Potential of isolated bacteria biostimulant from soil roots on white turmeric plants (Curcuma zedoaria)

S J R Lekatompessy*, Nuriyanah, T Widowati and R Simarmata

Research Center for Biotechnology – LIPI, Cibinong
Jl. Raya Bogor Km 46, Cibinong 16911, Indonesia
Email: sylviakohy@gmail.com

Abstract. The abundance of microbes around the roots of plants is an interesting thing where there is an interaction between plants and surrounding microbes. The purpose of this study was to obtain rhizosphere isolates from the soil of white turmeric roots, to determine the potential activity of rhizosphere bacteria from white turmeric as a biostimulating agent and biofertilizer agent that can help plant growth. The research method used was screening for potential growth hormone-producing bacteria (biostimulants), nitrogen fixation ability, enzyme production (protease, amylase, catalase) and biocontrol and bacterial colony PCR. The results showed that twelve rhizosphere bacteria isolates from white turmeric were isolated. Rhizosphere bacteria have the potential as a biostimulant agent, multipotential agent (biofertilizer agent, biodegradation agent and biocontrol agent) which is useful for helping plant growth, especially white turmeric plants. The twelve bacteria that had PCR amplification of the 16 S rRNA gene were performed, the results showed that the DNA bands could be continued for further identification. The results of the study by utilizing the potential of rhizosphere bacteria which have commercial value to be promoted into a safe and multi-functional biostimulant product based on superior isolates to support plant growth, especially white turmeric plants.

1. Introduction
The need for medicinal plants continues to increase along with the spread of corona disease and the tendency of people to reuse herbal medicines. The increasing need for medicinal plants requires appropriate and safe solutions to help increase plant productivity. Through the 2019 INSINAS activity, efforts to innovate biostimulant agents, biofertilizer technology specifically for white turmeric plants are expected to help increase the production of white turmeric plants.

Each plant actually has its own natural growth hormone, but in varying amounts. Under certain conditions, plants are not able to produce growth hormones optimally to assist plant growth. Giving chemical fertilizers that are commonly used on plants turns out to be given stimulants to help plant growth. The research conducted wanted to show that there was microbial activity in the soil of plant roots, especially white turmeric. The potential activity of microbes in spurring the process of root growth to support plant growth so that plant roots are strong and can carry out the process of optimal absorption of water and nutrients.

The application of potential bacteria-based biostimulant agents as well as other potentials as biological fertilizer agents can be one of the technological innovation efforts to increase plant production.
productivity, but this technology has not been widely applied, especially in white turmeric plants. Biostimulants are growth hormones produced by microbes and function as stimulants to help plant growth. Biostimulants are active compounds produced to help stimulate plant growth such as the IAA hormone [1]. The biostimulant product to be developed is based on potential bacteria producing growth hormone IAA from white turmeric.

Research Center for Biotechnology has developed LIPI’s superior biofertilizer products, namely BIOVAM, Bioplus, Biorhcin, and without realizing it, the development of this biofertilizer product as a biostimulant agent has also been carried out for a long time with the help of microbes. This potential microbe has more than one potential both as a biostimulating agent and biofertilizer agent. It is hoped that from this research, information on the potential activities of microbes in white turmeric as a biostimulating agent and biofertilizer agent will be obtained which can later be utilized and developed into a product plus biostimulant.

The white turmeric plant (Curcuma zedoaria) belongs to the zingiberaceae (ginger) rhizome family which is commonly used as herbal medicine. The content of white turmeric compounds, among others, curcuminoinds and essential oils, where the content of these curcumin compounds is used as an antioxidant that helps prevent cell damage; essential oils are used to maintain a healthy respiratory and digestive tract. The content of this white turmeric bioactive compound which makes white turmeric often referred to as anti-inflammatory, anti-diarrhea, anti-abdominal pain, anti-fart eradication, appetite enhancer, treat hepatitis, digestive disorders, antimicrobial, anticholesterol, and anti-HIV.

