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COMPATIBILITY STUDIES ON DIFFERENT ENDOPHYTIC MICROBES OF TOMATO ANTAGONISTIC TO BACTERIAL WILT PATHOGEN

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ABSTRACT
A mutual compatibility study was undertaken to develop the most effective endophytic bioconsortium for the management of bacterial wilt of tomato. The promising endophytes selected from *in planta* experiment were tested for their mutual compatibility to develop the microbial consortia. The interactions were studied among and between the three types of microorganism's *viz.* fungi, bacteria and actinomycetes. Among the 28 fungal combinations, five isolates VRF-1, VSF-3, CSF-1, MyRF-1 and ASF-3 were found highly compatible. When the five bacterial isolates selected from *in planta* experiment were subjected to mutual compatibility test by cross streak method, three isolates EkRB-1, VSB-1 and TRB-1 were found compatible. Both the actinomycetes, ORA-1 and VRA-1 were mutually compatible. Mutual compatibility between the selected fungi, bacteria, and actinomycetes was tested by co-inoculation in liquid media. Based on sporulation and growth of the organisms and further confirmation by plating on their respective media, the five fungal isolates *viz.* VRF-1, VSF-3, CSF-1, MyRF-1 and ASF-3, two bacteria, VSB-1 and TRB-1 and two actinomycetes, ORA-1 and VRA-1 were compatible to each other and therefore, all these isolates were selected for the development of different endophytic consortia.

KEY WORDS: Mutual compatibility, endophytic microbes, tomato, *Ralstonia solanacearum.*

INTRODUCTION

Bacterial wilt disease incited by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* is one of the most destructive diseases of solanaceous crops in tropics, subtropics, and warm temperate regions of the world. In Kerala, the yield loss due to the bacterial wilt incidence ranged from 20 to cent per cent depends upon the varieties (Sadhankumar, 1995). Biocontrol of plant pathogen is becoming an important component of integrated disease management. In view of the hazardous impact of pesticides and other agrochemicals on the ecosystem, biocontrol of plant diseases as an alternate strategy has received increasing attention in recent years. Therefore, the focus on the management of plant diseases has been shifted from chemical pesticides to more ecofriendly biopesticides to reduce environmental hazards and minimize the risk of development of pesticide resistant strains of plant pathogens. A novel method of biological control using endophytes has entered the arena of disease management with attempts to make the plant, defend itself from the pathogens. The beneficial effects that the endophytes can confer on plants have made their role highly significant in biological control of diseases in various crops (Bargabus *et al*., 2004; Kloepper *et al*., 2004). Recently, a greater thrust is given for the development of biological consortium since it consists of microbes with different biochemical and physiological capabilities, which permits interaction among themselves and will lead to the establishment of a stable and effective microbial community. It will further provide better management of diseases by way of synergistic effect and multiple mode of action. Therefore, a study was carried out on the compatibility of endophytes isolated from tomato antagonistic to bacterial wilt disease.

MATERIALS & METHODS

The endophytes isolated from tomato were subjected to both *in vitro* and *in vivo* experiment and the promising ones including fungi, bacteria and actinomycetes selected were tested for their mutual compatibility *in vitro* using PDA and selective media of the respective organisms. The interaction between the fungal endophytes was studied by dual culture technique. Eight mm mycelial discs of seven day old cultures of two fungi were placed at opposite ends in Petri dishes with PDA. Plates were kept in triplicates for each combination and incubated at room temperature for five days to observe the compatibility reaction. The selected bacterial endophytes were tested for compatibility by cross streak method. Two different bacterial isolates were streaked vertically and horizontally on NA mediated plates. The plates were incubated for 48 h at room temperature and observed for lysis at the juncture of the streaks. The compatibility between the selected actinomycetes was tested by cross streak method. The two actinomycetes were streaked vertically and horizontally on Kenknight's Agar medium. The plates were incubated for seven days, and observed for lysis at the juncture. The interaction between fungi and bacteria was studied adopting liquid culture co-inoculation method. Eight mm mycelial disc of one fungal endophyte was coinoculated with a loopful of one bacterial endophyte in 100 ml of PDB and individual endophytes were inoculated separately for comparison. The bacteria and

