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## CHARACTERIZATIONS OF *Trichoderma* sp. AND ITS EFFECT ON *Ralstonia solanacearum* OF TOBACCO SEEDLINGS

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### ABSTRACT

**Characterizations of *Trichoderma* sp. and its effect on *Ralstonia solanacearum* of tobacco seedlings.** This study aims to determine the molecular-based characteristics of *Trichoderma* sp. Tc-Jjr-02 and its effect as a biocontrol agent in protecting tobacco seedlings against bacterial wilt caused by *R. solanacearum*. The characterization of biocontrol agents was based on morphological and molecular data's observation using microscope and the key of determination. The in vivo experiments was consist of five treatments: (1) inoculation of *Trichoderma* isolates at six hours before *R. solanacearum* inoculation, (2) inoculation of *Trichoderma* isolates at six hours after *R. solanacearum* inoculation (3) simultaneous inoculation of *Trichoderma* isolates and *R. solanacearum*, (4) inoculated only with *R. solanacearum*, and (5) without any inoculation. The experiment was repeated six times. Based on BLAST's analysis, the Tc-Jjr-02 sequence is in accordance with *T. asperellum* with 100% Query Cover. Inoculation of *T. asperellum* Tc-Jjr-02 at six hours before and after and simultaneously with pathogens providing protection for young tobacco plants by slowing down the time for the onset of blight by 100–162%, reducing the symptom index by 56–63%, and increasing the dry weight of plant biomass by 39–53% compared to tobacco seeds which were only inoculated with *R. solanacearum*.

**Key words:** bacterial wilt, characterization, *Ralstonia solanacearum*, *Trichoderma* sp. Tc-Jjr-02

### INTRODUCTION

Tobacco has important role in the national economy as currently Indonesia is one of the biggest tobacco producers in the world (Raheem *et al.*, 2016). The use as a cigarette however, is increasingly suppressed (Levy *et al.*, 2017) and various alternative forms of substitution consumption continue to be developed (Farsalinos *et al.*, 2016), especially for health and pharmaceutical industries (Daniel, 2011; Fauzantoro *et al.*, 2017), as well as active ingredients for pesticides (Araka *et al.*, 2016).

The quality and productivity of tobacco cultivation is largely determined by the success in protecting plants from damage due to environmental stress and destructive organisms. *Ralstonia solanacearum* is a bacterium that causes Tobacco bacterial wilt disease which is very detrimental to tobacco cultivation (Li *et al.*, 2016). This bacterium can also damage various plants of the Solanaceae family including tomatoes (Huang *et al.*, 2013) and potatoes (Kheirandish & Harighi, 2015).

Moreover, it can even cause diseases in various important commodities in the world (Jiang *et al.*, 2017).

To date, the control of tobacco plant diseases has always relied on chemical pesticides which often fail to overcome severe disease attacks (Li *et al.*, 2014). Active ingredients of pesticides often cause disruption to the health of operators, develop pathogen resistance, and cause damage to non target organisms in plantations (Gava & Pinto, 2016), in addition to the impact of toxic materials pollution on the environment (Widmer, 2014) as well as the threat of exposure to hazardous substances for the public in around plantations (Damalas & Eleftherohorinos, 2011; Jallow *et al.*, 2017).

Utilization of biological control agents is one of the efforts to protect plants against pathogens that have good prospects in the future. However, the application must accompanied by the provision of nutrients and compounds that can encourage plant growth, so that plants get protection both directly and indirectly in the form of induced systemic resistance.

*Trichoderma* is a fungus that can be used as a biological controller and has the ability as a biofertilizer (Glare *et al.*, 2012; Buysens *et al.*, 2016). This fungus produces antibiotic, toxins, and enzymes that can inhibit pathogens (Pruksakorn *et al.*, 2010; Chowdappa *et al.*, 2013; Buysens *et al.*, 2016), it could damage the cell walls (Yedidia *et al.*, 2000) and degrade organic matter (Hu *et al.*, 2015; Saravanakumar *et al.*, 2016) of plant pathogenic fungi. The *Trichoderma* ability to produce extracellular compounds such as auxins and its derivatives (Vinale *et al.*, 2014), make it a superior biofertilization agent that can guarantee the nutritional needs and growth of plants while reducing the use of synthetic pesticides (Hu *et al.*, 2016).

