Searching for indigenous rhizobacteria from Solok Radjo coffee orchard at Aie Dingin, West Sumatera

I Chaniago¹*, Z Resti², R Yunita¹, M N Harefa¹, S R A Siregar¹

¹Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas, Padang 25163, Indonesia
²Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Padang 25163, Indonesia

Email: irawati@agr.unand.ac.id

Abstract. The coffee plant has been cultivated for a hundred years and is known for its use as a beverage that is widely accepted around the globe. Most of the coffee orchard in Indonesia has been managed conventionally and need to be improved to get a better quality of growth and yield. This experiment was conducted from July to November 2020 and aimed at identifying rhizobacteria associated with coffee plant rhizosphere that later can be applied to stimulate the early growth of coffee seedlings. The soil sample was collected from the Solok Radjo coffee orchard [var. Sigagar Utang] of Nagari Aie Dingin, Alahan Panjang, Municipality of Solok, the Province of West Sumatera. Four different conditions of coffee orchard were chosen for this experiment according to plant ages and soil fertility. They were 2-year-old and 6-year-old coffee plants with health and less healthy plant growth. One gram of soil was mixed with distilled water and was diluted prior to being cultured in Nutrient Agar media for 48 hours at ambient temperature. Bacterial colonies went through series of re-culture until pure isolate was obtained and were observed for their morphological and physiological characters. Most of the isolates responded negatively to a hypersensitive reaction test that brings about their potential to promote the growth of coffee plants. In contrast, phytotoxic activity was observed in 2 isolates of rhizobacteria collected from the 6-year-old coffee orchard with less healthy plant growth. This experiment found 46 isolates of rhizobacteria indigenous to coffee orchard var. Sigagar Utang. Future research needs to be directed to investigate the physiological characters of the rhizobacteria before application to the coffee plants.

Key words: coffee, rhizobacteria, Solok Radjo, morphological characters, phytotoxicity

1. Introduction

Coffee [Coffea spp.] plants are shrubs or small trees native to tropical and southern Africa and tropical Asia. They belong to the family Rubiaceae and genus Coffea and the product is used to flavor various beverages and other products. The coffee drink has been consumed by many people around the world. Coffee plants may grow in wide areas with various elevations. There are two major types of coffee plants grown in Indonesia. Coffee Robusta prefers low to medium elevation, meanwhile, coffee Arabica grows very well at high altitude.

Coffee orchards in Indonesia are mainly owned by the small-scale farming business [55%] with a high plant density of 2000-2500 plants per hectare. Most coffee orchard [45%] is located at areas with an elevation of 500-900 m above sea level. Some are found at a lower altitude of 500 m and some are grown at an elevation higher than 900 m above sea level; 28 and 27% respectively [1].
The yield of coffee in Indonesia reaches approximately 50-60% of its potential that is resulted from the use of low-yielded coffee varieties. Many farmers do not focus on their farming business. In other words, once the coffee seedlings are planted, farmers would seek other ways to make money while waiting to harvest the coffee beans. Good agricultural practices to grow coffee plants are not appropriately applied. Yet another problem is the delay in re-planting as some farmers do not want to root out their coffee plants with coffee beans attached to the trees [3]. Many farmers do not apply enough fertilizer due to the high price. Moreover, pest and disease management is very limited which may result in low quality of produce [1].

Good quality of coffee seedlings determines healthy growth and yield if followed by good agricultural practices in the field. Various approaches are needed such as choosing mother plants that are healthy and high yielding, resistant to pest and disease, and well adapted to the environment. Nature has provided plants with the ability to defend themselves from herbivores and predators through mechanical defense strategies such as a thorn, hairs on the leaf surface, thick bark and thick root epidermal layer, or through physiological defense such as the production of secondary metabolite.

The rhizosphere, as part of abiotic environmental factors, is a place where plant roots get access to soil nutrients and at the same time receive root exudates. Rhizosphere provides growth media to microorganisms living symbiotically with crops [Dosselaere et al., 1997]. A group of microorganisms living in association with plant roots is known as rhizobacteria and helps the plants to adapt to the environment where mineral nutrition is limited [7]. The aforementioned microorganism is known as plant growth-promoting rhizobacteria [PGPR] and accesses their nutrition from exudates of plant roots they're associated with [8]. They may protect plants against soil-born disease and increase the diversity of indigenous microorganisms [4-9].

