

IDENTIFICATION OF MICROBES ANTAGONISTIC AGAINST *Fusarium oxysporum* ISOLATED FROM RHIZOSPHERE ZONE OF WATERMELON

IDENTIFIKASI MIKROBA ANTAGONIS *Fusarium oxysporum* YANG DIISOLASI DARI RHIZOSFER TANAMAN SEMANGKA

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ABSTRACT

This research was aimed to isolate and identify microbes which antagonistic against *Fusarium oxysporum*, the causative agent of vascular wilt in watermelon plants. The antagonistic microbes were isolated from soil samples collected from rhizosphere of watermelon farm located at west Sanur village, South Denpasar, Bali. Isolation of fungi and bacteria were conducted on potato dextrose agar medium (PDA) and nutrient agar medium (NA), respectively. Fungal isolates were then observed under light microscope for its morphological characteristics before identification using a reference book. Bacterial isolates were characterized using various tests, such as gram stain reaction, existence of endospores, catalase reaction, and ability to ferment various sugars. Their characteristics were then compared with those described in a reference book. Two fungal species (*Trichoderma harzianum* and *Trichoderma viride*) and two bacterial antagonists (*Pseudomonas* sp. and *Bacillus* sp.) were found to have potential to be developed as biocontrol agents to inhibit the growth of *F. oxysporum*.

Keywords: *Bacillus* sp., *Pseudomonas* sp., *Trichoderma harzianum*, *Trichoderma viride*, Bali, Watermelon

INTISARI

Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi mikroba yang bersifat antagonis terhadap *Fusarium oxysporum* yaitu penyebab penyakit layu vaskular pada tanaman semangka. Mikroba antagonis diisolasi dari sampel tanah yang diambil dari rhizosfer tanaman semangka yang ditanam di perkebunan yang berlokasi di desa Sanur Barat, Denpasar Selatan, Bali. Isolasi fungi dilakukan pada *dextrose agar medium* (PDA) dan isolasi bakteri dilakukan pada *nutrient agar medium* (NA). Isolat fungi diamati dengan mikroskop cahaya untuk mengetahui ciri-ciri morfologi sebelum diidentifikasi menggunakan buku reference. Isolat bakteri dikarakterisasi menggunakan beberapa uji, meliputi, reaksi pewarnaan gram reaksi katalase dan kemampuan untuk melakukan fermentasi terhadap gula. karakteristik bakteri yang diamati kemudian dibandingkan dengan karakteristik bakteri yang tertera dalam buku reference. Dua spesies fungi (*Trichoderma harzianum* dan *Trichoderma viride*) dan dua spesies bakteri (*Pseudomonas* sp. dan *Bacillus* sp.) yang berpotensi untuk dikembangkan menjadi agen biokontrol penghambat pertumbuhan *F. oxysporum* berhasil diisolasi dalam penelitian ini.

Kata kunci: *Bacillus* sp., *Pseudomonas* sp., *Trichoderma harzianum*, *Trichoderma viride*, Bali, Semangka

INTRODUCTION

According to report Indonesian Statistical Agent in 2011, watermelon production in Indonesia reached 474,327 tons in 2009. However, production of this fruit decreased sharply in 2010 where its production was only 348,631 tons. The main cause of this decrease was attributed by vascular wilt due to *Fusarium oxysporum* infection (Budiastuti *et al.*, 2012). This fungus is a *soil borne pathogen* causing many severe problems in many agricultural crops, including water melon. Plants infected by this fungus will show specific characteristics, such as stem rot with brown color and drying leaves. Until

recently, watermelon farmers in west Sanur area rely only on the use of chemical-based fungicides to cope with this infection. Long term application of these fungicides has been claimed to have negative impact on ecosystems due to its long half-life (Djunaedi, 2009).

Based on the above background, a new and more environmentally friendly method, such as the use of natural enemy for this pathogen needs to be developed. Therefore in this research, microbes antagonistic against *F. oxysporum* were isolated from rhizosphere zone of watermelon farm located in West Sanur area. The main

objective of this research was to obtain potential antagonist isolates to be used to fight *F. oxysporum* infection in watermelon plants.

MATERIALS AND METHODS

Sample collection

Fungal and bacterial antagonists were isolated from soils collected randomly from rhizosphere zone of watermelon farm in west Sanur area. Soil samples (100 g each) were aseptically collected from 6 locations in this area, placed in plastic bags, and transported to Microbiology Lab in School of Biology, Udayana University where isolation of microbes antagonistic against *Fusarium oxysporum* was conducted.

Isolation of antagonists

Fungal and bacterial antagonists were isolated by applying dilution and spread method as specified in Ramona and Line (2002), Ramona (2005) Suryanti *et al.* (2013), and Wulansari *et al.* (2015). Samples were diluted up to dilution rate of 10^{-6} and spread on potato dextrose agar medium (PDA) for fungal isolation or on nutrient agar medium (NA) for bacterial isolation. All inoculated media were incubated at ambient temperature for 2-5 days until bacterial or fungal colonies grew. Colonies that showed antagonistic activities against *F. oxysporum* in *in vitro* dual culture assays were isolated and identified at least up to genus level.

