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Morphological Diversity of Endophytic Bacteria Peppermint (*Mentha Piperita* L.)

Rangga Ardiansyah[1], Sartika Wati Dwi Sapitri [1], Muhammad Naufal Dzakwan[1], Nisa Muhammad Thalib[2], Monika Thamara[1], Lia Putri Maharani[1], Risky Hadi Wibowo(*)[1,3], Sipriyadi Sipriyadi [1,2], Welly Darwis[1]

[1] Biology Department, Mathematics and Natural Sciences Faculty, Universitas Bengkulu
[2] Chemistry Department, Mathematics and Natural Sciences Faculty, Universitas Bengkulu;
[3] Magister Study Program of biology, Mathematics and Science Faculty, Universitas Bengkulu
Jl. W. R Supratman, Kandang Limun, 38122, Bengkulu, Indonesia

***Corresponding author:** rhwibowo@unib.ac.id

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Abstract

Background: *The peppermint plant (Mentha piperita L.) is a type of medicinal plant that is widely found in temperate regions, especially Europe, North America and Africa. This plant contains essential oils, menthol, and menthone, menthafuran, sesquiterpenes, triterpenes, flavonoids, carotenoids, tannins and various minerals are also widely contained in peppermint leaf oil (M. piperita L.). The purpose of this study was to isolate and determine the morphological diversity of endophytic bacteria from peppermint plants (M. piperita L.).* **Methodology:** *The method used in this study is to take isolation samples, pure culture, characterization and gram staining to determine whether it includes gram-negative or positive bacteria. The results of the study of 12 cultures of endophytic bacterial isolates from plants (M. piperita L.) were taken 10 isolates from each different culture media.* **Findings:** *The results of morphological observations of bacterial colonies show diverse characteristics in terms of surface, appearan, ceelevation, edges, and colour. Gram Staining Test of Endophytic Bacterial Isolates from the roots, leaves, and stems of mint plants against endophytic bacteria showed that there were gram-positive and gram-negative bacterial isolates. Then the results of bacterial cell shape morphology have a diversity of Cocobacil, Coccus, and Bacil forms so it can be concluded that endophytic bacterial isolates from peppermint plants (M. piperita L.) have varied morphological diversity.* **Contribution:** *Data on the morphological diversity of endophytic bacteria from M. piperita L. provides important basic data for further research on the potential of M. piperita L. endophytic bacteria.*

Keywords: *Bacteria; Endophytic; Mentha piperita L.; Morphology*

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INTRODUCTION

Peppermint (*Mentha piperita* L.) is a species of aromatic herbal medicinal plant that can produce essential oils (menthol, menthone, menthofuran) with high economic value and is often used in the pharmaceutical and cosmetic industries (Mucciarelli et al., 2007). Mint leaf oil is produced from the extraction of the stems, leaves, and flowers (Selina et al., 2019). This plant has leaves that are 4–9 cm long and 1.5–4 cm wide, dark green in colour with reddish veins, pointed tips, and coarse serrated edges. This plant has fine hairs on its leaves and stems. The rhizome roots and stems can grow to 30–90 cm. Peppermint flowers (*M. piperita* L.) are 6–8 mm long and purple in colour (Tiwari, 2016). The quality and quantity of secondary metabolites produced by this plant are influenced not only by genetic and environmental factors, but also by plant microorganisms, including endophytic bacteria that live asymptomatically in the plant's internal tissues and play an important role in the physiology of the plant host (Castronovo et al., 2021).

Endophytic bacteria are beneficial microbes because they can interact with host plants without damaging them. Several research results show that some species of endophytic bacteria can produce chemical compounds that can be utilised in the health sector. Active compounds are usually obtained by extracting plants, especially medicinal plants. The ability of endophytic bacteria to produce active compounds is a potential that can be developed (Semenzato & Fani, 2024). According to Puspita et al., (2018), endophytic bacteria have advantages for plants, including providing various nutrients, producing growth hormones, enhancing plant growth, and acting as control agents in plants. One species of medicinal plant that can be used as a source of endophytic bacteria is peppermint (*M. piperita* L.).

Exploring the diversity and characteristics of endophytic bacteria from peppermint plants is important for unlocking the potential for further research. Morphological approaches remain relevant as a first step in microbial bioprospecting, such as colony morphology, including colour, shape, surface, elevation, and colony edges, as well as cell morphology, including cell shape and arrangement, which can be used to identify potential isolates for further research (Mahlangu et al., 2022). Information and research on the diversity of endophytic bacteria in peppermint plants (*M. piperita* L.) are still very limited. Therefore, this study aims to isolate and determine the morphological diversity of endophytic bacteria from peppermint plants (*M. piperita* L.).

