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# Artikel 8

## Molecular Identification of Potential Rhizobacteria Isolated from Maize (Zea mays L.)

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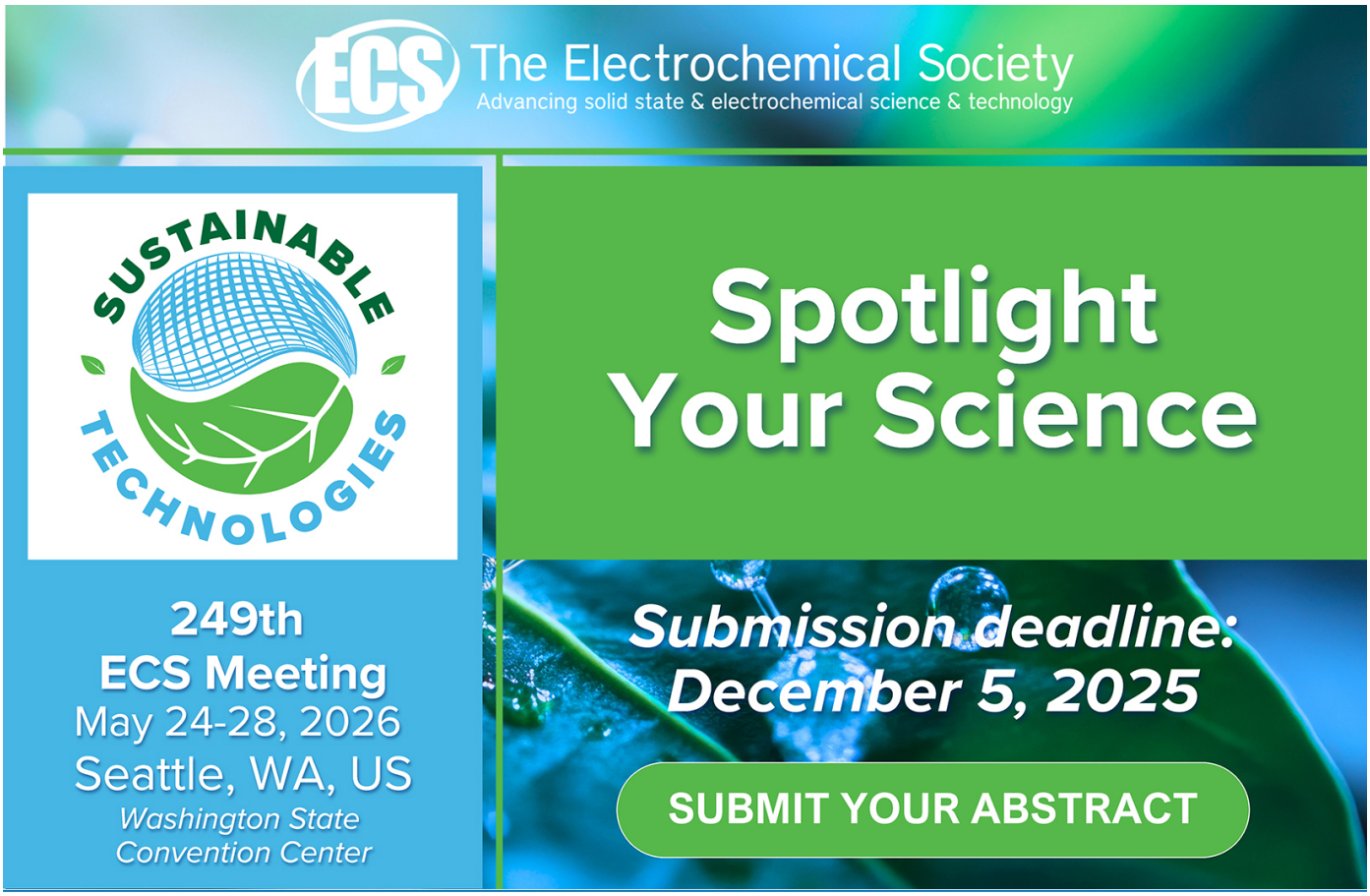
# Molecular Identification of Potential Rhizobacteria Isolated from Maize (*Zea mays* L.)





