Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 7 Issue 3 March 2025

Research Article

Unaltered Viability of *Lactobacillus Acidophilus* La-14 during Storage and After Marine Water Exposure in Fish Larvae Spray-Dried Microdiets

Eduardo Martínez-Angeles[1], María Gisela Ríos-Durán[1]*, Pamela Navarrete-Ramírez[2], Luciana Raggi[2], C Cristian Martínez-Chávez[1] and Carlos A Martínez-Palacios[1]

[1]*Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, México*

[2]*Consejo Nacional de Ciencia y Tecnología CONAHCyT, México - Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, México*

***Corresponding Author:** María Gisela Ríos-Durán, Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, México.

DOI: 10.31080/ASVS.2024.07.0964

Received: January 23, 2025

Published: February 17, 2025

Abstract

Probiotics have recently been an important supplement in in fish feeds showing positive effects on growth and survival in several freshwater and marine fish species. However, probiotic viability during the inclusion of these supplements in the diets has been a challenge, greatly depending on the elaboration process such as spray-drying for fish larvae microdiets. Importantly, scarce information exists about the influence of this process and microdiet handling conditions on the viability of probiotics, despite its potential to encapsulate and protect these microorganisms, with minimal chemical modification. This study evaluated the effect of the spray-drying process, storage time, and marine water exposure on the viability of *Lactobacillus acidophilus* encapsulated in microdiets. Spray-dried microdiets that including *L. acidophilus* La-14, can be stored for up to 6 months at 4 ºC maintaining an adequate CFU count (>10^9) and can be used as a probiotic supplement in microdiets for marine and freshwater fish larvae. This raises the possibility of implementing new feeding strategies, such as using enriched microdiets with probiotic strains capable of resisting salinity and long-term storage, which can have an essential application for the aquafeed industry.

Keywords: Microdiet; *Lactobacillus Acidophilus*; Viability; Fish Larvae

Abbreviations

CFU: Colony Forming Unit; LANMDA: Laboratorio de Nutrigenómica y Microbiómica Digestiva Animal; BHT: Butyl Hydroxytoluene; WPC-80: Whey Protein Concentrate 80%; LAB: Lactic Acid Bacteria

Introduction

The transition to exogenous feeding of fish larvae is a critical stage both in the natural environment and under culture conditions as many fish species depend on the consumption of live prey [1-3]. However, live food has the disadvantage of exhibiting variable nutritional profiles, requiring infrastructure investment with highly trained personnel, and increasing production costs [4,5]. The latter has prompted the development of economic and sustainable alternatives, such as the manufacture of microdiets (spray-dried) that provide adequate particle size and nutrition during the larval stages [6], which allow earlier weaning or complete live food replacement [7].

Furthermore, microdiets can be improved by adding dietary supplements such as probiotics, prebiotics, and antioxidants [8].

The dietary use of some probiotics, such as *Lactobacillus* spp., has shown positive growth and survival results in freshwater and marine fish species [9-17]. In particular, *Lactobacillus acidophilus* has also been shown to adhere to fish intestinal mucosa [18], tolerate wide pH ranges [19] and increase enzyme activity in the gastrointestinal tract [16]. But also, to produce antimicrobial compounds [20], and modulate the immune response [21]. In *C. estor* in particular, *Lactobacillus acidophilus* has shown positive effects in larvae growth and survival [22].

The spray-drying process generates microdiets (< 500 µm), encapsulating and protecting the ingredients with minimal chemical modification. Nevertheless, the viability of cells such as probiotics could be affected by the specific conditions (time, temperature, pressure, oxygen and moisture level) of the spray drying process, the specific strain [23,24], and possibly even the microparticle size. Microdiet handling conditions (*i.e.*, storage conditions and water salinity) may also alter probiotic viability before being ingested by fish larvae [25]. The influence of these factors on *L. acidophilus* viability in microdiets for feeding aquatic organisms is currently unknown and could be relevant for larviculture feeding.

Therefore, this study aims to evaluate the effect of the spray drying process, storage time and marine water culture medium on the viability of *the Lactobacillus acidophilus* probiotic strain encapsulated in small microdiets (23 µm average) for feeding fish larvae.

