

PROBIOTIC LACTIC ACID BACTERIA *Pediococcus* sp. IMPROVED THE GUT MICROBIOTA AND ENHANCED THE SURVIVAL OF MILKFISH *Chanos chanos* EARLY JUVENILES AGAINST THE PATHOGEN *Vibrio harveyi*

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ABSTRACT

Background

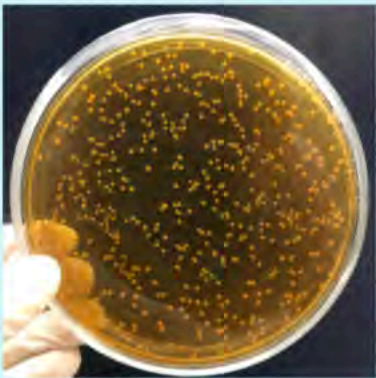
In the Philippines, milkfish aquaculture is seen as the industry's backbone. However, disease outbreaks induced by bacterial infection, notably Vibriosis disease caused by *Vibrio harveyi*, impede nursery output. Gut microbiota are important in aquatic organisms because they are symbionts that help the host's innate and adaptive immune responses, as well as the epithelial mucosal barrier. However, disruption of their colonization results in increased disease expression. Thus, probiotic supplements help support the gut microbiome, aiding host metabolic plasticity, immune responses, and metabolism.

Objective

*Pediococcus* sp., a lactic acid bacteria (LAB) isolated from a wild milkfish fry, was utilized to supplement milkfish early juvenile diet to boost the immunological response against *V. harveyi*.

Methodology

LAB isolation from wild milkfish fry gut.



Inoculating LAB suspension using NSS(10^6) to feeds.



Probiotic-treatment trial with early juvenile milkfish.



*Vibrio harveyi* immersion challenge test.

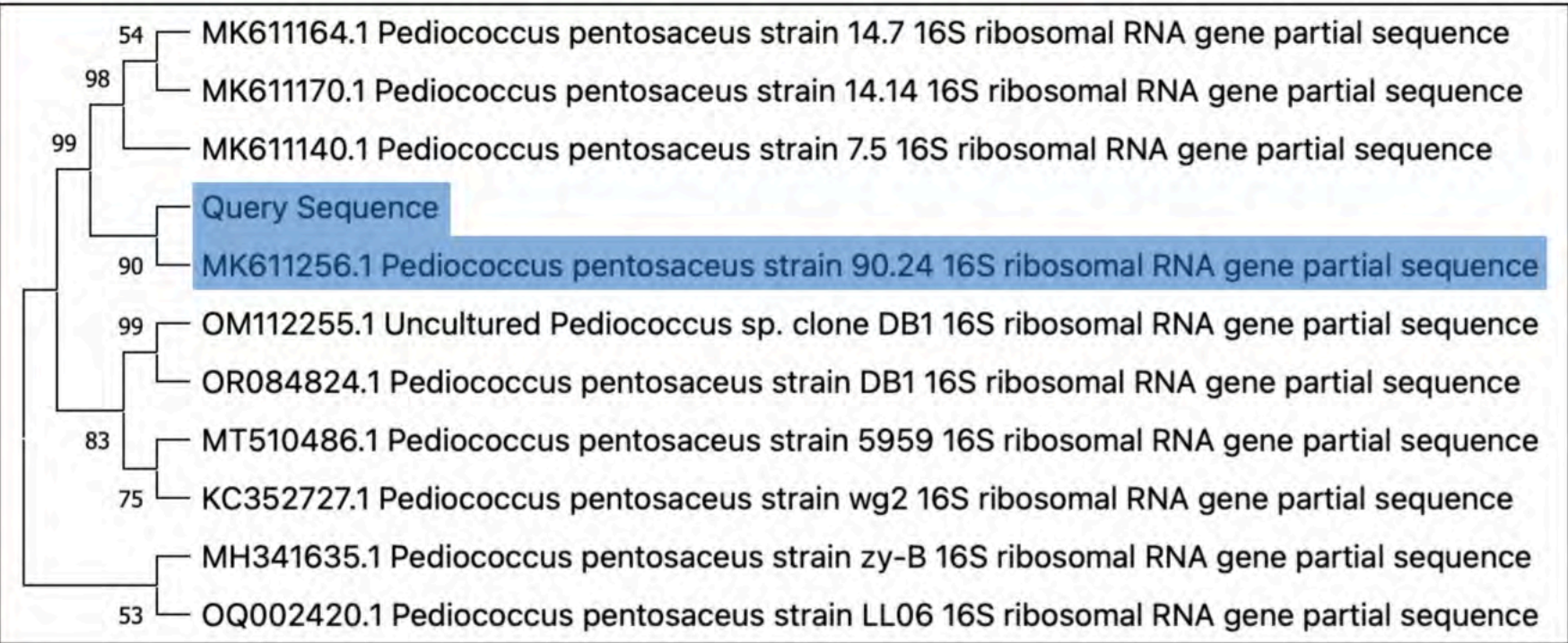


Conclusion

Lactic acid bacteria *Pediococcus* sp. in milkfish early juveniles diet inhibited *Vibrio* spp. through gut colonization after 12 days probiotic treatment. Fish survival against *V. harveyi* was also enhanced by 53.33% compared to the control with no probiotic, following a 14-day bacterial immersion challenge test.