# Molecular Identification of Potential Rhizobacteria Isolated from Maize (*Zea mays* L.)

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**Abstract**. Maize (*Zea mays* L.) is one of the highly demanding food plants in Indonesia but the production is currently not sufficient to meet the community's needs, so that efforts to import maize in Indonesia are still frequently carried out. Meanwhile, the maize production who mostly produced by local farmer mainly cultivated on marginal land which is threatened by drought stress. Efforts should be make to increase maize productivity on dry land by utilization of rhizobacteria isolated from maize rhizosphere. This study aimed to determine the types of rhizobacteria from the diversity of microbes that potentially increase the productivity of maize plants. This study used 2 isolation methods, namely isolation on gram-positive and gram-negative, followed by electrophoresis and PCR with primers of 27F and 1492R. Then the PCR results were sequenced and analyzed using Mega X. The sequencing results compared with Genbank at NCBI showed 10 isolates that have been shown to have closeness to several strains of bacteria, such as *Raoultella terrigena, Serratia marcescens, Serratia nematodiphila, Enterobacter hormaechei, Enterobacter cancerogenous, Enterobacter cloacae, Enterobacter asburiae, Citrobacter murliniae, Pseudomonas fluorescens.* depicted in phylogenetic analysis. Based on the phylogenetic analysis, the L5S2 $10^{-7}$ isolate has similarities with *the Enterobacter asburiae* strain, while the L2S1 $10^{-6}$ isolate is closely related to the *Enterobacter cancergenous* strain, Then the *Citrobacter murliniae* strains were closely related to L5S5 $10^{-8}$ and L1S3 $10^{-7}$ isolates. In addition, the *Enterobacter hormaechei* strain also has a close relationship with isolate L3S1 $10^{-8}$, and the *Raoultella terrigena* strain has a close relationship with isolate L5S2 $10^{-6}$. The L1S5 $10^{-6}$ and L3S5 $10^{-7}$ isolates had the same similarity based on genetic characters but had different abilities in helping the performance of plants belonging to the rhizobacteria group.

**Keywords**: Molecular, identification, phylogenetic, rhizobacteria

## 1. Introduction

Maize (*Zea mays* L.) is one of the food crops needed by the people in Indonesia and can be regarded as the second staple food after rice. Currently, maize production in Indonesia has not been able to meet the community's needs, so imports in Indonesia are still often carried out. Meanwhile, there are fewer maize farmers because the availability of land for farming is getting lower. According to Hipi et al.

[1], in 2011, Indonesia can meet domestic needs by importing 3.2 tons of maize. This import activity is carried out to increase production using quality hybrid seeds [2].

The problems that often occur in several regions in Indonesia are drought stress, which is very influential on the agricultural sector. According to Badami and Amzeri [3], drought stress is usually caused by biotic and abiotic factors. One of the causes of this frequent drought stress is the minimal N content in the soil so that plants find it difficult to meet the required nutrients. One of the efforts to overcome these problems is to create biological products used for maize plants on dry land. As stated Efendi et al. [4], a molecular approach can be used as an effort to obtain candidate inoculums that will be used in creating biofertilizers for drought-tolerant maize plants.

Efforts taken to increase maize plants' productivity include expanding the cultivation area, especially by making good use of dry or marginal land in Indonesia. According to Puslitbang Tanah dan Agroklimat [5], the government's efforts to increase the productivity of maize cultivation can be on dry or marginal land, of which 52.4 million hectares of land are still available in Indonesia. According to Moelyohadi et al. [6], it is said that marginal dry land has a low level of soil fertility so that the production of maize on marginal land can result in low productivity. So that there is a need for other efforts that can support increasing the productivity of maize on marginal land.

The next effort in cultivating maize plants on land with high drought stress could be utilizing rhizobacteria in the rhizosphere area of maize plants. Plant roots are the parts of plants that are known to contain the most microorganisms. According to Hartono and Jumadi [7], the highest population of microorganisms in plant roots is due to amino acids in the area, which is a source of nitrogen and carbon needed for the growth of plant root microorganisms. This research was carried out using molecular identification. Previous studies said that using only the characterization was not enough because they did not know the macroscopic morphology [8]. In the study, it was also that molecular analysis is said to find out information and genetic diversity in bacteria so that the morphological and biochemical characterization of bacteria can be identified and can support the classification and utilization of the bacterial isolates obtained.

