

## Analysis of Rhizosphere Bacterial Community in Suppressive and Conducive Soils to Basal Rot of Garlic Based on PCR-RISA

<sup>a</sup> Zainal D. Fatawi\*,

<sup>a</sup> Hadiwiyono,

<sup>a</sup> Salim Widono

<sup>a</sup> Study Program of Agrotechnology, Faculty of Agriculture, the University of Sebelas Maret (UNS), Surakarta, Indonesia

### ARTICLE INFO

#### Article history:

Received 31 October 2012

Revised 10 November 2012

Accepted 14 November 2012

Available online 03 December 2012

#### Keywords:

PCR,

RISA,

basal rot,

garlic,

rhizosphere bacterium.

### ABSTRACT

Basal rot (*Fusarium oxysporum* f.sp. *cepae*) is an important disease of garlic in Tawangmangu Karanganyar Central Java Indonesia. Disease-suppressive could be found in some lands around disease-conducive lands in Tawangmangu. The role of rhizosphere bacterial community to induce disease-suppressiveness has been studied extensively on many soil born pathogen. Among the many groups of such organisms are root-associated bacteria, which generally represent a subset of soil bacteria. Ribosomal Intergenic Spacer Analysis (RISA) is a Polymerase Chain Reaction (PCR)-based method as an useful assessment tool for the diversity analysis of microbes in the environment. Currently, the diversity analysis of rhizosphere bacterial community of garlic planted on suppressive and conducive soil has been conducted. Conducive soil is the land where the disease incidence of basal rot of garlic at 1% or lower, whereas suppressive soil at 40% or higher. The diversity analysis was done through independent cultureable approach of PCR-RISA using universal primers of operon gene region of rRNA between small subunit (16S) and large subunit (23S) called intergenic spacer region (ISR). The diversity of rhizosphere bacteria from suppressive soil was different from that of conducive soil. The rhizosphere bacteria in suppressive soil was more diverse than those of conducive soil and the bacteria in healthy garlic rhizosphere was also more diverse than those in the diseased one.

### Introduction:

Basal rot (*Fusarium oxysporum* f.sp. *cepae*) is an important disease in Tawangmangu Karanganyar Central Java Indonesia. In the fields, the disease incidence can reach over 60%. However in some fields the disease incidence are very mild whereas in other fields the disease incidence are very severe<sup>7, 8</sup>. The first land is called suppressive soil that is the land with existing pathogen but the disease is not establish whereas the second land is called conducive soil, that is the land with developing pathogen and the disease establish well<sup>4, 11, 12, 30</sup>.

The most important mechanism of suppressive soil is the involvement of the agents of biological control<sup>4, 14, 11, 12, 30</sup>. Klopper et al.<sup>13</sup> reviewed that Plant-associated microorganisms have been extensively examined for their roles in natural and induced suppressiveness of soilborn diseases. Among the many groups of such organisms are root-associated bacteria, which generally represent a subset of soil bacteria. Rhizobacteria are a subset of total rhizosphere bacteria which have the capacity, upon re-introduction to seeds or vegetative plant parts (such as potato seed pieces), to colonise the developing root system in the presence of competing soil microflora. By which it reports the results of DNA finger printing of bacterial community from the garlic rhizosphere (healthy and diseased) growing on suppressive and conducive soil in Tawangmangu Karanganyar Central Java. By purposing to study the relationship of community structure of the rhizosphere bacterial microbes to the suppressiveness. Molecular technique being culturable independent was used by a reason

**Corresponding author:** Zainal D. Fatawi\*,

**E-mail address:** [zainaldf@yahoo.com](mailto:zainaldf@yahoo.com)

**Citation:** Zainal D. Fatawi\* (Analysis of Rhizosphere Bacterial Community in Suppressive and Conducive Soil to Basal Rot of Garlic Based on PCR-RISA) BIOMIRROR: 10-13/ bm- 251114212

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that at about 99% microbes are unculturable [20] so the conventional techniques being culture dependent are less representative. *Polymerase chain reaction - Ribosomal intergenic spacer analysis* (PCR-RISA) was used for this study. PCR-RISA has been used by previous researches for determining bacterial community from rhizosphere and bulk soil<sup>2, 3, 19</sup>. The tool has been proved as one of accurate molecular techniques being culturable independent in addition to Denaturing Gradient Gel Electrophoresis (DGGE)<sup>15, 19, 24</sup>, Terminal Restriction Fragment Length Polymorphism (T-RFLP)<sup>1, 26</sup>, and PCR-Single Strand Conformism Polymorphism (PCR-SSCP)<sup>19, 21, 22, 23</sup>.