fungi were plated on their respective media after incubation periods of two and five days respectively. The microbial population in each plate was recorded and compared with that of individual cultures. The fungi and bacteria selected from the above experiment were further tested for their compatibility with actinomycetes using liquid culture co-inoculation method. Eight mm discs of a selected fungus and an actinomycete along with one loopful of bacteria were inoculated simultaneously in 100 ml PDB and incubated at room temperature for seven days. Individual isolates inoculated in PDB were maintained separately. The bacteria, fungi and actinomycetes were plated on their respective media after two, five and seven days respectively to check for their compatibility.

RESULTS

The promising endophytes selected from *in planta* experiment were tested for their mutual compatibility to develop the microbial consortia. The interactions were studied among and between the three types of microorganisms *viz.* fungi, bacteria and actinomycetes.

Compatibility between fungal antagonists

There were total of 28 combinations among the fungi (Table 1). The different interactions observed were intermingling of hyphae, presence of thick mycelial band, clear demarcation at the meeting point, heavy sporulation, and yellow pigmented band at the interaction site (Fig 1). Incompatible fungal combinations showed over growth, less sporulation and reduction in size of fungal colonies. Diffusion of metabolite was noticed in certain combinations. Other remarkable observations were the presence of thick brown pigmented band at the interaction point of CSF-1 and MyRF-1 on the reverse side of colonies, the colour change of spores of ASF-3 from green to yellow in ERF-1 x ASF-3 combination and the colour change from green spores of VSF-3 to olive green in MyRF-1 x VSF-3 combination. The highly compatible five isolates VRF-1, VSF-3, CSF-1, MyRF-1 and ASF-3 were selected for further studies.

