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A Review: Factors Affecting Outbreaks of Saprolegniosis on Aquatic Animals

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Abstract

In this paper, we review the current knowledge regarding factors affecting the outbreaks of saprolegniosis. Saprolegniosis is a serious disease which can cause significant economic losses in aquaculture. Oomycetes are considered to be responsible for the disease. Outbreaks of saprolegniosis occur when equilibrium between pathogenic oomycetes and host resistance is disturbed. This may be the result of an increase in numbers or virulence of the zoospores of oomycetes in the water, or an increase in the susceptibility of the host. The presence of oomycetes zoospores in the environment is regarded as one of the most important causes of saprolegniosis. However, important differences have been found between plant and animal pathogenic oomycetes and appear to be related not only to tactic responses to environment, but also to infection strategies to a host. In addition, fish are more susceptible to infection by pathogenic oomycetes when physically injured, stressed or infected. Furthermore, water environmental factors, such as temperature, salinity, and microorganisms, should be carefully considered since they play a key role in the outbreaks of saprolegniosis.

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Introduction

Saprolegniasis is a disease characterized by cotton-wool-like threads on eggs or the integument and gills of fish (Bruno et al., 1999). This disease can cause significant economic losses, threatening the aquaculture industry, by killing fish, fish eggs, amphibians and crustacians (Densmore and Green, 2007; Fernández-Benéitez et al., 2010; Hirsch et al., 2008; Kiesecker et al., 2010; Ruthig, 2009). Oomycetes are responsible for saprolegniasis (Bruno and Wood, 1999). Identification and classification of oomycetes have historically been a problem. Oomycetes were previously classified as fungi, because of their filamentous growth, and because they live on decaying matter as do fungi. However, several other characteristics, including combinations of cellulose cell wall, biflagellated zoospores, gametangial copulation, and biochemical analyses, suggest that the oomycetes are more closely related to algae (Phaeophyta and Chrysophyta) and higher plants, but are not true fungi (Paul and Steciow, 2004). With the advanced application of molecular techniques, our understanding of the taxonomy of oomycetes is clearer (Harper et al., 2005; Riisberg et al., 2009). At present, oomycetes are no longer considered to be true fungi but are classified within the kingdom Stramenopiles (Heterokonts), which also includes brown algae and diatoms (Baldauf et al., 2000; Tyler and Boore, 2006).

Taxonomically oomycetes are divided into three subclasses: Saprolegniomycetidae, Rhipidiomycetidae, and Peronosporomycetidae. Most animal pathogenic oomycetes including *Saprolegnia, Achlya and Aphanomyces* belong to the class Saprolegniomycetidae (Jiang et al., 2013; West, 2006). During infection, oomycetes translocate effector proteins and toxins into the tissues of the host which manipulate host immune responses. However, until recently, relatively little was known about the mechanisms of the translocation and it seems quite different between plant and animal pathogenic oomycetes (Ellis and Dodds, 2011; Kale and Tyler, 2011; Wawra et al., 2012).

For many years, malachite green oxalate was used to control outbreaks of saprolegniosis. However, in 2002, malachite green was banned due to potential teratogenic and mutagenic properties. As a result, there are only a limited number of chemical compounds available for fish farmers, and development of alternative control strategies to combat saprolegniosis is imperative (Liu, 2014). In this review, we provide a brief overview of some factors affecting outbreaks of saprolegniosis, including pathogenicity determinants of oomycetes, predisposing factors of hosts, and environmental factors. This is in order to better understand saprolegniosis which in turn may enable development of new strategies to control the infection in the future.

Pathogenicity determinants of oomycetes

The presence of oomycetes zoospores in the aquatic environment is regarded as one of the most important causes of saprolegniosis (Bly et al., 1992; Bly et al., 1993). Oomycetes zoospores are released during asexual reproduction stages and have several tactic responses, such as chemotaxis, which helps to find a suitable host. When in close proximity to a potential host, oomycetes establish disease by translocating effector proteins and toxins into the tissues of hosts to manipulate their immune responses.

Several special asexual reproduction stages which help to find a suitable host.