Land degradation occurs continuously and cannot be prevented. Therefore, soil bioresources are needed by utilizing potential bacteria in plant root areas to improve soil cultivation in an efficient and sustainable manner.

Rhizosphere bacteria play an important role in soil fertility by producing several essential compounds needed by plants, breaking down and recycling these compounds. According to Ernita et al. [2], indigenous rhizobacteria isolates are able to induce resistance, increase plant growth. Some researchers have also reported that rhizobacteria are capable of nitrogen fixation and synthesize IAA, cytokines and gibberellins [3,4].

This study aims to obtain rhizosphere isolates from the soil of white turmeric roots and to determine the potential activity of rhizosphere bacteria from white turmeric as a biostimulating agent and biofertilizer agent that can help plant growth.

2. Methods

2.1 Time and place
This activity was carried out from January 2nd 2020 to February 7th 2020, at the Symbiotic Plant Microbial Laboratory, Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong.

2.2 Isolation of white turmeric root soil samples
Sampling of the root soil of white turmeric from Karanganyar, Central Java was carried out randomly, then the soil samples were put together, stirred and then isolated by weighing 10 grams of soil samples put them in 0.85% NaCl solution for 1 hour, and incubated them in a shaker. One mL of each sample was and put it in a series of dilutions. Soil sample dilution at a dilution level of 10⁶ was then planted on nutrient agar (NA) media to obtain rhizosphere bacteria.

2.3 Regeneration of rhizosphere bacteria culture
The rhizosphere isolates obtained were then grown on NA media aseptically by the streak method.

2.4 Screening for potential rhizosphere bacteria from white turmeric
The bacterial activity test is one way to determine the potential of pure bacterial isolates through their physiological properties. Here are the details of the white turmeric rhizosphere microbial activity test:
2.4.1 Nitrogen fixation activity test
Nitrogenase enzyme activity plays a role in converting free nitrogen in the air into ammonia (NH₃) [5] and in the ability of bacteria to use malate as an energy source for growth. Nitrogenase activity test analysis was carried out by growing the bacteria on semi-solid NFB media then incubated. Observations were made every day for 7 days, the test was carried out at room temperature. The parameters for determining the activity of the nitrogenase enzyme qualitatively were the color change of the NFB media and the presence of white pellicle.

2.4.2 Protease, amylase and cellulase enzyme activity tests
2.4.2.1 Protease enzymes activity test.
This test is used to determine the ability of bacteria to degrade peptide bonds. The selective media used was skimmed milk agar (SMA). The SMA medium consists of 0.5 g peptone, 0.1 g NaCl, 2 g agar, 10 g skim milk, and 100 mL distilled water [6]. Bacterial isolates were inoculated on SMA media, then incubated at room temperature for 24-72 hours. Positive results were indicated by the presence of a clear zone around the bacterial isolate. This is an indicator of protein hydrolysis in SMA.

2.4.2.2 Cellulase enzyme activity test
This test was carried out on 1% CMC selective media. The bacteria were inoculated and incubated at room temperature for ± 24 hours. CMC medium contains 1% CMC (1 g CMC; 0.02 g MgSO₄.7H₂O; 0.075 g KNO₃; 0.002 g K₂HPO₄; 0.004 g CaCl₂.2H₂O; 1.5 g agar) in 100 mL phosphate buffer solution [7]. The growing isolate was then added with 5 mL of 0.1% congo red by pouring it evenly over the surface of the selective media and after 10 minutes the congo red solution was washed with 1M NaCl then observed.

2.4.2.3 Amylase enzyme activity test
The test was carried out using Strach Agar (SA) media which contains 4 g of Nutrient Agar, 2 g of starch, and 200 mL of distilled water. Isolates were grown on SA media. The cultures were incubated for 24 - 48 hours at room temperature. The growing bacteria were then stained by iodine reagent. If the bacteria had amylase enzyme activity, a clear zone would be formed around the bacterial colony, whereas bacteria that did not have amylase enzyme activity would not form a clear zone. The calculation of the clear zone around the isolate area uses the equation:

\[
\text{Clear Zone Area} = \frac{a}{b} \times 1 \text{ cm}^2
\]  
\[\text{(1)}\]

