別紙様式３

学位論文の要旨

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専　　攻 入学年度	宮崎大学大学院農学工学総合研究科博士後期課程 生物機能応用科学　　専攻 （西暦）2021 年度（　4 月）入学
学位論文 題　　目	Isolation and genetic characterization of lytic bacteriophages infecting bacterial fish pathogens and drug resistance mechanisms in *Lactococcus garvieae* serotype II （魚類病原細菌に感染するバクテリオファージの分離と遺伝的特性及びレンサ球菌の薬剤耐性機構）

【論文の要旨】（和文の場合 1, 200 字程度、英文の場合 800 語程度）

In Japan, intensive marine aquaculture is primarily characterized by the substantial production of *Seriola* species, which includes yellowtail, amberjack, and kingfish. However, these aquaculture operations face significant challenges due to bacterial infections, with pathogens like *Lactococcus garvieae* and *Nocardia seriolae* posing serious concerns. The overuse of antibiotics to control these pathogens has led to the emergence of drug-resistant strains. Notably, the vaccine developed to combat *L. garvieae* infections has shown limited efficacy and a short protection term, while a vaccine for *Nocardia seriolae* has not yet been introduced. To address this pressing issue, there is a growing need to explore alternative and eco-friendly options against these pathogens. One such method currently gaining attention in aquaculture operations is bacteriophage therapy, which offers potential solutions to combat these bacterial infections. The careful screening of the literature during this study did not find any lytic bacteriophage infecting these pathogens. Therefore, the primary objective of the present study was to first isolate and characterize the lytic bacteriophages that infect *L. garvieae* and *N. seriolae*.

A novel lytic siphophage named PLG-II, specifically targeting pathogenic *Lactococcus garvieae* serotype II strains, was successfully isolated from seawater samples collected in Miyazaki Prefecture, Japan. The whole-genome sequencing of PLG-II revealed a 32,271-bp double-stranded DNA molecule with an average GC content of 37.74%. It encompasses 69 open reading frames (ORFs), among which 43 currently lack reliable functional annotations for their products, along with a single tRNA. The lytic spectrum of phage "PLG-II" unequivocally demonstrated its strict lytic activity against serotype II strains, as it infected 17 out of 21 serotype II strains while showing no infectivity towards any of the 14 tested serotype I strains. The absence of any lysogenic and resistance genes in the phage DNA confirms its suitability for further experimentation. Moreover, initial challenge experiments provided confirmation that fish fed with bacteriophage-supplemented diets exhibited a 100% survival rate, in contrast to control fish fed with bacterial-supplemented diets only.

Nocardia seriolae also presents a significant threat to marine aquaculture operations. However, there is no phage isolated and characterized against this pathogen. In the present study, we also reported the first isolation of a lytic bacteriophage “NS-I” with a siphovirus morphotype from sea farm water samples collected from Miyazaki Prefecture, Japan. Its genome was a 43,361-bp double-stranded DNA molecule, exhibiting a GC content of 67%. Putative functions

could be assigned to only 39% (25/64) of the ORFs, whereas 61% (39/64) of the ORFs remained without any functional annotation. The lytic spectrum of NS-I showed its broad lytic range as it infected 15/15 *Nocardia seriolae* strains isolated from fish and 4/4 *Nocardia* sp strains isolated from non-fish sources. The absence of antimicrobial resistance genes, temperate markers, and virulence genes in the NS-I genome, makes it a promising candidate for further evaluation. Further experiments are required to confirm its suitability in real aquaculture settings.

Moreover, within the scope of this study, an in-depth exploration of drug resistance mechanisms was conducted for *L. garvieae* serotype II strains, with a specific focus on the *erm*(B) resistance plasmid. A total of 98 pathogenic strains of *L. garvieae* serotype II were collected from various prefectures in Japan over the period spanning 2018 to 2021. These strains exhibited notable resistance to erythromycin, lincomycin, and tiamulin. Subsequent PCR amplification revealed the consistent presence of *erm*(B) in all erythromycin-resistant strains. A conjugation experiment confirmed the transmissibility of *erm*(B) to recipient *Enterococcus faecalis* OG1RF, with transfer frequencies ranging from 10^{-4} to 10^{-6} per donor cell. The nucleotide sequencing analysis of the representative plasmid, pkh2101, isolated from an erythromycin-resistant strain unveiled a 26,850 bp DNA with an average GC content of 33.49%. This plasmid encompassed 31 CDSs, 13 of which remained devoid of any functional annotation. Interestingly, the plasmids isolated from *erm*(B) strains collected from three different prefectures confirmed the presence of the same (identical) plasmid. This confirms the prevalence of the same *erm*(B) plasmid around different prefectures. The comparative genomic analysis of this plasmid showed its highest similarity (97.57% identity) with the clinically isolated plasmid pAMbeta1, previously retrieved from *Enterococcus faecalis* DS-5.

In conclusion, this study reported the first isolation and characterization of lytic bacteriophage against *L. garvieae* serotype II and *N. seriolae*. Since the emergence of drug-resistance strains of *L. garvieae* and *N. seriolae* is on the rise, these phages could serve as potential therapeutic agents in an eco-friendly way. Nevertheless, further detailed experiments are required to fully evaluate their potential in real farming systems. This study also entailed drug resistance patterns and mechanisms of *L. garvieae* serotype II, confirming the presence of similar resistant plasmids in different farms around Japan. This comprehensive study underscores the importance of monitoring and understanding drug resistance patterns and mechanisms in aquaculture, providing valuable insights for the development of more effective strategies to manage and mitigate the growing issue of drug-resistant pathogens in marine aquaculture.

（注１） 論文博士の場合は、「専攻、入学年度」の欄には審査を受ける専攻のみを記入し、入学年度の記入は不要とする。
（注２） フォントは和文の場合 10.5 ポイントの明朝系、英文の場合 12 ポイントの times 系とする。
（注３） 学位論文題目が外国語の場合は日本語を併記すること。
（注４） 和文又は英文とする。