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ORIGINAL ARTICLE

Antagonistic fluorescent Pseudomonads: rhizobacteria with suppressive and plant growth promoting properties against *Phytophthora colocasiae*, the causal agent of taro leaf blight

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Abstract

Taro leaf blight caused by *Phytophthora colocasiae* affects plant health and is a major threat to taro culture in Cameroon. Chemical fertilizers used often harm the ecosystem. Plant growth-promoting rhizobacteria (PGPR) are better alternatives that increase plant growth promotion and suppress phytopathogens. In the present study, a total of 67 fluorescent *Pseudomonas* spp. was characterized by 17.91, 5.97, and 4.47% populations of *P. fluorescens*, *P. chlororaphis*, and *P. putida*, respectively, among the most represented. More than 36% of bacteria showed antagonistic potential through the production of both diffusible and volatile compounds. Some of them (03) exhibited antagonistic activity in dual culture against *P. colocasiae* with a diameter greater than 13 mm. These rhizobacteria produced a significant amount of siderophore, IAA, SA, HCN, protease, lipases, and cellulases. For the pot experiment, treatment by *Pseudomonas* significantly increased the enzymatic activity involved in the resistance of taro, such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL). The two antagonists also increased plant growth parameters of taro such as chlorophyll, plant height, shoot length, total leaf surface, fresh root biomass, and fresh leaf biomass. These findings showed that fluorescent *Pseudomonas* have an intriguing and undeniable potential in the fight against *P. colocasiae*, which could lead to the development of a biopesticide in the future.

Keywords: biocontrol, fluorescent *Pseudomonas*, *Phytophthora colocasiae*, rhizobacteria, taro

Introduction

Taro [*Colocasia esculenta* (L.) Schott] is a tuberous plant abundantly cultivated worldwide (Misra *et al.* 2008). Global production was estimated at 12 million tons on an area of 2 million hectares (Achancho 2013). Cameroon, according to MINADER, was the world's third-largest taro producer in 2009, with approximately 1.7 million tons (10 tons per hectare). Due to its agro-ecological importance and nutritional value, taro is cultivated, commercialized and consumed in many regions of Cameroon. Despite the socio-cultural and economic importance, its production is subject to several constraints. Among these, taro blight, which is the most devastating, is caused by the oomycete, *Phytophthora colocasiae* (Sameza *et al.* 2014). This disease can spread rapidly to other plant parts and has resulted in yield losses of up to 80–100% in many countries. Currently, synthetic pesticides like mancozeb and metalaxyl are used to control this disease (Mbong *et al.* 2013). Unfortunately, these substances have adverse consequences for the environment,

such as accumulation of residues and soil pollution (Shoaib *et al*. 2020). The use of biological inoculants could be an innovative and effective control strategy against this epidemic (Khan and Arshad 2022). The use of rhizobacteria as a biopesticide is a very promising alternative to synthetic pesticides due to their increased compatibility and specificity towards pathogens (Sharf *et al.* 2021). Some of these bacteria, including the fluorescent *Pseudomonas* genus, have a very high level of harmlessness for non-target organisms of the endogenous microflora, which bioprotective properties often depend on for multiple mechanisms and therefore, rarely trigger resistance phenomena in pathogens (David *et al*. 2018). Previous investigations have shown that *Pseudomonas* could be considered as true agricultural biological "compounds" because of their diversity, their fungal disease suppression properties and growth promotion properties of plants (Javed *et al*. 2021). Indeed, this phytobeneficial potential is linked to their ability to produce natural, diffusible antibiotics such as phenazines, pyoluteorin, pyrrolnitrin and DAPG (2,4 diacetylphloroglucinol), siderophores that are involved in the improvement of plant growth and health (Lemanceau *et al.* 2009). This then contributes to the acquisition of iron by plants, thus limiting the growth of some phytopathogenic microorganisms (Lemanceau *et al.* 2009). These and other unexplored properties of fluorescent *Pseudomonas* could make it possible to find antagonists of *P. colocasiae* in their biotope, which are able to reduce the severity of leaf blight. This study was aimed to determine the antagonistic potential of fluorescent *Pseudomonas* spp. by identifying plant-growth-promoting and biocontrol parameters involved in the suppression of *P. colocasiae*, the causal agent of taro blight.

Materials and Methods

Collection of soil samples

Soil samples were collected from bulk and rhizosphere soil of taro planted in a cultivated field in Dschang, West Region, Cameroon. The root systems of the plants were dug out and the rhizosphere soil that was attached to them was collected, placed in sterile plastic bags, labeled and transported in a refrigerator to the Biochemistry Research Laboratory, Littoral, Douala University . The collected samples were stored at –20°C for further analysis.

Isolation of rhizobacteria from rhizospheric soil

Isolation of rhizospheric bacteria from the taro rhizosphere was carried out using the agar plate method (Karnwal and Kumar 2012). Briefly, 1 g of the soil sample from the rhizosphere was collected aseptically and transferred to 4.5 ml of sterile physiological water (0.9% NaCl) in a 25 ml conical flask for 48 h. A volume of 0.1 ml from the stock was used for a serial dilution of 10^{-1}, 10^{-2}, and 10^{-3} on King B (KB) filled with 0.9 ml of sterile distilled water, and 0.1 ml of the suspension in test tubes was transferred from a suitable dilution on King B agar (Sigma-Aldrich, USA) plate in triplicate, and incubated at 28 ± 2°C for 24 h until colonies developed. After incubation, distinct colonies were collected with a sterile wire loop, streaked onto a presterilized King B agar plate, and incubated at 28 ± 2°C for 24 h for purification of the bacterial isolates.

Morphological and biochemical characterization of rhizobacterial isolates

Physiological characteristics of rhizobacteria isolates were identified on King B agar plates using purified bacterial culture as previously described. The color, shape, size, and pigmentation were recorded after incubation. The production of fluorescent pigment was investigated in the KB medium. After incubation for 24 to 96 h at 28°C, the development of the fluorescent pigment was revealed under a UV lamp (254 and 366 nm). Gram strain, levan oxidase, and oxidase tests were also recorded. The biochemical characteristics of the selected rhizobacteria isolates were identified using the API 20 NE gallery (BioMérieux, France). This gallery included eight conventional tests related to the activity of various enzymes (nitrate reductase or denitrification), tryptophanase or indole formation from tryptophan, glucose fermentation, arginine dehydrogenase or ammonia formation from arginine, urease, esculinase, gelatinase or gelatin liquefaction, and β-galactosidase, and 12 sugar assimilation tests (glucose, arabinose, mannose, mannitol, N-acetyl-D glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, and phenylacetate). Identification was carried out using the API WEB database. Identification of isolates was improved by the dichotomous keys used by Jacques (1994) and Digat and Gardan (1987).

Phytophthora colocasiae isolates

The *Phytophthora colocasiae* isolates used in this study came from the culture collection of the Laboratory of Biochemistry, University of Douala (Cameroon). The data regarding the characterization of these isolates was given by Sameza *et al*. (2014).

Dual culture antagonistic assay

Production of diffusible substances

The antagonism test was performed on solid culture media (PDA) using the method described by

Vincent *et al.* (1991). Briefly, an 18-h culture of each fluorescent *Pseudomonas* spp. was streaked for confirmation of antagonism and spot streaked (for screening of multiple isolates) with 20 µL of bacterial suspension on the opposite side of an agar plate. Then, a 2-mm explant of *P. colocasiae* from a 7-day culture was placed on top of, 2 cm from the end of the Petri dish. The latter was placed along a diametrical axis equidistant from each *Pseudomonas* spp. isolate. This confrontation of isolates with *P. colocasiae* was followed for 1 week at room temperature, and the distance between the tip of the colonies and that of the mycelium was measured and compared to the control containing only *P. colocasiae* mycelium. *Pseudomonas* spp. isolates with inhibitory power (zones of inhibition, ZI) greater than or equal to 2 mm were selected. These antagonists were sorted according to the diameter of the ZI into random groups, and all purified antagonist isolates were preserved as previously described.

