Involvement of Jasmonic Acid in the Induced Systemic Resistance of Tomato against Ralstonia syzigii subsp. indonesiensis by Indigenous Endophyte Bacteria

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Abstract. Endophyte bacteria colonize plant roots and exert a beneficial effect on plant growth are well known for their potential to reduce plant pathogen populations in the soil, thereby suppressing diseases. Elucidation of signaling pathways controlling disease resistance is a major objective in research on plant-pathogen interactions. It has been suggested that jasmonic acid (JA) could be an integral part of a general signal transduction system regulating inducible defense genes in plants. Recent studies had shown that Endophyte bacteria could elicited induce systemic resistance (ISR) of plants by jasmonic acid pathway. This research purposed to study the involvement of JA in ISR of tomato plants against Ralstonia syzigii subsp. Indonesiensis by indigenous endophyte bacteria. This research assayed the JA contained in the leaves and roots of tomato plants that treated with indigenous endophyte bacteria (seed and seedlings treatment) and inoculated with R. syzigii subsp. Indonesiensis with Gas Chromatography-Mass Spectrophotometry (GC-MS) in interval between 0 to 30 days after pathogen inoculation. Results shown that all selected indigenous endophyte bacteria can suppress R. syzigii subsp. Indonesiensis attack and increase JA contained in leaves and roots of tomato significantly until 12 days after pathogen inoculations. Strain Bacillus cereus EPL1.1.3 and Serratianematodiphila TLE1.1 respectively had the highest JA activivity both in roots and leaves of tomato. This indicated that one of the selected indigenous endophyte bacteria abilities to suppress pathogens attack mechanism related to JA pathway.

1. Introduction
Ralstonia syzigii subsp. Indonesiensis [1], formerly named R. solanacearum [2] (RS) is a gram-negative, soil-borne pathogen that causes bacterial wilt (BW), a disease affecting more than 50 plant families [3]. This disease is economically important for several vegetable crops including members of the Solanaceae family such as tomato. Bacterial Wilt caused by R. syzigii subsp. indonesiensis one of the important serious vascular diseases causes 15% to 55% crop losses around the world [4]. Despite inconsistency in field performance, biological control is considered as an alternative or a supplemental way of reducing root diseases in agroecosystem [4].

Certain strains of rhizosphere bacteria stimulate plant growth and are therefore called plant growth-promoting rhizobacteria (PGPR). One of PGPR are group as endophytic bacteria. Endophyte bacteria inhabit plant tissues, for part or all of their life without causing any apparent disease symptom [5]. Endophytes can be found in any plan and reside in any part of the plant organs such as roots. Bacterial endophytes known to not pose effect on the host plants and are considered as neutral. In
many cases, bacterial endophytes provide beneficial effects to the host such as improving plant tolerance to abiotic stresses [6]. Thus similarity ecological niche between endophytes and pathogens make them suitable as biocontrol agents of plant diseases [7]. The endophytes also had similar antagonistic effects as other bacteria such as competition, production of secondary antimicrobial metabolites, and induction of plant resistance.

Some of these biological controls trains are also able to reduce disease caused by foliar pathogens by triggering a plant-mediated resistance mechanism called induced systemic resistance (ISR)[8]. Induced resistance is a physiological ‘state of enhanced defensive capacity’ elicited by specific environmental stimuli, whereby the plant’s innate defenses are potentiated against subsequent biotic challenges. This enhanced state of resistance effective against a broad range of pathogens and parasites [9].

The widely recognized mechanism of biocontrol mediated by PGPR is competition for an ecological niche/substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens. Recently, research on mechanisms of biological control by PGPR revealed that several PGPR strains protect plants against pathogen infection through induction of systemic resistance, without provoking any symptoms themselves[10]. Rhizobacteria-mediated ISR has been reported for bean, carnation, cucumber, radish, tobacco, tomato and the model plant Arabidopsis thaliana, and is effective against different types of plant pathogens. In this respect, ISR resembles pathogen-induced systemic acquired resistance (SAR), which renders uninfected plant parts more resistant towards a broad spectrum of pathogens[11,12].

Recent advances in research on plant defense signaling pathways have shown that plants are capable of differentially activating distinct defense pathways depending on the type of invader encountered [13]. Upon pathogen infection, the production of JA is stimulated, leading to the activation of specific defense-related genes [14,15]. In this study, we investigated the activity of Jasmonate acid in tomato plants against *Ralstonia syzygii* sp. indonesiensis by indigenous endophyte bacteria.

