Seed bioprimering with indigenous endophytic bacteria isolated from Wakatobi rocky soil to promote the growth of onion
(*Allium ascalonicum* L.)

G A K Sutariati1*, A Khaeruni1, Muhidin1, A Madiki1, TC Rakian1, L Mudi1 and N Fadillah2

1Department of Agrotechnology, Faculty of Agriculture, Universitas Halu Oleo, Kendari Southeast Sulawesi 93212 Indonesia
2Graduated Student at Department of Agrotechnology, Faculty of Agriculture, Universitas Halu Oleo, Kendari Southeast Sulawesi 93212 Indonesia

Email: *sutariati69@yahoo.co.id*

**Abstract.** The realization of sustainable agriculture requires continuous research which can reduce the use of chemical fertilizers. This research was carried out to obtain the potential indigenous endophytic bacteria isolated from onion planted on rock soil of Wakatobi district in Southeast Sulawesi. The study conducted using a completely randomized design (CRD) consisting of 9 isolates. Germination was recorded daily until 14 days. At the same time, isolates evaluated for their ability to solubilize phosphate, fix N and synthesize IAA. Results showed that the seed treatments using endophytic bacteria significantly increased viability onion seeds, in term of germination increases in germination percentage. Almost all endophytic bacteria isolates tested were able to solubilize phosphate, fix N and synthesize IAA. From this study, Ke03 isolates showed the best performance and were able to increase germination percentage, root length and number of onion roots reaching 145%, 46% and 78% respectively, as compared to controls. The ability of Ke03 isolates to increase onion germination correlated with the ability of this isolate to synthesize IAA (80.33 ppm). Further research is needed to evaluate whether this isolate is effective in increasing the growth and yield of onions in the field.

1. **Introduction**

Wakatobi onion is one of the main crops of the people in Wangi-Wangi, Kaledupa, Tomia and Binongko Islands (abbreviated as Wakatobi), one of the districts in Southeast Sulawesi Province. Geographically, Wakatobi district has a dry area, with landscapes dominated by rock outcrops which are quite numerous and spread throughout the region. However, Wakatobi onion production ranks 3rd after North Kolaka and Buton [1]. One of the advantages of Wakatobi onions is their ability to adapt to dry land and rocky conditions. In addition, Wakatobi fried onions have a savorier flavor and more crispy with a more fragrant aroma compared to other onions, which are more popular.

The ability of Wakatobi onions to grow and develop on sub-optimal land (rocky dry land) is thought to be due to the involvement of the rhizosphere biotic communities and/or plant tissues microbes. Endophytic microbes that are beneficial fungi or bacteria found in plant tissues, generally in the form of diazotroph endophytic bacteria [2]. Endophytic microbes and rhizosphere inhabitants of
onion plants are mainly dominated by bacterial groups (Bacillus spp., Pseudomonas spp., Serratia spp.) and fungi (Trichoderma spp., Penicillium spp., Aspergillus spp.). This microbial group has the dual ability of being a biological agent which promotes plant growth and is also able to increase plant resistance to pests and diseases.

Microbial excellence as a growth promoters is caused by its ability to produce growth hormones (IAA, giberelin, and cytokines), which are naturally needed by plants to increase their growth and development [3-4]. In addition microbes can also chelate important elements from the area around the root of the plant so that it could be used by plants [5-6]. Microbial applications in plants also produce healthier and toxin-free products [7-13]. Endophytic bacteria that live in plant tissues, capable of fixing nitrogen, synthesizing siderophor and IAA hormones [14], and does not cause disease symptoms [15]. Endophytic bacteria as a promoter for plant growth as well as biological agents have advantages over other microbes because of their presence in plant tissues, making them more resistant to biotic and abiotic pressure [16-17].

