Effectiveness of endo-rhizobacteria as growth promoters and biological control of moler disease in shallots (*Allium ascalonicum* L.)

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**Abstract.** The study aims to evaluate the effectiveness of endo-rhizobacteria in spurring plant growth and controlling disease in local Wakatobi shallots. The study was conducted at the Agrotechnology Laboratory of the Agronomy Unit, and Screen House of the Faculty of Agriculture, the University of Halu Oleo, from March to June 2019. The study used a completely randomized design with eight treatments, namely without endo-rhizobacteria (control), Be03 (B) + W2R06 (R) isolates, B + R, isolate SWRII B02 (S) + Bacillus sp. CKD061 (C) (S + C), S + R, B + R + C, B + S + C, S + R + C and B + S + R + C. Each treatment was repeated 3 times so that there were 24 experimental units. The variables observed were plant height, number of tillers, number of leaves, the incidence of moler disease, and salicylic acid production. Observational data were analyzed using analysis of variance, followed by Duncan's multiple range test α 0.05 and regression analysis to see the relationship between salicylic acid production and the incidence of moler disease. The results showed that endo-rhizobacteria inoculation of Wakatobi local onion seeds was able to increase plant growth and was able to control disease in local Wakatobi shallots. Endo-rhizobacterial combination treatment of SWRII B02 + Bacillus sp. CKD061 (S + C) was able to increase plant height by 30.42%, number of tillers 72.03%, number of leaves 88.03%, salicylic acid production 24.36%, and reduce the incidence of moler disease by up to 100% when compared to controls. Seed treatment with a combination of endo-rhizobacteria isolates from SWRII B02 + Bacillus sp. CKD061 can be recommended as a growth booster and biological control of cancer in onions.

1. **Introduction**

Shallots (*Allium ascalonicum* L.) is a horticultural crop of high economic value that is widely used mainly as a spice in the kitchen, in addition to industrial raw materials and medicines. The price of shallots in Southeast Sulawesi is quite expensive because the shallots circulating in Southeast Sulawesi generally come from South Sulawesi and Bima, West Nusa Tenggara. In fact, Southeast Sulawesi has a type of local onion that is very potential to be developed, one of which is the local onion Wakatobi. One of the strengths of this local shallot is the stronger taste and aroma. In addition, from an agronomic
aspect, Wakatobi’s local shallots are able to grow and adapt to less optimal land conditions for plant growth, for example, dry land with rocky soil conditions. With these advantages, it is necessary to increase the production of Wakatobi’s local shallots so that they can supply the needs of shallots in Southeast Sulawesi. Wakatobi shallot production in 2017 only reached 40.4 t with an area of 40 ha. While onion production in Southeast Sulawesi reached 371.9 t with a production area of 184 ha. That is, the local onion Wakatobi only contributed 10.89% of the total onion production in Southeast Sulawesi [1].

The low production of local shallots is caused by various factors, one of which is the attack of pathogens that cause disease, especially moler disease. Moler or stem rot is the disease that most causes harm to the shallots plants. Moler disease is caused by *Fusarium oxysporum f.sp. cepae*, which can reduce yields by up to 50% [2]. *F. oxysporum f.sp. cepae* can also attack the tubers of shallots after harvest and during storage in warehouses [3].

Moler disease control efforts by farmers generally use synthetic pesticides that are systemic so that it is absorbed into the plant tissue. If consumed by humans, of course, it will have a negative impact on health. Another impact is the negative influence on the environment, among others, resulting in water and soil contamination. Therefore, environmentally friendly technology is needed in the control of this disease, among others, through the use of microbial biological agents in the form of bacterial endo-rhizome.

The use of biological agents in the form of rhizobacteria or endophytic bacteria independently has been reported to be effective in increasing plant growth and disease resistance. Endophytes are bacterial or fungal microorganisms that live in intercellular and intracellular colonies that spend part or all of their life cycle in the healthy tissue of their host plant [4]. Endophytic bacteria can be obligate, facultative, or passive associated with plants that act as fitostimulators, biofertilizers, and biocontrol agents so that they are beneficial to host plants [5]. Endophytic bacteria are able to produce hormones that promote plant growth in the form of IAA or auxin. Auxin hormone plays a role in stimulating plant growth [6-7]. Furthermore, endophytic bacteria can increase plant growth and act as biocontrol agents [8].

Like endophytic bacteria, rhizobacteria also act as plant growth promoters [9]. Generally, rhizobacteria increase plant growth through different mechanisms [10]. Rhizobacterial inoculation contributes to N2 fixation reaching 20-50% of the total plant nitrogen requirements [11]. Rhizosphere microbes have the ability to form nodules in the root region while at the same time increasing the resistance of plants to drought and plant diseases [12].

