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Antagonistic and antimicrobial activities of some bacterial isolates collected from soil samples

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Abstract Thirty seven bacterial cultures isolated from soil samples obtained from different locations were tested for their antagonistic activity against some fungal pathogens, viz., *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani*, causal agents of collar rot of sunflower, wilts and root rots, respectively. Among them, 5 bacterial strains, viz., A1 6 (*Bacillus sphaericus*), K1 24 (*Pseudomonas fluorescens*), M1 42 (*Bacillus circulans*), M1 66 (*Bacillus brevis*) and T1 22 (*Bacillus brevis*) showed positive antagonistic activity. M1 66 was the most effective in inhibiting mycelial growth of *S. rolfsii in vitro* followed by M1 42, T1 22, K1 24 and A1 6. Only one bacterial strain i.e. M1 42 exhibited antagonistic activity against *F. oxysporum*, and none of the bacterial strains gave positive activity against *R. solani*. Furthermore, antimicrobial activities of all the 5 strains were checked against different test organisms. These strains showed their extensive inhibition effect particularly against gram-positive test bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and the test fungal strain (*Candida albicans*). On the other hand, *B. brevis* M1 66 and *B. brevis* T1 22 strains had an inhibitory effect against gram positive and gram-negative test bacteria *(Escherichia coli* and *Proteus vulgaris*) as well as the test fungal strain.

Key words: Biocontrol · Sclerotium rolfsii · Antagonism · Bacillus · Pseudomonas

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The biological control of plant diseases with bacterial antagonism is a potential alternative of chemical control as it is expensive and also results in accumulation of toxic compounds in soil biota[1]. The phytopathogens can cause enormous loss of crop yields from 25–100%. Major fungal pathogen like *S. rolfsii* Sacc. [teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough] is a soil-borne plant pathogen with a wide range of hosts and world wide distribution[2]. It causes pre- and post-emergence damping off and collar rot of sunflower. The fungus spreads by mycelial contact with healthy plants and over-winters as sclerotia in soil. The sclerotia survive for a long period in soil and causes severe losses. The other fungal pathogen, *F. oxysporum*, an abundant and active saprophyte in soil and organic matter, has specific forms that are plant pathogenic[3] and cause wilts, root rots and damping off. Disease symptoms caused by *R. solani*, a very common soil borne pathogen with a great diversity of host plants, are referred to as damping-off, root rots and blights[4]. Due to high economic losses caused by fungal phytopathogens, biocontrol mechanisms are of great importance. In the present investigation, antagonistic effects of some bacterial strains have been examined against the above fungal pathogens with particular reference to antagonistic interaction. Also, the antimicrobial activities of these strains were checked against different test organisms.

The 37 bacterial strains were isolated from soil samples by serial dilution agar plate technique. The bacterial strains were characterized according to Bergey's Manual of Systematic Bacteriology[5]. All the strains were purified on Tryptic Soy Agar (TSA) and maintained as glycerol stock at –70°C. The fungal cultures used in the antagonistic studies were *S. rolfsii* (MTCC 288), *F. oxysporum* (MTCC 284) and *R. solani* (MTCC 4633) and the test / pathogenic microorganisms used in this study were *S. aureus* (MTCC 737),

Table 1 Antagonistic activity of some of the bacterial strains against fungal pathogens.

Bacterial Strains	Antagonism (*in vitro*) against		
	S. rolfsii	*F. oxysporum*	*R. solani*
	Growth inhibition (%)		
B. brevis M1 66	80.2	0.0	0.0
B. circulans M1 42	75.3	65.3	0.0
B. brevis T1 22	74.0	0.0	0.0
B. sphaericus A1 6	70.3	0.0	0.0
P. fluorescens K1 24	60.4	0.0	0.0

B. subtilis (MTCC 441), *E. coli* (MTCC 443), *P. vulgaris* (MTCC 426) and *C. albicans* (MTCC 277). These microorganisms were obtained from MTCC and Gene bank, IMTECH, Chandigarh, India. The fungal cultures were maintained in Potato Dextrose Agar (Hi-Media, Bombay, India) at 25ºC and the bacterial strains were maintained in Nutrient Agar (Hi-Media, Bombay, India) at 30ºC.