The main character of *Trichoderma* as a biocontrol and biofertilizer is indispensable for the protection of plants, including seeds, seedlings and young tobacco plants. However, selection of *Trichoderma* types and isolates are needed to choose the one that can provide a positive response to tobacco plants in inducing resistance to pathogen. Understanding the characteristics of *Trichoderma* fungus that is effective in controlling one type and/or several types of plants is important for the practice of formulations, the procurement of biological agents for crop protection, and for the development of their applications. For this reason, it is necessary to identify *Trichoderma* species based on molecular markers to provide adequate information for other researchers and executives engaged in plant protection.

*Trichoderma* sp. Tc-Jjr-02 is isolates collection of the UMSIDA Microbiology Laboratory that proved to be effective in controlling plants that have close kinship with tobacco, such as tomatoes (Huang *et al.*, 2013) and potato (Kheirandish & Harighi 2015; Sutarman, 2019). This study aims to determine the molecular-based characteristics of *Trichoderma* sp. Tc-Jjr-02 and its effect as a biocontrol agent in protecting tobacco seedlings against bacterial wilt caused by *R. solanacearum*. Molecular characterization of biocontrol agents was also used in this experiment to provide additional information supporting the morphological character information.

## MATERIALS AND METHODS

**Research Site.** This research was conducted at the Microbiology Laboratory and Greenhouse of the Departement of Agrotechnology, Faculty of Science and Technology and the Molecular Biology Laboratory of

the Faculty of Health Sciences, Universitas Muhammadiyah Sidoarjo in March–July 2019.

### Morphology and Molecular Characterization of Biocontrol Agent.

**Morphological Observations.** The two-week-old *Trichoderma* Tc-Jjr-02 isolate was propagated and placed on glass objects to be observed under a microscope at 400 times magnification. Microscopic structures to be observed were hyphal, phialid, conidiophores, and conidiospore branches. The morphological characteristics then compared with the description and key of determination by Gams & Bissett (2002).

**DNA Isolation and Preparation.** In total, 50 mg of propagules containing *Trichoderma* sp. Tc-Jjr-02 was put into 200  $\mu$ L of dH<sub>2</sub>O in the BashingBead™ tube and added 750  $\mu$ L of BashingBead™ buffer (Zymo Research, USA). The suspension then centrifuged at 10,000  $\times$  g for 1 min. The supernatant was then taken (400  $\mu$ L) and put into the Zymo-Spin™ III-F filter (Zymo Research, USA) and placed in a collection tube then centrifuge at 10,000  $\times$  g for 1 min. The filtrate then added with 1200  $\mu$ L genomic lysis buffer (Zymo Research, USA). A total of 800  $\mu$ L of the mixture was transferred into the Zymo-Spin™ IICR column (Zymo Research, USA) with collection tube and centrifuge at 10,000  $\times$  g for 1 min. Spill (flow through) was discharged until the liquid runs out. The mixture added with 200  $\mu$ L of DNA pre-wash buffer and put new collection tube, then centrifuged 10,000  $\times$  g for 1 min. A total of 500  $\mu$ L g-DNA wash buffer was added to the Zymo-Spin™ IICR column (Zymo Research, USA) and centrifuged at 10,000  $\times$  g for 1 min. Then the Zymo-Spin™ IICR column was transferred to a clean 1.5 mL microcentrifuge tube and 100  $\mu$ L DNA elution buffer was added directly to the center of the column and finally centrifuged at 10,000  $\times$  g for 30 s to elute the DNA.

**Polymerase Chain Reaction.** DNA amplification was performed on the Biorad T100 thermalcycler machine. The cycles used include pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and primer extension at 72 °C for 1 min. A total of 40 cycles were carried out with one cycle of final elongation for 10 min. The reaction used was 25  $\mu$ L PCR mixture (Bioline), 1  $\mu$ L each of 10 pmol primer ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White

et al., 1990; Fierer et al., 2005), 5 µL DNA template, and 18 µL sterile ddH<sub>2</sub>O.

