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Isolation and screening of *Pseudomonas fluorescens* isolates against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and their effects on seedling growth of *Paraserianthes falcataria*

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Abstract. *Yusran Y. 2023. Isolation and screening of* Pseudomonas fluorescens *isolates against* Fusarium oxysporum *f.sp.* radicis-lycopersici *and their effects on seedling growth of* Paraserianthes falcataria. *Biodiversitas 24: 2294-2301. Pseudomonas fluorescens* plays a major role in biological control of pathogens and plant growth promotion as well with various mechanisms. The aim of the present study was to determine the biochemical characteristics of several *Pseudomonas* spp. isolates and to screen them against root pathogenic fungus *Fusarium oxysporum* f.sp *radicis-lycopersici* (FORL) and to evaluate their effect on the growth enhancement of *Paraserianthes falcataria* (L.) I.C. Nielsen seedlings. About 28 *Pseudomonas fluorescens* isolates were isolated from the rhizosphere of several plants and identified based on their physiological and morphological characters as well as their biochemical reactions. All isolates were tested for their ability to suppress FORL growth *in vitro*. All isolates inhibited FORL growth with varying inhibition zones. Six *P. fluorescens* isolates showed highest inhibition zones, namely TMTP4, TMTP5, TMTP6, SCPA1, SCPB3 and Proradix. The results showed that eight *Pseudomonas* isolates, namely TMTP4, TMTP5, TMTP6, SCPA1, SCPB3, SMPB3, TMTA2 and Proradix had a significantly different effect compared to the control treatment in increasing the growth of *Paraserianthes* seedlings. Based on our findings, it was confirmed that some *P. fluorescens* isolates could be used as potential bio-inoculants for controlling plant diseases caused by FORL and as a biological fertilizer to increase plant growth.

Keywords: Antagonist, biocontrol, FORL, *Paraserianthes falcataria*, *Pseudomonas fluorescens*

INTRODUCTION

Microbial diversity in the soil plays an important role in maintaining soil fertility (Kumari et al. 2018), thus playing an important role in improving plant development and soil quality (Lugtenberg 2015). Microbes have a very important role in agriculture to promote the exchange of nutrients for plants and reduce the use of chemical fertilizers and pesticides as much as possible. One of them is bacteria, which live in abundance around the rhizosphere of plants. Plant rhizosphere interacts with soil and influences community and microbial populations and ecosystem productivity (Dries et al. 2021; Hakim et al. 2021).

Pseudomonas spp. have useful applications in plant growth promotion, biotechnology, bioremediation and biocontrol (Chellaiah 2018; Ghadamgahi et al. 2021; Sah et al. 2021). *Pseudomonas* spp. can increase plant growth through several mechanisms such as increasing the availability and uptake of mineral nutrients through phosphate dissolution, root growth through the production of phytohormones, or by increasing tolerance to abiotic stress (Giles et al. 2014; Höfte 2021; Pandey and Gupta 2021; Kaur et al. 2022). They can also be applied as a biofungicide. Soil-borne pathogens can be controlled using biological agents such as bacteria. In addition, these biological agents can also increase disease resistance (Damiri et al. 2019). In the rhizosphere area, this biological agent develops and adapts more easily, so it has the potential to be used as a biopesticide (Lahlali et al. 2022).

Chemical pesticides are well known and commonly used to protect crop and postharvest losses from pathogens and pests and to increase the quality and quantity of yields. However, it is very unfortunate that the use of these chemical pesticides has negative effects that are detrimental to human health and the environment. Therefore, there is a need for a method to protect plants from these pathogens that is compatible with the environment and human health. One solution that can be implemented is to use beneficial microorganisms known as biocontrol control method.

The effectiveness of biocontrol agents for commercial use, particularly in controlling plant diseases caused by soil-borne pathogens, has not been satisfactory for several reasons. One of them is that almost all biocontrol agents that are applied are only selected based on their antagonistic activity in vitro. Therefore, often the application of biocontrol agents in soil is not successful due to many influencing factors such as biotic factors and abiotic factors, so the effectiveness of biocontrol agents in controlling soil-borne plant pathogens is not sufficient (Bonaterra et al. 2022). Furthermore, the application methods require some improvements in order to increase the formation and colonization of biocontrol agents in the long term and in an appropriate population density.

