CHILEAN JOURNAL of AGRICULTURAL RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Microbial antagonism of *Pseudomonas fluorescens* and *Bacillus subtilis* to overcome *Ralstonia solanacearum* in potato seed production with an aeroponic system in Indonesia

Meksy Dianawati[1*], Hanudin Hanudin[1], Kiki Kusyaeri Hamdani[1], Wakiah Nuryani[1], Ineu Sulastrini[1], Yati Haryati[1], Ika Cartika[1], Indijarto Budi Rahardjo[1], Agus Muharam[1], and Ruth Feti Rahayuniati[2]

[1]National Research and Innovation Agency, Research Center for Horticulture, Cibinong 16911, Indonesia.
[2]Universitas Jenderal Soedirman, Faculty of Agriculture, Purwokerto 53123, Indonesia.
[*]Corresponding author (meksyd@yahoo.com).
Received: 1 February 2024; Accepted: 30 April 2024, doi:10.4067/S0718-58392024000400500

ABSTRACT

Potato (*Solanum tuberosum* L.) seed production using the aeroponic system has been widely implemented in Indonesia. However, aeroponic systems in tropical areas such as Indonesia faced obstacles in the form of high wilt attacks caused by the bacteria *Ralstonia solanacearum*. This research aimed to control *R. solanacearum* wilt disease in an aeroponic system using various microbes. The research was carried out from September 2022 to January 2023. This research consisted of four stages, namely exploration and isolation of microorganisms, identification of microbial antagonism, testing of in vitro potential microbial antagonism and testing of selected microbial antagonism in the aeroponic system. The nutrients used in the aeroponic system contained *R. solanacearum* with a concentration of 10^4 CFU mL^{-1}, while the concentration of the microbial solution used was 10^8 CFU mL^{-1}. *Pseudomonas fluorescence* was consistently the best microbe both in vitro and in aeroponics, whereas *Bacillus subtilis* could only overcome wilt in vitro and could not overcome wilt in aeroponic system. *Pseudomonas fluorescence* required 5 min soaking time and could increase wilt with increasing soaking time. The consortium between *B. subtilis* and *P. fluorescence* with 10 min soaking time could overcome wilt by 85% and increase the number of tubers and tuber weight by 79% and 85%, respectively, so this prospective microbial consortium could be applied to aeroponic systems environmentally friendly in tropical areas with high *R. solanacearum* opportunities.

Key words: Aeroponic, bacterial consortium, bacterial wilt, potato seed, soaking time, *Solanum tuberosum*.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are a leading horticultural commodity in the world (Osei et al., 2022) and Indonesia (Dianawati et al., 2023), which are a source of carbohydrates, protein, minerals and vitamins. Per capita potato consumption in Indonesia increased, due to the increasing small food industry made from potatoes and changes in potato consumption patterns as an alternative staple food (Dianawati and Yulyatin, 2021). However, the high national potato consumption per year is not yet supported by the national potato production capacity. Potato productivity in Indonesia in 2022 was 19.6 t ha^{-1} lower than the potential research results for potatoes of 25 t ha^{-1}, partly because of the low use of quality potato seeds (Dianawati et.al., 2023).

Potato seed production using the aeroponic system has been widely implemented in Indonesia, both by seed farmers, government seed centers, and the potato seed industry because of its higher production, healthier seed, and more efficient than other hydroponic and conventional systems. Its higher production is due to efficient nutrient absorption, tuber harvesting repeatedly, high stolon growth and development, and easy plant control (Dianawati et al., 2013; Raei et al., 2017), so that the use of aeroponic systems in the potato

seed production is expected to help realize national potato seed self-sufficiency in Indonesia (Dianawati and Wattimena, 2014) and other countries (Wang et al., 2017; Balena et al., 2021).

In its development, aeroponic systems in tropical areas faced obstacles in the form of high levels of wilt attacks caused by the bacteria *Ralstonia solanacearum* (Rs) (Nurbaya et al., 2013; Pathania et al., 2016). Nurbaya et al. (2013) reported that Rs infection in an aeroponic system caused wilting attacks varying between 43% and 100%. *Ralstonia solanacearum* is endophytic and can enter plant tissue without initial symptoms and is easily spread through contaminated water (Yuliar et al., 2015; Manda et al., 2020) in aeroponic nutrition, with closed circulation, so that it is only known when all the plants have wilted and then died (Mateus-Rodriguez et al., 2013). Potato plants infected with Rs in an aeroponic system can still produce, but the seed produced cannot be certified as breeder seed. These conditions have caused several seed breeders, government seed centers, and the potato seed industry in Indonesia to stop producing aeroponic potato seeds and return to implementing a hydroponic substrate system with a lower risk of failure.

Microbial antagonism can be a strategy to prevent Rs wilt in aeroponic systems because microbes can reproduce themselves after being established, self-sustaining, suppress disease in the long term in an environmentally friendly manner (Yuliar et al., 2015) and can be combined with other control methods (Nasir, 2016). Elsayed et al. (2020) stated that microbial antagonism is the best preventive biocontrol in endemic areas that are difficult to control. However, it is necessary to look for microbial antagonism that can live in aeroponic conditions because according to Stegelmeier et al. (2022), microbial antagonism isolated from soil cannot always survive in hydroponic systems due to limited rhizosphere diversity. Nurbaya et al. (2013) used the anaerobic bacteria *Clostridium* sp. to control Rs wilt by 85% in aeroponic system, while Raei et al. (2017), used a combination of *Azospirillum* sp. and *Pseudomonas* sp. bacteria to increase tuber production in an aeroponic system by 33%-54% depending on the strain.

Pseudomonas fluorescence (Pf) and *Bacillus subtilis* (Bs) are massive arsenals of secondary metabolites (Lyng and Kovács, 2023) in the form of aerobic endophytic bacteria which are widely used as biocontrol and biofertilizer in potato (Mamphogoro et al., 2020; Manda et al., 2020) and has potential for use in hydroponic systems (Stegelmeier et al., 2022; Chiaranunt and White, 2023). These two microbes are often associated with nutrient uptake, hormone production, and biocontrol activities improving by competing for nutrients and growth space, antibiosis, parasitism, and induced systemic resistance (Yuliar et al., 2015; Lal et al., 2022). This research aims to control *R. solanacearum* wilt disease in an aeroponic system by using various microbial antagonism at various soaking times.

MATERIALS AND METHODS

The research was carried out from September 2022 to January 2023. This research consisted of four stages, namely exploration and isolation of microorganisms in West Java province, identification of potentially microbial antagonism, in vitro testing of potential microbial antagonism in the Food and Horticultural Crop Protection Center (BPTPH) disease laboratory, Cianjur, and testing of selected microbial antagonist in the aeroponic system in the greenhouse, Potato Seed Center, Bandung, Indonesia.

Exploration and isolation of potential microbial antagonism

The research began with collecting microbial samples either from existing microbial collections or through microbial exploration in the field. Samples were taken from various plant parts, plant hosts, and locations. Isolation from the rhizosphere of soybean and edamame (*Glycine max* (L.) Merr.) plants (edamame is the edible, fresh seed of the soybean plant) using the serial dilution method (Sezen et al., 2016). Meanwhile, isolation of microbes from plantlets and cuttings of potato (*Solanum tuberosum* L.) plantlets as well as rice (*Oryza sativa* L.) stems and leaves were carried out by washing the plant tissue and then drying it in the air on tissue paper. Soil and liquid samples were streaked on nutrient agar (NA) and King's B media, then incubated at room temperature (19-29 °C) for 24 h, then the shape and color of the colonies growing on the media were observed.