**Sequencing and Phylogenetic Analysis.** Sequencing was performed using Sanger sequencing, 50 µL of the PCR product was sent to a commercial DNA sequencing service (1st Base; Singapore). Nucleotides were produced from sequencer machines (ABI 3730XL sequencers) and compared to GenBank using the Basic Local Alignment Search Tool (BLAST) program available at the National Center for Biotechnology Information (NCBI, 2020). Phylogenetic analyses were conducted in MEGA X (Kumar et al., 2018).

### Greenhouse Experiment.

**Isolates Preparation.** *Trichoderma* sp. Tc-Jjr-02 isolate used in this experiment was the collection of Microbiology Laboratory at Universitas Muhammadiyah Sidoarjo. *Trichoderma* isolate was propagated in PDA-chloramphenicol medium (Gil et al., 2009) with an incubation period of 14 days. After 14 days, the cultures of the isolate was harvested, washed, and mixed with water to produce a suspension with a population density of 10<sup>8</sup> cfu mL<sup>-1</sup>.

The pathogenic bacterial isolate of *R. solanacearum* that used in this study was a collection of the Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan (BBPPTP) Surabaya, Directorate General of Plantations-Ministry of Agriculture, Mojoagung-Jombang, East Java. Pathogenic bacterial isolate was propagated on NA media, after 4 days of incubation the culture was harvested and mixed with water to create an active spore suspension with population density of 10<sup>9</sup> cfu mL<sup>-1</sup>. For application purposes, each suspensions was diluted with sterile water to 10<sup>7</sup> cfu mL<sup>-1</sup> conidia of Tc-Jjr-02 isolates and 10<sup>8</sup> cfu mL<sup>-1</sup> active bacterial cells of *R. solanacearum* (Li et al., 2016). Each suspensions then stored in a 500 mL glass beaker; All isolation and propagation activities were done in aseptic conditions.

**Planting Medium and Plant Variety.** The soil that used as a planting medium was derived from land with a

height of ± 8.0 m asl in Jiken Village, Tulangan District, Sidoarjo Regency, East Java Province with basic characteristics: pH (H<sub>2</sub>O) 7.05, C-organic 0.56%, C/N ratio 14, CEC 29.64, and dusty clay texture. The soil was sterilized by autoclaving in 120 °C at 1 atm for 30 min.

In this experiment, Baleno variety of tobacco was used. This variety is usually planted in the dry season. Healthy seedlings were collected from planting tobacco seeds on sterile soil media for two months. The uniform and healthy seedlings showing no symptoms of necrosis, lesion, and/or other symptoms of the disease. About 30% of the root tip was cut using a sharp and sterile knife. Roots that have been cut off/injured were inserted into a suspension that contains biocontrol agent conidial suspensions and pathogenic bacterial cells or sterile water according to the desired treatment for 10 min. After the roots dry, the seedlings were transferred in to a new sterile growing media. Furthermore, seedlings were maintained in a nursery condition by watering with sterile water in the planting medium to maintain humidity according to the needs of the growth of tobacco plant seeds.

**Experimental Design.** The experiment in this study was arranged in a completely randomized design (CRD) consisting of co-inoculation of *Trichoderma* isolate and *R. solanacearum* in various inoculation methods. The treatments were: (1) inoculation of *Trichoderma* isolate at six hours before *R. solanacearum* inoculation, (2) inoculation of *Trichoderma* isolate at six hours after *R. solanacearum* inoculation, (3) simultaneous inoculation of *Trichoderma* isolate and *R. solanacearum*, (4) inoculated only with *R. solanacearum*, and (5) without any inoculation. The treatments were repeated six times, so that 30 experimental units were studied.

The observed variables in this study were: (i) incubation periode of bacterial wilt (hours); (ii) the bacterial wilt symptom index at 4 and 8 days after inoculation (DAI) using the formula which was determined based on the criteria in Table 1 (modified from Tahir et al., 2017); and (iii) dry weight of seedling stover at 6 days after inoculation (g).