Crown and root rot disease caused by *Fusarium* spp. are not uncommon (Wang and Jeffers 2000) and has been reported in several plants. *Fusarium oxysporum* f. sp.

radicis-lycopersici (FORL) is a common fungal pathogen found in the rhizosphere of many plant species. This pathogen has a wide range of host species but isolate host specialization is more limited especially in tomato plants (Jarvis and Shoemaker 1978; Szczechura et al. 2013). This disease is a major soil-borne systemic disease especially in soilless or hydroponic cultivation systems (Hibar-Beji et al. 2009).

Pseudomonas fluorescens is a dominant rhizosphere inhabitant of several plants and has been studied in the last 30 years as a biocontrol agent against soil-borne pathogens (Panth et al. 2020). This group of bacteria has also been successfully applied to control fusarium wilt in several plants under controlled conditions (Farfour et al. 2021; Yadav et al. 2021; Khalifa et al. 2022). The most frequently reported biocontrol mechanism of *P. fluorescens* is parasitism which may involve the production of extracellular cell wall degrading enzymes, for example chitinase and β-1,3-glucanase which can lyse the cell wall of pathogens (Veliz et al. 2017; de Medeiros et al. 2018; Jha 2019), antibiotics, bacteriocins, siderophores, chitinase, and other metabolites such as phytoalexins and induction of systemic resistance (David et al. 2018; Nerek and Sokolowska 2022).

The aim of present study was to determine the biochemical characteristics of several isolates of *Pseudomonas* spp. and to screen them against root pathogenic fungus FORL and to evaluate their effect on the growth enhancement of *Paraserianthes falcataria* (L.) I.C. Nielsen seedlings.

MATERIALS AND METHODS

Soil sample collection, isolation and identification of *Pseudomonas fluorescens*

Rhizosphere soil samples were taken from the rhizosphere of several plant species, namely Sengon (*Paraserianthes falcataria* (L.) I.C. Nielsen), cocoa (*Theobroma cacao* L.), corn (*Zea mays* L.), tomato (*Solanum lycopersicum* L.) and peanut (*Arachis hypogea* L.) which grows in the Palu Valley, Central Sulawesi, Indonesia. Rhizospheric soil samples were taken by uprooting the plants and shaking off the adhering soil so that only the soil attached to the roots was left. The soil was put into black plastic bags and then immediately taken to the laboratory and stored in the refrigerator until analysis. 1 g of rhizosphere soil was suspended in 9 mL of 0.1 M phosphate buffer + 0.1% peptone and stirred for 30 minutes, then allowed to stand for 10 minutes. Then the soil suspension was made serial dilution with the same buffer. A total of 100 µL of dilutions 10^2 and 10^3 were grown on King's B media and then incubated at room temperature for 48 hours. Colonies of *P. fluorescens* bacteria with fluorescent characteristics under UV light were taken with an ose needle and purified by placing them in 5 mL of sterile water and making a suspension. A total of 100 µL was taken and grown on King's B media using a petri dish, then incubated for 48 hours at room temperature. Single colonies that glowed were taken with a loop needle and streaked on King's B oblique media, then incubated for 48 hours at room temperature and stored as pure cultures. Colonies that formed were then selected and identified. Confirmation and identification of *Pseudomonas* species was carried out through a series of morphological observations and conventional biochemical reactions and characteristics of the bacterial colonies that appeared include; colony morphology, size, shape, and coloration were observed using Bergey's Manual of Determinative Bacteriology (Holt 1994).

Preliminary tests to determine the potential of *Pseudomonas* isolates were carried out by growing them on nutrient agar media enriched with 10% soil extract which had been sprinkled with *Fusarium* fungus suspension. Qualitative potential was evaluated by determining a positive score for isolates showing inhibition growth of the tested fungus and negative for the opposite.

Physiological and biochemical characteristics of *Pseudomonas fluorescens* isolates

Production of siderophores and extracellular enzymes

The physiological and biochemical characteristics of several *P. fluorescens* isolates were conducted according to the method of Maurhofer et al. (1994), with some modifications. The *P. fluorescens* isolates were grown in King's agar media in a petri dish for 24 hours at 28°C. After that, petri dishes were placed under UV light with a wavelength of 366 nm to observe bacterial fluorescence which indicates the presence of siderophores production.

