Original Article

Current Botany 2017, 8: 12-16
http://scienceflora.org/journals/index.php/jp/
doi:10.19071/cb.2017.v8.3122

New root endophytic waterborne conidial fungi from Kumaun Himalayas

S. C. Sati, Richa Pathak*

Department of Botany, D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India

Received: 14-10-2016
Accepted: 31-01-2017
Published: 04-02-2017

***Address for correspondence:**
Dr. Richa Pathak, Department of Botany, D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India. E-mail: richapathak35@gmail.com

ABSTRACT

Waterborne conidial fungi occurring in living roots of healthy plant growing in the wet and ravine areas of Dogaon and Ramgarh, Nainital, Kumaun Himalayas (India) were isolated as root endophytes. A total of 15 species (*Alatospora acuminata, Anguillospora longissima, Beltrania rhombica*, *Campylospora chaetocladia, Clavariopsis aquatica, Cylindrocarpon aquaticum, Flagellospora penicilloides, Helicomyces roseus, Helicosporium lumbricoides, Pleurophragmium sonum, Pestalotiopsis submersus, Seiridium* sp., *Setosynnema isthmosporum, Tetrachaetum elegans,* and *Tetracladium marchalianum*) of endophytic aquatic fungus were recovered as root endophytes of various host plants. Three species, namely, *H. lumbricoides, P. sonum,* and *S. isthomosporum* isolated from the roots of pteridophytes and grasses were found as new root endophytes. Among the studied host plants, five plant species were also found as new hosts.

KEY WORDS: Kumaun Himalayas, new hosts, root endophyte, waterborne conidial fungi

INTRODUCTION

Endophyte is an organism, often a bacterium or fungus, that lives within a plant for at least part of its life without causing apparent disease (Bacon and White, 2000). Endophytes are ubiquitous and have been found commonly in plants. Many economically important forage and lawn grasses carry fungal endophytes (*Neotyphodium* sp.) which may improve the ability of these grasses to tolerate abiotic stresses such as drought, as well as improve their resistance to insect and herbivores. The relationship between the plant and its endophytes is one of the symbiotic nature whereby the endophytes colonize the internal tissues of the plant (Schulz *et al.*, 1999; Stone *et al.*, 2004; Strobel, 2003; Zhang *et al.*, 2006). There is growing interest in endophytes and their origins, biodiversity, endophyte–host interactions, role in ecology, and the characterization of their secondary metabolites (Arnold, 2007; Saikkonen *et al.*, 2004). Strobel *et al*. (2004) stated that there is a need for new and beneficial compounds that can provide relief against ailments and diseases. The untapped source of biological diversity represented by microbial endophytes is a promising source of novel natural products for medicine and industry. Endophytic waterborne conidial fungi may also provide products useful in biotechnology and agriculture (Bills and Polishok, 1992; Petrini, 1991; Dreyfuss and Chapela, 1992).

Ingold (1942) described the occurrence of waterborne conidial fungi in abundance on submerged decaying leaf litter and foam from freshwater stream. Waterborne conidial fungi are also recorded as root endophytes in roots of living plants including grasses and pteridophytes from wet fields near ravine areas (Waid, 1954). Many riparian plants extend their roots into streams and rivers in search of water and nutrients. Since waterborne conidial fungi are the major fungal colonizers of plant detritus in running waters, they would also infect these healthy aquatic plant roots (Fisher *et al.*, 1991; Marvanova and Fisher 1991; Marvanova *et al*., 1992). Endophytic fungi have now been reported from diverse plants including grasses, mosses, ferns, conifers, and angiosperms (Bernstein and Carroll, 1977; Carroll and Carroll, 1978; Petrini, 1986; Clay, 1989, 1990; Petrini *et al.*, 1992a, b; Fisher and Petrini, 1989; Wilson and Carroll, 1994). Some reports on these fungi are also available from Indian continent (Sridhar and Barlocher, 1992a; Raviraja *et al*., 1996; Ananda and Sridhar, 2002; Sati and Belwal, 2005; Sati *et al.*, 2006). During the course of the present study, many waterborne conidial fungi have been isolated from high-altitude Kumaun Himalayan streams. In this paper, the species of

aquatic hyphomycetes occurring as root endophyte from this area are described.