*Note:*  
\(a\) Weight of zone formed (g)  
\(b\) Average weight of the 10 papers used (g)

The calculation of the clear zone in the isolate area was carried out by blotting the growing area of the isolate in a petri dish using paper then the pattern was cut according to the colonies formed. The paper pieces were then weighed as a zone formed by the strength of the active compound produced by the bacteria (a). Ten sheets of paper with a size of 1cm x 1cm were then weighed. The average of the paper weight became the b value. The clear zone area was calculated by comparing the zone weight and the average weight of 10 weighing papers. The larger the clear zone, the higher the ability of the bacteria to degrade the bonds of a polymer, whether in the form of protein, starch or cellulose [8]. The area of the clear zone that has been known is followed by calculating the zone value, so that the index value of the potential isolate can be found, with the equation.
2.4.2.4 Catalase enzyme activity test

The catalase enzyme test was carried out by giving a 3% H₂O₂ solution to the bacterial colonies. Bacteria that show the presence of the enzyme catalase are characterized by the formation of bubbles in the colony [9].

2.4.3 IAA (Indole 3 Acetic Acid) hormone activity test

As much as 0.5 mL bacterial isolates were grown on 0.5 mL tryptone medium, then incubated and shaken for 24 hours. Two mL of harvested bacterial culture was taken and then centrifuged at a speed of 10,000 rpm for 10 minutes at a temperature of 4 °C. Centrifugation aims to separate the supernatant and bacterial pellets. The test was carried out by taking the supernatant and given the Salkowsky reagent and then incubated in a dark room for 60 minutes. Incubation step in a dark room was done to prevent damage to IAA by visible light (photodegradation) [10]. Qualitative detection of IAA activity was observed through color changes, from a clear color to pink or reddish. Salkowsky reagent can react with IAA and produce a light color [11]. The basic principle of the reaction is the oxidation of the indole group IAA by iron (Fe) ions under acidic conditions. The color indicator that is formed is pink depending on the ability of the bacteria. The increase in color indicates the high level of IAA produced by bacteria. The color indicator was then measured on a spectrophotometer at a wavelength of 520 nm [12]. The higher the IAA concentration, the darker the resulting color.

2.4.4 Antagonistic test with the Fusarium oxysporum fungi

Further testing, rhizosphere isolates were grown together with F. oxysporum fungi on potato dextrose agar (PDA) media and incubated at room temperature for 14 days. From the results of the qualitative test, an analysis of the inhibition of Fusarium oxysporum was carried out using the equation:

\[
I = \frac{d_1 - d_2}{d_1} \times 100
\]

Note:
- \( I \): Percentage Index Inhibition
- \( d_1 \): Diameter of control fungi colonies
- \( d_2 \): Diameter of treatment fungi colonies

2.4.5 Amplification of 16S rRNA Encoding Gene

Identification was carried out on 12 rhizosphere bacterial isolates, where identification could be carried out to the species level if the 16S rRNA sequence was about 99% of the sequences in GenBank. Identification at the genus level is possible if the 16S rRNA sequence is approximately 97% similar to the GenBank sequence [13].

The PCR process involves three sequential stages of the temperature cycle, namely denaturation of DNA. Denaturation is the process of breaking the double strands of DNA into one. This usually occurs at a temperature of 95°C for 1-2 minutes, so that the DNA that is double stranded will separate into a single strand (single strand). The denaturation stage of DNA is followed by the process of DNA that is printed at 50°C for 1 minute. The primer will form hydrogen bonds with the mold in the complementary sequence region. The next stage of DNA synthesis will form new DNA based on printed DNA information with the help of the DNA polymerase enzyme. This synthesis stage occurs at a temperature of 72°C for 1-2 minutes. This stage is repeated from 25 to 35 cycles.