Production of volatile substances

The antagonistic potential of *Pseudomonas* spp. isolates through the production of volatile substances was investigated. For this purpose, each isolate was streaked on King B agar and then the lid of the previous dish was replaced by the bottom of another Petri dish containing PDA and a 7-mm explant of 7-day old *P. colocasiae*. Both dishes were sealed with parafilm. The control was prepared in the same way except that the bottom dish was free of bacteria. The dishes were incubated at 25°C and observations were noted after 7 days of incubation. The inhibition of fungal growth was calculated using the formula:

$$I = \frac{r1 - r2}{r1} \times 100,$$

where: I – represents inhibition percentage of radial growth of *P. colocasiae;* $r1$ – represents the radial growth of *P. colocasiae* control; $r2$ – represents that with *Pseudomonas* spp. (Trivedi *et al.* 2008).

Plant growth-promoting traits of fluorescent *Pseudomonas* rhizobacteria

Indole acetic acid (IAA) production

The quantification of IAA and its derivatives was evaluated by the colorimetric method described by Glickmann and Dessaux (1995). A volume of 100 µl of each fresh (18–24 h) culture (Absorbance 600 = 0.7), from each isolate, was inoculated into 100 ml of half-diluted TSB (tryptic soy broth). After incubation at 28°C, protected from light for 72 h and under constant agitation (150 rpm), the cells were separated by centrifugation (4000 g · 10 min^{-1}) and the culture supernatant was filtered. To 1 ml of filtrate, 2 ml of Salkowski's reagent (2% FeCl3 at 0.5M in 35% perchloric acid) were added. The suspension was then vortexed and incubated in the dark for 20 min, and the absorbance measured at 535 nm. The development of a pink coloration is an indicator of IAA production. The concentration of IAA was obtained against a standard curve established from a dilution series of IAA (Sigma-Aldrich) of 0–45 µg · ml^{-1} in TSB.

Salicylic acid (SA) production

The estimation of the production of salicylic acid was done by the method of Jacques *et al.* (1995) with some modifications. *Pseudomonas* from 18 h cultures were transferred to 50 ml of succinate (MS) liquid medium and then incubated with continuous shaking (150 rpm) for 40 h at 28°C. After centrifugation at 4000 rpm · 15 min^{-1}, the absorbance of the supernatant was determined at 400 nm and the amount of SA was estimated from a 3,5-dinitro-acid-salicylic acid calibration curve.

Siderophore production

Siderophore production by *Pseudomonas* rhizobacteria was estimated using the method of Schwyn and Neilands (1987). In brief, 18h bacteria colonies of pure culture were spotted on the Petri plates containing chrome azurol S media (Sigma) and incubated at 28 ± 2°C for 3 days. The catechol-type siderophore production was confirmed by the formation of a purple zone, whereas the hydroxamate-type siderophore gave an orange zone around the bacterial colonies.

Hydrogen cyanide (HCN) production

Cyanogenesis was evaluated on a liquid medium according to the method described by Meena *et al.* (2001). The culture of each isolate was incubated for 48 h at 28°C with constant agitation (180 rpm) in Meyer Erlens containing 50 ml of TSB. Whatman No. 1 paper (0.5/10 cm) saturated with sodium picrate solution was suspended vertically in these flasks. When the reaction was positive, the sodium picrate in the paper changed color according to the production of HCN.

Hydrolytic enzyme production

Cellulase production by *Pseudomonas* spp. was determined by the method described by Cattelan *et al.* (1999). A volume of 20 µl of suspension of each isolate was introduced into the wells of M9 agar supplemented with 10 g of cellulose and 1.2 g of yeast extract. Then, the plates were incubated for 8 days at 28°C. The development of a clear halo around the colonies indicated a positive reaction.

The research on lipase production was carried out by culturing the isolates on Tryptic Soy Agar (TSA) with 1% oleic acid. A positive reaction was marked by the appearance of a halo around the colonies (de Groot *et al.* 1991).

The proteolytic activity of the isolates was determined by the method of Smibert (1994). Each suspension (20 µl) of *Pseudomonas* spp. culture was introduced into the wells of skim milk agar (5%) and incubated for 48 h at 28°C. The development of a halo around the colonies indicated a positive reaction.

Pot experiment

The pot experiment was conducted in December 2018 in the Laboratory of Biochemistry at the University of Douala. The local taro cultivar (ebo coco variety), which is sensitive to *P. colocasiae*, was used as a test crop.

Soil preparation

The substrate used for the pot experiment was soil mixed with quartz in a proportion of 3 : 1 (w/w), and the whole was autoclaved twice at 120°C for 1 hour. It was then cooled for 24 h and placed in polyethylene pots (25 × 30 cm) with 3.5 kg of substrate per pot containing taro tubers (one tuber per pot).

Preparation of *Pseudomonas fluorescens* inoculants

For the inoculation of seedlings, both DS15V and DS17R bio inoculants were grown in King B medium overnight at 30°C and 120 rpm in an orbital shaker. After 18 h of growth, cells were harvested by centrifugation at 5000 rpm for 15 min, washed twice with sterile saline solution (0.9% NaCl), and resuspended in sterile saline solution to get an inoculum density of $1 \cdot 10^8$ CFU · ml^{-1}. The two sizes of inocula (DS15V and DS17R) were sprayed into the soil surface 1 week after the seedlings were transferred to pots.

Experimental design

The experimental device was done according to Shternshis *et al.* (2016) with some modifications. A randomized block design was used to assign treatments to three replicates. Plants were grown in rows 40 cm wide at a spacing of 2.5 m between rows. A total of 25 plants were planted in every meter, and the size of the buffer zone between plots was 2 m. The experimental set-up consisted of two blocks (healthy and infected plants) with two treatments and a control. For each set, three pots were used with three replicates. The block of healthy plants consisted of the control and the treatment with *P. fluorescens* DS17R and *P. chlororaphis* DS15V, respectively. The block of infected plants consisted of the plants treated with DS17R and DS15V isolates, respectively, after being infected by *P. colocasiae*. The control plots were left untreated. These were applied at a volume application rate of 0.5 l · m^{-2}. The concentration of both DS15V and DS17R suspensions was 3×10^8 CFU · ml^{-1}.

Inoculation of *Phytophthora colocasiae*

The effect of DS15V and DS17R on taro-induced resistance and severity rate was done on 21-day-old plants. The taro leaves were inoculated with 5 mm agar plugs of *P. colocasiae* 7-day-old culture. Then, the plants were incubated for 5 days, and the symptoms of taro leaf blight were measured. The plants were harvested 6 days later, and diameters of the necrosis were measured for determination of percentages of inhibition (I%) of taro leaf blight according to the formula:

$$I\% = \frac{Do - Dx}{Do} \times 100,$$

where: *D*o – the necrosis diameter on untreated plants with *Pseudomonas* and infected by *P. colocasiae*, *Dx* – the necrosis diameter on plants.

Thereafter, plants in each pot were washed, leaves removed, and stored in the refrigerator at –20°C for measurement of growth-promoting and resistance-related parameters.

Plant analysis

Plant growth-promoting parameters

After the harvest period, morphological and growth parameters were measured (fresh root biomass, fresh leaf biomass, plant height, shoot length, total leaf surface area, and chlorophyll content).

The size of the plant corresponds to the height of the petiole and was measured with a tape measure. The diameter of the collar is the circumference of the transitional part between the root system and the petiole. It was also measured with a tape measure. Biomass was assessed by weighing with a precision balance. The weighing was done on the root part (roots), the aerial part (leaves and stems), and the freshly harvested plant. The leaf area was obtained by applying the formula (Raunkiaer 1934) below:

$$S = 2/3L \times l,$$

where: *S* – leaf area, *L* – the average length of the leaf, *l* – average width of the leaf.

Chlorophyll content was determined by spectrophotometry. In brief, one gram (1 g) of the leaf was cut and then dried and ground in a mortar in the presence of a pinch of fine sand. The resulting grind was added to 3 ml of 90° ethanol and the mixture was allowed to stand in the air for 3 min. The resulting grindings were centrifuged at 5000 rpm for 10 min, and the recovered supernatant was used for chlorophyll determination. Chlorophyll content was determined by the standard formula:

Chlorophyll = chlorophyll a + chlorophyll b.

Resistance related parameters

Cystein content

A volume of 100 µl 10-fold diluted supernatant was prepared, mixed with 350 µl of ninhydrin, and covered with aluminum foil. The mixture was incubated in a boiling water bath at 100°C for 10 min to allow the reaction to proceed (pink staining). At the end of this operation, the mixture was cooled and 700 µl of 95° ethanol was added, and the optical density (OD) was read at 560 nm. The amount of cysteine was determined using a standard range (0 to 1 mg · l^{-1}) with a pure cysteine solution (0.1%).

