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Research Article

Elucidation of the mechanisms of antagonism of native fluorescent pseudomonads against *Xanthomonas axonopodis* pv. *punicae*, the causal agent of pomegranate bacterial blight

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ABSTRACT: Bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* is one of the most dreaded diseases of pomegranate. In the present study, an effort was made to identify the antagonistic mechanisms of native and efficient fluorescent pseudomonads isolates effective against the bacterial blight pathogen. A total of 99 fluorescent pseudomonads isolates were isolated from rhizosphere soil samples collected from different pomegranate-growing areas. Dual culture assay showed 83 isolates with inhibitory effect against the pathogen; among them, nine were found to be highly effective antagonists. These nine isolates were evaluated for production of siderophores, HCN, fluorescein, and pyocyanin. All the isolates showed siderophore, fluorescein and pyocyanin production, with three (FP-10, FP-79 and FP-87) showing higher iron chelation zones, two (FP-07 and FP-19) with strong fluorescein production and six (FP-03, FP-07, FP-10, FP-14, FP-19 and FP-79) with strong pyocyanin production. Three isolates (FP-64, FP-79 and FP-87) showed HCN production. The study identified efficient fluorescent pseudomonads with multiple modes of antagonism, which can further be evaluated under field conditions for management of pomegranate bacterial blight.

KEYWORDS: Fluorescent pseudomonads, fluorescein, hydrogen cyanide, siderophores, pyocyanin, *Xanthomonas axonopodis* pv. *punicae*

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, and it is called the 'Fruit of Paradise'. It is widely grown in Spain, Morocco and countries around the Mediterranean as well as in Egypt, Iran, Afghanistan, Arabia and Baluchistan. Apart from these countries, it is also cultivated in Myanmar, China, Japan, USA, USSR, Bulgaria and southern Italy. In India, pomegranate is cultivated on a commercial scale in Maharashtra, Karnataka, Andhra Pradesh, Gujarat, Madhya Pradesh and Uttar Pradesh.

Pomegranate crop is affected by several important diseases including bacterial blight, wilt, anthracnose, leaf and fruit spots, as well as pests like fruit and stem borer, fruit sucking moth, shot hole borer and various sucking insects. Among the diseases, the bacterial disease caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani & Singh, 1959; Vauterin *et al*., 1995) popularly known as 'bacterial blight' is a major threat for successful cultivation of pomegranate in India. Since 2002, the disease has become increasingly severe, posing a serious threat to the Indian economy and reducing the export potential of quality fruits. Pomegranate, 'the boon commercial fruit crop to the farmer, turned as a big bane' after the severe outbreak of bacterial blight.

In recent years, at the global level, there is an increasing and significant demand for the production of residue-free

organic pomegranate, apart from reducing the cost of cultivation of this crop. A number of strains of *Pseudomonas fluorescens*, *P. putida*, *P. cepacia*, *P. aeruginosa, P. aureofaciens* and *P. corrugata* have been widely used for the biological control of plant diseases (Leeman *et al.*, 1995; Vidhyasekaran & Muthamilan, 1999; Meena *et al*., 2001; Vidhyasekaran *et al*., 2001; Yenjerappa, 2009). Hence, the present study is focused on the exploitation of fluorescent pseudomonads to manage the disease, which can be used as one of the tools in eco-friendly management of this dreaded disease.

Fluorescent pseudomonads are the soil micro-organisms which are ubiquitous and are the common residents of the rhizosphere of crop plants (Cho & Tiedje, 2000; Misko & Germida, 2002; Ramette *et al*., 2003; Costa *et al*., 2006; Ayyadurai *et al*., 2007). In recent years, these soil microbes have attracted greater attention from the scientific community due to their significant roles in the biological control of plant pathogens and also the promotion of plant growth. The fluorescent pseudomonads suppress numerous fungal and bacterial plant pathogens by producing a variety of secondary metabolites such as pyrrolnitrin (Howell & Stipanovic, 1979), pyoluteorin (Howell & Stipanovic, 1980), Hydrogen Cyanide (HCN) (Voisard *et al.*, 1989) and 2,4-diacetylphloroglucinol (DAPG) (Nowak-Thompson *et al*., 1994; Validov *et al*., 2005).

Although the biological control of several plant diseases by using fluorescent pseudomonads has been studied in detail, there is still a great scope for studying the antimicrobial compounds produced by these microbes involved in suppression of plant pathogens. The purpose of the present study was to find out the antagonistic mechanisms involved in inhibition of *X. axonopodis* pv. *punicae* by the most efficient, selected antagonistic fluorescent pseudomonads.

