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Health & Welfare

Domestication of gut microbiota can improve shrimp aquaculture

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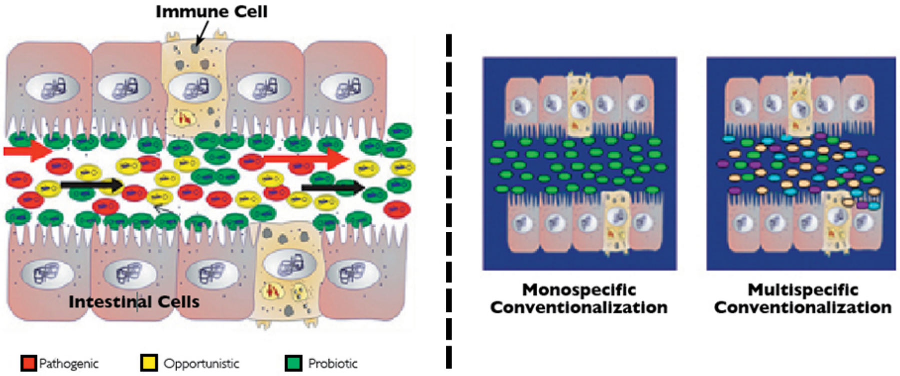
Bacteri, yeasts isolated from native microbiota of wild shrimp



Current larval-feeding practices for shrimp (left) present the risk of colonization of the shrimp gut by pathogenic bacteria that disrupt normal development of the digestive tract and associated immune system. In conventionalization (right), mono- or multispecies sets of selected bacteria and yeast are inoculated into the sterile digestive tract to improve digestive function.

The successful culture of shrimp at early stages may essentially depend on digestive tract colonization by beneficial microorganisms and exclusion of pathogenic ones. Native microbiota have been shown essential for correct development and function of the digestive tract and its associated immune system.

However, aquaculture production practices and systems do not always permit the process of digestive tract colonization with native microbiota. Water, eggs and nauplii are disinfected, but later contaminated with opportunistic bacteria present in the culture systems (microalgae, nauplii, zooplankton, feed) and not related to the native microbiota in cultivated animals.

With the support of FINCYT, domestication of native microbiota in white leg shrimp (*Litopenaeus vannamei*) has been investigated by the authors' teams at Marinazul in Peru and Concepto Azul in Ecuador. Microorganisms isolated from different parts of the shrimp digestive tract have been cultivated, molecularly characterized and evaluated according to a process known as "conventionalization." Conventionalized larvae – animals with global or partial native microbiota – showed better growth and/or survival in comparison to the normal hatchery-produced larvae.

Study setup

Bacteria and yeasts were isolated from the native microbiota of wild shrimp at larval, juvenile and adult life stages. Characterizations of the isolated bacteria and yeasts were based on metabolic assays of amylase and protease, antagonism tests with pathogenic bacteria and molecular criteria related to amplification and sequencing of 16S rDNA, rpoB and lactonase genes.

Mono- and multispecific conventionalization processes were performed by placing the shrimp in the respective suspensions of microorganisms. As a preliminary step, the authors established protocols for egg and larva disinfection before inoculation of microbial strains. Just after spawning, shrimp eggs were disinfected with formalin. A second disinfection process was applied to stage IV shrimp nauplii just before stocking them in sterilized seawater.

The selected microorganisms were administered directly into the culture water before and after the mouth-opening period of the shrimp. One inoculation was given every other day until the PL^{12} stage. Effective digestive tract colonization could be qualitatively and quantitatively monitored using laser-scanning confocal microscopy and real-time polymerase chain reaction testing.

At the end of the experimental period, growth and survival rates were estimated. In some cases, experimental infections with pathogenic bacteria were performed to estimate the protection efficiency of native microorganisms in conventionalized animals.

Results

Three specific collections of native microbiota microorganisms were established and molecularly characterized, corresponding to yeasts *(Saccharomyces, Moeszyomyces, Candida),* Gram-negative and Gram-positive bacteria belonging to the genera *Vibrio, Aeromonas, Acinetobacter, Oxalobacter, Photobacterium, Rhodobacter, Psychrobacter, Psychromonas, Pseudomonas, Shewanella, Proteus, Enterococcus, Micrococcus, Microbacterium, Bacillus* and *Lysinibacillus, Exiguobacterium* and a collection of unknown bacterial strains.