Table 1. Scores and categories of symptoms of tobacco leaf seedlings wilt disease

Score	Criteria for symptoms
0	There are no symptomatic/diseased plant parts
1	Symptomatic plant parts withering: 1–25% or one withered leaf
2	Symptomatic plant parts withering 25–50% or two withered leaves
3	Symptomatic plant parts withering 50–75% or two to three withered leaves

Source: Tahir et al. (2017)

Disease severity index was calculated using the following formula:

$$DSI = \frac{\sum n_i \times v_i}{N \times k} \times 100\%$$

- DSI = Disease severity index (%),  
 $n_i$  = Number of plants with a score of  $i$ ,  
 $v_i$  = Numerical value (score) of plants with appropriate symptoms (Table 1),  
 $N$  = Number of plants observed per unit of experiment,  
 $k$  = Value highest score of symptoms.

**Data Analysis.** The data's results of experiments on biological agents applications were analyzed using analysis of variance (ANOVA) at 5% and 1% significance levels and if the results of the analysis of variance were significantly different ( $p < 0.05$ ) and very significant ( $p < 0.01$ ), then the analysis was followed with the 5% Honestly Significant Difference (Tukey test).

## RESULTS AND DISCUSSION

**Observation of Morphology and Characterization of Biocontrol Agents.** Morphological observations of *Trichoderma* sp. Tc-Jjr-02 isolate in Figure 1 shows the appearance of colony and microscopic structures. The description of both macroscopic and microscopic characteristics were shown in Table 2.

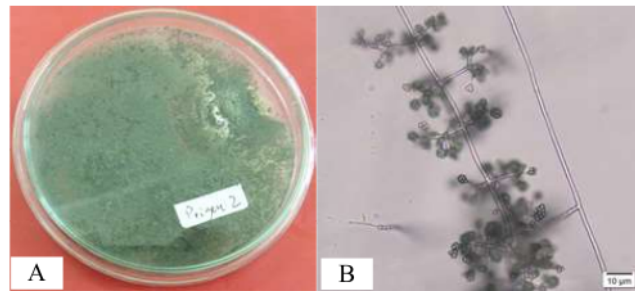


Figure 1. *Trichoderma* sp. Tc-Jjr-02 isolate. (A) Colony; (B) Microscopy structure

Table 2. Morphological description of *Trichoderma* sp. Tc-Jjr-02

Form	Descriptions
Colony (macroscopic)	At the beginning of growth, the colonies appear to have green stools with white edges, then the colonies appear green
Microscopic structures	Hyphae seeped to form elbows, hyaline conidiophore, shaped like a pyramid, upright branched, and slippery wall, phialid shaped like a thick pumpkin with size 5–7 µm; conidia are rather rounded or oval, slightly greenish color, size 2.5–3.5 µm

The result of electrophoresis DNA sequences of *Trichoderma* sp. isolate Tc-Jjr-02 for the biocontrol agent is shown in Figure 2. The results of BLAST search (<https://www.ncbi.nlm.nih.gov/>) showed 100% similarity with *Trichoderma asperellum* (isolate Tasum66, accession number MT102403.1) the dendrogram can be seen in Figure 3. It revealed that the *Trichoderma* sp. isolate Tc-Jjr-02 was placed in the same branch with reference strains of *T. asperellum*.

Homologous sequences obtained from the NCBI Gene Bank were reconstructed by MEGA 7 software (Kumar *et al.*, 2018) with the Neighbor Joining method so that a phylogenetic tree was produced (Figure 3). A similar method was used by Anam *et al.* (2019) on the RM-28 isolate whose data was stored in NCBI with the accession number MK092975 and identified as *T. asperellum*. The morphological and molecular characteristics of the Tc-Jjr-02 isolate are similar and consistent with *Trichoderma* sp. isolate T1 (accession number GenBank LC158827, KU497722 and KU497723) (Baiyee *et al.*, 2019) and with isolate TC01 (accession number GenBank MH752042 and MN813963) (Shang *et al.*, 2020), each of which is based on a phylogenetic tree determined as *T. asperellum*.