Total phenolic compounds

The determination of phenolic compounds was carried out using Folin's reagent according to the method described by Singleton and Rossi (1965). The plant material, consisting of taro leaves for 2-month old taro leaves, was harvested, washed, and then dried in the shade in a well-ventilated area away from moisture and at room temperature. After drying, the sample was then ground into a fine powder and stored in the dark. The polyphenols were extracted at 4°C to prevent polyphenol oxidases. The extraction of total phenols consisted of macerating 1 g of plant material in 10 ml of 70% v/v methanol for 1 h. After filtration, the filtrate was macerated once again for 1 h to maximize extraction. Both homogenates were centrifuged together at 3,000 rpm for 20 min. The supernatant obtained was recovered for the assay. The total amount of phenols was calculated from the standard range equation established with catechol (0–200 µg · ml^{-1}) and was expressed as micrograms of catechol equivalent (CAE) per gram of total plant dry matter.

Phenylalanine ammonia lyase (PAL) activity assay

Phenylalanine ammonia-lyase (PAL) activity was measured by spectrophotometry using the method described by Whetten and Sederoff (1992). A volume of 0.02 ml of crude enzyme extract was added to a tube containing 0.5 ml of phosphate buffer (0.05 M, pH 8.8) and 0.6 ml of L-phenylalanine (0.01 M). This mixture was incubated in the dark for 60 min. The reaction was slowed down by immersing the tube in an ice bath, and the optical density was read at 290 nm using a spectrophotometer. The specific activity was expressed as OD/hour/milligram of protein/gram of fresh material.

Polyphenol oxidase (PPO) activity assay

The polyphenol oxidase (PPO) activity was determined by the method described by Constabel *et al.* (1995). The reaction medium was obtained by mixing 500 µl of phosphate buffer (100 mM, pH 7.0) and 700 µl of catechol (0.01 M). The reaction was subsequently initiated by adding 20 µl of enzyme extract at 30°C. After 2 min of incubation, the tubes were immersed in an ice bath to stop the reaction. The absorption intensity was determined at 420 nm by spectrophotometry. Enzyme activity was expressed as OD/min/mg protein.

Peroxidase (PO) activity assay

Peroxidase activity was evaluated according to the method described by Hammerschmidt *et al.* (1982). A 2 ml volume of the reaction mixture (1v of 2% H_2O_2; 2V of 1% guaiacol and 5V of 0.066 M phosphate buffer pH 6) was introduced into test tubes and the reaction was initiated by adding 10 µl of extract. The time of the reaction was monitored, and the optical density (OD) was measured after each minute for 3 min of incubation at 420 nm and 25°C. The change in OD (ΔDO) between the third and first minute was noted. Enzyme activity was estimated as ΔDO/min/mg/g of fresh material.

Statistical analysis

Basic statistical analysis (means, standard deviation, bar graphs, and standard curves) was performed with MS Excel 2016. One-way analysis of variance (ANOVA) was used to determine any significant differences between the means of treatments with means in R 3.6.0 (R-Studio 2019) with $p < 0.05$. Correlation traits of antagonistic rhizobacteria were observed by a hierarchically clustered heatmap and dendrograms were defined using the ward method. Z-scores were derived using column scaling on the Seaborn package in Jupiter for anaconda navigator 2.0.3. For visualization of paired comparison, post hoc testing was performed using t-test independent samples with Bonferroni correction. Correlation coefficients were calculated among biocontrol and plant growth promoting (PGP) efficacy of antagonists with their *in vitro* assessed biocontrol and PGP potentials.

Results

Phenotypic characteristics of fluorescent *Pseudomonas* spp. isolates

The results in Table 1 show the biochemical characteristics of fluorescent *Pseudomonas* isolated from the taro rhizosphere. According to the identification scheme proposed by Digat and Gardan (1987), the *P. fluorescens*, *P. putida*, and *P. chlororaphis* populations were represented, respectively, at the proportions of 12 (17.91%), 3 (4.47%), and 4 (5.97%), respectively, of the characterized bacteria. The keys of Jacques and Bossis as well as the general scheme of Digat and Gardan (1987) allowed for the identification of species *P. fluorescens* and *P. putida* into their respective

biovars (Table 1). The population of *P. fluorescens* was represented by 12 isolates. Biovar I was recognized by its gelatin positive, levan negative, and denitrification negative reactions. It was the most dominant with 7 (58.33%), followed by biovar IV with 3 isolates (25%). Concerning *P. putida*, its biovars A or B were not identified. However, *Pseudomonas* spp., which can use L-tryptophan, is linked to biovar A, whereas tryptophan-negative isolates are associated with biovar B. On the other hand, *P. chlororaphis*, which belongs to biovar IV, revealed similarities with *P. fluorescens*.

Inhibition by diffusible and volatile substances

The antagonistic potential of dual confrontation was evaluated according to their capacity to produce diffusible substances (Table 2). The results showed that fluorescent *Pseudomonas* DS17R inhibited the growth of *P. colocasiae* with the highest inhibition rate of 17.80 mm, followed by DS15V (15.01 mm) and DS14N (14.67 mm) (Fig. 1B). The results of the volatile assay showed that the highest percentages of mycelium inhibition were observed with 91.66%, 87.08%, and 74.58% for DS17R, DS15V, and DS23 isolates (Fig. 1A), respectively. The lowest inhibition rate of 2.5% was observed for DS5D.

Antimicrobial traits of *Pseudomonas fluorescens* rhizobacteria

Enzyme production

The ability of isolates to produce cellulase, lipases, and proteases was evaluated in the culture media through the formation of clear halo zones indicating enzyme activity. Among the selected isolates, some of them (20) produced cellulase (69%), lipase (90%), and

Table 1. Biochemical characteristics of fluorescents *Pseudomonas* rhizobacteria

Biochemical tests	Bacteria strains/Biovars					
	P. fluorescens		*Pseudomonas* spp.		*P. putida*	P. chlororaphis
	bv IV	bv V	bv I	bv III	bv A/B	bv IV
	DS17R (3)	DS16V (2)	DS11 (7)	DS1 (5)	DS14N (3)	DS15V (4)
	reactions					
Denitrification	+	–	–	+	–	+
Tryptophanase	–	–	–	–	–	–
Glucose fermentation	–	–	–	–	–	–
Arginine dihydrolase	+	+	+	–	–	+
Urease	–	–	–	–	–	–
Esculinase (β glucosidase)	–	–	–	–	–	–
Gelatinase	+	+	+	+	–	–
β–galactosidase	–	–	–	+	+	–
Glucose	–	–	+	+	+	+
Arabinose	+	–	+	+	+	–
Mannose	+	+	+	+	+	+
Mannitol	–	–	+	–	–	+
N–acetyl–D glucosamine	+	+	+	–	–	+
Maltose	–	–	+	–	–	+
Gluconate	+	+	+	+	+	+
Caprate	+	+	+	+	+	+
Adipate	–	–	–	–	–	+
Malate	+	+	+	+	+	+
Citrate	+	+	+	+	+	+
Phenyl–acetate	–	–	–	–	–	–
Oxidase	+	+	+	+	+	+
Fluorescent pigmentation	+	+	+	+	+	+
Levan	+	–	+	–	–	+

(+) – positive; (–) – negative

Table 2. Rate of *Phytophthora colocasiae* mycelia inhibition by *Pseudomonas* rhizobacteria in dual culture and volatile substances