Rhizobacteria have been reported for the ability to improve plant growth and vigor through increasing Fe and Se uptake via roots in Arabidopsis thaliana [Wang et al., 2017], improved maize resistance to drought [Curá et al., 2017], induced plant tolerant to salinity stress [He et al., 2018], and induced plant immune system through interference with auxin production [11]. However, research on the growth improvement of coffee seedlings with indigenous rhizobacteria has not much been reported. Yet, rhizobacteria may be used to improve the growth of coffee seedlings if they can stimulate the production of auxin and gibberellin.

The exploration of rhizobacteria beneficial to plants will open the window to improve plant wellness in an environmentally friendly way. The research reported here aimed at exploring and identifying rhizobacteria indigenous to the coffee orchard at Aie Dingin, Alahan Panjang, the Province of West Sumatera. The isolate of rhizobacteria will be used for later work to improve the growth and wellness of coffee seedlings.

2. Material and Method
The soil sample was collected from the coffee orchard [var. Sigagar Utang] of Nagari Aie Dingin, Alahan Panjang, Municipality of Solok, the Province of West Sumatera. Four different orchard conditions were chosen i.e 6-year-old and 2-year-old coffee plants grown at fertile and less fertile land. The isolation and characterization of rhizobacteria were conducted at the Laboratory of Microbiology of Faculty of Agriculture, Andalas University Padang from July to November 2020. The soil was taken from 2-5 cm depth around the projection of the shoot canopy on the land.

Debris such as roots and twigs were removed from the soil. One g of soil was added to 10 mL of sterile distilled and vortexed for 3-5 minutes. This suspension was then marked as a 10² solution. One mL of the [10³] soil suspension was diluted with 9 mL of sterile distilled water then vortexed. The dilution process was repeated to get a 10⁶ solution. Then, 100 μL final soil suspension was poured into a test tube containing liquid NA medium before thoroughly mixed in a vortex. The mixture was then poured into a Petri dish, sealed, and incubated for 48 hours at ambient temperature.

The bacterial colony formed a transparent zone of the bacterial isolate. The isolate was then recultured onto fresh media several times until pure isolate was obtained. The pure isolates were then observed and identified for their morphological characters [color, shape, colony, colony surface, and
shape of colony edge]. The selected colony was then purified in streak plate using loop one and was incubated for 48 hours at ambient temperature. A single colony of bacteria was then aseptically moved into a microtube containing 1 mL of sterile distilled water for preservation purposes. The tubes were kept in a refrigerator for later use.

Gram test for bacteria was conducted to separate the rhizobacteria either Gram-positive or Gram-negative. Another bacterial character tested was the hypersensitive reaction [HR-pathogenesis] using leaves of four o’clock flower [Mirabilis jalapa] following Fahy and Persley [1983] with some modification. The isolate is pathogenic when causing necrotic on the leave of Mirabilis jalapa 24 hours after exposure. Data were not statistically analyzed as this research was more on exploration and observation of the morphological and physiological characters of the rhizobacteria.

3. Results and Discussion

Isolation and characterization of soil samples collected from the coffee orchard of coffee Arabica var. Sagagar Utang of Solok Radjo at Aie Dingin, Municipality Solok, the Province of West Sumatera found 46 isolates of indigenous rhizobacteria. Morphological and some physiological characters of the rhizobacteria are presented in Tables 1 to 4.

Table 1. Morphological and physiological characters of isolates of indigenous rhizobacteria from Solok Radjo coffee orchard of Coffee Arabica var. Sagagar Utang [6 years of age with healthy plant growth].

