

## Endophytic Bacteria Bengkulu's Coffee and Their Prospects

<sup>1</sup>Dadan Supardan, <sup>2</sup>Yosi Yulizah, <sup>3</sup>Fatkhanuddin Azis, <sup>4</sup>Fauziah Fitriana

<sup>1,2</sup> Institut Agama Islam Negeri Curup, Curup, Bengkulu, Indonesia

<sup>3,4</sup> Program Studi Sarjana Terapan Teknologi Veteriner, Departemen Teknologi Hayati dan Veteriner, Sekolah Vokasi Universitas Gadjah Mada, Yogyakarta, Indonesia

Corresponding author: [dadan.supardan@iaincurup.ac.id](mailto:dadan.supardan@iaincurup.ac.id)

### Abstract

Antimicrobial resistance (A.M.R.) is one of the main threats in the health sector. Most antimicrobials, including antibiotics, antifungals, antivirals, and antiparasitics, are derived from bioactive compounds found in bacteria, particularly the actinomycetes group. This study aimed to isolate and test endophytic bacteria on coffee plants against antibiotic-resistant pathogenic bacteria, namely penicillin-resistant *S. aureus*. This study used several methods: endophytic bacteria isolation, bacterial purification, gram staining, and endophytic bacteria antagonist test against antibiotic-resistant bacteria. Eight endophytic coffee isolates were obtained from the roots and pods of Robusta coffee. One bacterial isolate with the code AR4 had an antagonistic effect on tetracycline-resistant *S. aureus* bacteria.

**Keywords:** *Bengkulu's coffee, endophytic bacteria, bacterial isolation, antagonistic assay*

### 1. Introduction

Antimicrobial resistance (A.M.R.) is one of the main threats in the health sector. Each year, more than 2.8 million infections are caused by A.M.R. in the United States, and more than 35,000 people die. In addition, in 2017, there were 223,900 cases of *Clostridioides difficile*, and at least 12,800 people died (C.D.C., 2019; WHO, 2015). A.M.R. infections worldwide continue to increase, exacerbated by the uncontrolled misuse of antibiotics and the limited number of new antibiotics discovered. Around ten million people are estimated to die by 2050 (Webster, 2018; Fair and Tor, 2014).

Most antimicrobials, including antibiotics, antifungal, antiviral, and antiparasitic, are derived from bioactive compounds found in bacteria, particularly the actinomycetes group. Actinomycetes are widespread bacteria in various habitats: terrestrial habitats, fresh water, and seawater, associated with plants and animals, and some are pathogenic to humans, animals, and plants. One currently, the focus of research by researchers is bacteria associated with plant tissue (endophytes). Actinomycetes bacteria are a group of gram-positive bacteria that, until now, are very abundant in various habitats.

Various types of microbes, especially bacteria, are found in association/symbiotic with the internal tissues of plants known as endophytic bacteria, including one of them, is Actinomycetes. Endophytic bacteria are known to produce bioactive compounds, which have many benefits. Rai et al. (2014) mention that plant endophytic bacteria can produce compounds beneficial as insecticides, pesticides, and antimicrobials. A symbiosis between endophytic bacteria and plants in the long term can affect the metabolic pathways and biological systems of plants from these bacteria and can even affect their genetic

information to produce specific bioactive compounds that are similar to their hosts (plants) (Eyberger et al. 2006; Mitchell et al. 2010; Kumar et al. 2013; Chithra et al. 2014; Rai et al. 2014).

One plant that has many benefits for humans is the coffee plant. Indonesia is a country that is known to be one of the best coffee producers in the world, especially in Bengkulu Province. This plant has many varieties with different tastes according to geographical conditions and varieties. Coffee in each region has taste and aroma characteristics; this is influenced by many factors, one of which is the state of its metabolism. This condition is also influenced by the variety of endophytic bacteria contained in the coffee. This is a great opportunity to obtain unique bacterial isolates for these plants, and we hope to provide many benefits. So it is important to research to reveal the variety of endophytic bacteria in coffee that have the potential as antibiotic compounds against antibiotic-resistant bacteria. This study aimed to isolate and test endophytic bacteria on coffee plants against antibiotic-resistant pathogenic bacteria, namely penicillin-resistant *S. aureus*.