Oomycetes have several asexual reproduction stages not found in fungal pathogens, that help them to successfully infect hosts (Fig.1) (Phillips et al., 2008; West, 2006). When nutrient levels decrease, sporangia which are located at the top of hypha are formed. This is similar to many lower fungi, such as Chytridiomycota and Zygomycetes (Xing and Li, 1999). When the zoospores are formed in the sporangium, the entire sporangial release process takes place in less than a minute. It is considered one of the fastest developmental processes in any biological system (Walker and West, 2007). After their release, their unicellular biflagellate zoospores, named primary zoospores, swim only for a short time and produce primary cysts after shedding their two flagella and forming a cell wall within minutes (Berg et al., 2013; Bruno et al., 1999). This process can occur under laboratory conditions such as agitating the cultural solution or in the presence of nutrients such as peptone, dissolved fish mucus, or nutrient solutions such as bovine serum albumin, hemoglobin and hempseeds (Berg et al., 2013). Primary cysts can release new zoospores, called secondary zoospores that swim for many hours before

encysting to secondary cysts, and exhibiting chemotactic responses, electrotaxis, autotaxis, or autoaggregation and infecting new hosts (Bruno et al., 1999). Unlike true fungi, both the primary zoospores and secondary zoospores of oomycetes have two kinds of flagellae, one is posterior, and the other, anterior. The latter has a fibrous and ciliated structure. Although some true fungi, Chytridiomycota for example, also have motile zoospores, their flagellae are only a posterior whiplash type (Rossman, 2006). Once the secondary zoospores adhere to a host, and penetrate the root tissues, infection is initiated and secondary cysts form. Secondary cysts of some oomycetes, such as *Saprolegnia parasitica*, have long hooked hairs that are believed to increase attachment efficiency (Grandes et al., 2000; Ismail et al., 1979). Finally, the secondary cyst adheres and germinates to produce a hypha that may develop into a mycelium and invade the host. When secondary cysts do not adhere to a host, they will release new zoospores. This cycle of repeated zoospore emergence is defined as 'polyplanetism' and provides another opportunity to locate a suitable host.

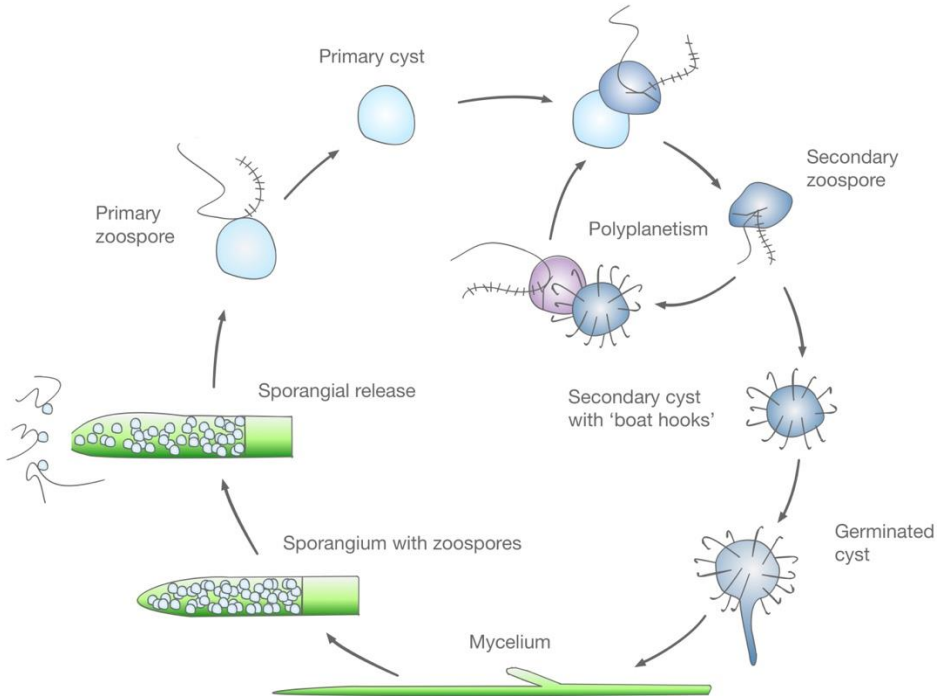

Fig.1 Schematic diagram of the asexual life cycle of *Saprolegnia parasitica*. This figure modified from van West (2006).