## 2. Materials and Methods

This research was conducted by observing the identification and molecular characterization of rhizobacteria isolates. The procedures were bacteria gram staining, DNA isolation, electrophoresis, DNA amplification using PCR, and data analysis.

### 2.1. Bacteria gram staining

Identification using gram staining is generally done to distinguish the type of bacteria from gram positive or gram negative. As stated by Nuraini et al. [9] that this gram test is carried out macroscopically with the help of a microscope, and it can be seen that bacteria that are gram-positive will look purple while gram-negative bacteria will look red. According to Delvia et al. [10] gram-positive bacteria are characterized by thick cell walls so that the spread of color from crystal violet absorbed in cells will persist even after washing.

### 2.2. DNA isolation

DNA isolation using a spin column Bacteria DNA Preparation kit (Jena Bio-science, Germany). Isolation of bacterial DNA begins with gram staining on bacterial isolates due to the procedural difference between gram-positive and negative bacteria.

### 2.3. Electrophoresis

Gel agarose was prepared from 2.25 g agarose gel, 15 ml TBE, and 150 ml aquades. The electrophoresis apparatus was set on 50V for 60 minutes. Then, remove the agarose gel and put it in the EtBr solution before being observed under gel documentator.

2.4. DNA amplification using PCR

After observing the documentation gel, the next step was PCR using primers 27F and 1492R. As for the steps, namely conducting primary dilution with the composition of $DH_2O$ 382l and Primer 12.5µl. Then the next step is to prepare a PCR tube, then put it into a PCR tube with a composition of $DH_2O$ 3.52l, PCR Mix 12.5µl, Forward primer 1.5µl, Reverse primer 1.5µl, DNA 3µl. Then the PCR tube was inserted into the PCR machine. Then the PCR was set with pre-denaturation 95ºC for 3 minutes, Denaturation 95ºC for 1 minute, Annealing 55ºC for 1 minute, 72ºC elongation for 1 minute (30 cycles). Post Elongation 72ºC for 7 minutes. The PCR result was observed using gel documentator after the electrophoresis process. The 3µl of PCR marker was inserted at the beginning of mold marker. The electrophoresis apparatus was set on 35V for 90 minutes.

2.5. Data Analysis

Analysis of the data used is by using phylogenetic analysis. Making analysis with phylogenetic tree by using MEGA software. The isolate sequences to be analyzed were compared with bacterial sequences that had the highest percentage of homology and were also compared with sequences of other types of bacteria. The phylogenetic tree was constructed using the Test Neighbor-joining tree and tested with the Bootstrap method [11].

## 3. Results

Based on phylogenetic analysis using 10 isolates that have been identified as having proximity to several bacterial strains such as *Raoultella terrigena, Serratia marcescens, Serratia nematodiphila, Enterobacter hormaechei, Enterobacter cancerogenous, Enterobacter cloacae, Enterobacter asburiae, Citrobacter murliniae, Pseudomonas fluorescens*. depicted in the phylogenetic analysis (Figure 1).