METHODS

Sample Collection

This research was conducted from March to May 2023 at the Microbiology Laboratory, Basic Science Building, Department of Biology, Faculty of Mathematics and Natural Sciences, Bengkulu University.

Tools and Materials

The equipment used in this study consisted of Petri dishes (Pyrex 150×25 mm), Erlenmeyer flasks (Pyrex 250 ml), a 13 cm mortar and pestle, screw-top tubes (Pyrex 10 ml), beakers (Pyrex 10 ml), measuring cylinders (Pyrex 100 ml), 15 cm dropper pipettes, spirit lamps, ose needles, Laminar Air Flow (Nuaire), colony counters, vortexes, binocular microscopes, autoclaves, hot plates, spiritus, tweezers, object slides, and cover slips. The materials used in this study were the roots, stems, and leaves of peppermint plants (*M. piperita* L.), Nutrient Agar (NA) medium, pure agar, nystatin, crystal violet, lugol, safranin, sodium hypochlorite (NaClO), cotton, cling wrap, aluminium foil, rubber, plastic, 70% alcohol, 96% alcohol, distilled water, tissue, disc paper, label paper, and immersion oil.

Research Procedure

Determination of Sampling Location

Peppermint plants (*M. piperita* L.) were collected from the yard of a house in the Bentiring Permai area, Muara Bangkahulu District, Bengkulu City, Bengkulu Province in a fresh state. The method of collecting samples is by taking root, stem and leaf samples from the peppermint plant (*M. piperita* L.) as much as 100 g each. Then, the samples were put into plastic to prevent contamination of fungi and other bacteria on the peppermint plant (*M. piperita* L.).

Isolation of Endophytic Bacteria

The method for isolating endophytic bacteria is by grinding, referring to the procedure described by Pramono *et al.* (2019). Peppermint plant samples (stems, leaves, and roots) were washed thoroughly with running water while fresh. Next, three replicates of each root, stem, and leaf section from different parts were cut and weighed at 1 gram and cleaned on the surface. Then, the sample pieces were soaked for 1 minute in 70% alcohol. Next, the sample pieces were placed in a sodium hypochlorite (NaClO) solution and left to stand for 5 minutes. Then, they were soaked again 3 times at 30-second intervals in 70% alcohol and then rinsed slowly with clean running water.

The sterilised samples of peppermint (*M. piperita* L.) roots, stems and leaves were then ground with a mortar and pestle until smooth. They were then diluted in stages to 10^{-5}. The dilution process involved placing the ground samples into a screw-top tube containing 9 ml of distilled water, vortexing, taking 1 ml and placing it into a screw-top tube for 10^{-1} dilution, vortexing again, taking another 0.1 ml and placing it into a screw-top tube for 10^{-3} dilution. Then, it is vortexed again and 0.1 ml is taken from each, then placed into a 10^{-5} dilution tube and vortexed again. Next, 0.1 ml of the 10^{-3} and 10^{-5} dilutions were taken and dripped into a 150×25 mm Petri dish containing Nutrient Agar (NA) medium to which nystatin (0.01% w/v) had been added, and spread using a spreader (spreading rod).

Next, the isolation results were incubated at a temperature of 25–30 °C for 24 hours. After incubation, bacterial colonies will grow. After the bacteria grow, the number of colonies is counted using a colony counter and the morphology of the bacterial colonies is observed, such as the shape, edges, surface, colour of the colony and elevation of the bacterial colony. After that, purification is carried out and then inoculated on a new medium (Radu & Kqueen, 2002).

Purification of Endophytic Bacterial Isolates

The method for purification of bacterial isolates is the quadrant scratch method. After incubating for 24 hours at 25-30 ˚C. The purified colonies were then used for further observation. After obtaining pure culture, endophytic bacteria are stored on Nutrient Agar slant agar media which will be used in further testing (Cappucino & Sherman, 2013).

Identification of Peppermint Endophyte Isolates

Observation of Colony Morphology

Pure culture isolates of endophytic bacteria of peppermint plants (*M. piperita* L.) were then identified based on macroscopic observations or colony morphology which included colony appearance, elevation of bacterial colonies, edges, colony surface shape, and colony colour (Austin & Austin, 2007).

Gram Staining

One ose of pure culture isolate was taken aseptically and mixed with distilled water on a glass slide. The preparation was then dried, and then fixed on the flame. After that, the glass object that has been fixed is dripped with crystal violet solution for 1 minute, then the preparation is rinsed with distilled water, then the preparation is dripped with lugol solution on the glass surface for 2 minutes, then the preparation is rinsed again with distilled water, then dripped with 96% alcohol, then rinsed. Then the preparation was dabbed with safranin solution for 30 seconds, then rinsed again with distilled water. The preparations were then observed under a microscope with a magnification of 10x100. The results of microscopic observations, can see the shape and placement of bacteria and can determine the type of bacteria both gram-positive bacteria to be observed with purple and gram-negative bacteria with red (Lay, 1994).