PCR products in the form of amplified DNA were then electrophoresed. DNA visualization was carried out under ultraviolet light exposure after the gel was immersed in TAE buffer solution.
Electrophoresis was carried out with agarose gel with a concentration of 1.2%. The results of electrophoresis from 12 rhizosphere bacterial isolates can later be seen in the form of bands which are amplified DNA fragments and show pieces of the number of base pairs. The results of electrophoresis can be seen with a UV transluminator.

3. Results and Discussion

3.1 Results of isolation of white turmeric root soil samples
Table 1 shows the isolates that were isolated from the root soil of the white turmeric plant. The morphology of rhizosphere isolates is diverse and the growth patterns of rhizosphere isolates tend to grow fast. According to Mariola et al [14], bacteria have the physical properties of protein in producing energy. In addition to the need for ATP for respiration, cells also carry out fermentation for the formation of ATP. The growth of bacteria that have an inner membrane surface area produces ATP per unit membrane area. The faster the growth of bacteria requires energy, which increases the ratio of [NADH] / [NAD +] to its metabolism.

| No | Isolate code | Morphology of Isolates | The color of isolate | Growth patterns |
|----|--------------|------------------------|----------------------|-----------------|
| 1  | TnTP1        |                        | Yellowish white      | Growing fast    |
| 2  | TnTP2        |                        | White                | Growing fast    |
| 3  | TnTP3        |                        | Yellowish white      | Growing fast    |
| 4  | TnTP4        |                        | Yellow               | Growing fast    |
| 5  | TnTP5        |                        | White                | Growing fast    |
| 6  | TnTP6        |                        | White                | Growing fast    |
| 7  | TnTP7        |                        | White                | Growing fast    |
| 8  | TnTP8        |                        | Thin yellow          | Growing fast    |
| 9  | TnTP9        |                        | White                | Growing fast    |
| 10 | TnTP10       |                        | Yellowish white      | Growing fast    |
| 11 | TnTP11       |                        | White                | Growing fast    |
| 12 | TnTP12       |                        | Yellowish white      | Growing slowly  |

According to Liu et al [15], the bacterial community present in plant roots is less diverse than the bacterial community in the soil around the plant roots because the bacteria that enter the plant roots have been selected so that the number of microbial populations is not abundant compared to the land.

Also added to the study [16], the population of bacterial cells from plant roots is only a small amount, around 10^4-10^9 / gram whereas if in the soil, the population of bacterial cells is around 10^6-10^9 bacterial cells / gram. The number of bacterial cell populations around plant roots showed the same number because the bacterial media colonized and the population numbers ranged from 10^6-10^9 bacterial cells / gram. This shows that the roots filter against effective habitat formation. To limit the existing bacterial community, the plant root tissue filters out certain microbes that can enter the tissue.
and become symbiotic. It is different from the bacterial community in the soil and around the roots because the nutrients and secretions released by plants through the roots will attract bacteria to get close. The exudate secretion that is released by the plant roots automatically makes the selection. The formed community of bacterial cells is beneficial to plants. Some researchers report that the benefits of the rhizobacteria community support plant growth because they have the ability to fix nitrogen and produce important hormones such as IAA and others.

3.2 Rhizosphere bacteria potential screening results
The nitrogenase enzyme activity test results obtained two isolates, namely TnTP 6 and TnTP 10, the isolates had the potential to produce nitrogenase enzymes. Bacteria that have the ability to fix nitrogen are characterized by the formation of white pellicles on the media and a change in media color. The discoloration of the media occurs due to an increase in bacterial pH due to bacteria binding to N₂ from the air and forming NH₄⁺ or other N compounds and releasing it. The formation of pellicles on the surface of the semisolidated NFB media indicates nitrogenase activity. According to Susilowati et al [17], the pellicle produced by rhizoforous bacteria on NFB media is due to anaerobic nature in the media, the oxygen diffusion rate is the same as the respiration rate of organisms for the activity of organisms. Nitrogenase enzymes help reduce acetylene to ethylene. Rhizobacterial activity plays an important role in soil fertility and plant productivity [18]. The ability of rhizobacteria to increase plant growth and break down compounds into simpler so that they are easily absorbed by plants.

