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Effectiveness of Endophytic Bacteria from Local Tomato Plants Against Wilt Disease Caused by *Fusarium oxysporum*

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Abstract

Fusarium wilt is a plant disease caused by *Fusarium oxysporum* that generates significant economic loss to crops. A method to sustainably control *F. oxysporum* is utilizing biological agents, such as endophytic bacteria. Therefore, this study aimed to isolate endophytic bacteria from tomato plant tissue, which could inhibit the pathogen of wilt disease (*F. oxysporum*). Endophytic bacteria were isolated from local tomato plants in Muna Regency, Indonesia. Morphological characteristics such as size, shape, color and height of bacterial colonies were then determined by Gram staining using potassium hydroxide (KOH). Endophytic bacterial isolates were evaluated for their ability to inhibit *F. oxysporum* through inhibition and hydrogen cyanide (HCN) production tests. Subsequently, analysis of variance was used to determine whether endophytic bacteria inhibited *F. oxysporum* growth, and if there was a significant effect, Duncan's test was conducted at 95% significance. HCN production was observed through qualitative methods. The results showed that four endophytic bacteria isolates, namely LBR I A03, SWR II B04, SDM II B05 and SWR I A02 inhibited the growth of *F. oxysporum* by more than 50%. It also revealed that four endophytic bacterial isolates were strong HCN producers and two were weak producers. Therefore, isolates showing antifungal activity in this study can be used as biopesticide agents to induce plant resistance to *F. oxysporum*.

Keywords: antagonistic bacteria; biocontrol; biopesticide; endophytes; HCN production

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INTRODUCTION

The tomato plant is a horticultural product with a high economic value that is widely farmed and consumed as one of the food sources worldwide. Tomato fruit is rich in nutrients and is used as an anticancer and anti-oxidative agent because it contains lycopene and flavonoids. This plant is auspicious (Abdel-Aziz et al., 2021), and due to its rich source of vitamins A and C, it is also one of the essential crops in the world (Alenazi et al., 2020; Çolak et al., 2020). The global cultivation area is 5.8 million ha, with a production of 244 million tons (Zia et al., 2021). Meanwhile, tomato production in Indonesia remains relatively low at 6.3 tons ha^{-1} when compared to Taiwan, Saudi Arabia and India, which produce 21, 13.4 and 9 tons ha^{-1}, respectively (Alwi et al., 2022).

Several pathogens significantly impact tomato plant production, including *Fusarium oxysporum*,

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which causes Fusarium wilt disease leading to suboptimal results (Rojas et al., 2020; Abdel-Aziz et al., 2021; Khalil et al., 2021; Bahroun et al., 2021; Másmela-Mendoza and Moreno-Velandia, 2022). Furthermore, *F. oxysporum* can cause 70% to 90% damage in infected tomato plants and up to 50% production losses (Jamil et al., 2021). The fungus damages plant tissues by blocking water and nutrient transportation in plants, ultimately resulting in permanent wilting and death (Hadiwiyono et al., 2020; Patel et al., 2022). Plants attacked by *F. oxysporum* appear wilted during the day, and the lower leaves of the plant turn yellow and wither at a later stage. Over several days, this symptom progresses to the top of the plant, and it eventually dries up and dies, resulting in a drop in tomato production (Vinchira-Villarraga et al., 2021).