Tactic responses of zoospores increase the chances of successfully finding a host

Oomycetes, though closer to algae and higher plants in phylogeny, show a totally different tactic to survive. Unlike plants, the light source is less important for oomycetes. A previous study has shown that predominantly unilateral illumination did not have a perceptible effect on the distribution of zoospores of *Phytophthora cactorum*, *Phytophthora nicotianae*, and *Phytophthora palmivora*, in a Petri dish (Cameron and Carlile, 1977). In addition, the potential aerotaxis of zoospore of *P. cactorum* and *P. nicotianae* has been evaluated when placed horizontally in capillary tubes filled with air, oxygen, nitrogen, or carbon dioxide (Cameron and Carlile, 1977). It seems that plant pathogenic oomycetes have no aerotaxis. Other tactic responses of oomycetes zoospores, such as chemotaxis, negative geotaxis, and electrotaxis, may also increase the success of finding a host (Cameron and Carlile, 1978, 1977; Cerenius and Söderhäll, 1984; El-Feki et al., 2003; Morris et al., 1992; Rand and Munden, 1993). Among them, chemotaxis is most widely studied and the most important response for oomycetes. It is noted that *P. palmivora* zoospores were repelled by many low molecular weight cations, such as H^+, K^+, Cs^+, Mg^{2+} and La^{3+}(Cameron and Carlile, 1977). It is suggested that negative chemotaxis of *Phytophthora* zoospores to cations is dependent on a general mechanism of cation interaction with a negatively charged membrane, and the

effectiveness of cations as repellents should parallel their ionic conductivities (Cameron and Carlile, 1977). This negative chemotaxis might help to protect oomycetes from being repelled by these low molecular weight cations. Inversely, zoospores can also be attracted to various organic compounds (positive chemotaxis), which greatly increase the chances of finding a suitable host. An early study reported that chemotaxis of *Aphanomyces astaci* Si zoospores toward the washed legs of all three species of crayfish was observed after a few minutes, and the exudate from crayfish legs attracted zoospores from three different strains of *A. astaci* (Cerenius and Söderhäll, 1984). In addition, the zoospores of *Saprolegnia diclina* showed a strong chemotactic response to the chorionic membrane prepared from live eggs of brook trout *Salvelinus fontinalis* (Rand and Munden, 1993). In addition, zoospores of *Saprolegnia parasitica* were attracted to fish tissue extracts (El-Feki et al., 2003). These results suggest that positive chemotaxis may have an important role in attracting zoospores of oomycetes towards aquatic animals. The ability of zoospores to swim towards a nutrient source greatly increases the chances of finding a suitable host.

There have been some reports about negative geotaxis and electrotaxis of zoospores of oomycetes. It has been reported that zoospores of *Phytophthora* tend to accumulate at the upper surface of a suspension. These indicate that the effect is due to negative geotaxis-active swimming as zoospores have a higher density than water (Cameron and Carlile, 1977). Furthermore, in a laboratory experiment by applying weak electrical fields and quantifying electrotaxis, it has been shown that *P. palmivora* zoospores exhibit anodal electrotaxis in electrical field ≥0.5V/m comparable in size to the physiological fields around roots (Morris et al., 1992). In conclusion, studies about tactic responses of zoospores will provide a reference to the further research of the infection strategy of oomycetes.

Candidate effector proteins and virulence proteins promote infection

Oomycetes often establish disease by translocation of effector proteins and virulence proteins into the tissues of the host to manipulate host immune responses (Jiang et al., 2013; Schornack et al., 2009; Whisson et al., 2007). However, until recently, relatively little was known about the mechanisms of translocation of effectors, and these seem to differ between plant and animal pathogenic oomycetes (Ellis and Dodds, 2011; Kale and Tyler, 2011; Wawra et al., 2012). Some effectors, such as inhibitor proteins of plant hydrolases, are thought to function in the plant apoplast, where they interfere with plant extracellular proteins involved in the pathogen defense (Damasceno et al., 2008). Other oomycete effectors from plant pathogenic oomycetes, namely members of the RXLR and Crinkler (CRN) families, are translocated inside host cells through binding to PI-3-P, relying on an intact RXLR-motif and involving a lipid raft-mediated endocytosis (Kale and Tyler, 2011; Kale et al., 2010; Plett et al., 2011; Schornack et al., 2009.) (Fig.2). However, these large families of Crinkler and RXLR effectors appear to be absent in the oomycete fish pathogen *Saprolegnia parasitica* (Jiang et al., 2013). Despite the absence of large RXLR effector families, a putative effector from *S. parasitica*, SpHtp1, which contain an N-terminal RXLR sequence, is translocated into fish cells during the interaction (West et al., 2010). However, the translocation of SpHtp1 is mediated by an interaction with tyrosine-O-sulfate–modified cell-surface molecules, not via PI-3-P, as has been reported for RXLR effectors from plant pathogenic oomycetes (Wawra et al., 2012) (Fig.2). Besides effector proteins, oomycetes often secrete a battery of virulence proteins to promote infection. It has been demonstrated that a serine protease (SPRG_14567) secreted from *S. parasitica* could degrade fish IgM, and it is indicated that this serine protease could be a virulence factor by suppressing initial immune responses of fish (Jiang et al., 2013). From the above, we can conclude that the immune response of the host may be avoided or even suppressed by effector proteins or virulence proteins of oomycetes during the initial stages of interaction thereby facilitating saprolegniosis.