Malic acid which is used in Nfb media can be utilized by bacteria to continue to grow. According to Borland et al [19], CAM induction in plants during stress positively affects the activity of enzymes involved in malate metabolism and glucogenesis. Increased activity of enzymes that function in malate metabolism and glucogenesis when experiencing drought stress. The ability of rhizosphere bacteria to utilize malate when applied to drought stress will help plants to carry out photosynthesis even though the stomata are not fully open. Bacteria can break down malate into CO₂ and pyruvate. CO₂ in the Calvin-Benson cycle is in the chloroplast stroma, while pyruvate will be used to reconstitute PEP (phosphoenol pyruvate/phosphoenol pyruvate) with the help of the PEP enzyme. The resulting carboxylase then forms oxaloacetate. This metabolic process is an acid metabolism that usually occurs in CAM plants (such as cacti etc.). The uniqueness of the rhizosphere bacteria from this test shows that rhizosphere bacteria have the ability to fix nitrogen and have the potential to survive even in drought stress conditions by carrying out acid metabolism in utilizing malate (CO₂ fixation).

![Figure 2. Test results for rhizosphere bacteria nitrogen fixation activity](image)

*Note: (1) Negative; (2) Negative Samples; (3, 4) Positive Samples; (5) White Pellicles.*
Figure 2 shows that the rhizofer bacteria with a positive code TnTP 6 and TnTP 10 have the potential to perform N fixation and CO₂ fixation during drought stress. These bacteria have the ability to act as acid metabolism bacteria (BAM). The ability of CAM which commonly occurs in plants is actually owned by bacteria so that it has the potential to help plants in drought stress conditions.

3.3 Protease, amylase and cellulase enzyme activity tests result
Proteases are enzymes that can degrade peptide bonds in proteins. The protease activity in bacteria aims to determine the ability of bacteria to degrade proteins by using proteolytic enzymes produced by bacteria to break down protein complex polymers so as to reduce environmental pollution.

Amylase-producing bacteria are able to degrade starch into simple sugars and make it easier for plants to absorb food [20]. Based on the results obtained, bacterial isolates with the codes TnTP3, TnTP4, TnTP5 and TNTP8 have the potential to be used as biodegradation agents and are also able to produce biostimulants. These four isolates have the ability to degrade complex polymers in the form of protein, starch and cellulases into simple molecules that are useful for stimulating plant growth.

In cellulose enzyme testing, a positive result is indicated by the formation of a clear zone in the isolate which has the ability to hydrolyze cellulose molecules to produce glucose. The ability of a rhizobacter to produce cellulase enzymes is an evaluation of the physiological properties of rhizobacteria and as a marker that rhizobacterial isolates have different abilities in producing extracellular hydrolase enzymes.

Based on the results of the research in Table 3, it can be seen that all rhizosphere isolates have catalase enzyme activity. The catalase enzyme activity was quite high, among others, in isolates TnTP3, TnTP5, TnTP7 and TnTP8. All rhizosphere bacterial isolates have the potential to have catalase enzyme activity. Bacterial isolates that have catalase enzyme activity are able to withstand environmental, chemical and mechanical stress conditions [21]. The mechanism of the catalase enzyme breaks down H₂O₂, namely during respiration, bacteria are able to break down H₂O₂ with the help of the catalase enzyme to form a defense system from the toxic H₂O₂ it produces itself and (O₂) is characterized by the presence of bubbles [22]. The gas bubbles in table 3. indicate that the rhizoferous bacteria also produce the enzyme catalase.

3.4 IAA (Indole 3 Acetic Acid) hormone production activity test result
Tryptophan is decarboxylated into tryptophan, then oxidized and deaminized to produce indole acetaldehyde. This molecule then undergoes oxidation and produces indole acetic acid. IAA is an important hormone in various aspects of plant growth to regulate physiological processes, such as cell division and differentiation and protein synthesis. The optimum concentration of auxin can form active cambium and then cleave and form the xylem layer. IAA supports cell elongation in plant stems by increasing cell size and cell division [23].