Fusarium infection is difficult to control because it is soil-borne, lives deep within the host tissue, and survives for extended periods (Bakker et al., 2020; Jamil et al., 2021). The pathogen, *F. oxysporum* can survive in the form of conidia for years and the spores can rapidly disseminate over large areas, causing an epidemic in plants (Patel et al., 2022). Prevention and control of *F. oxysporum* have generally been carried out through pest-resistant varieties, crop rotation and synthetic fungicides (Dukare and Paul, 2021). However, producing pest-resistant varieties is usually tricky due to the lack of dominant genes (Kamilova et al., 2006). The use of synthetic fungicides causes environmental pollution, interference with non-target organisms and resistance of pathogens to fungicides (Devi et al., 2022). Farmers have traditionally used synthetic fungicides such as carbendazim and benomyl to inhibit fusarium wilt disease (Yadav et al., 2018; Jamil et al., 2021; Attia et al., 2022). However, long-term use of synthetic fungicides on agricultural land can disrupt the balance of the ecosystem (Gutomo, 2007; Dukare and Paul, 2021) and produce residues that harm the environment and other organisms (Soesanto et al., 2020; Mon et al., 2021). Therefore, using endophytic bacteria as biopesticide agents is a promising alternative because they can induce plant resistance to pathogens and can be used in integrated agricultural activities to reduce pesticide use.

Biological control of pathogens is a non-chemical approach to plant disease management (Prasada and Masyhuri, 2020; Li et al., 2021; Listyowati et al., 2022). Endophytic bacteria act as biopesticide agents that stimulate and activate host plant responses more effectively compared to non-symbiotic plants (Card et al., 2015; Al Viandari et al., 2022). Furthermore, the bacteria inhabit the host plant tissue without causing disease symptoms in the host plant. In many hosts, endophytic bacteria act as plant growth promoters (Egamberdieva et al., 2017), induce plant resistance to drought (La Fua et al., 2021), and are biocontrol agents (Munakata et al., 2021). Several studies have reported that these bacteria can be useful in disease control. Endophytic bacteria such as *Lysinibacillus* sp. and *Paenibacillus dendritiformis* have been demonstrated to stimulate development in plants infected with *Rhizoctonia solani* and *Fusarium* sp. (Pal et al., 2022). Endophytic bacteria isolated from *Cicer arietinum* L. have also been shown to inhibit the growth of *F. oxysporum* f.sp. *radicis-lycopersici*. Furthermore, bacteria such as *Pseudomonas* sp. (Kamilova et al., 2006) and *Bacillus* spp., have demonstrated the ability of endophytic bacteria to produce antifungal chemicals against pathogenic fungi (Devi et al., 2022).

According to Duong et al. (2021), three of 50 isolates of endophytic bacteria from coffee plants in Vietnam demonstrated significant control over the growth of *F. oxysporum*, with an inhibition rate of more than 40%. Moreover, Jie et al. (2009) revealed that indigenous endophytic bacteria isolated from banana plants have the potential to control *F. oxysporum* f.sp. *cubense*, the causal agent of wilt disease in banana plants in the Guangdong region of China, with a 67% suppression rate. Previous similar results were reported by Chowdhury and Bae (2018); Verma and White (2018); Yanti et al. (2018). However, endophytic bacteria from local tomato plants have not been extensively investigated.

In this study, endophytic bacteria were isolated and their ability to inhibit the growth of *F. oxysporum* from local tomato plants in Southeast Sulawesi was evaluated. Southeast Sulawesi is located in the geographical area of Wallacea, with unique flora, fauna and microorganisms, including endophytic bacteria whose activities are strongly influenced by geographical location, genotype and soil properties, which have not been extensively studied. Exploration of endophytic bacteria in Southeast Sulawesi tomatoes is essential to determine the diversity of these bacteria that act as biocontrol agents in inhibiting and controlling

F. oxysporum. Therefore, this study aimed to isolate and identify indigenous endophytic bacteria that inhibit Fusarium wilt disease.

MATERIALS AND METHOD

Study area

The study was carried out from January to September 2021 at the Integrated Laboratory of the Institut Agama Islam Negeri (IAIN) Kendari, the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, and the Agrotechnology Laboratory of the Faculty of Agriculture, Universitas Halu Oleo, Kendari City, Indonesia. Kendari is located in the southern hemisphere, between Latitude 3º54'3" to 4º3'11" South and Longitude 122º23' to 122º39' East. It has only two seasons, which include the dry and rainy seasons. Furthermore, the maximum and minimum air temperatures are 33 ºC and 23.1 ºC, respectively, with an average humidity of 83.67% (Santi et al., 2019).