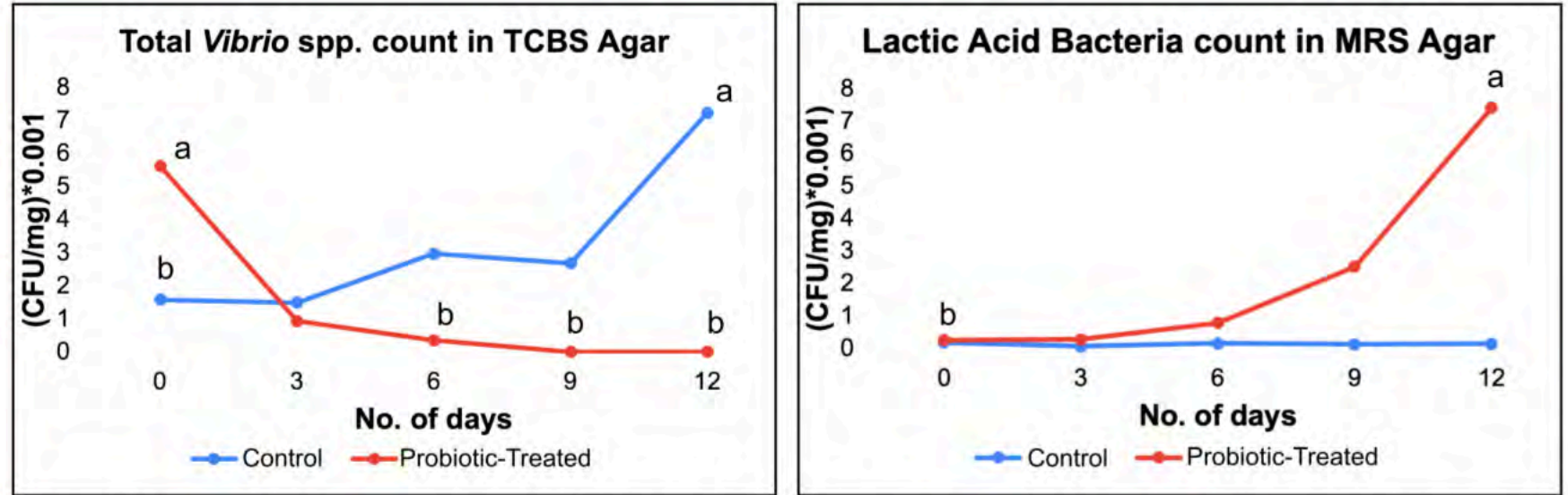
RESULTS

16S rDNA Gene Sequence Homology Alignment Analysis



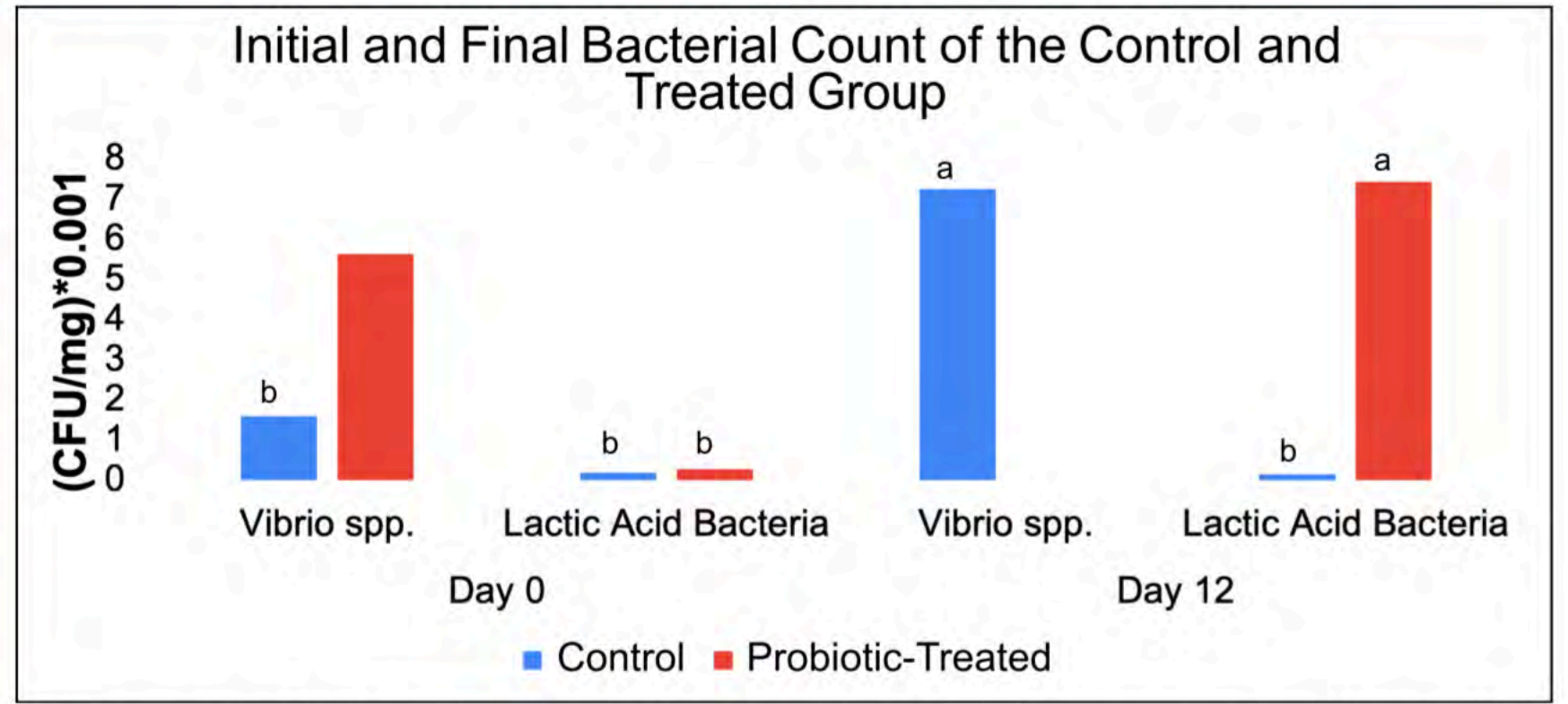
The top 10 NCBI blast nucleotide sequences producing significant alignment were processed in MEGA 11 to produce a neighbor-joining tree, showing a 90% likelihood of the query sequence being related to the MK611256.1 *Pediococcus pentosaceus* strain.