Identification of antagonistic microbial potential

Identification of previously isolated microbes was carried out on pure isolates of microbes resulting from exploration. Identification was carried out based on morphological characters according to AlAli et al. (2021), which includes shape, diameter, color, elevation, and edges and biochemistry. Next, gram testing was carried out on all selected isolates.

In vitro microbial antagonism testing

Testing the efficacy of microbial antagonism against *Ralstonia solanacearum* (Rs) in vitro used a completely randomized design with 20 treatments consisting of 18 microbial treatments and two control treatments. The positive control treatment used the antibiotic amoxycillin trihydrate with a concentration of 0.25%, while the negative control used sterile water. Each treatment was repeated twice. The Rs isolate came from the collection of the Disease Laboratory of the Vegetable Crops Research Institute, Indonesia. The test used a misting system that adopted the method of Kawaguchi et al. (2008). All treatments were misted by spraying the Rs bacterial suspension using a perfume sprayer with a concentration of 10^9 CFU mL^{-1}. All treatments were incubated for 24 h then the inhibition zone area was measured using a ruler. Data were analyzed using ANOVA and if significantly different, continued with the Duncan test at a confidence interval level of 95%.

Testing of selected microbial antagonism in aeroponic systems

The experimental design for testing selected microbes in aeroponics used a complete randomized block design with two treatment factors and one control. The first treatment factor was microbial antagonism, namely the four best microbes from in vitro microbial testing. The second treatment factor was the length of microbial soaking, namely 5, 10 and 15 min. The control treatment was without microbial antagonism. The treatment was repeated four times.

The nutrients used in the aeroponic system contain Rs with a concentration of 10^4 CFU mL^{-1}. The density of the microbial suspension used was adjusted to reach 10^8 CFU mL^{-1}. The microbial solution was applied to the roots of the cuttings by soaking them for the soaking time according to the treatment. The injection of the microbial solution was carried out on rockwool buffer media aged 1 and 2 wk after planting (WAP). Microbial solution spraying was carried out when the plants were 3, 4 and 5 WAP.

The research was carried out in a greenhouse with 0.5 mesh walls. The aeroponic tub was made of fiberglass with a width of 1.0 m, a length of 13.0 m, and a height of 0.6 m. The aeroponic tub was coated with Styroform with a thickness of 2.5 cm and covered with black and silver mulch. The 1000 L nutrient drum was connected to the aeroponic tank with a pipe 15 m long and 13 mm in diameter. Each tank consisted of two nutrient pipes with a distance between sprinklers of 60 cm. Nutrients were flowed from the nutrient drum with a plunger pump to be sprayed in a mist onto the roots via a sprinkler with a time span of 3 min of watering and 7 min of stopping. After hitting the roots, the nutrients fell by gravity and returned to the nutrient drum. Nutrients were maintained in the electrical conductivity (EC) range of 1-2 mmho using an EC meter and pH 6-7 using a pH meter.

The seeds used were 'Granola L' potato plantlet cuttings. Nursery by plantlet cuttings in trays used sterilized husk charcoal: Compost (1/1 v/v) planting medium. Two weeks later the seeds were transplanted into aeroponics. One experimental unit measured 1 m × 0.5 m with a planting distance of 20 × 20 cm^2 or 15 plants. Pest and disease control was carried out if there were indications of an attack. Harvest was carried out at 9 WAP.

The observed variable was the number of wilt plants every 2 wk until 8 WAP. The percentage of wilt plants was the percentage of the number of wilt plants compared to the total plants observed. The wilt cumulative percentage was the sum of the percentage of wilt plants up to the last week of observation. Inhibition power was the wilt reduction percentage of the control and treatments divided by the wilt percentage of control. Harvest observations were the fresh weight of stover, roots, tubers and total plants, the number of tubers based on tuber size and total tubers. Ratio of tuber and plant weight was comparison of tuber weight and total plant weight. Tuber size was differentiated based on the size of the tuber, namely small < 5 g, medium 5-10 g, and big > 10 g. Nutrient temperature, temperature and humidity both inside and outside the greenhouse were measured at 12:00 h. Data were analyzed using ANOVA and if there were significant differences, continued with the Duncan test with a confidence level of 95%. Testing between observed variables used the Pearson correlation test and principal component analysis (PCA) test with the RStudio program (PBC, Boston, Massachusetts, USA; http://www.rstudio.com).

RESULTS AND DISCUSSION

In vitro selection of microbial antagonism

Samples were obtained both from collections and exploration results (Table 1). Microbial collections came from the Faculty of Agriculture, Padjajaran University, and from farmers. This collection was the result of exploration in 2015 and has been tested for virulence due to continuous maintenance in storage. Based on the host from exploration results, isolates were taken from various plants such as soybeans, edamame (*Glycine max* (L.) Merr.), potato (*Solanum tuberosum* L.), and rice (*Oryza sativa* L.) (Table 1). Exploration samples came from the rhizosphere, plantlets, plantlet cuttings, stems or leaves (Table 1). Isolate M-18 was a combination of M-16 and M-17. All isolates were taken from three districts, namely Bandung, Cianjur, and Garut, in the West Java province of Indonesia with altitudes ranging between 279 and 1549 m a.s.l. or from six coordinate locations (Table 1).

Table 1. Host plants, origin of isolates, and location of isolate sample collection.

				Location of isolate sample collection			
Isolate code	Collection date	Host plant	Origin of isolate	Regency	Altitude m a.s.l.	Latitude (South)	Longitude (East)
M-1	12-09-2022	*Glycine max* (L.) Merr.	Rhizosphere	Cianjur	279	6°50'12"	107°2.15'48"
M-2	12-09-2022	*G. max*	Rhizosphere	Cianjur	279	6°50'12"	107°2.15'48"
M-3	12-09-2022	*G. max*	Rhizosphere	Cianjur	279	6°50'12"	107°2.15'48"
M-4	12-09-2022	*G. max*	Rhizosphere	Cianjur	279	6°50'12"	107°2.15'48"
M-5	12-09-2022	*G. max*	Rhizosphere	Cianjur	1100	6°45.1081'0"	107°2.78'89"
M-6	12-09-2022	*G. max*	Rhizosphere	Cianjur	1100	6°45.1081'0"	107°2.78'89"
M-7	12-09-2022	*G. max*	Rhizosphere	Cianjur	1100	6°45.1081'0"	107°2.78'89"
M-8	12-09-2022	*G. max*	Rhizosphere	Cianjur	1100	6°45.1081'0"	107°2.78'89"
M-9	12-09-2022	*Solanum tuberosum* L.	Plantlet	Bandung	1549	-7°12'7.067"	107°36'0.918"
M-10	12-09-2022	*S. tuberosum*	Plantlet cuttings	Bandung	1549	-7°12'7.067"	107°36'0.918"
M-11	12-09-2022	*S. tuberosum*	Plantlet cuttings	Bandung	1549	-7°12'7.067"	107°36'0.918"
M-12	12-09-2022	*Oryza sativa* L.	Stem, leaf	Cianjur	301	6°50'14.784"	107°16'25.932"
M-13	12-09-2022	*O. sativa*	Stem, leaf	Cianjur	301	6°50'14.784"	107°16'25.932"
M-14	12-09-2022	*O. sativa*	Stem, leaf	Cianjur	301	6°50'14.784"	107°16'25.932"
M-15 (A)	2015	*S. tuberosum*	Isolate collection from Padjajaran University	Garut	1.389	7°21'50.663"	107°44'4.668"
M-16 (B)	2015	*Bambusa* spp.	Isolate collection from farmer	Garut	1.389	7°21'50.663"	107°44'4.668"
M-17 (C)	2015	*Bambusa* spp.	Isolate collection from farmer	Garut	1.389	7°21'50.663"	107°44'4.668"
M-18 (D)	12-09-2022	*Bambusa* spp.	M16+M17	Cianjur	301	6°50'14.784"	107°16'25.932"