Materials and Methods

In the present study, two experimental isoproteic, isolipidic and isoenergetic microdiets were formulated and elaborated at Laboratorio Nacional de Nutrigenómica y Microbiómica Digestiva Animal (LANMDA), Universidad Michoacana de San Nicolás de Hidalgo, Mexico, as previously described in another publication [22]. The first microdiet was supplemented with *Lactobacillus acidophilus* La-14 (FloraFit, Dupont, Mexico), and the second was used as a control diet (without *L. acidophilus* supplementation). Both microdiets were formulated to contain 520 gKg-1 protein and 220 gKg-1 lipids (Table 1). Proximal analyzes of the ingredients and microdiets were performed according to the following AOAC (2000) methods and equipment: moisture (Fisher Scientific Isotemp oven), crude protein (Leco FP-528, Dumas method; Ebeling 1968), crude lipid (ether extract; Soxtec Avanti 2050), and crude ash (Fisher Scientific muffle furnace). All samples were analyzed in triplicate (Table 1).

	Experimental Diets	
	Control	***Lactobacillus acidophilus* La-14**
Ingredients (g Kg-1)		
Protein Ingredients [a]	673.8	673.8
Cod liver oil	97.9	97.9
Canola oil	90.7	90.7
Soy lecithin	19.3	19.3
Corn starch	31.4	31.4
Guar gum	20.0	20.0
Mineral premix [b]	15.0	15.0
Vitamin C[c]	3.4	3.4
Choline chloride [d]	3.0	3.0
Vitamin premix [e]	15.0	15.0
Others [f]	30.5	30.5
Lactobacillus acidophilus (CFU mL-1)	0.0	2.98 x 10^9
Chemical Composition* (g Kg-1) (n = 3)		
Crude Protein	539.3 ± 1.2	543.1 ± 2.3
Crude Lipids	223.5 ± 1.5	224.2 ± 2.2
Ash	58.2 ± 1.1	57.6 ± 0.5
Moisture	29.9± 0.8	30.7 ± 1.2

Table 1: Dietary formulation gKg-1 (protein / lipid level 520 / 220 gKg-1) and chemical composition (mean ± standard deviation) of the evaluated microdiets.

[a]Fresh California squid, fresh grouper fillets, dry krill (Tetra), egg albumin (Abaquim S.A.), Whey Protein

Concentrate (WPC80; América alimentos), calcium caseinate (Habacuq S.A. de C.V.), wheat germ.

[b]Mineral premix: macro elements and trace elements (DSM Nutritional products).

[c]L-Ascorbyl-2-Poliphosphate (AsPP), Rovimix®Stay C®35 (DSM Nutritional products).

[d]Choline Chloride (DSM Nutritional products).

[e]Vitamin premix (DSM Nutritional products).

[f]Butyl hydroxytoluene (BHT: antioxidant), Crystaline Taurine, Betaine, orozuz powder, apple extract.

[g]Chemical composition of the commercial microdiet was analyzed in LAMNDA, Universidad Michoacana de San Nicolás de Hidalgo.

*Dry basis

Micro-encapsulated diets were prepared by spray drying (Niro Atomizer, Copenhagen, Denmark, MOBILE MINOR™ 2014, MM-PSR model) with an inlet temperature of 185 °C, an outlet temperature of 75 °C, and a feeding rate of 30 mL min-1, as previously described in detail [22]. Before microdiet elaboration, the bacterial strain *L. acidophilus* was cultured in MRS Agar Lactobacilli media (Difco TM) at 37 °C for 24 hours. Subsequently, a sample of 1 mL was taken from the culture medium, diluted in saline water (1:3), and 0.1 mL of the dilution was placed inside a Neubauer chamber to count the number of bacteria per millilitre (CFU mL-1), using the following equation

CFU mL-1= (Counted bacteria)/(Counted area mm2 * Chamber depth mm))* dilution

The initial concentration of L. acidophilus (2.98 x 109 CFU mL-1) was directly added to the homogeneous liquid mixture of the supplemented microdiet ingredients before spray drying. After preparation, both microdiets were packed in hermetic plastic bags and stored at 4 °C. Probiotic viability was evaluated in the L. acidophilus supplemented diet after 24 h of the spray drying and every 15 days during six months of storage. The same procedure was performed in the control microdiet to evaluate the presence of lactic acid bacteria (LAB) during the trial to ensure no cross-contamination.

Two types of agar culture media specific for LAB (17.5 g MRS agar, Difco TM) were elaborated, one dissolved in 250 mL of ultra-filtered marine water (35 g L-1) and the other in 250 mL of distilled water. A 100 mL dilution (10-3) with 0.1 g of each microdiet (*L. acidophilus* and control) was carefully spread in Petri dishes in each culture media and incubated at 37 °C for 24 hours. Finally, bacterial colonies (CFU g-1) were counted on a dark field colony counter and calculated with the following equation

CFU g-1= (Number of colonies*Dilution factor)/Sample weight g.