The use of endophytic bacteria and rhizobacteria has the potential to increase growth as well as play a role in controlling plant diseases. This is caused by the ability of rhizobacteria that can protect from pathogenic attacks in the rhizosphere region. While endophytic bacteria can protect plants from plant tissues, so plants will be more resistant from biotic and abiotic attacks and increase crop yields. The endophytic and rhizobacterial consortium can provide various control mechanisms (competition, antibiotics, and induction of endurance) simultaneously, so it will be more effective in controlling pathogens [13-15]. Furthermore, the combination of microorganisms in the consortium can control various plant pathogens. Bacteria have more than one beneficial effect on the host, with different disease suppression mechanisms. Combining strains with suppressive mechanisms that have different diseases, can control pathogens more effectively [16].

Based on the concept of thinking, the research by combining the ability of endophytic bacteria and rhizobacteria is expected to have a dual effect in increasing the resistance of moler disease, which will further impact on increasing the yield of local shallots. This study aims to evaluate the effectiveness of bacterial endo-rhizome in stimulating the growth of local shallots plants while controlling disease in local Wakatobi shallots.
2. Methods

2.1. Location and time
The study was conducted at the Laboratory of Agrotechnology, and Screen House of the Faculty of Agriculture, the University of Halu Oleo, from March to June 2019.

2.2. Research design
The study design used was a completely randomized design, consisting of 8 treatments namely Control (K), Be03 (B) + W2R06 (R) isolates, SWRII B02 (S) + Bacillus sp. CKD061 (C), S + R, B + R + C, B + S + C, S + R + C and B + S + R + C. Each treatment was repeated 3 times so that in total there were 24 experimental units.

2.3. Planting media preparation
Planting media used for testing consists of a mixture of soil, manure, and husk charcoal. Before use, each is sterilized for 4 hours to avoid contamination. After sterilizing the three media is mixed with soil: manure: husk (2: 1: ½) and then filled in polybags.

2.4. Propagation of endo-rhizobacterial isolates and seed treatment
The media used to propagate the two types of bacteria (endo-rhizo) is TSA media. Isolate Be03, SWRII A02, Bacillus sp. CKD061 and W2R06 were grown on TSA media then incubated for 48 hours. Growing bacterial colonies were suspended in sterile aquades to a population density of 109 CFU / ml. Seed treatment was carried out by moisturizing the seeds with a bacterial endo-rhizo suspension for 18 hours. After treatment, the seeds are air-dried in a laminar airflow cabinet until they reach the initial weight, and the seeds are ready for use for testing.

2.5. Pathogen inoculation and planting
Pathogen F. oxysporum f.sp. cepae is grown on PDA media and incubated for seven days, then made a suspension and shaken on liquid GDP media for five days. Furthermore, the pathogen suspension is inoculated to the soil using a hand sprayer, then incubated for five days. Soil that has been infected with F. oxysporum f.sp. Cepa, after that, is planted with onion seeds which have been inoculated by endo-rhizome bacteria according to treatment.

2.6. Salicylic acid production in plants
Determination of salicylic acid content using a UV-Vis spectrophotometer. Test combination solution (9 mL) containing: 1 mL extract of shallot plant leaves (yield of leaf sample 1:10 g / v and 8 mL FeCl₃ 1g of sample leaf as a result of small pieces were put into Erlenmeyer, added 10% ethanol solution as much as 10 mL and shaken for 15 minutes at 150 rpm, then filtered, the filtrate was put into Eppendorf and centrifuged for 10 minutes at 12,000 rpm at 25 °C and added with 1% FeCl₃ solution, then the absorbance of the solution was measured at 525 nm wavelength [17].

2.7. Observed variable
The variables observed in this study included: plant height, number of tillers, number of leaves, disease incidence (KP), and salicylic acid production. The incidence rate is calculated by the formula: KP = y / N x 100%; where: KP = disease incidence (%), y = number of diseased plants, and N = total number of plants.

2.8. Data analysis
Data were analyzed using ANOVA, and if it showed a real effect, it was followed by Duncan's Multiple Range Test α 0.05.
3. Results and discussion

3.1. Results

3.1.1. Rhizo endo-bacterial effectiveness as a plant growth promoter. Seed inoculation with bacterial endo-rhizome was able to improve the growth of local shallot plants. Combination treatment of SWRII B02 + Bacillus sp. CKD061 was able to increase plant height better than control and other treatments at two weeks after planting and four weeks after planting, but at six weeks after planting, except control and BR treatment, all other combinations treatments had the same effect on plant height (Table 1).

