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Endophytic fungi associated with cacti in Arizona[1]

Trichur S. SURYANARAYANAN[1], Sally K. WITTLINGER[2] and Stanley H. FAETH[2]

[1] *PG & Research Department of Botany, Ramakrishna Mission Vivekananda College, Chennai 600 004, India.*
[2] *School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA.*
E-mail: drtss@vsnl.com

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21 cactus species occurring in various localities within Arizona were screened for the presence of fungal endophytes. 900 endophyte isolates belonging to 22 fungal species were isolated. *Cylindropuntia fulgida* had the maximum endophyte species diversity, while *C. ramosissima* harboured the maximum number of endophyte isolates. *Alternaria* sp., *Aureobasidium pullulans*, and *Phoma* spp. were isolated from several cactus species. The diversity of the endophyte assemblages was low and no host specificity among endophytes was observed. However, the frequencies of colonization of the few endophyte species recovered were high and comparable to those reported for tropical plant hosts. Species of *Colletotrichum*, *Phomopsis*, and *Phyllosticta*, which are commonly isolated as endophytes from plants of more mesic habitats, were absent from these cacti.

INTRODUCTION

Fungi that cause asymptomatic infections in aerial tissues of many different groups of plants have been called fungal endophytes. The majority of fungal endophytes are ascomycetes and their anamorphs. Basidiomycetes and zygomycetes rarely are isolated as endophytes (Petrini 1986). Recent studies have shown that some endophytic fungi are neither incidental residents nor merely latent pathogens of plant hosts (Ganley, Brunsfeld & Newcombe 2004). They may protect the plant from insect pests (Azevedo *et al.* 2000), fungal pathogens (Arnold *et al.* 2003, Dingle & McGee 2003), or increase host fitness in harsh environments (Redman *et al.* 2002), and possibly play a role in litter degradation (Aoki, Tokumasu & Tubaki 1990, Kumaresan & Suryanarayanan 2002).

There have been numerous studies on endophyte communities of temperate plant hosts (Bills 1996), and recently, several tropical plants have been investigated for their endophyte associations (Arnold, Maynard & Gilbert 2001, Cannon & Simmons 2002, Suryanarayanan, Murali & Venkatesan 2002, Suryanarayanan, Venkatesan & Murali 2003). However, very few plants growing in extreme or harsh habitats have been screened for fungal endophytes. These include marine angiosperms (Alva *et al.* 2002, Devarajan, Suryanarayanan & Geetha 2002) and halophytes (Fisher & Petrini 1987, Peláez *et al.* 1998, Suryanarayanan & Kumaresan 2000). There has not been a detailed study on endophytes of desert plants such as cacti (Bills 1996), with the exception of a preliminary study reporting the occurrence of endophytes in a single cactus species of *Opuntia* growing in Australia (Fisher *et al.* 1994). Desert plants have not been analysed for their endophyte associations perhaps because endophyte infections are thought to decrease rapidly with decreasing relative humidity and rainfall (Bills 1996). Cacti are native to the New World and are an important food source for wild animals; they are also used as forage, fodder, vegetable and human dietary supplements (Nobel 2002). We examined the endophyte communities of various cactus species growing in Arizona in order to compare the species composition and diversity to that reported for endophytes from more mesic habitats.

MATERIALS AND METHODS

21 cactus species collected from six different locations in Arizona were screened for their endophyte assemblages (Table 1). The collections were made during May and June 2004.

Stem tissue was collected for analysis from each cactus species at 0.6–1 m above the ground. 50 segments

[1] Dedicated to John Webster on the occasion of his 80th birthday.

Table 1. Cactus species studied for their endophyte assemblages.

Species	Code	Location[a]	County
Carnegiea gigantea	AC	A	Yavapai
Opuntia sp.	AO	A	Yavapai
Consolea sp.	BC	B	Maricopa
Cylindropuntia arbuscula	BC1	B	Maricopa
C. ramosissima	BC2	B	Maricopa
C. whipplei	BC3	B	Maricopa
O. engelmannii	BO	B	Maricopa
C. acanthocarpa	CC1	C	Pinal
C. bigelovii	CC2	C	Pinal
C. fulgida	CC3	C	Pinal
Echinocereus fasciculatis	CE	C	Pinal
Mammillaria viridiflora	CM	C	Pinal
C. californica	DC1	D	Yavapai
C. echinocarpa	DC2	D	Yavapai
C. imbricata	DC3	D	Yavapai
C. multigeniculata	DC4	D	Yavapai
C. versicolor	DC5	D	Yavapai
Cylindropuntia sp.	EC	E	Maricopa
O. ficus-indica	EO1	E	Maricopa
Opuntia sp.	EO2	E	Maricopa
Echinocereus engelmannii	FE	F	Yavapai

[a] A, Highway 17-Bumblebee Exit (34° 09.5′ N; 112° 09.8′ W; alt 800 m); B, Root Gorelick's Garden (33° 24.6′ N; 111° 56.0′ W; alt 360 m); C, Tonto National Forest (33° 16.6′ N; 111° 16.6′ W; alt 625 m); D, Mark Baker's Land (34° 46.5′ N; 112° 26.4′ W; alt 1440 m); E, Sally Wittlinger's Garden (33° 25.0′ N; 111° 56.7′ W; alt 365 m); and F, Highway 17-Sedona Exit (34° 41.6′ N; 111° 45.1′ W; alt 1155 m).