MATERIALS AND METHODS

For determining the endophytic waterborne conidial fungi, living roots of different healthy plants growing in the wet and ravine areas of two selected sites, namely Dogaon (1050 m a.s.l.) and Ramgarh (2050 m a.s.l), Nainital, Kumaun Himalayas (India), located in the outer Central Himalayas 28° 44' to 30° 49' N Latitude and 78° 45' to 81° 1'E Longitude, were collected. It comprises a temperate hilly zone, ranging 5-15°C temperature in winter while 25-35°C during summer having monsoon pattern of rainfall. The collected root samples were cut into 15-20 cm pieces with a sharp sterile knife and brought to the laboratory in sterile polythene bags for further processing.

Isolation and Culture

The collected root segments were washed under running tap water for 2-3 h and were dipped in 90% alcohol for 2-3 min for surface sterilization. After that, these root segments were rinsed with sterile water. These segments were then transferred into sterile Petri dish containing sterile water and incubated at 20°C ± 2°C for 10-15 days. For the detection of the conidia of endophytic fungi, incubated dishes were observed periodically under low power of microscope.

Some of the root segments were also immersed in 0.01% sodium hypochlorite solution for 3-6 min and then dipped in 90% ethanol up to 30 s. After surface sterilization, root segments were cut up to 1-2 cm long and rinsed with sterile water. These segments were placed into Petri dish containing 2% malt agar supplemented with antibiotics and incubated at 20°C ± 2°C for 10-15 days depending on the growth of emerging fungi. For sporulation, a small block of agar piece was cut from the periphery of the well-grown fungal colony and placed in sterile water. After 3-6 days, tremendous conidia were formed on the edge of submerged agar piece. This agar block was then examined under a microscope for different developmental stages of species, spore morphology, and method of spore release required for the identification of root endophytic fungi.

RESULT AND DISCUSSION

A total of 15 species representing 15 genera, namely, *Alatospora*, *Anguillospora*, *Beltrania*, *Campylospora*, *Claviriopsis*, *Cylindrocarpon*, *Flagellospora*, *Helicomyces*, *Helicosporium*, *Pleurophragmium*, *Pestalotiopsis*, *Seiridium*, *Setosynnema*, *Tetrachaetum*, and *Tetracladium* of waterborne conidial fungi were recovered as root endophytes (Table 1). This shows that 60% of root endophytes were recovered from a high-altitude stream of Ramgarh and 40% were recorded from a low-altitude stream of Dogaon. As evident from Table 1, 9 species were restricted to Ramgarh stream while two species were restricted to Dogaon stream. The variation in the occurrence of root endophytes may be due to the altitudinal difference and suggests that most of the root endophytes are low temperature loving.

Table 1: Root endophytes recorded from different plants growing in ravine areas of Nainital, Kumaun Himalayas

Root endophytes	Host plant	Locality	
		Ram	Dgn
A. acuminata Ingold	*C. viminea*, *E. adenophorum*	+	+
A. longissima (Sacc.and Therry) Ingold	*P. scripta*, *R. hastatus*	+	-
B. rhombica Penzing	*Elatostemma* sp.*, *Golfusia* sp.*, *P. scripta*, *R. hastatus*	+	-
C. chaetocladia Ranzoni	*C. viminea*, *E. adenophorum*, Pteridophytes, *R. alpina**, *V. wallichii*	_	+
C. aquatica de Wildeman	*C. viminea*, *E. adenophorum*, *Elatostemma* sp.*, *V. viminea**, UG	+	-
C. aquaticum (Nils.) Marv. and Descals	*C. viminea*, *P. scripta*, *V. wallichii*, *R. hastatus*, UG	-	+
F. penicillioides Ingold	*Elatostemma* sp.*, *Golfusia* sp.*, *P. scripta*, *V. wallichii*	+	+
H. roseus Link	*C. viminea*, *E. adenophorum*, *R. alpina**, *V. wallichii*	+	+
*H. lumbricoides*** Linder	*C. viminea*, Pteridophytes, *R. hastatus*, UG, *V. wallichii*	+	-
P. submersus Sati and Tiwari	*C. viminea*, *E. adenophorum*	+	-
P. sonum ** Sati and Tiwari	*P. scripta*, *V. canescens**	+	-
Seiridium sp.	*R. hastatus*, *V. wallichii*, UG	+	-
*S. isthmosporum*** Shaw and Sutton	*C. viminea*, *P. scripta*, *R. hastatus*	+	+
T. marchalianum de Wildeman	*C. viminea*, *Elatostemma* sp.*, *V. canescens**	+	-
T. elegans Ingold	UG, *V. wallichii*	+	-
Total		13	06