Table 1. Wakatobi local onion plant height inoculated with endo-rhizobacteria.

| Treatment | Plant height (cm) ± SD |
|-----------|------------------------|
|           | 2 wap      | 4 wap      | 6 wap      |
| Control   | 17.50 ± 0.67 a | 21.68 ± 0.82 b | 26.92 ± 0.99 c |
| SR        | 19.69 ± 1.30 a | 26.87 ± 0.78 b | 34.15 ± 1.71 a |
| SC        | 22.09 ± 1.11 a | 28.06 ± 0.98 a | 35.11 ± 1.09 a |
| BR        | 18.77 ± 0.64 b | 24.59 ± 0.50 c | 30.13 ± 1.25 b |
| BRC       | 20.63 ± 1.54 b | 25.50 ± 1.20 b | 33.55 ± 1.65 a |
| BSC       | 20.34 ± 0.53 ab| 26.10 ± 0.98 abc| 34.63 ± 1.64 a |
| SRC       | 19.51 ± 1.22 ab| 26.75 ± 1.65 ab| 33.86 ± 2.18 a |
| BRSC      | 19.27 ± 1.01 bc| 24.54 ± 1.57 c | 33.40 ± 1.31 a |

Note: The numbers followed by the same letter in the same column are not significantly different at UJBDα = 0.05

Combination treatment of SWRII B02 + Bacillus sp. CKD061 is able to increase the number of onion leaves better than the control and other treatments both at two weeks after planting, four weeks after planting, and six weeks after planting. The increase in plant height in these treatment combinations reached 56%, 62%, and 72%, respectively, at the age of 2 weeks after planting, four weeks after planting, and six weeks after planting compared with controls (table 2).

Table 2. The number of local leeks of Wakatobi inoculated with endo-rhizobacteria.

| Treatment | Number of Leaves (sheet) ± SD |
|-----------|-----------------------------|
|           | 2 wap      | 4 wap      | 6 wap      |
| Control   | 9.75 ± 1.30 b | 15.03 ± 0.61 c | 16.58 ± 0.85 d |
| SR        | 12.69 ± 1.98 abc | 18.64 ± 2.76 bc | 23.39 ± 0.79 b |
| SC        | 15.22 ± 1.07 a | 24.31 ± 1.17 a | 28.53 ± 1.63 a |
| BR        | 10.22 ± 0.51 a | 16.56 ± 1.90 a | 19.78 ± 2.50 c |
| BRC       | 13.83 ± 1.89 a | 20.08 ± 1.89 b | 26.08 ± 1.63 ab |
| BSC       | 14.94 ± 1.50 a | 19.56 ± 0.76 bc | 24.19 ± 2.48 b |
| SRC       | 13.83 ± 2.02 a | 21.08 ± 0.80 b | 25.17 ± 1.13 b |
| BRSC      | 13.22 ± 0.84 a | 19.36 ± 1.85 bc | 22.89 ± 2.26 b |

Note: The numbers followed by the same letter in the same column are not significantly different at UJBDα = 0.05

Bacterial endo-rhizome inoculation of the seeds also increases the number of shallots tillers. Among all treatments tested, the combination of SWRII B02 + Bacillus sp. CKD061 is able to increase the number of shallots tillers consistently compared to other treatments and controls both at two weeks after planting, four weeks after planting, and six weeks after planting. The increase in the number of tillers in each observation reached 98%, 58%, and 88% compared to controls (table 3).
Table 3. The number of local Wakatobi shallots tillers inoculated bacterial endo-rhizome.

| Treatment | 2 wap | Number of Tillers ± SD | 4 wap | | 6 wap |
|-----------|-------|------------------------|-------|-------|-------|
| Control   | 2.06 ± 0.42 c | 3.08 ± 0.14 b | 3.25 ± 0.46 c |
| SR        | 2.69 ± 0.49 bc | 4.08 ± 0.88 ab | 4.89 ± 0.84 b |
| SC        | 4.08 ± 0.38 a | 4.86 ± 0.99 a | 6.11 ± 0.32 a |
| BR        | 2.89 ± 0.51 bc | 3.44 ± 0.19 b | 4.78 ± 0.19 b |
| BRC       | 3.00 ± 0.90 b  | 4.50 ± 0.50 ab  | 5.42 ± 0.76 ab |
| BSC       | 3.64 ± 0.13 ab | 4.69 ± 0.49 a  | 5.58 ± 0.52 ab |
| SRC       | 3.67 ± 0.38 ab | 4.50 ± 0.75 ab  | 5.33 ± 0.14 ab |
| BRSC      | 2.72 ± 0.25 bc | 4.39 ± 0.35 ab  | 5.03 ± 0.29 b |

Note: The numbers followed by the same letter in the same column are not significantly different at UJBDα = 0.05.