RESULT AND DISCUSSION

Endophytic Bacteria Peppermint (*M. piperita* L.)

Endophytic bacteria that were successfully isolated from Peppermint plants (*M. piperita* L.) from home gardens in the Bentiring Permai area, Muara Bangkahulu District are shown in (Figure 1).

Figure 1. Plant Morphological *Mentha piperita* L.

The results of endophytic bacteria isolation from peppermint plants (*M. piperita* L.) were taken from the roots, stems, and leaves. Isolation was carried out using the grinding and serial dilution methods, which were grown on Nutrient Agar (NA) media incubated for 24 hours at 30 °C. The grinding method was used to break down plant cells and release endophytic bacteria located inside cells or intracellular spaces of plant tissues. According to Suhandono et al., (2016), endophytic bacteria are endosymbiotic microorganisms that reside within plant hosts and form mutually beneficial relationships. Endophytic bacteria live in intercellular spaces rich in carbohydrates, amino acids, and inorganic nutrients. Based on the result of the research that has been done, the calculation of the total number of bacterial colonies isolated from plant Peppermint (*M. piperita* L.) in (Figure 2).

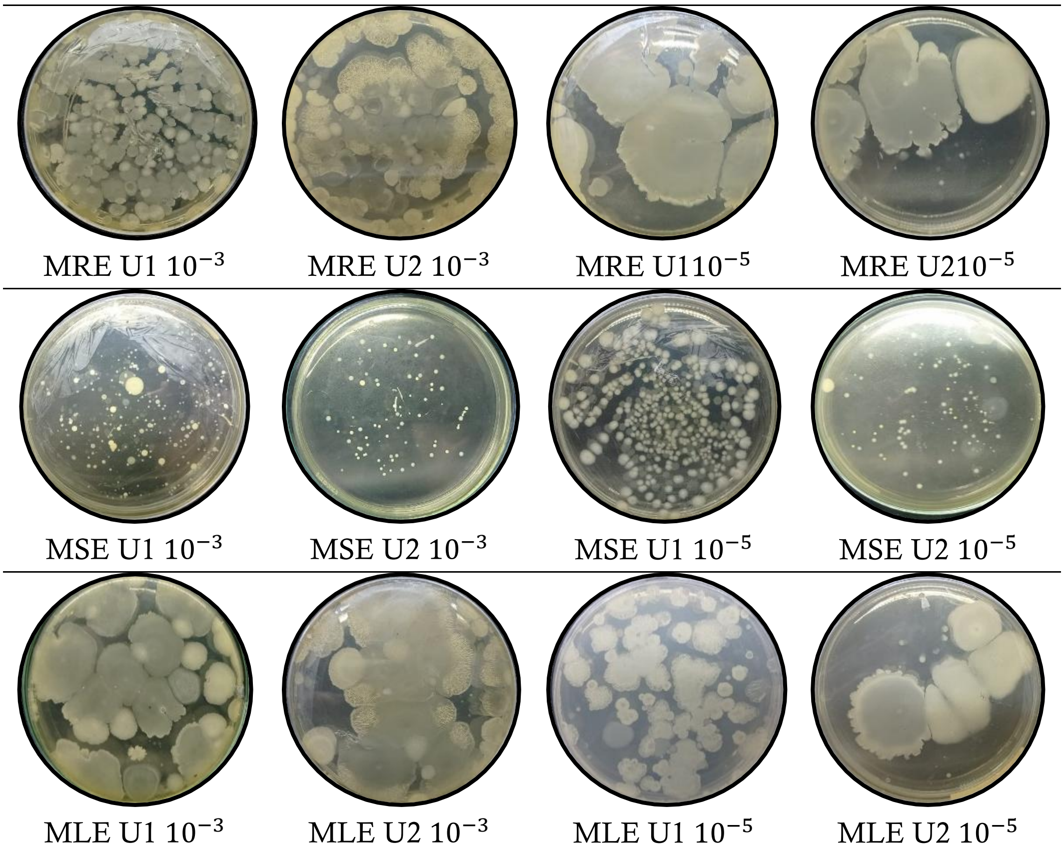


Figure 2. Results of Endophytic Bacteria Isolation from Several Parts of Peppermint Plants (*M. piperita* L.). Description: MRE = Mint Roots Endophytic, MSE = Mint Stem Endophytic, MLE = Mint Leaves Endophytic, U1 = Repetition 1, U2 = Repetition 2, 10^{-3} and 10^{-5} = dilution factors (petri dishes size 150 mm).

