PROBIOTICS IN LIVE FEED CULTURE: POTENTIAL AND CURRENT PERSPECTIVES

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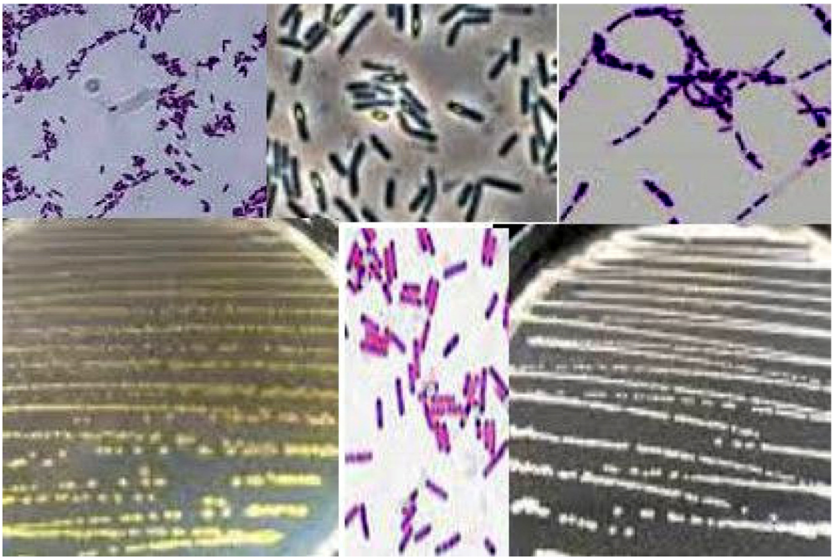
Probiotics are considered as an excellent tool for microbial management in mariculture. They play a major role in mariculture either to establish optimal microbial communities in live feed cultures or as direct feed supplementation for fish larvae. In the long run, adding probiotics to the raising water of live meals may benefit the larvae in addition to enhancing their quality and quantity. Bioencapsulation of various live feeds, includingcopepods, rotifers, and *Artemia*, improves the nutritional status and general health of fish. Enrichment of live feed with probiotics has been applied for many years as microbial adjuncts with promising effects on the growth, health and culture environment of aquatic organisms (FAO & WHO, 2002), that resulted in a growing interest in learning about the advantages of probiotics in live feed cultures. Much attention has been devoted to manipulate the composition of the microbial community in order to improve the stability of cultures and mitigate the proliferation of harmful bacteria. Application of probiotics in live feed cultures enhance the density, swimming behaviour and feeding performance of phyto and zooplankton which will be used as feed for fish and shrimp larvae. Aquaculture faces a significant challenge with regard to organic enrichment and nitrogenous wastes which encompass ammonium (NH_3^+) and ammonia (NH_3). The present chapter deals with the role of probiotics in mass culture of marine microalgae, rotifers and copepods.

Potential probiotic bacteria used in live feed culture

Several probiotics are reported as beneficial for the enhancement of population density of rotifers and copepods. Supplementation of probiotics in *Artemia* increased HUFA and total lipids levels of live feed. Potential probiotic bacterial species that can be used as co-culture with microalgae and as enrichment feed for rotifer, copepod and *Artemia* are *Lactococcus* sp., *Lactobacillus* sp. *Pediococcus* sp., *Carnobacterium* sp., *Bacillus* sp., *Enterococcus* sp., *Pseudoalteromonas* sp., *Microbacterium* sp., *Ruegeria* sp., *Streptococcus* sp., *Thermophilus* sp., and *Shewanella* sp.

Probiotic selection

Potential probiotics can be selected by isolation of native species from live feed or can be procured from commercial companies. Before application of these probiotics as enrichment in live feed, the probiotics can be tested for their antagonistic activity against pathogens and their efficiency in performance of other probiotic properties. The most efficient and potential probiotics can be selected as an enrichment medium for the culture of marine microalgae as water supplementation and as cofeed for rotifer, copepod and *Artemia* cultures.



Isolation and culture of beneficial bacteria

Probiotic enrichment methods in different live feed cultures

Probiotic enrichment in Rotifer culture

1. The rotifers should be washed with fresh seawater and stocked in a plastic bucket containing 50% volume of sterile seawater.

2. Probiotics (5 mg L^{-1}) are mixed with marine microalgae (single or in combination of two species with an approx. con of $1x10^8$cfu/ml) and are blended with 100 mL of deionized water.