Tomato plant sampling

Endophytic bacteria were collected in Muna Regency, Southeast Sulawesi, Indonesia. Muna Regency is located about 300 m above sea level at Latitude 4º06 to 5.15º South and Longitude 120.00º to 123.24º East. The minimum and maximum temperatures are 25.5 ºC and 28.6 ºC, respectively, with rainfall of 155.6 mm, and humidity of 85.4%. The local tomato plants used were healthy and mature plants (about 3 to 4 months old) with erect stems, green and fresh leaves, no infection, and no wilting (Singh et al., 2018). The utilization of mature plants was due to the development of endophytic bacteria in the vascular tissue. Plants were collected by pulling up to the plant roots, about 15 to 20 cm deep. The samples obtained, consisting of roots and stems of tomato plants, were placed in closed plastic and labeled. Furthermore, plastics containing tomato plants were placed in an ice box before being transported to the laboratory. The tomato plants were then cleaned in the laboratory with running water and separated into roots and stems before cutting to a size of 5 to 10 cm. The plant samples were then prepared for the isolation of endophytic bacteria (Kollakkodan et al., 2021).

Isolation of endophytic bacteria

Endophytic bacteria were isolated after carefully cleansing and drying the roots and stems for 30 minutes. The roots were then weighed up to 1 g, and the plant surface was sanitized with 70% alcohol for 3 minutes, followed by 4% NaOCl solution for 3 minutes, and then rinsed thrice with sterile distilled water. The roots were disinfected with 0.1 cc of distilled water and placed in 5% tryptic soy agar (TSA, Merck, Darmstadt, Germany) to determine the effectiveness of the surface sterilization. Subsequently, scratched roots and stems on 5% TSA medium were pulverized in a mortar and serially diluted to a 10^{-10} dilution. The 10^{-8} and 10^{-10} dilutions were distributed in a petri dish with a volume of 50 l of TSA medium. The suspension was incubated for two days before the growth of colonies was observed. Furthermore, colonies with distinct morphologies were further segregated. A single bacterial colony or pure culture was obtained by regularly isolating bacterial colonies from culturse media, storing them in an Eppendorf tube containing 0.9 ml of sterile 15% glycerol solution, and then storing them in a freezer at -20 ºC (Munif et al., 2012).

Isolation of *F. oxysporum*

F. oxysporum was isolated from Fusarium-infected tomato plants. The contaminated part, including the leaves, stems, or roots was isolated by severing it into 1 x 1-centimeter pieces. The fragments were then placed on potato dextrose agar (PDA, Himedia, India) and cultured for 4 to 5 days at room temperature. The morphological characteristics of the target pathogen were studied under a microscope and adapted to the growing mycelium before being isolated in PDA (Ayele et al., 2020).

Endophytic bacteria inhibition

Endophytic bacteria were tested *in vitro* for their ability to inhibit *F. oxysporum* by using dual culture in the PDA medium. A pathogenic hyphae-covered PDA medium with a diameter of 0.5 cm was used to inoculate a petri dish containing a new PDA medium. The inoculum pieces were placed 3 cm from the edge of the petri dish and incubated for 48 hours at a temperature of 26 to 28 ºC. The examined endophytic bacterial isolates were streaked longitudinally 3 cm from the edge of the cup in the opposite direction of the pathogen. Furthermore, observations on petri dishes were carried out on days 5 and 7 after inoculation with the Equation 1:

$$P = \frac{R1\text{-}R2}{R1} \times 100\% \qquad (1)$$

Where: P = the growth inhibition (%), R1 = the radius of *F. oxysporum* in the absence of bacteria (cm), R2 = the radius of *F. oxysporum* with test bacteria (cm) (Wiratno et al., 2019).