Based on the research, the results of the calculation of endophytic bacterial colonies of Peppermint plants (*M. piperita* L.) were obtained as listed in (Table 1).

Table 1. Calculation Results of Endophytic Bacterial Colonies from Peppermint Plants (*M. piperita L.*)

Isolate Codes	Colony Numbers			
	10^{-3}		10-5	
	U1	**U2**	**U1**	**U2**
MRE	TNTC	TNTC	16	10
MSE	TNTC	54	98	52
MLE	20	14	26	12

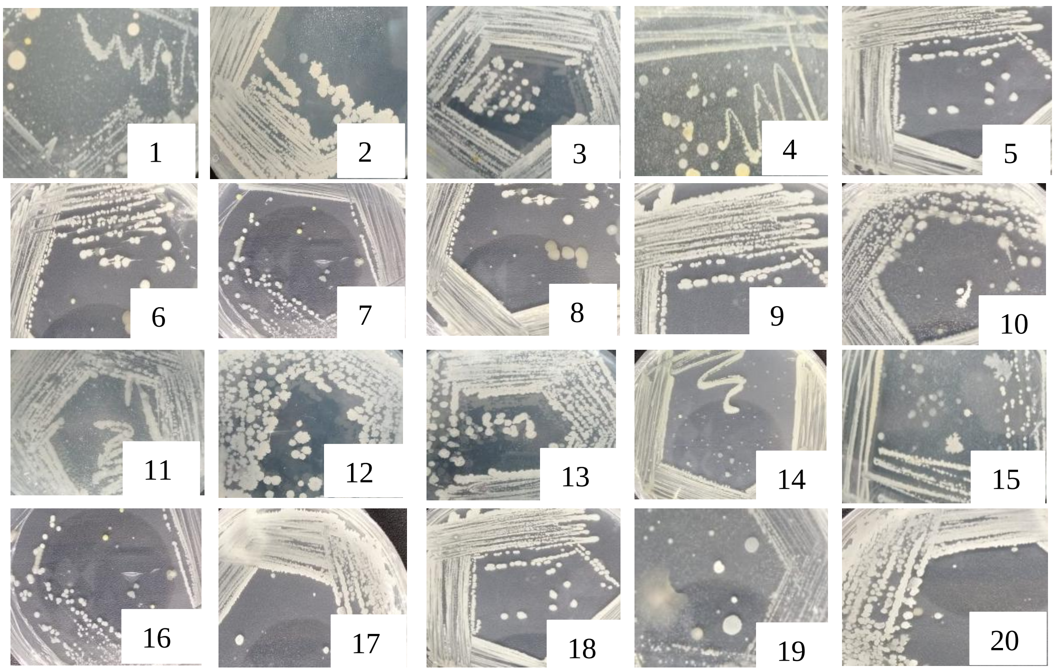
Description: Bacterial colonies from Peppermint plants (*M. piperita* L.), MRE = Mint Roots Endophytic, MSE = Mint Stem Endophytic, MLE = Mint Leaves Endophytic, U1 = Repetition 1, U2 = Repetition 2, 10^{-3} and 10^{-5} = dilution factors, TNTC = Too Numerous to Count.

Based on the results of endophytic bacterial isolation, 30 endophytic bacterial isolates (*M. piperita* L.) were obtained from peppermint plants, consisting of 10 isolates from the roots, 10 isolates from the leaves, and 10 isolates from the stems, as shown in (Figure 2). The diversity of colony count data obtained in Table 1 shows that as dilution increases, the number of microbial colonies decreases. This is in accordance with Sukmawati & Hardianti (2018), who stated that the distribution of the number of microbial colonies and the dilution factor for each sample showed that the number of microbial colonies increased with a higher dilution factor and was inversely proportional to the number of microbial colonies due to uniform data diversity and in accordance with the dilution factor principle.

If the number of colonies in a sample exceeds 300 cfu/g, the sample is categorised as turbidimetric (TNTC), as the number of microbial colonies that can be analysed ranges from 30 to 300 cfu/g. According to Bacon & Hinton (2006), endophytic bacteria can be distinguished by isolating them on agar media, and their numbers cannot be determined precisely in each plant. Nutrient agar (NA) medium can be used to isolate endophytic bacteria. This medium is rich in yeast extract, peptone, sodium chloride, and agar. Due to their complex characteristics and the composition of the culture medium, which is often comparable to the internal conditions of the plant used, endophytic bacteria can survive in Nutrient agar (NA) culture medium.

Results of Endophytic Bacterial Isolate Purification

Based on the research, the results of the purification of endophytic bacteria from Peppermint plants (*M. piperita* L.) were obtained as shown in (Figure 3).