C. viminea: *Carpessium viminea*, *E. adenophorum*: *Eupatorium adenophorum*, ESp.: *Elatostemma* sp., GSp.: *Golfusia* sp., *P. scripta*: *Pilea scripta*, P: Pteridophytes, *R. alpina: Roscoea alpina, R. hastatus: Rumex hastatus, V. canescens: Viola canescens, V. wallichii: Valeriana wallichii, A. acuminate: Alatospora acuminate, A. longissima: Anguillospora longissima, B. rhombica: Beltrania rhombica, C. chaetocladia: Campylospora chaetocladia, C. aquatic: Claviriopsis aquatic, C. aquaticum: Cylindrocarpon aquaticum, F. penicillioides: Flagellospora penicillioides, H. roseus: Helicomyces roseus, H. lumbricoides: Helicosporium lumbricoides, P. submerses: Pestalotiopsis submerses, P. sonum: Pleurophragmium sonum, P. sonum: Pleurophragmium sonum, S. isthmosporum: Setosynnema isthmosporum, T. marchalianum: Tetracladium marchalianum, T. elegans: Tetrachaetum elegans,* UG: Unidentified grasses, locality, Dgn: Dogaon, Ram: Ramgarh, +: Present, -: Absent, *: New host plants, **: New root endophytes

All these endophytic fungi were recorded from the roots of pteridophytes and grasses (*Carpessium viminea*, *Elatostemma* sp., *Eupatorium adenophyllum, Golfusia* sp., *Pilea scripta, Rumex hastatus, Roscoea alpina, Valeriana wallichii,* and *Viola canescens).* Four plant species, namely, *Elatostemma* sp., *Golfusia* sp., *Roscea alpina,* and *Viola canescens* are found as new host (Table 1) while *Helicosporium lumbricoides, Pleurophragmium sonum,* and *Setosynnema isthomosporum* are found for the first time as root endophytes.

All the species isolated as root endophytes possess different conidial shapes such as cylindrical, helical, rounded, sigmoid as well as tetraradiate (Figures 1 and 2). It is interesting to note that the root endophytes found in the present study belong to different group of fungi as *Beltrania rhombica* and *Pestalotiopsis submersus* belong to dematacious fungi while *Helicomyces roseus* and *H. lumbricoides* belong to helicosporous fungi and other represent to triradiate or tetraradiate conidial fungi. Earlier, these fungi have also been reported from submerged leaf litter in running freshwater bodies (Ingold, 1942; Marvanova, 1975; Sati *et al*., 1989, 1992, 2002a, b), Sati and Tiwari, 1993 a, b; Sridhar and Barlocher, 1992). Sati *et al*. represented 26 species of aquatic hyphomycetes as root endophytes from Kumaun Himalayas (Sati and Belwal, 2005; Sati *et al.,* 2006, Arya and Sati, 2008; Sati *et al.,* 2009). With the addition of these three new endophytes, the total record from Kumaun Himalayas, India, reached 29 species.