MATERIALS AND METHODS

Isolation of the pathogen

Pomegranate leaves exhibiting characteristic symptoms of bacterial blight were collected and used for the isolation of *Xanthomonas axonopodis* pv. *punicae* (*Xap*). The bacterial blight-infected portions of pomegranate leaves, along with adjacent healthy tissue, were cut into small pieces and surface sterilised with 1.0% sodium hypochlorite solution for 60 seconds. The leaf bits were then washed three times with sterile distilled water to remove residue of sodium hypochlorite. Subsequently, the washed leaf pieces were transferred to a Petri plate containing 1 mL of sterile distilled water, crushed using a sterile scalpel, and allowed to stand for 2 to 3 min. After sufficient bacterial ooze emerged from the crushed tissues, a loopful of the sap suspension was taken in a sterile inoculation loop and streaked onto Petri plates containing Nutrient Glucose Agar (NGA) medium. The inoculated plates were incubated at 28±1°C for 72 to 96 h. Since young, rapidly growing colonies were generally saprophytic in nature, observations were made for the appearance of well-separated, typical light-yellow bacterial colonies resembling *Xanthomonas* spp., which became evident after 96 h of incubation.

Purification and preservation of pathogen culture

Colonies of bacteria exhibiting typical characteristics of *Xap* were selected using a sterilised inoculation loop and streaked onto Petri plates containing NGA medium. The inoculated plates were then incubated at 28±1°C for 72 to 96 h, after which the development of well-separated, bright yellow, mucoid bacterial colonies was observed. These pure colonies were subsequently streaked onto NGA agar slants and incubated again at 28±1°C for 72 h. The resulting cultures were stored at 4°C in sterile polypropylene culture tubes containing sterile water for short-term use as stock cultures. For long-term preservation, a loopful of bacterial culture was suspended in 600 µl of Nutrient Glucose Broth in 2 ml cryovials, mixed with 600 µl of 50% glycerol, and stored at −80°C for future use.

Proving pathogenicity

Apparently healthy layered cuttings of the bacterial blight-susceptible pomegranate variety '*Bhagwa*' were planted in polybags filled with a standard nursery mixture. After one month, the pomegranate saplings were transplanted into earthen pots containing soil amended with the required nutrients. The seedlings were regularly watered and maintained under sufficient sunlight in a glasshouse.

To prove the pathogenicity of the isolated bacterium, the culture was multiplied in Nutrient Glucose Broth (200 ml) in Erlenmeyer flasks by inoculating a loopful of the bacterial culture. The inoculated flasks were incubated at 28±1°C for three days. Two-month-old susceptible pomegranate cultivar (*Bhagwa*) plants were first sprayed with sterile water and then covered with a polyethylene sheet for 24 h prior to inoculation. After this pre-incubation, the plants were sprayed with a bacterial suspension (4×10^7 cfu/ml), prepared by mixing broth culture and sterile distilled water in a 200:800 ratio. The suspension was applied using a

hand sprayer to ensure complete coverage of both the upper and lower leaf surfaces, as well as branches. Following inoculation, the plants were again covered with transparent polyethylene sheets and maintained in a humid chamber for 48 h under controlled conditions of >95% relative humidity and 28±1°C temperature. Control plants were sprayed with a mixture of sterile distilled water and nutrient broth. To maintain humidity, inoculated plants were sprayed with sterile distilled water twice a day. Observations were recorded daily to monitor the appearance and progression of bacterial blight symptoms. Once symptoms developed on the inoculated plants, the pathogen was re-isolated and compared with the original culture to confirm Koch's postulates. The experiment was repeated twice to ensure consistency of the results.

Isolation of fluorescent pseudomonads

Soil samples from the rhizosphere of 10 healthy pomegranate plants were collected at random from each field, and finally it was mixed and made into each sample consist of 100 g of soil. Fluorescent pseudomonads were isolated from pomegranate crop rhizosphere soil samples as per the method described by Weller and Cook (1983). The colonies grown on plates containing King's B medium fluorescing under UV light (365 nm wavelength) were picked up, purified and preserved. These isolates were maintained at 4°C with sterile distilled water (Liao & Shollenberger, 2003).

In vitro* screening of native fluorescent pseudomonads against *Xanthomonas axonopodis* pv. *punicae

Ninety-nine colonies of fluorescent pseudomonads were tested to know their capability to inhibit the growth of *Xap* by following the dual culture assay technique. Five loopfuls of 72 h old *Xap* culture multiplied on a nutrient agar plate were mixed with 2 ml of distilled sterilised water. Three hundred microliters (3×10^8 cfu/ml) of this suspension was spread on Petri dishes containing NGA. Two loopfuls of 72 h-old fluorescent pseudomonad cultures were mixed in 0.5 ml of distilled sterilised water; 10 ml the suspension of these suspensions were placed on the surface of NGA plates which were seeded with *Xap*. The inoculated plates were incubated at 28±1°C for 144 h.

Elucidation of the mechanism of action

Siderophore production

To study the involvement of siderophores in the inhibition of the growth of the pathogen, the different isolates of fluorescent pseudomonads were spotted on the King's B medium amended with 1 mM iron as $FeCl_3$ (Kumar & Dubey, 1993). Siderophore production by the isolates of fluorescent pseudomonad was tested following the method described by Schwyn and Neilands (1987).