Along the hepatopancreas, midgut and hindgut of the shrimp digestive tract, Gram-positive proteobacteria were predominant, with a majority of *Vibrio* species followed by *Photobacterium, Psychobacter* and *Shewanella*. *Rhodobacter* was only present in adult and juveniles. Gram-positive bacteria were principally represented by *Bacillus* species, followed by *Micrococcus, Exiguobacterium* and *Enterococcus* in adult and juveniles.

Low bacteria diversity was observed in shrimp larvae, where *Vibrio, Psychobacter* and *Bacillus* species were mostly present. Among *Vibrios*, the species *alginolyticus* was dominant (Table 1).

Emmerik, Cultivable bacterial distribution, Table 1

Bacteria Species	Adult Shrimp	Juvenile Shrimp	Postlarvae
Vibrio	62.35%	32.0%	42.0%
Photobacterium	17.65%	6.0%	–
Shewanella	4.71%	–	–
Bacillus	3.53%	21.0%	16.0%
Aeromonas	3.53%	8.0%	5.0%
Rhodobacter	2.35%	2.0%	–
Psychrobacter	2.35%	6.0%	37.0%
Micrococcus	1.18%	–	–
Leptolyngbya	1.18%	2.0%	–
Exiguobacterium	1.18%	13.0%	–
Proteus	–	3.0%	–
Psychromonas	–	3.0%	–
Microbacterium	–	2.0%	–
Enterococcus	–	2.0%	–

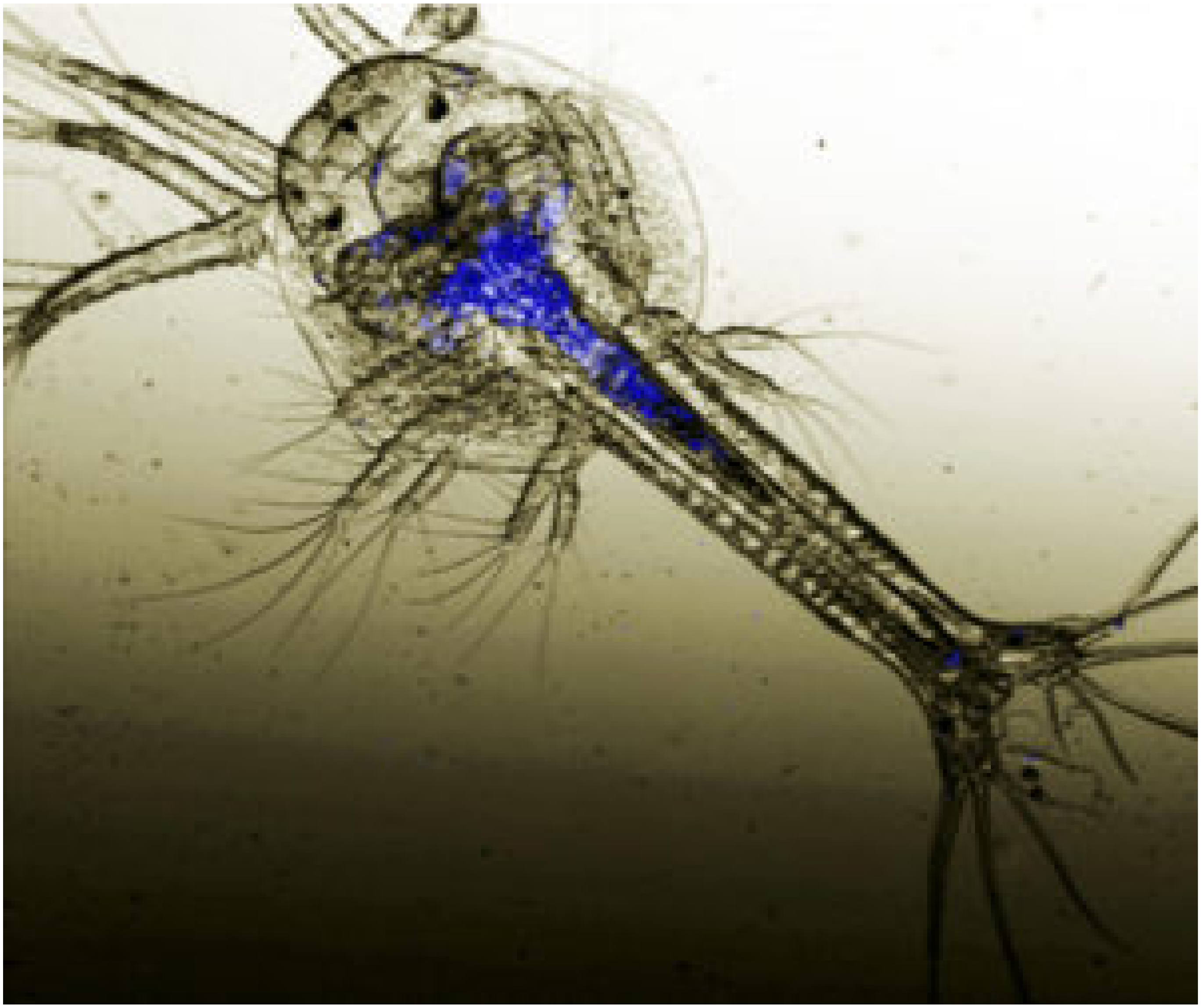
Table 1. Cultivable bacterial distribution in different stages of shrimp.

