The Potential Of Indigenous Endophyte Bacteria To Promote Local Tomato Growth Isolated From Dry Land In Muna, Southeast Sulawesi, Indonesia

Jumarddin La Fua, Laode Sabaruddin, La Ode Santiaji Bande, Sitti Leomo

Abstract : The exploration of endophyte bacteria as a growth promote has been done to find the best endophyte bacteria so that it can be used for the purposes of agriculture and food security in the future. Twenty-one endophyte bacteria isolates were isolated in the local tomato roots and stems in the dry land of Muna, Indonesia. Local tomato seeds treated by endophyte bacteria indicate an increasing dry weight of normal seeds, increasing germination and vigor index compared with seeds with non-endophyte bacteria. Of the 21 isolates of endophyte bacteria, twelve of them which are able to increase the viability and vigor index of local tomatoes belong to the Pseudomonas sp and Bacillus sp, such as isolates BU II 06, LAK II A02, SWR III B02, SWR I A05, SWR II B04, SWR II A03, SWR I A02, LBR I A03, SDM II A03, SDM II A04, MO II A02 and KS III A08. The analysis results whether endophyte bacteria isolates are potential to produce IAA indicates that from twelve isolates tested, ten of them can produce IAA. Isolates SWR II B04 has the highest ability to produce IAA, and the lowest was found in isolates SWR III. Besides, six other isolates can generate high enough IAA, namely IAA LI II A03, LI B04, SWR II A02, LAK II A02, SWR I A04 and SDM II. B05. The results indicate that endophyte bacteria isolated from the local tomato crops have the potential to promote plant growth, which makes this study very important and promising for the development of biological fertilizer on dry land.

Key Word: Endophyte Bacteria, Growth promote, IAA, Tomato.

1 INTRODUCTION

Tomato is one of the important horticultural crops cultivated by farmers and is one of the high economic value commodities with significant export potential. This plant contains β carotene, flavonoids, lycopene, vitamins, and becomes one of the most widely consumed vegetables in the world [1]. In Southeast Sulawesi, tomato cultivation is very strategic for the suitability of land for cultivation, and the social demand for these commodities is increasing every year. Of the 17 countries and cities in Southeast Sulawesi, 16 areas develop tomato plants, such as Buton, Muna, Konawe, Kolaka, South Konawe, Bombana, Wakatobi, North Kolaka, Buton Utara, North Konawe, East Kolaka, West Muna, Central Buton, South Buton, Kendari and Bau-Bau. Muna is a region in Indonesia characterized by its dry land and is dominated by rocks spread throughout the entire region. Nonetheless, Muna has featured plants cultivated by the local community, namely tomatoes. Local tomato is a plant cultivated by farmers for generations. These tomatoes can grow and develop on dry and rocky land and have different characteristics with other local tomatoes in which they are larger in size, distinctive taste and more tasty. In Southeast Sulawesi, tomato production in Muna was ranked first by the number of tomato production reached 7.5 t ha⁻¹ of total tomato production in Southeast Sulawesi 346.4 t ha⁻¹ and the area of cultivation of tomato reached 213 ha [2]. According to [3], cultivation of tomatoes in Muna mostly done on the ground are classified as rocky soil, dry with low soil fertility levels. Tomato plants growing in Muna have the ability to adapt, grow well, and have abundant harvests in extreme environments. According to [4] this is the case because of the involvement of microbes that have the potential to induce a lack of water, contribute to the health of plants [5], to support plant growth (6), to play a role in nutrient cycling process [7], as well as to develop the plant resistance in the extreme environment to meet the energy needs of its host [4]. Previous studies have shown that some endophyte bacteria have been observed and they contribute to promote the plant growth [7], are also environmentally friendly, and have been adapted with local ecological conditions. Endophyte bacteria are those which live and associate with plant tissues without causing any symptoms in plants [8]. The existence of bacteria endophyte in the tissue of plants involved in producing substances hyper-growth (9), anchoring nitrogen [10], mobilizing phosphate [11], and inducing plant resistance to pathogens disorders [12]. In addition, the endophyte bacteria play a role in helping plants to grow and adapt to environmental conditions gripped by drought to produce several compounds that can promote the plants’ growth helping them to adjust to environmental conditions [13]. It was further reported that the endophyte bacteria have the potential to improve the viability and vigor index [9]. Endophyte bacteria isolates were inoculated on rice and corn seeds proved to have advantages in promoting plant growth [14]. The endophyte bacteria also promote plant growth through the ability to produce IAA [15]. Endophyte bacteria isolates; MBN3, MJHN1, and MJHN10 are electrically insulated from plants Vigna radiata to produce very high amount of IAA and results in promoting plant growth in vitro way [16]. This indicates that the endophyte bacteria have the potential as a plant growth promoters. Exploration of endophyte bacteria is
expected to yield potential of endophyte bacteria growth promotes in dryland crops. Endophyte bacteria isolated samples selected are tested based on the ability to increase the viability and vigor index, and its physiological characterization. Through this research, it can be concluded that indigenous endophyte bacteria in dry and rocky land of Muna has the potential to promote tomato plants' growth on the dry land.