TABLE 1: Mutual compatibility of endophytic fungal isolates

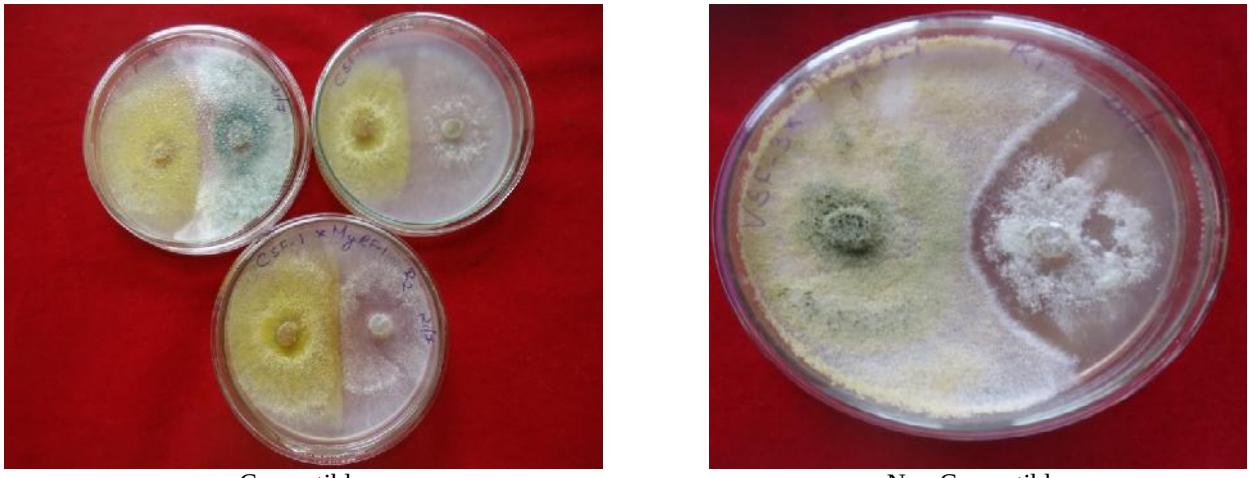
Sl. No.	Fungal combinations	Observations recorded
1	VRF-1 x PSF-1	Overgrowth, pigment diffuses to PSF-1
2	VRF-1 x VSF-3	Heavy sporulation at the meeting point
3	VRF-1 x KaRF-3	Overgrowth, pigment diffuses to KaRF-3
4	VRF-1 x CSF-1	Clear demarcation at the meeting point
5	VRF-1 x ERF-1	Clear demarcation at the meeting point, less sporulation in ERF-1
6	VRF-1 x ASF-3	Heavy sporulation at the interaction point
7	VRF-1 x MyRF-1	Heavy sporulation at the contact point, growth of MyRF-1 reduced
8	ERF-1 x PSF-1	Heavy sporulation at the interaction site, clear demarcation, heavy pigment production in PSF-1
9	ERF-1 x VSF-3	Intermingling of hyphae with heavy sporulation at the contact point, metabolite production in ERF-1
10	ERF-1 x KaRF-3	Overgrowth
11	ERF-1 x CSF-1	Overgrowth, growth of CSF-1 reduced
12	ERF-1 x ASF-3	Clear demarcation at the meeting point, pigment production in ASF-3, green coloured spores of ASF-3 turned yellow
13	ERF-1 x MyRF-1	Clear lytic zone at the meeting point, growth of MyRF-1 reduced
14	CSF-1 x PSF-1	Intermingling of hyphae with heavy sporulation at the contact point, metabolite production in CSF-1
15	CSF-1 x VSF-3	Clear demarcation at the meeting point
16	CSF-1 x KaRF-3	Overgrowth, growth of KaRF-3 reduced
17	CSF-1 x ASF-3	Clear demarcation at the meeting point, metabolite production in CSF-1
18	CSF-1 x MyRF-1	Thick brown pigmented band at the interaction point, less sporulation of CSF-1
19	PSF-1 x VSF-3	Clear lytic zone at the meeting point, yellow pigmented band present
20	PSF-1 x KaRF-3	Thick sporulation at the meeting point, growth of KaRF-3 reduced
21	PSF-1 x ASF-3	Yellow pigmented band at the interaction site, growth of ASF-3 reduced
22	PSF-1 x MyRF-1	Clear demarcation at the interaction point, growth of MyRF-1 reduced
23	KaRF-3 x VSF-3	Heavy sporulation at the meeting point, pigment production at the reverse side of VSF-3
24	KaRF-3 x ASF-3	Yellow pigmented band at the meeting point, growth of ASF-3 reduced
25	KaRF-3 x MyRF-1	Clear demarcation at the interaction point, growth of MyRF-1 reduced
26	ASF-3 x VSF-3	Clear demarcation at the meeting point, both cultures turned from dark to light green
27	ASF-3 x MyRF-1	Clear demarcation at the meeting point
28	MyRF-1 x VSF-3	Green spores of VSF-3 turned olive green, clear demarcation at the meeting point

Compatibility between bacterial antagonists

The five bacterial isolates selected from *in planta* experiment were subjected to mutual compatibility test by cross streak method (Fig 2). No lysis was observed at the juncture of TRB-1 x VSB-1, TRB-1 x EkRB-1 and VSB-1 x EkRB-1 combinations, which indicated the compatibility among the isolates (Table 2) and these three bacterial isolates EkRB-1, VSB-1 and TRB-1 were selected.

