学 位 論 文 の 要 旨

論文題目　Studies on the antimicrobial effect of *Mallotus japonicus* on growth of *Aeromonas hydrophila, A. salmonicida, Edwardsiella tarda* and *Vibrio anguillarum*

（アカメガシワ *Mallotus japonicus* の *Aeromonas hydrophila*、 *A. salmonicida*、*Edwardsiella tarda* および *Vibrio anguillarum* に対する抗菌効果について）

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Summary

The extensive use of antibiotic in aquaculture is primarily due to the growing cultivation of fishes in limited culture environments. Large quantities of culture fish products are being cultured in limited culture environments, and this predisposes them to a number of stressors which increase their susceptibility to several disease infections and/or pathogens. However, as a measure to control their spread, several synthetic antimicrobials are being used and this causes detrimental side effect to both culture animals and humans consumes them. So therefore, in order to replace antibiotic use in aquaculture, other alternatives method such as probiotic, vaccines and plant-derive bioactive compounds are being sought after. Nonetheless, unlike vaccines and probiotics, plant-derive bioactive compounds are cheaper and possess a broader spectrum of host range which make them an idea substitute to antibiotics.

1. Characterization of Aeromonas hydrophila infections in *Heterotilapia buttikoferi*

Heterotilapia buttikoferi is a freshwater fish species, which belongs to the family Cichlidae. The fish is native to West Africa and mostly found endemic to coastal rivers and streams in Guinea-Bissau and Liberia. Nevertheless, due to their high aesthetic value and rapid growth efficacy, they are mostly imported and bred as fishes for ornamental purposes and/or food for human consumption. In Japan, they are only bred for ornamental purposes.

Nonetheless in recent times, disease outbreak caused by bacterial pathogen *Aeromonas hydrophila* swept through the ornamental fish industry, hence resulting in large fish kills and morbidity. Nonetheless information on the pathogenicity of *A*. *hydrophila* to *H. buttikoferi* was not reported. So, in this study using the infection challenge trial, we described for the fish time the clinical signs and pathogenicity of *A. hydrophila* to *H. buttikoferi*. It was observed from the study that fish challenged with specific lethal doses (LD_{50}) of the bacterium elicited strong clinical signs ranging from exophthalmia, corneal opacity, ascites, liquefaction of internal organs and fin rot. The median lethal dose of the bacterium at which it is able to cause 50% mortality in the fishes was calculated at 2.0×10^{6}cfu/ml.

2. Antimicrobial effect of *Mallotus japonicus* against fish pathogenic bacteria

In this study the antimicrobial activity of the medicinal plant *Mallotus Japonicus* Müeller Argoviensis was evaluated against the fish pathogenic bacteria −*Aeromonas hydrophila*, *A. salmonicida*, *Vibrio anguillarum* and *Edwardsiella tarda*. It was observed from the study that the diethyl ether- , ethanol- and methanol extract of the plant, when challenged using the disk diffusion assay, minimum inhibitory concentration (MIC) assay and minimum bactericidal concentration (MBC) assay elicited strong antibacterial activities against the challenged bacteria species. It was however observed that, an extract of a diethyl ether group elicited the strongest bactericidal activity against the studied bacteria species at concentrations of 0.625, 2.5 and 5 mg ml-1, respectively. Also, some minor active compounds present within the plant extracts and detected by LC-MS such as citrinin, corilagin, fraxetin and furfuryl acetate demonstrated a corresponding strong antibacterial activity against the challenged bacterial species; hence confirming the potential of the plant as an alternative antimicrobial to antibiotics.

3. Immunomodulatory effect of *M. japonicus* on fish innate-immune response

Additionally, upon evaluating their role in improving the immune response of fish after dietary

intake, it was observed that the diethyl ether extract of the plant, when supplemented in the diet of fish significantly improve the innate-immune response against pathogenic disease infections of *A. hydrophila*. The lysozyme, serum bactericidal activity, globulin, total protein, albumin and phagocytic activity of the fish's blood was significantly improved after feeding on diet supplemented with the plant extract− *M. japonicus*.

3. Effect of *M. japonicus* supplemented diet on fish intestinal microbiota

The role of the extract on the microbial richness and diversity of the fish microbiota was also evaluated. It was observed from the study that feed supplemented with *M. japonicus*− diethyl ether extracts significantly enrich certain microbial taxa whereas diminishing others. For instance, the phylum Firmicutes, which was the second most predominantly identified OTUs; was significantly enriched after consumption of the supplemented plant diet contrarily to that of the phylum Proteobacteria which was the most dominant. It was observed that as the concentration of the plant additive increases, microbial richness and diversity skewed toward the phylum firmicutes contrary to the phylum proteobacteria. This was in correspondence to several other studies in which plant base diet was used as supplement in animal feed. We observed that the most commonly detected bacterial genus−*Bacillus*, often increases as fishes feed supplemented diet increases contrary to that of opportunistic pathogenic bacteria. Suggesting that the antimicrobial peptides in the plant additives might have counteract the proliferation of these taxa.

However, a fairly diverse microbial community was observed after feeding of plant supplemented diet. For instance, 5 phyla were identified (Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes and Actinobacteria), with 7 class (Actinobacteria, Flavobacteria, Bacilli, Fusobacteriia, α−proteobacteria, β−proteobacteria and γ−proteobacteria), 14 families (*Nocardiaceae, Flavobacteriaceae, Bacillaceae, Staphylococcaceae, Fusobacteria, Caulobacteraceae, Rhizobiaceae, Comamonadacea, Aeromonadaceae, Enterobacteriaceae, Pseudomonadaceae, Shewanellaceae, Xanthomonadaceae*) and 21 genera (*Rhodococcus,*

Nocardia, Flavobacterium, Chryseobacterium, Haloanella, Bacillus, Fictibacillus, Lysinibacillus, Staphylococcus, Cetobacterium, Brevundimonas, Shinella, Delftia, Diaphorobacter, Pelomonas, Aeromonas, Plesiomonas, Citrobacter, Pseudomonas, Shewanella, Pseudoxanthomonas). This effect of enrichment of specific taxa were observed not only in plant-supplemented diets but also in synthetic antimicrobial supplemented diet (Kokou *et al.,* 2020).

3. Conclusion

In conclusion, the present study demonstrated that the *M. japonicus* has a significant antimicrobial effect against some important pathogenic bacteria of fishes . Therefore, as an alternative to antibiotics, the plant extracts, particularly the diethyl ether-extract, can be considered as a prophylactic to disease control in fish. Also, the diethyl ether extract, can be used as a dietary supplement to improve the innate-immune response of the host. Since, the hematological parameters of the fish blood serum further confirms that the type of extract, dosage and duration of feeding significantly enhances the immune response of the fish against the challenged bacterial species. *M. japonicus* supplemented plant diet selected for beneficial microbial communities such as *Bacillus*, *Rhodococcus*, Cetobacterium and *Lysinibacillus*, whereas decreasing the abundance of pathogenic groups such as *Aeromonas, Pseudomonas, Staphylococcus* and *Nocardia* (Table 5.1). *Bacillus*, *Rhodococcus*, *Cetobacterium* and *Lysinibacillus* enriched within the fish microflora are involved in biodegradation, bioconversion and fermentation which helps in nutrient recycling whereas *Aeromonas, Pseudomonas, Staphylococcus* and *Nocardia* constitute important disease-causing pathogens of fish.

Keywords: microbiota, innate-immune response, pathogenecity, *M. japonicus* and *Heterotilapia buttikoferi*, *Aeromonas hydrophila*, *A. salmonicida*, *Vibrio anguillarum* and *Edwardsiella tarda*