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The effects of antifungal substances on some zoosporic fungi (Kingdom Fungi)

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Abstract Zoosporic fungi constitute a large group of true fungi which inhabit freshwater, brackish, marine and soil ecosystems. In general, very little is known about the effects of antifungal substances on the growth and survival of most species. This review focuses on experimental research with those isolates which have been studied, especially in some species of *Synchytrium*, *Olpidium*, *Batrachochytrium*, *Allomyces*, *Blastocladiella*, *Neocallimastix*. These genera represent genetically diverse groups. Although the research discussed here is restricted to a small sample, some general conclusions can be reached about zoosporic fungi as a whole. Like many other eukaryotic microorganisms, zoosporic fungi are sensitive to a large number of antibiotics, fungicides, surfactants, bacterial metabolites, metabolic poisons, proteins, heavy metals and other antifungal substances. These include substances commonly released into the environment for the control of plant and animal diseases, for increasing production of domestic animals and in the form of waste products from industry. It is possible that the release of antifungal substances into the environment might cause significant changes in the community structure of zoosporic fungi as well as of other groups of microorganisms which play significant roles in food web dynamics and ecosystem complexity. However, this needs documentation by quantitative studies. For these reasons, extensive research on the effects of antifungal substances is much needed.

Keywords *Synchytrium* · *Olpidium* · *Batrachochytrium* · *Neocallimastix* · Antibiotics · Fungicides · Ecological implications

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Introduction

For a long time, antifungal substances have frequently been used as tools in experimental mycology (Betina, 1985). Most of the research in this field has involved higher fungi, particularly pathogens and only occasionally has included zoosporic fungi. For example, cytochalasins, monensin and unsaturated carbonyl compounds have been used in developmental studies with *Allomyces macrogynus* and *A. arbuscula* (Sewall et al., 1986; Larsen et al., 1992; Nguyen Thi & Turien, 1993). Matsumae & Cantino (1971) used a large variety of these substances in experimental research on metabolic processes in

A. macrogynus, *Blastocladiella britannica, B. emersonii* and *B. cystogenes*.

Since their invention, fungicides and herbicides have commonly been applied to soils in agricultural practices. Fungicides are used to control plant diseases caused by true fungi [including zoosporic fungi (Fletcher et al., 2005; Widmer, 2006)] and stramenopiles, while herbicides are used to control herbaceous weeds. However, some herbicides, such as 2,4-D, diquat and paraquat, can also be toxic to fungi (Moubasher et al., 1981; Sahid et al., 1981). It is possible that both classes of chemicals as well as other kinds of antifungal substances may accumulate in the environment and target organisms other than those intended (Rodriguez-Kabara & Curl, 1980). For this reason, the importance of accumulation of antifungal substances in the environment is being reconsidered (Kim & Carlson, 2006).

Furthermore, possible roles of antifungal substances in microbial communities are being investigated (Weber et al., 2005). If high concentrations of antifungal substances are found in the environment any changes in species composition will need to be monitored carefully, because this could presumably alter the dynamics of the food webs and might lead to harmful consequences in the ecosystem. Therefore, this area of research is extremely important for our understanding of the ecological processes in general.

Recently, there has been renewed interest in antifungal substances because of their potential use in antifungal therapy in both human and veterinary diseases (Selitennikoff, 2001; Bishop et al., 2009; Vincente et al., 2009; Zairi et al., 2009). For example, the dermal glands of frogs produce large amounts of biologically active peptides and antimicrobial peptides such as magainins and dermaseptins S, which are similar to mammalian hormones. Most of these peptides exhibit antibiotic, fungicidal, virucidal and tumoricidal activities with a low cytotoxicity towards mammalian cells (Zairi et al., 2009). Clinical needs for novel antifungal agents have risen with the increasing incidence of emerging infectious diseases, and for that reason studies on mechanisms of action of antifungal agents have progressed rapidly (Odds et al., 2003; Durán et al., 2007). In addition, currently antifungal agents are being considered for the control of chytridiomycosis in amphibians which is caused by the zoosporic fungus *Batrachochytrium dendrobatidis* (Bishop et al., 2009).

In this review, we define antifungal substances as chemical compounds which negatively impact one or more stages of the life cycle of a fungus. Antifungal substances include antibiotics, fungicides, surfactants, bacterial metabolites, metabolic poisons, proteins, heavy metals and other chemicals. It is difficult to classify these substances because of overlap between categories. These substances can be either fungicidal, i.e. they lead to the death of fungi, or they can be fungistatic, i.e. they inhibit the growth of fungi.

This review will be focused on those antifungal substances which are known to negatively impact the growth and survival of zoosporic fungi. Most published studies on antifungal substances involve filamentous fungi and yeasts (terrestrial fungi), but there has also been some significant research with a few genera of zoosporic fungi. These include primarily plant and animal pathogens, a few saprophytic fungi and mutualistic rumen fungi. The effects of antifungal substances on the growth and survival of most other genera of zoosporic fungi are unknown.

A large number of species of zoosporic fungi are commonly found growing on substrates in marine, brackish, freshwater and soil ecosystems (Sparrow, 1960; Powell, 1993; Barr, 2001; Shearer et al., 2007). The zoospore of true fungi is characterized by a single posteriorly directed whiplash flagellum. Most of these fungi are currently placed into three phyla (Blastocladiomycota, Chytridiomycota and Neocallimastigomycota), while other genera such as *Olpidium* and *Rozella* have not yet been assigned to any phyla (James et al., 2006). The roles of these fungi in their environments are just beginning to be understood (Gleason et al., 2008).

Data on the effects of antifungal substances on zoosporic fungi are scattered throughout the literature and have never been reviewed. The main aim of the this review is therefore to provide valuable information necessary for future research into this group of fungi. We will discuss the effects of a wide range of antifungal substances on the growth and survival of *Synchytrium* and *Olpidium* (plant pathogens), *Batrachochytrium* (a vertebrate pathogen), *Neocallimastix* and other rumen fungi (symbionts in the digestive systems of herbivorous mammals) and two saprotrophic zoosporic fungi, *Allomyces* and *Blastocladiella* (Table 1). We will focus on experimental research with some zoosporic fungi rather than

technical reports on the diseases that they may cause. Then, we will consider the potential effects of the use of antifungal substances for the control of diseases on the ecology of non-pathogenic zoosporic fungi present in the same environment. Finally we will explore the possible roles of antifungal substances in interactions among species.