TABLE 2: Mutual compatibility between endophytic bacterial isolates

Sl No.	Bacterial endophytes	Observations recorded	Interpretation
1	TRB-1 x VSB-1	No change	Compatible
2	TRB-1 x EkSB-1	No change	Compatible
3	TRB-1 x MSB-1	Lysis at the juncture	Noncompatible
4	TRB-1 x EkRB-1	Lysis at the juncture	Noncompatible
5	VSB-1 x EkSB-1	Lysis at the juncture	Noncompatible
6	VSB-1 x MSB-1	Lysis at the juncture	Noncompatible
7	VSB-1 x EkRB-1	No change	Compatible
8	EkRB-1 x EkSB-1	Lysis at the juncture	Noncompatible
9	EkRB-1 x MSB-1	Lysis at the juncture	Noncompatible
10	MSB-1 x EkSB-1	Lysis at the juncture	Noncompatible



Compatible Non Compatible

FIGURE 1. Compatibility between fungal antagonists

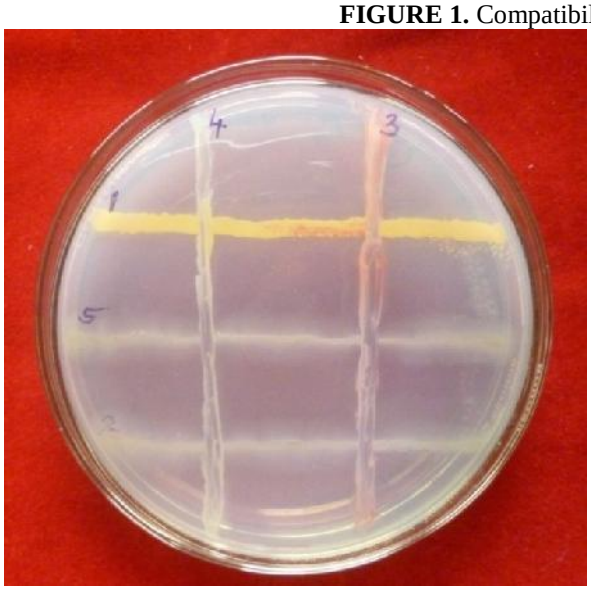


FIGURE 2: Compatibility between bacterial antagonists

FIGURE 3: Compatibility between actinomycetes

Compatibility between actinomycetes isolates

Two actinomycetes, ORA-1 and VRA-1 were found compatible, as no lysis was noticed at the juncture of the isolates (Fig 3).

Compatibility between fungi and bacteria

Growth characters of fungal and bacterial endophytes in liquid media were observed visually at second and seventh day of inoculation and the results are presented in Table 3.

It is observed from the table that, the growth of fungal and bacterial antagonists varied with different combinations. Fungal growth and sporulation were affected by the combinations with bacterial isolate, EkRB-1, which indicated the incompatibility of this isolate with fungal endophytes. Plating of the cultures on their respective media also yielded high population of both fungi and bacteria except EkRB-1. All isolates except bacterial isolate EkRB-1 were selected for further studies (Fig. 4).

Compatibility between fungi, bacteria and actinomycetes

Mutual compatibility between the selected fungi, bacteria, and actinomycetes was tested by co-inoculation in liquid

media (Fig. 5). Independent combinations with one each from the three organisms were inoculated in liquid medium. Sporulation and growth of the organisms were observed visually at two and seven days after inoculation for compatible combinations. It was further confirmed by plating on their respective media which also yielded high population of fungi, bacteria, and actinomycetes which indicated that the five fungal isolates *viz.* VRF-1, VSF-3, CSF-1, MyRF-1 and ASF-3, two bacteria, VSB-1 and TRB-1 and two actinomycetes, ORA-1 and VRA-1 were compatible to each other and therefore, all these isolates were selected for the development of different consortia.

