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Research article

Evaluation of the Effect of PGPR Strains on Tomato Growth and Suppression of Ralstonia Wilt Disease

Yulmira Yanti[1]*, Hasmiandy Hamid[1], R Reflin[1], Y Yaherwandi[1], Dede Suhendra[2],Doni Hariandi[3], and Ni Luh Suriani[4]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Indonesia
[2]Department of Agroecotechnology, Faculty of Agriculture, Universitas Andalas, Indonesia
[3]Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas, Indonesia
[4]Department of Biology, Mathematic and Natural Science Faculty, Udayana University, Indonesia

ORCID
Yulmira Yanti https://orcid.org/0000-0002-2853-3226

Abstract.
The tomato (*Lycopersicum esculentum* Mill.) has substantial scope for development due to its high economic value and large export potential. *Ralstonia syzygii* subsp. *Indonesiensis* (RSI) is the cause of bacterial wilt disease which attacks the vascular system in Solanaceae. It can cause large losses in yield and has caused global concern because of its widespread distribution and attack on many important crops. The aim of this research was to identifiy and characterize the ability of indigenous rhizobacterial isolates to control RSI and promote tomato growth. The PGPR traits studied were production of hydrogen cyanide, siderophores, biosurfactant, and ammonia, and protease activity. Bacterial identification was performed using 16S rRNA. Our findings revealed that the strains identified shared some similarities with *Bacillus thuringiensis* strain ATCC 10792 (IR.2.3.5), *B. mycoides* strain ATCC 6462 (IR.1.3.4), *Bacillus thuringiensis* strain IAM 12077 (IR.2.2. 1), *Serratia ficaria* strain DSM 4569 (IR.3.1.4), *Enterobacter oryzendophyticus* strain REICA_082 (IR.2.2.7), *Cronobacter dublinensis* subsp. *lausannensis* strain E515 (IR.2.2.5) and *S. rubidaea* strain DSM 4480 (IR.2.2.6). All of the isolates were tested for a variety of abilities related to growth promotion and biocontrol.

Keywords: Ralstonia wilt; 16S rRNA identification; PGPR traits

Corresponding Author: Yulmira Yanti; email: yy.anthie79@gmail.com

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1. Introduction

Tomato (*Lycopersicum esculentum* Mill.) is one of the horticultural commodities that is very potential to be developed due to its high economic value and large export potential [1]. Tomatoes are widely used as vegetables, spices, beverages, and industrial raw materials [2].

Ralstonia syzygii subsp. *Indonesiensis,* the causal of bacterial wilt disease is a vascular disease in solanaceae that can cause maximum yield loss, [4] ranging between 15-55% [4]. This disease had caused global concern because of its wide spread [5] and

attack on many important crops [6] from more than 200 species from 53 families [7]. The disease is hard to control due to the pathogens ability, high genetic diversity, diverse pathogenicity, soil-borne and physiological characteristics [6].

Various methods to control bacterial wilt have been carried out, including the use of pesticides, plant resistant varieties, sanitation, and crop rotation [8], however, the control is still not effective. Plant rotation is often ineffective because pathogens can survive in the soil even without hosts. Meanwhile, control by using bactericides or antibiotics is not only economically ineffective but also potentially causing undesirable effects, such as the death of natural enemies and the resurgence of new resistant strains [9].

A safer alternative method to control the pathogens is by utilizing microorganisms as biocontrol agents. Microorganisms such as Plant Growth Promoting Rhizobacteria (PGPR) group had reported to act as biocontrol agents[10]. The bacteria known to suppress pathogen growth through biocontrol activity in the form of the synthesis of protease and chitinase enzymes. Other characteristics, such as ammonia production, biosurfactants, siderophores, HCN and catalase activity, had an important role as biocontrol agents, promoting plant growth and increasing resistance[11].

Rhizobacteria ability as plant resistance inducer and strains ability to effectively control plant diseases were found to be better when introduced to the same host plant (indigenous) than to other plants [12]. Strain L115 of peanut rhizosphere were able to promote peanut growth and tolerate high soil temperatures [13]. Indigenous rhizobacteria were also able to suppress *Phytophthora capsici* that caused stem rot disease in chili plants with no visible symptoms and severity and increase the growth of chili[14]. Chili Rhizospheric indigenous rhizobacteria isolate, RZ.2.2.AG2 and RZ2.1. were also reported controlling wilt disease and growth promotion in chili plants of the chili plants [12].

The aim of this research were to characterize the ability and identify the indigenous rhizobacterial isolates capable of controlling *Rsi* and promote tomato growth..

2. Methodology

HCN production determined using color shifting by common methods using CDS solutions[15].

Siderophore production determined methods of Alexander and Zuberer[6].