### 2 MATERIALS AND METHODS

#### 2.1 Isolation of Endophyte Bacteria from Local Tomato Plants in Muna

The endophyte bacteria were isolated from the roots of plants. Plant roots are washed thoroughly and dried for ± 30 minutes. Then the roots were weighed as much as one gram and were sterilized using 70% alcohol for three minutes. Subsequent sterilization was done using a solution of 4% NaOCl for 3 minutes and rinsed with sterile distilled water three times. The roots of which have been carried out sterilization of 0.1 ml of distilled water last were rinsed smears on TSA media 5% to see the effectiveness of the sterilization. Roots that have been inscribed on TSA media 5% then crushed using a mortar and diluted to the dilution series 10-10. Serial dilutions of 10-8 and 10-10 were plated on TSA medium in a petri dish with the scatter volume 50μL. Scatter suspension incubated for two days, and then observation of the colony grows. Colonies that show morphological differences were further isolated. Isolation of bacterial colonies on the culture medium is done repeatedly (purification isolates) to obtain a single bacterial colony or a pure culture for further testing and stored in Eppendorf tubes containing 0.9 ml of sterile 15% glycerol solution and stored in the freezer with the temperature of -20°C.

#### 2.2 Propagation of Endophyte Bacteria Isolates and Seed Treatment

**Endophyte** bacteria isolates with pure cultures are then propagated using TSA media for the group of Bacillus sp. and King’s B, while a group of Pseudomonas sp was incubated for 48 hours. Furthermore, bacteria cell culture is harvested by suspending it. Local tomato seeds were subsequently incorporated into the suspension and were shaken with endophyte bacteria for 24 hours at a speed of 120 rpm.

#### 2.3. Seed Germination Test of Local Tomato in Muna

Local tomato seeds were nucolated with endophyte bacteria suspension and then germinated in a germination box of 20 cm x 15 cm x 10 cm (length x width x height) using sterile husk charcoal medium. 50 tomato seeds were planted in each box with three repeated treatments. Observations were made during the 14 days on viability and vigor index of local tomatoes including :

**Germination Percentage**

Germination percentage illustrates the viability of potential seed [17] and is calculated based on the percentage of normal seedling (KN) of the first measurement (I) at the age of 7 days after planting and the second measurement (II) is at 14 days after planting.

**Vigor Index**

Vigor Index describes the vigor growth rate [18], and is calculated based on the percentage of normal seedling on the first measurement by the age of 7th.

**Dry Weight of Normal Seedlings**

Normal weight for seedlings portraits of vigor as the ability to optimize seed food reserves in the seed itself formed by dry weight accumulation. Tests were carried out during the 14 days after the seeds germinate. After germination was revoked then wrapped and put into the oven for 72 hours. Then it is removed from the oven and weighed with the dry weight [19].

### IAA Synthesis Test

Measurement capability in synthesizing IAA of endophyte bacteria uses [20]. Endophyte bacteria isolates were planted in Kings B (for Pseudomonas sp.) and liquid TSB (for Bacillus sp.) For 24 hours, each contained 0.5 g l tryptophan, then centrifuged at 10,000 rpm for 10 minutes, and the supernatant was filtered with sterile filter paper. As the reagent was added to 1 ml of the sample solution in a 2 ml tube and was incubated in a dark room at normal temperature for 30 minutes. Then the sample is put into the microplate and observed with spectrophotometer on λ = 550 nm. The content of IAA in the sample is calculated by the regression are made of pure IAA at concentrations of 0, 6:25, 12.5, 25, 75, 100, 150, and 200μg / ml.

