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A Review of Saprolegnia Infection in Freshwater Fishes and Control of the Saprolegniosis

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Abstroct— Saprolegniosis is a fungal disease inflicted in wild and farm fish, caused by the water mold Saprolegnia sp. visible cotton-like white or grey patches on fish skin are often the first sign of infection. This ubiquitous saprophyte infects dead fish eggs and then spreads to healthy eggs resulting in major losses. Saprolegniosis is often a secondary infection; however, some virulent strains are known to cause primary infection on injured or stressed salmonids. Important economic losses in aquaculture are reported every year worldwide due to saprolegniosis. Infection by Saprolegnia sp. was well-controlled with the use of an organic dye with great antifungal efficacy; malachite green. Unfortunately, the use of malachite green was banned in 2002 worldwide due to its carcinogenic and toxicological effects. Up until now, no new treatment as effective as malachite green has been discovered.

SAPROLEGNIOSIS

Oomycetes are generally known as "aquatic mushrooms" are characterized by producing biflagellated and motile zoospores. These aquatic mushrooms are ubiquitous in freshwater ecosystems and feed on organic matter in water (Uhland et al., 2000). Among the members of oomycetes, the most important fungal pathogens of fresh and brackish water fish main include of family Saprolegniaceae (Khoo, 2000). The genus Saprolegnia, Aphanomyces and Achlya belonging to Saprolegniaceae can infect amphibians, molluscs, crustaceans (. Hulveyet al. 2007), fish and their eggs causing the disease known as saprolegnia (Khoo, 2000;Ramaiah, 2006; Van West, 2006).

The most frequently infected fish are salmonids family of fish vulnerable to saprolegnia (Neish, 1977; Stueland et al.,2005). The saprolegnia is often a secondary infection diagnosed by the appearance of white or grayish cottony tufts, when out of the water, have a rather mucoid appearance (Uhland et al., 2000). The infection begins from the head and fins of fish, spreading over the rest of the body especially on injured or stressed fish (Bruno et al, 1999).
The genus Saprolegnia is often isolated during a fungal infection in fish (Uhland et al., 2000), is one of the most devastating diseases in aquaculture (Branson, 2002; Molina et al.,1995; Ramaiah,2006) and is able to grow and reproduce throughout the year (Neish, 1977). Saprolegnia generally feeds on dead eggs as saprophyte but can spread to healthy eggs causing the death of these eggs. Several studies have shown that the most common strains of Saprolegnia parasitica are capable of causing infections primary (Neish, 1977, Van West,2006.). The Oomycete causing the most economic loss in infecting fish is species Saprolegnia parasitica (Torto-Alalibo et al., 2005; Brunoand Wood. 1999; Van West, 2006).

ECONOMIC IMPORTANCE

The first instance of Oomycete infection was reported in the European literature in 1877 and was named "salmon disease" (Neish and Hughes,1980). This infection that was first observed in rivers between England and Scotland quickly spread to the rivers of Great Britain. The Saprolegnia fungus was then identified as the causative agent of fungal infection(Neish and Hughes,1980). The Saprolegnia is partially responsible for the decline of the natural populations of salmonids. In recent years, several instances of infection with oomycetes have been reported in the literature of wild fish than of reared in fish farming (Bruno and Wood. 1999; Hatai and Hoshiai; 1992,Neish and Hughes,1980, Torto-Alalibo et al., 2005, Van West, 2006).
In aquaculture, fungal infections are the second source of loss economical especially in the cultivation of salmon and crustaceans (Meyer, 1991). In Japan, annual mortally up to 50% caused by S. parasitica is reported for coho and eel (Neish and Hughes,1980). S. parasitica is economically the most important pathogenic fungus in fish, especially in trout and salmon, causing losses of millions of dollars worldwide (Torto-Alalibo et al., 2005). Due to the increased susceptibility of the fish with infection during winter periods (winter kill), S. parasitica causes financial losses in the United States each year of around forty million dollars (Bly and Clem, 1992). Infection of Saprolegina on Channa punctatus was recorded from Wadali Lake Amravati, Maharashtra , India. The infected fishes in catch were identified with red spot on their body, damaged and their sluggishness (Pachade et al., 2014). In a study designed to investigate the Saprolegnia infection in fishes of Kolleru Lake, Andhra Pradesh and the percentage of infection of various species of fishes by Saprolegnia was recorded in Catla catla, Labeo rohita, Channa punctatus, Channa striatus, Clarias batrachus, Mastacembalus armatus, Mystus cavasius and M. seenghala (Mastan and Ahmad, 2018).