## 2. Material and Method

### a. Isolation and Purification of Endophytic Bacteria

The roots of the coffee plant as much as 200 grams are washed in running water three times to remove dirt attached to the roots, then the surface of the roots is sterilized using a combination of chemicals in the form of 5% sodium chlorate ( $\text{NaClO}_3$ ), 2.5% sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ), 75% ethanol and 10% sodium bicarbonate ( $\text{NaHCO}_3$ ) to clean and inhibit the growth of endophytic fungi then cleaned again with sterile distilled water and then cut into  $\pm 1 \text{ cm}^2$  aseptically. The roots were crushed using a mortar aseptically, then dissolved using sterile distilled water as much as 100 ml (stock sample solution) and homogenized.

Endophytic bacteria were isolated by first diluting the stock sample solution into four series of dilutions ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ ). Then, 0.5 ml was taken and spread on selective media from each dilution. S.C.A. with three repetitions and incubated for 48-96 hours. After 48 hours, the morphology was observed and purified using the quadrant four methods. After pure, the bacteria were rejuvenated and propagated using an N.A. medium. Furthermore, the selected isolates were identified morphologically and biochemically to obtain information on the type of bacteria that had been isolated.

### b. Bacterial Regeneration

Bacteria isolated from coffee roots were then re-grown or re-cultured on Starch Nitrate Agar media using the streak method, then incubated for 72-96 hours at  $30^\circ\text{C}$ . In addition, bacterial stock was also made using the same method, but the bacterial stock was grown on agar medium in a test tube.

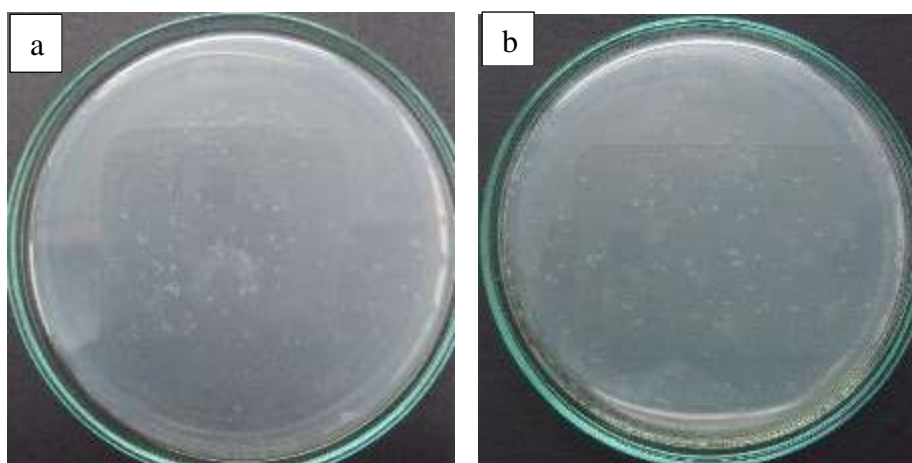
### c. Antibacterial Antagonistic Assay on *E coli* Antibiotic Resistant

The *E. coli* bacteria used were obtained from the Laboratory of Microbiology, Department of Biology, FMIPA, University of Bengkulu. Antagonist test in vitro used a dual culture assay (Suryanti et al., 2013). The test was carried out by growing in pairs a colony of pathogenic bacteria, namely *E. coli* with a diameter of 0.9 cm, and pieces of endophytic actinomycetes colony with a diameter of 0.9 cm in a petri dish containing N.A. media. then incubated at 35 oC for 48 -96 hours. Observations are made every 24 hours. The parameters observed were the percentage of inhibition in the form of clear zones formed.