Plant pathogens

Three genera of zoosporic fungi are known to be common pathogens of flowering plants, *Olpidium*, *Synchytrium* and *Physoderma* (Powell, 1993; Barr, 2001). Methods for control of the economically important diseases caused by these fungi are recommended in the literature provided by the manufacturers of fungicides and in technical reports. Some basic research on the effects of fungicides on these fungi is discussed below.

Synchytrium is a common parasite of potatoes and a few other vegetable crops (Karling, 1977; Powell, 1993). Widmer (2006) studied the effect of five fungicides on the release of active zoospores in *Synchytrium solstitiale*, a pathogen of *Centaurea solstitialis*, the yellow starthistle. Because no mycelium is produced and this fungus cannot be grown in culture apart from the host, active zoospore release was chosen as a method for testing survival and viability. Five fungicides (cycloheximide, benomyl, iprodione, mancozeb, and propionic acid) were chosen because they presumably represent different classes of fungicides with different modes of action. Infected leaf segments were placed in wells containing test solutions with fungicides. After incubation at 5°C for 24 h, the motility of newly released zoospores was tested under the microscope. Leaf segments were then washed in the test solution without fungicides and incubated at 10°C for 24 h. The motility of newly released zoospores was again observed. Streptomycin was present in all experiments to prevent the growth of bacteria. Cycloheximide, benomyl, iprodione, mancozeb and propionic acid all either prevented or inhibited the release of actively swimming zoospores at some concentrations either before and/or after the rinse. In some experiments, non-motile zoospore cysts were released, but Widmer (2006) concluded that these progagules were unable to infect healthy plants because no symptoms of disease appeared. Cycloheximide, benomyl and mancozeb proved to be particularly effective as fungicides at the dosages tested. Benomyl is commonly used to plant control diseases caused by true fungi and stramenopiles (e.g. Nan et al., 1992; Abdelzaher et al., 2004).

The roots of many host plant species contain a large number of resting spores of *Olpidium* which can persist in a viable state in the soil for years (Tomlinson & Faithfull, 1979). Lettuce big-vein disease is spread by the release of actively swimming zoospores of *Olpidium brassicae* into the soil, subsequent infection and growth of the thallus within susceptible host plants. *O. brassicae* zoospores function as vectors by carrying the lettuce big-vein virus (LBVV) which causes the disease. Zoospores are known to carry other viruses as well (Fletcher et al., 2005). Control of this disease has been directed at elimination of this fungus from the soil (Tomlinson & Faithfull, 1979).

Tomlinson & Faithfull (1979) tested boron, copper and zinc salts and carbendazim as fungicides to kill zoospores. Although all of these substances appeared to be toxic to zoospores at high concentrations, their application for disease control may not be practical due to the cost of application. Campbell et al. (1980) tested the effects of fenaminosulf, metalaxyl, pyroxychlor, captan, ethazole, triadimefon and benomyl on zoospore motility, infectivity and viability (subsequent growth and maturation of the thallus in vivo). The patterns of the responses differed but all of the seven fungicides caused at least some inhibition in one of more of the stages in the life cycle of *Olpidium*. Benomyl presumably affects the initial transfer of the virus from the fungus to the host cytoplasm.

Fletcher et al. (2005) tested six fungicides for controlling the spread of viral diseases caused by *Olpidium* but did not test the effects specifically on zoospores. Application of the antiviral agent ribavirin had no effect on zoospores but did reduce the severity of big vein of leaf in lettuce (Campbell, 1980). According to Fletcher et al. (2005) ribavirin interferes with the synthesis of big-vein virus, since this effect has been reported with other plant virus (Hansen, 1979; Shepard, 1977).

Tomlinson & Faithfull (1979) also studied the effects of various surfactants on the motility of zoospores of *O. brassicae*. The purpose of their study was to determine the methods of transmission of the

Table 1 Some antifungal substances which inhibit or kill zoosporic fungi

Genus	Name of substance	Reference
Synchytrium solstitiale	Benomyl	Widmer (2006)
	Cycloheximide	–
	Iprodione	–
	Mancozeb	–
	Proprionic acid	–
Olpidium brassicae	Boron salts	Tomlinson & Faithfull (1979)
	Copper salts	–
	Zinc salts	–
	Carbendazim	–
	Fenaminosulf	Campbell et al. (1980)
	Metalaxyl	–
	Pyroxychlor	–
	Captan	–
	Ethazole	–
	Triadimefon	–
	Benomyl	–
	Methyl bromide	–
	Deciquam	Stanghellini & Miller (1997)
	Ethylan CPX	–
	Hyanide 1622	–
	Manoxol/OT	–
	Agral	–
	Centrimide	–
Batrachochytrium dendrobatidis	Violacein	Brucker et al. (2008b); Harris et al. (2009)
	2,4-diacetylphloroglucinol	Brucker et al. (2008a)
	Chloramphenicol	Bishop et al. (2009)
	Itraconazole	Garner et al. (2009)
	Temporin A	Rollins-Smith et al. (2003)
	Ranatuerin-2 Ma	Woodhams et al. (2007); Rollins-Smith et al. (2002a); Rollins-Smith et al. (2002c)
	Ranatuerin-2 Mb	–
	Esculentin-1	Rollins-Smith et al. (2002a)
	Esculentin-2	–
	Brevinin-2	–
	Palustrin-3	–
	Ranalexin	–
	Temporin-1 M	–
	Mangainin I	Rollins-Smith et al. (2002b)
	Mangainin II	–
	Ranalexin	–
	CPF	–
	PGLa	–
	Dermaseptin	–
	Caerin 1.9	Woodhams et al. (2006)
	Caerin 1.1	Berger (2001)
	Maculatin 1.1	–
	Bradykinin	Rollins-Smith et al. (2006)

