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Isolation and screening of *Streptomyces* for biocontrol potential against *Rhizoctonia bataticola* infection of soybean

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Abstract: This study was carried out for the isolation of *Streptomyces* species having biocontrol potential against *Rhizoctonia bataticola*. *R. bataticola* causes many diseases in soybean. Total 21 *Streptomyces* were isolated from rhizospheric soil of different plants of fields in kanheri sarap. The diversity of *Streptomyces* was most wide in rhizospheric soil of Pomegranate. Fourteen isolates of *Streptomyces* exhibited zone of inhibition against *R. bataticola* by dual culture method. Out of fourteen, twelve isolates were isolated from soil of Pomegranate. *Streptomyces* ANSP2, ANSP4, ANSP12 and ANSN14 shows strong antagonistic activity against *R. bataticola*. These four isolates were also found compatible to *Bradyrhizobium japonicum*. Efficacy of ANSP4 treatment was tested on germination of soybean seed, challenged with *R. bataticola*. ANSP4 shows better results than chemical treated seeds.

Key words: Biocontrol agent, Pomegranate *(Punica granatum)*, Antagonistic *Streptomyces*, soybean, *Rhizoctonia bataticola*.

Introduction

Soybean is a one of the major crops in Akola district. Although many plant pathogens cause disease to soybean plant, *R. bataticola* is the most prevalent plant pathogen in Akola district. *R. bataticola* causes damping off, root rot, stem rot and charcoal rot resulting in reduction in crop yields (1,2). Exploring biocontrol agents for controlling these diseases is necessary considering the hazardous effects of chemical fungicides. Application of Actinomycetes for biocontrol of fungal phytopathogens has been explored earlier (3-5). *Streptomyces* sp. was shown to be effective biological control agent against plant pathogens. *Streptomyces* strain Di-944 was

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reported to strongly inhibit mycelial growth of virulent *R. solani* in dual culture method (6) and *Streptomyces lydicus* WYEC108 was reported to produce antifungal compound (5). Some *Streptomyces* sp. isolated from saline soil of Akola district were found to be antagonistic against *R. bataticola* (7).

Many studies have shown plant growth promoting activities of *Streptomyces* spp antagonistic against plant pathogens (5,8-15) Rhizospheric Actinomycetes has been employed for protection and plant growth promotion of different crops (16). Present study was focused on isolation of *Streptomyces* spp having biocontrol potential for *R. bataticola* infection of soybean seeds.

Material and Methods

Isolation of Streptomycetes

Soil sample were collected from rhizospheric part of different plants from fields of Kanheri sarap having non saline soil. Soil samples were collected from the rhizospheric part of Pomegranate *(Punica granatum)*, Cotton *(Gossypiun herbaceum)*, Pigeon pea (*Cajanus cajan)*, Soybean [*Glycin max*], Turmeric *(Curcuma Longa)*, Drumstick (*Moringeao leifera),* Sugarcane *Sachharam officinarum,* Capsicum (*Capssicum annum),* Neem (*Azadirachta indica)* from 15cm depth. Serial dilutions of soil samples were inoculated on Starch casein agar medium. Plates were incubated for 7 days at 28^0C and colonies were identified (17,18).

Isolation of Rhizoctonia bataticola

Infected soybean plants were collected from fields of Akola District. Samples were surface sterilised with 4% sodium hypochlorite. Tissues of surface sterilised infected plants were inoculated on potato dextrose agar and incubated for 8 days at room temperature (19). Fungal isolates were identified by microscopic examination (20).

Test for virulence

Test for virulence of *R. bataticola* was carried out by paper towel method by applying fresh broth of *R. bataticola* on surface sterilised soybean seeds (21).

Test for antagonism

Test of antagonism was carried out by dual culture method. *R. bataticola* was spread inoculated on Starch casein agar medium and over that inoculation spot inoculated isolated *Streptomyces*. The plates were observed for the zone of inhibition after incubation of 7 days at 30^0c (Fig. 3).

Compatibility with Bradyrhizobium japonicum

Bradyrhizobium japonicum was isolated from healthy nodules of soybean plant and spread inoculated on Starch casein agar medium and over that inoculation spot inoculated isolated *Streptomyces*. The plates were observed for the zone of inhibition and growth of both the cultures after 7 days incubation at 30^0C (Fig: 2).