Gram test (KOH)

Endophytic bacteria isolates were implanted on an object glass containing 1 to 2 drops of 3% KOH using an inoculation loop. The KOH was thoroughly mixed with the bacterium, and the mucus in the inoculation loop suggested a Gram-negative (-) response (Wiratno et al., 2019).

Hydrogen cyanide (HCN) production

The ability of endophytic bacterial isolates to produce HCN was evaluated using the technique developed by Alstrom and Burns, which involved culturing the isolates in petri dishes in a glycine medium. The middle of the lid was covered with filter paper saturated with a solution of picric acid consisting of 2 g picric acid, 8 g sodium carbonate, and 200 ml of aquadest. Furthermore, a change in the color of the filter paper from yellow to brownish orange suggested the production of HCN by endophytic bacteria (Alstrom and Burns, 1989).

Data analysis

The analysis of variance was used to examine the ability of endophytic bacteria to prevent the growth of *F. oxysporum*. Furthermore, Duncan Multiple Range Test (DMRT) with 95% significance was conducted if a significant effect was found. The qualitative data was then visually observed and descriptively assessed.

RESULTS AND DISCUSSION

Endophytic bacteria inhibition

F. oxysporum is one of the pathogens that negatively impact all phases of plant growth. Controlling Fusarium wilt is difficult because this pathogen is soil-borne (Haas and Défago, 2005). Endophytic bacteria are considered a promising biocontrol for sustainable agricultural development (Aldinary et al., 2021). Twelve isolates of endophytic bacteria were successfully isolated from local tomato plants' root and stem tissues. These bacteria were tested for their ability to inhibit *F. oxysporum* growth. Table 1 shows that all endophytic bacterial isolates could inhibit *F. oxysporum* growth and had a significant effect 5 and 7 days after inoculation (DAI).

The 12 isolates of endophytic bacteria from local tomato plants inhibited *F. oxysporum* on the 5th and 7th DAI. On the fifth DAI, the LBR I A03 treatment had the highest inhibitory power (46.90%). Table 1 shows that this result was not significantly different from the SDM II B05 and SWR I A02 treatments, with percentage inhibition of 43.87% and 41.02%, respectively. LBR I A03, SDM II B05 and SWR I A02 showed the highest inhibition of the mycelium growth of *F. oxysporum*. These observations indicate that endophytic bacteria isolated from local tomatoes can function as biocontrol agents against *F. oxysporum*. Inhibition by endophytic bacteria may be attributed to its ability to produce antibiotic or antimicrobial substances. One of its characteristics that act as antagonist is the production of antibiotic compounds that can inhibit pathogen growth (Ali et al., 2020). These results indicate that endophytic bacteria can inhibit the pathogen *F. oxysporum* directly. Additionally, the metabolites they produce can diffuse into the culture medium and suppress the growth of *F. oxysporum*, forming an inhibition zone. The inhibition zone formed indicated an antifungal production by endophytic bacteria. This study is consistent with the findings by Card et al. (2015) who stated that endophytic bacteria exhibit lytic activity and inhibit the growth of many pathogens.

On observation, 9 endophytic bacteria inhibited the growth of *F. oxysporum* on 7 DAI. SDM II B05 (53.37%) had the highest percentage inhibition, which was not significantly different from LBR I A03 (52.56%), SWR II B04 (51.54%) and SWR I A02 (50.16%) but significantly different from other treatments. Furthermore, SDM II B05, LBR I A03, SWR II B04 and SWR I A02 inhibited the growth of *F. oxysporum* above 50%. Similar findings were reported in the bacterium *Pseudomonas* sp. NS-1 and *Bacillus* sp. NS-22, which inhibited *F. oxysporum* germination (Dukare and Paul, 2021). The ability of endophytic bacteria to inhibit infections is attributed to their ability to produce cell wall-degrading enzymes and secondary metabolites. Fungal cell walls play a crucial role in cell division, hyphae growth and resistance to environmental stress. The mycelium appearance and the function of the fungus will be altered after cell wall disintegration (Kong et al., 2020).