Bacterial isolates	Antagonistic activity on PDA agar plate against *P. colocasiae*	
	Inhibition by diffusible substances [mm]	Inhibition by volatile substances [%]
DS1	9.00 ± 1.00 bc	35.41 ± 3.88 f
DS2M	11.00 ± 1.54 b	38.18 ± 3.18 e
DS3M	10.60 ± 2.20 bc	24.16 ± 5.27 g
DS4	12.76 ± 1.76 b	25.41 ± 4.44 g
DS5D	5.00 ± 1.00 c	2.50 ± 0.50 j
DS8	7.33 ± 0.44 c	11.42 ± 2.45 i
DS9	7.36 ± 1.72 c	5.00 ± 1.66 j
DSF	10.71 ± 0.58 bc	5.41 ± 1.11 j
DS10	10.00 ± 0.50 bc	73.75 ± 3.33 b
DS11	7.03 ± 0.66 c	35.41 ± 2.77 f
DS14	12.00 ± 2.00 b	30.41 ± 3.88 fg
DS14N	14.67 ± 2.84 a	65.29 ± 7.31 c
DS15D	10.66 ± 0.33 bc	41.66 ± 1.94 e
DS15E	12.00 ± 1.00 b	64.16 ± 2.22 c
DS15Q	8.20 ± 1.20 c	21.00 ± 1.66 g
DS15R	12.00 ± 1.00 b	72.91 ± 3.05 b
DS15V	15.01 ± 1.35 a	87.08 ± 5.55 a
DS16	12.66 ± 0.44 b	18.75 ± 4.16 h
DS16R	12.00 ± 2.00 b	32.91 ± 7.22 f
DS16V	7.15 ± 1.60 c	64.16 ± 6.38 c
DS17R	17.80 ± 2.20 a	91.66 ± 1.38 d
DS17W	11.71 ± 0.46 b	22.05 ± 2.50 g
DS21	11.00 ± 0.50 b	56.25 ± 12.5 d
DS23	10.55 ± 0.50 b	74.58 ± 4.72 b
DS24	11.29 ± 0.81 b	60.83 ± 6.38 c
DS25	10.00 ± 0.00 b	19.53 ± 3.21 gh
DS26	7.26 ± 1.11 c	52.91 ± 2.22 d

Data represents means ± standard deviation of three replicates. Means labelled with different letters are significantly different according to Student's t test at $p < 0.05$

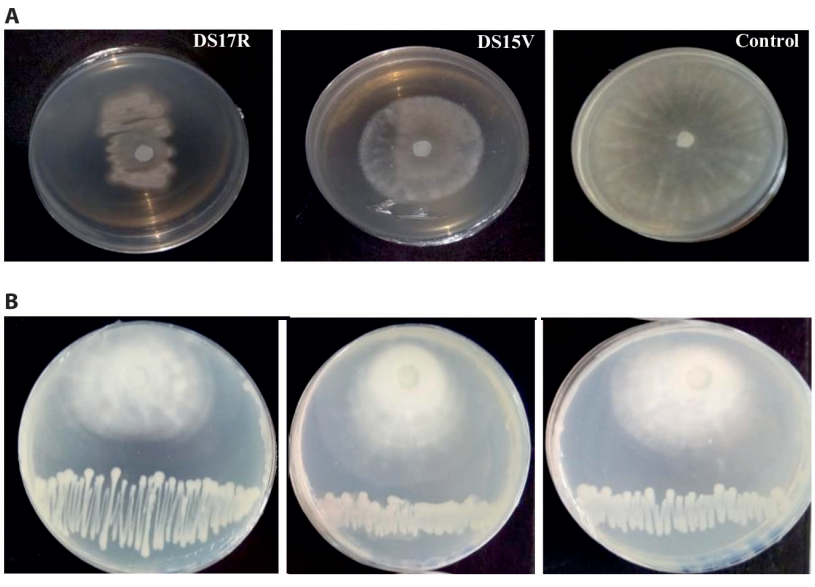

Fig. 1. Determination of antagonistic activity of *Pseudomonas fluorescents* against *Phytophthora colocasiae*. A – inhibitory effect of volatile metabolite produced by isolates DS15V and DS17R; B – antagonistic potential of DS17R, DS15V and DS14N isolates in dual culture test

protease (90%) (Table 3). However, the fluorescent *Pseudomonas* spp. DS15V and DS16R showed significant production of lipases. DS1M and DS8 produced more proteases. The production of cellulases in the cell was found to be highest with DS17R, DS14, DS11, and DS4, respectively.

Production of indole acetic acid (IAA)

From the table below, it can be seen that indole acetic acid (IAA) was produced significantly by isolates DS15V, DS17R, and DS26 at concentrations of 38.40, 42.29, and 41.45 $\mu g \cdot ml^{-1}$, respectively, ($p < 0.05$) compared to the reference strain PA012 (Table 3).

Production of salicylic acid (SA)

The capacity of isolates to produce salicylic acid (SA) was also observed (Table 3). We noted that four fluorescent pseudomonads DS14N, DS17R, DS16V, and DS15V showed significant production of SA with pick concentrations of 48.12, 50.38, 51.02, and 61.28 $\mu g \cdot ml^{-1}$, respectively.

Production of cyanuric acid (HCN)

The highest cyanuric acid (HCN) production was found with DS16V, DS17R, and DS30C isolates (Table 3). Additionally, except for five isolates, the majority of fluorescent antagonistic *Pseudomonas* spp. produced HCN.

Production of siderophore

The production of three classes of siderophore was evaluated in isolates that showed antagonistic potential (Table 3). From the results obtained, DS16R, DS16, DS16V, and DS15V produced significantly more hydroxamate, while DS11, DS16V, and DS30c showed high levels of carboxylates. However, DS8, DS21, and DS30c showed significant production of catecholate.

Relationships between antagonistic traits of inhibitions and characters of fluorescents *Pseudomonas*

Figure 2 shows the different links between the antagonistic traits of the isolates and their characteristics. In

Table 3. Plant growth promotion factors and hydrolytic enzymes production by fluorescents *Pseudomonas* rhizobacteria

Isolates	Production of								
	antimicrobial metabolites			siderophores			hydrolytic enzymzes		
	IAA [$\mu g \cdot ml^{-1}$]	SA [$\mu g \cdot ml^{-1}$]	HCN	Hydroxamates	Carboxylates	Catecholates	Cellulases	Lipases	Proteases
DS1	12.21 ± 2.12 e	41.00 ± 1.55 c	+	1.02 ± 0.02 h	–	1.38 ± 0.01 f	+	+	+
DS1M	27.53 ± 1.33 c	35.81 ± 2.57 d	+	0.98 ± 0.00 h	0.63 ± 0.00 k	1.36 ± 0.02 f	+	+	++
DS3M	13.71 ± 0.75 e	19.22 ± 1.12 e	+	2.12 ± 0.03 c	1.01 ± 0.01 j	1.33 ± 0.41 f	–	++	+
DS4	11.66 ± 2.21 e	32.15 ± 1.31 d	–	0.43 ± 0.00 j	nt	1.33 ± 0.03 f	++	++	+
DS8	6.33 ± 0.32 f	21.55 ± 0.33 e	*	0.46 ± 0.00 j	0.96 ± 0.00 j	1.88 ± 0.00 c	–	+	++
DS9	17.56 ± 1.89 d	9.33 ± 2.44 f	+	2.00 ± 0.02 d	1.67 ± 0.01 f	nt	–	+	+
DS11	22.12 ± 2.75 c	39.66 ± 5.71 c	+	1.59 ± 0.01 f	2.34 ± 0.40 b	1.71 ± 0.04 d	++	–	+
DS14N	19.74 ± 6.35 d	48.12 ± 3.67 b	+	1.04 ± 0.00 h	1.68 ± 0.02 f	1.13 ± 0.01 g	++	++	+
DS15V	38.40 ± 1.50 a	61.28 ± 4.10 a	+	2.27 ± 0.02 b	1.55 ± 0.33 g	1.36 ± 0.01 f	+	+++	+
DS15E	33.15 ± 3.26 b	42.65 ± 2.33 c	+	1.28 ± 0.01 g	1.29 ± 0.05 h	1.72 ± 0.02 d	+	++	+
DS15Q	27.43 ± 1.35 c	21.11 ± 2.59 e	+	1.78 ± 0.01 e	1.86 ± 0.32 e	1.12 ± 0.01 g	–	+	+
DS16V	25.33 ± 2.46 c	51.02 ± 2.50 b	++	2.29 ± 0.33 b	2.03 ± 0.30 c	1.10 ± 0.00 g	+	++	+
DS16R	22.45 ± 1.08 c	22.40 ± 0.25 e	–	2.41 ± 0.38 a	1.22 ± 0.01 i	0.58 ± 0.00 i	+	+++	+
DS16	26.13 ± 4.67 c	43.33 ± 4.78 c	+	2.26 ± 0.41 b	1.81 ± 0.21 e	0.24 ± 0.00 j	+	++	–
DS17R	42.29 ± 5.38 a	58.38 ± 5.66 b	++	0.56 ± 0.00 i	1.95 ± 0.01 d	1.38 ± 0.01 f	++	+	+
DS21	11.11 ± 0.66 e	nt	+	0.41 ± 0.00 j	0,65 ± 0.00 k	2.06 ± 0.06 b	–	++	–
DS23	18.86 ± 2.64 d	13.50 ± 1.33 f	–	0.56 ± 0.00 i	0.97 ± 0.00 j	0.82 ± 0.00 h	–	+	+
DS24	8.09 ± 0.57 f	10.96 ± 2.52 f	–	0.40 ± 0.00 j	0.66 ± 0.00 k	1.68 ± 0.25 d	–	+	+
DS26	41.45 ± 4.98 a	33.25 ± 6.92 d	–	1.82 ± 0.12 e	1.33 ± 0.23 h	1.51 ± 0.03 e	+	–	+
DS30C	14.33 ± 0.66 d	nt	++	0.41 ± 0.00 j	2.50 ± 0.50 a	2.33 ± 0.01 a	+	++	+
PA012	35.13 ± 2.15 b	40.29 ± 1.57 c	+	1.98 ± 0.04 d	1.65 ± 0.09 f	1.88 ± 0.05 c			