Test for effect on seed germination

Effect of different treatments was studies on percent germination and initial seedling growth of soybean on germination paper in sterile petriplates. The healthy surface sterilised seeds were treated with *R. bataticola* + selected *Streptomyces*, *R. bataticola* + Carbendazim and only with *R. bataticola*. The results were compared with proper control.

Result and Discussion

Total of twenty one *Streptomyces* were isolated from rhizospheric soil of different plants (Table 1) and *R. bataticola* was isolated from infected soybean plant (Fig. 1). Among different plants highest numbers of *Streptomyces* were isolated from rhizospheric soil of pomegranate followed by that of Capsicum. Out of twenty one cultures, fourteen *Streptomyces* were inhibitory to *R. bataticola*. Interestingly all the twelve cultures of *Streptomyces* from pomegranate soil were found to be antagonistic for *R. bataticola*. The colony characters of these species and their microscopic examination revealed the features of *Streptomyces spp.* (Table 2). ANSP2, ANSP4, ANSP12 and ANSN14 exhibited strong zone of inhibition as compared to others indicating their better biocontrol potential (Table 3, Fig. 2). All the four *Streptomyces* sp. showed compatibility with *Bradyrhizobium japonicum* which is an essential attribute for the growth of soybean plant.

R. bataticola treatment of soybean seed resulted in 30% reduction in percent germination as compared to control (table 4). However the growth seedlings were drastically retarded with the absence of secondary and tertiary roots. Treatment of *R. bataticola* challenged seeds with ANSP4 is very effective for increase in percent germination and this treatment had growth promoting impact the development of roots and shoots (Table 4, Fig 3).

The improvement in percent germination of Carbendazim treatment of *R. bataticola* challenged seeds was less as compared to ANSP4 treated seeds. ANSP4 treatment of the *R. bataticola* challenged seeds resulted in the formation of numerous secondary and tertiary roots making the seedlings healthier as compared to untreated control. This observation indicated the presence of significant plant growth promoting potential in ANSP4. Earlier reported that the presence of antagonistic and plant growth promoting attributes together in the same strains of *Streptomyces* (22,23,24,25). Plant growth promoting potential of some *Streptomyces* was also reported by some investigators (14,15,26,27). The finding of present work with respect to antagonistic and plant growth promoting activity of ANSP4 are in agreement with the above reports.

Table 1. Actinomycetes isolated from rhizospheric soil of different plants

S. No.	Rhizospheric Soil of Plants	Isolated Actinomycetes
1	Pomegranate *(Punica granatum)*	ANSP1-12
2	Drum stick (*Moringao leifera)*	ANSD13
3	Neem(*Azadirachta indica)*	ANSN14

4	Sugarcane *(Saccharum officinarum)*	ANSSu16
5	Turmeric (*Curcuma longa*)	ANST17
6	Cotton *(Gossypium herbaceum*)	ANSCo18
7	Pigeon pea((*Cajanus cajan*)	ANSPi-19
8	Soybean [*Glycine max*]	ANSS20
9	Capsicum *(Capsicum annuum)*	ANSC21

Table 2. Morphological Characteristic and microscopic examination of isolated *Streptomyces*

Sr. No.	**Isolate Number**	**Morphological characteristic of colony and microscopic examination**				**Gram Staining**	**Acid fast staining**
		Arial mass colour	**Substrate mycelium**	**Melanoid pigments**	**Spore chain morphology**	**Gram positive**	**No**
1	ANSP1	White	Pink	-	Rectus-Flexibilis (RF)	Gram positive	No
2	ANSP2	Gray	Yellow	-	Rectus-Flexibilis (RF)	Gram positive	No
3	ANSP3	Red	Brown	-	Rectus-Flexibilis (RF)	Gram positive	No
4	ANSP4	Gray	Light gray	-	Retinaculiaperti	Gram positive	No
5	ANSP5	Green	Yellow	Light Brown	Rectus-Flexibilis (RF)	Gram positive	No
6	ANSP6	black	Olive	-	Rectus-Flexibilis (RF)	Gram positive	No
7	ANSP7	Off white	Brown	-	Flexibilis (RF)	Gram positive	No
8	ANSP8	Gray	Yellow	-	Flexibilis (RF)	Gram positive	No
9	ANSP9	Yellow	Yellow	Pink	compact coils	Gram positive	No
10	ANSP10	Brown	Red	-	Open chain	Gram positive	No
11	ANSP11	Light yellow	Purple	Blue	Spira	Gram positive	No
12	ANSP12	Pink	Black	-	Spira	Gram positive	No

