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PRECISION PROBIOTICS IN AQUACULTURE: A SYSTEM AND METHOD FOR TAILORING BENEFICIAL MICROBES FOR SPECIES-SPECIFIC HEALTH AND PRODUCTION

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***ABSTRACT*:**

Aquaculture now supplies more than half of all fish consumed by humans worldwide, and its continued expansion is constrained by recurring disease outbreaks, inconsistent growth performance, and mounting pressure to curb antimicrobial use. Probiotic supplementation has long been proposed as an ecologically sound alternative to antibiotics, yet first-generation probiotic products often generic, terrestrially derived strains applied uniformly across species—have delivered inconsistent field results. This review examines the emerging paradigm of precision probiotics: host-adapted, mechanistically characterized microbial formulations designed around the physiology, gut ecology, immune architecture, and production environment of a specific aquatic species or life stage. We synthesize current understanding of host–microbiome interactions in finfish, crustaceans, and mollusks; describe the molecular and ecological mechanisms through which candidate probionts confer benefit; and outline how genomics, metabolomics, culturomics, machine learning, and genome-editing tools are converging to enable rational strain selection and formulation design. Species-specific case examples in salmonids, tilapia, carp, shrimp, and bivalves illustrate both the promise and the constraints of this approach. We also address the regulatory, ecological, and commercial barriers to scaling precision probiotics—including strain stability during processing, colonization competition with resident microbiota, biosafety of genetically modified strains, and the absence of harmonized international standards. We conclude that precision probiotics represent a necessary evolution beyond one-size-fits-all supplementation, but that translating laboratory-validated consortia into robust, farm-ready products will require sustained investment in mechanistic research, standardized efficacy testing, and regulatory frameworks that keep pace with the underlying science.

***Keywords**: Precision Probiotics; Aquaculture Microbiome; Host-Specific Probionts; Gut Microbiota; Synbiotics; Fish Immunity; Sustainable Aquaculture; Microbiome Engineering*

INTRODUCTION

Global aquaculture has grown from a marginal contributor to world fish supply in the mid-twentieth century into the dominant source of aquatic animal protein today, a transition driven by the stagnation of capture fisheries and rising demand for affordable, high-quality protein [1]. This growth, however, has been accompanied by a parallel rise in the density and intensity of farming systems, conditions that favor the emergence and rapid spread of bacterial, viral, and parasitic diseases. Historically, the aquaculture industry addressed disease pressure with antimicrobial compounds, a strategy whose limitations are now well documented: the emergence of multidrug-resistant pathogens, residues in edible tissue that threaten market access, and disruption of the resident microbiota that can paradoxically increase susceptibility to opportunistic infection. These concerns, combined with growing regulatory restriction of prophylactic antibiotic use in food animal production, have pushed the sector toward biological alternatives, among which probiotics occupy a central place [20].

The probiotic concept in aquaculture borrows heavily from human and terrestrial livestock nutrition, where defined live microbial supplements are used to influence gut ecology, nutrient assimilation, and immune competence. Early aquaculture probiotics largely followed this precedent, applying generic strains—frequently *Lactobacillus*, *Bacillus*, or yeast species originally isolated from mammalian or environmental sources—across a wide range of fish and shellfish species with only limited attention to whether those organisms could colonize, persist, or interact meaningfully with the target host's physiology [3]. The results of this approach have been

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genuinely useful in aggregate, with meta-analyses across many trials showing average improvements in growth, feed conversion, and survival following pathogen challenge [2]. Yet the same literature is characterized by considerable variance: the same probiotic strain that improves outcomes in one species, water chemistry, or rearing system frequently fails to replicate that effect elsewhere. This inconsistency has practical consequences for farmers, who face uncertain returns on probiotic investment, and for regulators, who must evaluate products whose mechanism of action is often poorly characterized [6].