Data represents means ± standard deviation of three replicates. Data with different letters in the same column are significantly different after ANOVA at $p < 0.05$ using Turkey's HSD test; IAA – indol acetic acid; SA – salicylic acid; HCN – hydrogen cyanide; (–) – absence; (+) – presence; (*) – very weak detection; nt – no tested

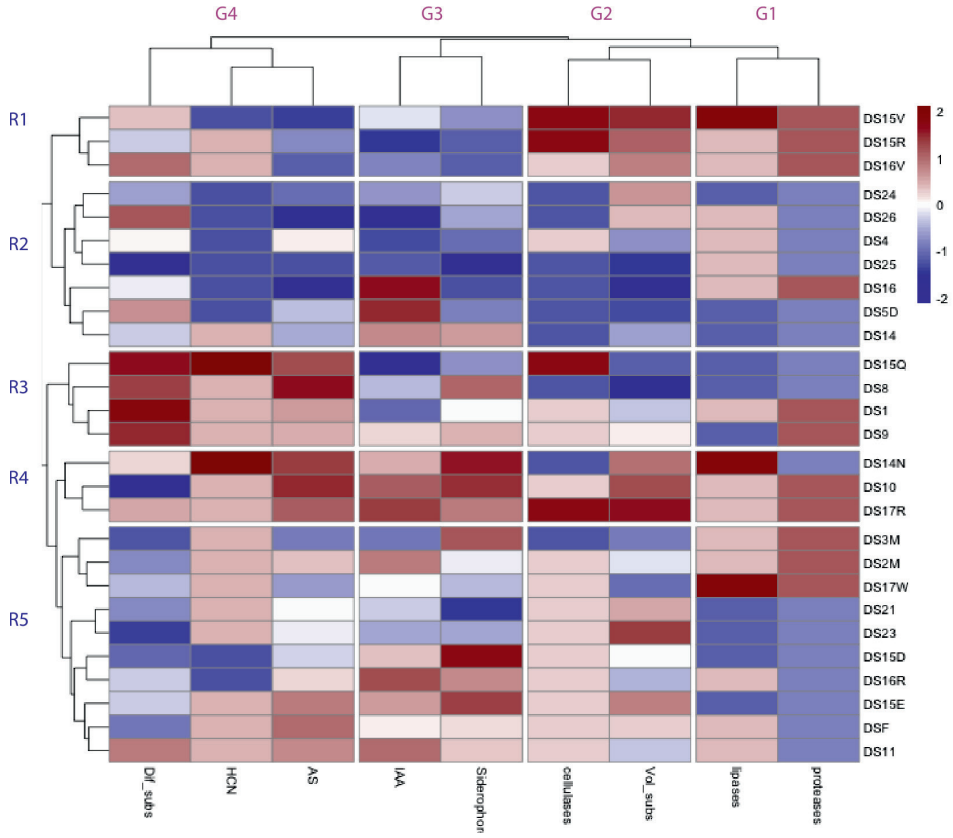

Fig. 2. Hierarchically clustered heatmap of antagonistic traits of 27 rhizobacteria isolates against *Phytophthora colocasiae*. Dendrograms were defined using the complete-linkage method and z-scores were derived using column scaling. The intensity of red color indicates high levels of antagonistic traits and blue color indicates low levels. G1, G2, G3 and G4 represent column clusters whereas R1,R2,R3,R4 and R5 indicate row clusters, respectively

this clustermap, a total of five (5) rows and four (4) clusters were identified. These are mainly R1 isolates (DS15V, DS15R and DS16V) which produce large quantities of substances in groups 1 and 2 (G1 and G2). Those of row 2 and 5 (R2 and R5) whose production of the substances involved in the antagonism was low. In addition, row 3 contained isolates (DS15Q, DS1, DS8, DS9) significantly producing the substances of group four while row four contained isolates (DS14N, DS10, DS17R) producing most of the compounds relating to the antagonist activity. This heatmap also demonstrated that the production of these substances was a function of the biochemical characteristics of each isolate. Indeed, biovars with similar characteristics were found in the same ranks. However, the complete taxonomy of these isolates will provide better information on the various links between the production of the substances involved in the suppression and their genotypic characteristics.

Effects of fluorescent *Pseudomonas* spp. on some physiological growth parameters of taro

Among the fluorescent *Pseudomonas* with major antagonistic traits, DS17R and DS15V were selected for pot experiments. Figure 3 presents the effects of the two biological inoculants on plant height (A), shoot length (B), total leaf surface area (C), and chlorophyll production (D). When DS15V and DS17R were inoculated on infected and non-infected or healthy taro seedlings, they significantly increased total leaf surface (Fig. 4), plant height, shoot length, and total leaf surface compared to the control (A, B, C). Similarly, the treatment with both DS15V and DS17R on infected seedlings significantly increased the chlorophyll rate compared to their control. However, treatment on healthy seedlings significantly decreased the chlorophyll level by 24.53% (D).

Bacterization of healthy taro seedlings showed effects on fresh root biomass (FRB) and fresh leaf biomass (FLB) (Fig. 5). The results showed that treatment of healthy and infected plants by DS17R and DS15V increased significantly FRB compared to the control. This result was similar in infected plants but with lower percentages (8.3% and 17.25%). Besides, both DS17R and DS15V inoculants stimulated a significant quantity of FLB (29.07 and 44.21%) in healthy plants.