The shape and dimensions of conidiospores and chlamydospores Tc-Jjr-02 also have similarities with the *T. asperellum* VM 100 (KY412854) endophytic isolate on *Vinca minor* stem which is able to control *R. solanacearum* (Leylaie & Zafari, 2018), and with

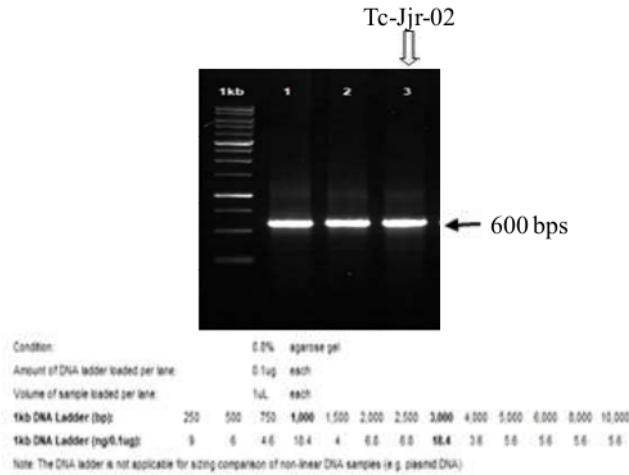


Figure 2. Electrophoresis of *Trichoderma* Tc-Jjr02 DNA fragments

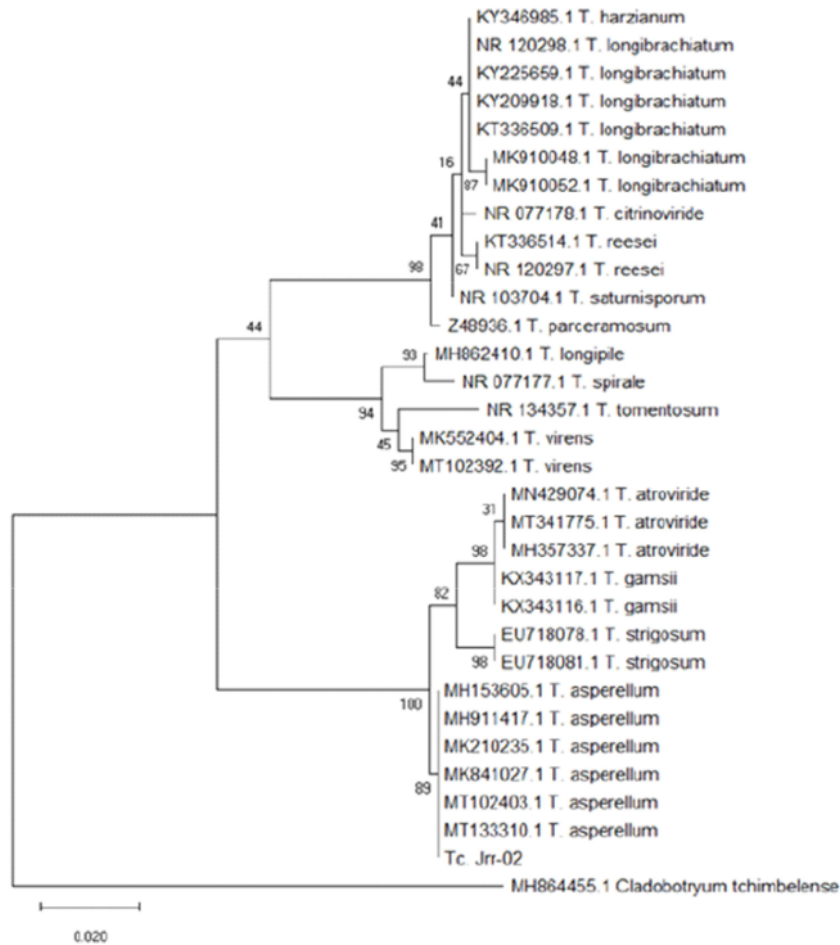


Figure 3. Phylogenetic tree of ITS region constructed with Neighbor Joining method on MegaX (Kumar *et al.*, 2018) with 1000x bootstrap showing *Trichoderma asperellum* as the most predominant homolog of Tc-Jjr-02 isolate.