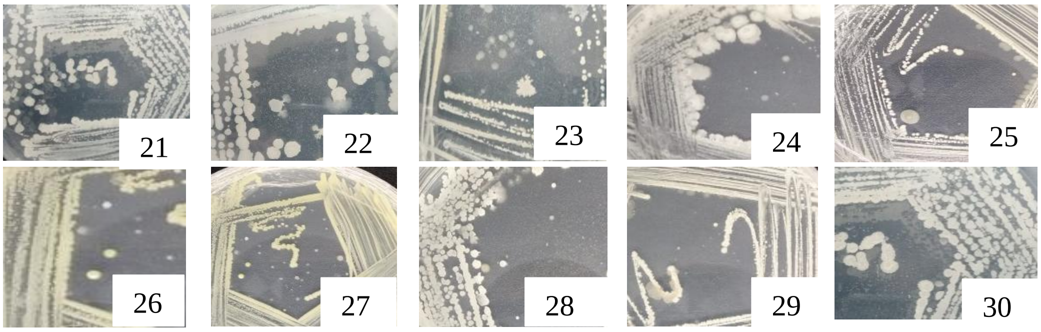


Figure 3. Morphological Observation of Colonies of Bacterial Isolates from Peppermint (*M. piperita* L.) Plants MRE = 1-10, MSE= 11-20, MLE= 21-30 (petri dishes size 150 mm).

Based on the research, the results of observations of the morphology of endophytic bacteria of Peppermint plants (*M. piperita* L.) were obtained as listed in (Table 2).

Table 2. Results of the Endophytic Bacteria Morphology from Peppermint Plants (*M. piperita* L.)

Isolate Codes	Species	Shape	Appearance	Elevation	Edge	Color
MRE	1	*Smooth*	*Circular*	*Raised*	*Entire*	White
	2	*Smooth*	*Irregular*	*Raised*	*Undolate*	Creamy White
	3	*Smooth*	*Circular*	*Convex*	*Undolate*	White
	4	*Smooth*	*Circular*	*Pulvinate*	*Entire*	Yellow
	5	*Smooth*	*Circular*	*Convex*	*Undolate*	White
	6	*Smooth*	*Circular*	*Convex*	*Entire*	White
	7	*Smooth*	*Circular*	*Convex*	*Entire*	White
	8	*Smooth*	*Circular*	*Convex*	*Entire*	White
	9	*Smooth*	*Circular*	*Convex*	*Undolate*	White
	10	*Smooth*	*Circular*	*Raised*	*Entire*	White
MSE	1	*Smooth*	*Irregular*	*Raised*	*Undolate*	White
	2	*Smooth*	*Circular*	*Raised*	*Lobate*	White
	3	*Wrinkled*	*Circular*	*Raised*	*Undolate*	White
	4	*Smooth*	*Circular*	*Convex*	*Entire*	Yellow
	5	*Smooth*	*Circular*	*Raised*	*Entire*	Yellow
	6	*Smooth*	*Circular*	*Convex*	*Entire*	White
	7	*Smooth*	*Circular*	*Flat*	*Lobate*	White
	8	*Smooth*	*Circular*	*Convex*	*Undolate*	Creamy
	9	*Smooth*	*Circular*	*Raised*	*Entire*	Creamy
	10	*Smooth*	*Irregular*	*Flat*	*Undolate*	White
MLE	1	*Wrinkled*	*Circular*	*Flat*	*Undolate*	White
	2	*Wrinkled*	*Circular*	*Flat*	*Undolate*	White
	3	*Smooth*	*Circular*	*Covex*	*Entire*	Yellow
	4	*Concentric*	*Circular*	*Flat*	*Undolate*	White
	5	*Smooth*	*Circular*	*Convex*	*Entire*	Yellow
	6	*Smooth*	*Circular*	*Convex*	*Entire*	Yellow

7	*Smooth*	*Circular*	*Convex*	*Entire*	Yellow
8	*Wrinkled*	*Circular*	*Flat*	*Undolate*	White
9	*Smooth*	*Circular*	*Raised*	*Undolate*	Yellow Orange
10	*Wrinkled*	*Circular*	*Raised*	*Undulate*	White

Remarks: MRE = Mint Roots Endophytic, MSE = Mint Stem Endophytic, MLE = Mint Leaves Endophytic

Observation of bacterial isolates was identified based on bacterial morphology, including appearance, surface, edges, colour, and elevation of bacterial colonies (Cappucino & Shermam, 2013). Based on morphological observations of 30 purified endophytic bacterial isolates (*M. piperita* L.), isolates MRE 6–7 were found to have the same colony shape and colour, namely a smooth surface, entire colony edges, white colour, convex elevation, and circular appearance. Isolates MRE 1 and 10 also had the same colony shape and colour, with a smooth surface, circular appearance, entire white colony edges, and raised elevation, while isolates MRE 2, 3, 4, and 5 had different shapes and colours. MRE2 has a smooth surface, irregular appearance, raised elevation, undulate colony edges, and a creamy white colour. MRE3 has a smooth surface, circular appearance, convex elevation, undulate colony edges, and a white colour. Isolate MRE4 has a smooth surface, circular appearance, pulvinate elevation, entire colony edges, and yellow colour. MRE5 has a smooth surface, circular appearance, convex elevation, undulate colony edges, and white colour.