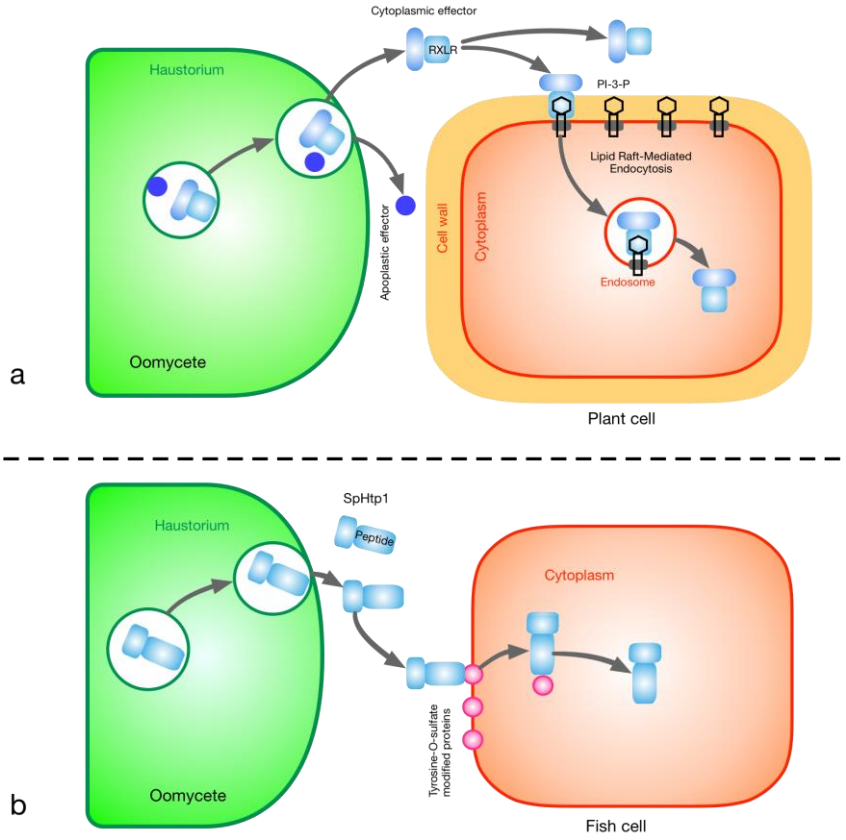

Fig.2 Oomycete pathogen effector proteins target different sites in host tissue. Schematic view of effectors secretion by a plant oomycete (a) and a fish oomycete (b). This figure modified from Kale and Tyler (2011) and Schornack et al. (2009).

(a) Apoplastic effectors are secreted into the extracellular space, where they interfere with apoplastic plant proteins involved in pathogen defenses. Cytoplasmic effectors translocate inside plant cells by binding to cell surface phosphatidyinositol-3-phosphate (PI-3-P) via the RXLR domain precedes entry via lipid-raft-mediated endocytosis.

(b) SpHtp1, a putative effector from the fish pathogenic oomycete, *S. parasitica*, is translocated into fish cells during the interaction. Cell surface binding and uptake of this effector protein is mediated by an interaction with tyrosine-O-sulfate–modified protein.

Predisposing factors of hosts

Many species of oomycetes are saprophytic opportunists, i.e. secondary infection agents, multiplying in injured, stressed or infected fish, and dead or unfertilized eggs (Pickering and Willoughby, 1982).

Injured, stressed or infected fish have higher risk of infection from saprolegniosis.