The selection of bacteria with metabolic characters potentially interesting for probiotic effects (protease, amylase and lactonase) was conceptualized to constitute several mixes for conventionalization.

Quorum quenching

Vibrio bacteria possess an elaborate communication system known as “quorum sensing,” which relies on extracellular production of lactone. High concentrations of lactone serve to induce such gene functions as bioluminescence, biofilm formation and toxin production. Nevertheless, some vibrio-antagonistic bacteria developed a system able to inhibit quorum sensing known as “quorum quenching” through degradation of lactone by the enzyme lactonase.

Most lactonase-producing bacteria are *Bacillus* or *Schewanella*. Identification of such bacteria was performed using polymerase chain reaction testing in order to detect the presence of the lactonase



This magnified photo shows a zoea I stage shrimp colonized with probiotic bacteria marked with ethidium bromide (blue areas).

gene sequence in the bacterial genome.

The efficiency of several strains considered potential probiotics able to inhibit three pathogenic vibrios was experimentally tested. Administration of a lactonase-producing bacteria mix to shrimp postlarvae experimentally infected by pathogenic vibrio was efficient and showed up to 10 times higher survival at three days post-infection than in infected vibrio without administration of probiotics.

Conventionalization effects

The use of strains able to produce extracellular amylase and protease could be very important to improve digestion and assimilation of balanced diets. Recent studies have shown that the shrimp domestication process resulted in a loss in capacity to process carbohydrates and favored selection of shrimp with greater capacity to digest proteins as an energy source.

Wild shrimp are characterized by their primary capacity to digest glucides, a “cheaper” energy source than protein. As a consequence, the selection and application of probiotics represent necessary tools to compensate for this drawback of domestication in feeds formulated with lower protein content without negative impacts on growth and survival rates. Moreover, simplification of ingested feeds by bacterial enzymatic activity should allow fast, optimized nutrient assimilation by shrimp digestive systems.

With the first eight bacterial mixes tested in triplicate, four mixes gave better survival in *Litopenaeus vannamei* than the control provided (Fig. 1). Results showed a 10 to 30 percent increase in survival with three combinations of *Bacillus* and *Vibrio alginolyticus* native strains. Microbial mixes IV and V, made mainly with Gram-negative pathogenic/opportunistic bacteria, showed strong negative impacts on survival.

Fig. 1: Survival indexes in Pacific white shrimp conventionalized with different mixes of bacteria.

Perspectives

It is important to consider that every animal and plant species has performed a coevolution process with its native microbiota. Aquaculture will have to consider such processes for present and future culture species. Because of the recent progress in molecular microbiology and confocal microscopy, applied research in aquaculture gnotobiology has been made possible, as showed by the present work with some model invertebrate and vertebrate species.

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