The results obtained in these tests were normally distributed and homoscedastic and were analyzed using one-way ANOVA, with a significance level of $\alpha = 0.05$. A Tukey posthoc test ($\alpha = 0.05$) was performed using Sigma Plot statistical software (ver. 14.5).

Results and Discussion

The spray-drying process of the microdiet supplemented with *L. acidophilus* did not impact the viability of the probiotic since bacterial concentration was 2.88 x 10^9 CFU g^{-1} after 24 hours, representing about 95% of the initial concentration added to the diet. The viability of the encapsulated strain in the microdiet steadily decreased but only significantly ($p < 0.038$) at 30 and 45 days, irrespective of the culture media (Figure 1). The viability of *L. acidophilus* was 1.99 x 10^9 CFU g^{-1} (69.1 %) and 1.94 x 10^9 CFU g^{-1} (67.4 %) in ultra-filtered marine and distilled water, respectively, after the 6-month storage period, compared to the microdiet 24 h after spray drying.

There is limited information on the effects of the spray drying process on the viability of probiotics such as *L. acidophilus* [26,27]. In this regard, ingredients such as whey protein isolates have been shown to allow the survival of *Lactobacillus plantarum* during the spray-drying process [28,29]. In our study, bacterial viability 24 hours after the spray-drying process was 95%, suggesting that the microdiet formulation was a stable matrix (arguably by the Whey Protein Concentrate (WPC-80), calcium caseinate and guar gum content) which may have protected *L. acidophilus* during and after the spray drying process, exposed to marine water, and up to 6 months of storage at 4 ºC. Interestingly, despite a recent report that confirms cell shearing of key surface factors of probiotics involved in the adherence to intestinal cells and mucus by the spray-drying process [30], better performance has previously been shown with the addition of *L. acidophilus* in spray-dried microdiets in pike silverside, *Chirostoma estor* larvae [22].

The results of the present study suggest that the probiotic strain *L. acidophilus* La-14 remained viable (10^9 CFU) in microdiets for at least six months, which are adequate bacterial counts to be used as probiotics in fish larviculture [31,32]. This increases the possibility of implementing new feeding strategies, such as using enriched microdiets with probiotic strains capable of resisting salinity and long-term storage, which can be applied in aquafeeds, live feed enrichment and human foods.

Conclusion

Lactobacillus acidophilus resulted viable after the spray drying process and can be stored for up to 6 months at 4 ºC maintaining an adequate CFU count (>10^9) for its use in marine and freshwater fish larvae microdiets.

Acknowledgements

We thank Consejo Nacional de Ciencia y Tecnología (CONACYT, grant number 591255 and Laboratorio Nacional de Nutrigenómica y Microbiómica Digestiva Animal, project number 315841) for the financial support.

Conflict of Interest

There is no financial convenience or conflict of interest between all authors in submitting this manuscript.