| No | Isolate code | Dilution | Shape | Elevation | Margin | Size [cm] | Number of colonies | Color | Gram | HR |
|----|--------------|----------|-------|-----------|--------|-----------|--------------------|-------|------|-----|
| 1  | L1 S1.1      | 10 [-5]  | Circular | Raised | Entire | 2.0       | 11                 | White | [+  ]| [-  ]|
| 2  | L1 S1.2      | 10 [-6]  | Irregular | Flat | Undulate | 1.8       | 19                 | White | [+  ]| [-  ]|
| 3  | L1 S1.3      | 10 [-5]  | Filamentous | Flat | Filamentous | 2.6       | 1                 | White | [+  ]| [-  ]|
| 4  | L1 S2.1      | 10 [-5]  | Circular | Raised | Entire | 0.6       | 6                 | Ivory  | [+  ]| [-  ]|
| 5  | L1 S2.2      | 10 [-5]  | Irregular | Flat | Undulate | 1.6       | 8                 | White  | [+  ]| [-  ]|
| 6  | L1 S2.3      | 10 [-5]  | Irregular | Umbonate | Lobate | 1.9       | 1                 | White  | [-  ]| [-  ]|
| 7  | L1 S3.1      | 10 [-6]  | Circular | Convex | Entire | 0.5       | 98                | White  | [+  ]| [-  ]|
| 8  | L1 S3.2      | 10 [-5]  | Irregular | Raised | Undulate | 0.7       | 30                | White  | [+  ]| [-  ]|
| 9  | L1 S3.3      | 10 [-6]  | Rhizoid | Flat | Filamentous | 2.0       | 2                 | White  | [-  ]| [-  ]|
| 10 | L1 S4.1      | 10 [-6]  | Circular | Flat | Entire | 0.5       | 130               | White  | [+  ]| [-  ]|
| 11 | L1 S4.2      | 10 [-6]  | Irregular | Raised | Undulate | 1.0       | 24                | White  | [-  ]| [-  ]|
| 12 | L1 S5.1      | 10 [-6]  | Circular | Raised | Entire | 0.4       | 7                 | Ivory  | [+  ]| [-  ]|
| 13 | L1 S5.2      | 10 [-5]  | Irregular | Raised | Undulate | 1.0       | 5                 | White  | [+  ]| [-  ]|

Total number of colony = 342 colonies, HR = hypersensitive reaction test.
Table 2. Morphological and physiological characters of isolates of indigenous rhizobacteria from Solok Radjo coffee orchard of Coffee Arabica var. Sigagar Utang [6 years of age with less healthy plant growth].

| No | Isolate code | Dilution | Shape | Elevation | Margin | Size [cm] | Number of colonies | Color | Gram | HR |
|----|--------------|----------|-------|-----------|--------|-----------|-------------------|-------|------|----|
| 1  | L3 S1.1      | 10 [-5]  | Circular | Convex  | Entire | 0.4       | 5                 | White | [-]  | [-]|
| 2  | L3 S1.2      | 10 [-6]  | Irregular | Flat   | Undulate | 1.4      | 17                | White | [+]  | [-]|
| 3  | L3 S1.3      | 10 [-5]  | Rhizoid | Flat    | Filamentous | 3.5       | 2                 | White | [+ ]| [-]|
| 4  | L3 S1.4      | 10 [-5]  | Irregular | Flat   | Lobate | 1.2       | 1                 | White | [+]  | [-]|
| 5  | L3 S1.5      | 10 [-5]  | Circular | Raised | Entire | 0.1       | 1                 | Yellow | [-]  | [-]|
| 6  | L3 S2.1      | 10 [-6]  | Circular | Convex | Entire | 0.3       | 1                 | White | [+ ]| [-]|
| 7  | L3 S2.2      | 10 [-5]  | Irregular | Flat   | Lobate | 1.6       | 6                 | Ivory | [-]  | [-]|
| 8  | L3 S2.3      | 10 [-5]  | Irregular | Convex | Undulate | 1.1      | 2                 | White | [+ ]| [-]|
| 9* | L3 S3.1      | 10 [-5]  | Irregular | Convex | Entire | 0.4       | 3                 | White | [-]  | [+ ]|
| 10 | L3 S3.2      | 10 [-5]  | Circular | Flat   | Lobate | 2.0       | 1                 | White | [+ ]| [-]|
| 11 | L3 S3.3      | 10 [-6]  | Circular | Convex | Entire | 0.3       | 118               | Ivory | [+ ]| [-]|
| 12 | L3 S4.1      | 10 [-6]  | Circular | Convex | Entire | 0.5       | 9                 | Ivory | [+ ]| [-]|
| 13 | L3 S4.2      | 10 [-5]  | Irregular | Flat   | Undulate | 1.1      | 16                | White | [+ ]| [-]|
| 14 | L3 S4.3      | 10 [-6]  | Rhizoid  | Flat    | Filamentous | 4.0       | 1                 | White | [+ ]| [-]|
| 15 | L3 S5.1      | 10 [-6]  | Irregular | Convex | Entire | 0.6       | 8                 | White | [+ ]| [-]|
| 16 | L3 S5.2      | 10 [-5]  | Circular | Flat   | Lobate | 1.0       | 31                | White | [+ ]| [-]|
| 17*| L3 S5.3      | 10 [-6]  | Rhizoid  | Flat    | Filamentous | 1.8       | 4                 | Ivory | [+ ]| [-]|

