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Full Length Research Paper

Evaluation of endophytic aquatic hyphomycetes for their antagonistic activity against pathogenic bacteria

Pratibha Arya[1]* and S. C. Sati[2]

[1]Botany Department, Govt. P. G. College Augustyamuni, Rudraprayag, Garhwal, Uttarakhand.
[2]Botany Department, Kumaun University, Nainital, Uttarakhand.

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Evaluation of some riparian endophytic aquatic hyphomycetous fungi viz., *Heliscus lugdunensis*, *Tetrachaetum elegans*, *Tetracladium marchalianum*, *T. breve* and *T. nainitalense* from surface sterilized roots of healthy riparian plants has been carried out for their possible antibacterial activity. Pathogenic test bacteria viz., *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Erwinia chrysanthemi*, *Escherichia coli* and *Xanthomonas phaseoli* were used. The antibacterial test was conducted by using agar-disc technique. Two aquatic hyphomycetes, *T. elegans* and *T. marchalianum* showed significant antagonistic activity against test bacteria.

Keywords: Antibacterial activity, root endophytes, *Tetrachaetum elegans*, *Tetracladium marchalianum.*

INTRODUCTION

Endophytes are microorganisms that inhabit the plant tissues in their life cycle without causing any apparent harm to their host (Petrini, 1991). Their presence implies a symbiotic interaction with the host plants (Azevedo *et al*. 2003). It has been known that endophytic fungi are important sources of bioactive compounds (Strobel, 2003; Pan et al., 2008). A number of new bioactive compounds from endophytes have been recognized as potential sources of antimicrobial substances (Strobel, 2003; Li et al., 2005).

Uses of microorganisms or their metabolites to prevent diseases offer an attractive alternative or supplement to disease management without the negative impact of chemical control (Gani and Ganesh, 2009). Many of the aquatic hyphomycetes have now been reported as root endophytes (Fisher et al., 1991; Sridhar et al., 1992; Sridhar and Raviraja, 1995; Sati and Belwal, 2005; Arya and Sati, 2010). Occurrence of aquatic hyphomycetes as root endophytes in healthy plants indicates that they may have beneficial role in plant health (Bills and Polishook, 1992; Dreyfus and Chapela, 1992; Singh and Waingankar, 2005). Endophyte is also well known to constitute a valuable source of secondary metabolites for the discovery of new potential therapeutic drugs (Miller, 1995). Recently, Sati and Arya (2010 a) reported the significant effects of two aquatic hyphomycetes viz., *Heliscus lugdunensis* and *Tetrachaetum elegans* in plant growth in pot experiments.

Interaction of aquatic hyphomycetes with bacteria and terrestrial fungi has been documented previously (Chamier et al., 1984; Gulis and Suberkropp, 2003). Platas et al. (1998) and Gulis and Stephanovich (1999) demonstrated the antagonistic activity of aquatic hyphomycetes due to release of diffusible inhibitory substances. According to Gloer (1995) the secondary metabolites of aquatic hyphomycetes could result in the discovery of new natural bioactive products of medicinal and agricultural importance. 'Quinapathin' from the aero-aquatic hyphomycetes (*Helicoon richonis* (Boud.) Linder (Fisher et al., 1988; Adriaenssens et al.,1994) and 'Anguillosporal', from *Anguillospora longissima* has resulted in the discovery of new metabolite (Harrigan et al., 1995). The earlier studies on antagonistic activity of ectomycorrhizal fungi and other endophytic fungi have shown positive results (Raviraja et al., 2006; Gbolagade et al., 2007; Vaidya et al., 2005; Gulis and Stephanovich, 1999).

Intra and interspecific interaction of aquatic hyphomycetes in relation to aquatic ascomycetes and release of diffusible inhibitory substances has also been reported (Shearer and Zare-Maivan, 1988; Barlocher, 1991). Sati and Arya (2010 b) also reported the

*Corresponding author. E-mail: arya.pratibha_82@yahoo.co.in

antagonistic effects of some root endophytic aquatic hyphomycetes against different plant pathogenic fungi.

The purpose of this paper was to study the antagonistic activity of five Aquatic Hyphomycetes viz., *Heliscus lugdunensis*, *Tetrachaetum elegans*, *Tetracladium breve*, *T. marchalianum* and *T. nainitalense* isolated from the roots of riparian plants against pathogenic bacteria (plants as well as animals).

MATERIALS AND METHODS

Isolation of riparian root endophytic aquatic hyphomycetes:

Roots of healthy riparian plants were collected from ravine areas near Nainital, Kumaun Himalaya, India (29.39° N 79.45° E). Root pieces were harvested, washed in sterile water, and surface sterilized by immersing it into 0.01% sodium hypochlorite solution (3-6 min) and then in 96% ethanol (30 s) following Fisher et al. (1991). Root pieces (1-2 cm) were then placed in 2% malt extract agar Petri dishes (90 mm diameter) and incubated at 20±2°C for 10-15 days in dark. Malt extract plates were also supplemented with 0.5 g/L Streptomycin to suppress the bacterial contamination. Isolation of Aquatic Hyphomycetous fungi was done from the hyphae that grew in agar (Figure 1). The isolates were identified with the help of relevant monographs and papers (Ingold, 1975; Marvanova, 1997). *Heliscus lugdunensis* Sacc. and Therry was isolated from *Strobilanthes alatus*, *Tetrachaetum elegans* Ingold from *Pilea scripta*, *Tetracladium breve* Roldan from *Eupatorium adenophorum*, *T. marchalianum* De Wildeman from *Geranium nepalense* and *T. nainitalense* Sati & Arya from *Eupatorium adenophorum*.