Gut Colonization of *Vibrio* spp. and *Pediococcus* sp. in Milkfish Early Juveniles



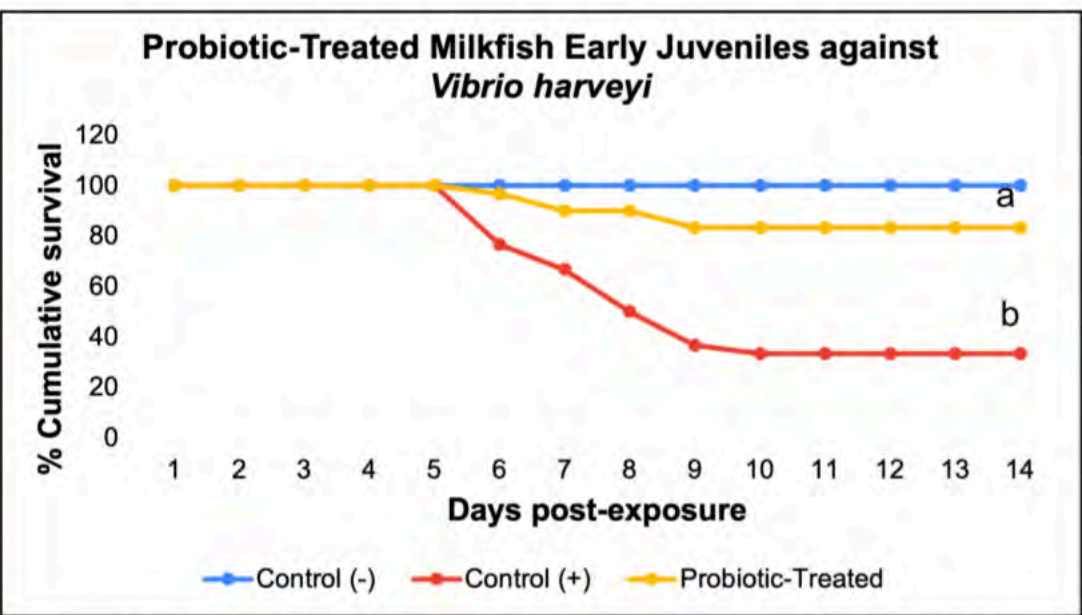
*Pediococcus* sp. completely colonized milkfish early juveniles gut after 12 days of treatment. \*Letters indicate significant difference within groups from the Day 0 count (Paired Samples t-Test,  $p < 0.05$ ).

Relationship Between the Treated and Control Group after the 12-day Probiotic Treatment Period



Results revealed that probiotic-treated fish have lower *Vibrio* spp. count and elevated LAB count after 12 days of treatment while fish untreated with probiotic showed otherwise. \*Letters indicate significant difference within groups (Paired Samples t-Test,  $p < 0.05$ ).

Survival of Milkfish Early Juveniles Treated with Probiotic against Pathogenic *Vibrio harveyi*



Fish not treated with probiotic, 14 days post-exposure to *V. harveyi*.



Probiotic-treated fish, 14 days post-exposure to *V. harveyi*.

*Pediococcus* sp. supplement in the early juvenile milkfish diet significantly improved survival compared to the control by 53.33% (Independent Samples t-Test,  $p < 0.05$ ). Fish with no probiotic supplement also showed more pronounced disease signs (e.g., fin rot, scale loss, lesions, and skin ulcerations).

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