To test whether bacterial isolates produce protease enzymes, specific medium for protease with a composition consisting of (g/L) K_2HPO_4 2.0, glucose 1.0, peptone 5.0, gelatin 15.0 and agars 15.0 were prepared in petri dishes (Abdel Galil 1992), then 24 µL of 24-hour-old bacterial culture was placed in the middle of each petri dish and then incubated for 4 days at 28°C. After incubation, the formation and size of clear zone around the bacterial colonies was recorded.

10% litmus milk and colloidal chitin agar in petri dishes were used to determine the production of proteinase and chitinase enzymes, respectively (Wood 1988). Furthermore, the production of hydrolyzed arginine was carried out by preparing Thorneley's 2A media containing 1% agar. Then as much as 10 mL of the media was added into each test tube. Bacterial culture aged 24 hours were inserted into a tube containing Thorneley's 2A media and plugged with mineral oil, and incubated for 2 days at 28°C. A change in color from faint pink to red indicates production of the enzyme arginine dihydrolase.

Production of salicylic acid

Production of Salicylic Acid (SA) from bacterial isolates was tested in KB-broth after 42 hours of incubation. The method used is based on Press et al. (1997). The bacterial culture was centrifuged at 2,600 g for 20 minutes. After that, 1 mL of the supernatant of each bacterial isolate sample was acidified (pH 2) and extracted with 2.0 mL of chloroform. Then, chloroform phase was dried with a nitrogen stream, and residue was re-suspended in 1 mL of 80% methanol. Total SA was measured by HPLC (Pharmacia Uppsala, Sweden) coupled with a fluorescent detector (LC 304, Linear Instruments, Reno, USA) at excitation/emission 304 and 408 nm (Press et al. 1997).

Production of cyanide

To determine the production of cyanide by effective bacterial isolates, culture cells of each 24-hour-old bacterial isolate were streaked on a petri dish containing KB media, sterilized filter paper was dipped in alkaline sodium picrate solution (5g Picric acid; 25g Na_2CO_3). H_2O 2l was placed inside the lid of the petri dish and then petri dish was closed carefully with parafilm. These petri dishes were incubated at 28°C and observed daily for a color change from yellow to reddish brown, which was due to the uptake of HCN by the picrate solution (Millar and Higgins 1970).

In vitro compatibility and antagonism test

The antagonistic effect of 28 *P. fluorescens* isolates was carried out on King's agar media (King et al. 1995) against the pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL). 24 hours old bacterial isolates were streaked in the middle of medium, with five replications. Three days later, FORL malt agar plugs (2 weeks old culture) were placed 30 mm away from the edge of bacterial streak, and then petri dishes were incubated at 26°C for one week. The inhibition distance from the edge of fungus to the bacterial layer was measured and the average of all replicates was calculated. For the compatibility test, 24-hour-old *P. fluorescens* isolates were streaked on KB media from the edge of the petri dish. A distance of 3 mm between the two opposing bacterial lines was left. Then the petri dish was incubated for 4 days at 26±2°C and antagonistic effect of the bacterial isolates was observed.

Antagonistic test of isolate Pseudomonas fluorescens *against FORL*

The antagonist test of *P. fluorescens* on the radial growth of FORL mycelium was determined by growing the fungus on King's medium containing *Pseudomonas* in a petri dish. *Pseudomonas* inoculum was added into 100 mL of Kings medium and then poured evenly into five petri dishes. Control treatment was made by replacing the same amount of *Pseudomonas* inoculum with sterile distilled water. Then pathogenic culture with a diameter of 0.5 cm was transferred aseptically to the center of King's media. The petri dish was then incubated for 7 days at 26ºC. Mycelial growth of fungi was measured on each petri dish, and growth on Kings B medium containing *Pseudomonas* was compared to growth on petri dishes containing only water (control). The experiment was repeated five times for each treatment and the average value was taken. The inhibitory effect of *Pseudomonas* on the mycelial growth of FORL fungus was recorded in terms of colony inhibition percentage and calculated according to the formula of Hmouni et al. (1996). The percentage of growth inhibition was determined as (1-Cn/Co) x 100, where Cn = the average diameter increase of fungal colony with *Pseudomonas* isolate treatment, and Co = the average increase in diameter of fungal colony in the control treatment.

