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Abstract

To better understand the development of colonies, the effect of nutrient availability and time on colony formation of a saprophytic species Rhizophydium sp. (AUS6) in the order Rhizophydiales and family Terramycetaceae in culture was studied. Biomass and zoospore production over a period of time was measured to predict changes within a colony. In culture, AUS6 sporangia develop from zoospore within 25 h at 24°C. Timing of development from encystment, germination, sporangial enlargement and mature sporangial formation was significantly different between synthetic medium (CSM), modified CSM (no carbon or nitrogen) and complex medium (1/2 YpSs). However, initial aggregation of thalli occurred within 24 h in all media at 24°C. The dynamic nature of the colony was highlighted by the fluctuation in colony biomass and zoospore production over time. An extracellular matrix was observed on the surface of the rhizoids and developing sporangia. Another matrix that appeared to reinforce the cell wall of enlarged sporangia was also identified in mature colonies. Both matrices facilitate the aggregation of thalli and continued maintenance of the colony structure. The entanglement of zoospores in branching rhizoids of older thalli also facilitates aggregation. The dynamics exhibited by AUS6 colonies and the ultrastructural features of thalli and the colony, indicate the need for further study on the function of chytrid colonies in general, especially under different environmental conditions.

Keywords: soil chytrid, colony, rhizoid, extracellular matrix.


Introduction

The phyla Blastocladiomycota and Chytridiomycota are ancient groups of zoosporic fungi commonly referred to as chytrids (Sparrow 1960, Barr 2001). There are six orders, the Blastocladiales, Chytridiales, Monoblepharidales, Neocallimastigales and Spizellomycetales and Rhizophydiales (James et al. 2000, Barr 2001, Letcher & Powell 2005, Letcher et al. 2006). Chytrids have been associated with disease in amphibians resulting in the decline of amphibian species (Berger et al. 1998, Longcore et al. 1999, Berger 2005). Anaerobic chytrids have been found in the gut of a number of economically important herbivores (Bauchop 1981, Li & Heath 1992). Halophytic chytrids have parasitic association with algae and phytoplankton (Sparrow 1960, Ibelings et al. 2004). Saprophytic soil chytrids make up a large portion of the phylum and are involved with biodegradation of plant and animal remains containing cellulose, chitin and/or keratin (Sparrow 1960, Powell 1993, Barr 2001, Longcore 2001). Morphological variability is influenced by multiple factors, including substrate, which makes categorization within the phylum difficult. Families within the phylum have undergone major revision with recent work considering not only the more stable ultrastructural
features of zoospore but also phylogenetic analyses of the 28 S rRNA and 5.8 S ribosomal gene sequence (Letcher & Powell 2005, Letcher et al. 2006).

In comparison to higher fungi relatively little is known about how chytrids interact and utilise nutrients from the environment. The phylum has a diversity of species, life cycles, morphologies and interactions with the environment (Powell 1993, Barr 2001, Longcore 2001). In general chytrids disperse via zoospores in water and upon settling form simple sporangia, with or without rhizoid and connecting ‘hyphae’ (Sparrow 1960, Barr 2001). It is not clear what structures are involved in growth and energy consumption and the functionality of established structures such as rhizoids at different stages of the life cycle. Chytrids accumulate on cellulose, chitin or keratin rich substrates (Sparrow 1960, Mitchell & Deacon 1986, Powell 1993,). The establishment of colonies follows attachment and encystment of zoospores, and initiation and elongation of rhizoids on different substrates. Rhizoids are hypothesized to convey nutrients back to the developing sporangium (Sparrow 1960). Growth involves extension and branching of rhizoids and enlargement of the sporangium (Sparrow 1960, Taylor & Fuller 1981, Li & Heath 1994, Longcore 2001).

The cycle of germination, sporangial maturation and sporulation in chytrids varies temporally and is largely dependent on species, biological interactions, substrate and environmental conditions (Sparrow 1960, Powell & Koch 1977, Barr 2001, Longcore 2001). Nutrient availability, for example, highlights the plasticity of chytrids species by altering the rate and pattern of the life cycle. The interaction as measured by biomass, zoospore release and infectivity of algal host, Asterionella formosa Hass., and chytrid parasite, Rhizophydiu planktonicum, for example, are influenced by the availability of phosphorus, light and temperature (Bruning 1991a, b, c, Bruning et al. 1992).

