学位論文内容の要旨

Dissertation Abstract

博士の専攻分野の名称：博士（水産科学）　　氏名：Karn Tippayakraisri

学 位 論 文 題 目
Title of dissertation

Studies on the Control of Water Mold Infection in Fish Eggs Using Glutaraldehyde
（グルタルアルデヒドを用いた魚卵におけるミズカビ病防除に関する研究）

Salmonid aquaculture and stock enhancement are critical components of the global fishery economy. A stable supply of healthy juveniles is essential to sustaining this industry. However, this production is severely threatened by water mold diseases caused by pathogenic oomycetes, mainly *Saprolegnia* species. These infections primarily affect incubating eggs, frequently causing mass mortality in hatcheries. Historically, control measures relied on malachite green and formaldehyde; however, these agents are now banned or heavily restricted due to carcinogenicity and human health risks. Furthermore, the commonly used alternative, bronopol, currently faces significant unstable supply. Therefore, there is an urgent need for safe and chemical alternatives effective agaisnt water mold infection. Glutaraldehyde (GA), a dialdehyde widely utilized as a high-level disinfectant in medical and industrial settings, has emerged as a promising candidate due to its potent, broad-spectrum biocidal activity and its status as not commonly classified as carcinogenic in regulatory frameworks. Despite its potential, its adoption for prevention of water mold infection has been limited due to the scarcity of studies demonstrating its efficacy and concerns regarding its high toxicity.

This study aims to evaluate the effectiveness of GA as a treatment for managing water mold infections in freshwater fish eggs, with particular emphasis on its anti-oomycete properties, practical application in aquaculture. Salmonids were selected as primary model species due to the severe economic losses caused by water mold infections in their production. Chapter I assessed the *in vitro* efficacy of GA against various *Saprolegnia* strains in comparison with conventional treatments (Malachite green, formaldehyde, and bronopol). Chapter II evaluated its effectiveness in preventing infections in salmonid eggs under laboratory conditions alongside an investigation into methods for neutralizing residual GA. Finally, Chapter III validated the efficacy and toxicity of GA in both a commercial salmonid hatchery and in ayu (*Plecoglossus altivelis*) eggs under laboratory conditions.

In Chapter I, this study assessed effectiveness of GA against various *Saprolegnia* strains. Seven *Saprolegnia* strains, including *S. parasitica*, *S. diclina*, and *S. hypogyna* were exposed to GA, malachite green, formaldehyde, and bronopol to evaluate their fungicidal effects. The growth of all tested strains was successfully inhibited by GA at concentrations ranging from 53 to 212 mg/L and temperatures between 10 and 20℃, with a minimum exposure duration of 30 min. The high efficacy of GA indicates its potential for controlling saprolegniasis in fish eggs. Additionally, one strain of *S. parasitica* was exposed to other aldehyde compounds (formaldehyde, glyoxal, acetaldehyde, isobutyraldehyde, malonaldehyde, and valeraldehyde) to assess the effectiveness of GA based on the presence of the aldehyde group. The fungicidal effects of these compounds were observed at higher concentrations (exceeding 405 mg/L) compared to GA, suggesting that the mere presence of aldehyde groups does not necessarily indicate fungicidal action.

Chapter II evaluated the efficacy of GA in controlling oomycete infections in rainbow trout and chum

salmon eggs and investigated its neutralization using common food additives. Biweekly treatment with GA at 100 mg/L for 30 min significantly reduced both oomycete infection and egg mortality, comparable to malachite green, formaldehyde, and bronopol, with no observed toxicity to fish. Among the tested neutralizing agents, sodium bisulfite, sodium pyrosulfite, potassium pyrosulfite, and sodium sulfite effectively neutralized GA solutions. Notably, sodium bisulfite at 3,300 mg/L neutralized GA within 15 min across a range of temperatures representative of aquaculture settings (10°C, 20°C, and 30°C). The 96-h 1% lethal concentration (LC_1) value following 30-min immersion of eyed chum salmon eggs exposed to sodium bisulfite for 15 min exceeded 3,300 mg/L, confirming that GA-treated eggs can safely undergo subsequent neutralization. These findings demonstrate that GA treatment, combined with efficient neutralization, offers a practical and environmentally responsible strategy for preventing water mold diseases in aquaculture.

Chapter III, which translates lab-scale results to commercial hatcheries, is often challenged by environmental factors such as flow-through water systems and high egg densities. To test this, a field trial was conducted at a commercial rainbow trout hatchery using a continuous flow-through system. Eggs were treated with GA at 50 mg/L (every 2 days), 100 mg/L (every 2–3 days), bronopol at 100 mg/L (every 2 days), and rearing water (every 2 days; control group). All GA-treated groups suppressed water mold infections to levels comparable to the bronopol-treated group, achieving high survival rates and hatching success without significant toxicity. Subsequently, to expand the potential application of GA, this study also investigated its efficacy against water mold infections and its acute toxicity in ayu under laboratory conditions. Ayu eggs were exposed to bronopol (50–100 mg/L; 30 min) or GA (25–50 mg/L for 30–60 min; 100–500 mg/L for 15–60 min) at daily, 2-day, or 3-day intervals. for 7 days at 17 °C. While bronopol effectively controlled infections in ayu without adverse effects, GA indicated the significant toxicity against ayu eggs. These results indicate that GA is practical reagent to prevent water mold infection in salmonid species while it is unsuitable for ayu.

In conclusion, this dissertation demonstrates GA treatment is a potent and practical solution for controlling water mold infection in salmonid aquaculture. An intermittent protocol—GA treatment at 100 mg/L for 30 min every two days— effectively suppresses water mold infections without inducing fish toxicity. Furthermore, GA containing treatment solution can be neutralized by sodium bisulfite, fascinating safe wastewater discharge and mitigating environmental impact. This research establishes GA as a high-potential alternative disinfectant, contributing to practical disease management in salmonid aquaculture.

Keywords: Glutaraldehyde, Water mold disease, *Saprolegnia*, Water mold treatment, Anti-oomycete, Glutaraldehyde neutralization,