Table 1 continued

Genus	Name of substance	Reference
Rumen Fungi	Monensin	Gordon & Philips (1998)
	Tetronasin	–
	Salinomycin	–
	Lasalocid	Stewart et al. (1987)
	Cycloheximide	–
	Narasin	Marounek & Hodrová (1989)
	Nitrovin	–
	Chitinase	Morgavi et al. (1994)
	Anticellulase Protein	Bernalier et al. (1993)
	Penthaclorophenol (PCP)	Hodrová & Marounek (1991)
Allomyces macrogynus	Chloramphenicol	Matsumae & Cantino (1971)
Blastocladiella britannica	Tetracycline	–
B. cystogena	Cycloheximide	–
B. emersonii	Nystatin	–
B. simplex	Trichomycin	–
	Endomycin	–

melon necrotic leaf spot virus. Agral, Centrimide, Deciquam, Ethylan CPX, Hyamine 1622, Manoxol/OT and lauryl sulphate were all toxic to zoospores at concentrations of from 1–10 μg/ml. Agral acts as an effective fungicide on this fungus. Tomlinson & Thomas (1986) continued with a more intensive study using Agral to kill zoospores of *O. radicale* at concentrations up to 20 μg/ml. Surfactants are known to destroy the permeability of cell membranes of zoospores of at least some species (Stanghellini & Miller, 1997).

Animal pathogens

Chytridiomycosis is an emerging infectious disease of amphibians, and this disease has been linked to significant declines in populations of many amphibian species worldwide (Rollins-Smith et al., 2003; Rollins-Smith & Conlon, 2005). The causative agent for this disease is the zoosporic fungus *B. dendrobatidis* (Rhizophydiales, Chytridiomycota) (Longcore et al., 1999).

A family of antimicrobial peptides (e.g. ranatuerin-2 Ma, 2 Mb, caerin I, temporin A and temporin-1 M) produced in granular glands in the dermal layer of many amphibians are highly effective inhibitors of infection by zoospores of this fungus (Berger, 2001; Rollins-Smith et al., 2002a, b, c, 2003, 2006; Rollins-Smith & Conlon, 2005; Woodhams et al., 2006, 2007). These peptides may provide some immunity to chytridiomycosis in amphibians. Rollins-Smith et al. (2003) tested temporin A and structurally related peptides produced in amphibian dermal granular glands and in wasp venom for inhibition of growth of *B. dendrobatidis*. Of these peptides two natural amphibian temporins, a wasp temporin, and six synthetic analogs effectively inhibited growth. The mechanism of action against the fungus involves the attachment to the membrane and finally its disruption by the folding of the temporins into an α-helical structure.

Natural microbiota appeared to be important in preventing disease in amphibians. Recently, Brucker et al. (2008b) and Harris et al. (2009) observed that the bacterium *Janthinobacterium lividum* isolated from the skin of the red-backed salamander produced the anti-fungal metabolite violacein. Violacein inhibited the growth of *B. dendrobatidis* at low concentrations in the laboratory. Brucker et al. (2008a) observed that another anti-fungal metabolite, 2,4-diacetylphloroglucinol, which is produced by *Lysobacter gummosus* and which is also found on the skin of the red-backed salamander, similarly inhibited the growth of this pathogenic chytrid. These two metabolites are produced by normal bacterial flora on the skin of this salamander and may play an important role in resistance to this disease.

Garner et al. (2009) tested itraconazole as a treatment for chytridiomycosis in larval frogs. The infection was cleared with low doses of this

antifungal substance, but depigmentation of the tadpoles was observed. Bishop et al. (2009) tested a method designed to remove *B. dendrobatidis* from the skin of adult frogs. This method involved the topical application of chloramphenicol. After treatment, the symptoms of chytridiomycosis disappeared and the infected frogs recovered.

Rumen fungi

Ionophores and other antibiotics have often been added to the feeds for cattle and sheep in order to improve production by inhibiting the growth of bacteria in the digestive system (Marounek & Hodrová, 1989). Ionophores are known to disrupt membrane function in fungi (Weete et al., 1989).

Populations of obligately anaerobic rumen fungi, bacteria and protozoa are universally present in the rumen and hindgut of herbivorous mammals (Trinci et al., 1994). Marounek & Hodrová (1989) tested the susceptibility of isolates in three genera of anaerobic rumen fungi, *Neocallimastix frontalis, Piromonas communis* and *Sphaeromonas communis,* to 14 antimicrobial feed additives by measuring substrate utilization. In all isolates the utilization of glucose was depressed by monensin, lasalocid, salinomycin and narasin (ionophores) and by nitrovin (a nitrofurone derivative). Unlike the other isolates *Piromonas communis* was also sensitive to nourseothricin, virginiamycin and avilamycin. All isolates were resistant to quinoxaline derivatives, avoparcin, bacitracin, tylosin and aureomycin at the dosages tested.

In general, pure cultures of all rumen fungi tested in laboratory studies have been found to be resistant to some antibiotics such as penicillin, streptomycin, avoparcin, bacitracin, tylosin, aureomycin and virginiomycin (Joblin, 1981; Marounek & Hodrová, 1989; Williams et al., 1994; Dehority & Tirabasso, 2000). In contrast, rumen fungi are very sensitive to ionophores, such as monensin, tetronasin, salinomycin, narasin, lasalocid, and cycloheximide both in pure culture and in the rumen, and research data indicate that these substances strongly inhibit the growth of rumen fungi at the dosages tested (Stewart et al., 1987; Phillips & Gordon, 1992; Gordon & Phillips, 1998; Dehority & Tirabasso, 2000).

