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Interspecific Interactions among Tropical and Subtropical Freshwater Fungi

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Abstract

Twenty-seven species of tropical and subtropical fungi isolated from freshwater were examined for evidence of interspecific interactions, which are important in determining the ecological roles of fungi. Evidence for interspecific interactions was examined by inoculating paired fungi 25 mm apart on the surface of agar plates. The antagonistic activities were different among different isolates and even between isolates of the same species, for example, *Ophioceras dolichostomum* isolated from different origins. *Pseudohalonectria longirostrum* and *Kirschsteiniothelia elaterascus,* which produced pigment in culture, were strongly inhibitory species. Several aquatic hyphomycetes seems to be less competitive and less likely to produce antagonistic substances. Competitive abilities were also influenced by the range of enzymes that a fungus produced. For example, *Verticillium* sp. and *Diaporthe* sp., which produced only one or two kinds of enzyme, were found to exhibit weak competitive abilities and were easily replaced. The results of competition experiments also showed that slow-extending fungi (e.g., *Pseudohalonectria longirostrum* and *Kirschsteiniothelia elaterascus*) were more competitive than early fast-extending fungi (e.g., *Ophioceras dolichostomum* and *Nectria haematococca*).

Introduction

Fungal interactions are important in determining the mode and pattern of growth of fungi in decaying wood and hence are likely to affect the organization, composition, and patterns of fungal colonization [16, 24, 25]. Interactions have been studied by a variety of techniques, including microscopic observations of hyphal interactions [3, 10], tests of inhibition on hyphal growth [1, 18], and examination of the reaction types for calculation of antagonism indices [11, 21, 26].

Interactions between fungal species have been reported in fungi that are found on different substrates, such as coprophilous fungi on dung [5, 9, 25]; fungi on agriculture products [13, 26]; basidiomycetes on wood [8]; marine fungi on submerged wood [11, 21]; and freshwater fungi on submerged wood [1, 18]. Since antifungal antibiotics may be produced by antagonistic fungi in some competitive interactions, studies of interaction patterns may produce effective

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strategies for biological control [2, 4], or may lead to new fungal metabolites [20, 23]. Fungal metabolites such as scytalidin from *Scytalidium* sp. [23] and culmorin from *Leptosphaeria oraemaris* [21] have been discovered.

Basidiomycetes, one of the most successful groups of wood inhibiting fungi in terrestrial habitats, have been examined extensively for their interaction abilities [7, 15, 22]. Previous studies have concentrated mainly on terrestrial basidiomycetes, as they were better known taxonomically. Basidiomycetes, however, rarely occur on submerged wood and their ecological importance in aquatic ecosystems is insignificant [6]. Ascomycetes and hyphomycetes appear to be more important in the decomposition of submerged wood. Cultural studies have been carried out on a small number of lignicolous marine fungi [11, 21] and freshwater fungi [1, 18]. Evidence for interference competition amongst some marine fungi have been observed and antibiosis has been involved where diffusible antibiotic compounds were produced by fungi [21]. The hyphal interactions of some freshwater fungi have been examined in pairs on agar plates, and these fungi were ranked according to their ability to inhibit and to resist inhibition [18]. Eight of 25 species produced zones of inhibition indicating that they are able to produce diffusible antifungal substances *in vitro* [18]. This suggests that competitive interactions are important determinants of fungal community structure in submerged wood. The main objective of this study was to determine the competitive activities among isolates of tropical and subtropical wood inhabiting fungi. The antagonistic activities were evaluated by interaction indices and indices of antagonism.

Materials and Methods

In Vitro Interaction Studies

Twenty-seven species of tropical and subtropical fungi isolated from freshwater were examined for evidence of interference competition (Table 2). All of them were isolated from submerged wood by single spore isolation and were able to grow on corn meal agar (Difco). The cultures were kept in the University of Hong Kong Culture Collection (HKUCC). Evaluation of antagonism in culture was calculated according to the method described by Asthana and Shearer [18]. Agar disks (5 mm diameter), from the leading edge of an actively growing colony of each fungus, were inoculated onto the agar at opposite sides of 90 mm diameter Petri dishes. Agar disks of opposing species were placed 25 mm apart from the challenge species. Isolates were categorized as slow, moderate, and fast growing fungi, according to their radial growth rates. Inoculum disks of slow growers were placed on agar 1 week in advance of moderate growers; moderate growers were placed on agar 4 days in advance of fast growers; and species with similar growth rates were placed on the agar simultaneously. Triplicate plates with fungal isolates in all possible combinations were inoculated. In other studies, fungi were found to attain maximal growth rates on CMA at 25°C in the dark; therefore, these were the incubation conditions [29].