Table 1. The ability of endophytic bacteria to inhibit the growth of *F. oxysporum*

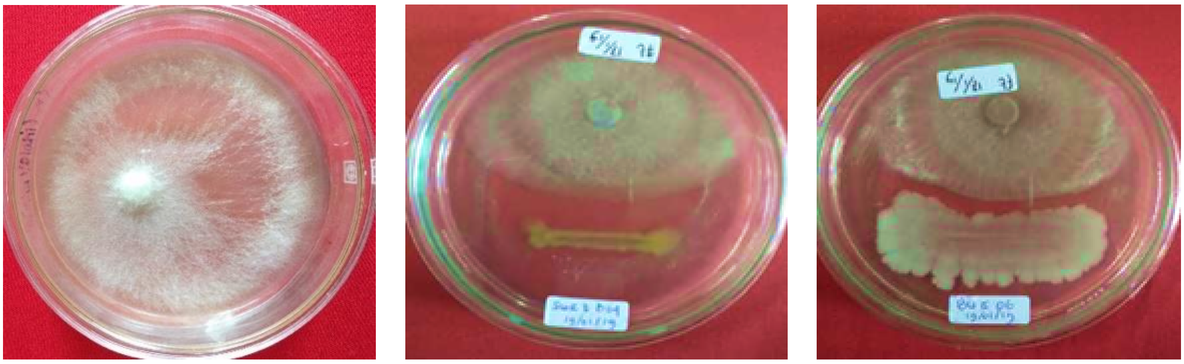
Isolate code	Isolation source	Gram strain	Isolate code	Inhibition (%)	
				5 DAI	7 DAI
LBR I A03	Root	-	LBR I A03	46.90^{a}	52.36^{a}
SWR II B04	Stem	-	SWR II B04	36.83^{bcd}	51.54^{a}
KS III A08	Root	+	KS III A08	9.15^{e}	0.00^{e}
BU II 06	Root	-	BU II 06	30.50^{d}	35.37^{d}
SDM II B05	Stem	-	SDM II B05	43.87^{ab}	53.37^{a}
SDM II A03	Root	-	SDM II A03	37.26^{bcd}	44.70^{bc}
SWR I A02	Root	-	SWR I A02	41.02^{abc}	50.16^{ab}
SWR III B02	Stem	+	SWR III B02	29.09^{d}	40.71^{cd}
MO II 02	Root	+	MO II 02	5.66^{e}	0.00^{e}
SDM I A02	Root	+	SDM I A02	4.76^{e}	0.00^{e}
SWR I A05	Root	+	SWR I A05	34.67^{cd}	35.75^{d}
LAK II A02	Root	-	LAK II A02	33.01^{cd}	44.38^{bc}

Note: The numbers followed by different letters in the same column are significantly different at the DMRT test level of $\alpha = 0.05$; (-) = gram-negative bacteria; (+) = gram-positive bacteria

Endophytic bacteria isolated from the roots and stems of local tomato plants can potentially inhibit the growth of *F. oxysporum in vitro*. On the seventh DAI, 9 of 12 endophytic bacterial isolates had significant biocontrol activity ($P < 0.05$). However, three isolates (KS III A08, MO II 02 and SDM I A02) could not inhibit the growth of *F. oxysporum*. This may be due to the inability of the bacterial isolates to suppress pathogen growth during the test period. Kollakkodan et al. (2021) stated that the biocontrol properties of endophytic bacteria are influenced by competition and antibiosis. Furthermore, inhibition can occur through one or several mechanisms that are thought to be defense mechanisms in competing with pathogens, such as the production of HCN compounds (Sehrawat et al., 2022), siderophores, hydrolytic enzymes, and mycoparasites activity (not tested in this study). This finding was also supported by Coombs et al. (2004) who stated that 6 of 17 endophytic bacteria, namely EN2, EN27, EN30, EN35, EN46 and EN60, were unable to inhibit the phytopathogenic fungi tested. This was attributed to the antibiosis compounds produced by endophytic bacteria, which are effective in suppressing pathogens.