In 16 strains of rumen fungi belonging to *N. frontalis*, *Neocallimastix joyonii, Piromonas communis* and *Sphaeromonas communis* the utilization of glucose and visible production of fungal biomass were depressed by pentachlorophenol but not by pentachlorobiphenyl (Hodrová & Marounek, 1991). The effect of pentachlorophenol on rumen fungi is related to its role as an uncoupler of electron transport and as a protonophore (Hodrová & Marounek, 1991). These substances are commonly used as component of paints and hydraulic liquids, wood preservatives, herbicides, insecticides and fungicides and are known to be toxic environmental pollutants.

Chitinases are extracellular enzymes produced by bacteria and protozoa in the rumen which are capable of digesting the cell walls of rumen fungi (Morgavi et al., 1994). It is unclear whether chitinases significantly reduce the number of viable fungi in the rumen. Bacterial and protozoan chitinases are known to digest fungal chitin outside the rumen under laboratory conditions (Morgavi et al. 1994). Some zoosporic fungi are parasites on other zoosporic fungi (hyperparasitism) (Sparrow, 1960) and presumably excrete chitinases during colonization of their hosts. The impacts of chitinases released into the environment on cell walls of living fungi are unknown.

Bernalier et al. (1993) detected an antagonistic factor produced by *Ruminococcus flavefaciens* which inhibits the ability of *N. frontalis* to hydrolyse cellulose but does not affect growth. This factor was partially characterized by Bernalier et al. (1993). Their data indicate that this factor is an extracellular protein but not a bacterial cellulase and probably not a bacterial protease. According to Bernalier et al. (1993) this protein presumably inhibits the activity of fungal cellulases in some way. However, the mechanism by which the bacterial protein inhibits fungal cellulases was not elucidated. Extracellular cellulases are released by rumen fungi into the rumen to facilitate the process of digestion of plant fibre (Trinci et al., 1994).

Joblin & Naylor (1993) studied the effect of bacterial fermentation products (formate, lactate, malate, ethanol, succinate, hydrogen, acetate, propionate and butyrate) on cellulose degradation by rumen fungi. Formate, lactate, ethanol and hydrogen, strongly inhibit growth particularly at high concentrations. Inhibition by acetate and malate also was documented but butyrate and propionate had no inhibitory effect. Since hydrogen production is a measure of fungal growth (Joblin & Naylor, 1989),

they found that hydrogen production together with pH correlated with changes in the solubilization of cellulose. Thus, fermentation products probably inhibit fungal growth rather than the expression or activity of cellulases (Joblin & Naylor, 1993).

Saprotrophic zoosporic fungi

Matsumae & Cantino (1971) tested the growth of wild types of *Allomyces macrogynous, B. britannica*, *B. cystogena*, *B. emersonii* and *B. simplex* and six mutants of *B. emersonii* on solid media with 48 antibiotics and other antifungal agents using zoospores as inocula. Penicillin G, oxacillin, phenethicillin, methicillin, viomycin, kanamycin, crestomycin, streptovaricin, leucomycin A, spiramycin, oleandomycin, erythromycin, and streptomycin appeared not to inhibit the growth of *Allomyces* or *Blastocladiella* (Matsumae & Cantino, 1971), however chloramphenicol, tetracycline, cycloheximide, nystatin, trichomycin, endomycin and blasticidin S were inhibitory (e. g. inhibited membrane function, mitochondrial protein synthesis or cell-wall synthesis). Differences in responses to some antibiotics were observed among the four species of *Blastocladiella*. The degree of antibiotic sensitivity appears to be species or isolate specific at the dosages tested. Matsumae & Cantino (1971) presented some evidence for different responses to antibiotics by different isolates. They also suggested that meiospores and mitospores of *Allomyces* differed in their responses to several antibiotics. However these are preliminary observations which need further research.

The DNA and RNA polymerase inhibitors, mitomycin C and actinomycin S, both inhibit the growth of *Allomyces* or *Blastocladiella* slightly (Matsumae & Cantino, 1971).

Like most other eukaryotic cells, respiration in *Allomyces* is sensitive to cyanide, a terminal oxidase inhibitor. Cyanide resistant pathways (SHAM sensitive pathways) are present as well since salicylhydroxamic acid also inhibits respiration (Heldt-Hansen et al., 1983). The cyanide sensitive pathway appears to be missing in the facultative anaerobe *Blastocladia* (Natvig & Gleason, 1983). Cyanide is produced naturally by many microorganisms including fungi in the soil (Knowles, 1976). In addition, *Allomyces* is sensitive to the antibiotic antimycin A, an electron transport inhibitor, and the metabolic poison rotenone. Matsumae & Cantino (1971) documented that in addition to *Allomyces*, four species of *Blastocladiella* were also sensitive to antimycin A.

Different stages of the life cycle of *Allomyces* were inhibited by five α, β unsaturated carbonyl compounds [four α, β unsaturated lactones (patulin, penicillic acid, parascorbic acid and tulipalin) and the naphtoquinone plumbagin]. Patulin and penicillic acid are produced by species of *Penicillium* and *Aspergillus* (Larsen et al., 1992).

Some herbicides can also be considered to be antifungal substances. The effect of metflurazon, a pyridazinone herbicide, on pigmentation in zoosporic fungi was studied by Vincent & Powell (1988). Metflurazon decreased visible pigmentation and altered pigment composition in two zoosporic fungi, *Rhizophlyctis rosea* and *Allomyces javanicus*, and in several higher fungi. This herbicide is known to inhibit carotene biosynthesis. The role of carotenoid pigments in zoosporic fungi is unknown.

Antifungal substances and competition

Some of the antifungal substances discussed previously can be produced naturally by microorganisms during interactions between species (chemical warfare). We will consider two examples here: (1) bacteria and protozoa compete with zoosporic fungi for food resources in the rumen. Bacteria and protozoa release chitinases into the rumen fluid which are capable of digesting fungal cell walls and, therefore, possibly limit the population size of viable zoosporic fungi (Morgavi et al., 1994). Proteins produced by bacteria inhibit fungal cellulase activity in the rumen (Bernalier et al., 1993). (2) Zoospores of the *B. dendrobatidis* compete for space with the normal bacterial flora on the skin of amphibians. Motile chemotactic zoospores reach the surface of the skin and attach quickly, but bacteria on the skin release antifungal substances which inhibit growth of this fungus (Harris et al., 2009). The production of the antifungal substance, violacein, by bacteria on the skin of amphibians is a clearly documented example (Brucker et al., 2008b).