MALACHITE GREEN

Malachite green is an organic dye that is also recognized for its activity as a disinfectant. This synthetic compound has been widely used to treat fish and their eggs against parasites internal and external (Sudova et al., 2007). The discovery of the antifungal effects of Malachite green in 1930s was of great help in limiting infections in fish farms (Bruno and Wood, 1999;Sudova et al., 2007, Torto-Alalibo et al., 2005). At a concentration as low as 1 ppm (1mg /L), three one hour Malachite green baths (24 hour interval between baths) is sufficient to treat

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infections. This simple and inexpensive treatment could be also used as a preventive measure, which for several years limited losses due to saprolegniosis. Subsequent studies have shown, however, that Malachite green, but especially its reduced form, leucomalachite green, can persist in fish tissue and thus easily enter the chain food for humans (Alderman, 1985, Sudova et al., 2007).

In 2002, the use of Malachite green in animals intended for consumption human has been banned around the world due to carcinogenic and toxic properties (Branson, 2002;Sudova et al., 2007, Torto-Alalibo et al., 2005). Since then, no new product is proposed that is as effective as malachite green and a revival of Saprolegnia infection have been observed (Bruno and Wood. 1999).

TAXONOMY OF SAPROLEGNIA SP.

The work of William Chambers Coker (Cocker, 1923) in 1923 on the taxonomy of Saprolegniaceae is the basis for the classification of aquatic fungi. Cocker first described S. parasitica: "Any parasite of the genus Saprolegnia, isolated from fish, which is asexual". Then onwards, the identification of species of the genus Saprolegnia has become quite complex due to the addition of several studies on these aquatic fungi. During these years, the identification of Saprolegnia species was done only using characteristics of the sexual organs (antheridia, oogonia and oospores) (Molina et al., 1995). In 2002, Johnson et al. published an elaborated systematic review of Saprolegniaceae and listed 17 genera and 122 species (Johnsonet al., 2002), specifying that several species overlapped. Indeed, many studies have demonstrated that it was difficult to identify solely on basis morphology of sexual structures, owing to many similarities between the species. In addition, some species of the genus Saprolegnia do not produce sex organs under laboratory conditions (Diéguez-Uribeondoet al.,2007;Keet al.,2009. Stuelandet al., 2005). The asexual organs therefore consolidate the identification of species (Hulvey et al.,2007, Stuelandet al., 2005). The species S. parasitica can be identified by the presence of long hooked hairs and indirect germination in a nutrient-poor environment (Stuelandet al., 2005).

Recently, studies on molecular characteristics have strengthened the identification of species. Amplification and sequencing of two regions encoding ribosomal RNA (Figure 1) have become useful tools for the identification of Saprolegnia species (Keet al.,2009, Steciow et al.,2007). The first sequence is one region of the 18S rRNA (A) gene and the second includes ITS (Internal Transcribed Spacer) and the 5.8S gene (B).

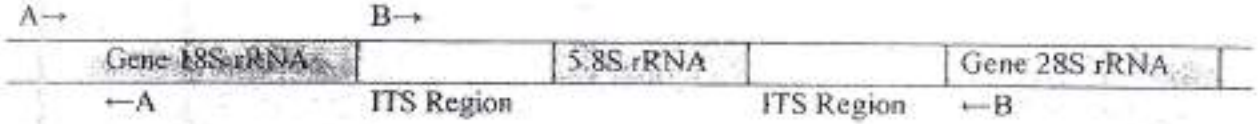

Figure 1. Diagrammatic representation of the 18S, 5.8S and 28S ribosomal RNA genes and ITS regions (Internal Transcribed Spacer). A and B two pairs of primers used to differentiate the species from Saprolegnia.

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LIFE CYCLE OF SAPROLEGNIA SP

The life cycle of Saprolegnia sp. is complex and includes the sexual and asexual reproduction (Figure 2). Reproductive organs are the oogonia (female) and the antheridia (male) found on the same hyphae (Raven et al,2000). The production of sexual organs in certain species of Saprolegnia is very rare, even absent in invitro conditions (Stuelandet al., 2005). The mode of asexual reproduction is most important for dispersal in aquatic environments. Zoosporangium is the main organ for asexual reproduction. It is formed at the tip of hypha and is releases several motile and biflagellate zoospores. These primary zoospores released swim for a short period of time with the help of apical flagella before encysting. Cysts can either germinate in hyphae to form mycelium or release a secondary zoospore having lateral flagella (Raven et al,2000). Dispersal and infection are mostly attributed to secondary zoospores which are motile than primary zoospores (Dieguezet al., 1994, Van West,2006). The secondary zoospores can have long hairs on their surface. Saprolegnia parasitica strains typically have long, hooked hairs on the surface of their spores, which would allow better attachment to the desired substrates (Van West,2006). These hooked hairs can be a sign of the pathogenicity of the strain because attachment to the host (Fregeneda et al, 2007). After a while, the secondary zoospores in turn encyst themselves to form a new zoospore or germinate under favourable conditions. This cycle of encystment and formation of zoospores can repeat itself several times depending on the strains names "polyplanetism" (Van West,2006). This adaptation therefore allows certain strains of Saprolegnia to make several attempts in order to find the ideal host (Bruno and Wood,1999).