Sridhar and Barlocher (1992) also reported some aquatic hyphomycetes as root endophytic fungi from the roots of *Acer spicatum, Betula papyrifera,* and *Picea glauca*. Sati and Belwal (2005) recorded 18 species as root endophytes, of which 10 species, namely, *Alatospora acuminata, A. pulchella, Acaulopage tetraceros, Anguillospora crassa, Campylospora chaetocladia, Lemonniera cornuta, L. Pseudofloscula, L. terrestris, P. submersus, and Tetrachaetum elegans* were reported for the first time. Sati *et al.* (2006) added three more species, namely, *Camposporium pellucidum, Diplocladiella scalaroides,* and *H. roseus* as root endophytes. Later, Arya and Sati (2008) also isolated five species of aquatic hyphomycetes as new root endophytes. By the addition of these three new root endophytes, i.e. *H. lumbricoides, P. sonum,* and *S. isthomosporum,* new root endophytes, i.e. *H. lumbricoides*, *P. sonum*, and *S. isthomosporum*, now there are 21 new root endophytic aquatic hyphomycetes from Kumaun Himalayas.

The endophytes have different biological niche with the host plant, and the study of root endophytes has also been suggested for the detection of many new products which are useful in biotechnology and agriculture (Bills and Polishok, 1992, Petrini, 1991; Dreyfuss and Chapela, 1992). Reports are available that the antimicrobial

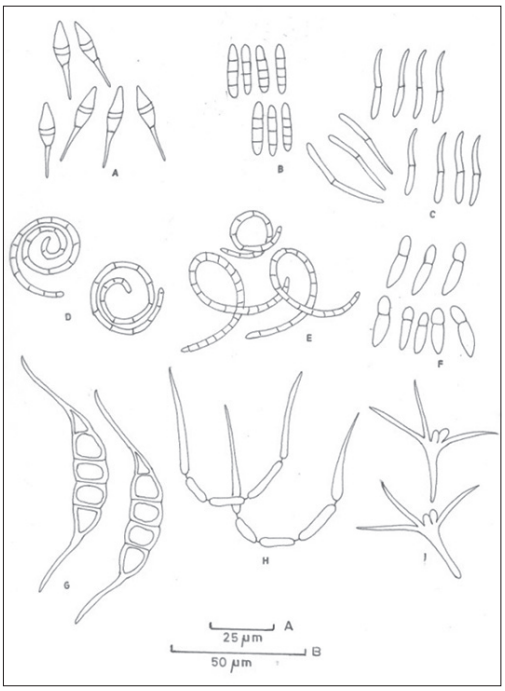

Figure 1: Camera Lucida drawings of root endophytes, A - *Beltrania rhombica,* B - *Cylindrocarpon aquaticum,* C - *Flagellospora penicilloides,* D - *Helicomyces roseus,* E - *Helicosporium lumbricoides*, F - *Pleurophragmium sonum,* G - *Seiridium* sp., H - *Setosynnema isthmosporum,* and I - *Tetracladium marchalianum* (G, H are in scale A, rest are in scale B)

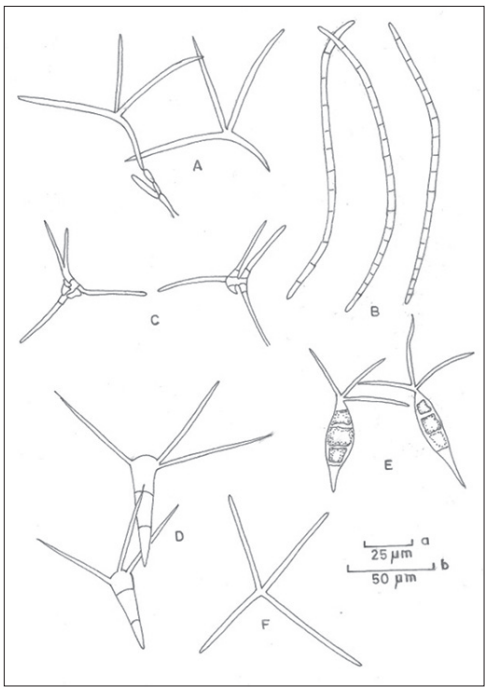

Figure 2: A - *Alatospora acuminata*, B - *Anguillospora longissima,* C - *Campylospora chaetocladia,* D - *Claviriopsis aquatica,* E - *Pestalotiopsis submersus,* and F - *Tetrachaetum elegans* (A is in scale b, rest are in scale a)

bioactive compounds are present in endophytic aquatic fungi. Quinaphthin was obtained from *Helicoon richonis* (Fisher *et al.*, 1988) and anguillosporal was recorded from *Anguillospora longissima* (Harrigan *et al.*, 1995).