Total number of colony = 223 colonies, HR = hypersensitive reaction test. *isolate with positive hypersensitive reaction with phytotoxic properties

Table 3. Morphological and physiological characters of isolates of indigenous rhizobacteria from Solok Radjo coffee orchard of Coffee Arabica var. Sigagar Utang [2 years of age with healthy plant growth].

| No | Isolate code | Dilution | Shape | Elevation | Margin | Size [cm] | Number of colonies | Color | Gram | HR |
|----|--------------|----------|-------|-----------|--------|-----------|-------------------|-------|------|----|
| 1  | L2 S1.1      | 10 [-5]  | Circular | Raised | Entire | 0.4       | 2                 | White | [+]  | [-]|
| 2  | L2 S1.2      | 10 [-6]  | Irregular | Flat   | Undulate | 1.0      | 49                | White | [+ ]| [-]|
| 3  | L2 S1.3      | 10 [-5]  | Rhizoid | Flat    | Filamentous | 2.0       | 2                 | White | [+ ]| [-]|
| 4  | L2 S1.4      | 10 [-5]  | Filamentous | Flat   | Filamentous | 1.5       | 1                 | White | [+ ]| [-]|
| 5  | L2 S2.1      | 10 [-6]  | Irregular | Flat   | Entire | 0.3       | 168               | White | [+ ]| [-]|
| 6  | L2 S2.2      | 10 [-6]  | Irregular | Flat   | Undulate | 0.7       | 120               | White | [+ ]| [-]|
| 7  | L2 S2.3      | 10 [-5]  | Filamentous | Convex | Lobate | 3.0       | 4                 | White | [-]  | [-]|
| 8  | L2 S3.1      | 10 [-5]  | Circular | Flat   | Entire | 0.4       | 52                | White | [+ ]| [-]|
| 9  | L2 S3.2      | 10 [-5]  | Irregular | Flat   | Lobate | 1.0       | 61                | White | [+ ]| [-]|
| 10 | L2 S4.1      | 10 [-5]  | Circular | Raised | Entire | 0.3       | 10                | White | [+ ]| [-]|
| 11 | L2 S4.2      | 10 [-5]  | Irregular | Flat   | Undulate | 1.0      | 13                | White | [+ ]| [-]|
| 12 | L2 S4.3      | 10 [-5]  | Rhizoid | Flat    | Filamentous | 2.0       | 1                 | White | [+ ]| [-]|
| 13 | L2 S5.1      | 10 [-6]  | Circular | Flat   | Entire | 0.3       | 7                 | White | [+ ]| [-]|
| 14 | L2 S5.2      | 10 [-6]  | Irregular | Flat   | Undulate | 1.5       | 17                | White | [+ ]| [-]|

Total number of colony = 507 colonies, HR = hypersensitive reaction test
**Table 4.** Morphological and physiological characters of isolates of indigenous rhizobacteria from Solok Radjo coffee orchard of Coffee Arabica var. Sigagar Utang [2 years of age with less healthy plant growth]

| No | Isolate code | Dilution | Shape    | Elevation | Margin | Size [cm] | Number of colonies | Color | Gram HR |
|----|--------------|----------|----------|-----------|--------|-----------|-------------------|-------|---------|
| 1  | L4 S1.1      | 10 [-5]  | Circular | Flat      | Entire | 0.4       | 2                 | White | [+|-]   |
| 2  | L4 S1.2      | 10 [-5]  | Irregular| Flat      | Lobate | 1.6       | 2                 | White | [+|-]   |

Total number of colony = 4 colonies, HR = hypersensitive reaction test

Morphological characters of isolates of rhizobacteria varied in shape such as circular, irregular, filamentous, and rhizoid. Most colonies are circular dan irregular in shape. The number of colonies ranged from 1 to 168 and was dominated by white color, whilst only 6 colonies were ivory and 1 colony was yellow. The size of rhizobacterial colonies varied from 0.1 cm to 4.1 cm. Isolates with various physiological characters may be used to improve the growth of coffee plants to whom the rhizobacterial be associated with. Future research will be directed to evaluate various physiological characters of the isolate and be applied to determine whether they can stimulate the growth of coffee plants.