The type of gram bacteria can also affect the growth-inhibitory activity of *F. oxysporum*. Bacterial isolates KS III A08, MO II 02 and SDM I A02 were found to be gram-positive. Gram-positive bacteria do not contain endotoxins in their cell walls. Bacterial endotoxins have properties such as heat resistance, the presence of phospholipids and carbohydrates (lipopolysaccharides), and the inability to be quickly neutralized by antitoxins (Validov et al., 2007). SDM II B05, LBR I A03, SWR II B04 and SWR I A02 were classified as gram-negative and had endotoxins in their cell walls, suggesting that they could inhibit the growth of *F. oxysporum*. This is consistent with the result of Prasetya et al. (2018), reporting that bacteria isolates AA2, AA8, AA9 and AA10 were gram-negative and produced chitinase enzymes to inhibit the growth of *F. oxysporum*, compared to AA7 bacteria which was gram-positive and could not inhibit fungi growth.

Figure 1 shows that the inhibition zone diameter of LBR I A03, SWR II B04, SDM II B05 and SWR I A02 was greater than 50%. The antibiosis mechanism formed a clear zone between the bacterial colonies and *F. oxysporum* when compared to controls. The formation of this clear zone occurred due to the presence of secondary metabolites produced by bacteria, which is a microbial defense mechanism to survive or compete by blocking the growth zone of pathogens (Egamberdieva et al., 2017). The appearance of differences in *F. oxysporum* inhibition by endophytic bacteria was due to the type and amount of antimicrobial compounds (Liu et al., 2020), concentration and quality of antimicrobial compounds (Liu and Zhang, 2021), and the presence of different inhibitory mechanisms against *F. oxysporum* (Devi et al., 2018). Endophytic bacteria can inhibit *F. oxysporum* through one or more mechanisms, which are considered defense mechanisms in competition with microorganisms (Kumar et al., 2020). Therefore, this study showed that endophytic bacterial isolates from local tomatoes effectively inhibited *F. oxysporum*.



a. b. c.

Figure 1. Inhibitory performance of endophytic bacteria on the growth of pathogenic fungal colonies on PDA. (a) Pathogen *F. oxysporum*, (b, c) Antagonistic test of endophytic bacteria isolates against the pathogen *F. oxysporum*

Hydrogen cyanide (HCN) production

Typically, bacterial strains utilized as biocontrols have various mechanisms to inhibit disease growth (Ramette et al., 2003). Endophytes are known to possess biocontrol features such as the generation of HCN, siderophores, hydrolytic enzymes and antibiotics, which are efficient in establishing disease resistance in plants (Sehrawat et al., 2022; Taheri et al., 2022). Several endophytic bacteria isolated from local tomato plants have been identified as HCN producers, and its production is essential for plant disease inhibition (Haas and Défago, 2005; Chaouachi et al., 2021). The isolates of endophytic bacteria that produce HCN were generally about 1 mm in size with various colony colors, including white, milky white and yellowish white. Table 2 shows that these isolates generally had small colonies with convex elevations, smooth edges and round forms. In addition, there were other elevations such as umbonate, plateau, convex and flat.