## 3. Results and Discussion

### 3.1. Results

The isolation was carried out after obtaining a suitable medium for 15 days. Eight endophytic bacterial isolates were obtained from coffee roots and coffee pod samples.

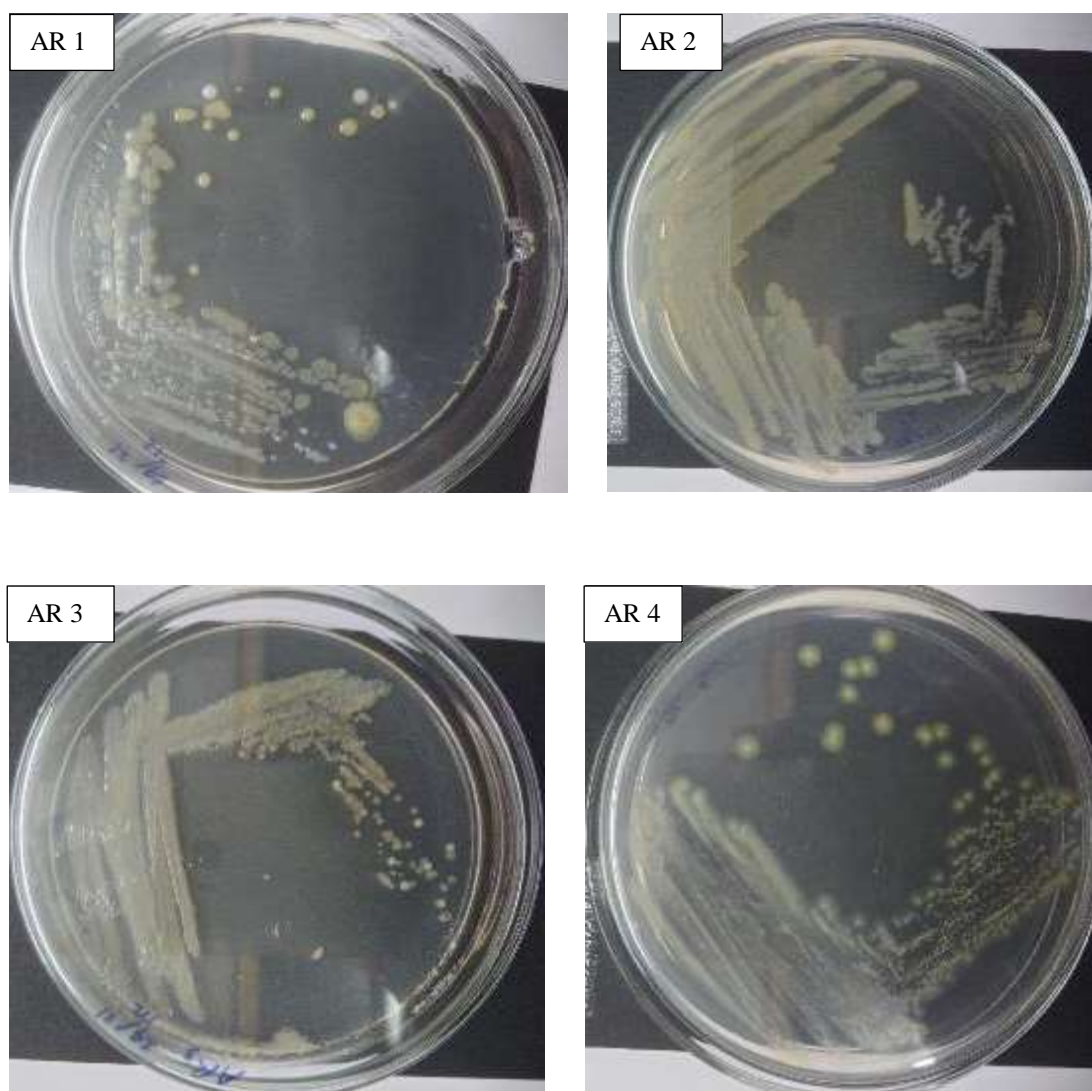