Deferration: Removal of contaminating Iron from glasswares

Glass is a good ion-exchange material and may adsorb or leach trace amounts of iron from its surface, leading to potential contamination. Therefore, to avoid interference from contaminating iron in siderophore production studies, proper treatment of glassware is essential. All glassware used in siderophore assays and in the preparation of Chrome Azurol S (CAS) agar medium was soaked in 2N HCl for 24 h. After acid treatment, the glassware was thoroughly rinsed with double-distilled water. This acid washing step proved crucial for obtaining consistent and accurate results.

Preparation of CAS for the detection of siderophore production

The CAS (Chrome Azurol S) medium is used to detect siderophore production by fluorescent Pseudomonad isolates (Schwyn & Neilands, 1987). To prepare one litre of Blue Agar CAS medium, 60.5 mg of dehydrated Chrome Azurol S (Himedia) was dissolved in 50 ml of distilled water and mixed with 10 ml of iron solution (1 mM $FeCl_3 \cdot 6H_2O$ in 10 mM HCl). This mixture was then slowly added to 40 ml of an aqueous solution containing 72.9 mg cetyltrimethylammonium bromide (CTAB) under continuous stirring. The final solution was autoclaved.

King's B agar medium was prepared by using PIPES buffer (30.2 g) and Difco agar (18.0 g). The pH was adjusted to 6.8 by using 50% (w/w) sodium hydroxide (NaOH) solution and autoclaved. After cooling, the CAS dye solution was added slowly along the inner wall of the flask with gentle agitation to avoid foam formation and ensure uniform mixing. Approximately 20 ml of CAS agar was then poured into each Petri plate. The plates were stored at 4°C for 24 h before use.

Spotting of fluorescent pseudomonad isolates

Fluorescent Pseudomonad isolates were grown overnight, and 10 µl of each culture was spotted onto CAS medium plates. The plates were incubated at 28±1°C for 48 h. Siderophore production was indicated by the formation of a yellow to orange halo around the colony. The extent of siderophore production was quantified by measuring the diameter (in mm) of the halo zone.

Hydrogen Cyanide (HCN) production

The ability of efficient isolates of fluorescent pseudomonads to produce HCN was assessed using the method described by Wei *et al*. (1996). Sterile Whatman No. 1 filter paper pads were placed on the lids of Petri plates, which were then sterilised by autoclaving. Sterile Tryptic Soya Agar (TSA) medium (HiMedia, Mumbai) amended with glycine (4.4 g/L) was prepared and poured into sterile Petri plates.

Twenty-four-hour-old active cultures of fluorescent pseudomonad isolates were streaked onto the medium. The filter paper pads in each plate were then soaked with 2 ml of sterile picric acid solution (prepared using 2.5 g picric acid, 12.5 g Na_2CO_3, and 1 litre distilled water) as described by Miller and Higgins (1970). The inoculated plates were sealed with parafilm to prevent the escape of volatile metabolites and incubated for one week at 28±1°C, allowing interaction between gaseous metabolites and the picric acid-impregnated filter paper.

After incubation, colour changes in the filter paper were recorded to determine HCN production. The interpretation was as follows: No colour change indicated no HCN production; brownish colour indicated weak production; brownish to orange indicated moderate production; and orange to reddish brown indicated strong HCN production.

Detection of fluorescein and pyocyanin

Pseudomonas agar 'F' favours the formation of fluorescein, whereas Pseudomonas agar 'P' stimulate the production of pyocyanin and reduces the formation of fluorescein (King *et al.*, 1954). All the isolates of fluorescent Pseudomonad were tested for production of fluorescein and pyocyanin by using Pseudomonas agar 'F' and Pseudomonas agar 'P', respectively.

Two loopfuls of 72 h-old fluorescent pseudomonad cultures were mixed in 0.5 ml of distilled sterilised water in a microcentrifuge tube; 10 ml of these suspensions were spotted onto Pseudomonas agar 'F' and Pseudomonas agar 'P' plates. The inoculated plates were incubated at 28±1°C for 48 h. The incubated plates were observed for fluorescence under UV light (365 nm wavelength) using a UV cabinet. The presence of characteristic bright yellow-green fluorescence and blue-green or blue-white fluorescence around the colony indicated the production of fluorescein and pyocyanin by the fluorescent pseudomonad isolates, respectively.

RESULTS

In vitro* screening of fluorescent pseudomonads against *Xanthomonas axonopodis* pv. *punicae

Fluorescent Pseudomonads (FP) isolated from rhizosphere soils of major pomegranate growing areas of northern Karnataka and border areas of Maharashtra were screened against *X. axonopodis* pv. *punicae* for inhibition of bacterial growth by dual culture technique *in vitro* as explained in 'Materials and Methods'. Inhibition zone was recorded as diameter in cm.