Biochemical test results showed that all microbial colonies tested were 2-3 mm in diameter with a round shape. The edges and elevations of the colonies of isolates M1 to M-16 were slippery and appeared somewhat rough, whereas isolate M-17 has raised and smooth edges. Isolates M1 to M-16 on NA media were cream colored, while M-17 was fluorescent green on KB media (Figure 1); M-1 to M-16 showed a gram positive (+) reaction, while M-17 had a gram negative (-) reaction. Based on morphological observations and biochemical tests, it was suspected that M-1 to M-16 had the same colony shape and color, but were different from M-17. The M-1 to M-16 were suspected to be *Bacillus subtilis* (Bs) because they reacted with positive cells with a cell wall consisting of one homogeneous layer, while M-17 was suspected as *Pseudomonas fluorescence* (Pf) because they reacted with negative cells with a cell wall consisting of three layers. In addition to these characteristics, M17 showed a fluorescent green color (Figure 1) as a specific characteristic of Pf which was thought to be caused by pyoverdines. Lyng and Kovács (2023) stated that pyoverdines was a Fe chelating agent produced by fluorescens group bacteria that grew in media that were poor in Fe ions.

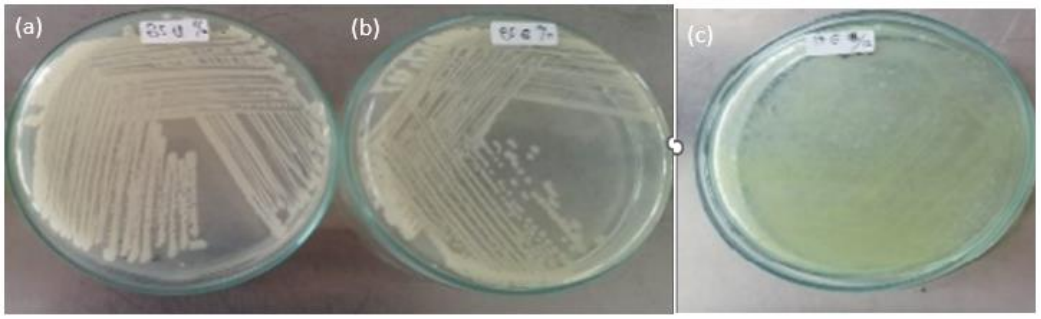

Figure 1. Colony shape and color of M-15 (a), M-16 (*Bacillus subtilis*) (b), and M-17 (*Pseudomonas fluorescens*) (c).

The in vitro antagonism test between the pathogen (Rs) and the test isolate showed that the test isolate was able to significantly influence the growth of the pathogen (Figure 2). The presence of a clear inhibition zone indicated that the tested isolate could produce secondary metabolites that were capable of inhibiting or killing pathogens (Lal et al., 2022) (Figure 3). The highest inhibition zone was M-18 as a combination treatment of Pf and Bs of 1.95 mm and was not significantly different from the positive control (bactericide) (Figures 2 and 3). Thus, the inhibition zone of the consortium treatment between Pf and Bs (M-18) was the highest in inhibiting Rs compared to when the isolates were given alone. The use of microbial consortia could suppress pathogen attacks compared to single microbes, because of the synergistic effect of each microbe which complements each other by using available nutrient sources (Soesanto et al., 2019). *Pseudomonas fluorescence* could grow rapidly in various conditions (Nasir, 2016; Wai et al., 2022), but could not withstand environmental stress, while Bs could produce endospores when facing environmental stress, both hot and dry (Radhakrishnan et al., 2017), but it took longer to colonize roots (Majid, 2016) with a longer adaptation time (Hashem et al., 2019). Apart from that, Pf was offensive because it produced bactericidal or bacteriostatic molecules, while Bs was more defensive, because it could neutralize offensive molecules or prevent being overtaken by a competitor, so that the two could work together well (Lyng and Kovács, 2023).

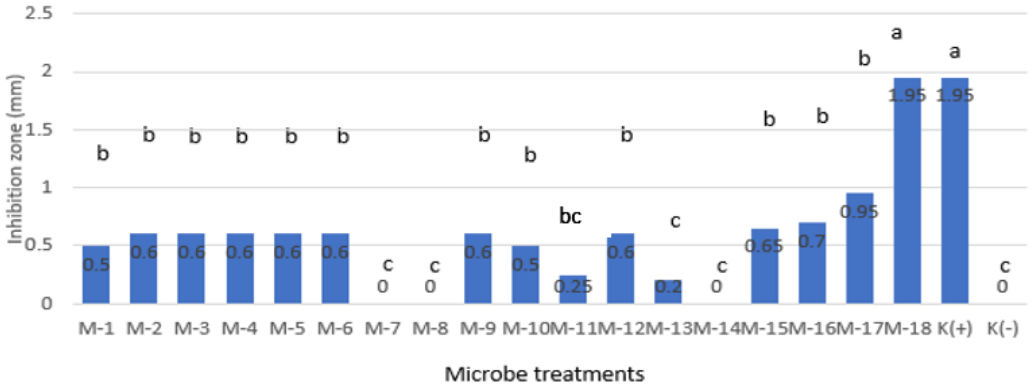

Figure 2. Inhibition zones of various isolates tested. Value of inhibition zones of microbe treatment followed by the same letter with other treatments are non significantly different using Duncan's test ($p < 0.05\%$).

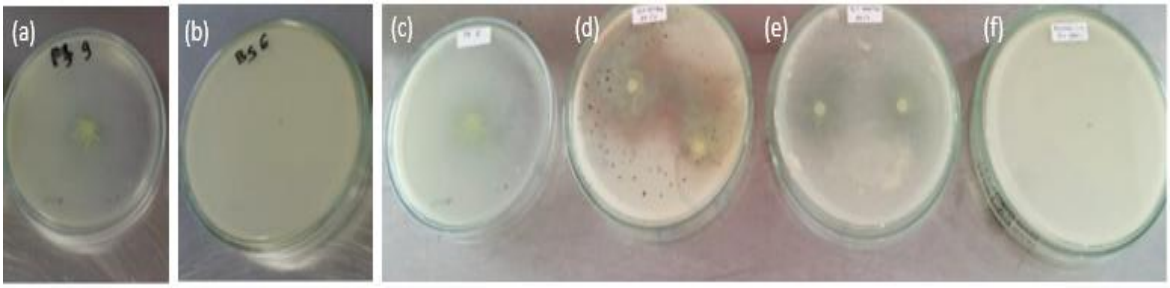

Figure 3. Inhibition zones produced by in vitro testing between *Ralstonia solanacearum* and M 15 (a), M16 (b), M17 (c), M18 (d), M19 (K+) (e), and M20 (K-) (f).

