Viability and biodiversity of freshwater hyphomycetes in foam at Ton Nga Chang Wildlife-Sanctuary, Songkhla, southern Thailand

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A survey of freshwater hyphomycetes was conducted at Ton Nga Chang Wildlife-Sanctuary, Songkhla, Thailand. The viability of aquatic hyphomycetes trapped in fresh and old foam was estimated using tetrazolium bromide (MTT) staining. Percentages of conidia with at least one viable cell was in the range of 44–77% and 42–69% in fresh and old foam, respectively. Viability of conidia in fresh and old foam was not significantly different at (P>0.05). *Anguillospora* sp., *Helicomyces* sp., *Thozetella* sp. and *Volutella* sp. were tested for their viability under laboratory conditions. The conidial viability after aeration in water remained at 83–88%, after seven days. However only 3–45% remained viable after drying for 10 hours. Sixty-two fungi were recorded during this study, including 48 species in 34 genera and 14 unidentified taxa.

Key words: biodiversity, conidial viability, freshwater hyphomycetes, southern Thailand, tetrazolium bromide.

Introduction

Freshwater hyphomycetes are a specialised group of anamorphic fungi that play an important role in the degradation of leaf litter or other submerged substrata in aquatic habitats (Bärlocher, 1992). Members of this group are intermediaries in energy and food webs in aquatic ecosystems and are distributed worldwide. Aquatic hyphomycetes grow on leaf litter in streams, releasing large numbers of conidia (Bärlocher, 1982). Typically, these conidia are branched or sigmoid. They are often trapped by air bubbles and accumulate in persistent foam below waterfalls or rapids (Iqbal and Webster, 1973; Ingold,

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1975; Hyde and Goh, 1993). Tubaki *et al*. (1983) and Sivichai *et al*. (1998) have undertaken preliminary studies of this group in Thailand. Although the presence of conidia of aquatic fungi in foam is well documented (Ingold, 1975), their viability under these conditions has been investigated only once in a temperate region (Sridhar and Bärlocher, 1994). Little is known about the taxonomy and ecology or viability of conidia in foam of aquatic hyphomycete in Thailand.

The tropical rain forest at Ton Nga Chang Wildlife-Sanctuary is one of the primary forests in southern Thailand and harbours a rich diversity of plants, animals and microorganisms. Our objective was to assess the viability and diversity of freshwater hyphomycetes in foam. In laboratory experiments, we examined how conditions present in the natural stream environment might affect conidium viability.

Materials and methods

Study sites and sampling procedures

Ton Nga Chang Wildlife-Sanctuary was chosen for the study, located in Songkhla and Sathun provinces, southern Thailand (6°5′ to 7°3′ North and 100°8′ to 100°16′ East). Four collecting sites were located along the 5-10m wide river of the Ton Nga Chang waterfall at approximately 50m intervals. The samplings sites were located in the direction from upstream to downstream. Foam forming on the surface below the waterfalls (Fig. 1), was sampled eight times at bi-weakly intervals between June and September 1998.

Collection of foam samples

Two types of foam were distinguished: fresh foam (colour and consistency similar to whipped egg white) and old foam (yellow-brown, partly dried up). Foam was scooped into plastic bottles, sealed, placed in an icebox and returned to the laboratory within an hour.

Viability testing

The viability of conidial cells for all species was tested with tetrazolium bromide (MTT, Sigma 2128). A few drops of foam diluted with sterile distilled water were transferred to clean slides in Petri dishes, mixed with an equal quantity of MTT (0.5 mg/ml), and incubated in the dark for 3 hours at 25 C. A total of 200 conidia per sample were examined under a compound microscope.

Fig. 1. Appearance of old foam in stream. **Fig. 2.** Conidium of *Flabellospora multiradiata* treated with tetrazolium bromide (MTT) with living cells stained purple to deep blue (arrowed). Non-living cells were colourless. Bar = 20 µm.

Conidia were considered to be viable when at least one cell had reduced the stain and formed the red-coloured formazan product. Non-living conidia or cells were colourless (Sridhar and Bärlocher, 1994). One-way analysis of variance (ANOVA) was used by SPSS 4.0 for Windows.