Many isolates of soil bacteria especially from the genus *Streptomyces* and other related genera (Actinobacteria) are known to produce a large

number of antibiotics (Watve et al., 2001). Since some of these antibiotics, such as cycloheximide, violacein and chloramphenicol, also inhibit the growth of zoosporic and other fungi, they can, therefore, be classified as antifungal substances. Cyanide is also produced by many soil bacteria (Knowles, 1976). Furthermore, a number of soil bacteria, such as *Pseudomonas aeruginosa*, are known to produce and excrete rhamnolipids (Stanghellini & Miller, 1997). These compounds are naturally occurring biosurfactants which cause the lysis of fungal zoospores. The mechanisms of action of surfactants are discussed by Stanghellini & Miller (1997).

Willoughby (1983a, b) studied the interaction between *R. rosea* and bacteria on cellulosic baits. Willoughby never isolated either antifungal or antibacterial substances produced by these microorganisms but the results of his research suggest that chemical warfare between zoosporic fungi and other microorganisms is an important determinant of population levels, but this remains to be proven.

Zoosporic fungi are considered to be primary colonizers of fibrous plant material (Sparrow, 1960; Edwards et al., 2008). Pollen grains are often used as baits for zoosporic fungi because they are excellent food resources (Sparrow, 1960; Barr, 1987). A variety of different species are usually observed growing on pollen grains placed in a Petri dish with water and soil. Because all zoospores are actively motile and at least some are chemotactic (Mitchell & Deacon, 1986) we expect that they would reach the uncolonized baits relatively quickly. Species which produce larger numbers of zoospores would be expected to colonize a larger number of pollen grains. However, the zoospores of different species of zoosporic fungi, stramenopiles and bacteria which reach the pollen grains simultaneously must compete for space on the surface of the pollen grains along with other microorganisms. The food resources in the cell walls and within cytoplasm are subsequently digested by extracellular enzymes released by rhizoids of zoosporic fungi.

The dynamics of initial colonization of perennial ryegrass by rumen fungi has been studied by Edwards et al. (2008). It differs from that of bacteria, and is primarily mediated by the time taken for fungal zoospores to locate, attach and encyst on plant material. Six genera of rumen fungi have been identified in the rumen and hindgut of various mammals (Kown et al., 2009). The rhizoids of these fungi penetrate the fibre matrix and release a large number of extracellular enzymes. Some of the functional genes involved in this process have been analysed in *N. frontalis* (Kown et al., 2009).

Are antifungal substances involved in these processes? This is suggested by some preliminary evidence as previously discussed, but the roles of antifungal substances in interactions between fungal and bacterial species need thorough investigation.

Isolation of zoosporic fungi into pure culture

Some antibiotics, such as penicillin and streptomycin, which inhibit the growth of bacteria, have been used frequently during isolation of zoosporic fungi into pure culture (Barr, 1987; Joblin, 1981). Although it has been assumed that these antibiotics do not affect the growth of zoosporic fungi, Barr (1987) urges caution. Before the use of antifungal substances for isolation into pure culture is attempted, a thorough knowledge of their effects on the target zoosporic fungi and the other microorganisms present is mandatory.

If penicillin and streptomycin are effective in inhibiting the growth of bacteria, zoosporic fungi can grow at a faster rate than bacteria. However, other groups of fungi and stramenopiles can also grow faster. Other antibiotics, such as chloramphenicol, may inhibit the growth of zoosporic fungi as well as bacteria (Matsumae & Cantino, 1971) and therefore should not be used during isolation. After isolation the antifungal substances should be removed from the cultures as quickly as possible as they could have undesirable long term effects. Furthermore, the studies by Matsumae & Cantino (1971) suggest that at least some antibiotics can cause genetic changes in zoosporic fungi. Therefore, care should be taken to prevent the release of these genetically modified isolates into the environment.

Ecological implications

If antibiotic substances are released into terrestrial and aquatic environments and if they accumulate, we would expect these substances to have inhibitory effects on zoosporic fungi at high concentrations.

Some of these substances are used to control plant and animal diseases caused by zoosporic fungi. Other antifungal substances may be released into the environment in agricultural, industrial and municipal wastes. Kim & Carlson (2006) have recently developed methods for measurement of the accumulation of ionophore antibiotics (monensis, salinomycin and narasin) in the environment and have presented data from three sites in Colorado. The substances can be used as markers for other antifungal substances.

Many zoosporic fungi play important roles in both aquatic and soil ecosystems. These include decomposition of plant material in detritus (Sparrow, 1960; Gleason et al., 2008), natural regulation of population sizes of invertebrates and phytoplankton and food resources for grazing zooplankton and filter feeders in aquatic ecosystems (Kagami et al., 2007; Gleason et al., 2008). We predict that many, if not all, zoosporic fungi are sensitive to the antifungal substances discussed in the present review. Therefore, it is possible that the accumulation of antibiotic and antifungal substances in the environment might cause significant changes to the species composition, density and abundance in communities of zoosporic fungi. Furthermore, the presence of these substances might stimulate the development of resistance to antifungal substances in microorganisms. The measurement of inhibition of growth of zoosporic fungi by antifungal substances at levels present in the environment awaits further research.

Conclusions

The studies published on the effects of antifungal substances on zoosporic fungi have focused primarily on plant and animal pathogens and rumen fungi with only a few exceptions such as the study by Matsumae & Cantino (1971) on antibiotics, the study by Heldt-Hansen et al. (1983) on respiration, the study by Vincent & Powell (1988) on pigmentation and the studies by Sewall et al. (1986) and Nguyen et al. (1993) on gametogenesis. The studies with plant pathogens were conducted to explore the potential uses of fungicides to control plant diseases. The studies with rumen fungi were conducted to remove zoosporic fungi from the rumen in order to determine the role of these fungi in fibre digestion and ultimately to find methods to increase animal production. The studies with *B. dendrobatidis* were part of research designed to understand natural mechanisms in amphibians for resistance to chytridiomycosis.