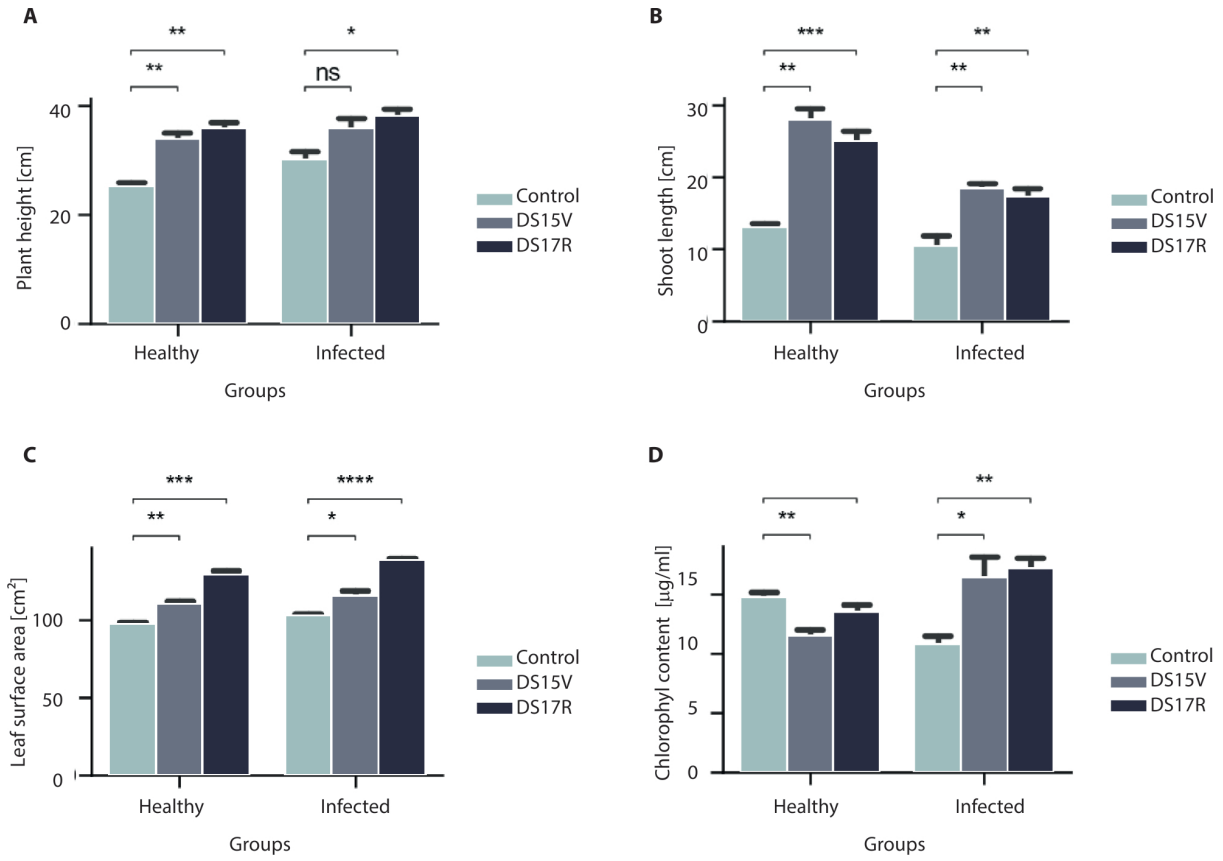

Fig. 3. Plant height (A), shoot length (B), total leaf area (C) and chlorophyll content (D) after DS15V and DS17R inoculation on infected and healthy taro plants. Errors represent the standard deviation; means labelled by different bulled (*) are significant according to t-test independent samples with Bonferroni correction; ns – non significant; (*) – $p < 0.05$; (**) – $p < 0.01$; (***) – $p < 0.001$ and (****) – $p < 0.0001$

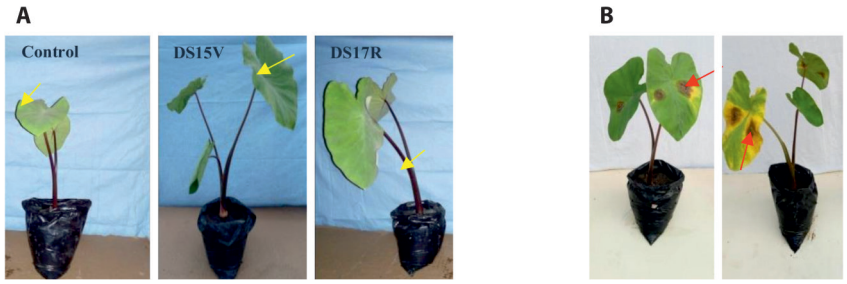

Fig. 4. A – plant leaf area surface and plant height after DS15V and DS17R inoculation on healthy taro plants; B – disease symptoms (necrosis) on the plant surface

Efficacy of fluorescent *Pseudomonas* spp. as a biocontrol agent against *Phytophthora colocasiae*

Effect on total protein and cysteine content

Treatment of healthy taro plants with *P. fluorescens* DS17R induced decreased protein levels, while *P. chlororaphis* DS15V showed a similar protein level compared to the control (Fig. 6). In infected plants, we noted a significant increase in both treatments, while cysteine content decreased in healthy plants treated with the two inoculants. However, inoculation of infected taro revealed increased cysteine content.

Effect of *Pseudomonas* spp. isolates on phenylalanine ammonia lyase (PAL) and polyphenol content

Figure 7 shows the effects of our two antagonists on the polyphenol content of taro plants. Application of inoculants on infected taro seedlings caused the highest increase in polyphenols compared to the control.

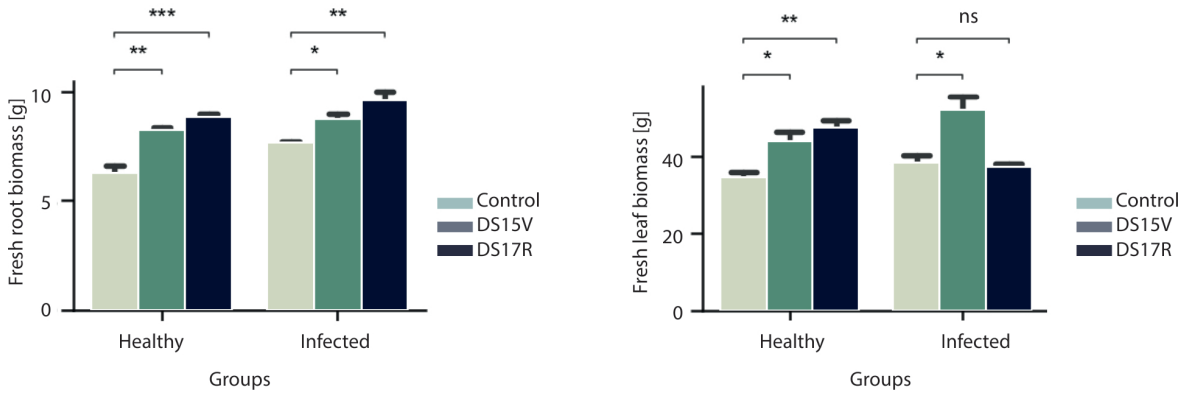

Fig. 5. Fresh root and fresh leaf biomass ratios after DS15V and DS17R inoculation on infected and healthy taro plant. Errors represent the standard deviation; means labelled by different bulled (*) are significant according to t-test independent samples with Bonferroni correction; ns – non significant; (*) – $p < 0.05$; (**) – $p < 0.01$; (***) – $p < 0.001$ and (****) – $p < 0.0001$

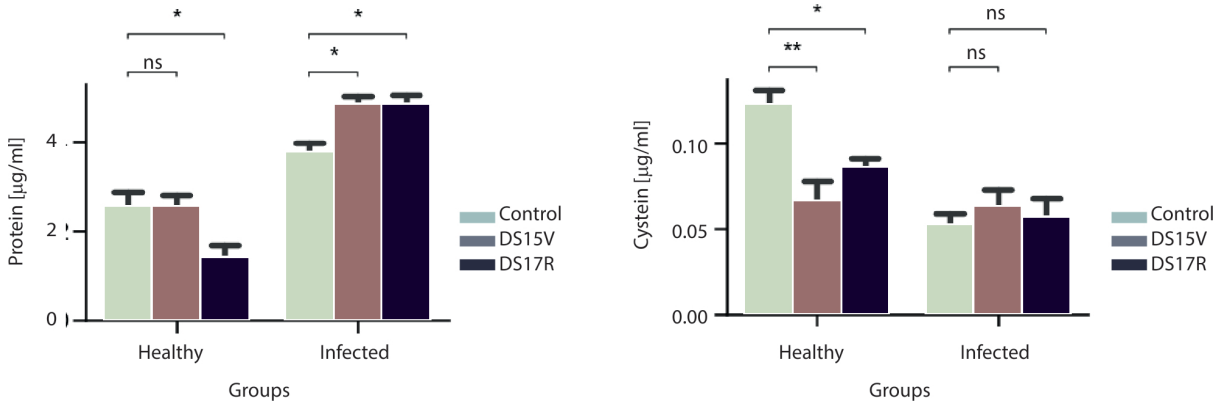

Fig. 6. Protein and cystein content (µg · ml^{-1}) after DS15V and DS17R inoculation on infected and healthy taro plants. Errors represent the standard deviation; means labelled by different bulled (*) are significant according to t-test independent samples with Bonferroni correction; ns – non significant; (*) – $p < 0.05$; (**) – $p < 0.01$; (***) – $p < 0.001$ and (****) – $p < 0.0001$

However, this polyphenol level remained stable in healthy seedlings treated with *P. fluorescens* DS17R. The activity of phenylalanine ammonia lyase (PAL) increased when healthy plants were treated with *P. chlororaphis* DS15V and induced a significant increase when plants were infected and inoculated with both DS15V and DS17R.