Among single isolates, the inhibition zone of Pf (M-17) was higher than that of all Bs isolates (M-1 to M-16) (Figure 2); Pf could produce many types of antibiosis such as hydrogen cyanide, 2,4-diacetylphloroglucinol and pyoluteorin, which could directly interfere with the growth of pathogens. *Pseudomonas fluorescence* was offensive because it produced bactericidal or bacteriostatic molecules (Lyng and Kovács, 2023). The superiority of Pf over Bs was also reported by Elsayed et al. (2020). *Pseudomonas fluorescence* could grow quickly in various conditions, so it could colonize roots quickly (Nasir, 2016). Yuliar et al. (2015) stated that Pf was the dominant bacteria that was virulent against Rs, followed by Bs and other species.

The best Bs treatments were M-15 and M-16, meanwhile there were three Bs isolates that did not form an inhibition zone at all, namely M-7, M-8, and M-14 (Figure 2). Lyng and Kovács (2023) stated that strain responses would vary in controlling Rs. This was because the success of biocontrol depended on the right selection process and its ability to fight specific pathogens on plants (Hashem et al., 2019). Differences in Bs strains and species showed differences in biocontrol mechanisms, so it was necessary to understand how to select, formulate, and practically apply them.

Based on the test results, the four best treatments recommended for further experiments were selected, namely treatments with inhibition zones ranging from 0.65-1.95 cm, respectively, namely treatments M-15 (Bs), M-16 (Bs), M-17 (Pf), and M-18 (Bs+Pf consortium). All tested isolates were not phytopathogenic based on tests on tobacco plants, because all isolates did not cause disease symptoms. Thus, the bacteria selected in this test were safe for plant growth and could be used as biofungicides.

Microbe selection in aeroponic systems

The application of microbes in this study when transplanting from the nursery to the aeroponic system coincided when the plants began to be exposed to nutrients contaminated with Rs. Transplanting was done by cleaning the roots from the planting medium, so there was a possibility of injury. This wound was thought to be the place where Rs entered the plant in the aeroponic system; Rs easily entered through wounds at the root tips while growing or through natural openings or lenticels of tuber in aeroponic (Pathania et al., 2016). Once Rs entered the plant, it would multiply in the xylem and produced exopolysaccharides which would block the flow of water and nutrients, causing the plant to wilt (Nasir, 2016).

The average nutritional temperature at 12:00 h was 23.32 °C lower than the temperature inside and outside the greenhouse (25.95 and 25.49 °C). Placement of nutrient storage containers underground in this study could help reduce nutrient temperature. Meanwhile, the temperature conditions inside the greenhouse in this study at 12:00 h (25.95 °C) were relatively higher (25.49 °C) and the humidity inside the greenhouse (41.43%) was lower than outside the greenhouse (42.24%). The description of the microclimatic conditions in this study showed that the research was carried out at temperatures during the day that were not too high (< 25 °C) with low air humidity below 50%. Daytime temperatures still below 25 °C caused tuber yield not to be influenced by temperature as in aeroponic research conducted by Chiipanthenga et al. (2013). The low air humidity in this study was attempted to be overcome by routinely spraying nutrients intermittently to the roots with an interval of 3 min of watering and 7 min of stopping watering.

The growing range for Rs was at 15-35 °C, but optimum temperature for Rs was 30-35 °C and could still survive at low temperatures below 4 °C (Nasir, 2016). This caused the wilt percentage in the control at 2 WAP to not be immediately high above 80% (Figure 4), because the nutrient temperature and temperature inside and outside the greenhouse during the day were still below 30 °C. Apart from that, the wilt attacked in this study occurred gradually during the day on young leaves and in the early morning they looked fresh again, like the wilting symptoms presented by Mamphogoro et al. (2020). After a few days, permanent wilting occurred because the young leaves began to turn yellow and spread to the old leaves. This type of wilt attack by Manda et al. (2020) was called a hidden infected plant and could spread the infection easily to other healthy plants in aeroponics with a closed nutritional system or by Mamphogoro et al. (2020) it was known as "green wilt" disease because the leaves of infected plants remained green when the plant showed symptoms. The Rs wilt attacks often occur in hot temperatures or in hot lowlands or in the dry season (Elsayed et al., 2020).

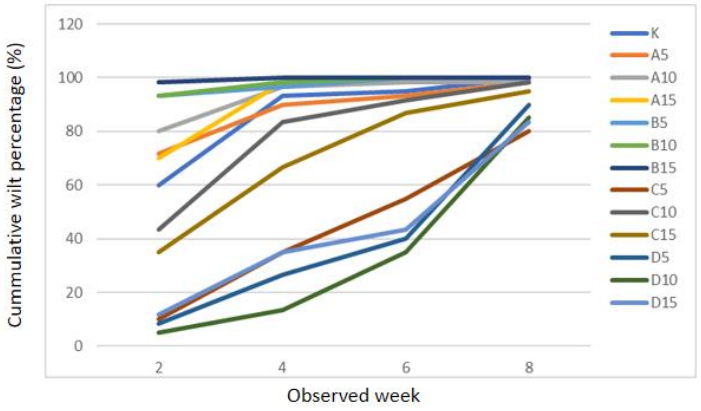

Figure 4. Cumulative wilt percentage at various weeks of observation. K: Control; A/B/C/D5/10/15: microbe A/B/C/D with 5/10/15 min soaking time.

The cumulative wilt percentage in this study was immediately high in the 2nd week above 50% for microbes A and B at all soaking times compared to the control (Figure 4). This showed that the microbe Bs found in treatments A and B at the beginning of growth actually caused wilting to develop more quickly compared to controls. Wilting in controls began to increase with microbes A and B above 90% in the 4th week (Figure 4). Wilt attacks in treatment C with microbe Pf exceeding 50% in 10 and 15 min soaking occurred in the 4th week, while in 5 min soaking occurred in the 6th week. Thus, a longer soaking time for microbe C could increase the attack of Rs. Meanwhile, wilt attacks were above 50% in treatment D (combination of Pf and Bs) starting in the 8th week. Thus, treatment D with various soaking times and treatment C with 5 min soaking could withstand wilt attacks (below 50%) until the 6th week. In the 8th week, all treatments had wilt attacks above 80%. Even though the Rs concentration given was 10^4 CFU mL^{-1}, it was considered low according to Elsayed et al. (2020), but because the nutrient circulation was closed, the plants were continuously exposed to Rs, so all the plants eventually wilt. This was because Rs easily spreads quickly in contaminated water (Mamphogoro et al., 2020).

There was an interaction between microbes and its soaking time on the wilt percentage of 2 and 6 WAP, the cumulative wilt percentage in all observations, the percentage of inhibition power, stover weight and total plant weight (Table 2). The best percentage of inhibition power was found in three treatment combinations, namely microbe C soaking for 5 min and microbe D with various soaking times (Table 2). This showed that to obtain the highest inhibition power, microbe C required a short soaking time (5 min), while the soaking time for microbe D has no effect. This phenomenon was in line with the initial observation of wilt attacks in Figure 4. The Bs and Pf microbes in aeroponics had different responses to Rs or there were differences in the responses of Bs in vitro and in aeroponics to Rs (Figure 2 and Table 2). Apart from that, Pf and consortium microbes (Pf and Bs) responded differently to the soaking time (Table 2). These two discoveries could be new to the world of microbial antagonism in aeroponics.

Table 2. Interaction between microbes and its soaking times on observed variables. Data was transformed with *Log (x+4), **log (x+6), ***log (x+10). WAP: Weeks after planting, CV: coefficient of variance. Numbers followed by the same letter in a column are non significantly different using Duncan's test ($p < 0.05$).