(each a wedge of 0.5 cm^2 of the epidermal tissue along with about 0.3–0.5 cm of internal tissue and mucilage) were cut from the stem tissue of each host individual and screened for endophytes (Fisher *et al.* 1994). The segments, bereft of spines, were surface sterilized by immersing them in 75% ethanol for 20 s followed by treatment with sodium hypochlorite (4% available chlorine) for 90 s; they were then washed in sterile water and plated on potato dextrose agar (PDA) medium amended with 0.5 $g\,l^{-1}$ streptomycin sulfate (Sigma Biochemicals, Sigma-Aldrich, St Louis, Mo). Sterilized tissue segments were pressed on to the surface of PDA medium to check the efficacy of surface sterilization procedure. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective in removing the surface fungi (Schulz *et al.* 1993). Petri dishes were incubated in a light chamber at 27°C and observed for 10 d. The cultures are deposited in the School of Life Sciences, Arizona State University, USA.

All the statistical analyses were performed for the number of isolates obtained from each host. Fisher's α was calculated using the method of Fisher, Corbet & Williams (1943). The Relative Importance values were calculated as described by Ludwig & Reynolds (1988), with the dominant endophyte of an assemblage being assigned a value of 100% and computing the relative importance of each additional taxon as a percentage of the most abundant taxa.

RESULTS AND DISCUSSION

In all, 1050 segments of stem tissues from 21 species of cacti were screened for endophytes; 900 endophyte isolates belonging to 22 fungal species were isolated from these segments (Table 2). A maximum of seven species were isolated from *Carnegiea gigantea*, whereas *Cylindropuntia ramosissima* yielded a maximum of 114 isolates. *C. fulgida* showed the maximum endophyte diversity, with a value of 2.3 (Table 2). Seven of the cactus species had a low diversity value for their endophyte assemblages (Table 2). In contrast, the leaves of some tropical plant hosts may typically harbour about 20 species of endophytes (Lodge, Fisher & Sutton 1996, Arnold *et al.* 2000, Kumaresan & Suryanarayanan 2001, Suryanarayanan *et al.* 2003). In the present study, both the number of endophyte species isolated from cacti (2 to 7) and the species diversity of the endophyte assemblages of cacti were low compared with the foliage of trees of tropical forests (Suryanarayanan *et al.* 2002, 2003). This difference likely reflects both the different environmental conditions of the two habitats, as well as structural adaptations of the cacti to reduce water loss. In the majority of the hosts examined to date, endophyte colonization has been positively correlated with humidity and precipitation (Bills 1996). This is especially true of plants in arid environments (Faeth & Hammon 1997). Although the desert is a xeric habitat, precipitation may be seasonally abundant, providing regular periods in which conditions are favorable for spore dispersal and infection to occur. Leaves of several mesophytic plant hosts have been shown to harbour fewer endophytes in the dry season than in the wet season (Rodrigues 1994, Wilson & Carroll 1994, Suryanarayanan, Kumaresan & Johnson 1998, Suryanarayanan & Thennarasan 2004). Furthermore, the thick and waxy cuticle and the low frequency of stomata in cacti, adaptations to reduce evaporative water loss, may pose additional barriers that inhibit infection by parasitic fungi (Zimmermann & Granata 2002). Such structural features may also reduce the colonization of cacti by some endophyte species resulting in lesser endophyte diversity. Finally, the widely-spaced distribution of cacti, and of desert plants in general, relative to more mesic plant communities may serve to restrict dissemination of spores from plant to plant. Suryanarayanan *et al.* (2003) showed that the endophyte diversity in a tropical forest in southern India was positively correlated with host abundance.

Although the number of endophyte species isolated from cacti was relatively low, their frequency of colonization (i.e. the proportion of sampled segments colonized) was high and comparable to that reported for some tropical plant hosts (Suryanarayanan *et al.* 2002). Thus, while it appears that cacti harbour only a few species of endophytic fungi, these few species occur at relatively high densities. Endophytes such as *Alternaria* sp., *Aureobasidium pullulans*, *Ascochyta* sp., and *Phoma* spp. were isolated from different species of

Table 2. Number of isolates, species and species diversity of fungal endophytes recovered from different cactus species. For host codes see Table 1.