3. This mixture is then poured into the bucket containing rotifers. Based on the stocking densities of rotifer, the feeding rate of this mixture can be adjusted.

4. Rotifers can be fed to fish larvae after 6 hours of enrichment

Enrichment of probiotics in Copepod and *Artemia* culture

Newly hatched copepod and *Artemia* are soaked in the probiotic suspension (10 mg L^{-1}) with strong aeration.

This enrichment process should be carried out for 1 h in order to avoid nutrient losses in copepod and *Artemia*.

The enrichment protocol is as follows:

1. The Artemia that has just hatched is not completely developed and therefore cannot consume food from water. However, they are capable of absorbing water that contains probiotics.
2. Consequently, the *Bacillus* has the ability to inhabit both the gastrointestinal tract and the external surface of Copepod/*Artemia.*
3. In Long-term probiotic enrichment, the retention of probiotics in Copepod/*Artemia* nauplii is compared over a long-term period of 24 hours which coincides with the commercial EFA enrichment period.
4. After the 22-hour hatch phase, copepod and *Artemia* nauplii are enriched with S. presso (INVE Aquaculture®, Belgium) and probiotics (1×10^{10} cfu/ ml).
5. Analyze *Artemia* and copepod samples at 10 min, 15 min and 24 hours probiotic enrichment following the methods described above.
6. Disinfection of samples with sodium hypochlorite (5 ppm) for two-time intervals, 10 and 15 minutes gives best results during enrichment process.
7. In short-term probiotic enrichment, the Copepod and *Artemia* are rinsed and concentrated.
8. The stock is inoculated with probiotics at concentration of 1mg/L.
9. The density of the Copepod and *Artemia* can be determined by diluting the culture 1:20 in aerated seawater and counting eight 100 µL samples of *Artemia* under a microscope.

10. *Artemia* and copepod nauplii can be disinfected with sodium hypochlorite (5 ppm) for 15 minutes prior to enrichment. Sodium thiosulphate (10 ppm) is used to neutralise the sodium hypochlorite.

11. Then the cultures are inoculated with the bacterial concentrate to attain 10^6 CFU/mL and remained at room temperature for 30 minutes after inoculation.

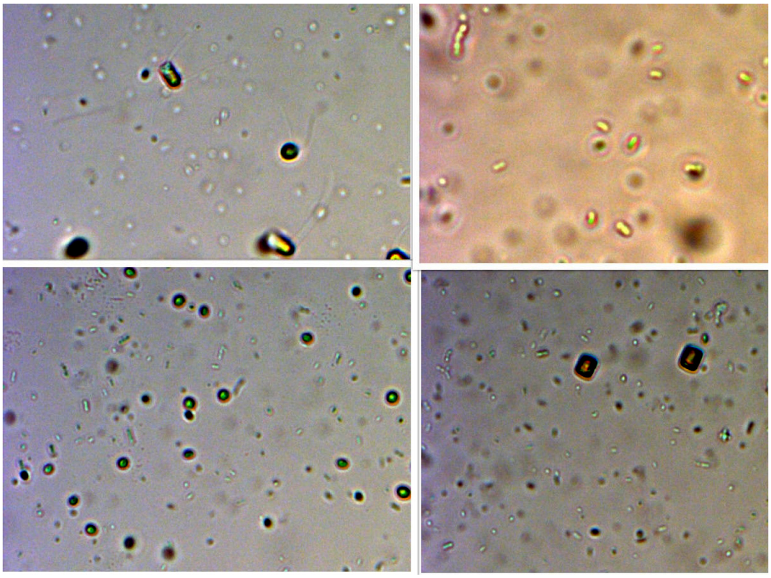
12. The enriched culture is stored in a refrigerator (4 p C) for 6 hours

13. Samples (water, copepods, *Artemia*) are taken at 0.5 and 6 hours for observation of enrichment and for determination of density, survival and also for elimination of pathogens in water.

Application of enriched probiotic consortium in larval rearing of finfish

1. Probiotic consortium (1.6 × 10^{10} CFU.g"1) which is already tested for its potential antagonistic and other probiotic characters towards the cultured larvae is supplemented with live feed diet to the fish larvae as a multi-strain probiotic.

2. The strains are kept in Modified Marine Broth, grown at 35æ%C for 24 h, and then centrifuged at 1800g for 15 min and resuspend in a sterile saline solution.