The concept of "precision probiotics" has emerged from this recognition of variance as a symptom rather than noise. Rather than treating probiotic efficacy as a property of a strain in isolation, the precision paradigm treats efficacy as an emergent property of the interaction between a specific microbial genotype, a specific host genotype and life stage, a specific resident microbial community, and a specific rearing environment. Under this framework, strain selection begins not with a catalog of previously marketed probiotic species but with characterization of the target host: its digestive physiology, its innate and adaptive immune architecture, the composition and successional dynamics of its native microbiota, its principal pathogens, and the physicochemical conditions under which it is farmed. Candidate microorganisms are then selected, and where appropriate engineered, to match this host-specific profile, with efficacy validated through mechanistic assays before large-scale trials are undertaken [18].

This shift has been made possible by the same technological advances that have transformed microbiome science more broadly. High-throughput sequencing has made it feasible to characterize the gut and water-column microbiota of commercially important aquaculture species at a resolution that was unattainable a decade ago. Culturomics the systematic, high-throughput isolation of previously unculturable microbial taxa has expanded the pool of candidate probionts beyond a handful of well-studied genera. Metabolomic and transcriptomic profiling allow researchers to move from correlational associations between microbial taxa and host health toward mechanistic explanations grounded in specific metabolites, signaling pathways, and immune transcripts. Meanwhile, computational tools, including machine learning classifiers trained on multi-omic datasets, are beginning to allow in silico prioritization of candidate strains before they are tested in vivo, compressing what was once a years-long empirical screening process.

This review has four aims. First, we describe the foundations of host–microbiome interaction in aquatic animals, emphasizing features that distinguish aquatic hosts from terrestrial livestock and that must inform any precision approach. Second, we examine the principal mechanisms through which probiotic microorganisms exert beneficial effects in aquatic hosts, organized around competitive exclusion, metabolic contribution, immunomodulation, and water-quality mediation. Third, we survey species-specific considerations across major aquaculture taxa salmonids, warm-water finfish such as tilapia and carp, penaeid shrimp, and bivalve mollusks illustrating how host biology shapes probiotic design. Fourth, we examine the technological toolkit enabling precision design, the delivery systems required to translate laboratory findings into farm-ready products, and the regulatory and ecological challenges that must be resolved before precision probiotics can be deployed at scale. We conclude with a perspective on the research priorities most likely to accelerate responsible adoption of this technology.

THE AQUACULTURE MICROBIOME: FOUNDATIONS OF HOST–MICROBE INTERACTION

2.1 A fundamentally open microbial system

The single most consequential difference between the microbiome of an aquatic animal and that of a terrestrial livestock species is the degree of openness between the host-associated microbiota and the surrounding environment. A pig or chicken interacts with its microbial environment primarily through ingested feed, contact with conspecifics, and the built environment of a barn. A fish, shrimp, or oyster, by contrast, is bathed continuously in water that is itself teeming with microorganisms, and it exchanges microbes with that water across the gill epithelium, the skin, and the gut with every respiratory and feeding cycle. The gut microbiota of most aquatic species is therefore only loosely "resident" in the sense familiar from mammalian microbiome science; it is better understood as a dynamic subset of the surrounding water and sediment microbial pool, filtered and selected by gut physiology, diet, and host immune surveillance, but continuously reseeded from the external environment.

This openness has two major implications for precision probiotic design. First, water-column and sediment microbial management is not a peripheral concern but a core determinant of gut microbiota composition, meaning

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that probiotic strategies for aquatic species often must operate simultaneously at the level of the feed and the level of the rearing water. Second, because the external reservoir of microorganisms varies enormously between farming systems—recirculating aquaculture systems, flow-through raceways, earthen ponds, and open net pens each present a distinct microbial background—a probiotic strain validated in one system cannot be assumed to behave identically in another, even when the host species is held constant.