Table 1. Type of interactions and numerical values assigned [modified from 18, 21][a]

Categories	Reaction types	Points
A	Hyphae of response and challenge species intermingling freely with little or no reduction in hyphal growth rates of either species.	0
B_1	Response species overgrows challenge species. Growth rate of challenge species reduced.	1
B_2	Response species grows up to and around challenge species.	1
C	Colonies of both species approach each other until almost in contact when growth of both species ceases.	2
D	Mutual inhibition at a distance between response and challenge species.	3
E_1	Challenge species overgrows response species. Growth rate of response species reduced.	4
E_2	Challenge species grows up to and around response species.	4

[a] The indices of antagonism (IA) were calculated for each isolate by summing the assigned points for each category:

$$IA = B_1(n \times 1) + B_2(n \times 1) + C(n \times 2) + D(n \times 3) + E_1(n \times 4) + E_2(n \times 4),$$

where n = number of times a fungus shows this category of antagonism.

Inhibition Ability of Each Isolate

Reactions were recorded after 2 weeks, or when the colonies met, or when one of the colonies had fully grown to the edge of the Petri dish. Radial growth of the response species both toward and away from each other were measured on a line through the center of the Petri dish. The overall ability of each challenge species to inhibit response species was determined by summing the percentage inhibition of growth of all response species paired with that challenge species.

Indices of Antagonism

Antagonistic activities of each of the isolates were also evaluated by indices of antagonism. The type of hyphal interaction was recorded using a key as described by Shearer and Zare-Maivan [18] and numerical values were assigned as in Table 1. The indices of an-

Table 2. The inhibition ability and the index of antagonism of each isolate

Symbol	Isolate	Collection site[a]	Σ % of inhibition[b]	Categories							IA[c]
				A	B1	B2	C	D	E1	E2	
A1	*Aeroaquatic* sp.—HKUCC#236	1	1201	18	1	0	5	2	1	0	21
A2	*Aniptodera lignalitis*—HKUCC#812	7	1108	18	2	1	0	4	2	0	23
A3	*Annulatascus velatisporus*—HKUCC#808	1	986	15	2	0	3	4	2	1	32
B1	*Brachydesmiella anthostomelloidea*—HKUCC#175	3	1308	9	6	1	4	2	2	3	41
B2	*Byssothecium* sp.—HKUCC#195	3	1009	15	3	3	2	1	3	0	25
C1	*Camposporidium antennatum*—HKUCC#527	4	1338	13	3	0	3	4	2	2	37
C2	*Cateractispora aquatica* I—HKUCC#794	7	1288	11	8	2	2	2	0	2	28
C3	*Cateractispora aquatica* II—HKUCC#795	7	1251	11	7	3	4	0	1	1	26
C4	*Chaetosphaeria* sp.—HKUCC#377	7	956	5	8	3	3	1	7	0	48
D1	*Dactylaria* sp.—HKUCC#386	4	694	12	5	3	0	2	5	0	34
D2	*Diaporthe* sp.—HKUCC#563	2	809	12	6	1	3	2	3	0	31
D3	*Dictyosporium* sp.—HKUCC#753	4	1284	8	2	0	6	3	6	1	51
D4	*Didymosphaeria* sp.—HKUCC#182	3	1107	10	3	0	7	3	3	1	42
K1	*Kionochaeta australiensis*—HKUCC#172	3	1405	2	8	2	5	2	6	2	58
K2	*Kirschsteiniothelia elaterascus*—HKUCC#390	4	1050	10	5	2	0	6	4	0	41
M1	*Massarina bipolaris*—HKUCC#167	3	1309	16	3	0	3	3	2	0	26
N1	*Nais aquatica*—HKUCC#177	7	1374	3	6	3	5	3	5	2	56
N2	*Nectria haematococca*—HKUCC#555	7	687	9	6	4	1	2	3	2	38
O1	*Ophioceras dolichostomum* I—HKUCC#562	2	1127	10	5	0	4	4	3	1	41
O2	*Ophioceras dolichostomum* II—HKUCC#564	6	1136	8	6	1	3	4	4	1	45
O3	*Ophioceras dolichostomum* III—HKUCC#537	5	374	11	4	2	5	0	5	0	36
O4	*Ophioceras dolichostomum* IV—HKUCC#461	7	1059	9	8	3	3	2	2	0	31
P1	*Penicillium* sp.—HKUCC#153	5	857	11	5	0	6	3	1	1	34
P2	*Pseudohalonectria longirostrum*—HKUCC#1846	7	732	7	0	3	5	6	4	2	55
T1	*Tiaroporella paludosa*—HKUCC#539	5	1347	2	14	4	0	5	2	0	41
V1	*Verticillium* sp. I—HKUCC#541	6	947	11	6	0	1	1	7	1	43
V2	*Verticillium* sp. II—HKUCC#700	7	713	9	8	3	5	1	1	0	28