Conidial viability during aerated and after drying

Four species: *Anguillospora* sp., *Helicomyces* sp., *Thozetella* sp. and *Volutella* sp. isolated from foam were maintained on corn meal agar at 25 C.

To induce conidium production, agar disks were cut from the margin of the 2-week old colony and placed into a sterile conical flask containing sterile distilled water. Flasks were connected with an air pump. Airflow into the flasks was adjusted and equilibrated to be the same rate for each flask. Cultures were aerated for 24-48 hours until the conidia produced. The number of conidia was estimated, approximately 10^3 conidia per ml was used. The aerated conditions were run for another 3, 5 and 7 days. Viability of newly produced conidia was tested with MTT.

Cultures from the same four species were prepared as described above. The estimated conidial suspensions were transferred to a clean glass slide, left in a laminar-flow cabinet for 1, 5, 15, 30 minutes, 1, 5 and 10 hours, respectively and conidial viability was determined using MTT. The light illumination, temperature, airflow rate and relative humidity were estimated at the same rate. One-way ANOVA and regression analysis were used by SPSS 4.0 for Windows and GenStat, respectively. Four types of decay curves were tested in order to interpret the conidial viability under both conditions: Exponential decay, Logistic, Generalized logistic and Gompertz curve.

Fungal identification

Fungal diversity was examined directly from the foam samples under a microscope according to the conidial morphology as described by Carmichael *et al*. (1980), Ingold (1975) and Marvanová (1997).

Results

Conidial viability in foam

Viable cells of the conidia stained with MTT initially turned bright red, later became deep blue and could easily be distinguished from non-viable cells (Fig. 2). Percentages of conidia with at least one viable cell varied between 44 to 77% and 42 to 69% in fresh and old foam, respectively (Fig. 3). One-way ANOVA comparing different age of foam, different sites and 8 collecting times was made. The conidial viability in fresh and old foam for all sites and times was not significantly different at $P>0.05$.

Percentage viability in fresh foam decreased from the first collection to week 8 (early August), then increased again from week 10 to the end of the study. The viability of conidia in old foam fluctuated without apparent pattern throughout the study period.

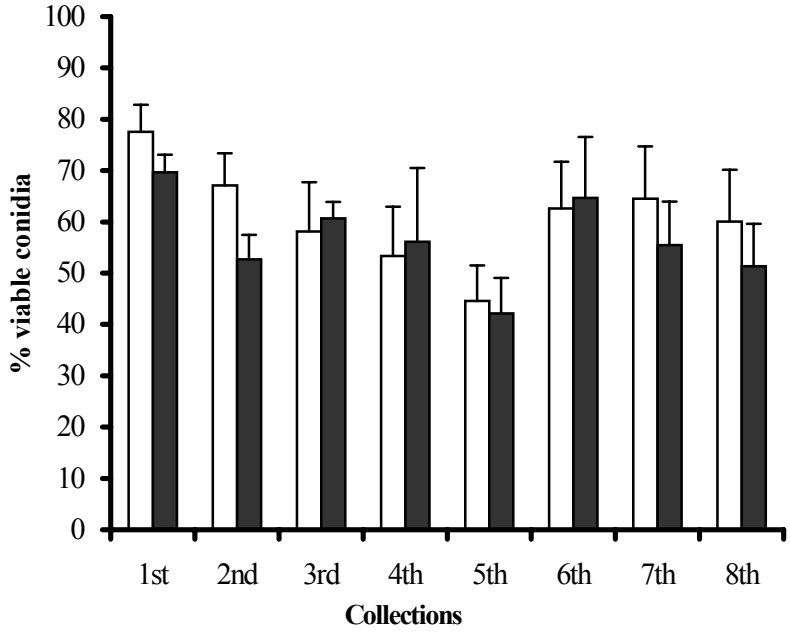

Fig. 3. Percentage viable conidia in fresh and old foam for eight collections. Total of 200 conidia counted. Bars represent standard error. □ fresh foam; ■ old foam.