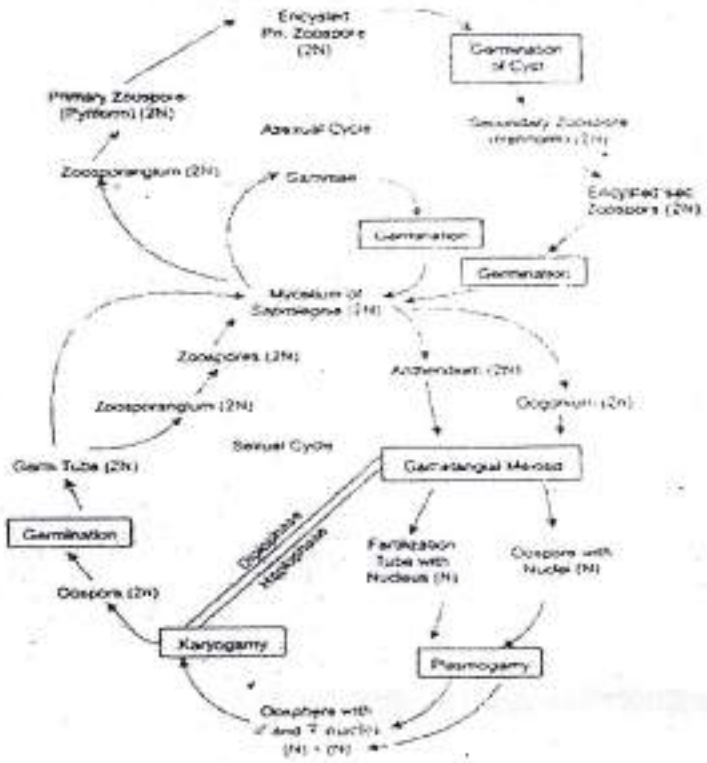

Figure 2 Life cycle of Saprlegnia parsitica

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THE DISEASE CAUSED OF SAPROLEGNIA SP

Saprolegnia is an opportunistic pathogen that is ubiquitous in freshwater environments. The fish are consequently exposed to it as in fish farming as in lakes and rivers. Infection caused by this aquatic mold is therefore common. Several incidents of saprolegnia have been reported worldwide especially in salmonids (Meyer, 1991). This disease is devastating for salmonid populations in their natural environments than in fish farming. It is important to know the causes and its mode of action in order to prevent and treat saprolégniosis.

PREDISPOSING FACTORS

The development of saprolegnia is by a combination of factors and not just the presence of the parasite (Robertset al., 2001). To better understanding of the outbreaks of saprolegnia, three important components must be taken into consideration: the pathogen, the host and the environment. The pathogen, is aquatic mold Saprolegnia, must be a virulent to cause infection (Stuelandet al., 2005). Several strains of this fungus does not cause infection on healthy fish. The fish as the host, is more vulnerable to infection as it already has a primary infection (Pickering and Willoughby, 1982, Robertset al., 2001) or if it is injured in the epidermis, which is the first external barrier against parasites. The secretion of mucus covering the epidermis is an important physical barrier of the fish and also prevents the attachment of spores (Pickering and Wiloughby, 1982). In case of weakened immune defenses, the susceptibility of the host is increased. In addition, many changes in the epidermis are made during sexual maturation of fish and this condition predisposes him to infections (Pickering and Willoughby, 1982, Robertset al., 2001).The environment is another component that should not be underestimated when analysis of factors predisposing to infections are studied. Several factors stress such as poor water quality, temperature variations, declining oxygen level and high density of fish (Stuelandet al., 2005) are closely related to development of saprolegnia infection.

EVOLUTION OF THE INFECTION

Saprolegniosis is an infection that can develop at any stage of fish life (Pickering and Willoughby, 1982). In eggs, mold is detected by the thick layer of mycelium that spreads from dead eggs to healthy eggs. This leads to causing death by suffocation if infected eggs are not removed or treated quickly (Bruno and Wood,1999).