**Figure 2.** Isolates of endophytic bacteria coffee a) Root samples, b) Fruit samples

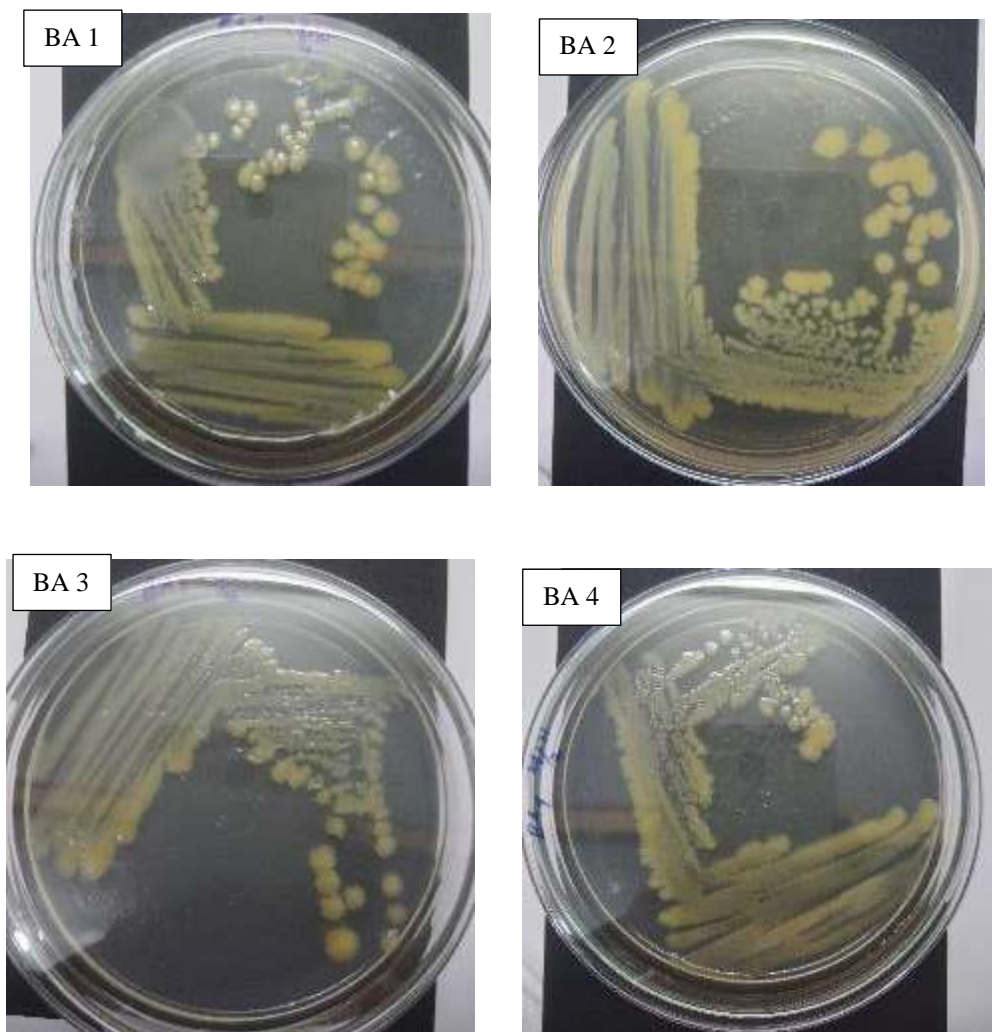
Based on Figure 2, 8 robusta coffee endophytic bacteria isolates were obtained from root and fruit samples.