Conidial viability during aeration and after drying

The viability of conidia aerated under water for up to seven days was fitted in the Exponential decay curve (Fig. 4). All four species showed a steady decrease in viability over time. It decreased from 95–98% after three days to 83–88% at day 5 to day 7.

The Hyperbolic curve was chosen as the most appropriate model to interpret the viability after drying (Fig. 5). The viability of conidia when allowed to dry out for 10 hours was 3-45%. Viability decreased after five minutes drying and more rapidly after 15 minutes. Differences were observed between the species: *Anguillospora* sp. and *Helicomyces* sp. had a viability of 37–45%, whereas it was only 3–7% in *Thozetella* sp. and *Volutella* sp. (significantly difference at $P<0.05$).

Anguillospora sp. and *Triscelophorus* sp. were found consistently at all collecting sites, and throughout the period of observation. Commonly found genera were *Anguillospora*, *Articulospora*, *Beltrania*, *Brachiosphaera*, *Campylospora*, *Clavariopsis*, *Condylospora*, *Diplocladiella*, *Dwayaangam*, *Flabellospora*, *Helicomyces*, *Helicosporium*, *Isthmotricladia*, *Laridospora*, *Phalangispora*, *Tricladium*, *Triscelophorus* and *Varicosporium*. Examples of commonly found genera are illustrated in Fig. 6.

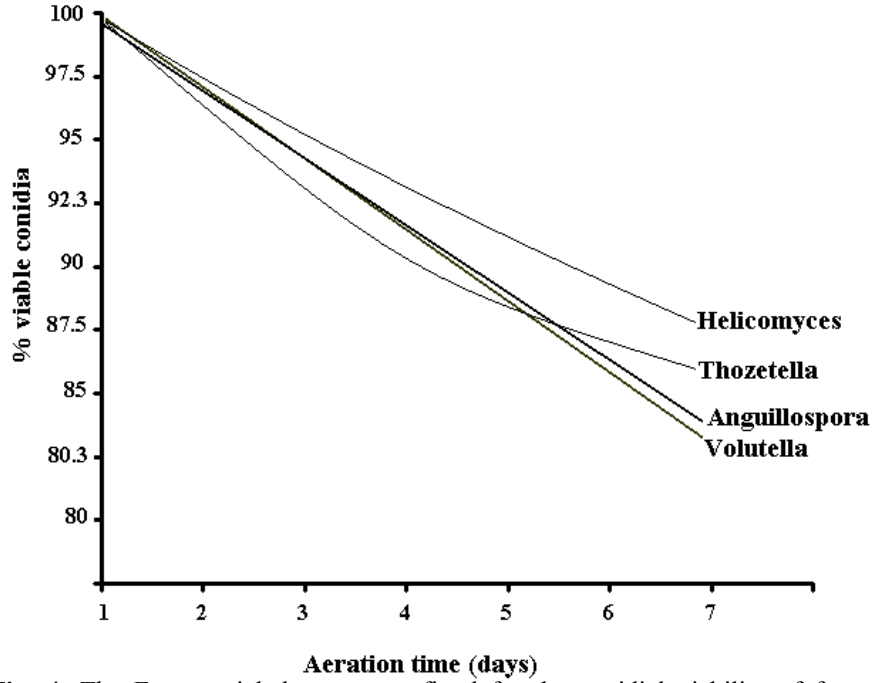

Fig. 4. The Exponential decay curve fitted for the conidial viability of four species after aeration for seven days.