A total of 99 colonies were collected from different rhizospheres of pomegranate and screened for antagonistic activity; 83 colonies were found inhibitory against the pathogen. The zone of inhibition ranged from 1.47 cm to 4.35 cm. The top nine FP isolates which showed the maximum inhibitory zone and where the pathogen failed to show regrowth in the inhibitory zone after 144 h after inoculation, were selected for further studies. Among the nine selected (Figure 1) isolates, FP-87 showed a maximum

Table 1. Efficient isolates of fluorescent pseudomonad in terms of zone of inhibitions

Fluorescent pseudomonad isolates	Zone of inhibition (Diameter in cm)	Remarks (Numerical ranking)
FP-03	3.48	4
FP-07	3.47	5
FP-10	3.25	7
FP-14	3.18	9
FP-19	3.58	3
FP-64	4.07	2
FP-79	3.40	6
FP-87	4.35	1
FP-94	3.22	8
S.Em. ±	0.14	-
CD @1 %	0.59	-

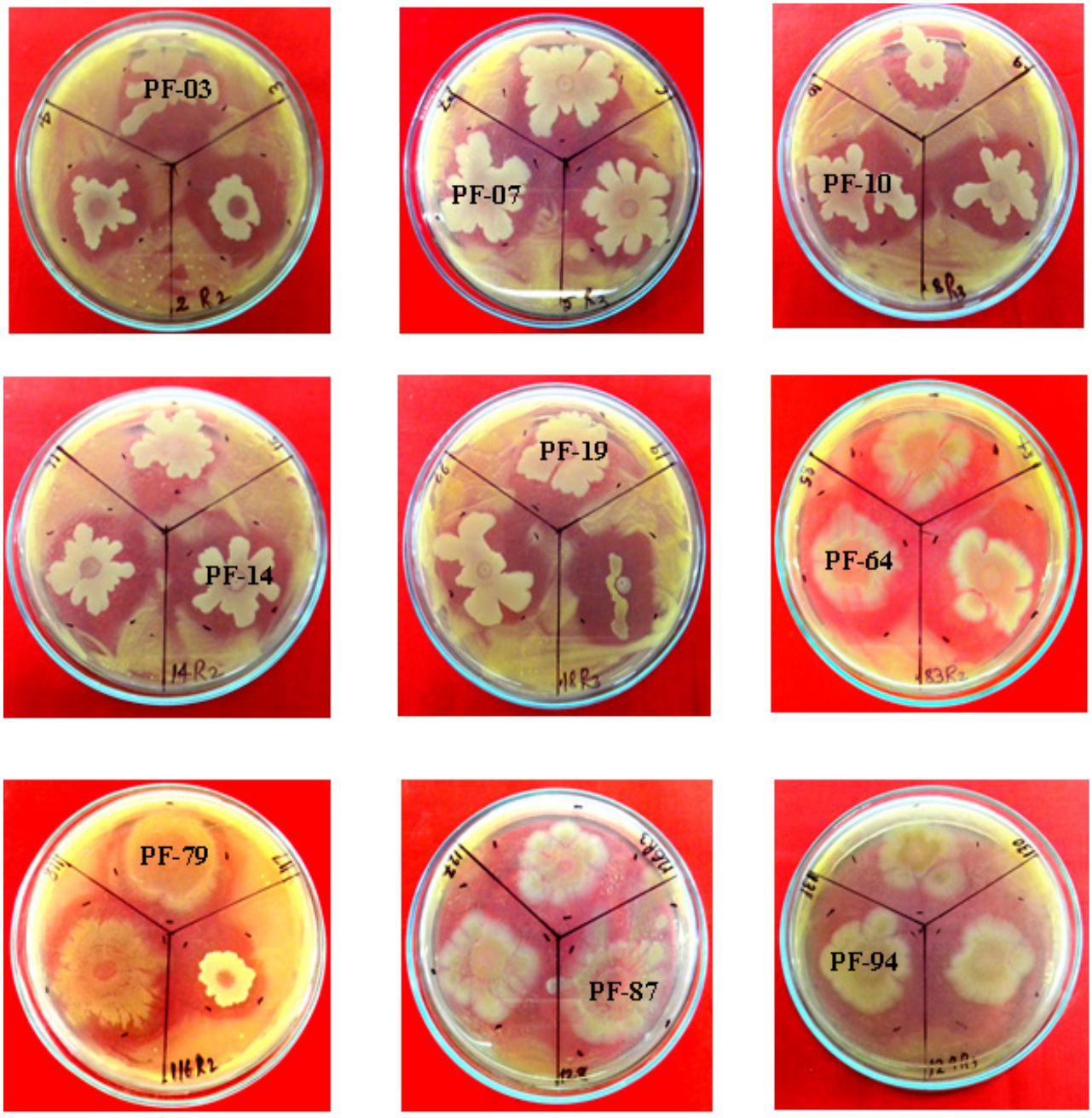


Figure 1. *In-vitro* screening of fluorescent pseudomonads against *X. axonopodis* pv. *punicae.*

zone of inhibition of 4.35 cm in diameter, followed by FP-64, FP-19 and FP-03 with zones of inhibition of 4.07 cm, 3.58 cm and 3.48 cm in diameter, respectively (Table 1). However, the inhibition zone produced by the FP-87 isolate was significantly superior over the remaining isolates except FP-64, where they were on par with each other. The rest of the isolates remained on par with each other.