Antagonistic properties of all bacterial strains were tested against *S. rolfsii*, *R. solani* and *F. oxysporum* on TSA plates using a dual culture technique[6]. Agar blocks (5 mm dia.) containing 5 days old mycelia were placed at the

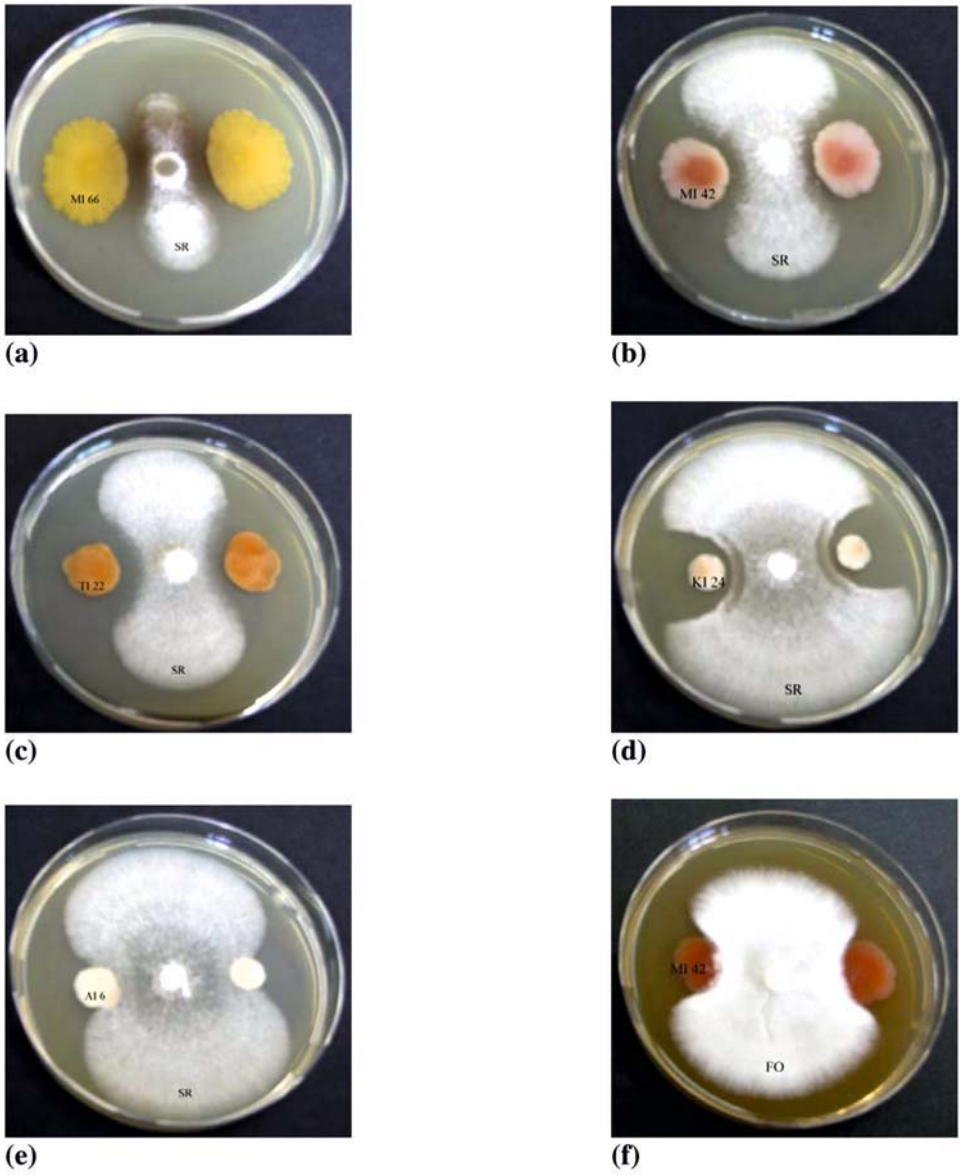

Fig. 1 Dual culture showing colony interaction.

