and Yellow Podzolic soils, which have low pH, low fertility, and low organic matter, making them less ideal for crop cultivation. However, several types of plants, such as areca nut, show the ability to grow and produce well even though they have never received fertilization applications.

The result showed that the isolation of microbes from the rhizosphere of areca plants was proven to obtain several rhizobacterial isolates that could potentially be developed as PGPR. From four different
sampling locations (Sub-districts of Angata, Landono, Palangga and Wolasi), it was found that rhizobacterial isolates had different abilities to increase seed viability and vigor, although in general all isolates were able to improve seed viability and vigor much better than control. Several previous studies also reported the same results [28-31].

Among the 7 rhizobacterial isolates isolated from Angata sub-district, 5 isolates had the potential to be PGPR, namely AG1, AG2, AG3, AG4 and AG7. From Landono sub-district, among the 7 isolated rhizobacteria isolates, there were 6 potential isolates, namely LD1, LD2, LD3, LD4, LD5 and LD7. From Palangga sub-district, there were 4 potential isolates (PL1, PL2, PL5, PL6), while from Wlasi sub-district, all isolated isolates had the potential to be PGPR (WL1, WL2, WL3 and WL4).

The ability of rhizobacteria isolates as growth promoters is generally due to their ability to fix nitrogen, dissolve phosphate, and synthesize growth hormone. The study of the rhizobacteria isolates, there were 6 potential isolates, namely LD1, LD2, LD3, LD4, LD5 and LD7. The rhizosphere of seeds after being inoculated with rhizobacteria was due to their ability to fix nitrogen [6,20,33]. Several studies also reported that the increased viability of seeds after being inoculated with rhizobacteria was due to the ability of the rhizobacteria to solubilize phosphate [6,34-35].

4. Conclusions
The rhizosphere of areca nuts has the potential to obtain superior rhizobacterial isolates which could promote plant growth. The research found that 19 isolates which have the potential to promote plant growth were selected, namely AG1, AG2, AG3, AG4 and AG7 (Angata), LD1, LD2, LD3, LD4, LD5 and LD7 (Landono), PL1, PL2, PL5, PL6 (Palangga) and WL1, WL2, WL3 and WL4 (Wolasi). Field-scale experiments is required to evaluate these potential isolates as biofertilizers.

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