### Purification of Endophytic Bacteria

The purification process was carried out using the quadrant four methods, and several different bacterial morphologies were obtained, both in color, the shape of the colony edges, elevation, and the pigments produced (Figure 3).



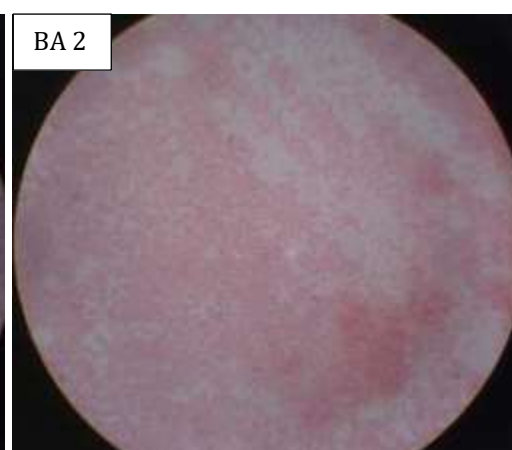
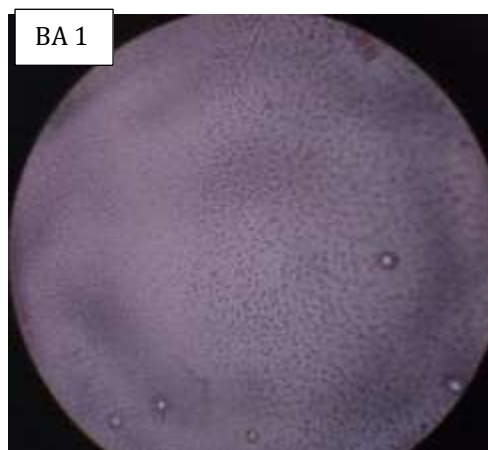
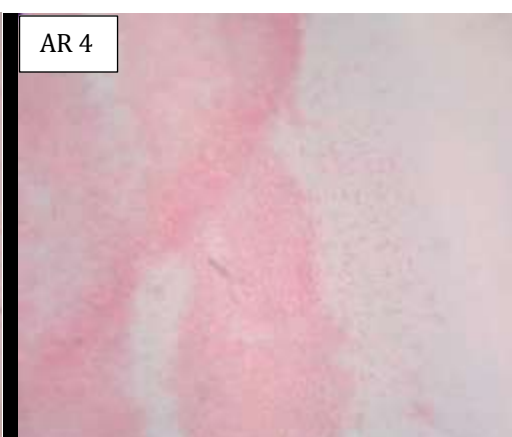
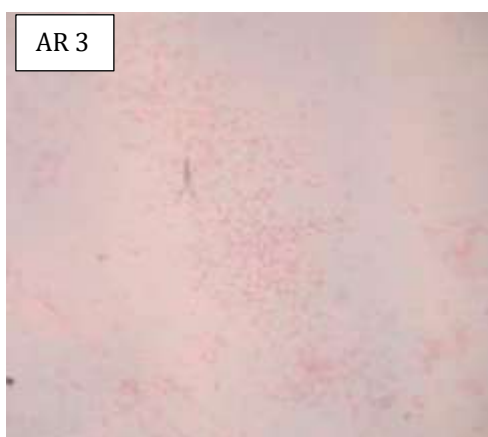
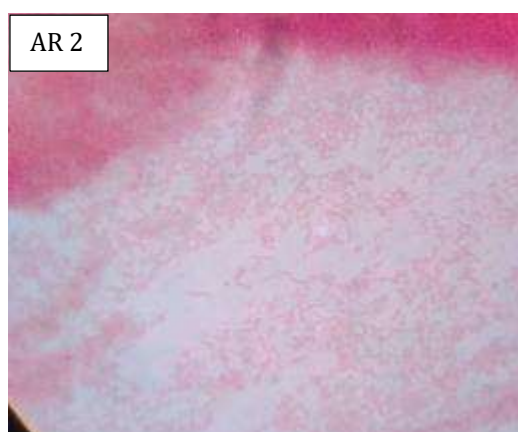
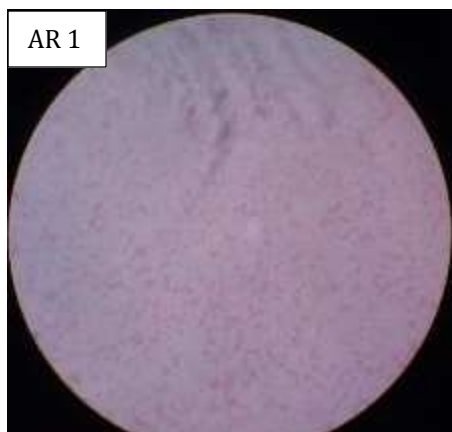
**Figure 3.** Bacterial isolates AR1 and AR2 isolated from roots