The endophytic bacteria have ability to increase plant growth through the capability in synthesizing of IAA, nitrogen fixation and phosphate mobilization which play a role in promoting and increasing plant vigor [18-19]. In addition, endophytic bacteria could also synthesize antimicrobial compounds which play a role in controlling pathogens [16]. This study aims to obtain endophytic bacterial isolates which effectively promoting onion plant growth on sub-optimal land.

2. Material and Methods

2.1. Location and time
The study conducted at the Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University in February-June 2018.

2.2. Design research
The study was arranged in completely randomized design (CRD) using 10 treatments, that are one control, nine endophytic bacteria comprising three isolates originated in Wangi-Wangi (Wa05, Re01, and Re05), two isolates in Kaledupa (Ke03 and Ke05), two isolates in Tomia (Te01 and Te05), and two isolates in Binongko (Be02 and Be03), all isolates were the best selection done in 2017.

2.3. Endophytic bacterium isolates preparation
The endophytic bacteria propagated in petri dishes using TSA media TSA. The bacterium colony was suspended until the population density reached 10^9 cfu/ml [20] and kept in a refrigerator at 5ºC.

2.4. Assay for ability endophytic bacterium synthesize Indole-3-acetic acid (IAA)
The Bacillus spp. and P. fluorescens isolates ability to synthesize IAA was analyzed [21]. Bacillus spp. grown for 24 hours in nutrient broth, while P. fluorescens in liquid King’B [22]. The 0.5 g/l amino acid tryptophan was added in each medium to stimulate auxin synthesis. The bacterial culture was centrifuged for 10 minutes at 10,000 rpm. The supernatant was separated from bacterial cells, filtered with millipore filter paper (0.2 μm), and analyzed for its IAA content. The content of IAA in bacterial culture filtrate was detected using FeCl₃12 g/l reagent in H₂SO₄ 7.9 M. FeCl₃ (1 ml) reagent and bacterial culture filtrate (1 ml) were added to the eppendorf tube (2 ml volume), and the mixture was incubated in space dark at 26 °C for 30 minutes. After the incubation period, the absorbance value of the mixture is read by a spectrophotometer at a wavelength of 550 nm. The standard curve based on the absorbance value of pure IAA solutions with concentrations of 0, 6.25, 12.5, 25, 50, 75, 100, 150, and 200 μg/ml was used to calculate the contents of IAA in bacterial culture filtrate.

2.5. Phosphate solubilizing ability of endophytic bacterial isolates
Phosphate dissolving rhizobacteria ability evaluated using Pikovskaya's agar test media with the addition of tri-calcium phosphate (TCP) as a phosphate source. The media was sterilized and the pH
was set to 7.2 using KOH 5 N. The test medium was poured into a petri dish (ϕ 9 cm), made a hole with a cork hole and filled with 0.2 ml suspension of the tested rhizobacteria isolate. Test media with bacteria were incubated for 3 days in an incubation chamber with a temperature of 28 °C. The solubilize phosphate ability was evaluated qualitatively on the bacterial suspension [23].

2.6. N fixation ability
The ability of to fix nitrogen was analyzed qualitatively using Burk salt media. Burk salt media (stock) is made by mixing MgSO₄ (2 g), K₂HPO₄ (8 g), KH₂PO₄ (2 g) and CaSO₄ (1.3 g). Then mixing FeCl₃ (0.145 g) and Na₂MOO₄ (0.0235 g) solubilized in 100 mL of distilled water (stock of Fe-Mo solution). Then 1.3 g of Burk salt media were mixed with 1 mL of stock of Fe-Mo solution plus 10 mL of distilled water and 10 g of sucrose then solubilized in 1000 mL of sterile distilled water and then sterilized (reagent solution). For observation, one ounce of isolate was put into the reagent solution (in a test tube) then incubated in a shaker using a speed of 150 rpm for 48 hours. Positive isolates as nitrogen fixation if the bacteria are able to grow in Burk salt solution which is characterized by turbidity of the media in the test tube. Isolates that show a positive reaction (grow) are given a + (positive) sign, while those who react negatively (not grow) are marked - (negative).