*T. asperellum* GDFS1009 isolate used by Karuppiyah *et al.* (2019) in testing the control power of *Fusarium granarium* and its ability to promote wheat plant growth. Likewise, the dimensions of conidiospores and hyphal branching and the appearance of similar colonies between Tc-Jjr-02 and *Trichoderma* Ta1 and Ta2 isolates were also shown by Hewedy *et al.* (2020) based on the kinship analysis defined as *T. asperellum*.

**Greenhouse Experiment.** The analysis of variance showed that *Trichoderma* influenced the initial emergence of wilting symptoms in tobacco seedlings ( $p < 0.01$ ) (Table 3). The appearance of each tobacco plant seedling at 96 hours after inoculation was shown

in Figure 4. It also showed that *Trichoderma* sp. influenced on the bacterial wilt symptom index of tobacco seedlings ( $p < 0.01$ ). The mean index of disease symptoms at 4 and 8 DAI was presented in Table 4. The analysis results showed that *Trichoderma* sp. influenced on the dry weight of stover tobacco seedlings ( $p < 0.01$ ). The average dry weight of stover tobacco seedlings was presented in Table 5.

Overall, *Trichoderma* sp. inoculation at 6 hours before pathogen inoculation, six hours after pathogen inoculation, as well as simultaneous inoculation with pathogen could: (i) slowing down the time to onset of blight symptoms 100, 162, and 154%, (ii) suppressing the symptom index of 63, 56, and 56%, and (iii)

Table 3. Mean effects of *Trichoderma* sp. Tc-Jjr-02 on the initial appearance of symptoms of tobacco bacterial wilt

Treatment	Time at which the initial symptoms appear (hours)
<i>Trichoderma</i> inoculation 6 hours before inoculation of pathogen	52.00 ± 6.20 b
<i>Trichoderma</i> inoculation 6 hours after inoculation of pathogen	68.00 ± 6.20 c
<i>Trichoderma</i> inoculation together with pathogen inoculation	66.00 ± 10.64 c
Only pathogen inoculation	26.00 ± 4.90 d
Without <i>Trichoderma</i> and pathogen inoculation	92.00 ± 9.80 a

The mean followed by different letters shows significantly different effect (HSD;  $\alpha = 0.05$ )

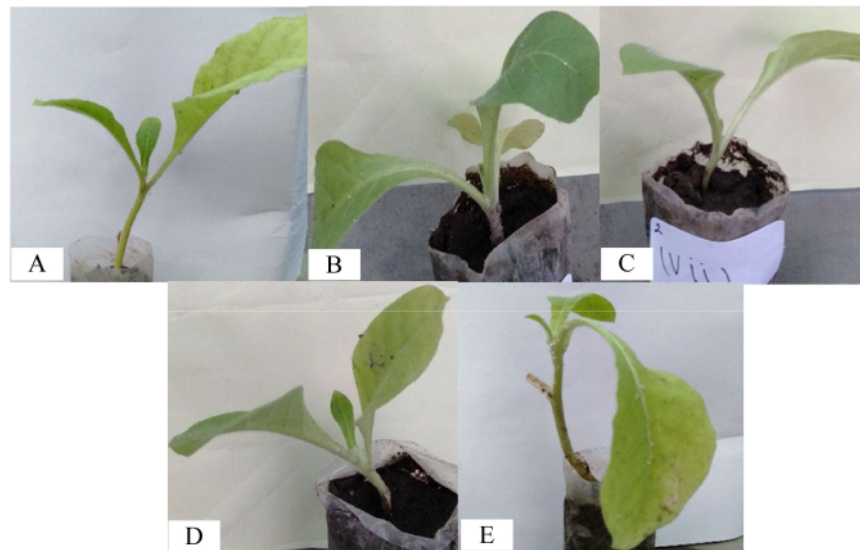


Figure 4. Appearance of tobacco seedlings at 90 hours after inoculation. (A) *Trichoderma* sp. inoculation 6 hours before inoculation of pathogens; (B) *Trichoderma* sp. inoculation 6 hours after inoculation of pathogens; (C) *Trichoderma* sp. inoculation together with pathogens; (D) Only inoculated pathogens; (E) Without inoculation.

increasing the biomass dry weight of tobacco seedlings was 39, 42, and 53% compared to tobacco seedlings which were only inoculated with *R. solanacearum*.