#### 2.4 Data Analysis

Data from the observation on the ability of endophyte bacteria on the viability of local tomato seeds were analyzed using analysis of variance. While the observation ability of endophyte bacteria to produce the hormone IAA was analyzed descriptively. If the results of the analysis of variance showed a real influence, then it was continued by Duncan Multiple Range Test (DMRT) on the actual level of 95%.

### 3 RESULTS

A total of 21 isolates of endophyte bacteria have been isolated from tissues of roots and stems of tomato plants obtained from several villages in Muna, Southeast Sulawesi, Indonesia. Isolation of endophyte bacteria found in tomato plants is dominated by the genus Pseudomonas sp taking up to 15 isolates and genus Bacillus sp up to six isolates. The ability of endophyte bacteria test results in improving the viability and vigor of local tomatoes is presented in the following table:

**Table 1. Effect of seed treatment with endophyte bacteria on normal dry weight (NDWS), germination percentage (GP), and the vigor index (VI).**

<table>
<thead>
<tr>
<th>Endophyte Bacteria Isolates</th>
<th>Isolate Code</th>
<th>NDWS (mg)</th>
<th>GP (%)</th>
<th>VI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-endophyte bacteria</td>
<td>Control</td>
<td>295.03 ± 5.19 fg</td>
<td>89.33 ± 8.74 b</td>
<td>38.67 ± 5.81 fg</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>TB I B06</td>
<td>377.87 ± 9.23 cg</td>
<td>100.00 ± 0.24 a</td>
<td>74.67 ± 5.33 abcd</td>
</tr>
</tbody>
</table>
LAK II A02 isolate has better value compared with other isolates in promoting the viability and vigor index of Local tomato plants. DMRT test results indicate that the highest viability of local tomatoes was found in the isolates LAK II A02 which had no significance with the isolates of SWR II A02, SWR III B04, SDM II B05, LI I A03, MO II A06, SWR I A04, and LBR II B02, but they are significantly different from other isolates, especially those without endophyte bacteria. DMRT test results in Table 1 indicate that the highest value of seed germination was found in isolate SWR I A04 which was significantly different from the SDM I A02 and SWR I A05 especially without endophyte bacteria as its treatment process, but they were not significant with other isolates. Duncan Multiple test results in Table 1 indicate that the highest value for viability and vigor index of local tomato seed was found in SDM II B05 which was significantly different from SDM I A02, SDM II A03, KS III A08. Seed treatment using endophyte bacteria could significantly promote the normal dry weight, germination, and vigor index of seeds. Twelve isolates of endophyte bacteria have potential as plant growth promoters namely isolates BU II 06, LAK II A02, SWR III B02, SWR I A05, SWR II B04, SWR II A03, SWR I A02, LBR I A03, SDM II A03, SDM II A04, MO II A02. To determine the ability of endophyte bacteria in promoting the viability and vigor of local tomatoes, physiological analysis of endophyte bacteria was conducted to investigate its ability to synthesize IAA. Of the 12 isolates, ten isolates of endophyte bacteria can synthesize IAA. The test results in the ability of endophyte bacteria synthesize IAA are presented in the following table. IAA is the main hormone in plants controlling various physiological processes in plants such as cell division, network division, and response to light [21]. In general, the endophyte bacteria electrically insulated from local tomato plants have the potential to vary the synthesis process of IAA. The analysis results of the potential bacteria to produce IAA show that of the 12 isolates tested, ten isolates of bacteria can produce IAA with different concentration.