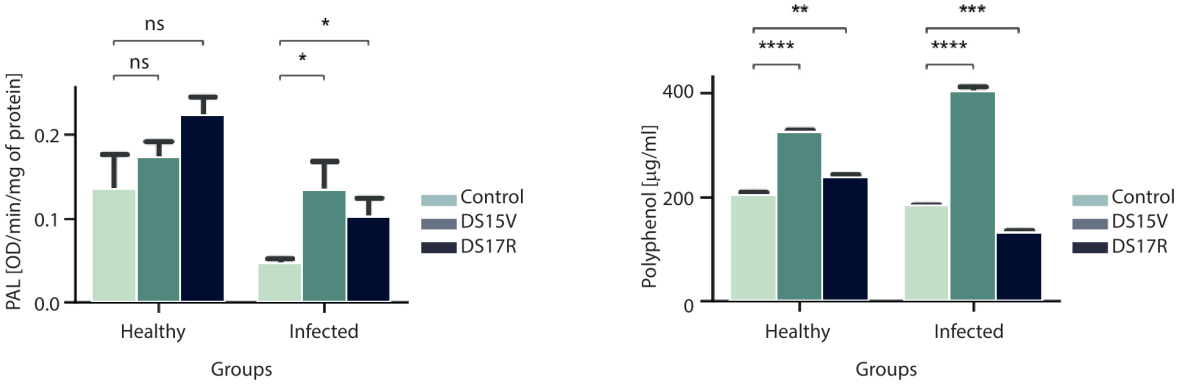

Fig. 7. Phenylalanine ammonia lyase (PAL) activity (OD/min/mg) and polyphenol (Poly p) concentration (µg · ml^{-1}) after DS15V and DS17R inoculation on infected and healthy taro plants. Errors represent the standard deviation; means labelled by different bulled (*) are significant according to t-test independent samples with Bonferroni correction; ns – non significant; (*) – $p < 0.05$; (**) – $p < 0.01$; (***) – $p < 0.001$ and (****) – $p < 0.0001$

Defense-related enzyme activity changes

Effect of fluorescent Pseudomonas isolates on polyphenol oxidase (PPO) activity

The maximum polyphenol oxidase (PPO) activity was noted in healthy and infected plants treated with DS15V and DS17R compared to the control (Fig. 8). As compared to the positive control, this activity varied in both isolates, and inoculation with DS17R showed great PPO activity.

Effect of antagonists on peroxidase (PO) activity

The induction of peroxidase (PO) activity in taro plants treated with the two antagonists varied significantly, as observed in Figure 9 below. Indeed, the treatment improved enzymatic activity in both healthy and infected plants treated with DS17R and DS15V. These seedlings were much more responsive to induction by the DS17R isolate.

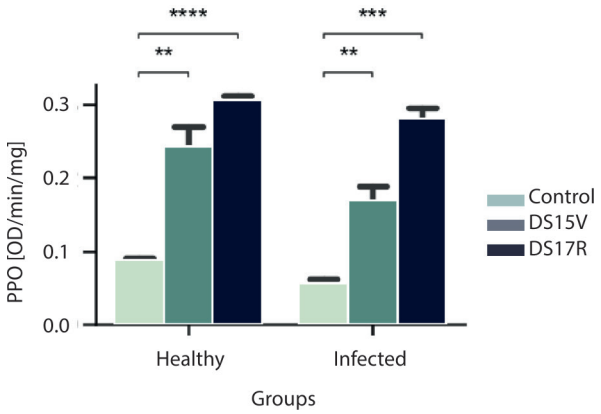

Fig. 8. Polyphenol oxidase (PPO) activity (OD/min/mg) after DS15V and DS17R inoculation on infected and healthy taro plants. Errors represent the standard deviation; means labelled by different bulled (*) are significant according to t-test independent samples with Bonferroni correction; (**) – $p < 0.01$; (***) – $p < 0.001$ and (****) – $p < 0.0001$

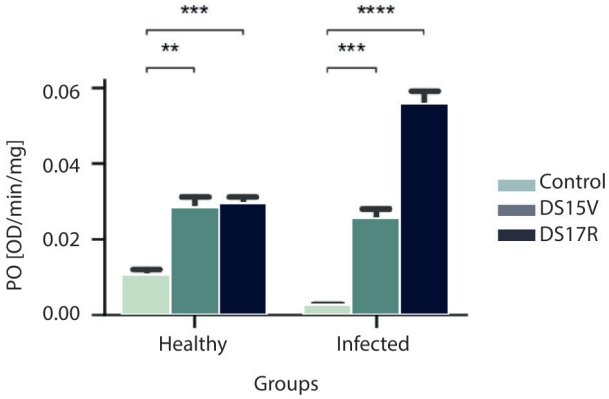

Fig. 9. Peroxidase (PO) activity (OD/min/mg) after DS15V and DS17R inoculation on infected and healthy taro plants. Errors represent the standard deviation; means labelled by different bulled (*) are significant according to t-test independent samples with Bonferroni correction; (**) – $p < 0.01$; (***) – $p < 0.001$ and (****) – $p < 0.0001$

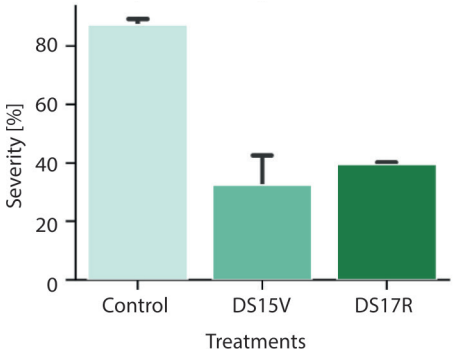

Fig. 10. Severity (%) of infected taro plants after DS15V and DS17R inoculation

Effects of Pseudomonas spp. on the severity of taro leaf blight

The bioprotective effect induced by these two antagonists was evaluated on 5-week-old seedlings inoculated at the root level, and the ability of the two antagonists to reduce the severity of late blight was also evaluated. Based on the results obtained from this experiment, we recorded a significant reduction in the severity (57.14% and 44.92%) of DS15V and DS17R inoculations, respectively (Fig. 10).

The correlation analysis (Tables 4 and 5) showed a significant correlation between plant growth parameters (leaf surface, shoot length, chlorophyll, fresh leaf biomass) and severity. Similarly, resistant related parameters such as polyphenol oxidase, phenylalanine ammonia-lyase, cystein, and protein also presented a significant correlation with the severity of taro leaf blight.

Discussion

Reports on the potential use of bacteria as biocontrol agents against plant pathogens have increased in recent decades. Plant growth promoting rhizobacteria may promote plant growth either directly by facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development, that is, by acting as biocontrol bacteria. Recently, we demonstrated how *P. fluorescens* rhizobacteria can use a wide range of organic and inorganic compounds under diverse environmental conditions.

Table 4. Pearson correlations between antagonistic parameters of bacteria with disease suppression and plant growth promoting rhizobacteria (PGPR) activity

	PGP parameters					
	PH	SL	LS	Chl	FRB	FLB
Severity	0.3 ns	0.67*	0.60*	0.71**	0.68*	0.29 ns

*correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level;
ns – correlation is non-significant; PH – plant heigh; SL – shoot length; LS – leaf surface; Chl – chlorophyll; FRB – fresh root biomass; FLB – fresh leaf biomass

Table 5. Pearson correlations between antagonistic parameters of bacteria with disease suppression and PGPR activity

	Resistance related-parameters					
	PPO	PO	PAL	Cystéine	Phénols	Protéines
Severity	0.60*	0.45 ns	0.69**	0.50*	0.25ns	0.92**

*correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level;
ns – correlation is non-significant; PPO – polyphénol oxydase; PO – peroxydase; PAL – phénylalanine-ammonia-lyase

The growth, antimicrobial metabolite content, and activity against *P. colocasiae* were influenced by different abiotic as well as osmotic stresses (Ntyam *et al.* 2018). In this study, these *Pseudomonas* rhizobacteria revealed a broad range of additional antagonistic traits, giving them the capacity to promote growth and the resistance of taro against *P. colocasiae* in field experiments.

Among the *Pseudomonas* rhizobacteria used in this study, 75% of them came from the rhizosphere and rhizoplane of healthy taro plants. This supports the fact that the rhizosphere zone helps in selecting strains of bacteria from the natural environment where they are eventually used. The variability in the metabolism of fluorescent *Pseudomonas* spp. confers significant plasticity on these bacteria in their adaptation to various environments, such as the rhizosphere. Mezaache-Aichour *et al.* (2012) obtained similar results where a population of *P. fluorescens* was elevated in the rhizosphere compared to other species.