The antimicrobial activity of some root endophytic fungi has been studied by Arya and Sati (2011) and they found good antifungal and antibacterial activities of these root endophytic fungi. Recently, Singh and Sati (2014) studied the antimicrobial activity of a root endophytic fungus *Cylindrocarpon aquaticum* and reported that it can inhibit the growth of some pathogenic bacteria. Thus, the antibacterial potentiality of root endophytic fungi can be used in pharmaceutical companies for the production of useful compounds.

Nowadays, more attention is being given to the biology of endophytes as these microorganisms have now been noticed for their potentiality as the source of new bioactive compounds (Strobel, 2003). Besides the use of endophytes for new bioactive compounds required in the preparation of drugs, these may be also useful in industrial or agricultural applications.

ACKNOWLEDGMENTS

This work is supported by a grant from SAP (DRS) UGC, New Delhi, India.

REFERENCES

Ananda K, Sridhar KR. Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. Can J Microbiol 2002;48:871-8.

Arnold AE. Understanding the diversity of foliar endophytic fungi: Progress, challenges, and frontiers. Fungal Biol Rev 2007;21:51-66.

Arya P, Sati SC. Evaluation of endophytic hyphomycetes for their antagonistic activity against pathogenic bacteria. Int J Microbiol 2011;2:343-7.

Arya P, Sati SC. Occurrence of aquatic hyphomycetes as a root endophyte in some riparian plants roots of Nainital, Kumaun Himalaya. Edited volume published by UCOST, Dehradoon, 2008.

Bacon CW, White JF. Microbial Endophytes. New York: Marcel Deker Inc; 2000.

Bernstein ME, Carroll GC. Internal fungi in old growth Douglas fir foliage. Can J Bot 1977;55:644-53.

Bills GE, Polishook JD. Recovery of endophytic fungi from *Chamaecyparis thyoides*. Sydowia 1992;44:1-12.

Carroll GC, Carroll FE. Studies on incidence of coniferous needle endophyte in the Pacific Northwest. Can J Bot 1978;56:3034-43.

Clay K. Clavicipitaceous endophytes of grasses: Their potential as biocontrol agents. Mycol Res 1989;92:1-12.

Clay K. Fungal endophytes of grasses. Annu Rev Ecol Syst 1990;21:275-97.

Dreyfuss M, Chapela IH. The potential of fungi in discovery of novel, low molecular weight pharmaceuticals. Discovery of Novel Natural Products with Therapeutic Potential. London: Butterworth Publications, Biotechnology Series; 1992.

Fisher PJ, Anson AE, Webster J. Quinaphthin, a new antibiotic, produced by *Helicoon richonis*. Trans Br Mycol Soc 1988;90:499-502.

Fisher PJ, Petrini O, Webster J. Aquatic hyphomycetes and other fungi living aquatic and terrestrial roots of *Alnus glutinosa*. Mycol Res 1991;95:543-7.

Fisher PJ, Petrini O. Fungal saprobes and pathogens as endophyte of Rice (*Oryzae sativa*). New Phytol 1992;120:137-43.

Fisher PJ, Petrini O. Two aquatic hyphomycetes as endophytes in *Alnus glutinosa* roots. Mycol Res 1989;92:367-68.

Harrigan GG, Armentrout BL, Gloer JB, Shearer CA. New bioactive natural products from two *Anguillopsora* species. In: The VI International Marine Symposium (Incorporating Freshwater Mycology). A Meeting of the British Mycological Society. 8-15 July, 1995. Programme and Abstracts. England: University of Portsmouth; 1995. p. 135.

Ingold CT. Aquatic hyphomycetes of decaying alder leaves. Trans Br Mycol Soc 1942;25:339-417.

Marvanova L, Fisher PJ, Aimer R, Segedin BC. A new *Filosporella* from alder roots and foam water. Nova Hedwigia 1992;54:151-8.

Marvanova L, Fisher PJ. A new endophytic hyphomycetes from alder roots. Nova Hedwigia 1991;52:33-7.

