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Isolation and Characterization of Saprolegniaceae from Rainbow Trout (*Oncorhynchus mykiss*) Eggs in Iran

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Abstract: The goal of the this study was to identify Saprolegniaceae fungi isolated from affected rainbow trout eggs in six hatcheries in Mazandaran Province (Northern Iran) from December 2006 to February 2007. The isolated oomycetes were classified according to their morphological characteristics on hemp seed media at 18-24°C. Seven fungal species belonging to three genera of *Saprolegnia*, *Achlya* and *Brevilegnia* were identified. The fungal species were morphologically characterized as *S. parasitica*, *S. mixta*, *S. monilifera*, *Saprolegnia* sp., *A. oblongata*, *Achlya* sp. and *Brevilegnia* sp. This is the first recorded oomycetes infection in rainbow trout hatcheries in Iran.

Key words: *Saprolegnia*, *Achlya*, *Brevilegnia*, rainbow trout hatchery, morphology

INTRODUCTION

Aquaculture production now represents more than 30% of total fish production. According to West (2006), the majority of this production comes from freshwater aquaculture. Aquaculture industry, particularly rainbow trout farming has been rapidly developed in many areas of Iran. One of the most serious problems in trout hatcheries is oomycete infection from Saprolegniaceae family (Bruno and Wood, 1999; Kitanchaen and Hatai, 1998). Unfertilized fish eggs are susceptible to fungal infection particularly from the family Saprolegniaceae. During egg incubation, these fungi produce mycelia which grow and spread from the nonviable to the healthy eggs suffocating them and causing mortality (Post, 1987; Lilley and Inglis, 1997; Hussein and Hatai, 2002).

From taxonomical standpoint, Saprolegniaceae belongs to a division of Oomycota that is not considered to be true fungi. This is due to presence of a little chitin in its cell walls. Unlike most Eumycetes, the members of this group remain diploid throughout their lifecycle with meiosis occurring in gametangia before fertilization (Soderhall and Unestam, 1970; Baldauf *et al.*, 2000). Saprolegniaceae genera, particularly *Saprolegnia* and *Achlya* are generally considered as opportunistic pathogens for fish and egg. Bruno and Wood (1999) suggested that these fungi are unspecific and capable of infecting number of different species; however some strains of these fungi are particularly virulent for fish and are capable of causing severe infections in fish (Willoughby and Pickering, 1977; Stueland *et al.*, 2005). These fungi affect the fish eggs by adhesion mechanism and penetration into the egg membranes (Willoughby, 1994). Such virulent mechanism can cause a mass morbidity and mortality during hatching period. Losses are particularly severe at lower water temperature (e.g., in trout hatcheries). Despite the economically significant losses during the egg incubation in Iranian trout hatcheries, minimum data is available concerning the specifics of the involved causative agents. While in most trout hatcheries, the affected eggs show typical clinical fungal infection signs, there is no information on the actual fungal species involved. This information is particularly important for providing a suitable

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prevention method and control of the conditions faced by the fish farmers. The goal of this study was set to identify Saprolegniaceae isolated from rainbow trout eggs.

MATERIAL AND METHODS

A total of 900 rainbow trout (*Oncorhynchus mykiss*) eggs infected by fungi were collected in Mazandaran Province in Northern Iran from December 2006 to February 2007. As showed in Table 1, broodstocks age that used for propagation in the studied hatcheries were about 2 to 6 years. The collected samples were transported to the laboratory in sterile screw capped 40 mL plastic bottles at environment temperature of 10-14°C. Each egg was placed in a 20 mL sterilized petri dish containing several halves of sterilized hemp seeds and Sterilized Tap Water (STW). Each Petri dish was incubated at 18-24°C for 24-48 h under natural light. Microscopic slices were taken from the growth fungi and were examined under compound microscope. In order to obtain new colonies and better inducing sexual organs, some hyphae were aseptically taken out with the help of sterile needles and were transferred to GPA (glucose peptone agar) containing 250 µg mL⁻¹⁰ penicillin and 250 µg mL⁻¹⁰ streptomycin (Willoughby and Pickering, 1977) at 18-24°C for a minimum of 48 h. Once the growth of the oomycete in the GPagar plates was observed, a block of agar from the edge of each colony was cut and placed in sterile Petri plates containing STW and hemp seed. At this stage, morphological characteristics, microscopic observations and all required measurements were completed. Identification was performed using the methods described by Coker and Matthews (1937), Johnson (1956), Beakes *et al.* (1994) and Khulbe (2001).