Four significant issues arise from our review of the effects of antifungal substances on zoosporic fungi based on published research. First, only a few species have been studied, although they represent genetically diverse groups. Second, the techniques, ranges in concentrations of antifungal substances and species used in each study are so very different that comparisons of the effects of dosage levels between antifungal substances are often not possible. Third, for the same reason, quantitative comparisons of the response to antifungal substances between different taxonomic groups is not possible. Fourth, the research on zoosporic fungi currently in progress is limited. In general, very little is known about the effects of antifungal substances on zoosporic fungi as a group because of the limited amount of data available. Nonetheless, we suggest that all zoosporic fungi may respond to antifungal substances in ways similar to other groups of fungi and other eukaryotic microorganisms. All available evidence suggests that zoosporic fungi are not unique in this respect.

Some differences in the sensitivity of different isolates of zoosporic fungi to different antifungal substances have been observed under identical conditions, but remain to be carefully documented. Whether antifungal substances can be used to target specific groups of zoosporic fungi and not negatively impact other groups is not known. Also, it is not known if particular stages in the life cycle of zoosporic fungi can be targeted, although preliminary evidence with *Allomyces* and *Olpidium* suggests that this may be possible.

The mechanisms of action of antifungal substances are often known from research with other groups of prokaryotic and eukaryotic microorganisms. We can only assume that the mechanisms of action are similar in all groups of eukaryotic microorganisms including fungi because such a genetically diverse range of organism has been studied (Odds et al., 2003).

In conclusion, preliminary research suggests that the growth and survival of many zoosporic fungi are probably significantly impacted by a large number of antifungal substances which are released into

the environment. The nature and magnitude of the responses of zoosporic fungi to antifungal substances await careful quantitative studies. Before antifungal substances can be used to control plant and animal diseases, the ecological consequences must be carefully considered. Because of the relative lack of knowledge of the effects of antifungal substances on growth and survival of zoosporic fungi, considerable further research is urgently needed. The recent concern about the spread of chytridiomycosis among amphibians highlights the importance of the issues discussed in this review. This review provides a summary of much of the significant research on the effects of antifungal substances on zoosporic fungi. Hopefully, this will be used as a basis for future studies.

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References

Abdelzaher, H. M. A., M. A. Shoulkany & M. M. Yasser, 2004. Effect of benomyl and metalaxyl on reproduction of the plant parasite (*Pythium deliense*) and the mycoparasite (*P. oligandrum*). Archives of Phytopathology and Plant Protection 37: 307–317

Barr, D. J. S., 1987. Isolation, culture and identification of Chytridiales, Spizellomycetales and Hyphochytriales. In Fuller, M. S. & A. Jaworski (eds), Zoosporic fungi in teaching and research. Southeastern Publishing Corporation, Athens: 118–120.

Barr, D. J. S., 2001. Chytridiomycota. In McLaughlin, D. J., E. G. McLaughlin & P. A. Lemke (eds), The Mycota, Vol VII. Part A. Systematics and Evolution. Springer-Verlag, New York: 93–112.

Berger, L., 2001. Diseases in Australian frogs. PhD thesis, James Cook University, Townsville.

Bernalier, A., G. Fonty, F. Bonnemoy & P. Gouet, 1993. Inhibition of the cellulolytic activity of *Neocallimastix frontalis* by *Ruminococcus flavefaciens*. Journal of General Microbiology 139: 873–880.

Betina, V., 1985. Antifungal agents as tools in experimental mycology. Folia Microbiologia 30: 80–89.

Bishop, P. J., R. Speare, R. Poulter, M. Butler, B. J. Speare, A. Hyatt, V. Olsen & A. Haigh, 2009. Elimination of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* by Archey's frog *Leiopelma archeyi*. Diseases of Aquatic Organisms 84: 9–15.

Brucker, R. M., C. M. Baylor, R. L. Walters, A. Lauer, R. N. Harris & K. P. C. Minbiole, 2008a. The identification of 2, 4-diacetylphloroglucinol as an antifungal metabolite produced by cutaneous bacteria of the salamander *Plethodon cinereus*. Journal of Chemical Ecology 34: 39–43.

Brucker, R. M., R. N. Harris, C. R. Schwantes, T. N. Gallaher, D. C. Flaherty, B. A. Lam & K. P. C. Minbiole, 2008b. Amphibian chemical defense: antifungal metabolites of the microsymbiont *Janthinobacterium lividum* on the salamander *Plethodon cinereus*. Journal of Chemical Ecology 34: 1422–1429.

Campbell, R. N., 1980. Effects of benomyl and ribavirin on the lettuce big vein agent and its transmission. Phytopathology 70: 1190–1192.

Campbell, R. N., A. S. Greathead & F. V. Westerlund, 1980. Big vein of lettuce: infection and method of control. Phytopathology 70: 741–746.

Dehority, B. A. & P. A. Tirabasso, 2000. Antibiosis between ruminal bacteria and ruminal fungi. Applied and Environmental Microbiology 66: 2921–2927.

Durán, N., G. Z. Justo, C. V. Ferreira, P. S. Melo, L. Cordi & D. Martins, 2007. Violacein: properties and biological activities. Biotechnology and Applied Biochemistry 48: 127–133.

Edwards, J. E., A. H. Kingston-Smith, H. R. Jimenez, S. A. Huws, K. P. Skøt, G. W. Griffith, N. R. McEwan & M. K. Theodorou, 2008. Dynamics of initial colonization of nonconserved perennial ryegrass by anaerobic fungi in the bovine rumen. FEMS Microbiology Ecology 66: 537–545.

Fletcher, J. D., C. M. France & R. C. Butler, 2005. Virus surveys of lettuce crops and management of lettuce big-vein disease in New Zealand. New Zealand Plant Protection 58: 239–244.

Garner, T. W. J., G. Garcia, B. Carroll & M. C. Fisher, 2009. Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. Diseases of Aquatic Organisms 83: 257–260.