Plants that were not inoculated with pathogens and *Trichoderma* sp. appeared to be wilt starting from 92 hours after inoculation (Table 3, Figure 4) with symptom indices of 15.63 and 18.75, respectively at 4 and 8 DAI (Table 4). The rather withering symptoms were not plant infections, but the seedlings response to stressful environmental temperatures. The temperature and humidity in the greenhouse in this experiment (22–30 °C and 70–80%) could cause symptoms of wilting in post-overspine tobacco seedlings in all treatments; and could accelerate the increase in intensity of wilting symptoms (Li *et al.*, 2017). In hot conditions throughout the day, tomato plants were usually more susceptible to infection by pathogens as indicated by the appearance of leaves that look wilted (EMPPPO, 2004).

Fungi released secondary metabolites such as peptaibols and harzianolides (Zeilinger *et al.*, 2016), acid phosphatase that play a role in phosphate solubilization (Zhao *et al.*, 2017) and various other active secondary metabolites to the rhizosphere that promote root branching and nutrient absorption for plants (Martínez-Medina *et al.*, 2014; López-Bucio *et al.*, 2015). The growth and development of root was strongly influenced by the availability of hormones (Giehl & von Wirén, 2014). *Trichoderma* could increase lateral root

development and encourage plant growth (Rubio *et al.*, 2014), increasing shoot and root dry weight as well as chlorophyll content (Colla *et al.*, 2015).

In the treatment that was inoculated only with *R. solanacearum*, wilting quickly appeared with the highest average disease index (61%). Withered occurred due to a blockage in the root vessel transport network by bacterial cells and polysaccharides (Mutimawurigo *et al.*, 2019) which could threaten the plant to death. This pathogenic bacterial infection could increase root exudate in the form of various organic acids which can create a rhizosphere environment that was less favorable for plants (Li *et al.*, 2018). *R. solanacearum* produced various enzymes effector outside its cells and in the plant cells which were indicated by the activity of acetyltransferases, phosphatases, and proteases in the framework of plant infection (Wei *et al.*, 2017). With the intensity of attack symptoms that exceeded 50%, the Baleno variety of tobacco could be categorized as susceptible to bacterial wilt diseases (Laeshita & Arwiyanto, 2017). In line with this study, *T. harzianum* which was formulated as a biofertilizer made from compost carriers, was able to suppress the tobacco seedlings disease index by 26.8%, while in the control the seedling disease index could reach 68.2%, (Yuan *et al.*, 2016). Another study showed that, until the plants were ready to harvest, no application of biocontrol agents could raises the incidence of bacterial wilt disease up to

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Table 4. Mean effects of *Trichoderma* sp. Tc-Jjr-02 on the index of symptoms of tobacco bacterial wilt

Tretament	Disease symptoms index	
	4 DAI	8 DAI
Trichoderma inoculation 6 hours before inoculation of pathogen	21.88 ± 3.42 ab	22.92 ± 3.23 ab
Trichoderma inoculation 6 hours after inoculation of pathogen	20.83 ± 3.23 ab	27.08 ± 3.23 b
Trichoderma inoculation together with pathogen inoculation	25.00 ± 5.59 b	27.08 ± 3.23 b
Only pathogen inoculation	42.71 ± 2.55 c	61.46 ± 4.70 c
Without Trichoderma and pathogen inoculation	15.63 ± 3.42 a	17.71 ± 2.55 a

The mean followed by different letters in the same column shows significantly different effect (HSD;  $\alpha=0.05$ ).

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Table 5. Mean effects of *Trichoderma* sp. Tc-Jjr-02 on the dry weight of the tobacco seedlings stover

Tretament	Weight of dry stover (g)
Trichoderma inoculation 6 hours before inoculation of pathogen	10.33 ± 2.42 a
Trichoderma inoculation 6 hours after inoculation of pathogen	10.50 ± 2.51 a
Trichoderma inoculation together with pathogen inoculation	11.33 ± 1.97 a
Only pathogen inoculation	7.42 ± 1.56 b
Without Trichoderma and pathogen inoculation	12.83 ± 2.32 a

The mean followed by different letters shows significantly different effect (HSD;  $\alpha=0.05$ )



62.67–62.89% (Wu *et al.*, 2020). Susceptibility of tobacco seedlings showed that plants lack the ability to inhibit enzyme activity in the infection process (Khan *et al.*, 2016).