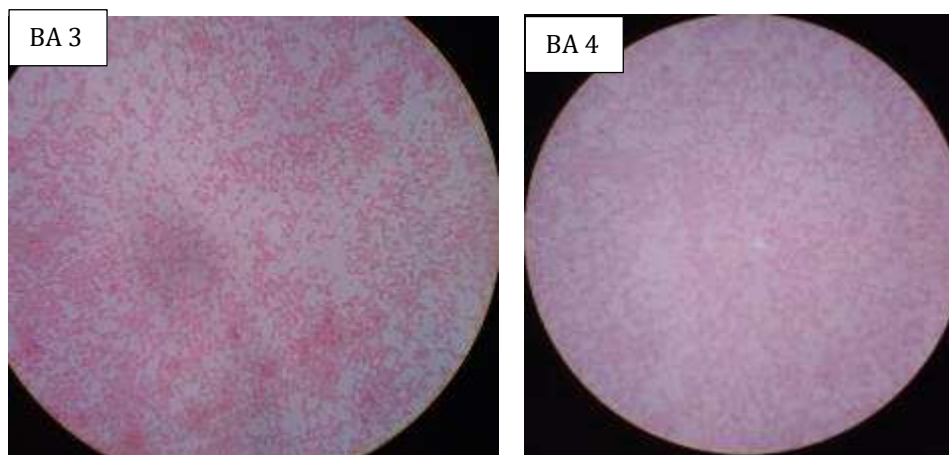


**Figure 4.** Isolate BA1, BA2, BA3 and BA4 isolated from fruit samples

### **Gram Staining**

Gram staining was performed on eight bacterial isolates from roots and fruit. Based on the results of gram staining, it was found that all bacterial isolates were gram-positive bacteria marked by the presence of a pink color on the bacteria (Figure 5).





**Figure 5.** Gram stain endophytic bacteria

### 3.2. Discussion

Endophytic bacteria are defined as bacteria that spread to healthy plant tissues without causing apparent symptoms or causing adverse effects on the host plant. The endophytic bacteria spread over many plants but are asymptomatic and undetectable and can only be detected by isolating them. Endophytic bacteria actively spread in plant tissues, form and associate long-term, lifelong natural associations, and are generally not specific to any particular plant organ. Thus, endophytic bacteria can be isolated from roots, leaves, and stems, and some from inflorescences and fruits.