Microorganisms in the natural environment exist within a community or colony in order to protect themselves against harmful environmental changes, colonize new territories, allocate limited nutrient resources, and form robust and/or specialized subpopulations (Palkova & Vachova 2006). It is likely that many species of chytrids form colonies, but very little is known about if and how they would form and their interaction with the environment. The chytrid life cycle, rate of colony growth and zoospore production are important aspects in understanding this interaction.

AUS6 is found in culture as clumps, where sporangia of varying sizes have interwined rhizoids. Zoospores are released and probably wash away from the parent sporangium. Thus the complex colony may reflect competitive interactions within the colony. Alternatively, the sporangia may be of different ages, and the colony a complex community, which functions as a unit in its interactions with the environment. The main aim of this study was to observe how nutrient availability and time influence colony formation of a saprophytic species Rhizophydium sp. (AUS6) in the order Rhizophydiales and family Terramyctaceae (Letcher et al. 2006) in culture. The investigation was based on morphology, dry biomass measurements and zoospore counts of AUS6 over time.

Materials and Methods

Experimental Organism

Rhizophydiu sp. (AUS6) [kindly provided by P. Letcher, University of Alabama, Tuscaloosa] is a soil fungus that was isolated from wet sclerophyll using onion skin (Gleason et al. 2004). The purified culture was grown on synthetic Chytrid Specific Medium (CSM) [K2PO4 (600 mg), MgSO4 (200 mg), NH4NO3 (200 mg), CaCl2.2H2O (50 mg), FeEDTA (500 mg), thiamin (133 mg), L-alanine (900 mg), L-methionine (100 mg), D-glucose (4 g), agar (Sigma) (15 g) and 2.5 L micronutrients (10 μM MnCl2.4H2O, 10 μM ZnCl2, 33 μM H3BO3, 1 μM CuSO4.5H2O and 0.2 μM (NH4)2MoO4) in 1 L of distilled H2O (d H2O)] (Midgley et al. 2006) and incubated at 24°C.

Harvesting zoospores for the experimental protocol

To optimise zoospore numbers 1.5 mL dH2O was placed on a healthy mature culture. After 3 h another 1.5 mL dH2O was added and spread over the plate to allow zoospore...
dispersal. Cultures were incubated at 24°C for a further 24-48 h to allow sporangial maturation. The process of adding distilled H₂O (1.5 mL) was repeated and the cultures left for an additional 3 h at 24°C. The plates were then tilted at a 5-10° angle to allow accumulation of released zoospores to one side of the Petri dish for easy collection. A drop of the collected zoospores was placed on haemocytometer and counted using an Olympus CH-2 compound microscope fitted with a 40x Olympus objective (E A40, 0.65).

**Culture media for experimental protocols**

Synthetic CSM, containing monosaccharides and inorganic nitrogen, was prepared as described above. Modified CSM (mCSM) [K₂PO₄ (600 mg), MgSO₄ (200 mg), NH₄NO₃ (200 mg), CaCl₂.2H₂O (50 mg), FeEDTA (500 mg) and agar (15 g) in 1 L of dH₂O] was also prepared, containing no carbon or nitrogen. Growth on synthetic media was compared to growth on a defined stage, immediately after transfer to solid mCSM, CSM and YpSs media of CSM, mCSM and YpSs were prepared as described above but without agar. Modified CSM (mCSM) [soluble starch (10 g), yeast extract (0.5 g), K₂HPO₄ (0.5 g), MgSO₄.7H₂O (0.25 g) and agar (15 g) in 1 L of dH₂O], containing polysaccharide and organic nitrogen. Liquid media of CSM, mCSM and 1/2 YpSs were prepared as described above but without agar.

**Experimental protocol for rate of Rhizophydium sp. (AUS6) maturation**

The time taken for AUS6 zoospores to mature to a defined stage, immediately after transfer to solid mCSM, CSM and 1/2 YpSs media, was recorded by observing 10 random sightings of a particular stage using a 10x Olympus objective (E A10, 0.25). The four stages of thallus development being recorded were encystment (an enlarged zoospore head without a tail); germination (an immature sporangium with a bifid axis and initiation of rhizoid branching); sporangial enlargement (expanded sporangium containing small number of zoospores); and, mature sporangium (extensive rhizoid branching and filled with zoospores). Two-way analysis of variance (ANOVA) was performed at a significance level of 0.05 to determine whether there was any initial influence on the growth rate during establishment in the three media and also whether the rate of development was heterogeneous within a population.