Gleason, F. H., M. Kagami, E. Lefèvre & T. Sime-Ngando, 2008. The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. Fungal Biology Reviews 2: 17–25.

Gordon, G. L. R. & M. W. Phillips, 1998. The role of anaerobic gut fungi in ruminants. Nutritional Research Reviews 11: 133–168.

Hansen, A. J., 1979. Inhibition of apple chlorotic leaf spot virus in *Chenopodium quinoa* by rivavirin. Plant Diseases Report 63: 17–20.

Harris, R. N., R. M. Brucker, J. B. Walke, M. H. Becker, C. R. Schwantes, D. C. Flaherty, B. A. Lam, D. C. Woodhams, C. J. Briggs, V. T. Vredenburg & K. P. Minbiole, 2009. Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME Journal 3: 818–824.

Heldt-Hansen, H. P., N. G. Grant & L. W. Olson, 1983. Respiration of gametangia of the aquatic phycomycete *Allomyces macrogynus*. Plant Physiology 73: 111–117.

Hodrová, B. & M. Marounek, 1991. Sensitivity of anaerobic rumen fungi to pentachlorophenol and pentachlorobiphenyl. Acta Veterinaria BMO 60: 335–338.

James, T. Y., P. M. Letcher, J. E. Longcore, S. Mozley-Standridge, D. Porter, M. J. Powell, G. W. Griffith & R. Vilgalys, 2006. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98: 860–871.

Joblin, K. N., 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in roll tubes. Applied and Environmental Microbiology 42: 1119–1122.

Joblin, K. N. & G. E. Naylor, 1989. Fermentation of woods by rumen anaerobic fungi. FEMS Microbiological Letters 65: 119–122.

Joblin, K. N. & G. E. Naylor, 1993. Inhibition of the rumen anaerobic fungus *Neocallimastix frontalis* by fermentation products. Letters in Applied Microbiology 16: 254–356.

Kagami, M., E. von Elert, B. W. Ibelings, A. de Bruin & E. van Donk, 2007. The parasitic chytrid, *Zygorhizidium*, facilitates the growth of cladoceran zooplankter, *Daphnia*, in cultures of the inedible alga, *Asterionella*. Proceedings of the Royal Society B 274: 1561–1566.

Karling, J. S., 1977. Chytridiomycetarum Iconographia. J. Cramer, Montecello.

Kim, S.-C. & K. Carlson, 2006. Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. Water Research 40: 2549–2560.

Knowles, C. J., 1976. Microorganisms and cyanide. Bacteriological Reviews 40: 652–680.

Kown, M., J. Song, J. K. Ha, H.-S. Parl & J. Chang, 2009. Analysis of functional genes in carbohydrate metabolic pathway of anaerobic rumen fungus *Neocallimastix frontalis* PMA02. Asian-Australasian Journal of Animal Science 22: 1555–1565.

Larsen, J., L. Lange & L. W. Olson, 1992. Mastigomycotina developmental sensitivity to α, β unsaturated carbonyl compounds. Journal of Phytopathology 134: 336–342.

Longcore, J. E., A. P. Pessier & D. K. Nichols, 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91: 219–227.

Marounek, M. & B. Hodrová, 1989. Susceptibility and resistance of anaerobic rumen fungi to antimicrobial feed additives. Letters in Applied Microbiology 9: 173–175.

Matsumae, A. & E. C. Cantino, 1971. Sensitivity of the spores of *Blastocladiella emersonii* and related fungi to antibiotics and some other drugs. The Journal of Antibiotics 23: 77–84.

Mitchell, R. T. & J. W. Deacon, 1986. Selective accumulation of zoospores of chytridiomycetes and oomycetes on cellulose and chitin. Transactions of the British Mycological Society 86: 219–223.

Morgavi, D. P., M. Sakurada, Y. Tomita & R. Onodera, 1994. Presence in rumen bacterial and protozoal populations of enzymes capable of degrading fungal cell walls. Microbiology 140: 631–636.

Moubasher, A. H., F. T. El-Hissy & M. I. A. Abdel-Kader, 1981. Selective effects of three herbicides on the fungus flora of Egyptian soil. Folia Microbiologica 26: 37–44.

Nan, Z. B., P. G. Long & R. A. Skipp, 1992. Prochloraz and benomyl drenches to assess the effects of fungal root pathogens on growth of red clover under field conditions. Australasian Plant Pathology 21: 98–103.

Natvig, D. O. & F. H. Gleason, 1983. Oxygen uptake by obligately-fermentative aquatic fungi: absence of a cyanide sensitive component. Archiv fur Microbiologie 134: 5–8.

Nguyen Thi, B. N. & G. Turien, 1993. Comparative inhibitory effects of cytochalasins on the gametangial differentiation in *Allomyces arbuscula*. Folia Microbiologia 38: 205–208.

Odds, F. C., A. J. P. Brown & N. A. R. Gow, 2003. Antifungal agents: mechanisms of action. Trends in Microbiology 11: 272–279.

Phillips, M. W. & G. L. R. Gordon, 1992. Fungistatic and fungicidal effects of the ionophores monensin and tetronasin on the rumen fungus *Neocallimastix* sp. LM1. Letters in Applied Microbiology 15: 116–119.

Powell, M. J., 1993. Looking at mycology with a Janus face: a glimpse at chytridiomycetes active in the environment. Mycologia 85: 1–20.

Rodriguez-Kabara, R. & E. A. Curl, 1980. Chemical control of plant diseases: problems and prospects. Annual Review of Phytopathology 31: 403–421.

Rollins-Smith, L. A. & J. M. Conlon, 2005. Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations. Developmental and Comparative Immunology 29: 589–598.

Rollins-Smith, L. A., C. Carey, J. E. Longcore, J. K. Doersam, A. Boutte, J. E. Bruzgal & J. M. Conlon, 2002a. Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. Antimicrobial agents and chemotherapy 26: 471–479.