Observation of fungal hyphae lysis was carried out directly on the mycelium of fungal cultures that had been inoculated with each bacterial isolate in a petri dish under a microscope.

Effect of Pseudomonas fluorescens *isolates on the growth of seedlings*

Furthermore, *Pseudomonas* isolates which were effective in controlling FORL in vitro, were tested for their effect on the growth of *P. falcataria* seedlings in a greenhouse.

The experiment was arranged in a completely randomized design, consisting of nine treatments, namely without the application of *Pseudomonas* isolates (Control), Proradix, TMTP4, TMTP5, TMTP6, SCPA1, SCPB3, SMPB3, TMTA2. *P. falcataria* seeds were soaked in sterile hot water (100°C) for 30 seconds, then soaked in cold water (25°C) for 24 hours. Furthermore, these seeds were put in petri dish and soaked in *Pseudomonas* isolates and one commercial *Pseudomonas*, namely *Pseudomonas* sp. "Proradix®" (produced by Sourcon-Padena GmbH & Co. KG, Tübingen, Germany) in the amount of 1.5×10^{10} cfu l^{-1}. For control treatment, *Paraserianthes* seeds were only treated with sterile water. Furthermore, each pot was filled with 1.5 kg of sandy loam soil used as a growing medium, which had the following characteristics: pH 6.1, P 13 mg, K 22 mg and Mg 35 mg 100 g^{-1} soil, Mn 137 mg, Zn 5 mg, B 0.45 and Fe 236 mg kg^{-1} soil (analyzed according to VDLUVA, 2007). The soil was mixed with sand (3:1) before being used as a planting substrate. The addition of N, P, K, Mg and Fe as basic fertilizer was 50, 50, 100, 50 and 0.06 mg kg^{-1} of substrate respectively.

The pots were prepared using a completely randomized design in a greenhouse and maintained for fourteen weeks at 75% humidity. Additional light was provided during the low light intensity phase and the plants were irrigated as needed according to soil moisture 15% on a dry weight basis and harvested 14 weeks after planting. At the time of harvesting, the roots and shoots of the seedlings were separated and then washed clean. Shoots and roots were dried at 65°C for 72 hours to determine their dry weight. Elemental mineral P was analyzed by photo-spectrophotometry method.

Data analysis

Data were analyzed using Analysis of Variance (ANOVA). Data for inhibition of FORL mycelium growth by *Pseudomonas* isolates and growth of *Paraserianthes* seedlings are presented as means ± standard deviation. The differences between the treatments were compared using honest significant difference test at the 5% level.

RESULTS AND DISCUSSIONS

Physiological and biochemical characteristics of *Pseudomonas fluorescens* isolates

The results of isolation showed that 29 *Pseudomonas* isolates positively produced fluoresce on King's B medium under ultraviolet light at a wavelength of 366 nm, indicating that all of these isolates produced siderophores in Fe-deficient media (Table 1 and Figure 1). Siderophores produced by *Pseudomonas* spp. directly involved in biocontrol activities through competition with pathogens for iron, direct antagonism or induced systemic resistance (Höfte 2021; Pieterse et al. 2021; Bonaterra et al. 2022; Kuhl-Nagel

2022). Most strains of *Pseudomonas* spp. produce a compound called fluorescent pyoverdine (Rehm et al. 2022).

The concentration of cyanide produced by *Pseudomonas* spp was detected by intensity of color change from yellow to reddish brown in the media. All the tested *Pseudomonas* isolates produced cyanide, except SMPB1 isolate. TMTP4, TMTP5 and SCPA1 isolates were strongly produced cyanide compared to other isolates (Table 1). Hydrogen Cyanide (HCN) is a poison that can inhibit cytochrome c oxidase, which is a terminal component of the respiratory chain in many organisms (Höfte 2021).