Identification of bacterial and fungal antagonists

Fungi and bacteria that showed antagonistic activities against *F. oxysporum* in the *in vitro* assays were identified up to genus or species level when necessary based on their morphological and physiological characteristics. Fungal isolates were observed under a light microscope for their hypha morphology, conidiophore, their spore structures, and colour on PDA. Their characteristics were then compared with that specified in a reference book of *Fungi and Food Spoilage* (Pitt and Hocking, 1997). For bacterial identification, their characteristics observed included gram stain reaction, endospore existence, catalase reaction, ability to ferment various sugars (glucose, lactose, maltose and sucrose), reaction on indol, and motility on SIM medium. The characteristics of each bacterial isolate were then compared with that specified in reference book of *Bergey's Manual of Determinative Bacteriology 9th Edition* (Holt *et al.*, 1994).

RESULTS

Two potential fungal and two bacterial antagonists were successfully isolated in this project. The microscopic and macroscopic (visualized with a Yazumi light microscope

with 400 x magnification) characteristics of *T. Harzianum* and *T. viride* at 5 days of aged on PDA medium are shown in Figure 1 and the microscopic characteristic of these two bacterial isolate is shown in Figure 2.

DISCUSSION

The two potential fungal antagonists were identified as *Trichoderma harzianum* and *Trichoderma viride*. The mycelium of the *Trichoderma harzianum* was initially white, and as a function of time it changed to green with grey pigmentation. Their Fialid grew on each branch tip where their clustered konidia with pale green were found. The size of their konidia, the length of their fialid, and the diameter of their hyphae ranged 2.63-3.07 μm , 7.28-8.54 μm , and 2.67-3.71 μm , respectively. Those characteristics matched with those specified in the identification reference book of *Fungi and Food Spoilage* (Pitt and Hocking, 1997). While the *Trichoderma viride* initially had pale green colonies and then changed into dark green. Their konidia are round and green, and located on the tips of their fialid. The size of their konidia, the length of their fialin, and the diameter of their hyphae ranged from 3.58 to 5.46 μm , from 7.62 to 8.46 μm , and from 2.60 to 2.74 μm , respectively. According to Pitt and Hocking (1997), both *T. viride* and *T. harzianum* are morphologically similar. The difference between them is the size of their konidia, where the size of *T. viride* konidia is bigger (ranging from 3.5 to 5.5 μm) than that of *T. harzianum*. The microscopic and macroscopic (visualized with a Yazumi light microscope with 400 x magnification) characteristics of *T. harzianum* and *T. viride* at 5 days of aged on PDA medium are shown in Figure 1.

T. harzianum and *T. viride* successfully isolated in this project were found to dominate the six locations of sampling points in the watermelon farm, in west Sanur area. This results is in line with that reported by Otadoh (2011) who stated that *Trichoderma* spp. are saprophytic fungi widely spread in soil samples. This type of fungi can grow rapidly in the rhizosphere zone of plants (Saba *et al.*, 2012). When grown on a medium *in vitro*, their growth is characterized by green konidia (Steyaert, 2007).

Fungi *Trichoderma* spp. has ability to produce cell wall degrading enzymes, such as cellulase (Raut *et al.*, 2014), chitinase (Sharma *et al.*, 2012), and glucanase (Saba *et al.*, 2012). These enzymes help their hyphae to penetrate lumen and assimilate the cell wall of fungal pathogens. Furthermore, Sharma *et al.* (2012), Hassan *et al.* (2013) and Benitez *et al.* (2004) reported that *Trichoderma* spp. also has the ability to produce antibiotics, such as harzianic acid, alamethicins, tricholin, peptaibols, alkil piron, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid, inhibiting the growth of other fungi around them.