## Materials and Methods:

Purposive sampling method was used for determining rhizosphere soil of garlic sample at Tawangmangu Karanganyar Central Java. The criteria of suppressive soil is the land area of garlic in which the disease incidence of basal rot at 1% or lower, where as conducive soil at 40% or higher, at generative stage of garlic planting, no symptom caused by other diseases or pests, and cultivar Tawangmangu Baru. Each area was sampled 10 plants and each criteria soil (suppressive and conducive) was sampled from 2 area. Bacterial community analysis was applied on composite samples from 10 plants of sample.

The extraction of BDB and rhizosphere bacteria was done using DNAMITE Kit. Intergenic Spacer Region (ISR) between gene of small-subunit (SSU) and large-subunit(LSU) rRNA were amplified in 25 µL PCR mix of final concentration, total amount 50 ng DNA of bacteria, 2.5 mL of 10mg mL<sup>-1</sup> BSA (Bovine Serum Albumin), 12.5 Mega Mix Royal (Microzone<sup>RM</sup>) and 1.25 µL of 100 pmol of each primer. The primers were S926f and L189r with running program of PCR-RISA as described by Yu and Mohn<sup>28</sup> by a little modification consisted of pre-denaturizing at 95°C for 2 minute, 30 cycles consisted of denaturizing at 94°C for 0,5 minutes, annealing at 47°C for 0,5 minutes, extension at 72°C for 5 minutes.

Electrophoresis of amplified DNA was done using 6% polyacilamid gel electrophoresis (PAGE), made from 5.30 mL of 30% polyacrilamid, 1 mL 10XTBE buffer, 2.70 mL dH<sub>2</sub>O, 5 µL Temmed, and 2 mL of 10% Ammonium Persulfate (APS). The electrophoresis was run at 50 V current for 135 minutes in 1xTBE buffer by using the machine of mini vertical electrophoresis (BioRad<sup>TM</sup>).

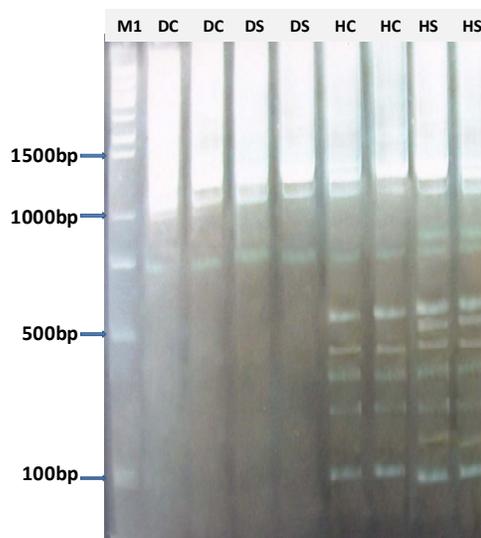
## Results and Discussion:

Generally the results were obvious that DNA finger printing using PCR-RISA generated the bacterial community structure of garlic rhizosphere from suppressive soil being different from those of conducive one. Bacterial community from suppressive soil were significantly more diverse than those of conducive soil, and the healthy garlic was significantly more diverse than the diseased garlic one (Figure 1).