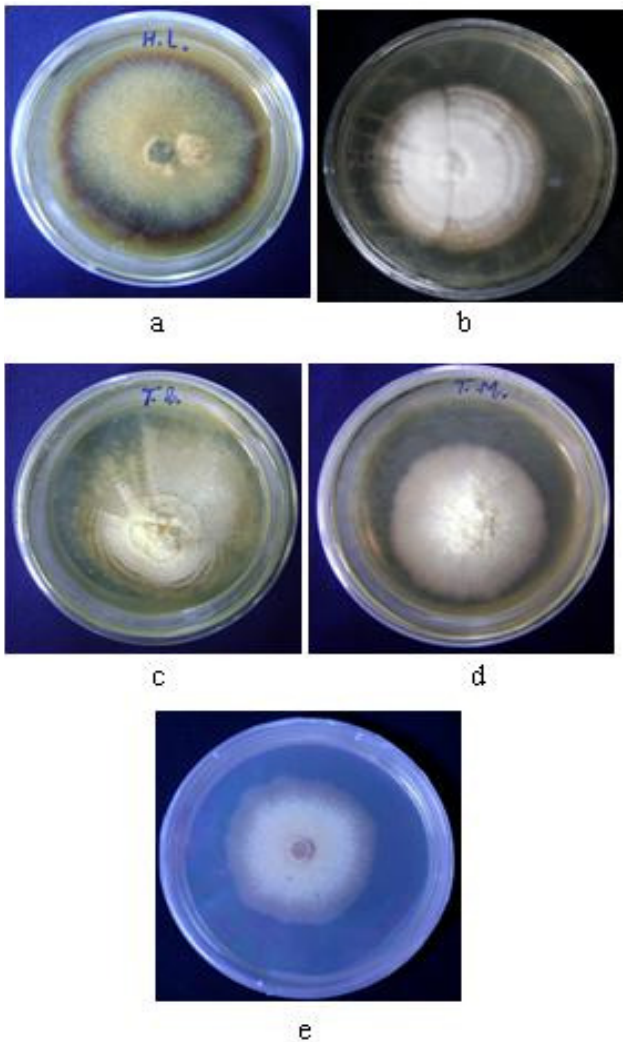

Figure 1. Culture plates of different Aquatic Hyphomycetes. a: *Heliscus lugdunensis;* b: *Tetrachaetum elegans;* c: *Tetracladium breve;* d: *T. marchalianum;* e: *T. nainitalense.*

Antagonistic activity against pathogenic bacteria

Antagonistic activity of aquatic hyphomycetes against pathogenic test bacteria viz., *Erwinia chrysanthemi*, *Xanthomonas phaseoli*, *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Escherichia coli* was tested by using agar-disc technique. Bacterial species were grown in Nutrient Agar broth (beef extract = 1 g, yeast extract (oxoid) = 2 g, peptone (bacteriological) = 5 g, sodium chloride = 5 g and distilled water = 1 l). Nutrient agar broth was seeded with test bacteria at 37 ± 2°C for 48 h to prepare the bacterial suspension.

The mycelial disks (7 mm diam.) of actively growing colonies of aquatic hyphomycetes were cut from the periphery of the culture plates and aseptically placed in the centre of the assay plates (Figures 2-3). These assay plates were prepared by 15 ml Nutrient Agar (NA) medium poured in 90 mm Petri plates. Nutrient Agar surface was seeded with bacterial suspension of *A. tumefaciens*, *E. coli*, *B. subtilis*, *E. chrysanthemi* and *X. phaseoli*. This experiment was performed in three replicates and the plates with bacterial growth without the aquatic hyphomycetes were used as control. Plates were inspected after 24 h of incubation for the presence of clear inhibitory zones around the agar disks indicating the antagonistic activity of the aquatic hyphomycetes used in this experiment.

Analysis of data

One way Analysis of Variance (ANOVA) was performed for *T. elegans* and *T. marchalianum*. *T. elegans* showed significant variations ($P < 0.001$) while *T. marchalianum* was found not significant in antagonistic activity against bacteria.

Table 1. Inhibition of bacterial growth (mm) by Aquatic Hyphomycetes (± SEM based on 3 replicates).

