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Phenotypic Identification of Promising Rhizospheric Antagonistic Microbes of Chilli (*Capsicum annuum* L.)

A. Thoyajakshi bai[1*], Ch. Ruth[1] and K. Dinesh[2]

[1]Department of plant pathology, College of Horticulture, Dr. Y. S. R. Horticultural university, Anantharaju peta, Y. S.R Kadapa, Andhrapradesh- 516105, India
[2]Department of plant pathology, College of agriculture, Central agricultural university, Imphal-795004, India

**Corresponding author*

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A B S T R A C T

Biological control is known to be effective eco-friendly method for the management of crop diseases (Cook and Baker, 1983). Rhizosphere antagonists were isolated from healthy rhizosphere soil samples of chilli collected from major chilli growing areas of Andhra Pradesh. A total of 20 rhizosphere microbes were isolated. Out of which (eight fungal antagonists, ten bacterial antagonists and two fluorescent Pseudomonads) were found to exhibit antagonism against chilli wilt pathogen. On further *in vitro* evaluation, nine isolates including four fungi, four bacteria and one *pseudomonas* sp. were found to be most efficient against chilli wilt pathogen. Those rhizosphereic bacterial antagonists (RBA1, RBA 2, RBA 3 and RBA 4) and rhizospheric fluorescent pseudomonads (RFP1) which were found to be extremely efficient against Fusarium wilt pathogen in dual culture were further phenotypically identified based on the production of Siderophores, HCN and ammonia. Among them fluorescent pseudomonads RFP 1 was positive to siderophore, HCN and ammonia production.

Introduction

Chilli (*Capsicum annum* L.) is one of themost important vegetable grown in India. Andhra Pradesh is major producer of chilli followed by Karnataka and Tamil Nadu. As Biocontrol of pathogen is a promising strategy for the replacement of chemical treatments (Dubey *et al*., 2007). A roving survey conducted to isolate beneficial rhizosphere antagonistic microbes from healthy chilli plants. The rhizospheric soil samples of chilli which upto 2 cm depth near to root zone were collected to extract beneficial rhizospheric antagonistc microbes. All the beneficial microbes, a total of 20 rhizosphere microbes were isolated. Among which, 20 isolates (eight fungi, ten bacteria and two fluorescent Pseudomonads) were found to exhibit antagonism against chilli wilt pathogen. On further *in vitro* evaluation, nine isolates including four fungi, four bacteria and one *Pseudomonas* sp. were found to be more efficient antagonists. They were tested *in vitro* for their antagonism

against chilli wilt pathogen *Fusarium oxysporum*. Then the rhizospheric antagonistic microbes which are highly efficient were assessed *in vitro* for their ability to produce hydrogen cyanide (HCN), siderophores and ammonia.

Materials and Methods

The efficient rhizosphere bacterial antagonists RBA 1, RBA 2, RBA 3 and one rhizospheric fluorescent pseudomonad RFP 1 were phenotypically tested *in vitro* for the production of siderophores, ammonia and HCN.

Siderophore production

The chrome azurol sulfonate assay agar was used for the qualitative assay. The chrome azurolsulfonate (CAS) assay (Schwyn and Neilands, 1987) was used since it is most responsive and convenient. The cultures were spot inoculated onto the blue agar (CAS agar) and incubated at 28°C for 3-5 days. The results were interpreted based on the color change due to transfer of the ferric ion from its intense blue complex to the siderophore. The sizes of yellow-orange halo around the growth indicated total siderophore activity. The result was scored either negative or positive.

HCN production

HCN production by bacterial isolates was detected by the method of Baker and Schipper (1987). The King's B agar was amended with 4.4 $gm^{-1}$of glycine and sterilized. The sterile medium was poured into dishes and allowed to solidify and the bacterial isolates were inoculated. Whatman No.1 filter paper disc (90 mm diameter) was soaked in picric acid solution (2.5 g picric acid + 12.5 g Sodium carbonate in 1000 ml of water) and placed on the lid of each plate. Three replications were maintained for each isolate. Petri dishes were sealed with parafilm and incubated at room temperature for four days and the uninoculated plate served as control. An observation on colour change of filter paper from deep yellow to orange brown and to red indicates the production of HCN.

Ammonia production

Selected antagonistic rhizosphere bacterial isolates were tested for their potential for production of ammonia following the method of Dye (1962). The bacterial isolates were grown in 10 ml of peptone water and incubated at 30°C for four days. Three replications were maintained for each bacterial isolate. After incubation, 50 µl of Nessler's reagent was added to the broth. The change in the colour of the broth from faint yellow to deep yellow or brown colour indicated the production of ammonia. The reaction was scored as nil, low, medium and high in 1-4 scale based on intensity of colour.

