Optimizing Microbial Management in Live Food: Bio-Encapsulation of Functional Microorganisms in Rotifers and *Artemia*

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Introduction

Robust hatchery performance increasingly relies on advanced microbial management strategies. Rather than merely limiting the proliferation of harmful microorganisms, modern approaches aim to actively shape beneficial microbial ecosystems that can support animal health, improve rearing conditions and strengthen overall biosecurity. Within this framework, the targeted use of beneficial bacteria has become a key tool, contributing to a paradigm shift from corrective disease treatments towards proactive, efficiency-oriented microbial management. Live food organisms such as rotifers and *Artemia* play a critical role in early larval rearing, but they can also act as vectors for opportunistic and potentially pathogenic bacteria. Optimizing microbial control during live food production is therefore essential to reduce microbial risks at the larval stage. In this study, different strategies for the application of Sanolife MIC-F were compared in order to modulate the bacterial flora of ready-to-feed rotifers and *Artemia*, with the objective of improving bio-encapsulation efficiency of functional microorganisms and overall microbial stability of live food.

Materials and methods

Application in rotifers

Rotifers were cultured on a mixed diet consisting of VitAlgae Nanno, RoBoost and fresh baker's yeast. At the end of the culture period, rotifers were enriched with Easy Dry Selco (EDS) to optimize their nutritional profile prior to feeding. Following enrichment, several microbial management strategies were evaluated. These strategies combined the use of a natural *Vibrio* controller (Sanocare SURE) with Sanolife MIC-F, which were applied either with or without a prior germination step, and at different concentrations ranging from 2×10^7 to 2×10^8 CFU ml^{-1}. In one treatment, *Bacillus* spp. were additionally administered on a daily basis directly in the culture tank at a concentration of 2×10^4 Bacilli ml^{-1}. To characterize the microbial community associated with the rotifers, bacterial plate counts were performed on a general culture medium (Tryptic Soy Agar (TSA)) and on a selective medium for *Vibrio* spp. (Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS)). The presence and growth of Bacilli were assessed after incubation of TSA plates for 24 h at 37°C. In parallel, rotifer samples were analyzed using Illumina-based 16S rRNA gene sequencing in order to determine the most abundant bacterial phyla.

Application in Artemia

EG SEP-Art® D-FENSE cysts were hatched and the *Artemia* nauplii were harvested after 21h, after which they were transferred to enrichment tanks. In one treatment, Sanolife MIC-F was added directly to the enrichment

water after an appropriate germination step (1 h at 30°C), at a dosage of 1 g of germinated Sanolife MIC-F per liter of enrichment water. *Artemia* nauplii were then introduced into this matured water and subsequently enriched with EDS.

In additional treatments, enriched *Artemia* were first harvested, cleaned and concentrated before the addition of probionts. Different dosages of germinated Bacilli were tested at varying nauplii concentrations, up to a maximum of 8 million nauplii.l^{-1}. The incorporation of the different Bacilli species into the *Artemia nauplii* was evaluated at different time intervals following administration. As for rotifers, microbial plate counts were carried out and 16S rRNA gene amplicon sequencing was performed to characterize the bacterial communities associated with the *Artemia* nauplii.

Results

The results indicate that the functional & beneficial Bacillus species, when combined with the appropriate use of bacteriostatic products during live food preparation, can be effectively bio-encapsulated in both rotifers and *Artemia*. Across all tested application strategies, a clear modulation of the microbial community was observed. In particular, all treatments led to a reduced presence of Proteobacteria in the live food, accompanied by an increased relative abundance of Firmicutes, including *Bacillus* spp.

The use of technologies controlling bacterial development in live food, specifically SURE for rotifers D-FENSE for *Artemia*, contributed to a reduction of *Vibrio* loads in the live food. This reduction appeared to facilitate the encapsulation of the beneficial Bacillus species resulting in a more stable microbiota associated with the live food. Multiple application routes proved successful, highlighting the possibility to adapt microbial management protocols to different operational conditions in hatcheries.

Discussion

This study demonstrates that targeted microbial management strategies are effective tools for modulating the bacterial communities associated with rotifers and *Artemia* nauplii. Across all evaluated application scenarios, the combined use of technologies designed to control microbial development together with the application of selected Bacillus strains consistently reduced the relative abundance of Proteobacteria, including *Vibrio* spp., while promoting an enrichment of Firmicutes, particularly *Bacillus* spp. within the live food microbiota. The use of SURE and D-FENSE not only limited the proliferation of opportunistic bacteria but also enhanced the uptake and bio-encapsulation efficiency of the administered *Bacillus*. Effective incorporation of germinated *Bacillus* cells was achieved through both enrichment-water application and post-harvest treatment of live food, highlighting the flexibility and robustness of the proposed approaches. Overall, these results support the integration of selective microbial management strategies with optimized bioencapsulation protocols as a proactive approach to control live food microbiota, enhance biosecurity and promote more stable microbial conditions in marine larviculture, in line with previous findings on *Bacillus*-based microbial management.

References

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