Hemolytic assay determined by halo zone using agar diffusion technique [17].

Biosurfactant production assayed by biofilm formations in the surface of the medium NB.

Ammonia production assayed by the color shift to brown yellow as a positive result using Nessler's reagent [18].

Protease activity assayed by halo zone on Luria Bertani Broth medium [19]

Bacteria Identification Using 16S rRNA identified by 16S RNA using universal primer 27F and 1492R and sequence data compared with GenBank.

3. Result and Discussion

3.1. Rhizobacteria identification

The seven rhizobacterial isolates were identified molecularly with the 16S rRNA encoding gene. Rhizobacterial isolates rRNA amplified using 27F and 1492R and showed a base size of approximately 1,500 bp (Fig 1).'

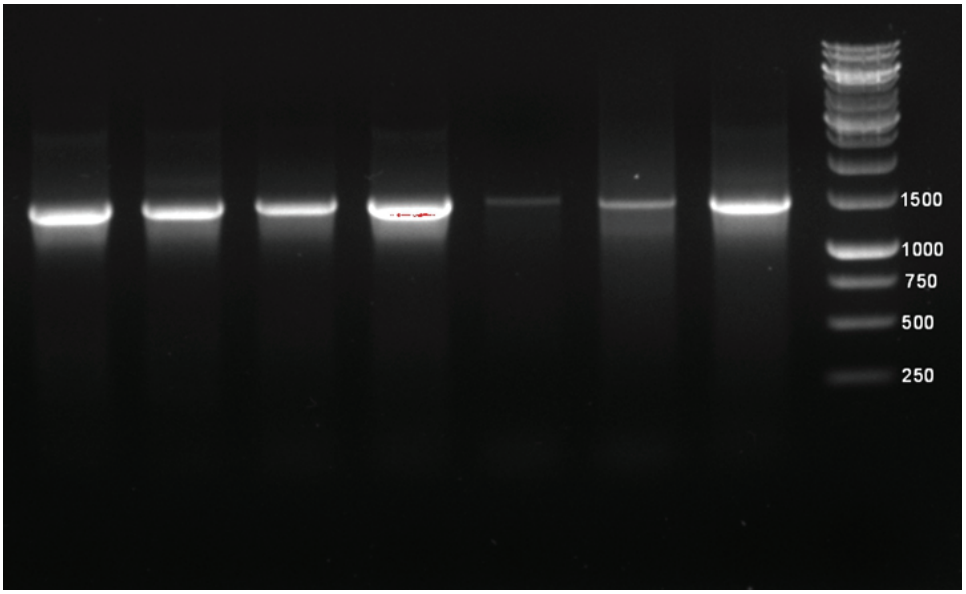

Figure 1: of 16S rRNA gene amplification from rhizobacterial strains.

BLAST analysis showed that isolate IR.2.3.5 had 100% similarity with *Bacillus thuringiensis* strain ATCC 10792; isolate IR.1.3.4 had 100% similarity with *B. mycoides* strain ATCC 6462; isolate IR.3.1.4 had 99% similarity with *Serratia ficaria* strain DSM 4569; isolate IR.2.2.1 had 99% similarity with *Bacillus thuringiensis* strain IAM 12077; isolate IR.2.2.7 had 99% similarity with *Enterobacter oryzendophyticus* strain REICA_082; isolate IR.2.2.5 had 99% similarity with *Cronobacter dublinensis* subsp. *lausannensis*

strain E515; and isolate IR.2.2.6 had 99% similarity with *Serratia rubidaea* strain DSM 4480 (Table 1)

Table 1: Identification of 16S rRNA of indigenous rhizobacteria using BLAST.

Isolate	Total base	Sequence analysis result	% of similarity
IR.2.3.5	1154	*Bacillus thuringiensis* strain ATCC 10792	100
IR.1.3.4	1214	*Bacillus mycoides* strain ATCC 6462	100
IR.3.1.4	1198	*Serratia ficaria* strain DSM 4569	99
IR.2.2.1	1216	*Bacillus thuringiensis* strain IAM 12077	99
IR.2.2.7	1195	*Enterobacter oryzendophyticus* strain REICA_082	99
IR.2.2.5	1184	*Cronobacter dublinensis* subsp. *lausannensis* strain E515	99
IR.2.2.6	1025	*Serratia rubidaea* strain DSM 4480	99