**Morphological observations using light microscopy**

Sterile Aclar® plastic films (8x8 mm square) in 24-well plates (ProSciTech) were inoculated with 2 x 10⁶ zoospores and incubated in liquid mCSM, CSM or 1/2 YpSs (n=6) to observe Rhizophydium sp. (AUS6) development at 24°C for 72 h. At various intervals according to media and Fig. 1, the Aclar® inoculated films were mounted on a slide in a drop of liquid medium. Differential interference contrast (DIC) images were taken using a Nikon Eclipse E800 microscope fitted with a HCX PL APO 63x/1.20 W CORR objective and DIC filters, using Sensicam 12 bit Cooled Imaging (PCO CCD Imaging), Camwire v 1.21 (PCO) software and processed with Adobe Photoshop 7 (Adobe Systems).

**Morphological observations using transmission electron microscopy**

Four Petri dishes with mCSM, CSM or 1/2 YpSs agar were each inoculated with 2 x 10⁶ zoospores. The inoculated plates were incubated at 24°C for 72 h. Cultured agar squares (5x5 mm) were excised from the plates at various intervals according to media and Fig. 1 and fixed in 3% glutaraldehyde (ProSciTech, Australia) in 0.1M Na Cacodylate buffer, pH 7.2 at 24°C for 2h. The fixed specimens were washed three times for 15 min each with 0.1 M Na cacodylate buffer before post-fixing with 1% OsO₄ (ProSciTech) in 0.1 M Na cacodylate buffer for 60 mins in the dark at room temperature. The specimens were then washed once with 0.1 M Na cacodylate buffer for 15 min and twice with dH₂O for 15 min each. Specimens were then stained with 4% uranyl acetate (ProSciTech) in dH₂O for 10 min and washed three times for 15 min each with dH₂O and dehydrated in 10, 30, 50, 70, 80, 90, 95% ethanol for 15 min each and 100% ethanol for 20 mins. Ethanol was removed from the cultures by continuous rotation in 1:3 LR White in ethanol (Sigma) at 23°C overnight followed by 2:3 LR White in ethanol and twice in 100% LR White. Specimens were orientated for flat embedding (ProSciTech) and polymerised at 50°C overnight.

Ultrathin sections were cut using a Leica Ultracut UCT and placed on 100 mesh nickel
Figure 1. Morphological changes of Rhizophydium sp. (AUS6) in mCSM, CSM and 1/2 YpSs over time. Encystment of AUS6 on all substrates occurred within 3-4 h of inoculation with zoospores. Germination occurred 7-8 h after inoculation. Sporangial enlargement and maturation occurred 11-13 h and 23-25 h respectively, after inoculation.

Experimental protocol for biomass measurements

Polyurethane 5 mL test tubes (n=4) containing 4 mL of liquid mCSM, CSM or 1/2 YpSs were inoculated with 2.7x10^5 zoospores and incubated for either 4, 7, 10, 14 or 18 days at 24°C. Absorption of water from the atmosphere was minimized by drying the filter paper, used to measure biomass, at 60°C for at least 2 h and cooling in a desiccator. Initial weight measurements of the filter papers were made prior to use. The incubated test tubes were scraped gently with a small spatula to dislodge any culture from the sides before filtering, drying (60°C) overnight and storing in a desiccator. Exposure to the atmosphere was reduced by removing each filter paper directly from the desiccator and weighing. Two-way analysis of variance was performed at a significance level of 0.05 to determine whether there was any difference in biomass of AUS6 in the three media and whether this changed over time.

Experimental protocol for zoospore release measurements

Five 12-well plates were set-up with agar media. Each plate contained 4 wells of mCSM, CSM and 1/2 YpSs. Each well was inoculated with 2.7x10^5 zoospores and incubated for either 4, 7, 10, 14 or 18 days at 24°C. Cultures in the incubated plates were then induced to sporulate by adding 0.5 mL dH_2O and incubating at 24°C for 3 h. The plates were then tilted and counted as described previously. Two-way analysis of variance was performed at a significance level of 0.05 to determine whether there was any difference in zoospore release from AUS6 colonies in the three media and whether this changed over time.