![Figure 3. Potential for IAA-producing white turmeric rhizosphere bacteria](image-url)
The IAA hormone is a type of phytohormone that stimulates cell division, cell differentiation, and protein synthesis. The hormones produced by plants are sometimes not optimal. For this reason, an innovation is where bacteria are able to produce abundant IAA in the stationary phase, when the carbon source (C) is limited by acidic pH conditions. Biosynthesis of IAA microbes in the soil can be triggered by the presence of L-tryptophan from root exudates or damaged cells.

Hormone synthesis occurs through the presence of tryptophan and the intermediate compound indolepiruvic acid [24]. Based on the qualitative test conducted, all isolates produced various IAA hormones. Five rhizosphere bacterial isolates that had a high IAA production capacity were TnTP1, TnTP2, TnTP7, TnTP10 and TnTP12 isolates. Qualitative test results were carried out using a spectrophotometer to measure the absorbance of the color produced from bacterial isolates with a wavelength of 520 nm [25].

Table 2 IAA levels produced by isolates were analyzed by making IAA standard solutions, then the formula for regression equations for IAA levels produced by bacteria was analyzed in order to obtain an estimate of the IAA concentration content of rhizosphere isolates.

| No | Isolate code | Color       | IAA concentration (ppm) |
|----|--------------|-------------|------------------------|
| 1. | TnTP1        | Pink        | 2.588                  |
| 2. | TnTP2        | Pink        | 1.311                  |
| 3. | TnTP3        | Pink yellow | 0.554                  |
| 4. | TnTP4        | Pink        | 1.339                  |
| 5. | TnTP5        | Pink        | 1.255                  |
| 6. | TnTP6        | Pink yellow | 0.175                  |
| 7. | TnTP7        | Pink        | 4.158                  |
| 8. | TnTP8        | Pink yellow | 0.133                  |
| 9. | TnTP9        | Pink yellow | 0.189                  |
| 10.| TnTP10       | Pink        | 2.321                  |
| 11.| TnTP11       | Pink yellow | 0.596                  |
| 12.| TnTP12       | Pink        | 2.391                  |

From this standard curve we get the regression equation:

\[
\frac{y + 0.0035}{0.0713} = 0.027x - 0.003
\]

\( IAA \text{ concentration} = \text{where } y \text{ is the absorbance value} \)
Hormones, which are commonly added in commercial chemical fertilizers, are available from bacterial isolates. Rhizosphere bacteria have the potential as biostimulant agents to directly provide the growth hormone needed by plants. The biostimulant potential of rhizosphere bacteria from the white turmeric plant has multifunctional potential so that this potential can also be used as a biofertilizer as a capital.

According to Lestari et al [26] the age of bacteria will affect the production of IAA, this is due to a decrease in nutrient content. The difference in the ability of bacteria to use nutrients in the media supports the metabolic rate and affects the growth rate of these bacteria. Rhizosphere bacteria aged 24 hours have high metabolic activity in the production of IAA and have not been affected by a decrease in nutrition.

According to Lestari and Tallapragada [27,28], bacteria synthesize IAA using the L-Tryptophan metabolic pathway. Production of IAA through the indole-3-pyruvate (IPA) and indole-3-acetamide (IAM) pathways uses tryptophan as a precursor. Tryptophan is a physiological precursor of auxin biosynthesis in plants and microbes.

3.5 Results of antagonist test with the fungi of Fusarium oxysporum

The results of the antagonistic test of rhizosphere bacteria isolates with plant pathogenic fungi to optimize the use of biofungicide-based bioagents so that they become the right alternative for controlling pathogenic microbes that cause disease in cultivated plants.

![Figure 5](image.png)

**Figure 5.** Results of isolate antagonist test with *F. Oxysporum* fungi.

*Note:* (A) Negative control, (B) Positive Test with TNTP 5 Isolate, (C) Positive Test with TNTP 7 Isolate

Biofungicides are commonly used to inhibit the growth of pathogenic fungi that cause disease in cultivated plants which are expected to be effective in controlling the attack of the pathogenic fungus *F. oxysporum* on plants and safe for plant cultivation. Based on tests conducted on 12 bacterial isolates, two types of rhizosphere isolates were selected which could potentially be used as biofungicidal agents against plant diseases caused by *F. oxysporum* fungi.