HKUCC# = The Culture Collection number of the University of Hong Kong.
[a] Collection site: 1 = CowBay stream, Australia, 2 = Lipur Hertary stream, Malaysia, 3 = Mt. Lewis stream, Australia, 4 = Mt. Makiling stream, Philippines, 5 = Palmiet River, South Africa, 6 = Sungai Belaong, Brunei, 7 = Tai Po Kau stream, Hong Kong.
[b] Σ % of inhibition represents the ability of each isolate to inhibit other species. The higher the value, the more inhibitory it is.
[c] IA represents index of antagonism. The higher the IA, the stronger the ability to compete and dominate a range of competitors.

tagonism reflect the ability of an individual fungus to compete and dominate a range of competitors.

Results

Inhibition Ability

The abilities of the freshwater fungi tested in the cultural studies to inhibit other isolates are shown in Table 2. Generally, the greater value of the percentage of inhibition of a fungus, the more strongly competitive it is. *Brachydesmiella anthostomelloidea, Kionochaeta australiensis,* and *Kirschsteiniothelia elaterascus* were the most inhibitory species. *Ophioceras dolichostomum* IV and *Verticillium* sp. II were the least inhibitory. The competitive ability of the same species from different origins varied. For example, all *Ophioceras dolichostomum* were weakly inhibitory species, but *Ophioceras dolichostomum* I collected in Malaysia was much stronger than *Ophioceras dolichostomum* IV collected in Hong Kong.

Indices of Antagonism

The inhibition ability of each isolate was also evaluated by their reaction types with other fungi (Table 2). The indices of antagonism reflect the ability of an individual fungus to compete and dominate a range of competitors. A lower index of antagonism indicated that the species was weaker in terms of its inhibition of other species. With the exception of *Massarina bipolaris, Brachydesmiella anthostomelloidea,* and *Dactylaria* sp., all species were self-inhibiting. Species with high indices of antagonism (e.g., *Pseudohalonectria longiro-*