The consensus is that healthy fish are at low risk of pathogenic oomycetes infections (Berg et al., 2013). However, fish are more susceptible to infection by pathogenic oomycetes when physically injured, stressed, or infected (Pickering and Willoughby, 1982). Physical injury comes largely from mechanical damage due to high stocking densities of farmed fish and tegument damage sustained during spawning (Bruno et al., 1999). Skin lesions of physically injured fish could be the initial predisposable condition for colonies. Reports found that injured fish have a higher susceptibility to oomycetes under experimental conditions (Srivastava and Srivastava, 1977). The skin of fish plays a fundamental role against oomycetes infections as it is the first point of contact for the infective organism and has at least three lines of defenses against oomycetes infection following infection of fish with zoospores (Bruno et al., 1999). Firstly, the mucosal layer covering the fish epithelia acts primarily as a physical barrier to colonization by oomycetes or other infectious agents and serves to reduce the number of propagules on the fish surface by increased secretion of mucus when in contact with oomycetes (Bruno et al., 1999). Secondly, the epidermal layer also acts as an important protective barrier.

Minor injuries to the epidermal layer of fish are often 'hotspots' for Saprolegnia infection and it suggests that this layer has a central role in protecting fish from infection when it is intact (Phillips et al., 2008). Thirdly, club cells that lie on the surface layers of the epidermis release chemical alarm cues when encountering attack (Phillips et al., 2008) and have antipathogenic role during oomycetes infection (Chivers et al., 2007). Research has found that fathead minnows infected with cysts of *Saprolegnia* had a greater number of club cells within the epidermis than uninfected minnows (Chivers et al., 2007). This indicates that club cells of fish may provide a defense during oomycetes infection. In general, the skin of fish acts as an important protective barrier when coming into contact with oomycetes. When the epidermal integrity of fish is destroyed, this may assist oomycetes to clone and cause outbreaks of saprolegniosis.

Environmental stressors, including poor water quality, temperature shocks, over-handling, overcrowding, and other stressors, can result in immunosuppression of fish and increased occurrences of oomycete infections (Bailey, 1984; Huang et al., 2010; Tao et al., 2016). In fact, many studies have demonstrated that sudden decrease in water temperature was associated with immunosuppression of fish and a higher risk of saprolegniosis (Bly and Clem, 1991; Bly et al, 1993). Immunosuppressed minnows that had suffered cadmium (Cd) exposure were not able to produce club cells in response to *Saprolegnia* infection, and this led to a higher incidence of saprolegniosis (Chivers et al. 2007). It is confirmed that environmental stressors can result in the immunosuppression of fish making them more susceptible to infection by oomycetes.

Hosts infected with a primary pathogen are more susceptible to infection by oomycetes. Primary bacterial infection associated with *S. parasitica* has been recorded in the Japanese eels, *Anguilla japonica* (Egusa, 1965; Egusa and Nishikawa, 1965) but this was inhibited by adding antibiotic dihydrostreptomycin to the water (Egusa, 1965). Furthermore, concurrent infestations with *Saprolegnia* sp. were also observed in wild Atlantic salmon infected with *Gyrodactylus salaris* and *Gyrodactylus* sp. which damaged the skin of the host (Heggberget and Johnsen, 1982; Johnsen, 1978). In general, these findings demonstrated that hosts with a primary pathogen were more susceptible to oomycetes.

Small, dead or unfertilized eggs have higher risk of infection

Size differences between fish eggs are thought to be one of the factors of *Saprolegnia* infection. In general, small eggs from species such a common carp (*Cyprinus carpio*) and catfish (*Pelteobagrus fulvidraco*) were infected within days, whereas Saprolegnia infections occurred much later in salmonid eggs (Cao et al., 2012; Chukanhom and Hatai, 2004).

Dead or unfertilized eggs are at higher risk of infection with saprolegniosis. In an experiment with live and dead Atlantic salmon eyed eggs which were infected with eight different *Saprolegnia* isolates, all isolates were only able to grow on dead eggs (Thoen et al., 2011). However, this differs from the results of Songe et al. (2016) who claimed that *S. parasitica* was wildly attracted to eyed eggs of *Salmo salar* L., believing that there might be a possible facultative biotrophic mechanism by *S. parasitica*.