Phenotypic characterization has been used to identify and classify isolates according to some morphological criteria, pigmentary requirements, reaction with some dyes, and nutritional requirements. However, these properties do not allow total and accurate taxonomic identification. The biochemical profile of these isolates reported a high population of biovar I, characterized by its gelatin positive, levan and denitrification negative reactions. As related by Latour and Lemanceau (1997), this characteristic was observed in varying species and biovars. Besides, *P. fluorescens* biovars I, V, and VI, as well as *P. putida*, reduce nitrate through a nitrate reductase compared to *P. chlororaphis* and *P. fluorescens* biovars II, III, and IV. Similar results were also observed by Palleroni (1984). Furthermore, *P. fluorescens* biovar V includes strains that are difficult to classify because the essential properties that would allow their differentiation from other biovars have not been identified. This multiple biovar system reveals high phenotypic heterogeneity and reflects the high genetic diversity of *Pseudomonas* rhizobacteria obtained.

Antibiosis is one of the most important mechanisms used by plant growth promoting rhizobacteria (PGPR) to limit the invasion of pathogens into the host plant tissue. *Pseudomonas fluorescens* revealed their antagonistic characteristics against *P. colocasiae* through the production of antimicrobial substances. *P. putida* DS14N, *P. chlororaphis* DS15V, and *P. fluorescens* DS17R exhibited highest mycelial growth rates. The genus *Pseudomonas* is well known for producing large arrays of antifungal metabolites such as volatile and diffusible organic compounds. The results showed that *P. fluorescens* DS17R and *P. chlororaphis* DS15V produced a large amount of HCN, siderophore, cellulases, proteases, and lipases. These isolates were more effective than others in inhibiting the mycelial growth of *P. colocasiae* by volatile (91% and 82%) and diffusible (17 and 15 mm) compounds, respectively. Similarly, Anand *et al.* (2020) have shown that the antagonistic potential of the bioagents could be attributed to the HCN, siderophores, and hydrolytic enzyme production capability of the stains. Furthermore, HCN has been postulated to play an important role in the biological control of pathogens, and hydrolytic enzymes are involved in the cell wall degradation of fungal pathogens (Oni *et al.* 2022).

Iron plays a crucial role in most redox enzymes involved in the electron transport chain of intermediary metabolism, and despite its abundance in nature, its bioavailability remains extremely low. In this study, we noted the presence of three types of siderophores. Meyer and Abdallah (1978) reported that this production was also observed in other strains of fluorescent

Pseudomonas such as *P. aeruginosa*, *P. putida*, *P. chlororaphis*, and *P. aureofasciens*. In addition, salicylic acid (SA) also acts as a precursor for the biosynthesis of pyochelin, another siderophore capable of stimulating a defensive response in plants (Audenaert *et al.* 2002). Systemic acquired resistance (SAR) in plants is associated with an increase in the concentration of endogenous SA produced by *Pseudomonas* spp. both locally at the site of infection and systemically in distant tissues.

Based on previous observations, we further explored specific antagonistic mechanisms behind its antimicrobial efficiency through a pot experiment. The application of DS15V and DS17R to healthy taro plants significantly promoted the growth of leaf area, height, crown diameter, and plant root biomass. This ability of fluorescent *Pseudomonas* has already been reported by several authors. The growth stimulation may be related to their ability to colonize roots, thereby reducing the colonizable space available to the plant pathogen and uptake of some nutrients in the root environment. Furthermore, their capacity to secrete indole-3-acetic acid (IAA) phytohormone could increase root and length growth. The synthesis of indole acetic acid (IAA) is widespread in rhizobacteria, resulting in increased plant growth. The increase in chlorophyll content in seedlings inoculated with *P. fluorescens* DS17R could be associated with its positive effect on taro cell physiology and photosynthesis. This suggested that interactions between hosts and microbes lead to physiological changes and the translocation of sugars, resulting in changes in the photosynthetic rate of leaves and their metabolic activities. The significant growth of taro and chlorophyll content observed was also related to biocontrol agents such as *P. fluorescens* being inoculated into diseased tomato plants.

The synthesis of a range of different antibiotics is the PGPB trait that is most often associated with the ability of the bacterium to prevent the proliferation of plant pathogens. In this work, the beneficial effect of DS15R and DS17R bio inoculants was observed through the reduction of the incidence percentage of *P. colocasiae* on taro leaf blight. The treatment by antagonists showed low levels of total protein in taro plants. This result could explain the "priming" phenomenon. This mechanism allows the plant to "prepare" itself for a future infection without making heavy investments in metabolic and energetic resources (to the detriment of fundamental cellular processes) while no aggression has yet to be fought (van Hulten *et al.* 2006). The same phenomenon appears in pea treated with *Bacillus pumilus* SE34, where a rapid deposition of callose and phenolic material on the walls at the penetration site is observed only after the addition of the pathogen *Fusarium oxysporum* (Benhamou *et al.* 1996). However, the observed increase in PAL activity can be explained by its role in the synthesis and accumulation of secondary metabolites such as phenolic compounds following the presence of rhizobacteria. PAL is the key enzyme in the metabolic pathway of phenylpropanoid synthesis which converts L-phenylalanine by deamination into trans-cinnamic acid, which is the precursor of the synthesis of most phenolic compounds such as phenols, flavonoids, coumarins, tannins, and lignin.

The high content of phenolic compounds in the plants treated with both rhizobacteria could explain the role of PAL in promoting cell death via damage to cell membranes.

Furthermore, the application of the two antagonists to infected taro seedlings increased cystein levels in plant tissues. Cystein is involved in the synthesis of methionine and glutathione, which are active molecules that play a role in the plant's response to stress, protection against oxidative stress, in the detoxification of heavy metals, and participate actively in defense (Couturier *et al.* 2013).

The effects of rhizobacteria significantly increased the level of proteins that played an important role in taro defense mechanisms. Indeed, after infection, the plant synthesizes a variety of structural proteins and enzymes to ensure its defense and integrity. Ramamoorthy *et al.* (2002) explained the role of high protein content induced by *Pseudomonas* rhizobacteria and the resistance against *Venturia inaequalis* and *Fusarium vascularis*. Inoculated taro seedlings show increased polyphenol oxidase (PPO) and phenol oxidase (PO) enzymatic activity. PPO catalyzes the oxidation of phenols into o-quinones, compounds considered to be biochemical markers of plant resistance. It also accelerates cell apoptosis and limits the progression of pathogens in infected organs through their proteolytic activity. Phenol oxidase activity is responsible for the degradation of hydrogen peroxide (H_2O_2) in the plant, leading to the mass production of free radicals which can in turn react with the parietal components of the pathogen and cause its destruction. The reactive oxygen species produced could also attack and damage the pathogen's biomolecules, triggering the hypersensitivity response, which is a form of an amplified version of immunity (Garcia-Brugger *et al.* 2006).

These findings imply that some biochemical and morphological factors are related to the severity of taro leaf blight. The activities of phenylalanine ammonia-lyase, polyphenol oxidase, phenol oxidase, proteins, and cystein content, as well as shoot length and fresh leaf biomass, are all essential variables for effective resistance to *P. colocasiae* infection. Similar results were reported by Ali *et al.* (2020), who used two strains of *Pseudomonas* spp. and *B. subtilis* to reduce *Pythium* damping-off and root rot of cucumber seedlings, as well as an 84% increase in plant fresh weight.

Conclusions

Overall, the findings of the present study showed that many species colonized the taro rhizosphere, especially *P. putida* and *P. chlororaphis*, with a predominance of *P. fluorescens* species. Among the most potent rhizobacteria, *P. chlororaphis* DS15V, *P. putida* DS14N, and *P. fluorescens* DS17R exhibited strong inhibition activity against *P. colocasiae* in dual culture and volatile assay. These bacteria were also able to produce plant-growth-promoting substances such as siderophore, salicylic acid, indole acetic acid, cyanuric acid, and hydrolytic enzymes. Field experiments showed that DS15V and DS17R significantly reduced disease severity. Biochemical (phenylalanine ammonia-lyase, polyphenol oxidase, cysteine, soluble proteins) and morphological (leaf surface, shoot length, fresh leaf biomass) parameters may have critical functions for the taro leaf severity. It is suggested that these fluorescent *Pseudomonas* rhizobacteria have beneficial characteristics to promote growth and protect taro against *P. colocasiae*.

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