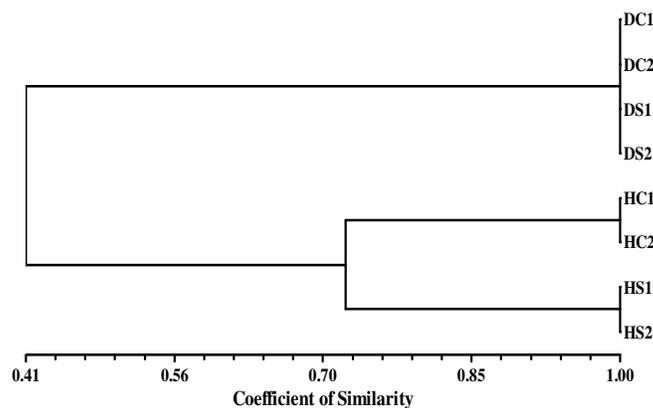
There are some fragments (8 bands) at a range of 100 bp to 1000 bp founded from suppressive soil. The fragments show thick and visualized conveniently. It means that population of the rhizosphere bacteria is abundant in those samples. Possibly, the bacteria having the fragment are involved in the exhibition of suppressiveness of the

rhizosphere soil. At list 8 fragments belong to some species of bacteria were founded in the suppressive soil, which was not found in the conducive soil. According to the result of clustering analysis based on similarity coefficient of the communities and UPGMA dendrogram in Figure 2, it is clear that the bacterial community structure of garlic rhizosphere from suppressive soil is different from conducive soil.

**Figure 1. The DNA fragment pattern of rhizosphere bacteria from conducive (C1, C2, C3) and suppressive (S1, S2, S3) soil to basal rot of garlic generated by PCR-RISA with the primer of S926f and L189r**



**Figure2: UPGMA Dendrogram of clustering analysis based on coefficient similarities of the rhizosphere bacterial community of garlic planted on conducive soil (C), suppressive soil (S), D(diseased garlic), and H (healthy garlic) of basal rot of garlic generated by PCR-RISA with the primer of S926f and L189r**



As a tool to study on the bacterial community analysis of rhizosphere bacteria of garlic, PCR-RISA is a discriminative tool. It can be interpreted that from a number of 10 composite samples of garlic rhizosphere soil could delineate 3 deferent DNA patterns. It means that bacterial

community structure of the rhizosphere bacteria in garlic is significantly diverse. Each sample could delineate two to eleven convenient DNA fragments.

Based on the present works, a speculation can be made about the role of rhizosphere bacterial community of plant in the disease suppressiveness, since it is interesting that a fragment positioned at the range of 100 bp and 1000 bp are the most frequently recovered from suppressive soil. The relationship of bacterial community of garlic rhizosphere to plant healthiness against some pathogens had been studied extensively in many plants<sup>3, 14, 27, 15, 13, 29, 30</sup>. Some of rhizosphere bacteria were reported to induce disease-suppressive of some plants from certain soil born and foliage diseases<sup>5, 14, 17, 27, 15; 13, 14, 29, 30</sup>. In addition biological factor, it is possible that the activity of bacterial community from disease-suppressive of basal rot of garlic in Tawangmangu was also effected by other soil properties including organic matter, physical and chemical condition<sup>9, 10</sup>.

This study was the first attempt to use culture-independent method to evaluate the presence and activity of the dominant populations of bacterial community in relation to exhibition of infected garlic by basal rot pathogen. PCR-RISA was used for this study. The results show that PCR-RISA can be used to detect the bacterial community of garlic rhizosphere. PCR-RISA has been an alternative widely applying to diversity analysis of microbial community including rhizosphere bacteria<sup>2, 3, 6, 17, 19</sup>, in addition to Denaturing Gradient Gel Electrophoresis (DGGE)<sup>15, 19, 24</sup>, Terminal Restriction Fragment Length Polymorphism (T-RFLP)<sup>1,19,26</sup>, and PCR-Single Strand Conformism Polymorphism (PCR-SSCP)<sup>19,21,22, 23</sup>.

## Conclusion:

The present study suggested that based on PCR-RISA, the rhizosphere bacterial community of garlic growing on suppressive soil was more diverse than the conducive soil, and similarly the rhizosphere bacterial community of healthy garlic was more diverse in comparing to diseased one.

## Acknowledgments:

This research was founded by a project of "Hibah Fundamental" from DP3M, Directorate-General of High Education, the Ministry of Education and Culture, the Republic of Indonesia through Contract No: 2339/UN27.16/PN/2012.

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