The results extracellular enzymes production showed that protease enzymes was strongly produced by TMTA3, SCPA1, SCPA2, SCPB2, SMPA2, SMPA3, SMPA4, SPAA1 and SPAA2 isolates, SMPB1 and SPAB1 isolates did not produce protease enzymes. Arginine dihydrolase enzymes were produced by all isolates except for TMTA1 and TMTA2. Further, all isolates were also produced proteinase enzymes, but SMPB1 and SPAB1 isolates did not produce it. However, all *Pseudomonas* isolates produced chitinase enzymes, and some isolates produce it strongly, namely TMTA1, TMTA2, TMTA3, TMTA4, TMTP1 and TMTP2 (Table 1). Many factors affect the production of enzymes by *Pseudomonas*, for example temperature (Alves et al. 2018), growth medium (Dutta et al. 2016) and glutamine as a source of carbon or nitrogen to induce proteinase (Lundgren et al. 2021).

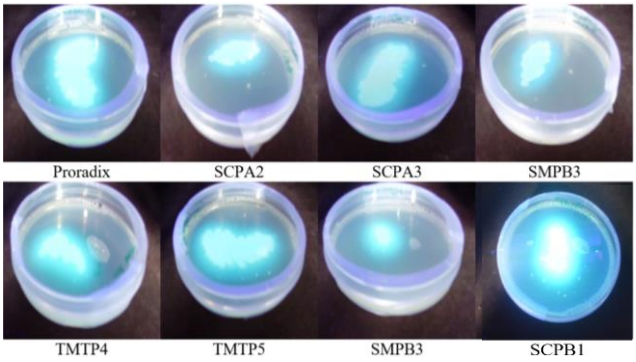

Figure 1. Characteristics of several *Pseudomonas* isolates on King's B media under ultraviolet light

The production of Salicylic Acid (SA) in King's B liquid medium by several *Pseudomonas* isolates was varied, the highest production was observed in TMTP4 isolate (297.3 µg/L) which was higher than the commercial *Pseudomonas proradix* (269 µg/L) followed by isolates TMTP5 (213.6 µg/L), SMPB3 (132.7 µg/L) and SCPA1 (106.4 µg/L). Five *Pseudomonas* isolates, namely TMTA2, TMTA4, SCPA3, SMPB1 and SPAB1 did not produce SA (Table 1). Various biochemical and molecular events associated with the process of inducing disease resistance in plants are influenced by a phenolic compound called salicylic acid.

Table 1. Physiological and biochemical characteristics of *Pseudomonas fluorescens* isolates

Isolates	Cyanide production	Antagonistic effect	Protease	Arginine dihydrolase	Proteinase	Chitinase	Salicylic acid (µg/L)
TMTA1	+	+	++	Negative	+	+++	12.5
TMTA2	+	+	++	Negative	+	+++	0
TMTA3	+	+	+++	Positive	+++	+++	26.3
TMTA4	+	+	+	Positive	+	+++	0
TMTP1	+	+	++	Positive	++	+++	9.5
TMTP2	+	+	++	Positive	++	+++	43.6
TMTP3	+	+	++	Positive	++	++	12.9
TMTP4	+++	++ (lysis)	+	Positive	+	+++	297.3
TMTP5	+++	+++ (lysis)	+	Positive	+	+++	213.6
TMTP6	+	++	+	Positive	+	+++	37.3
TMTP7	+	+	+	Positive	+	++	17.3
SCPA1	+++	+++ (lysis)	+++	Positive	+++	+++	106,4
SCPA2	+	+	+++	Positive	++	++	4.7
SCPA3	+	++ (lysis)	++	Positive	+	+++	0
SCPB1	+	++	++	Positive	+	+++	3.5
SCPB2	+	+	+++	Positive	++	++	9.2
SCPB3	++	+++ (lysis)	++	Positive	++	+	97.9
SMPA1	+	+	++	Positive	+	+	14.7
SMPA2	+	+	+++	Positive	++	++	25.3
SMPA3	+	+	+++	Positive	++	+	21.2
SMPA4	+	+	+++	Positive	++	++	17.4
SMPB1	-	+	-	Positive	-	+	0
SMPB2	+	+	++	Positive	+	+	5.7
SMPB3	++	++ (lysis)	++	Positive	+	+++	132.7
SPAA1	+	+	+++	Positive	++	+	25.3
SPAA2	+	++	+++	Positive	++	++	13.3
SPAB1	+	+	-	Positive	-	+	0
SPAB2	+	+	++	Positive	+	+	10.7
Proradix	++	+++ (lysis)	++	Positive	+	+++	269