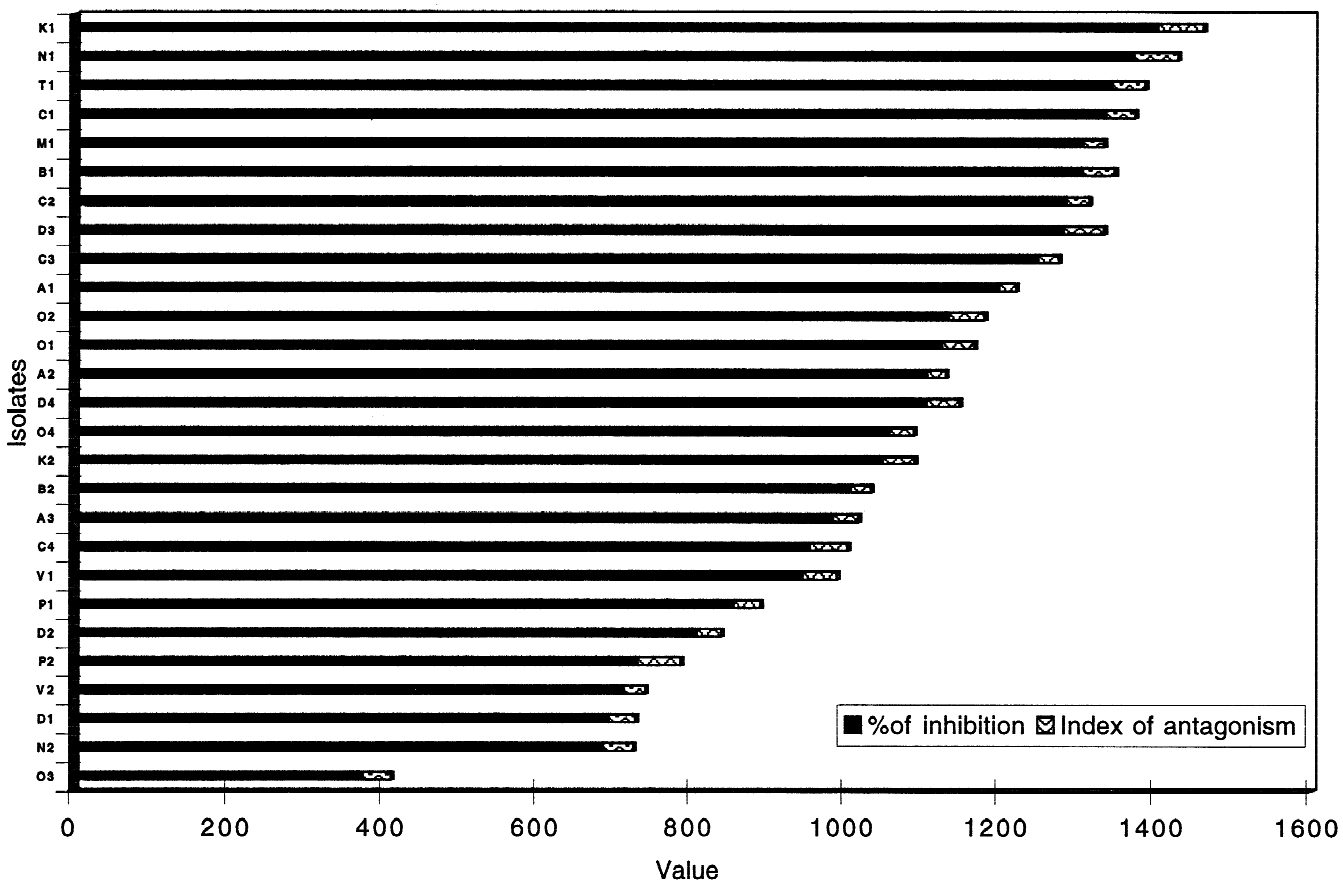

Fig. 1. The inhibition ability of each isolate represented by index of antagonism and percentage of inhibition.

strum and *Kirschsteiniothelia elaterascus*) were involved mostly in mutual inhibition at a distance from other competitors (category D).

Evaluation of Indices

Many isolates showed similar antagonistic abilities in both interaction assessing methods (Fig. 1). *Pseudohalonectria longirostrum, Kionochaeta australiensis, Chaetosphaeria* sp., *Brachydesmiella anthostomelloidea, Kirschsteiniothelia elaterascus, Tiaroporella paludosa,* and *Camposporidium antennatum* were strong inhibitory species. *Ophioceras dolichostomum* IV, *Diaporthe* sp., *Cateractispora aquatica* I and II, *Verticillium* sp. II, and *Byssothecium* sp. were weak inhibitory species. However, the results for *Ophioceras dolichostomum* II, *Aniptodera lignalitis,* and *Aeroaquatic* sp. were contradictory.

Discussion

Fungi isolated from submerged wood were tested to evaluate their *in vitro* interaction abilities. Most of these fungi inhibited the growth of other fungi to some degree, and stimulation of growth was not observed. The antagonistic activities were different among isolates and even between isolates of the same species isolated from different origins. *Pseudohalonectria longirostrum, Kionochaeta australiensis, Chaetosphaeria* sp., and *Brachydesmiella anthostomelloidea* were consistently shown to be the most strongly inhibitory species in both interaction-assessing methods. The four species of *Ophioceras* were less inhibitory than *Pseudohalonectria longirostrum,* which is in agreement with the results of other studies [1, 18]. Asthana and Shearer [1] found that *Ophioceras* species were less antagonistic than *Pseudohalonectria* species. The high percentage of mutual intermingling with reduction in hyphal growth recorded in this study is similar to that reported by Strongman et al. [21] and Miller et al. [11] in marine fungi. Shearer and Zare-Maivan [18], however, reported that only 3.1% of the interactions involved hyphal intermingling in temperate freshwater fungi.