Elucidation of mechanism of biocontrol

Siderophore production

The formation of a yellow halo zone around colonies of fluorescent pseudomonads on dark blue agar plates indicated siderophore production. The size of the yellow halo varied among isolates. These siderophore-producing isolates chelated iron from the Chrome Azurol S/Fe^{2+}/

Table 2. Detection of siderophore, HCN, fluorescein and pyocyanin produced by the efficient isolates of fluorescent pseudomonad

Fluorescent pseudomonad isolates		Siderophore production			HCN production	Fluorescein production	Pyocyanin production
		Colony diameter in cm	Iron chelation zone (Diameter in cm)	Reaction			
FP-03	2.41	0.38	++	-	++	+++	
FP-07	2.70	0.62	++	-	+++	+++	
FP-10	2.18	0.80	+++	-	++	+++	
FP-14	2.71	0.33	+	-	++	+++	
FP-19	2.26	0.45	++	-	+++	+++	
FP-64	3.65	0.40	++	++	+	++	
FP-79	3.61	0.90	+++	+	++	+++	
FP-87	3.96	0.75	+++	++	++	++	
FP-94	2.75	0.47	++	-	+	++	
S.Em±		0.10					
CD @ 1%		0.40					

- No production + Low production
++ Medium production +++ Strong production

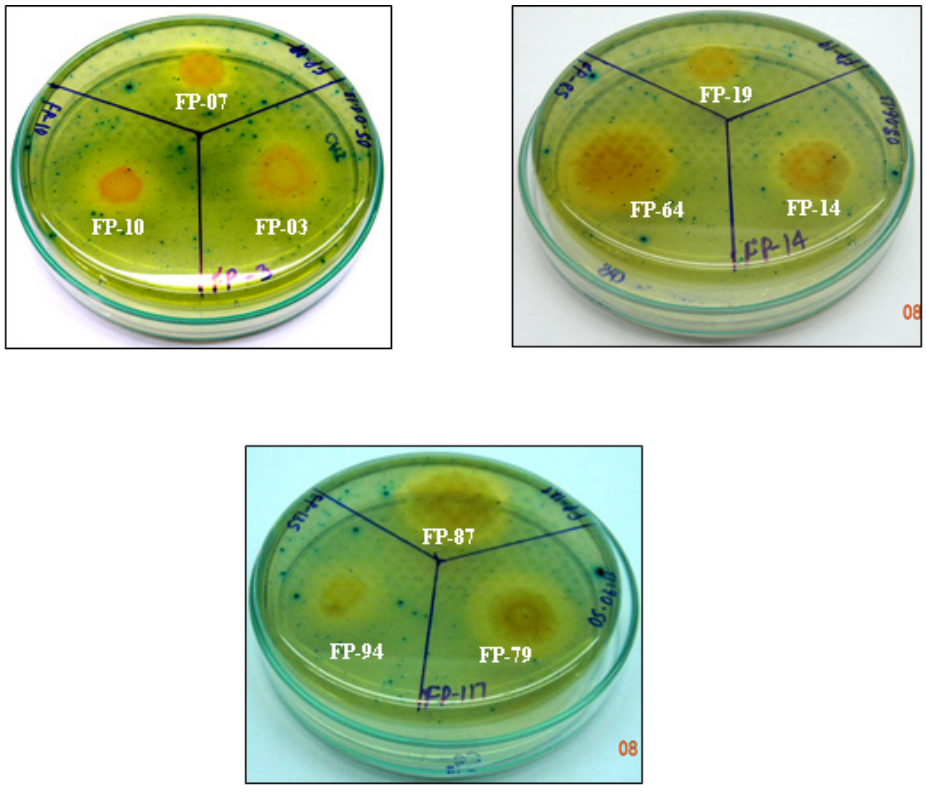


Figure 2. Siderophore production in fluorescent pseudomonads.

hexadecyltrimethyl ammonium bromide complex, resulting in a colour change from dark blue to yellowish fluorescence in nearly all isolates. Among them, isolates FP-79, FP-10, and FP-87 showed higher siderophore production, with zones of 0.90 cm, 0.80 cm and 0.75 cm, respectively, as shown in Table 2 and Figure 2. Iron chelation zones produced by FP-10, FP-79 and FP-87 were significantly superior over the remaining isolates, and they were also on par with each other.

HCN production

Among nine FP isolates tested, only three were found positive for HCN production. Among these HCN-producing isolates, FP-64 and FP-87 changed the yellow colour of the filter paper to a medium brown colour and were scored as medium (++) indicating a medium level of HCN production, whereas isolate FP-79 scored as a low (+) producer of HCN (Figure 3).

Fluorescein and pyocyanin production

The FP isolates grown on Pseudomonas agar 'F' were surrounded by a yellow to greenish yellow zone, whereas FP isolates grown on Pseudomonas agar 'P' were surrounded by a blue to green zone due to the production of fluorescein and pyocyanin, respectively. FP isolates tested showed varied proportions of fluorescein and pyocyanin production after 48 h of incubation. The isolates FP-07 and FP-19 showed strong production of fluorescein (Figure 4), and isolates FP-03, FP-07, FP-10, FP-14, FP-19 and FP-79 showed strong production of pyocyanin (Figure 5).