Therefore, endophytic bacteria are microbes that live in association with plants, forming asymptomatic biotrophic associations between cells. Endophytic bacteria differ from bacteria temporarily associated by chance, which do not last long. Endophytic bacteria are associated with plants as biotrophic symbionts, and these bacteria can be obligate or facultative, encompassing thousands of host plants, although some endophytes are restricted to plant families. Some bacteria are associated only with grasses, whereas others are symbiotic with various plant species, including monocots and dicots. Most studies indicate that infection occurs through breaks, weeds, or wounds on the roots of seedlings either during germination or afterward (Dobereiner & Pedrosa, 1987; Patriquin, Dobereiner, & Jain, 1983; Patriquin & Dobereiner, 1978; Chaintreuil, Giruad, Prin, Lorquin, Gillis, De Lajudie, Dreyfus, 2001; Bacon & Hinton, 2002). Airborne infection can occur through stomatal cells and other natural infections through pores, but this also does not represent a natural route of infection by endophytic bacteria. Based on McCully (2001), undamaged roots do not allow indiscriminate entry of microbes.

Endophytic bacteria have been found in every plant species ever studied. Thus, it is infrequent to find plants without endophytic bacteria (Partida-Martinez and Heil, 2011). In fact, plants without endophytic bacteria are more susceptible to phytopathogenic infections and more vulnerable to stress conditions (Timmusk et al.,

2011). The type of diversity of endophytic bacteria found in plants can depend on several factors, as shown in Fig. 6.

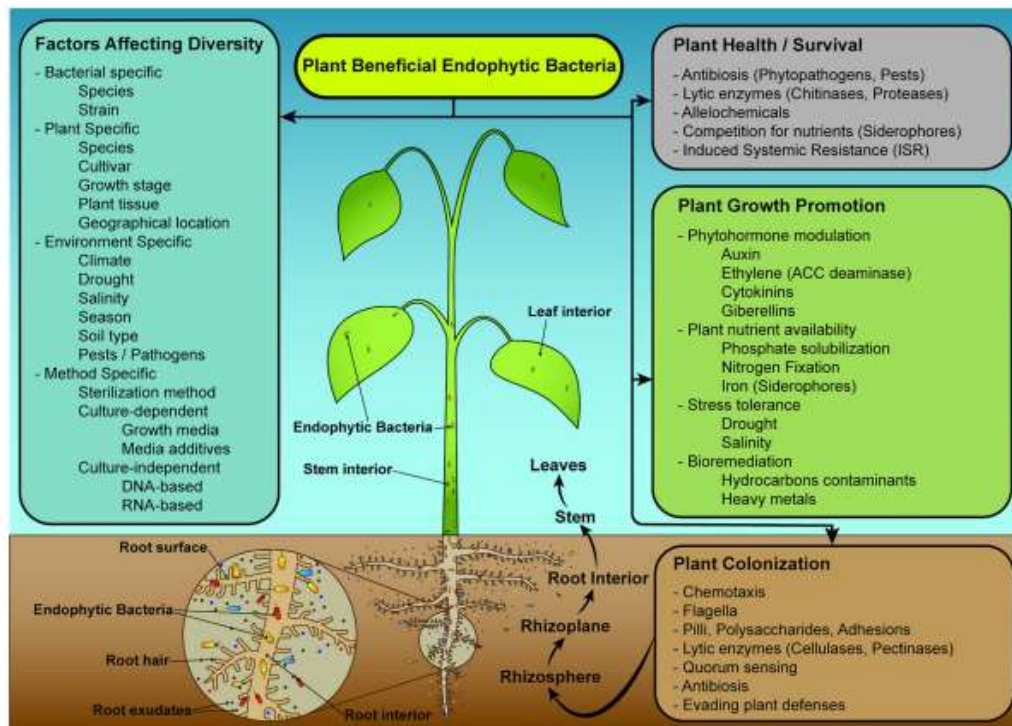


Figure 6. Factors affecting the diversity of endophytic bacteria in plants

## Conclusion

Endophytic bacteria is one of the bacteria that is relatively difficult to isolate, each plant has its level of difficulty, both in providing bacterial growth media and isolation techniques. Based on the results, it can be concluded that: 8 bacterial isolates were successfully isolated from samples of robusta coffee roots and pods, and AR4 bacterial isolates had an antagonistic effect on *S aureus* bacteria Tetracycline resistance.

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