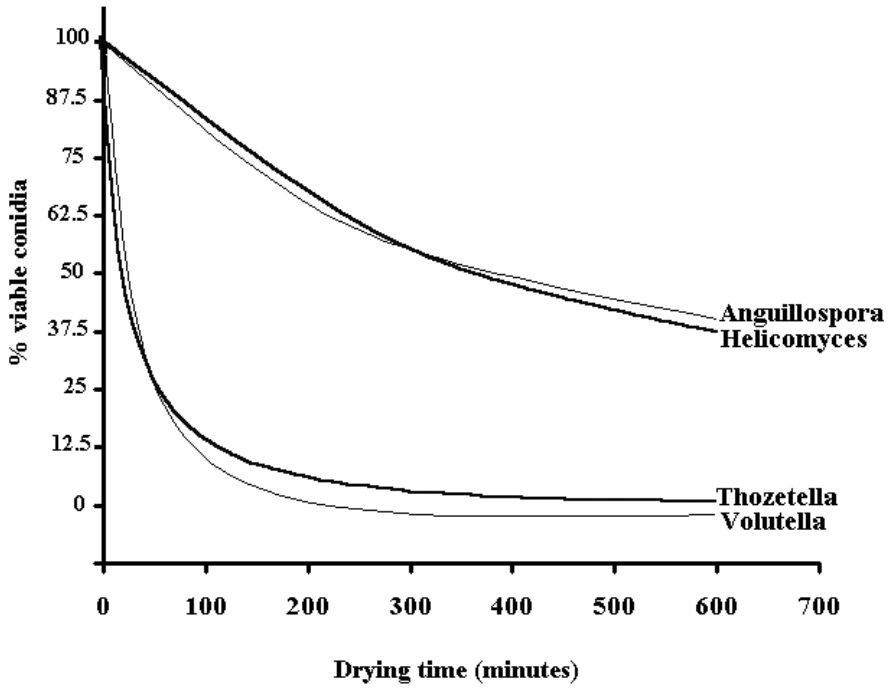

Fig. 5. The Hyperbolic curve fitted for the conidial viability of four species after drying conditions for 10 hours.

Fig. 6. Examples of commonly found genera. **a.** *Isthmotricladia gombakiensis*. **b.** *Triscelophorus acuminatus*. **c.** *Campylospora chaetocladia*. **d.** *Lunulospora cymbiformis*. **e.** *Diplocladiella appendiculata*. **f.** *Flabellospora verticillata*. **g.** *Tricladium aciculum*. **h.** *Helicomyces* sp. **i.** *Beltrania rhombica*. **j.** *Ingoldiella hamata*. Bars = 20 µm.

Table 1. List of identified fungal species found in foam.

Name	Name
Hyphomycetes	*Nawawia* sp. 2
Anguillospora sp.	*Nawawia* sp. 3
Anguillospora-like sp.	*Phalangispora constricta*
Beltrania rhombica	*Pseudobeltrania* sp.
Brachiosphaera tropicalis	*Quadricladium*-like sp.
Campylospora chaetocladia	*Scutisporus* sp.
C. filicladia	*Sporidesmium tropicale*
Camposporium-like sp.1	*Tetrachaetum*-like sp.
Camposporium-like sp.2	*Thozetella* sp.
Clavariopsis sp.	*Tricladium aciculum*
Condylospora spumigena	*Trinacrium*-like sp.
Dendrospora sp.	*Triscelophorus acuminatus*
Diplocladiella appendiculata	*Varicosporium giganteum*
D. scalaroides	*V. macrosporum*
Dwayaangam sp. 1	*Volutella* sp.
Dwayaangam sp. 2	*Wiesneriomyces* sp. 1
Flabellospora crassa	*Wiesneriomyces* sp. 2
F. multiradiata	
F. verticillata	**Coelomycetes**
Helicomyces sp.	*Chaetospermum camelliae*
Helicosporium sp.1	*Chaetospermum*-like sp.
Helicosporium sp.2	*Pestalotia* sp.
Isthmotricladia gombakiensis	*Robillarda* sp.
I. laeensis	
Laridospora appendiculata	**Basidiomycetes**
Lunulospora cymbiformis	*Ingoldiella hamata*
Nawawia sp. 1	
Total	34 genera, 48 species

Biodiversity of freshwater hyphomycetes

Sixty-two fungal taxa, distributed among 34 genera were observed, among them 48 fully identified species (Table 1). Hyphomycetes dominated; in addition, there were three genera of coelomycetes and one basidiomycete genus. The most abundant species in the foam belonged to the genera