Results

The nutrient source significantly effects Rhizophydium sp. (AUS6) maturation.

Two-way ANOVA showed that in the presence of media containing abundant monosaccharides and inorganic nitrogen (CSM), polysaccharide and organic nitrogen
(1/2YpSs) or no carbon and nitrogen (mCSM) there was a significant difference in the maturation of AUS6 ($F_{2,119}=5.3$, $p=0.006$). There was also a significant difference in the time taken to develop to the four observed stages ($F_{3,119}=3149$, $p<0.05$). The combined effects of media and time were also significant ($F_{6,119}=3.0$, $p=0.008$). These results imply that although zoospores have an endogenous energy source their subsequent encystment and development on a substrate is dependent on the individual effects of nutrient availability and time but also the combined effects of nutrient source and time. The significant difference in time for different stages indicates the time dependent nature of AUS6 thallus maturation and consistency in the rate of maturation in a culture exposed to different nutrient sources (Fig. 1).

Figure 2. DIC image of *Rhizophydium* sp. (AUS6) colony in liquid media. Sporangia at different stages of development are visible. The rhizoids (R) from each thallus branch extensively. The lipid bodies (L) are visible in immature sporangia. Zoospores associate with the lipid body. The more mature sporangia have large quantities of zoospores (arrow) (Bar=20 $\mu$m).

Figure 3. *Rhizophydium* sp. (AUS6) colony formation on solid media. (a-c) Examples of colony structures during formation on solid agar. The sporangia (S) mature in rings, which eventually become filled-in with settled zoospores (arrowhead). There are diverse stages of development in the colony. The sporangia that release zoospores (arrows) are surrounded by sporangia (Bar=200 $\mu$m). (d) Close-up of leading edge of colony showing rhizoids (R) extending from the sporangia (S) and trap released zoospores (arrowhead) so they encyst on the edge and thereby increase the mass of the colony (Bar=100 $\mu$m).
Morphological changes in *Rhizophydium* sp. (AUS6) occurred hourly with colony formation occurring within 25 h of inoculation

Encystment of zoospores occurred in the first 3 h after inoculation of liquid and agar cultures. By 7-8 h the immature sporangia or germlings in media had fine rhizoids extending from the axis. By 12 h, encystment had occurred with initial branching of rhizoids from the axis in both liquid and agar cultures. Maximal diameter of the rhizoids was 2 µm. Extensive branching and elongation of the rhizoids occurred as sporangia matured and enlarged.

In some cases zoospore production was visible inside the sporangia and often observed in close association with a lipid body (Fig. 2). By 24 h, mature sporangia were visible in the colony (Fig. 3) and by 48 h the majority of sporangia were filled with zoospores. Branching of rhizoids entangled thalli and therefore the formation of colonies in liquid and agar. By 72 h many of the sporangia had released their zoospores and colonies consisted of different developmental stages (Fig. 4). The colonies contained enlarged mature sporangia whose cell walls were thicker than the average sporangium. In some cases, sporangial germination occurs within the larger structure. Fibrous extracellular extensions on the surface of rhizoids and multiple branching of rhizoids entangled released zoospores (Fig. 5).

*Rhizophydium* sp. (AUS6) biomass was influenced by nutrient availability and time, but zoospore release was primarily influenced by time of stimulation.

Comparison of biomass and zoospore release was made between consecutive treatment times and indicated as a percentage change. AUS6 zoospores cultured on mCSM encysted by day 4 and biomass had increased by 110% from day 0 but matured slowly as reflected by the 73% reduction in released zoospore number in comparison to the initial inoculated zoospore number (Fig. 6). By day 7, the biomass had decreased by 65% in comparison to day 4. Despite the initial encystment and germination some sporangia were reabsorbed. However further investigation is required, due to the large standard deviation, to confirm this trend. The surviving sporangia had matured sufficiently by day 7 to produce a 170% increase in zoospore numbers in comparison to day 4. By day 10, the biomass of the colony of sporangia had increased by 54% and matured sufficiently to allow a 25% increase in zoospore yield compared to day 7. The 80% increase in biomass between day 10 and day 14 resulted in a 110% increase in zoospore numbers. Colony biomass appeared to be increasing with the production of zoospore and maturation of sporangia. However by day 18, there was a 70% reduction in biomass and a 98% reduction in zoospore count. The reduction in zoospore production suggests that reabsorption of culture compensates for the lack of nitrogen and carbon source in the substrate.