Table 3 shows the multipotency of rhizosphere bacteria more than one other potential that can support plant growth, especially white turmeric, except that TnTP9 isolate only has one potential as a biostimulant agent. The natural multipotency of the rhizosphere bacteria from the white turmeric plant is an inner value that can be promoted for biostimulant plus products.
Table 3. Potential rhizosphere bacteria from white turmeric to support plant growth.

| No | Isolate code | IAA Concentration (ppm) | Enzyme activity | Anti fungi |
|----|--------------|-------------------------|-----------------|------------|
|    |              |                         | N. fix | Protease | Amilase | Selulase | Katalase |            |
| 1. | TnTP1        | 2.588                   | -      | -        | -       | -        | +        | -          |
| 2. | TnTP2        | 1.311                   | -      | 1.31     | -       | -        | +        | -          |
| 3. | TnTP3        | 0.554                   | -      | 1.17     | 0.33    | 2        | +        | -          |
| 4. | TnTP4        | 1.339                   | -      | 2.49     | 0.28    | 1.2      | +        | -          |
| 5. | TnTP5        | 1.255                   | -      | 0.09     | 0.58    | 1.24     | +        | +          |
| 6. | TnTP6        | 0.175                   | +      | -        | 0.82    | -        | +        | -          |
| 7. | TnTP7        | 4.158                   | -      | -        | -       | -        | +        | +          |
| 8. | TnTP8        | 0.133                   | -      | 1.44     | 5.3     | 0.58     | +        | -          |
| 9. | TnTP9        | 0.189                   | -      | -        | -       | -        | +        | -          |
|10. | TnTP10       | 2.321                   | +      | -        | -       | -        | +        | -          |
|11. | TnTP11       | 0.596                   | -      | -        | -       | -        | +        | -          |
|12. | TnTP12       | 2.391                   | -      | 0.50     | -       | -        | -        | +          |

Of all the rhizosphere bacteria of white turmeric in table 3. The highest IAA producer was the rhizosphere bacterial isolate TnTP7 (4,158ppm) with the potential for biodegradation and biocontrol agents as well as the lowest IAA in isolate TnTP8 (0,133ppm) with potential as a biocontrol agent.

3.6 Result of Amplification of 16S rRNA Encoding Gene

Based on the visualization of the 16S rRNA gene, the size of about 1550 base pairs and about 500 bases is a hypervariable region. This area is the part that differentiates between organisms. Primers used in sequence amplification recognize continuous areas and amplify hypervariable regions, thus obtaining a unique sequence for the organism. The results of PCR amplification of 16S rRNA gene were used for molecular identification of organisms by sequencing methods.

Figure 6. Visualization results of 16S rRNA gene electrophoresis.

Note: (A) DNA Ladder; (1) TnTP 2; (2) TnTP 3; (3) TnTP 4; (4) TnTP 5; (5) TnTP 6; (6) TnTP 7; (7) TnTP 8; (8) TnTP 9; (9) TnTP 10; (10) TnTP 11; (11) TnTP 12

The results of identification of rhizosphere isolates could not be continued due to the covid pandemic conditions. This information on bacterial identification can later complement the identity of rhizosphere isolates if later it will be developed into a multi-functional biostimulant product that
has commercial value where the type of bacteria and its potential are clearly known.

4. Conclusion
Twelve rhizosphere bacteria isolate from white turmeric were isolated. Rhizosphere bacteria have potential as biostimulant and multipotential agents (biofertilizer agents, biodegradation agents and biocontrol agents) which are useful for helping plant growth, especially white turmeric plants. The twelve bacteria that had been PCR showed DNA bands that could be continued for further identification. The results of simple biotechnology research in utilizing the natural potential of rhizosphere bacteria which is of deep value to be promoted into a safe, multi-functional biostimulant product based on superior isolates to support plant growth, especially white turmeric plants.

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