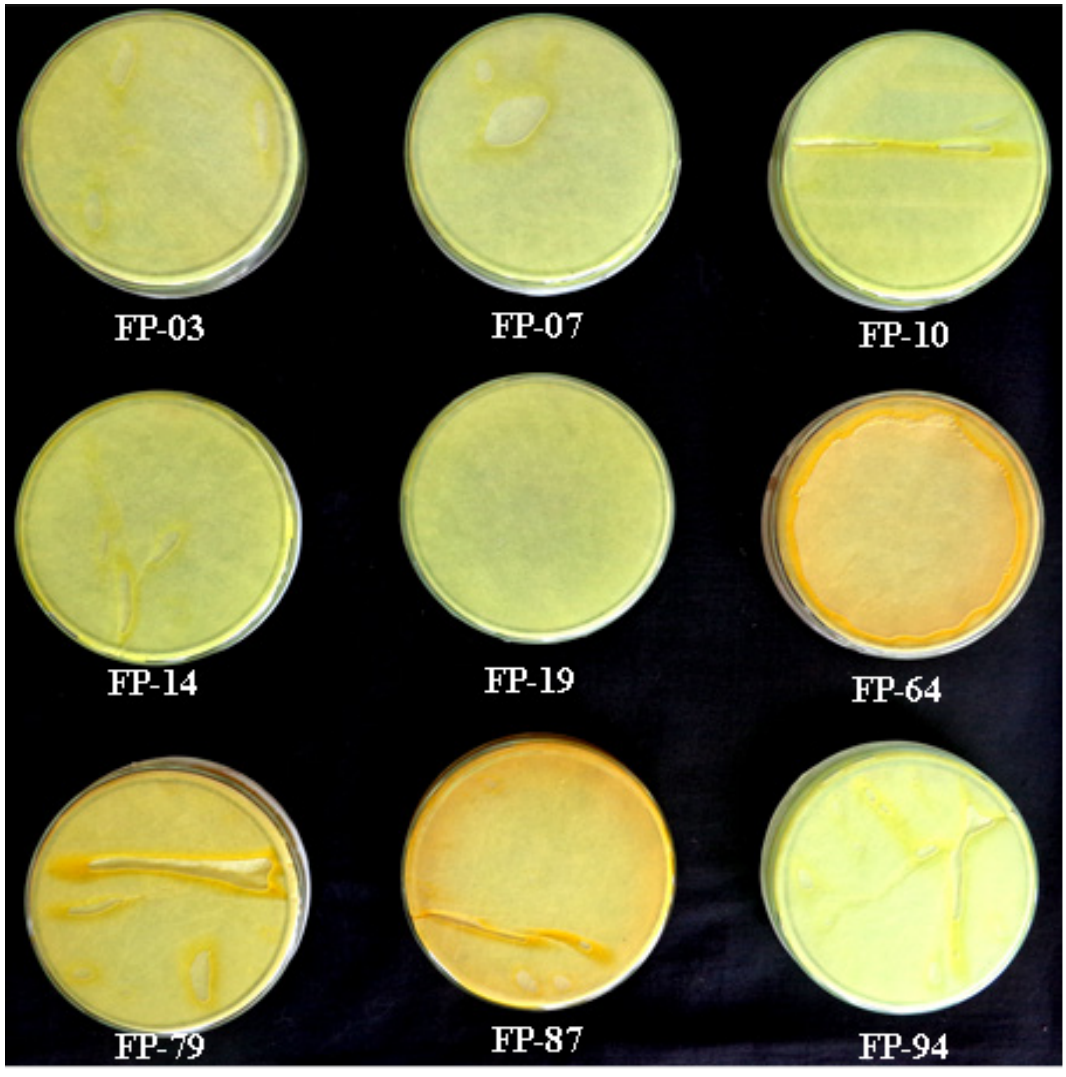


Figure 3. HCN production by efficient fluorescent pseudomonads.