3.1.2. Endo-rhizobacteria effectiveness as a biological control moler disease. Evaluation of the effectiveness of endo-rhizobacteria as biological control of disease in shallots was observed using variables of disease incidence and salicylic acid production. Inoculation of bacterial endo-rhizome in seeds can reduce the incidence rate of disease, and on the other hand, increase salicylic acid production. The effectiveness of the bacterial endo-rhizome combination against the incidence rate of the disease is presented in Figure 1a, while its effectiveness in increasing salicylic acid production is presented in Figure 1. The combination of endo-rhizobacteria isolates SWR II A02 + Bacillus sp. CKD061 can reduce the incidence rate of cancer by up to 100% compared to controls. Meanwhile, in the salicylic acid production variable, inoculation of the combination of SWRII B02 + Bacillus sp. CKD061 (SC) was able to increase salicylic acid production by 24% compared to controls (figure 1).

![Figure 1](image_url)

Figure 1. Moler disease (a) and salicylic acid production, (b) local onion, which is inoculated endo-rhizobacteria. The numbers followed by the same letter in Figure are not significantly different at UJBDα = 0.05.

3.2. Discussion
The results showed that the inoculation of bacterial endo-rhizome isolates in the seeds was able to increase the growth of shallot plants. This is evident from an increase in plant height, number of leaves, and the number of onion seedlings in seed treatment with a combination of bacterial endo-rhizome isolates compared to controls. Some previous research results also show that the application of two or more types of microbes provides a better plant growth performance compared to using only one type of microbe. Application of a bacterial combination of Bacillus spp. and the Pseudomonas spp. able to
increase the growth of soybean plants [18]. Furthermore, it was reported that the application of Azotobacter spp. + Bacillus spp. + Trichoderma spp. Also better able to increase growth and production of sweet corn. The application of the consortium of three types of microbes was able to increase N and P uptake of sweet corn plants by 153% and 204%, respectively. The application of the microbial consortium was also able to increase plant height, prone dry weight, weight, and production of fresh sweet corn cobs, respectively, by 24%, 64%, 29%, and 29% [19]. Other relevant studies also reported that a rhizobacteria consortium combined with mycorrhizae was effective in increasing the growth and production of local upland rice [20]. An endophytic bacterial consortium of Bacillus sp. SJI + S. marcescens JB1E3 was able to increase the growth of Chile plant height by 38% and the number of leaves of Chile plants by 70% compared to control [15].

Improvement of plant growth caused by microbial activity caused by the ability of these microbes to produce various compounds or growth-promoting components. Bacteria from the Bacillus spp. and P. fluorescens are able to fix nitrogen, dissolve phosphate, and produce growth hormones in the form of IAA [21]. Furthermore, it was reported that the improvement in plant growth was due to the ability of a consortium of 3 NABP1 bacteria to fix nitrogen, producing better IAA and GA3 compared to other isolates [22].

Rhizobacterial isolates isolated from the local shallots rhizosphere of Wakatobi were able to dissolve phosphate [23]. The combination treatment of 75% S. epidermidis BC4 bacteria with 25% P. fluorescens RH4003 can stimulate the increased dry weight of tomato plants [24]. It was further reported that the combined application of B. subtilis + P. fluorescens bacteria gave the best results in increasing the tuber weights of potato plants to reach 86.01% and 103.81% when compared to without bacterial inoculation [25].

Other studies have also shown that the use of microbial consortia can reduce the incidence of disease in plants [26-29]. The combined application of B. subtilis + P. fluorescens bacteria gives the best results in increasing plant resistance to R. solanacearum with suppression of attacks by 44.75% and 67.30% compared to controls [25]. Furthermore, it was reported that the combination of Azoto II-1 75% + Endo-5 25% could suppress smallpox [14]. In addition, a consortium of endophytic bacteria was also reported to be able to reduce the mortality of Pratylenchus Coffee, up to 65.8% [30].

The low severity of disease in maize plants is caused by an increase in total phenol and salicylic acid [31]. Furthermore, it was reported that the treatment of biological agents bio free in a solid formulation combined with soy compost gave the best results in increasing plant resistance to bacterial pustules. Induction of plant resistance occurs systematically, which is characterized by an increase in salicylic acid of 0.3 mg and an increase in the peroxidase enzyme of 0.07 mL-1 units in plant samples [32]. This shows that increased production of salicylic acid in local conditions that are inoculated with bacterial endo can reduce the incidence rate of molar disease.

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4. Conclusion

Inoculation of endo-rhizome bacterial combinations in seeds was able to increase the growth of Wakatobi's local shallot plants as indicated by an increase in plant height, a number of leaves, and a number of tillers compared to control. Inoculation of bacterial endo-rhizome in seeds is also able to increase plant resistance to cancer as indicated by a decrease in the incidence of disease and an increase in salicylic acid production. The combination of endophytic bacteria SWRII B02 and rhizobacteria Bacillus sp. CKD061 is the best treatment that effectively increases growth and is able to control cancer by up to 100% compared to control.

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