Microbes	Soaking time	Wilt percentage		Wilt cumulative percentage				Inhibition power	Stover weight	Total plant weight
		2 WAP	6 WAP	2 WAP	4 WAP	6 WAP	8 WAP			
	min	——%——		——%——				—%—	—g—	—g—
Control	0	36.67b	1.67d	60.00d	93.33ab	95.00ab	100.00a		0.00b	0.00b
Microbe A	5	65.00ab	3.34cd	71.67cd	90.00ab	93.33ab	98.33a	1.67c	23.00b	45.50b
	10	60.00ab	1.67d	80.00cd	96.67ab	98.33a	98.33a	1.67c	66.75b	83.75b
	15	46.67ab	1.67d	70.00cd	98.33a	100.00a	100.00a	0.00c	0.00b	0.00b
Microbe B	5	83.33a	3.34cd	93.33ab	96.67ab	100.00a	100.00a	0.00c	0.00b	0.00b
	10	81.67a	1.67d	93.33ab	98.33a	100.00a	100.00a	0.00c	0.00b	0.00b
	15	71.67ab	0.00d	98.33a	100.00a	100.00a	100.00a	0.00c	0.00b	0.00b
Microbe C	5	10.00c	20.00a	10.00f	34.98d	55.00c	80.00c	20.00a	224.07a	308.48a
	10	43.33ab	8.33bcd	43.33e	83.33b	91.67ab	98.33a	1.67c	82.25b	101.00b
	15	35.00b	20.00ab	35.00e	66.67c	86.67b	95.00a	5.00bc	168.50ab	224.46ab
Microbe D	5	8.33c	13.33ab	8.33f	26.67d	40.00d	90.00ab	10.00ab	127.75ab	209.67ab
	10	5.00c	21.67a	5.00f	13.33e	35.00d	85.00bc	15.00a	185.25a	265.86a
	15	10.00c	8.33bcd	11.67f	35.00d	43.33d	83.33bc	16.00a	197.13a	299.63a
CV, %		13.65*	27.09*	21.27	11.93	8.80	6.40	22.72*	29.66**	32.22***

Microbe C in the form of Pf bacteria were endophytic bacteria that work to enter cells systematically, so it was thought that they did not require a long soaking time to enter plant tissue. Lyng and Kovács (2023) stated that Pf could grow quickly in the rhizosphere, so that Pf was offensive and attacked Rs quickly. Javandira et al. (2018) reported that the best concentration of Pf to suppress *Erwinia carotovora* in potato tubers was the lowest, namely 10^5 CFU mL^{-1} compared to 10^7 and 10^9 CFU mL^{-1}, while this study used a high Pf concentration of 10^8 CFU mL^{-1} compared to the best concentration according to research by Javandira et al. (2018). This condition raised the suspicion that long soaking times at high concentrations caused the concentration to become too high and poison the plants. Like the phenomenon of feedback inhibition in continuous liquid culture that growth could be hampered by the resulting metabolism, namely too many compounds produced, thereby poisoning the plant. Stegelmeier et al. (2022) stated that high concentrations were not necessarily effective because the microbial response in artificial systems was very different.

Microbe D was a consortium treatment between two bacteria Pf and Bs. Majid (2016) stated that Pf could colonize quickly but was sensitive to environmental stress, especially high temperatures, while Bs took longer to colonize roots, but was resistant to environmental stress by forming endospores. In this way, the collaboration between the two could synergize and complement each other in producing secondary metabolites which were useful both for suppressing wilting and increasing plant growth and production. This synergy was proven by the longer the soaking time for microbe D tended to increase the wilt inhibition power although it was not significantly different (Table 2). Stegelmeier et al. (2022) suggested the priority of microbial consortia in hydroponic systems because diversity in artificial systems was limited so that they complement each other. The results of consortium research between Pf and Bs could reduce Rs in potatoes by 75%-81% (Istifadah et al., 2019). Tkachenko et al. (2023) reported that consortium bacteria had a greater effect after planted in aeroponic condition from adverse environmental factors.

The high inhibition power percentage of the best treatment (microbe C soaked for 5 min and D at all soaking times) was shown by the cumulative wilt percentage at 2, 4, 6, and 8 WAP and the low wilt percentage at 2 WAP (Table 2). The high inhibition power percentage caused the stover weight and the total plant weight to be the highest (Table 2). This was because microbes C and D with the best soaking time could not only help overcome wilt, but also increased plant growth and produced high plant production. Soesanto et al. (2021),

reported that secondary metabolites produced by antagonistic microbes would suppress the development of pathogens and stimulate plant growth so that the plant's ability to carry out photosynthesis increased and was followed by rapid plant growth and cell elongation so that stem, leaf and root growth was also high. This was because microbial antagonism could play a role in stimulating plant growth through various mechanisms, namely increasing the solubility and uptake of N, P, K and Fe nutrients, increasing the synthesis of the phytohormones auxin, cytokinin and gibberellin, and improving plant roots (Yuliar et al., 2015; Lal et al., 2022).

Microbes A and B (Bs) with various soaking times could not inhibit wilt attacks. This was indicated by the high wilt attack of 2 WAP, the cumulative wilt percentage of 4, 6, and 8 WAP, so that microbes A and B had lower stover and total plant weight compared to the best treatment of microbe C (Pf) soaking 5 min and microbe D (consortium Bs and Pf) with various soaking times (Table 2). However, microbes A and B (Bs) in this study showed different characteristics against the initial attack of wilt 2 WAP (Table 2). Microbe B was attacked by wilt more quickly than microbe A after soaking for 15 min. This showed that each Bs strain was different responding to wilt disease (Stegelmeier et al., 2022; Lyng and Kovács, 2023).

In vitro test results showed that microbes A and B (Bs) could overcome wilt (Figure 2), but when tested in an aeroponic system they were not successful, and even had the effect of increasing wilt at the beginning of growth (Figure 4). This was because microbe isolates from soil could not always survive on the same plant under hydroponic conditions (Stegelmeier et al., 2022; Chiaranunt and White, 2023) or because variations in the biotic and abiotic environment were difficult to consider when selecting in vitro (Lal et al., 2022). Research into appropriate in vitro isolate selection methods by adapting artificial aeroponic systems could be a topic for further research.

The aeroponic system allowed the roots to absorb oxygen from the air at a higher rate, so that the energy resulting from root respiration could be used to increase nutrient uptake (Dianawati et al., 2013). The low reducing sugar and total sugar content in roots using an aeroponic system was a consequence of increased respiration due to the abundance of O_2 for the roots. However, the high oxygen in this aeroponic system not only increased nutrient uptake, but was also thought to increase Rs attack on the roots. *Ralstonia solanacearum* included aerobic microbes whose virulence increased with increasing oxygen (Elsayed et al., 2020; Manda et al., 2020). Meanwhile, the two microbes used in this research, Pf and Bs, were aerobic bacteria that could grow optimally in conditions where oxygen was abundant (Lyng and Kovács, 2023). However, Pf was reported to be able to grow under anaerobic conditions on nitrite and ammonium media by (Soesanto et al., 2011). Some Pf strains could use nitrate as an electron acceptor to replace oxygen. Meanwhile, competition between Bs and Rs for oxygen in aeroponics showed that Bs was unable to compete and actually disrupted plant growth (Table 2). Elsayed et al. (2020), reported that the competition ability of Bs was lower than that of Pf, even though it was widely found around the rhizosphere, because its position was far from the roots. Meanwhile, according to Wai et al. (2022), Bs consumed more C, so less C was conserved, causing Bs to be unable to compete with Pf. Stegelmeier et al. (2022) also stated that *Bacillus* species did not grow well in hydroponics, unlike *Pseudomonas*. In addition, the microbial soaking which was almost the same as the Rs inoculation when transplanting to aeroponics meant that Bs did not have much time to adapt against Rs. Hashem et al. (2019) stated that Bs needs time to form endospores when environmental stress occurred. Thus, improving the initial application time for Bs could be considered for further research in overcoming Rs in aeroponics.