Note: -: undetected, +: weak, ++: moderate, +++: strong, TMTA/TMTP: Isolates from tomato rhizosphere, SCPA/SCPB: Isolates from cacao rhizosphere, SMPA/SMPB: isolates from maize rhizosphere, SPAA: isolates from *Paraserianthes* rhizosphere, SPAB: isolates from peanut rhizosphere

In terms of their effect on FORL fungi, seven isolates namely TMTP4, TMTP5, SCPA1, SCPA3, SCPB3, SMPB3 and *P. proradix* caused lysis in FORL fungal hyphae when in contact with these bacterial cells, while the other isolates did not cause lysis (Table 1). This may be caused by the presence of enzymes and salicylic acid produced by the seven isolates, which play a role in inhibiting fungal growth.

The fluorescent *Pseudomonas* isolates tested had varied biochemical and physiological characteristics. The antifungal compounds produced by these bacterial isolates also varied in their action on the FORL mycelium from fungistatic to fungicidal depending on the age of fungal and bacterial cultures, the time interval between bacterial inoculation and placement of the fungal plug on the agar plate and the type of media used. Based on this, it can be expected that the variation in the antagonistic effect of these isolates may be greater under greenhouse or field conditions, if the biological control of FORL mainly depends on the production of in vitro determined antifungal compounds.

There was a positive correlation between cyanide production and antagonistic effects against plant pathogenic fungi (Anand et al. 2020). This is confirmed by several researchers that the antimicrobial compounds produced by *P. fluorescens* isolates were effective in playing a decisive role in suppressing soil-borne plant pathogens such as pyoluterin (Suresh et al. 2022), phenazine (Biessy and Filion 2018), phenazine-1-carboxylic acid (Wan et al. 2021) and 2,4-diacetylphloroglucinol (Gu et al. 2022).

Antagonistic and compatibility effects in vitro

The test results showed that six *Pseudomonas* isolates namely TMTP4, TMTP5, TMTP6, SCPA1, SCPB3 and *P. proradix* were strongly and significantly different in inhibiting FORL growth on King's B media compared to other isolates (Figure 2 and 3). However, all tested *Pseudomonas* isolates were significantly different from the treatment without isolate (control).

Pothiraj et al. (2018) also found that several *Pseudomonas* isolates can control the dry root rot pathogen (*Macrophomina phaseolina*) in black gram plants. Similarly, Rathore et al. (2020) reported that isolate *Pseudomonas fluorescens* pf-5 was more effective than the other 9 isolates in inhibiting the growth of the pathogenic fungus *Fusarium oxysporum* f. sp. *cumini*, which cause wilt in *Cuminum cyminum* L. (Cumin) plants.

Pseudomonas also synthesizes lytic enzymes involved in controlling plant diseases caused by soil-borne pathogens (Khalil et al. 2022). *P. fluorescens* antagonist activity against soil-borne pathogens correlated with high levels of β-1,3-glucanase and chitinase (Tapia et al. 2020; Nerek and Sokolowska 2022). Confirmed by Bjelic et al. (2018) that the secretion of hydrolytic enzymes such as chitinase, glucanase, lipase, and protease allows hydrolysis of the cell walls of pathogenic fungi. These enzymes are involved in the degradation of fungal cell walls. Likewise, salicylic acid is widely used as an elicitor to stimulate the synthesis of secondary metabolites (Li et al. 2016).

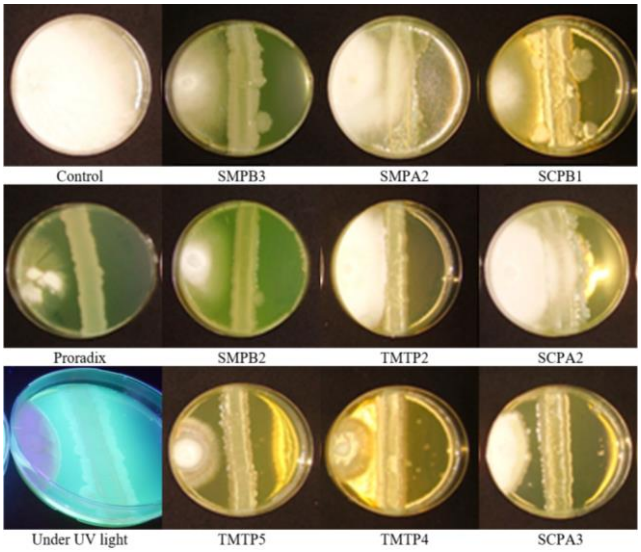

Figure 3. Effect of inhibition by several *Pseudomonas* isolates on FORL mycelium growth in vitro