The bacterial colonies in the Mint Stem Endophytic (MSE) isolates have varying colours and shapes. MSE1 has a smooth surface, irregular appearance, raised elevation, undulate colony edges, and a clear white colour. MSE2 has a smooth colony surface, circular appearance, raised elevation, lobate colony edges, and a white colour. MSE3, 5, 6, and 7 have the same colony shape, namely a wrinkled surface, circular appearance, raised elevation, undulate colony edges, and a white colour. MSE4 has a smooth surface, circular appearance, convex elevation, entire colony edges, and a yellow colour. MSE8 has a smooth surface, circular appearance, convex elevation, undulate colony edges, and a cream colour. MSE9 has a smooth surface, circular appearance, entire colony edges, white colour, and raised elevation. MSE10 has a smooth surface, irregular appearance, flat elevation, undulate colony edges, and white colour.

The results of observations of Mint Leaves Endophytic (MLE) isolates for isolates MLE1 and MLE2 have the same shape, namely a wrinkled surface, circular appearance, flat elevation, undulate colony edges, and white colour. MLE3 has a smooth surface, circular appearance, convex elevation, entire colony edges, and yellow colour. MLE4 has a concentric surface, circular appearance, flat elevation, undulate colony edges, and white colour. MLE5 isolate has a smooth surface, yellowish-white colour, entire colony edges, convex elevation, and circular appearance. MLE6 has a smooth surface, circular appearance, convex elevation, entire colony edges, and yellow colour. MLE7 has a smooth surface, circular appearance, convex elevation, entire colony edges, and yellow colour. DTM has a wrinkled surface, circular appearance, flat elevation, undulate colony edges, and white colour. MLE9 had a smooth surface, circular appearance, raised elevation, undulate colony edges, and yellow-orange colour. MLE10 had a wrinkled surface, circular appearance, raised elevation, undulate colony edges, and white colour. Based on the research conducted, there are various morphologies of each part of the peppermint plant (*M. piperita* L.), namely the roots, stems, and leaves.

Gram Staining Results of Endophytic Bacterial Isolate

Based on the research, the results of the research that has been done, the coloring result of bacterial isolates from plants (*M. piperita* L.) are shown in (Figure 4).