Single-handedly, differences in microbe treatment influenced all observed variables, while differences in soaking time influenced wilt percentages at 1, 4, and 8 WAP (Table 3). Microbe D had a number of tubers with various tuber sizes, roots and tuber weight, as well as the highest ratio of tuber weight and a lower wilt percentage at 1 WAP compared to control and microbes A, B, and C (Table 3). However, treatment D was not significantly different from treatment C in terms of wilt percentage of 1 WAP, root weight, number of large and medium tubers, and ratio of tuber weight (Table 3). The difference between treatments C and D could be seen from the peak of C wilt attacks at 4 WAP, while the peak attack of D microbes was at 8 WAP or later than C microbes. This was thought to be the cause of changes in the tuber weight, the number of small and total tubers of microbe D were higher than of microbe C. When related to the interaction influence of microbe and soaking time, the toxic effect of microbe C during long soaking times was thought to cause singly, microbe C to have lower variables than microbial D.

A single microbial soaking treatment could reduce wilt attacks at 1 and 4 WAP and increase wilt attacks at 8 WAP, but did not affect the root and tuber weight, the number of tubers of various sizes, and the ratio of tuber and total plant weight (Table 3). This showed that microbial soaking could overcome wilt and could delay

the peak of attack later at 8 WAP compared to the control. There were nonsignificant differences in the variables of tuber weight, number of tubers, and ratio of tuber and total plant weight due to differences in microbes, which occurred due to differences in plant growth phase response which then became the same between microbes after forming tubers.

Table 3. Effect of various microbes and various soaking periods on the observed variables. Data was transformed with *Log (x+4), ***log (x+10). WAP: Weeks after planting, CV: coefficient of variance. Numbers followed by the same letter in a column are non significantly different using Duncan's test ($p < 0.05$).

	Wilt percentage (%)			Weight (g)			Number of tubers			Ratio of tuber and total plant weight
Treatments	1 WAP	4 WAP	8 WAP	Root	Tuber	Big	Medium	Small	Total	
Microbe treatments										
Control	23.33^{a}	33.33^{a}	5.00^{bc}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Microbe A	16.67^{a}	21.11^{b}	1.67^{c}	4.50^{b}	8.67^{b}	0.33^{ab}	0.08^{b}	0.50^{b}	0.92^{b}	4.28^{b}
Microbe B	16.11^{a}	3.33^{c}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Microbe C	0.00^{b}	32.22^{a}	13.33^{b}	41.15^{a}	11.90^{b}	0.43^{ab}	0.33^{ab}	0.42^{b}	1.18^{b}	7.00^{ab}
Microbe D	0.55^{b}	16.67^{b}	46.67^{a}	50.72^{a}	37.63^{a}	0.83^{a}	0.88^{a}	3.34^{a}	5.06^{a}	20.55^{a}
Soaking time treatments (min)										
0	23.33^{a}	33.33^{a}	5.00^{b}	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	4.17^{b}	16.25^{b}	20.00^{a}	32.30	14.91	0.38	0.41	0.92	1.72	5.85
10	7.92^{b}	17.50^{b}	14.17^{ab}	13.93	15.16	0.46	0.18	1.55	2.19	9.35
15	12.92^{b}	21.25^{b}	12.01^{ab}	26.04	13.57	0.35	0.38	0.73	1.45	8.69
CV, %	27.72*	18.17*	21.79*	22.34***	22.73***	10.09*	8.55*	15.43*	19.74*	18.23***

Harvest variables, both weight and number of tubers, had a significantly negative effect on the wilt cumulative percentage and a significantly positive effect on the percentage of inhibition power (Table 4). The wilt percentage variable at a longer observation time (6 and 8 WAP) had a positive effect on the harvest variable, while the wilt percentage at the beginning of the observation (1 and 2 WAP) had a negative effect on the harvest variable. This showed that a high wilt percentage at the beginning of the observation had a negative effect on harvest variables compared to if wilting occurred at the end of the observation. Thus, the beginning of the wilt attack greatly influenced the harvest variables. This was in accordance with what was stated by Lal et al. (2022), early attacks of disease could cause crop failure, so applying microbes as early as possible could prevent plant disease attacks. Microbial antagonism could weaken pathogens, but if the pathogen had already entered the plant, then protection against the pathogen would still fail. Meanwhile, the wilt percentage at 4 WAP had no effect on all observed variables.

There were five clusters based on a fairly high PCA value of 86.2% (Figures 5a and 5b). The first cluster was microbe D soaking for 10 min with a high percentage of inhibition power, number of tubers and tuber weight. The second cluster was microbe D soaking for 5 and 15 min and microbe C soaking for 5 min with a high percentage of inhibition power and high stover and total plant weight. The third cluster was microbe A soaking time for 10 and 15 min and control with a high cumulative wilt percentage of 2, 4, 6, and 8 WAP. The 4th cluster was microbe B with a high wilt percentage of 1 and 2 WAP and a high cumulative wilt percentage of 2 WAP. The 3rd and 4th clusters were clusters with high wilt attacks and had low plant weight, tuber weight, number of tubers, and vice versa for clusters 1 and 2. Meanwhile, the 5th cluster was a transition cluster between the two with an indicator of wilt percentage at 4 WAP, namely microbe A soaking for 5 min and microbe C soaking for 10 and 15 min. The difference the 5th cluster with others was seen in the opposite wilt cumulative percentage and inhibition power (Figures 5a and 5b) which was also shown by the negative correlation value (Table 4). Thus, PCA sharpened the conclusion that the best isolate for aeroponics was cluster 1, namely microbe D with a soaking time of 10 min with a higher number of tubers and tuber weight per plant by 79% and 85%, respectively.

Table 4. Correlation among observed variables. *Significantly different from the Pearson correlation test ($p < 0.05$). SBSB: stover weight; SBSA: root weight; SBU: tuber weight; SBT: total plant weight; SJU: number of total tuber; SJB: number of big tuber; SJS: number of medium tuber; SJK: number of small tuber; SUB: ratio of tuber and total plant weight; L1/2/4/6/8: wilt percentage at 1/2/4/6/8 wk after planting; K2/4/6/8 cumulative wilt percentage at 2/4/6/8 wk after planting; DH: inhibition power.