Table 2. The ability of endophyte bacteria in synthesizing IAA

<table>
<thead>
<tr>
<th>No.</th>
<th>Endophyte Bacteria Isolates</th>
<th>Isolate code</th>
<th>Synthesis of IAA (µg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas sp.</td>
<td>SWR III B04</td>
<td>0,00</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas sp.</td>
<td>TB I B06</td>
<td>0,00</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas sp.</td>
<td>SWR III B02</td>
<td>3,83</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus sp.</td>
<td>M0 II 06</td>
<td>6,59</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas sp.</td>
<td>LBR II B02</td>
<td>8,87</td>
</tr>
<tr>
<td>6</td>
<td>Pseudomonas sp.</td>
<td>LAK II A02</td>
<td>13,50</td>
</tr>
<tr>
<td>7</td>
<td>Pseudomonas sp.</td>
<td>SWR II A02</td>
<td>23,42</td>
</tr>
<tr>
<td>8</td>
<td>Pseudomonas sp.</td>
<td>SWR I A04</td>
<td>13,10</td>
</tr>
<tr>
<td>9</td>
<td>Bacillus sp.</td>
<td>LI B04</td>
<td>24,32</td>
</tr>
<tr>
<td>10</td>
<td>Pseudomonas sp.</td>
<td>SDM II B05</td>
<td>13,02</td>
</tr>
<tr>
<td>11</td>
<td>Pseudomonas sp.</td>
<td>LI II A03</td>
<td>34,40</td>
</tr>
<tr>
<td>12</td>
<td>Pseudomonas sp.</td>
<td>SWR II B04</td>
<td>56,67</td>
</tr>
</tbody>
</table>

produced ranged from 3.83 to 56.67 µg/ml. Endophyte bacteria on isolates SWR II B04 have the highest ability to produce IAA with a concentration of 56.67 µg/ml, while the lowest in producing IAA was found in isolates SWR III B02 with a concentration of 3.83 µg/ml. In addition to SWR II B04 isolate, there are six isolates that could produce IAA concentrations, such as isolates LI II A03, LI B04, SWR II A02, LAK II A2, SWR I A04 and SDM II B05 with a concentration of IAA 34.40 µg/ml, 24.32 µg/ml, 23.42 µg/ml, 13.50 µg/ml, 13.10 µg/ml and 13.02 µg/ml respectively.
dry weight, germination percentage, and vigor index.