Discussion

Conidial viability

Several factors may affect conidial viability in natural habitats. These include temperature, water pH, desiccation, dissolved oxygen, conidial

abrasion in fast flowing water, seasonal leaf fall and rainfall (Tubaki *et al*., 1983; Sridhar and Kaveriappa, 1984; Chauvet, 1991; Tan and Koh, 1995; Rajashekhar and Kaveriappa, 1998). These parameters may also apply to the viability of conidia in foam. Our results show that conidial viability in fresh and old foam was not significantly different in the range 44 to 77% and 42 to 69%, respectively ($P>0.05$). By contrast, a study in a temperate stream by Sridhar and Bärlocher (1994) recorded values of 76–91% and 20–43% in fresh and old foam, respectively. The rate of conversion from fresh to old foam affect the conidial viability. In the temperate streams, the transition can be as rapid as one week in summer (Sridhar and Bärlocher, 1994). In winter, due to ice formation it can be delayed for 2–4 weeks. This condition could make the conidia survive in fresh foam at a higher rate (79–91%), possibly because of depressed metabolic activities at low temperatures. In our study the conversion period was even less than one week as can be seen from the depressed viability of the conidia. The reason may be because of the rainfall during our study. This may have increased the water current in the particular stream. The conversion from fresh to old foam may be as quickly as within a week or even less. Thus the younger conidia were probably continuously being trapped in old foam, resulting the viability of conidia in fresh and old was not different.

Under aerated conditions the viability of newly released conidia of the four test species in our study was 83–88% (Fig. 4). This is similar to the laboratory-produced conidia of *Articulospora tetracladia* and *Heliscus lugdunensis* (85 and 89%, respectively) reported by Sridhar and Bärlocher (1994). This may be because air bubbles under laboratory conditions provide a good source of oxygen, giving a higher survival rate than that of conidia in natural foam, which gave the value of 42–77% for both types of foam.

Under drying conditions, the viability of *Anguillospora* sp. and *Helicomyces* sp. was clearly higher than those of *Thozetella* sp. and *Volutella* sp., significantly different at $P<0.05$ (Fig. 5).

This may be related to their conidial morphology. *Anguillospora* sp. and *Helicomyces* sp. are sigmoid and helicoid, respectively, and both are multicellular, their nutrient storage capacities might be greater than those of *Thozetella* sp. and *Volutella* sp. The conidia of these two species are smaller, single-celled, ovoid and sickle-shaped, respectively. Thus, the probability of surviving under drying conditions, (i.e. at least for one cell forming formazan complexes) is much higher in *Anguillospora* sp. and *Helicomyces* sp. than in the two single-celled species.

Biodiversity of freshwater hyphomycetes

Several genera identified were similar to those reported from northern and central regions of Thailand. These include *Anguillospora*, *Campylospora*, *Clavariopsis*, *Flabellospora*, *Isthmotricladia*, *Lunulospora*, *Phalangispora* and *Triscelophorus* (Hywel-Jones, unpubl. obs.; Tubaki *et al*., 1983). The number of species increased steadily from June to August, followed by a slight decrease (data not shown). The availability of substrata and frequency of rainfall may be the most important parameters affecting species diversity in tropical freshwater ecosystems (Tan and Koh, 1995). In this study, the higher number of species was correlated with the heavy rainfall, recorded from July to September, with a concomitant increase in water flow, and consequently the release of more conidia from the leaf litter.

Our results demonstrated the high diversity and viability of aquatic hyphomycete conidia present in the foam of a tropical freshwater stream. The fate of these conidia is not known. It is possible that the conidial entrapment in foam may be temporary and that they will eventually attach themselves and develop on suitable substrata. This implies that foam may represent a good inoculum for fungal dispersal. Our study showed that conidial viability in foam is likely to remain high, but that exposure to drying conditions may quickly become fatal. Multi-celled conidia appear to have a greater chance to survive unfavorable condition. However, more study sites, longer periods of study and different seasons should be the subject of further focus.

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