3.2. Biochemical characteristics

The strains Biochemical character showed that two strains of *B. thuringiensis* strain IR.2.3.5 and *S. ficaria* strain IR.3.1.4 were able to produce siderophores. Five isolates of *B. mycoides* strain IR.1.3. 4, *B. thuringiensis* strain IR.2.3.5, *E. oryzendophyticus* strain IR.2.2.7, *S. ficaria* strain IR.3.1.4, and *C. dublinensis* subsp. *lausannensis* strain IR.2.2.5 were able to produce salicylic acid. Three isolates of *B. mycoides* IR.1.3.4, *B. thuringiensis* IR.2.3.5, and *S. ficaria* strain IR.3.1.4 were able to produce proteases.Four isolates of *B. thuringiensis* strain IR.2.3.5, *B. mycoides* strain IR.1.3.4, *E. oryzendophyticus* strains IR.2.2.7, and *C. dublinensis* subsp. *lausannensis* strain IR.2.2.5 were able to produce ammonia. All isolates produced biosurfactant, however, they did not produce cyanide acid and hemolysis (negative) (Table 2).

Table 2: Biochemical characteristics of selected indigenous rhizobacteria.

No	Isolates	Siderophore	HCN	Salicylic acid	Protease	Ammonia	Biosurfactant	Haemolysin
1	IR.2.2.6	-	-	+	-	-	+	-
2	IR.2.3.5	+	-	+	+	++	+	-
3	IR.2.2.1	-	-	-	-	-	+	-
4	IR.1.3.4	-	-	+	+	+++	+	-
5	IR.2.2.7	-	-	-	-	+	+	-
6	IR.3.1.4	+	-	+	+	-	+	-
7	IR.2.2.5	-	-	+	-	+	+	-

The isolates of indigenous rhizobacteria were identified as different strains, consisting of Bacillus, Serratia, Enterobacter and Cronobacter genera. Most isolates were identified

from the *Bacillus* spp group. Bacillus is known as a genus that has been widely reported as PGPR and biocontrol agents. *B. pumilus* and *B. mycoides* were reported being able to induce sugar beet plant resistance to *Cercospora beticola* [20]. *Bacillus* spp. wa reported being able to control *Ralstonia solanacearum* in mulberry plants [21] and control *Xanthomonas euvesicatoria* and *Xanthomonas perforans* in tomato plants [22]. *B. thuringiensis* was reported being able to induce systemic resistance to *Ralstonia syzygii* subsp. *indonesiensis* in tomato plants [23]. *B. cereus* AR156 was also reported to induce *Arabidopsis thaliana* systemic resistance through mechanism of salicylic acid signaling pathways and jasmonic-ethylene signaling pathways [24]. Serratia and Enterobacter strains were able to control *R. solanacearum* and *F. oxysporum* f.sp *solani* wilt disease of tomato[12]. *Enterobacter* sp. was reported being to suppress the *R. solanacearum* and promote tomato plants growth and yields [25].

Biochemical characterization of indigenous rhizobacteria isolates is related to the mechanism of their ability to control plant pathogens. In this study, some isolates produced siderophore.Siderophore productions are one of vital PGPR abilities, because siderophore can bind iron ion (Fe3 +) to Fe compound and make it available for plants [26]. Rhizobacteria isolates that produced proteases were identified from the *Bacillus* sp.. Some Bacillus species can produce various enzymes, including proteases, penicillinase, nucleases, phosphatases, lipases, phospholipase C, thiamaminases, and bacteriolytic enzymes [27]. *Bacillus* sp. strains B8 and B11 produce protease and chitinase enzymes that can interfere with the development of pathogens [28]. Indigenous rhizobacteria isolates do not produce secondary metabolites of hydrogen cyanide that are generally produced by the *Pseudomonas fluorescens* group and also by other Pseudomonas groups that are toxic to plant pathogens [29]. All isolates were able to produce biosurfactants. The higher the viscosity value of the bacteria, the faster the bacteria to divide and develop [30]. Thus, it can be stated that the rhizobacteria are increasingly effective as biocontrol agents. This because the surfactant compounds produced affect the nature and effectiveness of the rhizobacteria. All isolates did not form a haemolysis reaction in the blood medium, indicating that the isolates were not pathogenic to animals and humans

4. Conclusion

From the results of the study, it can be concluded that the isolates obtained were identified, and they shared similarities with *Bacillus thuringiensis* strain ATCC 10792 (IR.2.3.5), *B. mycoides* strain ATCC 6462 (IR.1.3.4), *Bacillus thuringiensis* strain IAM 12077

(IR.2.2. 1*), Serratia ficaria* strain DSM 4569 (IR.3.1.4), *Enterobacter oryzendophyticus* strain REICA_082(IR.2.2.7), *Cronobacter dublinensis* subsp. *lausannensis* strain E515 (IR.2.2.5), and *Serratia rubidaea* strain DSM 4480 (IR.2.2.6). All isolates were characterized, showing various abilities related to growth promoters and biocontrol abilities.

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