Fungal Isolates	Inhibition (mm) of Test Bacteria				
	A. tumefaciens **(Gram-ve)**	***E. coli*** **(Gram-ve)**	***X. phaseoli*** **(Gram-ve)**	***B. subtilis*** **(Gram +ve)**	***E. chrysanthemi*** **(Gram -ve)**
T. elegans	9.0 ± 0.00	8.33 ± 0.33	8.0 ± 1.15	8.67 ± 0.67	—
H. lugdunensis	—	—	—	—	—
T. marchalianum	8.0 ± 0.00	12 ± 2.00	9.33 ± 0.33	8.33 ± 0.88	8.67 ± 0.33
T. breve	—	—	—	—	—
T. nainitalense	—	—	—	—	—

— = No activity.

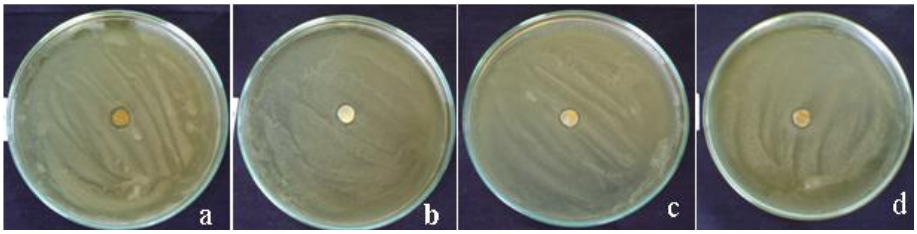

Figure 2 a-d. Inhibition of plant pathogenic bacteria by *Tetrachaetum elegans: a-A. tumefaciens; b-E.coli; c-X.phaseoli; d-B.subtils.*

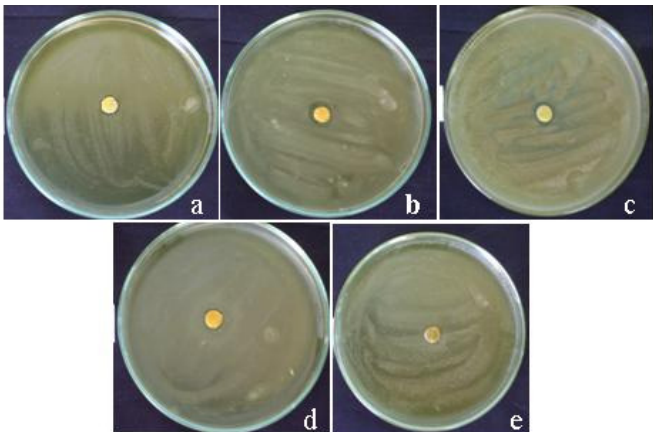

Figure 3 a-e. Inhibition of plant pathogenic bacteria by *Tetracladium marchalianum: a-A. tumefaciens; b-E.coli; c-X.phaseoli; d-B.subtils; e-E. chrysanthemi.*

RESULTS

Activity against pathogenic bacteria

The result of antagonistic activity against five pathogenic bacteria is presented in Table 1. It is evident that out of the five fungal species taken in the current study, *T. elegans* showed activity against four pathogenic bacteria namely *A. tumefaciens*, *E. coli*, *X. phaseoli* and *B. subtilis* (Figure 2, a-d) showing a clear zone of inhibition (Table 1). *T. marchalianum* showed its activity against all the test bacteria viz., *A. tumefaciens*, *E. coli*, *X. phaseoli*, *B. subtilis* and *E. chrysanthemi* (Figure 3, a-e). However, *H. lugdunensis*, *T. breve* and *T. nainitalense* showed no inhibitory effect against all the test bacteria (Table 1).

DISCUSSION

In this study the antagonistic activity of aquatic hyphomycetes against pathogenic bacteria was screened by using agar disc technique. In a test performed by Gulis and Stephanovich (1999), they observed *H. lugdunensis* as biologically inactive, showing no activity against Gram

–ve and Gram +ve bacteria, yeast and Hyphomycetes in “agar well” technique using its metabolite. It is interesting to note that in the present screening test also *H. lugdunensis* showed no antagonistic activity against pathogenic bacteria (Table 1). In the present experiment, *T. marchalianum* showed activity against all the test bacteria (Table 1). This supports the work of Gulis and Stephanovich (1999).

Gulis and Suberkropp (2003) screened 28 isolates of aquatic hyphomycetes belonging to *Alatospora acuminata* (6 isolates), *Anguillospora filiformis* (5 isolates), *Articulospora tetracladia* (10 isolates), *Tetrachaetum elegans* (4 isolates) and *Tricladium chaetocladium* (3 isolates) against 16 bacterial isolates. These aquatic hyphomycetes inhibited the bacterial growth by forming the clear zones in the bacterial loans around wells containing the fungal culture broth. In the present study *T. elegans* also showed inhibitory activity against 4 test bacteria (Figure 2, Table 1). It is worth mentioning here that all the previous studies on antibacterial activity were determined by using metabolites of endophytic aquatic hyphomycetes but the present investigation was conducted to determine the antagonistic role of these fungi by using agar disc technique for the first time.

CONCLUSION

Aquatic hyphomycetes occurring as endophytes not only help in developmental and physiological activity of plants but also antagonize their bacterial pathogens. Endophytic aquatic hyphomycetes with potential as antibacterial can be used in pharmaceutical companies for the large scale production of useful compounds.

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