There was only an 80% increase in biomass from day 0 to day 4 when AUS6 zoospores were transferred to CSM (Fig. 7). The colony was relatively immature as reflected by the 50% decrease in zoospore release in comparison to initial numbers (2x10^5 zoospores). By day 7, the 240% increase in zoospore count suggests that the cultures had matured sufficiently to produce zoospores. In contrast, the overall biomass had decreased by 64%. This implies the initial encystment and germination was followed by reabsorption of some sporangia and the maturation of others. Recovery of biomass and zoospore numbers in CSM between days 7 and 10 was slow (27 and 1% respectively). In contrast between days 10 and 14, biomass and zoospore numbers had increased by 500% and 6% respectively. The relatively low zoospore increase implies slow sporangial maturation. By day 18, the 50% reduction in biomass suggests reabsorption of biomass and a decline of 123% in zoospore release. On day 4, biomass of 1/2 YpSs liquid cultures had increased by only 33% from day 0. However the decline in zoospore release was not as pronounced (32%) (Fig. 8). By day 7, the biomass had decreased by 44% in comparison to day 4 indicating some reabsorption of culture. The 240% increase in zoospore release suggests the remaining sporangia had matured sufficiently to produce zoospores. There was a further 50% decline in biomass between days 7 and 10 with a decline of 70% in zoospore production. The ten-fold increase in biomass between days 10 and 14
was associated with only a 50% increase in zoospore production at day 14. The increase in biomass implies the release, encystment and development of sporangia prior to day 14 but after day 10. By day 18, there was a 65% decline in biomass implying reabsorption of some sporangia while others continue to mature, hence the 64% reduction in zoospore release. Two-way ANOVA indicated a significant difference in biomass when the day of sampling was considered alone ($F_{4,44}=45.6$, $P<0.5$). There was no significant difference in AUS6 biomass when media (mCSM, CSM and 1/2 YpSs) was considered alone ($F_{2,44}=0.2$, $P=0.2$) but there was a significant difference when the interaction between the day of sampling and media are considered ($F_{8,44}=9.2$, $P<0.05$). This indicates that biomass fluctuates over time and the nutrients in media influence the fluctuation. Analysis of zoospore release showed that there is a significant difference when considering time only ($F_{4,44}=8.5$, $P<0.05$). However there is no significant difference when considering media alone ($F_{2,44}=1.0$, $P=0.4$) or when looking at the combined effects of time and media ($F_{8,44}=1.0$, $P=0.4$). This suggests that AUS6 compensates for any changes in nutrient availability. Despite differences in nutrient media, biomass and zoospore production was seen to fluctuate in all media over time.
Discussion
This study suggests that maturation of *Rhizophydidum* sp. (AUS6) is dependent on nutrient availability. Utilization of endogenous and exogenous energy sources varied according to the AUS6 life cycle in culture. Encystment and maturation of individual AUS6 zoospores in the absence of carbon and nitrogen (mCSM) indicate the use of an endogenous energy source. The association of the lipid globule in zoospores with the microbody-lipid globule complex imply its use as an endogenous energy source (Powell 1978, Powell & Roychoudhury 1992). Variable utilization of endogenous energy by established communities of AUS6 in mCSM is also implied by the observed fluctuation in biomass and zoospore production over time. Biomass and zoospore production readings on day 4 suggest that AUS6 established itself quickly by using most of its resources at the expense of zoospore production. This build-up
Figure 7. Mean Biomass and % change in zoospore production of *Rhizophydium* sp. (AUS6) in CSM. (a) A fluctuation in biomass with major peak on day 14. (b) % change in zoospore production recovered from an initial 48% reduction from the original inoculum and peaked on day 14.

in biomass suggests the aggregation of thalli. Aggregation ensures the survival of the colony under adverse conditions such as nutrient depletion. Reduction in biomass on day 7 suggests reabsorption of part of the colony to provide energy for sporulation. The high yield in zoospores on day 7 is essential for expansion of the colony. The utilization of endogenous energy may partly account for the fluctuation in AUS6 biomass over time in a nutrient depleted environment. Although fluctuations in biomass and zoospore production were observed, they were asynchronous and can be used to predict the composition of the colony at any point in time. For example, a low relative yield in biomass and a high zoospore release implies partial reabsorption of the colony, but the survival of mature, sporulating AUS6 sporangia. In contrast, high biomass and low zoospore
Figure 8. Mean Biomass and % change in zoospore production of *Rhizophydium* sp. (AUS6) in 1/2 YpSs. (a) Biomass remained relatively low with the exception of day 14 where there was three-fold increase followed by a drop on day 18. (b) % change in zoospore production was negative on day 4 with a peak 240% increase from the original inoculum on day 7. A secondary peak was observed on day 14.