Results and discussion:

Production of siderophores

Qualitative assay for detection of siderophore production by *Pseudomonas fluorescens* has done by streaking the bacteria on CAS agar media and was observed for production of siderophores in vitro. The clear orange zone formation around the bacterial colony on the CAS agar was noticed after 72 hrs. of inoculation, which is an indication for the good production of siderophores. Among the fluorescent pseudomonads, RPF 1 was found to be positive for siderophore production (Plate 1). Whereas all other bacterial isolates RBA1, RBA 2, RBA 3 and RBA 4 were negative to the production of siderophores. These results were in agreement with Alka Gupta and Murali Gopal (2008), who also observed orange halo around the bacterial colony. The similar results were obtained by

El-Azeem *et al.*, (2007) who have qualitatively assessed for siderophores. Correspondingly, many workers reported that the Pseudomonads can be known by their ability to produce siderophores such as pyoverdine by exhibiting yellow-green color fluorescence under UV light (Sharma and Johri, 2003; Ramya Smruthi *et al.*, 2012). Bhakthavatchalu *et al.*, (2013) tested the siderophore producing ability of *P. aeruginosa* FP6 and recorded the maximum production of siderophore (85.70 µM) after 36 hrs of incubation (Table 1 and Plate 1).

Several scientists Deshwal and Kumar (2013), Sreedevi *et al.*, (2014), Kumar *et al.*,1996) and Kloepper *et al.*, (1980) from their results concluded that siderophore producing *Pseudomonas* strains exhibit more inhibitory effect against Fusarium and other pathogens under iron limiting condition.

Table.1 Mechanism of action of promising rhizosphere bacterial antagonists and fluorescent pseudomonads

Sl. No.	Bacterial antagonist	Siderophore production	HCN production	Ammonia production Color change
1	RBA 1	-	-	Yellow
2	RBA 2	_	_	Yellow
3	RBA 3	_	_	Yellow
4	RBA 4	_	_	Yellow
5	RFP1	++	++	Brownish orange

- nil production
++ good production

Plate.1 Siderophore production by RFP 1

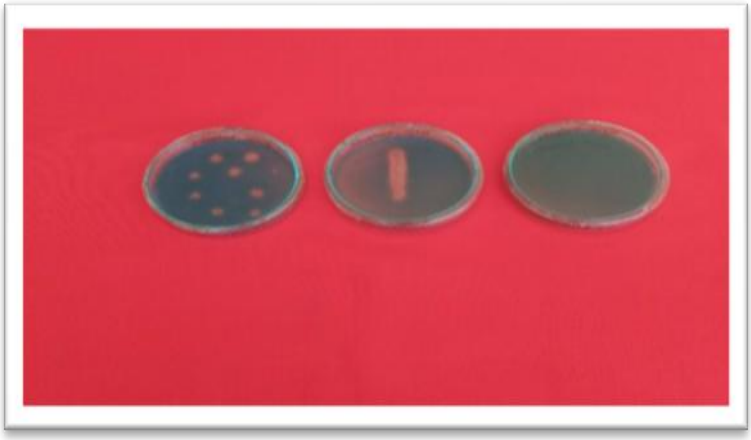

Plate.2 Ammonia production by RFP 1

Plate.3 HCN production by RFP 1

Production of ammonia

A dark orange brown colour has been appeared after addition of Nessler's reagent to the four days old *Pseudomonas* inoculated peptone broth thus confirming the production of ammonia by the isolate RFP 1 and bacterial isolate RBA 1, but all other isolates RBA 2, RBA 3 and RBA 4 found to be negative to ammonia production, while the uninoculated control remained in light faint yellow colour (Plate 2). Likewise, Baligh *et al.*, (1996) in their findings reported that the production of volatile ammonia by *Pseudomonas* sp. has been indicated as a possible mechanism to control soil borne pathogens.

Production of HCN

The efficient rhizosphere bacteria antagonists were tested for HCN production. Out of all the tested isolates, only *Pseudomonas* isolate (RPF 1) showed its ability for production of HCN by transformation of filter paper from yellow to brown. All the bacterial antagonists were not able to produce HCN hence the color of filter paper remained to be same color as that of unioculated control (Plate 3). Hence, it could be affirmed from the findings that isolates RFP 1 significantly suppresses the growth of soil borne pathogens. The production of HCN by *Pseudomonas* is involved in the suppression of various

pathogens in particular fungi (Verma *et al.*, 1989) and could act as an inducer of plant resistance (Kumar *et al.*, 2012).

The results are in agreement with Cappuccino and Sherman (2005), Castric (1975) who determined production of HCN by *Pseudomonas* by observing a color shift of filter paper from yellow to orange brown.

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