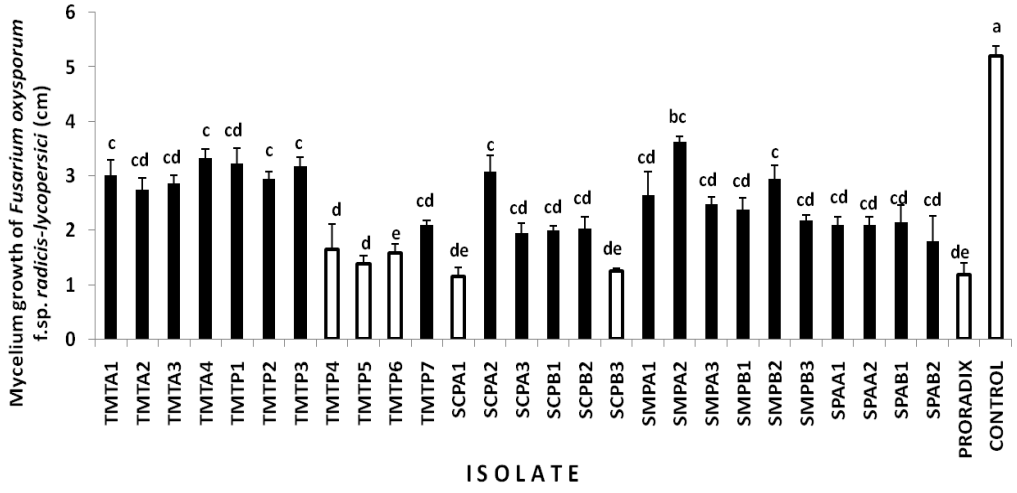

Figure 2. In vitro antagonistic effects of several *Pseudomonas fluorescens* isolates against the fungus FORL on King's B medium. Different letters above the bars indicate significant differences between the treatments

Effect of *Pseudomonas fluorescens* isolates on the growth of *Paraserianthes falcataria*

The effect of several isolates of *P. fluorescens* on the growth of seedlings of *P. falcataria* is presented in Figures 4, 5 and 6. The results of the present study indicate that the treatment of *Pseudomonas* isolates had a significant effect on dry root weight, dry shoot weight and P content of *P. falcataria* seedlings. All isolates showed significantly different effect compared to the treatment without *Pseudomonas* isolates (control). However, isolate TMTP5 showed the best effect and was almost the same as *P. proradix* (commercial *Pseudomonas*). Although statistically the TMTP5 treatment did not significantly different from other isolates.

Pseudomonas can increase plant growth through several mechanisms, for example its ability to produce growth hormones, such as auxins (Swarnalakshmi et al. 2020), indole-3-acetic acid (Marathe et al. 2017), gibberellins (Gusmiaty et al. 2018) as well as induce high P solubility (Ghadamgahi et al. 2022). *Pseudomonas* has a dependency on the nutritional effect of the environment on various types of plants, including *Nicotiana tabacum* (Giles et al. 2014), *Solanum lycopersicon* (Pastor et al. 2014), *Oryza sativa* (Habibi et al. 2014), *Andrographis paniculata* (Thakur et al. 2023) and *Malus domestica* (Kurek et al. 2013). It was reported that some *Pseudomonas* strains can convert some forms of insoluble nutrients into soluble forms that are easily absorbed by plants, for example phosphorus (Liu et al. 2019; Yu et al. 2022), potassium or zinc (Saha et al. 2016). The high content of phosphorus in the shoots of *Paraserianthes* seedlings applied with *Pseudomonas* isolate may be related to the solubilization of phosphate by the bacterial isolates through the production of organic acids such as gluconic, acetic, lactic, fumaric and succinic acids. The production of organic acids results in a decrease in soil pH, producing H^+ which replaces Ca^{2+} and releases HPO_4^{2-} into the soil solution. According to Zhu et al. (2011) phosphate solubilizing microorganisms increase the bioavailability of soil phosphorus for plants. Further, Alori et al. (2017) stated that phosphorus is one of the essential elements needed for plant growth and development; and accounts for about 0.2% of the plant's dry weight. In general, the different effects of *Pseudomonas* isolates in increasing seedling growth were influenced by differences in the physiological and biochemical characteristics of each isolate.