Enhanced maturation of AUS6 in CSM and 1/2 YpSs compared to mCSM in the first 24 h of inoculation indicate the use of exogenous energy source by germinating AUS6 thalli. Maturation of individual AUS6 sporangia was enhanced by the availability of exogenous carbon and nitrogen. Initial establishment of the colony in the presence of exogenous carbon and nitrogen was slow but in the long-term, day 7-18, surpassed the yields of a nutrient poor medium. Complex carbon and

release indicate the composition of the colony to be either primarily of young sporangia or spent sporangia. If the next harvest, within 72 h, indicated high zoospore release this would suggest the former to be true. In the absence of exogenous carbon and nitrogen in this closed system, it is predicted that overall reduction in biomass and fertility will occur over an extended period of time.
predicted that the aggregation of AUS6 thalli mucopolysaccharide but is likely to contain glycoprotein and extracellular matrix is not known at this stage, wall is being produced. The composition of the periods, such as rhizoid elongation and part AUS6 may have a similar role. This function is microbial infection. The extracellular matrix on the surface of rhizoid thalli. The presence of an extracellular matrix entangled in the rhizoid branches of mature colonies. Released zoospores become aggregate thalli and facilitate the formation of rhizoids of AUS6, and possibly other chytrids, made in this study in culture, suggests nutrient uptake the structure beyond that of anchorage and rhizoids support the variable functionality of colony dynamics.

The survival and development of zoospores entangled in amongst the finely branched rhizoids support the variable functionality of the structure beyond that of anchorage and nutrient uptake (Sparrow 1960). Observations made in this study in culture, suggest that rhizoids of AUS6, and possibly other chytrids, aggregate thalli and facilitate the formation of colonies. Released zoospores become entangled in the rhizoid branches of mature thalli. The presence of an extracellular matrix on the surface of rhizoids and sporangial cell wall facilitates aggregation. The matrix lacks organelles and has been shown to entangle proximal thalli. Similar fibrillar material extensions have been observed on the thalli of Chytridium sphaeroteca and Monoblepharella sp. (Held 1974, Powell 1982, 1994, Shields & Fuller 1996). The extracellular matrix on the surface of Rozella allomycis, Allomyces javanicus and Rhizophyldium sp. is thought to inhibit microbial infection. The extracellular matrix in AUS6 may have a similar role. This function is particularly important during vulnerable periods, such as rhizoid elongation and sporangial enlargement, when nascent cell wall is being produced. The composition of the extracellular matrix is not known at this stage, but is likely to contain glycoprotein and mucopolysaccharide (Powell 1994). It is predicted that the aggregation of AUS6 thalli will buffer developing thalli within the colony from external environmental factors by forming a stable microclimate. Similar matrices and functions have been identified in yeast. Saccharomyces cerevisiae for example forms fluffy structured colonies which consist of cells embedded in a matrix network (Kuthan et al. 2003). Candida species also have an extracellular matrix present in the colony which contains cross-linked carbohydrates (Joshi et al. 1975).

Mature AUS6 colonies also contain large structures that appear to be enlarged mature sporangia containing encysted zoospores or immature sporangia. Similar large structures have been noted in Batrachochytrium dendrobatidis (Berger 2005). The surface of these structures is composed of layers of extracellular membrane. The deposition of these layers is likely to have occurred during colony formation. The matrix is distinct in structure from that previously described on the surface of rhizoids and immature sporangia and whether it has similar functionality is not clear. It would be interesting to compare the function and composition of these matrices to microbial matrices. Morphological studies on chytrids have been largely based on the life cycle of the thallus however it is important also to consider the morphology of the colony. Like yeast and other filamentous fungi the colony has additional functions that allow the species to survive. Chytrids are found in a diversity of habitats and therefore determining whether chytrids form colonies and if so how a colony functions in those habitats is important.

References