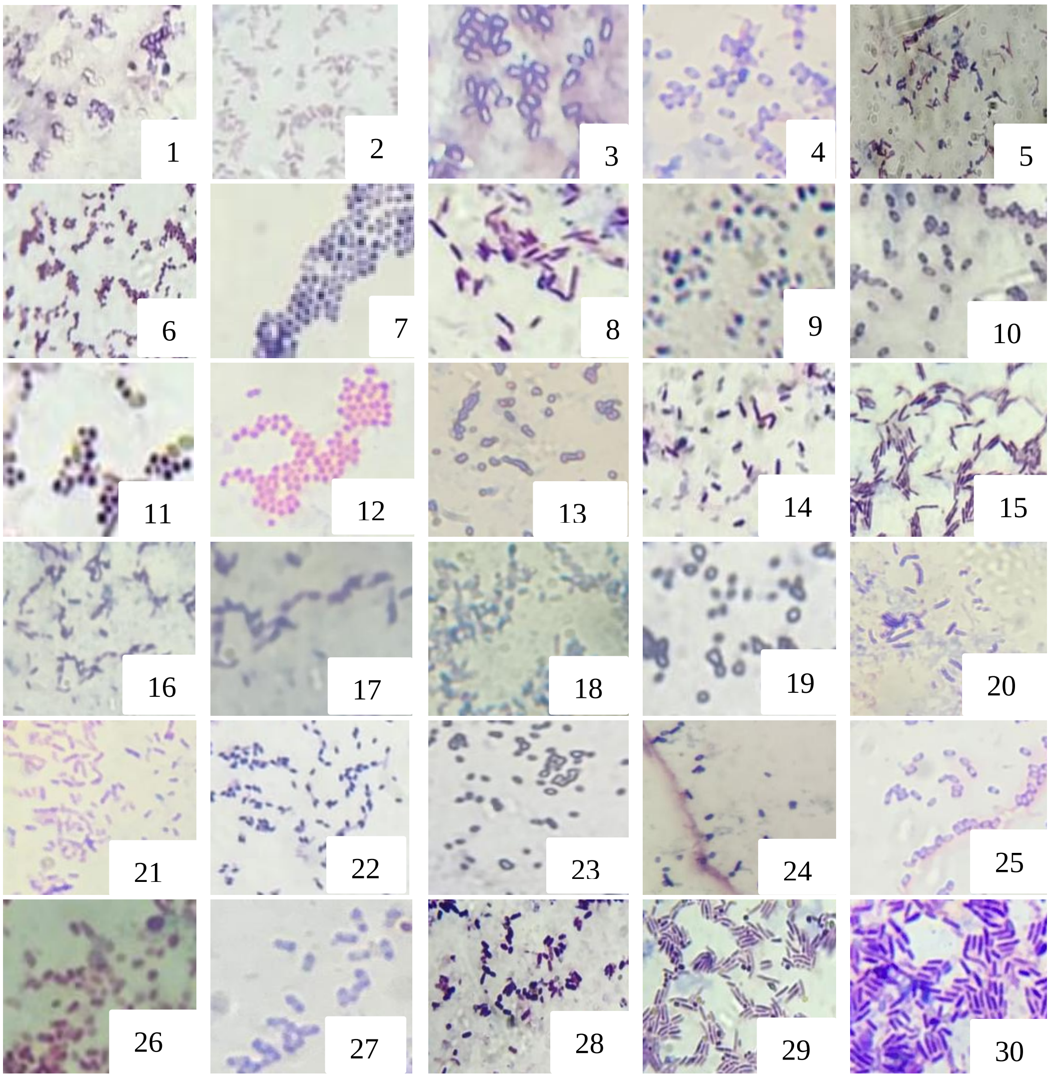


Figure 4. Gram Staining of Endophytic Bacterial Isolates from Peppermint (*M. piperita* L.) Plants (100×). MRE = 1-10, MSE= 11-20, MLE= 21-30.

Based on the research, the results of microscopic observations of endophytic bacterial isolates of Peppermint plants (*M. piperita* L.) were obtained as listed in (Table 3).

Table 3. Results of Microscopic Observations of Endophytic Bacterial Isolates from (*M. piperita* L.)

Isolate Codes	Species	Gram Staining		Cell Arrangement
		Positive (+)	Negative (-)	
MRE	1	✓		*Cocobacil*
	2	✓		*Bacil*
	3	✓		*Cocobacil*
	4	✓		*Cocobacil*
	5	✓		*Bacil*
	6	✓		*Bacil*
	7	✓		*Coccus*
	8	✓		*Bacil*
	9	✓		*Cocobacil*
	10	✓		*Cocobacil*
MSE	1	✓		*Coccus*
	2		✓	*Coccus*
	3	✓		*Cocobacil*
	4	✓		*Bacil*
	5	✓		*Bacil*
	6	✓		*Bacil*
	7	✓		*Bacil*
	8	✓		*Cocobacil*
	9	✓		*Coccus*
	10	✓		*Bacil*
MLE	1	✓		*Bacil*
	2	✓		*Cocobacil*
	3	✓		*Cocobacil*
	4	✓		*Bacil*
	5	✓		*Cocobacil*
	6	✓		*Cocobacil*
	7	✓		*Cocobacil*
	8	✓		*Bacil*
	9	✓		*Bacil*
	10	✓		*Bacil*

After purification, Gram staining was performed to observe the shape and arrangement of the papermint endophytic bacteria and to determine whether they were Gram-positive or Gram-negative bacteria. As shown in (Table 3), all MRE isolates were Gram-positive bacteria. MRE isolates 2, 5, and 8 were bacilli, MRE isolates 1, 3, 4, 9, and 10 were cocobacilli, and MRE isolate 7 was a coccus. MSE isolates 1 to 10, except for 2, are Gram-positive bacteria, while MSE 2 is a Gram-negative bacterium. MSE isolates 4, 5, 6, 7, and 10 are bacilli, MSE isolates 1, 2, and 9 are cocci, and isolates 3 and 8 are coccobacilli. All MSE isolates are Gram-positive bacteria, MLE isolates 1, 4, and 10 are bacilli, and MLE isolates 2, 3, 5, 6, 7, 8, and 9 are coccobacilli. Gram bacteria can be distinguished based on their colour after staining. According to Nurhidayati et al., (2015), Gram-negative bacteria cell walls appear red in Gram staining, while Gram-positive bacteria cell walls appear purple. This difference is due to Gram-positive bacteria having low lipid content in their cell walls, making the cell walls susceptible to dehydration due to the influence of alcohol in reducing the size of cell pores and decreasing permeability, resulting in the production of crystal violet pigment as the main colouring agent. Gram-negative bacteria have an outer layer of cell walls consisting of lipopolysaccharides (lipids) that can be washed away by alcohol, turning red when stained with safranin. Aglinia et al., (2020) elucidate that the distinctions between Gram-positive and Gram-negative bacteria arise from differences in their cell wall structures. Specifically, Gram-positive bacteria possess a substantially thick peptidoglycan layer, which enables them to retain the crystal violet stain during the Gram staining process. In contrast, Gram-negative bacteria have a comparatively thin peptidoglycan layer situated near the cytoplasm, rendering them unable to retain the crystal violet stain during the alcohol-based decolorization phase.