	SBSA	SBU	BT	SJU	SJB	SJS	SJK	SUB	L1	L2	L4	L6	L8	K2	K4	K6	K8	DH
SBSB	92*	76*	99*	69*	69*	83*	59*	70*	-77*	-84*	26	88*	76*	-90*	-89*	-83*	-92*	92*
SBSA		65*	94*	55	54	86*	42	50	-72*	-83*	24	78*	78*	-88*	-87*	-84*	-89*	89*
SBU			80*	98*	93*	88*	92*	95*	-65*	-73*	-4	65*	87*	-78*	-87*	-88*	-80*	80*
SBT				73*	72*	88*	62*	72*	-77*	-86*	23	87*	81*	-92*	-92*	-87*	-94*	94*
SJU					89*	79*	98*	96*	-59*	-70*	-12	65*	86*	-74*	-85*	-86*	-74*	74*
SJB						73*	82*	89*	-71*	-62*	13	60*	72*	-71*	-73*	-72*	-63*	63*
SJS							68*	74*	-64*	-80*	4	61*	87*	-83*	-89*	-92*	-92*	92*
SJK								93*	-49	-63*	-21	60*	81*	-65*	-79*	-79*	-65*	65*
SUB									-58*	-63*	-6	66*	73*	-68*	-76*	-74*	-69*	69*
L1										56*	-22	-79*	-64*	75*	74*	67*	66*	-66*
L2											-43	-76*	-86*	97*	90*	88*	84*	-84*
L4												21	-1	-41	-7	-2	-8	8
L6													69*	-84*	-84*	-73*	-77*	77*
L8														-88*	-96*	-99*	-83*	83*
K2															94*	91*	87*	-87*
K4																99*	92*	-92*
K6																	91*	-91*
K8																		-100*

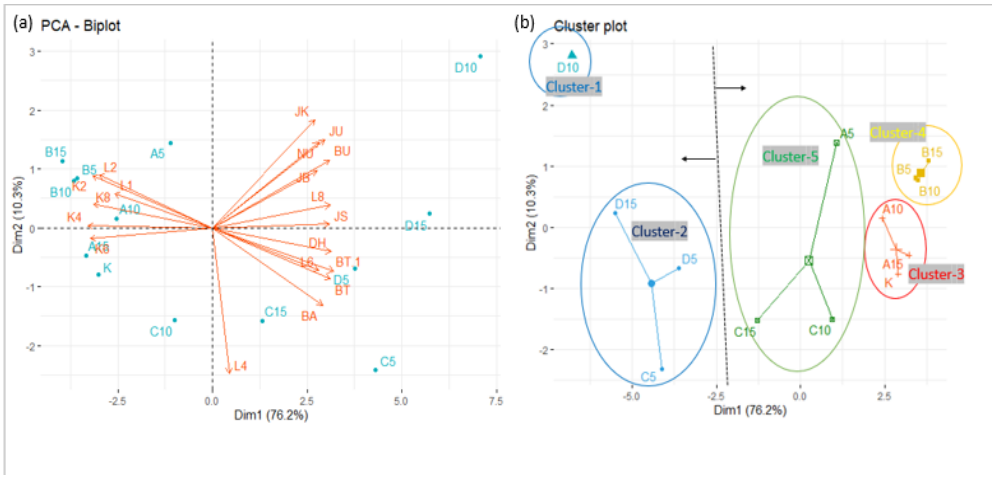

Figure 5. PCA biplot (a) and clusters plot (b) for various treatment combinations and observation variables. K: Control; A/B/C/D5/10/15: microbe A/B/C/D with 5/10/15 min soaking time; SBS: stover weight; SBSA: root weight; SBU: tuber weight; SBT: total plant weight; SJU: number of total tuber; SJB: number of big tuber; SJS: number of medium tuber; SJK: number of small tuber; SUB: ratio of tuber and total plant weight; L1/2/4/6/8: wilt percentage at 1/2/4/6/8 wk after planting; K2/4/6/8 cumulative wilt percentage at 2/4/6/8 wk after planting; DH: inhibition power.

Sterile conditions in the greenhouse and the use of plantlet from tissue culture in the aeroponic system still required the addition of wilt prevention technology using microbial consortium, especially in aeroponic system in the tropics. It is not guaranteed that workers and the tools used are always sterile, while on the other hand, opening of tuber lenticels which often happen in aeroponics are easily invaded by wilt bacteria (Pathania et al., 2016). In such conditions the use of microbial antagonism is very necessary and can be combined with other control methods. Our microbial consortium treatment showed that it could not only overcome wilt by Rs

environmentally friendly, but also increase the number of tubers in the aeroponic system. The selection of consortium microbes in this study was also considered safer in various soaking times compared to single Pf microbes which were susceptible to wilt attacks in the long soaking times.

CONCLUSIONS

Pseudomonas fluorescence was consistently the best both in vitro and in aeroponics, whereas *Bacillus subtilis* could only overcome wilt in vitro and could not overcome wilt in aeroponic system. *Pseudomonas fluorescence* required 5 min soaking time and could increase wilt with increasing soaking time. The consortium between *B. subtilis* and *P. fluorescence* with 10 min soaking time could overcome wilt by 85% and increase the number of tubers and tuber weight by 79% and 85%, respectively, so this prospective microbial consortium could be applied to aeroponic systems environmentally friendly in tropical areas with high *Ralstonia solanacearum* opportunities.

Author contribution

Conceptualization: M.D., H.H. Methodology: M.D., H.H., W.N. Prepared material: H.H., W.N. Data curation: M.D., H.H., K.K.H., W.N., I.S., Y.H., I.C., I.B., A.M., R.F.R. Analyzed data statistically: M.D., H.H., K.K.H. Writing-original draft: M.D., H.H. Writing-review & editing: M.D., K.K.H., Y.H., A.M., R.F.R. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

Thanks are conveyed to the Rumah Program Bibit Unggul Pertanian dan Pangan Tanaman Ternak project, fiscal year 2022, by National Research and Innovation Agency (BRIN) which has funded research activities. Thank are also extended to Noor Istifadah for her isolate collection and BPTPH Cianjur and UPTD BBK Pangalengan for their facilities doing this research.

References

AlAli, H.A., Khalifa, A., Al-Malki, M. 2021. Plant growth-promoting rhizobacteria from *Ocimum basilicum* improve growth of *Phaseolus vulgaris* and *Abelmoschus esculentus*. South African Journal of Botany 139:200-209. doi:10.1016/j.sajb.2021.02.019.

Balena, L., Malanchuk, J.P., Junior, C.T., Ribas, E.L., Eschemback, V., Kawakami, J. 2021. Growth, yield and dormancy of aeroponically produced potato minitubers as a function of planting density and harvesting date. Horticultura Brasileira 39(2):161-68. doi:10.1590/S0102-0536-20210206.

Chiaranunt, P., White, J. 2023. Plant beneficial bacteria and their potential applications in vertical farming systems. Plants 12(2)400. doi:10.3390/plants12020400.

Chiipanthenga, M., Maliro, M., Demo, P., Njoloma, J., Khumar, N. 2013. Performance of different potato genotypes under aeroponics system. Journal of Applied Horticulture 15(2):142-46. doi:10.37855/jah.2013.v15i02.28.

Dianawati, M., Cahyaningrum, H., Suwitono, B. 2023. The determination of nitrogen concentration in the G2 potato seed production on fertigation system. p. 1-7. In Proceeding International Conference of Technology on Community and Environmental Development. Universitas Muhammadiyah Yogyakarta (UMY), Yogyakarta, Indonesia. doi:10.1051/e3sconf/202342501005.

Dianawati, M., Ilyas, S., Wattimena, G.A., Susila, A.D. 2013. Minituber production of potato aeroponically by determining optimum rate of nitrogen foliar fertilizer. Journal of Horticulture 23(1):47. doi:10.21082/jhort.v23n1.2013.p47-55.

