

The efficacy of *Moina micrura* enriched with probiotic *Bacillus pocheonensis* in enhancing survival and disease resistance of Red Hybrid Tilapia (*Oreochromis* spp.) larvae

ABSTRACT

The administration of probiotics via live feeds, such as *Artemia* and rotifers, has gained significant attention. Moreover, indiscriminate use of antibiotics in conventional aquaculture practices in order to prevent or control disease outbreaks has resulted in the occurrence of residues and antimicrobial resistance. Thus, the application of eco-friendly feed additives, such as probiotics, as a safer alternative has received increasing attention in recent years. However, only minimal information on the administration of probiotics via freshwater cladoceran *Moina micrura* is available despite being commonly used for larval and post-larval feeding of freshwater crustaceans and fish. Thus, this study aimed to evaluate the application of *Bacillus pocheonensis* strain S2 administered via *M. micrura* to red hybrid tilapia (*Oreochromis* spp.) larvae. *Bacillus pocheonensis* that has been previously isolated from *Spirulina* sp. was subjected to preliminary *in vitro* evaluation of antagonistic properties. The agar well-diffusion assay revealed that this probiont could inhibit the growth of *Streptococcus agalactiae* and *Aeromonas hydrophila*. The size of inhibition zones ranged from 8.8 ± 0.2 to 18.2 ± 0.4 mm. *Moina micrura* was later used as a biological model in preliminary *in vivo* bacterial challenge assays to evaluate the efficacy of *B. pocheonensis* in protecting the host from diseases. *Moina micrura* was pre-enriched with *B. pocheonensis* at 104 and 106 CFU mL⁻¹ before *S. agalactiae* and *A. hydrophila* were introduced into the culture. The study revealed that *B. pocheonensis* at 104 CFU mL⁻¹ was able to significantly enhance the survival of *M. micrura* after being challenged with both pathogens ($63 \pm 3\%$) in comparison to the control group. The relative percentage survival (RPS) of *M. micrura* was highest ($p < 0.05$) when treated with *B. pocheonensis* at both concentrations 104 and 106 CFU mL⁻¹ (38.33) after being challenged against *S. agalactiae*. To assess the efficacy of *B. pocheonensis* in protecting red hybrid tilapia against streptococcosis, the larvae were fed with either unenriched (control) *Moina* or probiont-enriched *Moina* daily for 10 days. A significantly ($p < 0.05$) higher survival rate ($77 \pm 3\%$) was observed in larvae fed with probiont-enriched *M. micrura* compared to other treatments, and the RPS was recorded at 62.90. In addition, the *S. agalactiae* load was suppressed in larvae fed probiont-enriched *M. micrura* (6.84 ± 0.39 CFU mL⁻¹) in comparison to the control group (7.78 ± 0.09 CFU mL⁻¹), indicating that the probiont might have contributed to the improvement of tilapia health and survival. This study illustrated that *M. micrura* was suitable to be used as a vector for probiotics in freshwater fish larvae as an alternative to hazardous antibiotics for disease control.

Keyword: Live feed; *Moina micrura*; Tilapia; Enrichment; Probiotic; *Streptococcus agalactiae*; Streptococcosis infection