

**STUDIES ON SAPROLEGNIASIS IN SALMONIDS IN HOKKAIDO**

(北海道のサケ科魚類の水カビ病に関する研究)

**MORTADA MOHAMED ABDEL-HAMID HUSSEIN MOHAMED**

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**NIPPON VETERINARY AND SCIENCE UNIVERSITY  
TOKYO, JAPAN.**

**Under supervision of Professor Dr. KISHIO HATAI**

指導教授 畑井喜司雄

## Summary

Outbreaks of water-borne fungal infections of fish, amphibians, and reptiles are a common problem, especially in fish farms and hatcheries. Of particular concern is saprolegniasis, which is an infectious fungal disease that is widespread in all stages of the life cycle of fish. The disease causes serious losses in fish farms and hatcheries, especially among salmonid species. The overall aims of this study are to gain a clearer understanding of the incidence and prevalence of saprolegniasis especially among salmonid fishes in Japan. Therefore, the author attempted to work on the objectives to investigate the *Saprolegnia* species associated with fungal infection in salmonids, with the goal of finding additional taxonomic criteria for such pathogenic species. Moreover, special efforts were done to develop devices for control of such infection either by using antifungal agents as substitute for malachite green and formalin or by biocontrol technique.

In chapter 2, the prevalence of *Saprolegnia* species associated with the epizootics of saprolegniasis that occurred in freshwater-cultured salmon and their eggs at some hatcheries in Hokkaido (Japan) were investigated. In almost all cases, the initial clinical sign was characterized by the growth of cotton-like mycelia on the body surface, especially the head, adipose fin, and caudal fin, but the mycelia were not visible to the naked eye in the internal organs.

As a result 42 isolates, all of the genus *Saprolegnia*, were isolated from lesions and classified in the genus *Saprolegnia* according to their morphological and biological characteristics on hemp seed cultures at various temperatures. *Saprolegnia salmonis* was a new species added to the genus as well as the most widely and dominant species in all sites investigated in Hokkaido. Other *Saprolegnia* species were found to be involved in saprolegniasis incidence (in

Hokkaido) and identified as *Saprolegnia parasitica* and as *S. australis*.

Chapter 3, describes the pathogenicity and the pathology of of *Saprolegnia salomonis* NJM 9851 and *S. parasitica* NJM 9868, isolated from saprolegniasis outbreaks, against immature stages of five salmonids species belonging to three genera. The investigation was carried out on fingerlings of rainbow trout, *Oncorhynchus mykiss* (Walbaum), masuo salmon, *O. masou* (Brevoort), sockeye salmon, *O. nerka* (Walbaum), brown trout, *Salmo trutta* (Linnaeus), and Japanese char, *Salvelinus pluvius* (Hilgendorf). The cumulative mortality rates of the tested fish groups that exposed to high zoospore concentrations of *S. salomonis* NJM 9851 were 90, 93.3 and 100% for brown trout, sockeye salmon and the other tested salmonid species, respectively. On the other hand, all salmonid species exposed to high zoospore concentrations of *S. parasitica* NJM 9868 experienced cumulative mortality of 100%.

The histopathological changes of the saprolegniasis lesions found in all sites of infection were loss of the epidermis, edema of the hypodermis and different degrees of degenerative changes in the underling musculature. It is clear from these results that *S. salomonis* NJM 9851 and *S. parasitica* NJM 9868 are highly pathogenic *Saprolegnia* species to three genera of salmonid fishes.

Chapter 4, aims to examine the possibility of using alternative antifungal agents of natural origin that may have no impact either on fish or on ecosystem as substitutes for other harmful ones. Eugenol, a major essential oil of clove, and thymoquinone, one of the major carbonaceous compounds of *Nigella sativa* oil, were investigated for their antimycotic activity against some fish pathogenic species of Saprolegniaceae as well as the toxic effect of both compounds on selected fishes. Two eugenol solutions, eugenol 10% v/v solution in dimethyl sulfoxide (DMSO) and FA 100<sup>®</sup>, which contains 10 % v/v

eugenol were used. On the other hand, thymoquinone solution was prepared in tween distilled water (TDW, 25µL/mL) (v/v). The fungal species investigated for the antimycotic effect of eugenol were *Saprolegnia parasitica*, *S. diclina*, *S. ferax*, *S. salmonis*, *Aphanomyces piscicida*, and *Achlya klebsianna* while the antimycotic effect of thymoquinone was investigated only on *Saprolegnia parasitica* H 2 and *S. salmonis*. The minimum inhibitory concentrations (MICs) of eugenol in DMSO against *Saprolegnia* spp., *A. klebsiana* and *A. piscicida* were 500, 250, and 125 µg/mL, respectively, while the fungicidal effects (complete killing) were 1,000, 500, and 250 µg/mL respectively. In contrast, the MICs of FA100<sup>®</sup> against *S. parasitica*, other *Saprolegnia* spp., *A. klebsiana*, and *A. piscicida*, were 250, 125, 250, and 63 µg/mL, respectively, while the fungicidal effects were 1,000, 1,000, 1,000, and 63 µg/mL, respectively. On other hand, The MICs of thymoquinone against *Saprolegnia parasitica* H2 and *S. salmonis* were 125 and 63 µg/mL, respectively, while the fungicidal effect for both species was 160 µg/mL. Zoospores of the *Saprolegnia* spp. tested and of *A. klebsiana* could not germinate in the presence of 250 µg/mL of both eugenol solutions, while those of *A. piscicida* were killed by 125 µg/mL of both eugenol solutions. Tested zoospores of *S. parasitica* H 2 and *S. salmonis* could not germinate in 4 µg/mL of thymoquinone when they exposed for 30 minutes. The toxicity of eugenol was higher than that of thymoquinone to salmonids but both compounds were less toxic to cyprinids.

Chapter 5, deals with selecting of some natural competitors (bacteria) of *Saprolegnia* that may be used for biocontrol of *Saprolegnia* infection in fisheries field. In this study 5 of 47 bacterial isolates were found to be have the capability to inhibit the growth of *Saprolegnia parasitica* H2 and *S. salmonis* NJM 9851 on solid media. The author also examined culture media for these

bacteria that were effective in enhancing this activity. Among 8 media examined, BHI, HI, and to a lesser extent NA agar were suitable for demonstrating the antifungal activity. The inhibitory activity against the tested *Saprolegnia* spp. did not reside in supernatant of bacterial broth culture of the selected strains. The strains, which produce antifungal substances, were found to belong to the genera *Alteromonas*, *Pseudomonas*, and *Aeromonas*. These results suggest that the selected bacteria are natural competitors of *Saprolegnia*, and may be used for biocontrol of *Saprolegnia* in aquaculture.