	AC	AO	BC	BC1	BC2	BC3	BO	CC1	CC2	CC3	CE	CM	DC1	DC2	DC3	DC4	DC5	EC	EO1	EO2	FE
Alternaria sp.			2	8	38	8	4	10		2	2	10	32	48	20	46	10	8			2
Ascochyta sp.				4				2		2	2							10			4
Aureobasidium pullulans	10	6		4	18	2	2	2		8	6		4	14	42	6	10				
Chaetomium sp.																			8	10	
Cladosporium sp.													2				2	2		4	
Coniothyrium sp.								8		8											
Drechslera sp.			2																		
Epicoccum nigrum	4																				
Fusarium sp.						24															
Nigrospora oryzae						4															
Pestalotiopsis sp.																		4			
Phoma sp. 1												2	16			38	8				8
Phoma sp. 2	4												8	10	6		12	8			
Phoma sp. 3	36																	10			4
Phoma sp. 4	2																				
Phoma sp. 5		2	24	22	24	6	38														
Phoma sp. 6				2	34	18	2														
Stemphylium sp.							6														
Ulocladium sp.									2												
Sterile form 1	2		4				2		2	4									4	6	2
Sterile form 2	2																				
Sterile form 3										4	6	20									
Species	7	2	4	5	4	6	6	4	2	6	4	3	5	3	3	3	5	6	2	3	5
Isolates	60	8	32	40	114	62	54	22	4	28	16	32	62	72	68	90	42	42	12	20	20
Fisher's Alpha	2.1	0.9	1.2	1.5	0.8	1.6	1.7	1.4	1.6	2.3	1.7	0.8	1.3	0.6	0.6	0.6	1.5	1.9	0.7	1	2.1

Table 3. Relative importance values for the two dominant endophytes isolated from different hosts. For host codes see Table 1.

Code	Dominant endophyte	RI	II Dominant endophyte	RI
AC	*Phoma* sp. 3	100	*Aureobasidium pullulans*	28
AO	*Aureobasidium pullulans*	100	*Phoma* sp. 5	33
BC	*Phoma* sp. 5	100	Sterile form 1	17
BC1	*Phoma* sp. 4	100	*Alternaria* sp.	36
BC2	*Alternaria* sp.	100	*Phoma* sp. 6	89
BC3	*Fusarium* sp.	100	*Phoma* sp. 6	67
BO	*Phoma* sp. 5	100	*Stemphylium* sp.	16
CC1	*Alternaria* sp.	100	*Coniothyrium* sp.	80
CC3	*Aureobasidium pullulans*, *Coniothyrium* sp.	100	Sterile form 1, Sterile form 3	50
CE	*Aureobasidium pullulans*, Sterile form 3	100	*Alternaria* sp., *Ascochyta* sp.	33
CM	Sterile form 3	100	*Alternaria* sp.	50
DC1	*Alternaria* sp.	100	*Phoma* sp. 1	50
DC2	*Alternaria* sp.	100	*Aureobasidium pullulans*	29
DC3	*Aureobasidium pullulans*	100	*Alternaria* sp.	48
DC4	*Alternaria* sp.	100	*Phoma* sp. 1	83
DC5	*Phoma* sp. 2	100	*Alternaria* sp., *Aureobasidium pullulans*	83
EC	*Ascochyta* sp., *Phoma* sp. 3	100	*Alternaria* sp., *Phoma* sp. 2	80
EO1	*Chaetomium* sp.	100	Sterile form 1	50
EO2	*Chaetomium* sp.	100	Sterile form 1	60
FE	*Phoma* sp. 1	100	*Ascochyta* sp., *Phoma* sp. 3	50

cacti and also dominated the endophyte assemblage of many species of cacti (Table 2). It is pertinent that in only seven of the 21 cactus species studied, the second most dominant fungus of the endophyte assemblage had a relative importance of >50% (Table 3). This indicates that the endophyte assemblages of the majority of cacti are dominated by a single species.

Many of the fungal genera we isolated from various species of Arizona cacti also were reported to be endophytic in *Opuntia stricta* growing in Australia (Fisher *et al.* 1994). Furthermore, plurivorous endophyte genera commonly reported from plants of more mesic habitats, such as *Colletotrichum*, *Phomopsis*, and *Phyllosticta*, were not found in cacti of Arizona and Australia (Fisher *et al.* 1994). We did not observe any host specificity among the endophytes the Arizona cacti. Although some endophytes such as *Epicoccum nigrum*., *Fusarium* sp., *Phoma* sp. 4, *Stemphylium* sp., and *Nigrospora oryzae* were exclusive to some hosts (Table 2), they may not be host-specific as they are ubiquitous fungi, and excepting *Stemphylium* sp., the other fungi are isolated as common endophytes (Bills & Polishook 1992, Suryanaryanan *et al.* 2002). These results suggest that the endophyte assemblages of the plants of arid zones are low in diversity and some fungal species appear cosmopolitan in distribution. Our results, coupled with those of Fisher *et al.* (1994), strengthens the view that endophyte colonization depends more on the availability of inoculum and host plants than on the geographical location of the host plants (Fisher *et al.* 1994, Suryanarayanan & Kumaresan 2000).

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Corresponding Editor: J. K. Stone