Intensive research on *Pseudomonas* biocontrol has been carried out, but only a few strains of *Pseudomonas* have been commercialized. In general, *Pseudomonas* works inconsistently, which can be attributed to poor adaptation to environmental conditions and specific host responses to microorganisms (Höfte 2019; Oni et al. 2022). The results show that *Pseudomonas* bacterial isolates with their multifunctional properties will attract more attention in the field of bio-fertilization and bio-control in the future.

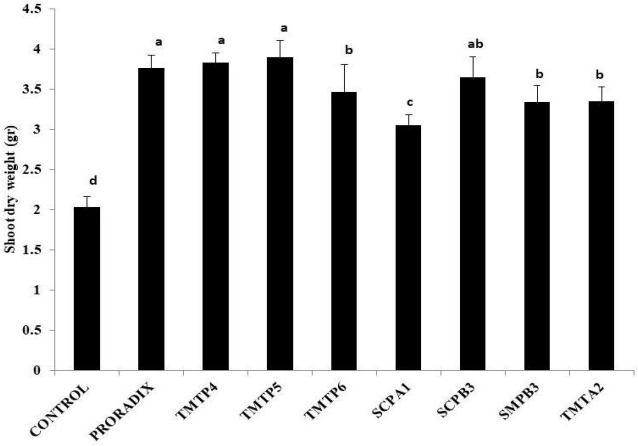

Figure 4. Effect of *Pseudomonas* isolates on shoot dry weight 14 weeks after planting. Different letters above the bars indicate significant differences between the treatments

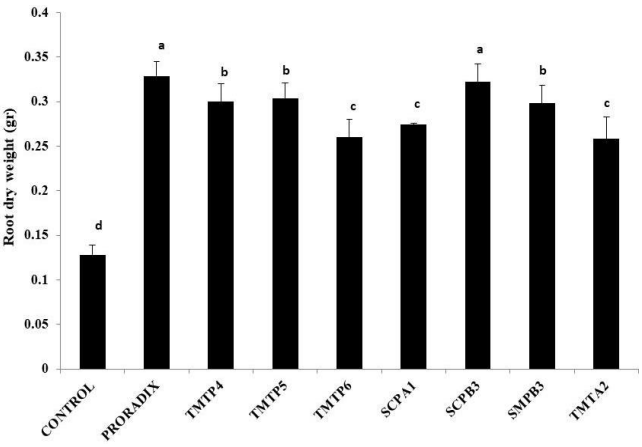

Figure 5. Effect of *Pseudomonas* isolates on root dry weight 14 weeks after planting. Different letters above the bars indicate significant differences between the treatments

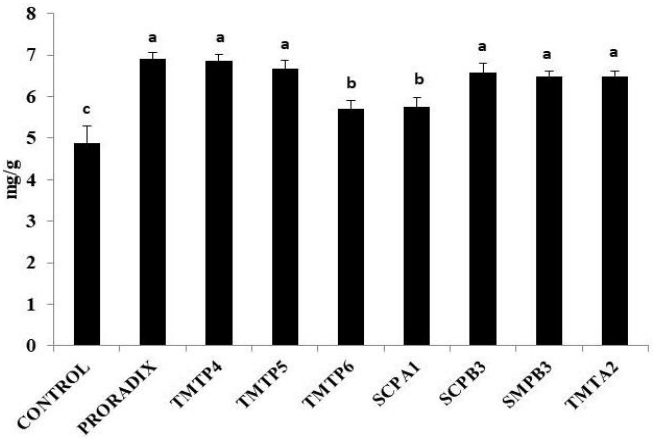

Figure 6. Effect of *Pseudomonas* isolates on phosporus content of *Paraserianthes* seedlings 14 weeks after planting. Different letters above the bars indicate significant differences between the treatments

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