Water environmental factors affecting outbreaks of saprolegniosis

The possible contribution of water quality to the outbreaks of saprolegniosis has been discussed in several earlier studies (Bly et al., 1992; Bly et al., 1993; Oláh and Farkas, 1978). Along these lines, abundant laboratory and field evidence demonstrates that differing levels of pH, oxygen, total ammonia nitrogen, and un-ionized ammonia appeared to have no effect on the development of saprolegniosis (Bly et al., 1992; Bly et al., 1993; Bly and Clem, 1991). However, other water environmental factors, such as water temperature, salinity, and microorganisms, have a great effect on outbreaks of saprolegniosis (Bly et al., 1992; Bly et al., 1993; Taylor and Bailey, 1979; Mifsud and Rowland, 2008; Bly et al., 1997; Hussein and Hatai, 2001; Lategan et al., 2004a; Lategan et al., 2004b).

Water temperature

Outbreaks of saprolegniosis often occur in early spring and in late autumn when the water is colder (Oláh and Farkas, 1978). Channel catfish cultured in earthen ponds in the southern U.S. frequently suffered from saprolegniosis, named 'winter kill', from October to March as observed by farmers during severe cold weather where the pond water temperature decreased from 20℃ to 10℃ within 24h and remained low for at least one

week (Bly et al., 1993). It appears that temperature might be a key factor to these 'winter kills'. To understand the interrelationships between environmental temperature and saprolegniosis, several experiments were conducted. Bly et al. (1993) monitored two commercial catfish ponds for over one year to determine if a rapid drop in pond water temperature could be correlated with outbreaks of winter saprolegniosis and noted that it was associated with an immunosuppression of the catfish in these ponds and the *Saprolegnia* sp. zoospore levels were highest, reaching ≥ 5 spores/ml during the winter months in contrast to less than 1 spore/ml during most of the summer months. Moreover, channel catfish were immunosuppressed by a rapid decrease in water temperature under controlled laboratory conditions, and this caused 92% infection and 67% mortality within 21 d post oomycetes challenge (Bly et al., 1992). It therefore seems that the onset of saprolegniosis might occur due to a combination of a rapid drop in water temperatures and the length of time these low water temperatures persisted. It also seemed that if one of these factors was missing, the fish in the ponds remained healthy (Bly et al., 1993). These results confirmed that water temperature played a fundamental role in the outbreaks of saprolegniosis, and winter saprolegniosis was an immunodeficiency disease caused by oomycetes.

Salt

Saprolegniosis is widely reported in fresh water fish, but rarely in seawater. It seems that seawater or salt may play an important role in the control of outbreaks of this disease. It was clearly demonstrated as early as 1979 that Daily 2-3 h treatments with seawater could control *S. diclina* in eggs of pink salmon safely and effectively (Taylor and Bailey, 1979). They argued that seawater was an effective substitute for malachite green in the control of saprolegniosis (Taylor and Bailey, 1979). Seawater, salt (NaCl) is a safe, relatively inexpensive parasiticide and osmoregulatory aid that is widely recommended for the prevention of saprolegniosis (Mifsud and Rowland, 2008). The effectiveness of salt in controlling saprolegniosis may vary with fish species, salt concentration, and the strain of oomycetes (Marking et al., 1994; Mifsud and Rowland, 2008; Chukanhom and Hatai, 2004). The control of Saprolegnia infection of trout eggs and improved hatching rate was observed at a salt concentration of 3% (Marking et al., 1994). It has been reported that the survival rate of silver perch harvested from a pond and treated with 2 or 3 g/L salt was 100% compared to untreated fish (0g/L salt) whose survival rate was only 66.7% after infection with *S. parasitica* (Mifsud and Rowland, 2008). However, isolate of *S. diclina* was able to tolerate up to 3.0% of NaCl in the environment, whereas the isolates of *A. klebsiana* and *Al. arbuscula* grew poorly in 1.0% NaCl (Chukanhom and Hatai, 2004). The effectiveness of salt control of saprolegniosis may be different depending on the species of oomycetes. In conclusion, seawater or salt (NaCl) can be used to control saprolegniosis, however the effectiveness of salt in controlling saprolegniosis may vary with fish species, salt concentration, and strain of oomycetes.