TABLE 3: Mutual compatibility of endophytic fungal and bacterial isolates

Sl. No.	Isolates	Mycelial growth	Sporulation	Bacterial growth	Filtrate colour
1	VSF-3 x VSB-1	+++	+++	+++	Colourless
2	VSF-3 x TRB-1	+	+	+++	Colourless
3	VSF-3 x EkRB-1	-	-	++	Colourless
4	VSF-3 (control)	+++	+++	-	Colourless
5	ASF-3 x VSB-1	+++	+++	+++	Colourless
6	ASF-3 x TRB-1	+	+	+	Colourless
7	ASF-3 x EkRB-1	-	-	+	Colourless
8	ASF-3 (control)	+++	+++	-	Colourless
9	CSF-1 x VSB-1	++	++	++	Brown
10	CSF-1 x TRB-1	++	++	+++	Light yellow
11	CSF-1 x EkRB-1	-	-	++	Brown
12	CSF-1 (control)	+++	+++	-	Brown
13	VRF-1 x VSB-1	++	+	++	Dark yellow
14	VRF-1 x TRB-1	+	+	+++	Light yellow
15	VRF-1 x EkRB-1	-	-	+	Light yellow
16	VRF-1 (control)	+++	+++	-	Dark yellow
17	MyRF-1 x VSB-1	+	+	++	Colourless
18	MyRF-1 x TRB-1	+	+	++	Colourless
19	MyRF-1 x EkRB-1	-	-	++	Colourless
20	MyRF-1 (control)	++	++	-	Colourless

Growth			Sporulation		
+++	-	Good	+++	-	Good
++	-	Average	++	-	Average
+	-	Poor	+	-	Poor
--	-	No growth	--	-	Nil

FIGURE 4: Compatibility between fungi and bacteria

FIGURE 5: Compatibility between fungi, bacteria and actinomycetes

DISCUSSION

It is likely that, most cases of naturally occurring biological control results from mixtures of antagonists, rather than from high population of a single antagonist. Mixtures of antagonists are considered to account for protection in

disease suppressive soils. Combinations of biocontrol agents for plant diseases include mixtures of fungi and mixtures of fungi and bacteria. Most of the reports on bioconsortia showed that, combinations of antagonists resulted in improved biocontrol. However, there are also reports of combinations of bioagents that do not result in improved suppression of disease compared with the individual antagonist. Incompatibility of the coinoculants can arise because biocontrol agents may also inhibit each other as well as the target pathogen. Thus an important prerequisite for successful development of microbial consortia appears to be the compatibility of the coinoculated microorganisms. Therefore, the endophytes selected from *in planta* experiment were subjected to mutual compatibility test for the development of efficient microbial consortia. Fifteen endophytes including eight fungi, five bacteria, and two actinomycetes were tested *in vitro* for their compatibility. The interactions among the eight fungal endophytes showed observations like heavy sporulation at the meeting point, diffusion of metabolite, intermingling of hyphae, yellow pigmented band at the interaction point, clear demarcation at the meeting point, and dark green spores turned olive green. Similar interactions were observed between endophytic *Trichoderma* isolates of black pepper by Mathew (2007). Among the eight fungi tested, three were noncompatible of which one showed suppression of growth and two exhibited overgrowth mechanism. The noncompatibility among the fungal isolates was also noticed by Mohammed *et al* (2011) in a composting process of oil palm waste where they observed the combinations of *T. viride* and *Penicillium* sp., *T. viride* and Basidiomycete M1, *T. reesei* and *P. tigrinus* may interact as compatible, while *A. niger and T. viride*, *A. niger* and *T. reesei*, *T. viride* and *T. reesei* and *Penicillium* sp. and *P. tigrinus* were partially compatible and the other combinations were incompatible or inhibited by each other. Likewise, among the five bacteria, two isolates were incompatible with bacterial isolates and one with fungal isolate and both actinomycetes were found compatible with each other. Similar observations have been reported previously also. For example, *T. viride*/*T. harzianum* and *P. fluorescens* were reported to be compatible and improved plant growth,as well as suppressed seedling disease of chilli and tomato significantly when these were applied together (Rini and Sulochana, 2006; Chaube and Sharma, 2002). Later Rini and Sulochana (2007) also reported compatibility between *Trichoderma* spp. and fluorescent pseudomonads in dual cultures testing their efficacy against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato.

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