13	ANSD-13	White	Pink	Yellow	Rectus Flexibilis	Gram positive	No
14	ANSN14	white	orange	-	Spiral	Gram positive	No

Table 3. Zone of Inhibition of Streptomycetes against *Rhizoctonia bataticola*

Sr. No.	Streptomyces spp.	Zone of inhibition in mm
1	ANSP1	5
2	ANSP2	6
3	ANSP3	5
4	ANSP4	7
5	ANSP5	3
6	ANSP6	4
7	ANSP7	5
8	ANSP8	4
9	ANSP09	5
10	ANSP10	5
11	ANSP11	4
12	ANSP12	6
13	ANSD13	-
14	ANSN14	6
15	ANSSu15	-
16	ANSSu16	-
17	ANSSu17	-
18	ANST18	5
19	ANSCo19	-
20	ANSCo20	-
21	ANSPi21	-

Table 4. Effects of different treatments on soybean seed germination and growth of seedlings.

S. N.	Treatments	Germination	Shoot	Roots	Secondary and tertiary roots	Status
1	Rb	70%	Infected with retarded growth	Infected	Absent	Infected
2	Rb+ANSP4	99%	length between 5 to 14 cm	Healthy	Numerous	Well developed and Healthy
3	Rb+ Carbendazim	80%	length between 3 to 9 cm	Weaker	Scanty	Weak
4	Control	95%	length between 4 to 10 cm	Healthy	Scanty but healthy	Healthy

Note- Rb –*Rhizoctonia bataticola*, ANSP4 – a culture of *Streptomyces* ANSP4

Fig. 1 Microscopic examination of *Rhizoctonia bataticola*

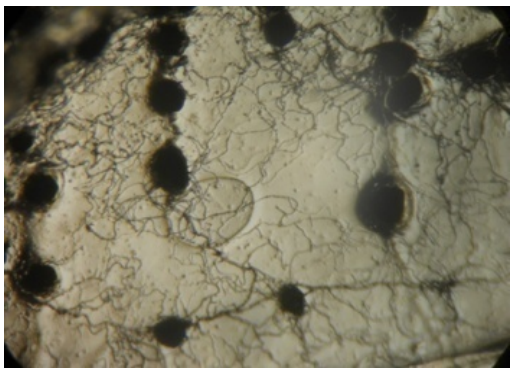

Fig. 2 Zone of Inhibition against *Rhizoctonia bataticola*

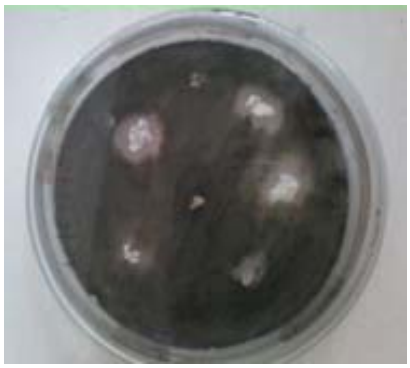

Fig. 3 Effect of ANSP4 on germination of soybean seeds

1 2 3 4

1-Streptomyces ANSP4 and *Rhizoctonia bataticola* treated seeds

2-*Rhizoctonia bataticola* treated seeds

3- Chemical (Carbendazim) and Rhizoctonia bataticola treated seeds

4- Untreated seeds (Normal seeds)

Conclusion

Streptomyces ANSP4 able to inhibit *R. bataticola* and increases growth parameters of soybean. *Streptomyces* ANSP4 could be explored in future for the applied biocontrol and PGPR prospects for soybean.

Conflict of interests

There is no conflict of interests.

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