Bibliography

1. Yúfera M and MJ Darias. "The onset of exogenous feeding in marine fish larvae". *Aquaculture* 268 (2007): 53-63.
2. Hamre K., *et al.* "Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing". *Reviews in Aquaculture* 5 (2013): S26-S58.
3. Hu, J., *et al.* "Feeding and development of warm water marine fish larvae in early life". *Emerging Issues in Fish Larvae Research*, edited by M Yúfera, Springer, Cham (2018): 275-296.
4. Blair T., *et al.* "Evaluation of microdiets versus live feeds on growth, survival and fatty acid composition of larval haddock (*Melanogrammus aeglefinus*)". *Aquaculture* 225 (2003): 451-461.
5. Conceição LE., *et al.* "Live feeds for early stages of fish rearing". *Aquaculture Research* 41(2010): 613-640.
6. Alam MS., *et al.* "Growth performance, survival and body composition of southern flounder *Paralichthys lethostigma* larvae fed different formulated microdiets". *Aquaculture Research* 46 (2015): 1924-1936.
7. Campoverde C., *et al.* "Early weaning in meagre *Argyrosomus regius*: Effects on growth, survival, digestion and skeletal deformities". *Aquaculture Research* 48 (2017): 5289-5299.
8. Waiyamitra P., *et al.* "Probiotics modulate tilapia resistance and immune response against tilapia lake virus infection". *Pathogens* 9 (2020): 919.
9. Taoka Y., *et al.* "Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system". *Fisheries Science* 72 (2006): 310-321.
10. Al-Dohail MA., *et al.* "Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling". *Aquaculture Research* 40 (2009): 1642-1652.
11. Wang Y. "Use of probiotics *Bacillus coagulans, Rhodopseudomonas palustris* and *Lactobacillus acidophilus* as growth promoters in grass carp (*Ctenopharyngodon idella*) fingerlings". *Aquaculture Nutrition* 17 (2011): 372-378.
12. Salaghi Z, Imanpuor M and V Taghizadeh. "Effect of different levels of probiotic primalac on growth performance and survival rate of Persian sturgeon (*Acipenser persicus*)". *Global Veterinaria* 11 (2013): 238-242.
13. Lara-Flores M and MA Olvera-Novoa. "The use of lactic acid bacteria isolated from intestinal tract of Nile tilapia (*Oreochromis niloticus*), as growth promoters in fish fed low protein diets". *Latin American Journal of Aquatic Research* 41(2013): 490-497.
14. Rodrigues JB., *et al*. "High protein microparticles produced by ionic gelation containing *Lactobacillus acidophilus* for feeding pacu larvae". *Food research international* 63 (2014): 25-32.
15. Hoseinifar SH., *et al.* "Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review". *Reviews in Aquaculture* 8 (2016): 89-102.
16. Akter MN., *et al.* "Effect of *Lactobacillus acidophilus* supplementation on growth performances, digestive enzyme activities and gut histomorphology of striped catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) juveniles". *Aquaculture Research* 50 (2019): 786-797.
17. Dawood MA., *et al.* "Probiotic application for sustainable aquaculture". *Reviews in Aquaculture* 11 (2019): 907-924.
18. Liu W., *et al.* "Comparison of adhesive gut bacteria composition, immunity, and disease resistance in juvenile hybrid tilapia fed two different *Lactobacillus* strains". *Fish and Shellfish Immunology* 35 (2013): 54-62.
19. Gebara C., *et al.* "Viability of *Lactobacillus acidophilus* La5 in pectin-whey protein microparticles during exposure to simulated gastrointestinal conditions". *Food Research International* 51 (2013): 872-878.

20. Todorov SD., *et al.* "Bacteriocin production and resistance to drugs are advantageous features for *Lactobacillus acidophilus* La-14, a potential probiotic strain". *New Microbiologica* 34 (2011): 357-370.

21. Villamil L, Reyes C and MA Martínez-Silva. "*In vivo* and *in vitro* assessment of *Lactobacillus acidophilus* as probiotic for tilapia (*Oreochromis niloticus*, Perciformes: Cichlidae) culture improvement". *Aquaculture Research* 45 (2014): 1116-1125.

22. Martínez-Angeles E., *et al.* "Effects of formulated microdiets on weaning success and larval performance of pike silverside *Chirostoma estor* (Jordan, 1879)". *Animal Feed Science and Technology* 288 (2022): 115288.

23. Tripathi MK and SK Giri. "Probiotic functional foods: Survival of probiotics during processing and storage". *Journal of Functional Foods* 9 (2014): 225-241.

24. Terpou A., *et al.* "Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value". *Nutrients* 11 (2019): 1591.

25. Broeckx G., *et al.* "Enhancing the viability of *Lactobacillus rhamnosus* GG after spray drying and during storage." *International Journal of Pharmaceutics* 534 (2017): 35-41.

26. Paéz R., *et al.* "Effect of heat treatment and spray drying on lactobacilli viability and resistance to simulated gastrointestinal digestion". *Food Research International* 48 (2012): 748-754.

27. Soukoulis C., *et al.* "Impact of milk protein type on the viability and storage stability of microencapsulated *Lactobacillus acidophilus* NCIMB 701748 using spray drying". *Food and Bioprocess Technology* 7 (2014): 1255-1268.

28. Rajam R., *et al.* "Effect of whey protein-alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions". *Journal of Functional Foods* 4 (2012): 891-898.

29. Khem S, Small DM and BK May. "The behaviour of whey protein isolate in protecting *Lactobacillus plantarum"*. *Food Chemistry* 190 (2016): 717-723.

30. Kiekens S., *et al.* "Impact of spray-drying on the pili of *Lactobacillus rhamnosus* GG". *Microbial Biotechnology* 12 (2019): 849-855.

31. Hai NV. "The use of probiotics in aquaculture." *Journal of Applied Microbiology* 119 (2015): 917-935.

32. Wang A., *et al.* "Use of probiotics in aquaculture of China-a review of the past decade". *Fish and Shellfish Immunology* 86 (2019): 734-755.