Six endophytic bacterial isolates produced HCN, namely LBR I A03, LAK II A02, SDM II B05, SDM II A03, SWR I A02 and SWR III B02. HCN production was observed by a color change on the Whatman filter paper from yellow to brown. Based on the difference in the color of the filter paper, the strains were classified as +++ (very strong), ++ (strong) and + (weak) for each color, namely brown, orange-brown and light brown, respectively. From the results, two isolates changed the color of the filter paper to light brown, four isolates produced an orange-brown color, and three isolates did not show any color change. This color change occurred due to the presence of picric acid, which reacts with free cyanide produced by bacteria present on the filter paper (Pathak et al., 2021). Furthermore, six HCN-producing isolates inhibited *F. oxysporum* growth by 40.71% to 53.37%. The effectiveness of endophytic bacterial isolates in controlling the pathogen *F. oxysporum* is related to its HCN-producing ability (Aydi-Ben-Abdallah et al., 2020; Bahmani et al., 2021). HCN is a volatile secondary metabolite synthesized by many bacteria (Attia et al., 2022). It inhibits electron transport and interferes with the cell's energy supply, resulting in pathogen growth inhibition (Sehrawat et al., 2022). Cyanide also forms stable complexes with essential elements (Cu^{2+}, Fe^{2+} and Mn^{2+}) that are toxic to most living organisms (Blumer and Haas, 2000). The production of HCN by LBR I A03 and LAK II A02, SDM II B05, SDM II A03, SWR I A02, and SWR III B02 contributed to the suppression of pathogens. This is possible because HCN inhibits *F. oxysporum* by disintegrating cellular structures, causing hyphae to lyse, and inhibiting pathogen development, particularly spore growth (Dukare et al., 2019).

HCN-producing endophytic bacteria showed mixed results in *F. oxysporum inhibition.* LBR I A03 and LAK II A02 were weak producers of HCN, while SDM II B05, SDM II A03, SWR I A02 and SWR III B02 were strong producers. This difference was due to the concentration and quality of HCN compounds produced after the bacteria reached the stationary phase (Ramette et al., 2003). HCN is released in the stationary phase, as a secondary metabolic product by microorganisms, which affects organisms by inhibiting cytochrome oxidase-mediated ATP synthesis (Sehrawat et al., 2022). HCN produced by endophytic bacteria can lyse some parts of the cell wall of pathogenic fungi to inhibit the spread of pathogens.

Table 2. Morphological characteristics and HCN production of endophytic bacterial isolates from local tomato plants

Isolate code	Characteristic colony				HCN production	
	Color	Form	Elevation	Size		
LBR I A03	White	Round	Flat	Small	+	Light brown
SWR II BO4	White	Round	Plateau	Large	-	Yellow
BU II 06	White	Irregular	Umbonate	Large	-	Yellow
SDM II B05	Yellow	Irregular	Convex	Small	++	Orange brown
SDM II A03	Yellow	Round	Flat	Small	++	Orange brown
SWR I A02	White	Round	Convex	Small	++	Orange brown
SWR III B02	White	Round	Convex	Small	++	Orange brown
SWR I A05	White	Irregular	Plateau	Small	-	Yellow
LAK II A02	White	Round	Plateau	Small	+	Light brown

Notes: (-) = Does not produce HCN; (+) = weak HCN production; (++) = strong HCN production

SDM II B05 was the most effective in inhibiting *F. oxysporum* growth due to its consistent inhibition at 53.37%. HCN production was also higher than that of the other isolates. The efficiency of SDM II B05 against the pathogen *F. oxysporum* demonstrated its potential as a biocontrol agent. Furthermore, specific secondary metabolites produced by some isolates of endophytic bacteria from local tomato plants must be identified to establish their importance in plant defense against pathogenic diseases.

CONCLUSIONS

Endophytic bacteria isolated from tomato plants have the potential to induce plant resistance in *F. oxysporum*. Four endophytic bacterial isolates, namely isolate LBR I A03, SWR II B04, SDM II B05 and SWR I A02, were able to inhibit *F. oxysporum* growth by over 50%. Furthermore, 4 endophytic bacterial isolates were strong producers of HCN, while 2 were weak producers. This research requires further identification and characterization to confirm the promising effects observed *in vitro* through plant experiments, which will lead to biopesticides development to increase agricultural crop productivity.

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