Co culture of probiotic consortia with marine microalgae

3. Then, the respective suspensions are sprinkled on the artificial feed.
4. The quantity of feed should be replenished every seven days on a weekly basis and stored at 4 C.

Advantages of Probiotic supplementation in live feed

Probiotics consist of live microbial supplements designed to optimize the host's microbial equilibrium for its potential benefit. Most high-intensity live food culture systems see a decrease in yield due to the accumulation of organic carbon, nitrates and phosphorous, which can cause high levels of these nutrients to be released, which can eventually cause the culture to break down. The removal of particulate matter and excess nutrients continues throughout the harvesting period in many culture systems. Hence, to improve the live feed culture condition, probiotically enriched system will be the best solution for constant collapse of the live feed cultures. Therefore, the constant collapse of live food cultures may be remedied by a bacterially mediated mechanism for enhancing the condition of the culture and maximizing the utilization of nutrients. Adding carbon in this system can restore the correct C: N ratio, enabling bacteria to transform solid waste into biomass.

Bacterial communities have the potential to shape the environmental conditions and population expansion of live food cultures. When bacteria and microalgae are combined, the resulting population growth of rotifers is significantly higher compared to using bacteria alone as the primary diet. In the case of larger live food sources, single bacteria cells may prove to be an inadequate diet, and the process of flocculation can be employed to boost the growth of these organisms. Enhanced utilization of microalgae due to its increased digestibility of probiotic bacteria which can enhance amictic reproduction with the availability of increased food.

Enrichment of microalgae with probiotic bacteria enhances number of eggs, population density, locomotion and swimming behaviour of rotifers. Enrichment of probionts helps in dominating their population in culture systems and decreases or inhibits growth of other microbes. Probiotics play a significant role in balancing microbial community of rotifer/copeod culture systems and also on the population density. Probiotic application enhances rotifer/copeod density significantly and the concentration of probiotics and strains vary significantly with initial density of zooplankton and also mode of culture.

Rotifer enrichment with probiotic consortium

The density of copepod nauplii increase significantly (2.4 times) in tanks supplemented with probiotics. Water quality of live feed culture systems also improve with probiotic inoculation in culture tanks. A decrease in *Vibrio* count and increase in the density with increase of probiotic loads and in the mass culture tanks of copepod and rotifers supplemented with probiotic enriched diets. The total bacterial loads and ammonia levels of water in copepod tanks decrease in probiotic treated tanks and eliminates *Vibrios* from tanks fed with marine microalgae supplemented with probiotics.

Conclusion

The usage of probiotics as supplementary diet along with microalgae is suggested to improve the quality of water and density of copepod/rotifer in mass scale production which in turn helps to improve the survival rates of finfish larvae in early stages of development.

References

Douillet, P. A. 2000. Bacterial additives that consistently enhance rotifer growth under synxenic culture conditions - Evaluation of commercial products and pure isolates. *Aquaculture*, 182: 249-260.

Jayasree Loka, S. M. Sonali, purbali saha, K. Devaraj and K. K. Philipose. 2016. Use of commercial probiotics for the improvement of water quality and rotifer density in outdoor mass culture tanks. Indian J. Fish., 63(4): 145-149, 2016. DOI: 10.21077/ijf.2016.63.4.60352-24

Lara-Lores. M. 2011. The use of probiotic in aquaculture: an overview International Research Journal of Microbiology, 2 (2011), pp. 471-478

Verdonck, L., Swings, J., Kersters, K., Dehasque, M., Sorgeloos, P. and Leger, P. H. 1994. Variability of the microbial environment of rotifer (*Brachionus plicatilis*) and *Artemia* production systems. *J. World Aquac. Soc.*, 25: 55-59.

Vine, N. G., Leukes, W. D. and Kaiser, H. 2006. Probiotics in marine larviculture. *FEMS Microbiol. Rev.*, 30: 404-427.

Yun-Zhang Sun, Hong-Ling Yang, Kun-Peng Huang, Ji-Dan Ye, Chun, Xiao Zhang. 2013. Application of autochthonous *Bacillus* bioencapsulated in copepod to grouper *Epinephelus coioides* larvae 2013. Aquaculture 392–395: 44-50

Zink, I. C., Douillet, P. A. and Benetti, D. D. 2013. Improvement of rotifer, *Brachionus plicatilis* population growth dynamics with inclusion of *Bacillus* spp. probiotics. *Aquac. Res.*, 44: 200-211.