*Trichoderma* does not interfere with the stability of bacterial cells, but the enzyme chitinase produces by bacteria could disturb the stability of the fungal cell wall (Chowdappa *et al.*, 2013). The chitinase enzyme could trigger *Trichoderma* to defend itself by realizing antimicrobial compounds such as 6-Pentyl-a-pyrone, harzianolide harzianopyridone (Vinale *et al.*, 2008) phenyl ammonia lyase (PAL), peroxidase, polyphenol oxidase, lipoxigenase (Fan *et al.*, 2011) related to the activity of regulating plant growth and plant resistance to pathogens (Singh *et al.*, 2018).

*Trichoderma* use most of the organic material from cells that die as substrate for its activities (Hu *et al.*, 2015; Sutarman *et al.*, 2018). Decomposition of organic matter by *Trichoderma* will contribute as nutrients for plant to grow (He *et al.*, 2019) which reflected by stover dry weights and root dry weights (Table 5). In treatments using *Trichoderma*, the root weights were 10.33–11.33 g. This result were not significantly different from the dry weight of stover seedlings that were not inoculated by any microorganism.

The treatment that inoculated with *Trichoderma* sp. showed a low symptom index, which is 22.92–27.08 at 6 DAI. In the treatment of *Trichoderma* sp. application 6 hours before the pathogen inoculation, the disease index did not differ significantly from the treatment without any inoculation. This fact shows that this fungus possesses the ability to compete in the use of space (Lu *et al.*, 2016).

*Trichoderma* has the ability as an endophyte which is shown by its protective ability in plant tissue (Abdulmyanova *et al.*, 2015) and the anti-microbial compounds it produces (Zeilinger *et al.*, 2016) making it able to suppress pathogenic bacteria as shown by *T. asperellum* against *R. solanacearum* in *Vinca* minor plants (Leylaie & Zafari, 2018) and *T. asperellum* isolate Tc-Jjr-02 on *R. solanacearum* on tobacco seedlings in this experiment. The suppression by biological agents will have an effect on decreasing the density of pathogenic bacterial cells according to Wei *et al.* (2015) will have an effect on decreasing the intensity and incidence of plant diseases.

The similarity of the Tc-Jjr-02 isolate nucleotide sequence with *T. asperellum* is also shown by the similar role in promoting plant biomass. Tc-Jjr-02 was able to increase the biomass of tobacco seedlings, also similar to *T. asperellum* isolate RM-28 which increases sorghum-sudangrass seedling biomass (Anam *et al.*,

2019). The characteristics of Tc-Jjr-02 in enhancing plant growth and resilience were represented by the suppression of a similar symptom index shown by *T. asperellum* TI which induces plant resilience to leaf spot fungi in lettuce (Baiyee *et al.*, 2019) and *T. asperellum* TC01 on *Camellia sinensis* seedlings (Shang *et al.*, 2020), and as a control for stem rot in carnation (Vinodkumar *et al.*, 2017).

Information on the characteristics of *Trichoderma* sp. both morphologically and molecularly is important as a consideration in controlling bacterial wilt caused by *R. solanacearum*, considering that, this pathogen is also proven to cause severe attacks not only on tobacco plants but also on tomato and potato plants (Gutarra *et al.*, 2017).

## CONCLUSION

The molecular identification results showed that *Trichoderma* sp. Tc-Jjr-02 is *Trichoderma asperellum*. This fungus is influential in providing protection for young tobacco plants by slowing down the initial appearance of blight symptoms by 100–162%, reducing the symptom index of 56–63%, and increasing the dry weight of plant biomass 39–53% compared to tobacco seeds which are only inoculated with *Ralstonia solanacearum*. Inoculation of *T. asperellum* Tc-Jjr-02 at six hours before and after and simultaneously with pathogens were not significantly different in response to plant resistance.

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