(a) M1 66 (*B. brevis*) vs. *S. rolfsii*
(b) M1 42 (*B. circulans*) vs. *S. rolfsii*
(c) T1 22 (*B. brevis*) vs. *S. rolfsii*
(d) K1 24 (*P. fluorescens*) vs. *S. rolfsii*
(e) A1 6 (*B. sphaericus*) vs. *S. rolfsii*
(f) M1 42 (*B. circulans*) vs. *F. oxysporum*

Table 2. Antimicrobial activity of some of the bacterial strains on test organisms.

Bacterial Strains	Inhibition zone (diameter, mm) against test organisms				
	S. aureus MTCC 737	*B. subtilis* MTCC 441	*E. coli* MTCC 443	*P. vulgaris* MTCC 426	*C. albicans* MTCC 277
B. brevis M1 66	25	25	20	24	22
B. circulans M1 42	11	12	0	0	14
B. brevis T1 22	9	0	0	0	10
B. sphaericus A1 6	24	23	14	0	20
P. fluorescens K1 24	9	0	0	0	14

center of TSA plates. A loopful culture (24 h old) of bacterial strain was inoculated at 2 cm juxtaposed to the pathogen on each plate. The fungal pathogen was inoculated centrally on TSA plate. Uninoculated plates served as control. All the plates were incubated at 28±1°C for 5 days and colony growth inhibition (%) was calculated by using the formula: C-T/C x 100, where C is the colony growth of pathogen in control, and T is the colony growth of pathogen in dual culture.

Most of the bacterial strains isolated were identified as *Bacillus* spp. Out of these 37, only 5 bacterial strains i.e. A1 6 (*B. sphaericus*), K1 24 (*P. fluorescens*), M1 42 (*B. circulans*), M1 66 (*B. brevis*) and T1 22 (*B. brevis*) showed positive antagonistic activity. As evident from Table 1, maximum growth inhibition exhibited by M1 66 and M1 42 was 80.2% and 75.3% respectively, was recorded against *S. rolfsii* after 5 days of incubation (Figs. 1a, 1b). The other three bacterial strains i.e. T1 22 (*B. brevis*), K1 24 (*P. fluorescens*) and A1 6 (*B. sphaericus*) caused 74.0%, 70.3% and 60.4% growth inhibition respectively (Figs. 1c, 1d, 1e). Among 5 bacterial strains, only one i.e. MI 42 (*B. circulans*) showed positive antagonistic activity (65.3%) against *F. oxysporum* and none of the bacterial strains were positive against *R. solani* (Fig. 1f).

To study the antimicrobial activity of 5 bacterial strains (A1 6, K1 24, M1 42, M1 66 and T1 22), all these were cultured on NB medium and incubated at 30°C for 24h. Nutrient agar medium was poured into each sterile petridish (90 mm in diameter). 100 μL of cell suspensions of target strains viz. *E. coli* and *P. vulgaris* (gram-negative bacteria), *S. aureus* and *B. subtilis* (gram-positive bacteria) and *C. albicans* cultured for 24 h were spread on the plates and wells of 5 mm diameter were punched in the agar with a sterile cork borer. The bacterial cultures were centrifuged at 10,000 rpm for 15 minutes to remove cell debris. After centrifugation supernatant samples (100 μL) were filled into the wells of agar plates. The inoculated plates were incubated for 24h at their optimum growth temperatures[7].

As evident from the Table 2, all the strains showed good antimicrobial activity against one or the other test organism. *B. brevis* M1 66 showed antimicrobial activity against all the test organisms. *B. sphaericus* A1 6 had inhibitory activity against all the test organisms except *P. vulgaris*. *B. circulans* M1 42 showed antimicrobial activity particularly against gram positive test bacteria (*S. aureus* and *C. albicans*) whereas *B. brevis* T1 22 and *P. fluorescens* K1 24 showed antimicrobial activity only against *S. aureus* and *C. albicans*.

Members of the genus *Bacillus* are well known as producers of a large variety of peptide antibiotics. Cyclic peptides such as gramicidin S, tyrocidin and bacitracin and lipopeptides such as iturines, bacillomycins and fengycins are characteristic secondary metabolites, which have been isolated from this group of microorganisms[8,9,10]. Isolation and chemical characterization of the antimicrobials determined is the subject of further studies.

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