Rollins-Smith, L. A., J. K. Doersam, J. E. Longcore, S. K. Taylor, J. C. Shamblin, C. Carey & M. A. Zasloff, 2002b. Antimicrobial peptide defenses against pathogens associated with global amphibian declines. Antimicrobial agents and chemotherapy 26: 63–72.

Rollins-Smith, L. A., L. K. Reinert, V. Miera & J. M. Conlon, 2002c. Antimicrobial peptide defenses of the Tarahumara frog, *Rana tarahumarae*. Biochemical and Biophysical Research Communications 297: 361–367.

Rollins-Smith, L. A., C. Carey, J. M. Conlon, L. K. Reinert, J. K. Doersam, T. Bergman, J. Silberring, H. Lankinen & D. Wade, 2003. Activities of temporin family peptides against the chytrid fungus (*Batrachochytrium dendrobatidis*) associated with global amphibian declines. Antimicrobial agents and chemotherapy 47: 1157–1160.

Rollins-Smith, L. A., D. C. Woodhams, L. K. Reinert, V. T. Vredenburg, C. J. Briggs, P. F. Nielsen & J. M. Conlon, 2006. Antimicrobial peptide defenses of the mountain yellow-legged frog (*Rana muscosa*). Developmental and Comparative Immunology 30: 831–842.

Sahid, I. B., A. J. E. Lyon & S. N. Smith, 1981. The effect of bipyridyl herbicides on the loss of nutrients from fungi. New Phytologist 89: 401–409.

Selitennikoff, C. P., 2001. Antifungal proteins. Applied and Environmental Microbiology 67: 2883–2894.

Sewall, T., L. Olson, L. Lange & J. Pommerville, 1986. The effect of monensin on gametogenesis and zoosporogenesis in the aquatic fungus, *Allomyces macrogynus*. Protoplasma 133: 129–139.

Shearer, C. A., E. Descals, B. Kohlmeyer, J. Kohlmeyer, L. Marvanová, D. Padgett, D. Porter, H. A. Raja, J. P. Schmit, H. A. Thornton & H. Voglmayr, 2007. Fungal biodiversity in aquatic habitats. Biodiversity and Conservation 16: 49–67.

Shepard, J. F., 1977. Regeneration of plants from protoplasts of potato virus X-infected tobacco leaves. II. Influence of virazole on the frequency of infection. Virology 78: 261–266.

Sparrow, F. K. Jr., 1960. Aquatic Phycomycetes, 2nd ed. University of Michigan Press, Ann Arbor.

Stanghellini, M. E. & R. M. Miller, 1997. Biosurfactants. Plant disease 81: 4–12.

Stewart, C. S., C. A. McPherson & E. Cansunar, 1987. The effect of lasalocid on glucose uptake, hydrogen production and solubilization of straw by the anaerobic rumen fungus *Neocallimastix frontalis*. Letters in Applied Microbiology 5: 5–7.

Tomlinson, J. A. & E. M. Faithfull, 1979. Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*. Annals of Applied Biology 93: 13–19.

Tomlinson, J. A. & B. J. Thomas, 1986. Studies on melon spot virus disease of cucumber and on control of the fungus vector (*Olpidium radicale*). Annals of Applied Biology 108: 71–80.

Trinci, A. P. J., D. R. Davies, L. K. Gul, M. I. Lawrence, B. B. Nielsen, A. Rickers & M. K. Theodorou, 1994. Anaerobic fungi in herbivorous animals. Mycological Research 98: 129–152.

Vincent, M. A. & M. J. Powell, 1988. Effects of the pyridazinone herbicide Sandoz 6706 (metflurazon) on pigmentation in fungi. Mycologia 80: 673–678.

Vincente, F., A. Basilio, G. Platas, J. Collado, G. F. Bills, A. G. Del Val, J. Martin, J. R. Tormo, G. H. Harris, D. L. Zink, M. Justice, J. N. Kahn & F. Pelaez, 2009. Distribution of the antifungal agents sordarins across filamentous fungi. Mycological Research 113: 754–770.

Watve, M. G., R. Tickoo, M. M. Jog & B. D. Bhole, 2001. How many antibiotics are produced by the genus *Streptomyces*? Archives of Microbiology 176: 386–390.

Weber, R. W. S., A. Meffert, H. Anke & O. Sterner, 2005. Production of sordarin and related metabolites by the coprophilous fungus *Podospora pleiospora* in submerged culture and in its natural substrate. Mycological Research 109: 619–626.

Weete, J. D., A. Ei-Mougith & J. M. Touze-Soulft, 1989. Inhibition of growth, lipid and sterol biosynthesis by monensin in fungi. Experimental Mycology 13: 85–94.

Widmer, T. L., 2006. Cryopreservation of *Synchytrium solstitiale* in planta. Plant Disease 90: 429–432.

Williams, A. G., K. N. Joblin & G. Fonty, 1994. Interactions between the rumen chytrid fungi and other microorganisms. In Mountfort, D. O. & C. G. Orpin (eds), Anaerobic Fungi. Marcel Dekker Inc, New York: 191–227.

Willoughby, L. G., 1983a. The bacterial antagonism of *Karlingia rosea*: further observations, from Spain and Cyprus. Nova Hedwigia 38: 113–127.

Willoughby, L. G., 1983b. The bacterial antagonist of *Karlingia rosea*: Observations from mainland Spain. Transactions of the British Mycological Society 81: 435–438.

Woodhams, D. C., L. A. Rollins-Smith, C. Carey, L. Reinert, M. J. Tyler & R. A. Alford, 2006. Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. Oecologia 146: 531–540.

Woodhams, D. C., L. A. Rollins-Smith, R. A. Alford, M. A. Simon & R. N. Harris, 2007. Innate immune defenses of amphibian skin: antimicrobial peptides and more. Animal Conservation 10: 425–428.

Zairi, A., F. Tangy, K. Bouassida & K. Hani, 2009. Dermaseptins and magainins: Antimicrobial peptides from frogs' skin—new sources for a promising spermicides microbicides—a mini review. Journal of Biomedicine and Biotechnology. doi:10.1155/2009/452567.