This research found 2 isolates with phytotoxic properties from the 6-year-old coffee orchard with a less healthy growth condition. This result was revealed from hypersensitive reaction [HR] with Mirabilis jalapa plants as target species. The sign of necrotic in the leaves of Mirabilis jalapa was observed at 48 hours after exposure to the isolate. As for their phytotoxic effect, these two isolates cannot be used for the experiment. They are isolate L3 S3.1 and L3 S5.3. Photographs of the different colony of of rhizobacteria isolated from the soil of Solok Radjo coffee orchard are presented in the following figures:

**Figure 1.** Rhizobacteria isolated from soil of 6-year-old healthy coffee plants, shape: rhizoid, margin: filamentous, color: white

**Figure 2.** Rhizobacteria isolated from soil of 6-year-old healthy coffee plants, shape: irregular, margin: undulate, color: white

**Figure 3.** Rhizobacteria isolated from soil of 6-year-old less healthy coffee plants, shape: circular, margin: entire, color: yellow

**Figure 4.** Rhizobacteria isolated from soil of 6-year-old less healthy coffee plants, shape: irregular, margin: undulate, color: ivory
4. Conclusion
Various isolates of rhizobacteria found in association with the rhizosphere of Solok Radjo coffee orchard were identified with various morphological characters. Most of the isolates responded negatively to the hypersensitive reaction test that brings about their potential to promote the growth of coffee plants. In contrast, phytotoxic activity was observed in 2 isolates of rhizobacteria collected from a coffee orchard of 6-year-old with less healthy plant growth. Future research needs to be directed to investigate the physiological characters of the rhizobacteria before application to the coffee plants.

5. Acknowledgement
We are very grateful for the research grant provided by the Faculty of Agriculture through grant number 01/PL/SPK/PNP/FAPERTA-Unand, dated 14 May 2020. We would like to express our gratitude to the Dean of Faculty of Agriculture, Universitas Andalas, Padang for providing us with the opportunity to conduct this research.

References
[1] Byrareddy, V., Kouadio, L., Mushtaq, S., and Stone, R. 2019. Sustainable Production of Robusta Coffee under a Changing Climate: A 10-Year Monitoring of Fertilizer Management in Coffee Farms in Vietnam and Indonesia Agronomy 9 499;
[2] Curá, J. A., Franz, D. R., Filosofia, J. E., Balestrasse, K. B., and Burgueño, L. E. 2017 Inoculation with Azospirillum sp. and Herbaspirillum sp. bacteria increases the tolerance of maize to drought stress Microorganisms 5 41
[3] Direktorat Jenderal Perkebunan 2014 Statistik Perkebunan Indonesia 2013-2015 December Jakarta
[4] Driouich, A., Follet-Gueye, M. L., Vicré-Gibouin, M., Hawes, M. 2013 Root border cells and secretions as critical elements in plant host defense Curr. Opin. Plant Biol. 16, 489–495
[5] Fahy, P. C., and Persley, G. J. 1983 Plant bacterial diseases: a diagnostic guide Academic Press Australia 393 pp
[6] He, Ao-Wei, Shu-Qi Niu, Qi Zhao, Yong-Sheng Li, Jing-Yi Gou, Hui-Juan Gao, Sheng-Zhou Suo, and Jin-Lin Zhang 2018 Induced salt tolerance of perennial Ryegrass by a novel bacterium strain from the rhizosphere of a desert shrub Haloxylon ammodendron Int. J. Mol. Sci. 19 469
[7] Kloepper, J. W., Rodriguez-Kabana, R., Zehnder, G. W., Murphy, J., Sikora, E., and Fernandez, C 1999, Plant root-bacterial interactions in biological control of soil-borne diseases and potential extension to systemic and foliar diseases Aust. J. Plant Pathol. 28 27–33
[8] Ryan, P., Delhaize, E., and Jones, D. 2001, Function and mechanism of organic anion exudation from plant roots Annu. Rev. Plant Phys. 52 527–560
[9] Timmusk, S. 2003 Mechanism of Actions of The Plant-Growth-Promoting Rhizo Bacterium Paenibacillus polymyxin [Dissertation] Uppsala Sweden Departement of Cell and Molecular Biology Uppsala University
[10] Wang, Jianfei, Cheng Zhou, Xin Xiao, Yue Xie, Lin Zhu, and Zhongyou Ma 2017 Enhanced Iron and Selenium Uptake in Plants by Volatile Emissions of Bacillus amyloliquefaciens [BF06] Appl. Sci. 7 85
[11] Zhou, Cheng, Lin Zhu, Zhongyou Ma, and Jianfei Wang 2017 Bacillus amyloliquefaciens SAY09 Increases Cadmium Resistance in Plants by Activation of Auxin-Mediated Signaling Pathways Genes 8 173