According to Wilson et al., (2017), endophytic bacteria are dominated by the Proteobacteria group and include Gram-negative bacteria. The results of research by Nursanty & Suhartono (2012), show that the antimicrobial activity of endophytic bacterial isolates from plants can inhibit the growth of *S. aureus* alone, out of five microbes, namely *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, and *Pseudomonas aeruginosa*. It is possible that endophytic isolates from plant roots that do not exhibit antimicrobial properties contain other potential compounds. Additionally, several studies have shown that endophytic bacteria can produce antioxidants. Muntean et al., (2019), stated that in previous studies, the endophytic bacteria identified in mint plants were *Staphylococcus aureus, Pseudomonas aerogenosa, Pseudomonas aureus, Bacillus substillus*, and *Serratia marcescens*. Peppermint extract (*M. piperita* L.) is bacteriostatic against *Escherichia coli*, *Mycobacterium avium*, *Streptococcus aureus*, *Streptococcus pyrogens*, and *Serratia marcescens.*

Based on microscopic observations, it is suspected that coccus-shaped, Gram-positive bacteria such as MRE 1, 3, 4, 9, and 10, MSE 1, 3, 8, and 9, MLE 2, 3, 5, 6, and 7 are *Staphylococcus aureus* bacteria. According to Herlina (2015), *Staphylococcus* is a Gram-positive bacterium, coccus-shaped, short-chain, paired, clustered, and 0.5-1.0 mm in diameter. Meanwhile, Gram-positive bacilli-shaped bacteria such as MRE 2, 5, 6, and 8, MSE 4, 5, 6, and 7, MLE 1, 4, 8, 9, and 10 are suspected to be *Bacillus subtilis* bacteria. According to Graumann (2007), *Bacillus subtilis* bacteria are rod-shaped, aerobic, Gram-positive, have motile and diverse oxidation activities, and have endospores that function as a response to extreme environments and are formed from vegetative cells. *Bacillus subtilis* bacteria have positive starch hydrolysis and catalase tests and an optimal growth temperature of 37 ºC.

The roots and stems of mint plants are not usually used for medicinal purposes. In general, it is the leaves of mint plants that are used, especially older leaves. However, endophytic bacteria in the roots and stems of mint plants also have the potential to exhibit morphological diversity and determine Gram-negative and Gram-positive bacteria in mint plants. For example, research conducted by Hadipoentyanti (2012); Padila (2013), to determine the main components of peppermint (M. piperita L.) successfully isolated and identified the contents of peppermint (*M. piperita* L.), such as essential oils, menthol, menthone, menthafuran, sesquiterpenes, and triterpenes. Flavonoids, carotenoids, tannins, and various minerals are also abundant in peppermint leaf oil (*M. piperita* L.).

Based on the research results, endophytic bacteria were successfully isolated from peppermint plants (*M. piperita* L.), with 10 isolates obtained from each part of the plant: roots, stems, and leaves of M. piperita L. Gram staining tests of endophytic bacterial isolates from Mint Roots Endophytic (MRE) and Mint Leaves Endophytic (MLE) showed that all were Gram-positive bacteria with various shapes, namely bacilli, cocci, and coccobacilli. Meanwhile, Mint Stem Endophytic (MSE) isolates showed a variety of Gram-negative bacteria in the second isolate with a cocci shape. Thus, further research is needed to identify the species of potential endophytic bacterial isolates and isolate the compounds they produce.

CONCLUSION

Based on the results of the research conducted, 30 endophytic bacterial isolates were obtained from peppermint plants (*M. piperita* L.), with 10 isolates from the roots, 10 isolates from the stems, and 10 isolates from the leaves of peppermint plants (*M. piperita* L.). Based on the results of morphological identification, the colonies were very diverse in shape (circular and irregular), surface (smooth, wrinkled, concentric), elevation (convex, pulvinate, raised, flat), edge (entire, undulate, lobate), and colour (white, yellow, cream, and orange). Based on the results of microscopic identification using Gram staining, there was a diversity of Gram-positive and Gram-negative cells with Cocobacillus, Bacillus, and Coccus shapes. Further research is important to identify and isolate potential isolate species and to determine the compounds that can be produced by these isolates for antimicrobial production or use as medicine.

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