2. Materials And Methods

2.1. Bacterial Preparations

9 rhizobacteria isolates used in this study was the best isolates that could control *R. syzigii* sp. indonesiensis. Rhizobacterial isolates used were B. cereus EPL1.1.3, B. cereus TLE2.3, B. toyonensis EPL1.1.4, S. nematodiphila TLE1.1, B. anthracis SNE2.2, B. cereus E1.AB1.2, B. cereus E1.AB2.1, E. cloacae subsp. dissolvens TLE2.2, S. marcescens KLE3.3 the isolates cultured in Nutrient Agar (NA) media in petri-dish by streaking each of the strains to the agar and incubated for 72 h. One pure colony of each strains was inoculated into 25mL of NB in culture bottle (50mL) and incubated in rotary shaker 110 rpm for 24 hours. 4 mL of the culture then was transferred to 400 mL of sterile coconut water in Erlenmeyer flask for main culture and incubated for 2x24 hours [16]. Suspension of rhizobacteria strains in the coconut water culture was diluted with comparison to McFarland scale 8 (Density estimated 108 CFU/mL).

2.2. Tomato plants sample preparation

Surface sterilized tomato seeds' were soaked in rhizobacterial strain (108 cfu/ml) suspensions separately for 15 minutes. Seeds soaked in sterilized distilled water were treated as control. The seed were then directly plant to seed tray contain sterile growth medium (soil: cow dung manure = 3:1). Three weeks old seedlings then planted to polybag contain same soil and treated without synthetic fertilizer application, and watered routinely until harvested.

2.3. Pathogen inoculation

The *Ralstonia syzygii* sub sp. indonesiensis was isolated from infected plants by dipping the stem in sterilized water and the suspension then streaked in Tryphenyl tetrazolium (TZC) agar medium. The isolates were then assayed on 2 weeks old tomato plants to select the most virulence pathogens by injecting 1 ml of Ralstonia suspension (108 CFU/ml) to the tomato plant base stem. The most
virulence bacteria (the fastest wilt disease development) was then re-cultured in TZC agar and used for plant disease infection. The pathogen was inoculated on the 2 weeks old plants by root wounding methods described by Yanti et al., [16]. The roots were cutted in 2 sides of the plants using scissors and poured with 10 mL (108 CFU/mL) of the R. syzigii sub sp. indonesiensis suspensions. Pathogen inoculation were done After 14 days after planting by cutting the roots and then pouring the suspension of R. syzigii sub sp. indonesiensis (106 cells / ml). Root and leaves were harvested after 0, 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28 and 30 days after pathogen inoculation.

2.4. Extraction and quantification of JA

Tomato leaves were frozen in liquid nitrogen and pulverized with mortar and pestle. 1 g of fresh weight was used to extract JA. 100 ng of 9,10-dihydrojasmonic acid were added per gram of fresh weight as an internal standard. Extraction and GC-MS quantification of JA was carried out as described by Mueller and Brodschelm [17].

![Fig 1. Jasmonate acid activity on root of tomato leaves introduced with indigenous endophyte bacteria.](image)

3. Result and Discussions

Jasmonate acid introduced with indigenous endophyte bacteria strains had shown increased compared to control. Jasmonate acid on leaves that introduced with indigenous endophyte bacteria strains increase significantly since 1 day after innoculations (dai). Highest jasmonate acid activity observed on the leaves that introduced with B. cereus EPL1.1.3 on 12 day after innoculations (3.6 ng.g^-1) Jasmonate acid activity on the tomato roots introduced with indigenous endophyte bacteria were shown higher compared with the activity in the leaves. Highest jasmonate acid concentrations were observed on tomato roots introduced with B. cereus EPL1.1.3on 17 dai (4.37 ng.g^-1), and then shown decreased on 19-30 dai. Those increased activity of jasmonate acid on tomato leaves and roots that introduced with indigenous endophyte bacteria shown increased defense response.
Increased concentration of jasmonate acid on leaves and roots of tomato introduced with endophyte bacteria shown increased defense response activity on tomato. Jasmonate acid, methyl jasmonate and other similar compound are playing important role in various regulation in cellular process like on lessions and defense response [18, 19]. Jasmonate acid production on plants were a complicated regulation process and the concentrations on undisturbed plants often in low concentrations [20]. Jasmonate acid accumulated on plants that getting injured or in plants treated with pathogen elicitors. Jasmonate acid function as gene expression signal activation on various gene like proteinase inhibitor, thionin and phytoalexin enzyme metabolism [21].

Acknowledgement
The author was grateful; this research was funded by ‘Penelitian Unggul Perguruan Tinggi’ Batch 2018 Contract No. 050/SP2H/LT/DRPM/2018 January 30th, 2018 from Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

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