RESULTS

In this study, based on the morphological characteristics(zoo sporangium and sexual organs), a total of seven species in three genera of Saprolegniaceae fungi were isolated from the infected eggs. As shown in Table 2 about 20 to 39% of eggs in studied hatcheries were infected. The isolated species belonged to *Saprolegnia parasitica*, *S. mixta*, *S. monilifera*, *Saprolegnia*, sp., *Achlya* sp., *Brevilegnia* sp. The most frequency of isolated fungi were belonged to *Saprolegnia parasitica* with 47.8 frequency, whereas *S. monilifera* and *A. oblongata* with 2.4% were shown the lowest frequency. In Table 3 frequency of isolated saprolegniaceae fungi in this study are shown.

Table 1: Hatchery conditions during sampling

Hatchery conditions	Values
pH	7.5-8.5
Salinity (ppt)	0.30-0.58
Water temp. (°C)	9-12.8
Density of eggs (No. cm ⁻²)	42-65
Broodstock age (year)	2-6

Table 2: Percentage of infected eggs in studied hatcheries and No. of fungi species

Factors	Values
Infected eggs (%)	20-39
Fungi species	
<i>Saprolegnia parasitica</i>	20
<i>Saprolegnia</i> sp.	8
<i>S. mixta</i>	3
<i>S. monilifera</i>	1
<i>Achlya oblongata</i>	1
<i>Achlya</i> sp.	7
<i>Brevilegnia</i> sp.	2

Table 3: Individual percentage of each Saprolegnia genus

Fungi species	Percentage
<i>Saprolegnia parasitica</i>	47.61
<i>Saprolegnia mixta</i>	7.20
<i>Saprolegnia monilifera</i>	2.40
<i>Saprolegnia</i> sp.	19.00
<i>Achlya</i> sp.	16.60
<i>Achlya oblongata</i>	2.40
<i>Brevilegnia</i> sp.	4.80
Total	100.00

DISCUSSION

In this study, for the first time, seven species of Saprolegniaceae were isolated and identified in rainbow trout eggs from salmonid hatcheries in Northern Iran. *Saprolegnia parasitica* with 47.6% isolation was observed in most of hatcheries under this study (Table 3). It is being suggested that *S. parasitica* is the most important fungus species among infected Rainbow trout eggs in Mazandaran Province. This finding does not support previously considered facts that the greatest losses on fish eggs are due to *Saprolegnia* species (Willoughby, 1970; Ogbonna and Alabi, 1991; Czezug and Kiziewicz, 1999; Hussein *et al.*, 2001).

The other saprolegnia species which was isolated is *S. mixta* that according to Lartseva (1986) causes great losses in acipenserid hatcheries in Russia.

According to Steciow (2003) all varieties of genus *Brevilegnia* have been isolated mainly from soil. Only one unidentified species has been isolated from the mud of an eel culture pond in Japan (Hoshina *et al.*, 1960). Also, Czezug and Kiziewicz (1999) reported *Brevilegnia unisperma* on the egg of *Carassius carassius*. However, in this study *Brevilegnia* sp., was isolated from the rainbow trout eggs.

Ecological differences from different hatcheries conditions (chemical factors, age of bloodstock, eggs crowded) may have played a role in the type of fungi developed in rainbow trout eggs in this study. Although, environmental variables were not studied directly, they are known to influence the growth, reproduction and intensity of aquatic fungal infection (Richards and Pickering, 1978; Willoughby, 1994). There is no doubt that ecological differences play an important role in the species diversity of the fungi that develop on both fish and eggs (Wood and Willoghby, 1986; Hussein *et al.*, 2001). Alabi (1971) also observed that the occurrence of Saprolegniaceae is correlated with some water parameters (i.e., temperature, pH, ionic concentration and organic content). Saprolegniaceae are saprophytic fungi that can affect fish and fish eggs in special condition.

In this study for the first time, isolation of *S. mixta*, *S. monilifera*, *A. oblongata* and *Brevilegnia* sp., from rainbow trout hatcheries from Iran has been reported.

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