Dianawati, M., Wattimena, G.A. 2014. The potential of aeroponic technology in supporting national potato seed self-sufficiency. p. 260-266. In Proceeding National Seminar PERHORTI, Malang, Indonesia.

Dianawati, M., Yulyatin, A. 2021. Alternative potential of various processed potato varieties in the highland. p. 3-9. In Proceeding International Conference on Agribusiness and Rural Development. Universitas Muhammadiyah Yogyakarta (UMY), Yogyakarta, Indonesia. doi:10.1051/e3sconf/202131603003.

Elsayed, T.R, Jacquiod, S., Nour, E.H., Sørensen, S.J., Smalla, K. 2020. Biocontrol of bacterial wilt disease through complex interaction between tomato plant, antagonists, the indigenous rhizosphere microbiota, and *Ralstonia solanacearum*. Frontiers in Microbiology 10:1-15. doi:10.3389/fmicb.2019.02835.

Hashem, A., Tabassum, B., Abd_Allah, E.F. 2019. *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi Journal of Biological Sciences 26(6):1291-1297. doi:10.1016/j.sjbs.2019.05.004.

Istifadah, N., Fatiyah, N., Fitriatin, B.N., Djaya, L. 2019. Effects of dosage and application frequency of microbial consortium mixed with animal manure on bacterial wilt and late blight diseases of potato. IOP Conference Series: Earth and Environmental Science 334:012038. doi:10.1088/1755-1315/334/1/012038.

Javandira, C., Purnomo, A., Rosyidah, E. 2018. Agricultural dictionary. Unusida, Sidoarjo, Indonesia.
Kawaguchi, A., Inoue, K. Ichinose, Y. 2008. Biological control of crown gall of grapevine, rose, and tomato by nonpathogenic *Agrobacterium vitis* strain VAR03-1. Biological Control 98(11):1218-1225. doi:10.1264/jsme2.ME13014.
Lal, M., Kumar, A., Chaudhary, S., Singh, R.K., Sharma, S., Kumar, M. 2022. Antagonistic and growth enhancement activities of native *Pseudomonas* spp. against soil and tuber-borne diseases of potato (*Solanum tuberosum* L.) Egyptian Journal of Biological Pest Control 32:22. doi:10.1186/s41938-022-00522-w.
Lyng, M., Kovács, A.T. 2023. Frenemies of the soil: *Bacillus* and *Pseudomonas* interspecies interactions. Trends in Microbiology 31(8):845-857. doi:10.1016/j.tim.2023.02.003.
Majid, A. 2016. The potential of *Pseudomonas fluorescence* and *Bacillus subtilis* bacteria to control bacterial leaf blight in soybeans (*Pseudomonas syringae* pv. *glycinea*). p. 66-71. In Proceeding National Seminar on Research Results and Community Service. Jember State Polytechnic, Jember, Indonesia.
Mamphogoro, T.P., Babalola, O.O., Aiyegoro, O.A. 2020. Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other solanaceous crops. Journal of Applied Microbiology 129(3):496-508. doi:10.1111/jam.14653.
Manda, R.R., Addanki, V.A., Srivastava, S. 2020. Bacterial wilt of solanaceous crops. International Journal of Chemical Studies 8(6):1048-1057. doi:10.22271/chemi.2020.v8.i6o.10903.
Mateus-Rodriguez, J.R., de Haan, S., Andrade-Piedra, J.L., Maldonado, L., Hareau, G., Barker, I., et al. 2013. Technical and economic analysis of aeroponics and other systems for potato mini-tuber production in Latin America. American Journal of Potato Research 90(4):357-68. doi:10.1007/s12230-013-9312-5.
Nasir, S. 2016. Review on major potato disease and their management in Ethiopia. International Journal of Horticulture and Floriculture 4(5):239-246.
Nurbaya, Kuswinanti, T., Baharuddin. 2013. Bacterial antagonist isolates in controlling bacterial wilt disease of potato (*Ralstonia solanacearum*) in aeroponic cultivation system. International Journal of Agriculture System 1(1):49-54.
Osei, R., Yang, C., Cai, F., Boamah, S. 2022. Mechanisms underlying salicylic acid on root parameters and antioxidant enzyme activity of potato plant under *Lelliottia amnigena* stress. Chilean Journal of Agricultural Research 82:22-233. doi:10.4067/S0718-58392022000200225.
Pathania, N., Trevorrow, P., Hughes, M., Jovicich, E. 2016. Optimization of aeroponic technology for future integration in quality potato seed production systems in tropical environments. Acta Horticulturae 1118:31-38. doi:10.17660/ActaHortic.2016.1118.5.
Radhakrishnan, R., Hashem, A., Abd Allah, E.F. 2017. *Bacillus*: a biological tool for crop improvement through bio-molecular changes in adverse environments. Frontiers in Physiology 8:1-14. doi:10.3389/fphys.2017.00667.
Raei, Y., Weisany, W., Panahian, M. 2017. Effect of different biological fertilizers on yield of potato cultivars mini-tubers under aeroponic system. p. 22-27. In Proceeding 8th ICSA International Conference on Sustainable Agriculture for Food, Energy, Industry and Environment in Regional and Global Context. 21 November 2017. Islamic Azad University of Science and Research Branch, Tehran, Iran.
Sezen, U.U., Barney, J.N., Atwater, D.Z., Pederson, G.A., Pederson, J.F., Chandler, J.M., et al. 2016. Multi-phase US spread and habitat switching of a post-Columbian invasive, *Sorghum halepense*. PLOS ONE 11(10):e0164584. doi:10.1371/journal.pone.0164584.
Soesanto, L., Dwijayanto, H., Mugiastuti, E., Manan, A. 2021. Application of secondary metabolites of two *Pseudomonas flourescens* isolates to control bacterial wilt of potato. KnE Life Sciences 2022:278-93.
Soesanto, L., Mugiastuti, E., Khoeruriza, K. 2019. Granular formulation test of *Pseudomonas fluorescens* P60 for controling bacterial wilt (*Ralstonia solanacearum*) of tomato in planta. Agrivita 41(3):513-23. doi:10.17503/agrivita.v41i3.2318.
Soesanto, L., Mugiastuti, E., Rahayuniati, R.F. 2011. Morphological and physiological features of *Pseudomonas fluorescens* P60. In Proceeding 4th International Seminar of Indonesian Society for Microbiology. Udayana University, Denpasar, Indonesia.
Stegelmeier, A.A., Rose, D.M., Joris, B.R., Glick, B.R. 2022. The use of PGPB to promote plant hydroponic growth. Plants 11(20):2783. doi:10.3390/plants11202783.
Tkachenko, O.V., Evseeva, N.V., Kargapolova, K.Y., Denisova, A.Y., Pozdnyakova, N.N., Kulikov, A.A., et al. 2023. Rhizobacteria increase the adaptation potential of potato microclones under aeroponic conditions. Microorganisms 11(7):1866. doi:10.3390/microorganisms11071866.
Wai, C.W.N.G., Yan, W.H., Tsim, K.W.K., So, P.S., Xia, Y.T., To, C.T. 2022. Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* as the soil amendment. Heliyon 8(11):e11674. doi:10.1016/j.heliyon.2022.e11674.
Wang, K., He, W., Ai, Y., Hu, J., Xie, K., Tang, M., et al. 2017. Optimizing seed potato production by aeroponics. Philippine Journal of Crop Science 42(1):69-74.
Yuliar, Nion, Y.A., Toyota, K. 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes and Environments 30(1):1-11. doi:10.1264/jsme2.ME14144.