Microorganisms

Numerous aquatic bacteria are antagonistic to pathogenic oomycetes. Three out of seven *Pseudomonas fluorescens* from commercial channel catfish pond water inhibited *saprolegnia*'s hyphal growth (Bly et al., 1997). In addition, cyst germination was inhibited by each of seven *P. fluorescens* subcultures at ratios as high as 1 : 0.5 to 1 : 10, whereas *Escherichia coli* (as a control) occurred only at a ratio of 1 : 500 (Bly et al., 1997). Results indicated that some strains of *P. fluorescens* in the pond could inhibit hyphal growth and cyst germination of *Saprolegnia* strains. Another report from Hussein and Hatai claimed that some bacteria isolated from the lesions of salmonid fish with saprolegniosis, which belonged to the genera *Alteromonas*, *Pseudomonas*, and *Aeromonas,* could produce antifungal substances (Hussein and Hatai, 2001). These results demonstrated that these bacteria may be used for biocontrol of saprolegniosis in aquaculture. The potential therapeutic application of *Aeromonas media* strain UTS A199 as a candidate for the biocontrol of saprolegniosis in the eel, *Anguilla australis*, has been evaluated (Lategan et al., 2004a; Lategan et al., 2004b). It was found that the presence of A199 delayed the progress of the disease by inhibition of hyphal growth within lesions and cyst germination but could not protect the fish from the disease under these

conditions (Lategan et al., 2004a). In general, these bacteria appear to be the natural competitors of oomycetes and thus may be used for biocontrol of oomycetes in the future. However, a potential problem in using live bacteria for biological control is that the bacteria themselves may be a disease causative agent in fish. *A. hydrophila, A. sobria,* and *P. fluorescens* are now recognized as causal agents of bacterial hemorrhagic septicemia in almost all types of fish species (Sahoo et al., 2016). In view of this, further studies in biocontrol of saprolegniosis should concentrate on both safety and effectiveness of bacteria.

Perspectives

Outbreaks of saprolegniosis occur when the equilibrium between pathogenic oomycetes infection and the host's resistance is disturbed. This may be a result of an increase in numbers or virulence of the oomycetes zoospores in the water or an increase in the susceptibility of the host (Pickering and Willoughby, 1982).

The presence of oomycetes zoospores in the environment is regarded as one of the most important causes of saprolegniosis of channel catfish (Bly et al., 1992; Bly et al., 1993). Oomycetes zoospores are released during asexual reproduction stages and have several tactic responses, such as chemotaxis, that helps find a suitable host. Once close to a potential host, oomycetes establish disease by translocation of effector proteins and toxins into the tissues of the host to manipulate host immune responses. The most famous oomycete in aquaculture is *S. parasitica*. SpHtp1 is a putative effector of *S. parasitica* and interacts with tyrosine-O-sulfate–modified cell-surface molecules to translocate into host cells (Wawra et al., 2012; West et al., 2010).

Emerging evidence suggests that fish are more susceptible to infection by pathogenic oomycetes when physically injured, stressed, or infected (Pickering and Willoughby, 1982). The skin of fish plays a fundamental role and has at least three lines of defense against oomycetes infection by zoospores (Bruno et al., 1999). When the fish's epidermal integrate is destroyed, it helps oomycetes to form clones and results in higher risk of saprolegniosis (Srivastava and Srivastava, 1977). Several reports suggest that environmental stressors, such as poor water quality, temperatures shock, handling, and overcrowding, can all result in immunosuppression in fish, and those immunocompromised fish are more susceptible to oomycetes (Bailey, 1984; Bly et al., 1993; Chivers et al., 2007). In addition, many studies have demonstrated that the hosts with a primary pathogen are more susceptible to be infected by oomycetes (Egusa, 1965; Egusa and Nishikawa, 1965; Heggberget and Johnsen, 1982; Johnsen, 1978). Furthermore, many studies have shown that water environmental factors, such as water temperature, salinity, and microorganisms, have a great effect on outbreaks of saprolegniosis (Bly et al., 1992; Bly et al., 1993; Bly and Clem, 1991).

In summary, outbreaks of saprolegniosis are closely connected with the lack of equilibrium between pathogens and hosts. This is relevant to the increasing number and/or virulence of the oomycetes zoospores in the water and higher susceptibility of fish to this pathogen. The possible contribution to ability of oomycete zoospores to infect, predisposing factors of hosts, and water environment to outbreaks of saprolegniosis has been discussed in several earlier studies referred to above. However, until recently, relatively little was known about the mechanisms which explained how effector proteins of oomycetes could enter the host cells. As well as the possible virulence proteins that combat the activity of fish immunoglobulins against pathogenic oomycetes need to be investigated. Today, with the ban on malachite green, only a limited number of chemical compounds are effective in the control of saprolegniosis. Generally, a combination of farm management, husbandry practices, and chemical bath treatments may be an effective strategy in preventing or limiting outbreaks of saprolegniosis (Bruno et al., 1999).

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