2.7. Treating seed with bio-priming
Prior to treatment application, seeds were disinfected with natrium hypochlorit 2%. Seed treatments with bio-priming were conducted by mixing the seeds and endophytic bacteria suspension into culture bottle and covered with plastic. Seeds which had been conditioned and control (those only submerged in sterile deionized water) incubated for 24 hours in 28-30 °C. After treatments the seeds were dried for 2 hour in the laminar flow.

2.8. Seed viability and vigor test
The effect of endophytic bacteria on the onion seed viability and vigor were tested using bio-priming treatment on sterile burned rice husk. Twenty-five seeds were grown per treatment each of which was replicated three times. The effects of endophytic bacteria on the viability and vigor of onion seed were evaluated using germination percentage, root length and number of roots parameters.

1. Germination percentage (GP) was measured the seed viability [24]. It was measured based on the percentage of normal seedlings (NS) at 7 days after planting (dap) and 14 dap, using the following formula:

   \[ GP = \frac{\sum NS \text{ at observation 1} + \sum NS \text{ observation 2}}{\sum \text{seeds planted}} \times 100\% \]  

2. Root length (RL), was observed at 14 days after planting.
3. Number of roots (NR), was observed at 14 days after planting.

2.9. Data analysis
The data analyzed using of analyze of variance (Anova) and further tested with Duncan’s Multiple Range Test (DMRT) at α=0.05.

3. Results and Discussion

3.1. Results

3.1.1. The effects of seed bio-priming with endophytic bacteria isolates on germination percentage of onion. Inoculation of endophytic bacteria isolates on seeds significantly affected the onion seed
germination percentage. Among the 9 isolates tested, there were 6 isolates (Ke03, Be02, Be03, Ke05, Te05 and Wa05) which were able to increase seed germination percentage compared to controls and other isolates. The Ke03 isolate indicated the best germination percentage compared to the control and other isolates. Increase in germination percentage reached 145% compared to controls (Figure 1).

Inoculation of endophytic bacteria isolates on seeds through bio-priming treatment also significantly affected the root length and the number of onion roots. Among the 9 isolates tested, only 2 isolates (Ke03 and Be02) were able to increase the root length of onion compared to the controls and other isolates. Ke03 isolates produced the best root length compared to controls and other isolates. Similarly Ke03 produced the highest number of roots compared to controls and other isolates. The root length increase reached 46% compared to the control, while the increase in root number only reached 78% (Figure 2).

Figure 1. The endophytic bacteria isolates effect on germination percentage of onion seed

Figure 2. The endophytic bacterial isolates effect on root length and number of onion roots

3.1.2. The ability of endophytic bacteria isolates to solubilize phosphates, fix N and synthesize IAA. The endophytic bacteria isolates ability in dissolving phosphate, fixing N and synthesizing IAA could be seen in Table 1. Among the 9 isolates tested, 8 isolates were able to solubilize the phosphate. It was
indicated by the formation of clear zones (halo) on the media containing TCP. Only one isolate cannot solubilize phosphate, namely Te05. Similarly, almost all isolates tested were able to fix N, except Te05. The ability of N-fixing endophytic bacteria isolates was indicated by turbidity on the Burk salt media. All isolates tested has ability to synthesize the IAA in the media containing tryptophan. Ke03 isolates synthesize the highest IAA content reaching 80.33 ppm (Table 1).

The use of indigenous microbes in promoting of plant growth is one of the contributions of biotechnology in an effort to increase the productivity of agricultural crops. The biological microbe’s ability in improving plant growth and yield, is connected to their role in mobilizing nutrients in soil and plants, synthesis of growth hormone, nitrogen fixation or activation of the mechanism of induction of plant resistance to disease [25]. Endophytic bacteria are beneficial microorganisms that interact with host plants without negative impact on plants [15]. Endophytic bacteria also are able to provide nutrients, produce IAA hormones, and produce extracellular enzymes, cyanide production, phosphate solvents and fluorescence activity [26].