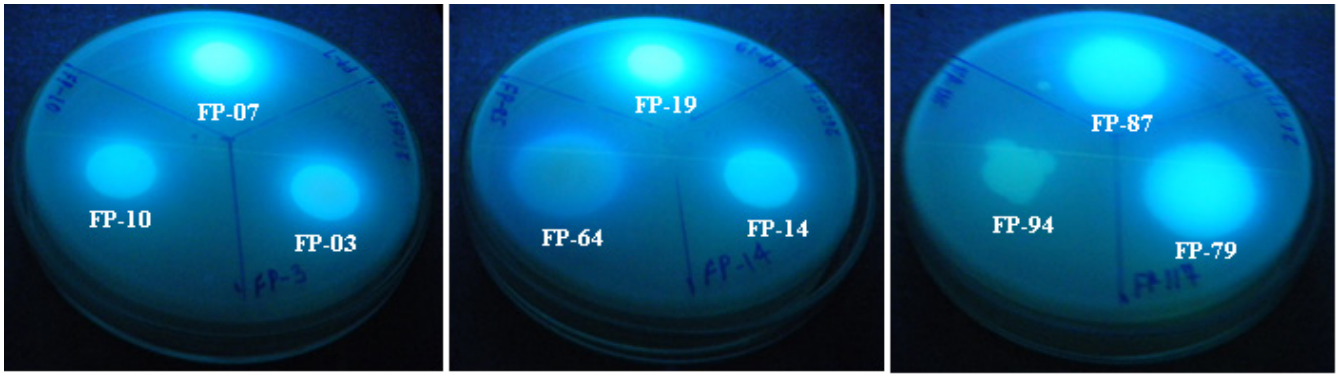


Figure 4. Detection of fluorescein production by fluorescent pseudomonads under UV light (365 nm).

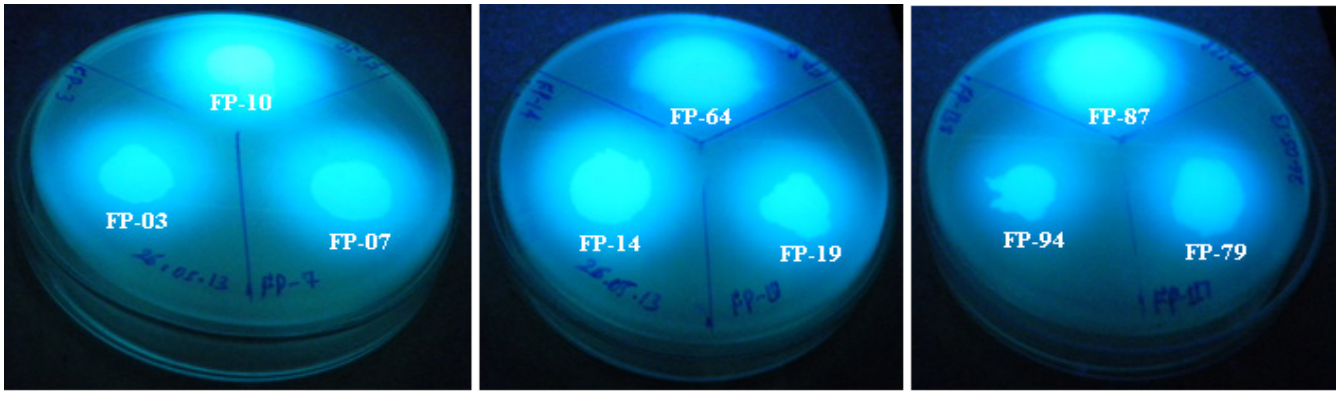


Figure 5. Detection of pyocyanin production by fluorescent pseudomonads under UV- light (365 nm).

DISCUSSION

The fluorescent pseudomonads are known to produce a wide range of secondary metabolites, which play a crucial role in the biological control of plant diseases caused by different groups of pathogenic bacteria and fungi (Keel *et al*., 1992; Chin-A-Woeng *et al.*, 2000, 2001; de Souza *et al*., 2003; Nagarajkumar *et al*., 2005; Weller *et al*., 2006)

A total of 99 colonies were collected from different rhizosphere soils of pomegranate growing areas of northern Karnataka and border areas of Maharashtra. Further, they were screened for their antagonistic activity against *Xanthomonas axonopodis* pv. *punicae* under *in vitro,* 83 colonies showed inhibition. Nine isolates were found to be potent antagonists. Dual culture assay indicated that the inhibition zone produced by the FP-87 isolate was significantly superior over the remaining isolates, except FP-64, where they were on par with each other. Various research workers also demonstrated the role of fluorescent pseudomonads in the inhibition of different species of *Xanthomonas*.

A similar observation was made by Jagadeesh (2000) wherein out of 431 rhizobacterial isolates screened against *Ralstonia solanacearum*, 38 isolates were found to be powerful antagonists with the variable zones of inhibition of the pathogen. Further, studies indicated that the antibacterial mechanism was due to the production of antibiotic siderophore or HCN. A study conducted by Suneesh (2004) revealed that out of 48 different strains of fluorescent pseudomonads collected from Western Ghats of Uttar Kannada district, only two isolates were found to be potential antagonists against *R. solanaceaium*. Megha (2006) reported that out of 52 strains of fluorescent pseudomonads collected from Western Ghats of Uttar Kannada district, only 14 were inhibitory to *Ralstonia solanacearum* and 10 were inhibitory to *Xanthomonas campestris*.

It is imperative to prove the antibacterial mechanism of each FP isolate, because the production of antimicrobial compounds, especially the quantity of secondary metabolites are often specific for an isolate (Fravel, 1988). Hence, all the selected effective antagonistic FP isolates were tested

for the production of HCN, siderophores, fluorescein and pyocyanin.