Ophioceras dolichostomum II was strongly inhibitory according to the percentage of inhibition, but weakly inhibitory according to the index of antagonism. Asthana and

Shearer [1] reported that the interaction index of *Ophioceras* species was lower than that of *Pseudohalonectria* species, but the type of reaction was not mentioned. Lack of comparable data makes it difficult to state whether this fungus is a strongly or weakly inhibitory species. Clearly, more data are needed.

Several species used in this study were found to produce a variety of enzymes, including amylase, caseinase, laccase, tyrosinase, peroxidase, phenol oxidase, exoglucanase, and endoglucanase [29]. Phenol oxidase and exoglucanase production were significantly correlated ($P < 0.05$) with the percentage of inhibition (correlation coefficients $r = 0.53$ and 0.48, respectively). Fungi with weakly antagonistic abilities produced only one or two kinds of enzyme. For example, *Verticillium* sp. II and *Diaporthe* sp. only produced polyphenol oxidase and amylase, respectively, and the resulting inefficient use of substrate may place them at a competitive disadvantage. The ability of a fungus to produce a variety of enzymes would, on the other hand, be a competitive advantage [18]. *Heliscus lugdunensis,* which is not an effective degrader of complex substrata, was found to be intermediate in inhibition [18]. Not many of the isolates tested here can produce caseinase, and those that did were found to be less inhibitory species, such as *Nectria haematococca* and *Ophioceras dolichostomum* III. It is probable that these latter fungi only utilize simple substrates and so they are frequently overgrown by other species.

Nectria haematococca was found to be weakly inhibitive (Table 2). Shearer and Zare-Maivan [18] reported a similar result. They found that *Nectria haematococca* was particularly susceptible to inhibition, and this fungus is thought to be an early successional species. Their observation is also correlated to its ability to produce amylase, caseinase, and exoglucanase, but not polyphenol oxidase and endoglucanase [29]. It is not very effective in degrading complex substrates, such as lignin. This fungus, therefore, may have a nutritional requirement for readily available substrates such as sugars [28].

Species of *Pseudohalonectria* and *Lasiosphaeria* were reported to produce diffusible pigments in culture and on natural substrata. These fungi were also strongly inhibitory species at a distance [18]. In this study, *Pseudohalonectria longirostrum* and *Kirschsteiniothelia elaterascus,* which produced pigment in culture, were also found to be strongly inhibitory species, and they inhibited other fungi by mutual inhibition at a distance.

Several aquatic hyphomycetes were found to be weakly inhibitory fungi, and they did not produce any pigment in culture. Therefore, these aquatic hyphomycetes seem to be the least inhibitory fungi and may be less likely to produce antagonistic substances [18]. It is probable that persistent and late colonizers are more likely to produce antagonistic substances so as to inhibit the growth of early colonizers.

Statistical analysis showed that the percentage of inhibition was significantly correlated ($P < 0.05$) with growth rate (correlation coefficient $r = 0.4$). Slow-extending fungi or late colonizers (e.g., *Pseudohalonectria longirostrum* and *Kirschsteiniothelia elaterascus*) were more competitive than early colonizers or fast-extending fungi (e.g., *Ophioceras dolichostomum* and *Nectria haematococca*). However, *Brachydesmiella anthostomelloidea,* a fast-extending and strongly competitive fungus, is known only from North Queensland [19]. When a fast-growing fungus becomes dominant, resources may be rapidly extracted and other species excluded from utilizing the resources [12]. The result in terms of the antagonistic activity of *Brachydesmiella anthostomelloidea* supported the hypothesis that a fast-extending fungus can colonize a larger area and be more competitive than other fungi by production of branching hyphae.

Data indicated that there are competitive interactions between fungi *in vitro.* Under laboratory conditions, fungi were inoculated at different times, according to their growth rates, in order to prevent the occurrence of primary resource capture. However, in the natural environment, inhibition would be the result of competition for nutrients and space or production of antagonistic compounds or direct hyphal interference [18]. It is unlikely that inhibition involves nutrient depletion in this experiment because of the rich medium and short duration of the experiment [27]. Under high concentrations of soluble nutrients and the free movement of metabolites in agar media, the visible effects of interactions between species could be intense. Many factors such as inoculum potential, germination efficiency, growth rate, and substrate utilization patterns would affect the outcome of competitive interactions *in vivo.* Extrapolation of results from *in vitro* studies to natural substrates may not be appropriate [14, 17]. However, these data act as a guide to the outcome of interactions in nature, and these fungi have been further studied in the natural environment (unpublished data).

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