The results of this study indicated that endophytic bacteria isolated from Wakatobi onion plants were able to stimulate onion seed germination up to 145% compared to controls. Similarly, a root length and number of roots increased after application of endophytic bacteria reached 46% and 78% respectively compared to controls. This shows that endophytic bacteria isolated from rocky soil in the Wakatobi islands have considerable potential to be further developed as biological fertilizers. In line with this study, other researcher also found that the endophytic bacteria inoculation could stimulate the growth of lateral roots, adventitious roots, and primary roots of corn plants [27]. Istiqomah and Joko [28] also reported that inoculation of endophytic bacteria in corn plants could increase plant height by 47.69%, canopy wet weight 68.09%, root wet weight 62.9%, canopy dry weight 35.19 % and root dry weight of 52.93%.

The evaluation of the ability of endophytic bacteria showed that the endophytic bacteria isolates tested were able to solubilize the phosphate, fix N and synthesize IAA. Ke03 isolates synthesize the highest IAA at 80.33 ppm, isolates Re01 solubilize phosphate better than other isolates, which are 1.35 cm. While isolates Re01, Te01 and Be03 fixed N better than other isolates. The ability of Ke03 isolates to increase onion germination percentage (81.48%) correlated with the ability of these isolates to synthesize IAA (80.33 ppm). However, Be02 isolates were able to increase the onion germination percentage (74.07%) even though the synthesis of IAA was low (34.67), it was thought that growth promoters used a different mechanism. As previously explained, although in general the mechanism of endophytic bacteria in promoting plant growth correlates with its ability to solubilize phosphate, fix N

### Table 1. The endophytic bacteria isolates ability in solubilize phosphate, fix N and synthesize IAA

| Isolate Code | Solubilize phosphate (cm) | Fix N* | IAA content (ppm) |
|--------------|---------------------------|-------|-------------------|
| Wa05         | 1.03                      | +     | 52.50             |
| Re01         | 1.35                      | +++   | 74.33             |
| Re05         | 1.10                      | +     | 40.50             |
| Ke03         | 1.18                      | ++    | 80.33             |
| Ke05         | 1.20                      | +     | 31.50             |
| Te01         | 1.20                      | +++   | 23.83             |
| Te05         | 0.00                      | -     | 53.50             |
| Be02         | 1.28                      | ++    | 34.67             |
| Be03         | 1.25                      | +++   | 41.00             |

* For N fixation activity: + positive reaction, N fixation occurs with turbidity level after observation + (slightly cloudy), ++ (cloudy), +++ (very cloudy); - negative reaction, no N fixation
and synthesize growth hormone (IAA), but the correlation is not always positive, it is thought that there are still other mechanisms which need to be studied further [29].

Endophytic bacteria are found in almost all internal plant tissues. The mechanism of plant growth promoters carried out by endophytic bacteria is similar to the mechanism used by the rhizosphere bacteria [15]. It was further stated that the association of endophytic bacteria and plants is mutually beneficial for both parties. Endophytic bacteria give a positive impact on the plant. on the other side, the plants could facilitate micro biomass (endophytic bacteria) are beneficial, so they can live in plant tissues. In diverse and fluctuating environmental conditions, endophytic bacteria have advantages compared to rhizosphere bacteria that live freely in the environment of rooting plants in terms of efficiency in communicating and interacting with their host plants [30].

4. Conclusion
It concluded that seeds bio-priming treatment using endophytic bacteria isolates could improve germination percentage, roots length and number of roots of onion. Ke03 isolates showed the best performance and were able to increase germination percentage, root length and number of onion roots reaching 145%, 46% and 78% respectively, as compared to controls. The ability of Ke03 isolates to increase onion germination correlated with the ability of this isolate to synthesize IAA (80.33 ppm). Further research is needed to evaluate whether the isolate could a positive impact on the growth and yield of onions in the field.

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