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Bacteriophage therapy of *Pseudomonas plecoglossicida* infection in ayu (은어의 *Pseudomonas plecoglossicida* 감염에 대한 박테리오파아지 요법)

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Introduction

A newly emerging bacterial disease, named as bacterial hemorrhagic ascites caused by *Pseudomonas plecoglossicida*, has posed a threat for ayu *Plecoglossus altivelis* culture industry in Japan for this decade (Nakatsugawa and Iida, 1996; Nishimori *et al.*, 2000; Wakabayashi *et al.*, 1996). The disease occurs with high mortalities through almost all rearing period of this fish species. In particular, the fact that there no licensed chemotherapeutic agent available for this disease has been the cause of intensification in industrial damage. Furthermore, there is a dilemma in use of antimicrobial agent such as florfenicol or sulfisozole for treatment of cold water disease caused by *Flavobacterium psychrophilum*, another serious disease for cultured ayu (Wakabayashi *et al.*, 1994; Iida and Mizokami, 1996), because *P. plecoglossicida* infection abruptly emerges and results in heavy mortality after such chemotherapy.

Theoretically, bacteriophage can be used to treat infectious disease, but little attention has been paid to phage therapy and prophylaxis of bacterial infections in fish. Our recent studies, however, suggest that phage could be useful for controlling bacterial disease of fish (Nakai *et al.*, 1999; Park *et al.*, 2000). The present study was conducted to examine *in vitro* and *in vivo* anti-bacterial activities of phages specific *P. plecoglossicida*.

Materials and Methods

Two types of bacteriophage (PPpW-3; *Myoviridae* and PPpW-4; *Podoviridae*) specific to *P. plecoglossicida* were used in this study. *In vitro* antibacterial activity of these phages, ten mL of Trypto soy broth was inoculated with *P. plecoglossicida* PTH-9802 (4.8×10^2 CFU mL^{-1}) and with serially 10-fold diluted PPpW-3, PPpW-4, or a mixture of PPpW-3 and PPpW-4 (10^6 to 10^2 PFU mL^{-1}) and then incubated with gentle agitation at 20℃. Bacterial growth was measured by spectrophotometer

(530 nm). *In vivo* activity of phage was examined with ayu weight 2.7g fish were fed *P. plecoglossicida*-impregnated pellet (10^7 CFU g^{-1}) and then fed with PPpW-3, PPpW-4, or the mixture of two phages. Also, phage (PPpW-3/PPpW-4) therapy was evaluated under co-habitation condition with fish which had been previously injected with *P. plecoglossicida* and commercial fish pond where *P. plecoglossicida* infection was severely prevailing.

Results

PPpW-4 phage was stronger than PPpW-3 phage and the mixture of two phages exhibited the highest inhibitory (or lytic) activity in *in vitro* antibacterial activity. In the phage therapy, where fish were orally challenged with *P. plecoglossicida* and then receiving oral treatment with phages, the cumulative mortality of fish in the control group received phage-free feed was 93.3% in 2 weeks. A significantly lower mortality was obtained in fish treated with PPpW-3 (53.3%), PPpW-4 (40.0%), and the phage mixture (20.0%). When phage therapy was evaluated under co-habitation, significantly lower mortalities (26.7% in both trials) were produced in phage-treated groups compared with those of phage-untreated controls (90.0% and 100%). For 2 weeks prior to phage treatment, daily mortality decreased at a constant level (average 5.0%) from Day 3 to Day 15 and then reached a lull state at about 6 kg day^{-1} mortality from 18 kg day^{-1}. These results suggest the potential for phage control of disease.

Reference

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