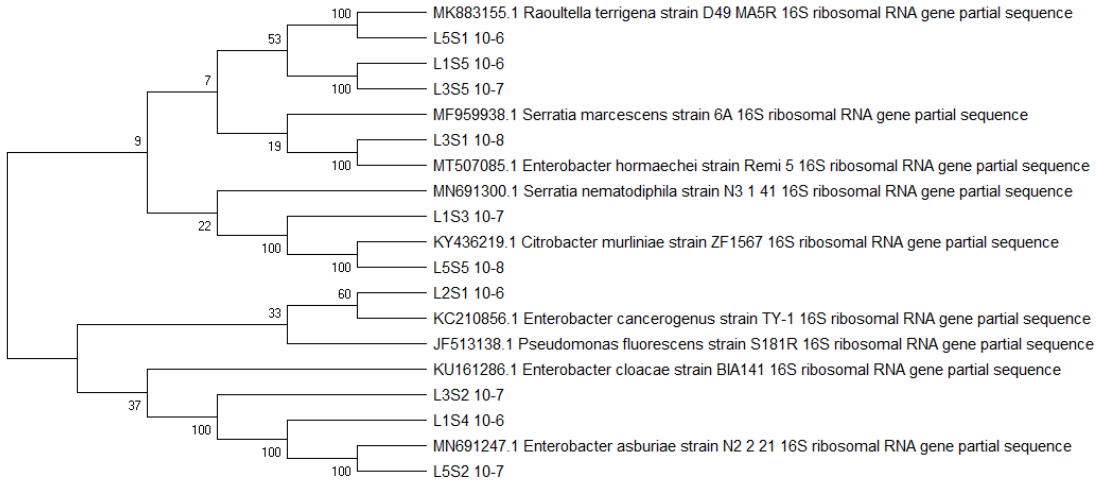


**Figure 1.** Phylogenetic analysis

Based on the phylogenetic analysis, isolates L5S2 10-7 had similarity with strains of *Enterobacter asburiae*, while isolates L2S1 10-6 had closeness with strains of *Enterobacter cancergenous*, then strains of *Citrobacter murliniae* had proximity to isolates L5S5 10-8 and L1S3 10-7. In addition, the *Enterobacter hormaechei* strain also has a close kinship with the L3S1 isolate 10-8, and the *Raoultella terrigena* strain has a close relationship with the L5S2 isolate 10-6. The isolates L1S5 10-6 and L3S5 10-7 have the same similarities based on genetic characters but have different abilities in helping the performance of plants belonging to the rhizobacteria group.

## 4. Discussion

Rhizobacteria can be found in the plant rhizosphere, a thin layer of soil that covers the root surface and positively influences plant growth. Several genera of rhizobacteria are reported as PGPR, namely *Pseudomonas, Enterobacter, Azospirilum, Azotobacter, Burkholderia, Bacillus, and Serratia* [12]. Based on the phylogenetic analysis of the characteristics of the bacterium *Raoultella terrigena* Schoebitz et al. [13] stated that this bacterium belongs to the rhizobacteria class that can provide plant protection against drought stress. In addition, *Serratia marcescens* is also one of the organisms that can also produce chitinase enzymes and is one of the most effective bacteria to degrade chitin [14]. As it is known that the cell wall structure of fungi is composed of chitin, thus chitinase from *Serratia marcescens* can be a biopesticide to control plant-disturbing organisms caused by fungi [15].

*Enterobacter* sp., which belongs to the rhizobacteria group and comes from the Enterobacteriaceae family, produces protease enzymes with proteolytic activity. Aeron et al. [16] revealed that Enterobacter sp. produces commercially important enzymes such as amylase, protease, gelatinase, lipase, deoxyribonuclease, phosphatase, and urease. In addition, bacteria belonging to the Enterobacteriaceae group that have proteolytic activity have the ability to produce protease enzymes that are secreted into their environment. This protease enzyme then works to hydrolyze protein compounds into oligopeptides, short-chain peptides, and amino acids.

In addition, the ability to stain Pseudomonas sp. This belongs to the rhizobacteria group, which can dissolve phosphate. The bacteria can be a fungal antagonist because it produces various antifungal antibiotic compounds such as phenazine compounds pyrolnitrin, pioluteorin, diacetyl phloroglucinol, and rhamnolipids [17]. Furthermore Schmidt et al. [18] proved that the rhizobacteria strain Pseudomonas fluorescens showed antagonistic activity against the fungus P. oryzae due to its ability to produce siderophores, protease enzymes, and chitinases. Some strains also produce hydrolytic enzymes, which may also play a role in direct antagonism. Biological agents can control plant pathogens because they have the ability to produce siderophores, hydrogen cyanide (HCN), antibiotic compounds, and enzymes and induce systemic resistance in plants [19].

## 5. Conclusion

10 isolates that have been identified as having proximity to several bacterial strains such as *Raoultella terrigena, Serratia marcescens, Serratia nematodiphila, Enterobacter hormaechei, Enterobacter cancerogenous, Enterobacter cloacae, Enterobacter asburiae, Citrobacter murliniae, Pseudomonas fluorescens*. The further research can be done based on this study to understanding the characteristic and potential use of the rhizobacteria isolates.

## Acknowledgement

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