4 DISCUSSION

The endophyte bacteria enter the plant tissue primarily not only through the root zone but also through the flowers, stems, and cotyledons. In particular, these bacteria enter the plants' tissue through main roots, secondary roots, stomata, or the damaged leaves [22]. The endophyte bacteria can promote the plant growth functions in its role as PGPB (Plant Growth Promoting Bacteria) that they directly affect the metabolism of plants by providing the necessary substances [23]. Endophyte mechanism in stimulating the growth of plants is done by anchoring capacity N2 and dissolving P, which is bound to be available through the organic acids [24]. Moreover, these bacteria can penetrate and become systemic in the plant host, actively inhabit apoplast, vessels, and sometimes the intracellular space. Colonization presents the ecological niches, similar to the one occupied by the plant pathogens, and therefore, these bacteria act as biological control agents against pathogens [25]. Therefore, endophyte bacteria thought to have potential in the development of environmentally sustainable agriculture. The viability and vigor index testing result indicates that of 21 isolates of endophyte bacteria, twelve of them show an increase in viability and vigor index compared to control and other treatments, such as BU II 06, LAK II A02, SWR III B02, SWR I A05, SWR II B04, SWR II A03, SWR I A02, LBR A03, SDM II A03, SDM II A04, MO II A02. The inoculated seeds of local tomatoes with twelve isolates have higher viability and vigor index compared to the control plants with normal-dry weight which is from 369 to 715.10 mg, germination percentage 96-100%, and vigor index from 73.33 to 92%. In the variable of normal dry weight, some isolates such as LAK II A03, LBR II B02, SWR III B04, and LII A03 showed a statistically better than the other treatments. However, isolates MO II A02, KS III A05 and SDM I A02 do not show an increase of normal dry weight that compared with controls that are not inoculated with endophyte bacteria, as well as for variable of isolates germination on the SDM I A02 and SDM II A03. This may be caused by inoculation of endophyte bacteria that has not helped the growth of seeds, and environmental conditions are not favorable [26]. However, in general inoculation of endophyte bacteria on the normal dry weight, germination percentage, and the vigor index shows significant effects, The results are consistent with several previous studies that the use of endophyte bacteria contribute to increased growth, increased seed viability, yield, and tolerance to diseases [27]. As endophyte bacteria isolates SW521-L21, KW7, KW7-S22, KW7-S06, HS-R01, and CB R05 which was applied to rice seed and it can increase the dry weight of the leaves and roots significantly compared with the control seed [21]. An increase in yields between 10% and 20% through the application of PGPR on some agricultural crops [28]. In addition, it was reported that the isolated NEB4, NEB5, and can increase the weight of soy NEB17 when it is inoculated with endophyte bacteria compared with controls [29]. The stimulation of plant growth associated endophyte bacteria can happen with a variety of mechanisms One of which is the production of phytohormones as IAA hormone [30]. Phytohormones presence can improve various stages of plant growth and can modulate the growth and development of plants [31]. In addition, the role of phytohormones in plant growth and development is fundamental, varied, and complex because it plays an important role in response to feedback from the environment and is used as molecular signaling between bacteria and host [22]. The results showed that of the 12 isolates of endophyte bacteria tested, ten isolates could produce IAA with varying concentrations ranging from 3.83 to 56.67 μg/ml. However, isolates SWR III B04 and TB 1 B06 does not produce IAA. This is in accordance with [32] who point out that the stimulus plant growth include increased growth, yield, and production plants can be through the expression of one or more characteristics that encourage the growth of plants, such as those found in corn plants that bacteria that encourage the growth of corn is not necessarily produced by bacteria that produce a lot of IAA. In addition, the production of IAA by bacteria can vary also affected species or strain, phase of growth, and the availability of media [33]. Endophyte bacteria isolates SWR II B04 has the highest ability to produce IAA with a concentration of 56.67 μg/ml, while the lowest in producing IAA was found in isolates SWR III B02 with a concentration of 3.83 μg/ml. In addition there are six isolates that could produce IAA concentrations such as isolates LI II A03, LI B04, SWR II A02, LAK II A2, SWR I A04 and SDM II B05 with a concentration of IAA 34.40 μg/ml, 24.32 μg/ml, 23.42 μg/ml, 13:50 μg/ml, 13:10 μg/ml and 13.02 μg/ml respectively. The ability to produce IAA as in SWR II B04 and other isolates considered to be related to the promotion of growth in plants by bacteria [34]. This is because the bacteria IAA works in conjunction with a host of endogenous auxin to stimulate plant growth promote [35]. Regression analysis showed that the hormone IAA less significant results against normal dry weight, germination, and vigor index. [36] suggest that the presence of IAA provides the same germination index control but increase the lateral root growth in media containing

Figure 1. The regression analysis between hormone IAA a Normal Dry Weight of Seeds (NDWS), Germination Percentage (GP), and Vigor Index (VI) of local tomatoes.
IAA. Based on the content of IAA, isolates SWR II B04 and LI II A03 is considered the best in producing IAA. Therefore, it is necessary to test it to get the best endophyte bacteria isolates in promoting plant growth.

5 CONCLUSION
Endophyte bacteria isolates electrically insulated from local tomatoes in Muna has potential to produce substances of plant growth promoters and in particular to produce IAA and increase the viability and vigor index of the tomato, so it is very promising in the development of biological fertilizer for the improvement and growth of plants. Of the 21 isolates of endophyte bacteria, 12 potential bacteria could increase the viability and vigor index of local tomatoes belonged to the Pseudomonas sp and Bacillus sp, such as isolates BU II 06, LAK II A02, SWR III B02, SWR I A05, SWR II B04, SWR II A03, SWR I A02, LBR I A03, SDM II A03, SDM II A04, MO II A02 and KS III A08. Meanwhile, ten isolates were able to produce growth hormone IAA and isolates SWR II B04 and LI II A03 IAA produce the highest amount compared to other isolates that can be developed as isolates that can spur the growth of plants. Further study of the environmental factors that affect the growth and survival of endophyte bacteria need to be done in terms of precise identification of bacterial strains and promising as potential bacteria that can be used as a growth promoters agent through field trials. Through field trials, best endophyte bacteria can be obtained to promote plant growth, which can be applied for agricultural cultivation.

6 REFERENCES


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