All fluorescent pseudomonad isolates consistently produced siderophores, as evidenced by the formation of yellowish-orange halo zones on CAS plates. Among them, isolates FP-79, FP-10, and FP-87 exhibited higher siderophore activity compared to the others. Iron chelation zones produced by FP-10, FP-79 and FP-87 were significantly superior over the remaining isolates, and they were on par with each other. Many of the microorganisms growing under iron-limiting conditions are known to produce iron-chelating compounds called siderophores. It has been postulated that siderophores act as biostatic compounds through drastic reduction of the quantity of ferric ions available to phytopathogenic bacteria. The role of siderophores in the control of some plant bacterial diseases has been identified. The siderophores are known to suppress the multiplication of *Xanthomonas axonopodis* pv. *malvacearum,* causing bacterial blight of cotton crop (Mondal *et al.*, 2000). Jagadeesh (2000) described the role of fluorescent siderophore in the biological control of *Ralstonia solanacearum* causing bacterial wilt of tomato. Similarly, Mallesh (2008), who screened PGPR strains against soil-borne pathogens of Coleus and Ashwagandha, observed that all the tested PGPR strains invariably produced siderophores. But, strains RB50, RB31 and RB43 produced strong siderophores. Though siderophores are part of primary metabolism (as iron is an essential element), sometimes they also act as antibiotics, which are commonly considered to be secondary metabolites (Haas & Defago, 2005). Therefore, siderophore-producing fluorescent pseudomonad isolates could definitely serve as one of the most potent biocontrol agents against plant pathogens compared to non-producing isolates. Out of 56 isolates of fluorescent pseudomonads isolated from cotton rhizosphere, 19 isolates showed strong production of siderophore in the *in-vitro* assay against *Xanthomonas axonopodis* pv. *malvacearum* (Fallahzadeh & Ahmadzadeh, 2010). Mishra and Arora (2012), Velusamy *et al.* (2013), Spago *et al.* (2014) and Vasconcellos *et al.* (2014) reported that the inhibition of different species of *Xanthomonas* by different species of rhizosphere bacteria was due to antibiotics and siderophore production.

HCN production is an almost universally observed trait within the group of *Pseudomonas* present in the rhizosphere, as some studies revealed that about 50% of pseudomonads isolated from potato and wheat rhizosphere are able to produce HCN *in vitro* (Bakker & Schippers, 1987; Schippers *et al*., 1990). The production of HCN by rhizosphere bacteria has been hypothesised to play a very crucial role in the biological control of plant pathogens (Defago *et al.,* 1990). In the current study, among the nine fluorescent pseudomonad isolates tested, only three were found positive for HCN production. Among these HCN-producing isolates, FP-64 and FP-87 changed the yellow filter paper to a medium brown colour, indicating a medium level of HCN production. Jagadeesh (2000) tested all the strains of rhizobacteria antagonistic against *Ralstonia solanacearum* for HCN production. Results revealed that only one strain (fluorescent pseudomonad RBL 102) was found to be a strong, six strains were moderate; five strains and two reference strains (*P. fluorescens* and *P. fluorescens* CHAO) were weak HCN producers. Similarly, Mallesh (2008) stated that all seven PGPR strains effective against *Ralstonia solanacarum,* one of the wilt complex pathogens of Coleus and Ashwagandha, were positive for HCN production. PGPR strains RB13 and RB50 were strong, RB1 and RB31 were moderate strain RB10 and RB43 were weak, whereas RB22 scored as nil with respect to HCN production. The production of HCN by antagonistic bacteria has been reported by many other researchers (Saxena *et al.,* 1996; Rangeswaran & Prasad, 1998), and HCN is known to inhibit electron transport, disrupting the energy supply to the cells, ultimately leading to the death of the pathogen (Knowles, 1976).

In the present study, all FP isolates were found to produce fluorescein and pyocyanin in varying proportions on Pseudomonas agar medium. FP-07 and FP-19 showed strong production of fluorescein, and isolates FP-03, FP-07, FP-10, FP-14, FP-19 and FP-79 showed strong production of pyocyanin. Similar results were obtained by Rachid and Ahmed (2005), who reported the specific production of fluorescein and pyocyanin by the *Pseudomonas*. Mallesh (2008) reported that most of the PGPR strains antagonistic against Coleus and Ashwagandha wilt complex pathogens produced fluorescein and pyocyanin. RB13 showed strong production of fluorescein, whereas RB31 and RB-50 exhibited strong pyocyanin production.

CONCLUSION

The findings of this study provide evidence that efficient native isolates of fluorescent pseudomonads produced siderophores, HCN, fluorescein and pyocyanin against *X. axonopodis* pv. *punicae*. This study advances our understanding towards biocontrol of plant pathogens by using native isolates of fluorescent pseudomonads with different mechanisms of antagonism.

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AUTHOR CONTRIBUTION

Madhu S. Giri: Carried out the research and prepared the manuscript. V. I. Benagi: Supervised and mentored the research. V. B. Nargund: Designed the experiments and also involved in the preparation and creation of initial draft of the manuscript. P. Jones Nirmalnath and S. Jahagirdar: Supplemented the ideas and methodology of the experiments. All authors have read and agreed to the published version of the manuscript.