The young fish at their first feeding will feed everything within their reach, including dirt that may contain spores (Roberts and Sheperd, 1997). This results in an infection of the digestive tract and the yolk sac. The infection spreads quickly, causing the death of these. The infection spread over the entire surface of the epidermis andcan be seen with the mycelium completely covered the dead fish. In case of adult fish, the infection usually begins on the epidermis at the level of the head and the fins. The mycelium appears in form of cottony tuft and that can be observed as spread on the 80% of the surface of the fish (Bruno and Wood,1999).

Saprolegnia causes necrosis of cells which leads to the destruction of dermis and epidermis (Torto-Alalibo et al., 2005) of fish that become even more susceptible to secondary infections (Neish and Hughes, 1980). The most virulent strains can penetrate organs causing damage to the underlying muscles and to the blood vessels. The infection can sometimes spread very quickly in fish with no apparent immune response. A once infected, salmonids cannot be cured from the infection caused by saprolegnia (Pickering and Willoughby, 1982). A loss of balance is often observed in fish infected as well only breathing difficulties shortly before death (Uhland et al., 2000). Death is caused by osmotic imbalances that appear due to the destruction of large tissue areas on fish (Pickering and Willoughby, 1982, Van West,2006). The survival time of the fish is variable but it has been described that in trout, only three days separate the appearance of the first signs and death (Pickering and Willoughby, 1982).

ALTERNATIVE TREATMENTS

Since the ban on the use of malachite green as a treatment against saprolegnia, a lot of research has been done to find a new remedy (Bly et al., 1997, Pottinger and Day, 1999,). The essential criteria for finding a good candidate as an aquatic fungicide are:

- Elevated anti-Saprolegnia activity
- Low toxicity to eggs and fish
- Large difference between the minimum inhibitory concentration (MIC) of fungus and that harmful to eggs and fish
- Non toxic for human consumption
- Inexpensive to use

Several alternative treatments have been proposed and are currently used in fish farms to treat saprolegnia on eggs and fishes. The best known products are bronopol, formalin, peroxide hydrogen and iodine-free salt. The specifics of each are explained below. Bronopol (2-bromo-2-nitropropane-1,3-diol) is used as a preservation in medical, pharmaceutical, cosmetic and also in shampoos (Pottinger and Day, 1999). This broad spectrum antimicrobial is a product non-toxic and does not represent a danger for the environment. In order to process saprolegnia in eggs, it is recommended to use it in a bath of 30 minutes at concentrations between 30 and 50 mg / ml, and from 15 to 20 mg / ml for fish (Morin, 2006). However, it is not recommended for young fishes because the bronopol is toxic to fish at this stage of life (Sudova et al., 2007). Formalin is an aqueous solution that is used for storage but also as a disinfectant and antimycotic. It is used to treat several external parasites of fish as well as saprolegnia (Van West,2006).

Hydrogen peroxide (H_2O_2) is an interesting compound because of broad spectrum of activity. It is effective against many microorganisms such as bacteria, mold, yeast and viruses (Bruno and Wood,1999). It is a non-polluting product that can be used as a preventive as well as curative. In order to treat saprolegnia, it is advisable to use it in doses ranging from 500 to 1000 mg / L for 15 minutes (Bruno and Wood,1999, Morin, 2006), which is a fairly high MIC for a treatment.

The margin between the MIC and the toxicity being low, it is not suggested to use this product as a treatment with fish (Pottinger and Day, 1999).

Iodine-free salt (NaCl) is undoubtedly the oldest of the treatments for fish and it is very safe. Not causing danger for the environment, it is the only product that can be used freely for treat fish infected with saprolegnia (Morin, 2006). Salt is a good treatment for use as a preventative because it is effective in limiting incidences of saprolegnia. However, in order to treat an infection already present, large amount of salt is required which can be problematic fish farming. None of these substitutes meet all the criteria essential to be a good antifungal compound to treat saprolegnia. Indeed, these treatments are either too expensive (bronopol), toxic (formalin, peroxide hydrogen) or ineffective (salt). Work has been done to explore new opportunities such as treatment option like vaccines and bacterial antagonists. Vaccine development is a less explored option to prevent saprolegniosis. However, may not be effective considering as the majority of fish are already exposed to Saprolegnianaturally and no immunity seems to protect them from an outbreak. (Bruno and Wood,1999).

CONCLUSION

Several bacterial antagonists of Saprolegnia have been discovered in the aquatic environment (Bruno and Wood,1999). The one that was most often found in the literature is Pseudomonas fluorescens, a Gram negative ubiquitous bacteria in freshwater environments that exhibits anti-Saprolegnia characters (Bly et al., 1997). Use as a treatment for bacteria of the genus Pseudomonas is not possible because they are responsible for diseases ulcerative bacteria causing sudden and high mortality in fish (Uhlandet al., 2000). An alternative would be to isolate the inhibitory agent produced by the bacteria for use as a treatment.

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