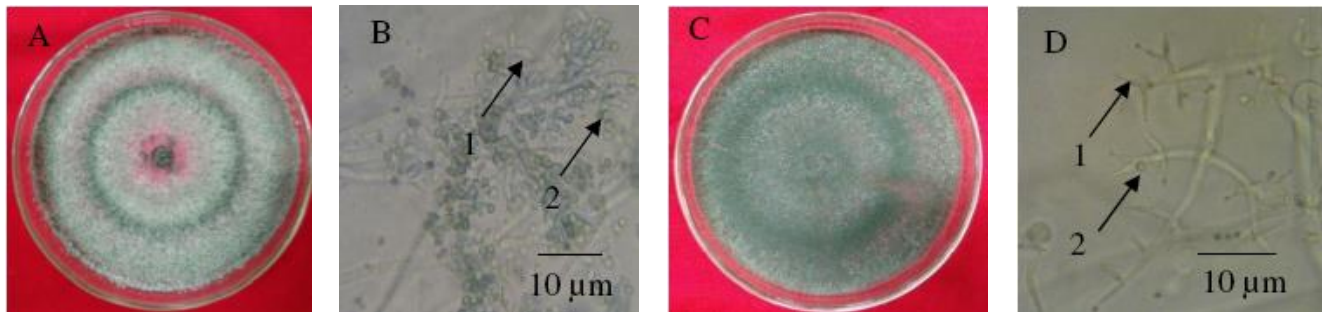


Figure 1. Macroscopic and microscopic characteristics of *T. harzianum* (A and B) and *T. viride* (C and D). Their Fialid and Konidia are shown by numbers 1 and 2, respectively.

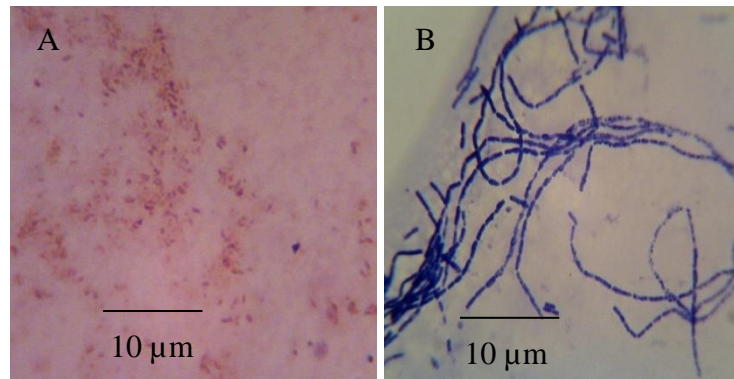


Figure 2. Microscopic characteristic of *Pseudomonas* sp. (A) and *Bacillus* sp. (B) observed under a light microscope (Yazumi) with magnification of 1000x

In addition to hydrolytic enzymes and antibiotics, some species of *Trichoderma* also produce *siderophores* for chelating Fe^{3+} ions in the soil so that the availability of these ions is limited for fungal pathogens (Benitez *et al.*, 2004). Due to those characteristics, many species of *Trichoderma* spp. have been developed as biocontrol agents to control infections of *Fusarium* (Ojha and Chatterjee, 2012), *Rhizoctonia* (Seema and Devaki, 2012), and *Phytophthora* (Raut *et al.* 2014).

This research showed two bacterial antagonists were successfully isolated in this and they were identified as *Pseudomonas* sp. and *Bacillus* sp., based on the characteristics specified in the reference book of *Bergey's Manual Determinative Bacteriology 9th Edition* (Holt *et al.*, 1994). *Pseudomonas* sp. isolate has greenish yellow pigment when grown on a solid medium *in vitro*. Other characteristics of this isolate observed in this research were Gram negative with cell size ranging from 0.86 to 1.91 x 0.2 to 0.67 μm , no endospore, catalase positive, ferments sugars (glucose, maltose, and sucrose), motile on SIM medium, unable to ferment laktosa, and showed neative result for indol test. Whilst, *Bacillus* sp. isolate showed some characteristics, such as Gram positive, chain cells with size of 2.44 to 2.90 x 0.61 to 1.73 μm , produced endospore, catalase positive, fermented sugars (glucose, maltose, and sucrose), motile on SIM medium, negative indol test, and did not ferment lactose. The

microscopic characteristic of these two isolate is shown in Figure 2.

In the *in vitro* bioassays, these two bacterial antagonists inhibited the growth of *Fusarium oxysporum* (the causative agent of wilt in watermelon plant). The main cause of this inhibition was inconclusive and need to be further elucidated. Although it was inconclusive, some researchers reported that inhibition of *Pseudomonas* or *Bacillus* isolates may be due to sideropore production (Karimi *et al.*, 2012), antibiosis (Sallam *et al.* 2013), competition (Jenifer *et al.*, 2013), or other toxic compounds, such as HCN (Chaur and Lo, 1998). Ozaktan *et al.* (2015) and Vijayaraghavan and Vincet (2012) stated that antibiosis mechanism by *Pseudomonas* sp. and *Bacillus* sp. on fungal pathogens can happen through production of hydrolytic enzymes, such as cellulase, glucanase and chitinase. Toua *et al.* (2013) and Chaur and Lo (1998) reported that *Pseudomonas* sp. are also capable to produce antibiotics, such as phenazines, pyrrolnitrine and 2,4-diacetylphloroglucinol, in addition to the above hydrolytic enzymes.

CONCLUSION

Based on the morphological and physiological characteristics of the isolates, the two fungal and bacterial isolates were identified as *T. harzianum*, *T. viride* and *Pseudomonas* sp. and *Bacillus* sp. Those microbes appeared to be potential for bio control agent development to control the causative agent of wilt vascular disease in watermelon farm.

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