Marvanova L. Concerning *Gyoerffyella* Kol. Trans Br Mycol Soc 1975;65:555-65.

Petrini O, Fisher PJ, Petrini LF. Fungal endophyte of bracken (*Pteridium aquilinum*), with some reflections on their use in biological control. Sydowia 1992b;4:282-93.

Petrini O, Sieber TN, Toti L, Viret O. Ecology, metabolite production and substrate utilization in endophytic fungi. Nat Toxins 1992a;1:185-96.

Petrini O. Fungal endophytes of tree leaves. In: Andrews JA, Hirano SS, editors. Microbial Ecology of Leaves. New York: Springer; 1991. p. 179-97.

Petrini O. Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, den Henvel JV, editors. Microbiology of the Phyllosphere. Cambridge: Cambridge University; 1986. p. 175-87.

Raviraja NS, Sridhar KR, Barlocher F. Endophytic aquatic hyphomycetes of roots of plantation crops and ferns from India. Sydowia 1996;48:152-60.

Saikkonen K, Wali P, Helander M, Faeth SH. Evolution of

endophyte-plant symbioses. Trends Plant Sci 2004;9:275-80.
Sati SC, Arya P, Belwal M. *Tetracladium nainitalense* sp. nov. a root endophyte from kumaun Himalaya, India. Mycologia 2009;101;692-5.
Sati SC, Belwal M. Aquatic hyphomycetes as endophyte of riparian plant roots. Mycologia 2005;97:45-9.
Sati SC, Mer GS, Tiwari N. Occurrence of water borne conidial fungi on *Pinus roxburghii* needles. Curr Sci 1989;58:918-9.
Sati SC, Pargaien N, Belwal M. Three species of aquatic hyphomycetes as new root endophytes of temperate forest plants. Nat Acad Sci Lett 2006;29:9-10.
Sati SC, Tiwari N, Belwal M. Conidial aquatic fungi of Nainital, Kumaun Himalaya, India. Mycotaxon 2002b;81:445-55.
Sati SC, Tiwari N, Belwal M. Species diversity of water borne conidial fungi in running freshwater bodies of Kumaun Himalaya, Uttaranchal. In: Tiwari SC, Sharma GD, editors. Microbial Diversity, Status and Potential Applications. New Delhi, India: Today and Tomorrow Printer and Publisher; 2002a. p. 26-35.
Sati SC, Tiwari N, Mer GS. Colonization of aquatic hyphomycetes on pine needles. Indian Phytopathol 1992;45:106-7.
Sati SC, Tiwari N. A new species of *Pestalotiopsis* on submerged leaf litter. Nova Hedwigia 1993a;56:543-7.
Sati SC, Tiwari N. Ingoldian Aquatic Hyphomycetes from Two Temperate Fresh Water Streams of Nainital, in Kumaun Himalayas, India, No. 2382. Tokyo, Japan: Abstract in XV International Botanical Congress; 1993b. p. 324.
Schulz B, Rommert AK, Dammann U, Aust HJ, Strack D. The endophyte-host interaction: A balanced antagonism? Mycol Res 1999;103:1275-83.
Singh L, Sati SC. Bio-prospecting of root endophytic aquatic fungus *Cylindrocarpon aquaticum* for its antibiotic potential. J Pure Appl Microbiol 2014;8:4903-8.
Sridhar KR, Barlocher F. Aquatic hyphomycetes in spruce roots. Mycologia 1992a;84:580-4.
Stone JK, Polishook JD, White JF. Endophytic fungi In: Mueller GM, Bills GF, Foster MS, editors. Biodiversity of Fungi. Boston: Elsevier Academic Press; 2004. p. 241-70.
Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J Nat Prod 2004;67:257-68.
Strobel GA. Endophytes as sources of bioactive products. Microbes Infect 2003;5:535-44.
Waid JS. Occurrence of aquatic hyphomycetes upon the roots surface of beech grown in woodland soil. Trans Br Mycol Soc 1954;37:420-1.
Wilson D, Carroll GC. Infection studies of *Discula quercina*, an endophyte of *Quercus garryana*. Mycologia 1994;86:635-47.
Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. Nat Prod Rep 2006;23:753-71.