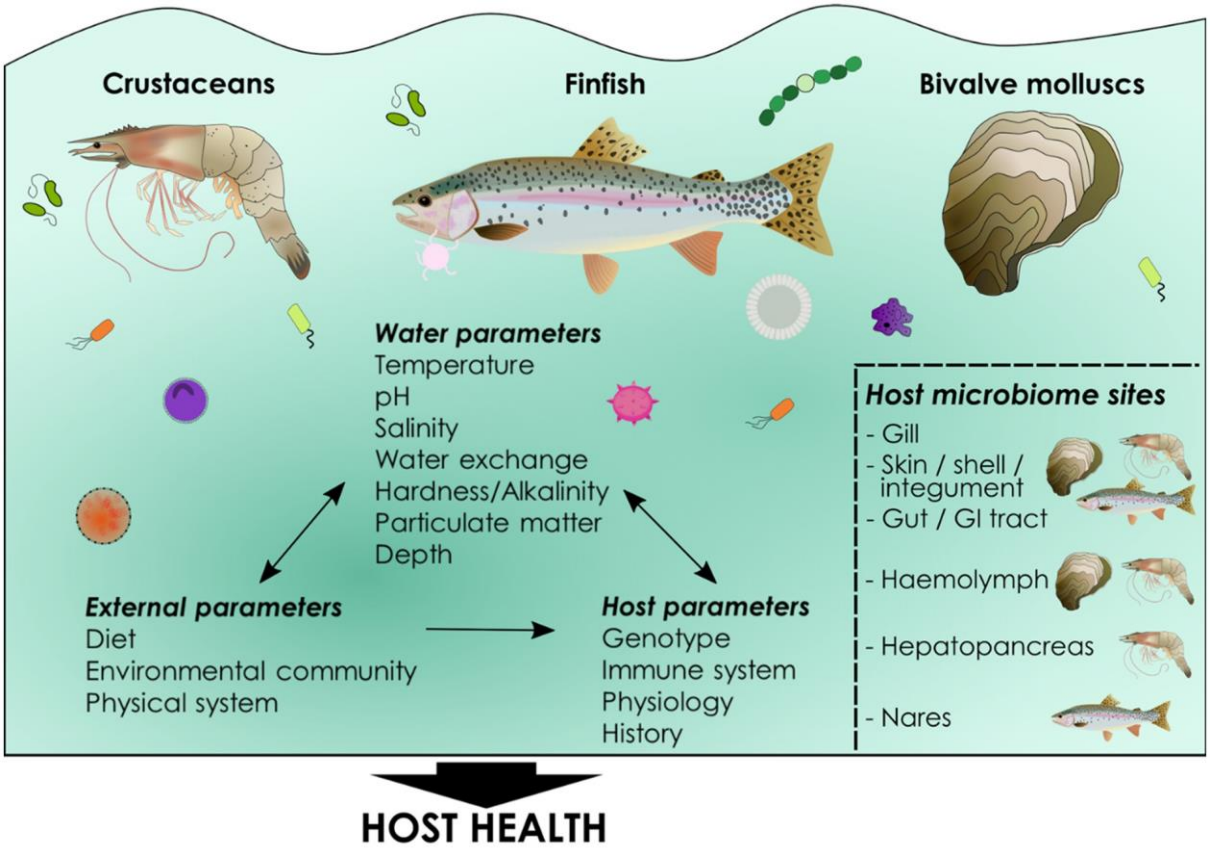

Figure 1. Host- and water-parameter determinants of microbiome composition across crustaceans, finfish, and bivalve molluscs, and the principal host microbiome sampling sites for each group. Source: adapted from published aquaculture microbiome literature.

2.2 Ontogenetic and life-stage variation

Aquatic animals typically pass through more pronounced developmental transitions than most terrestrial livestock, and the microbiota shifts substantially at each transition. Larval fish and crustaceans frequently lack a fully differentiated gut and stomach, rely on yolk reserves and then live feeds such as rotifers and Artemia, and harbor a microbiota dominated by whatever organisms are introduced with those live feeds or present in the rearing water. As the digestive tract matures and animals transition to formulated diets, the microbiota undergoes a marked succession, often with a narrowing of diversity as host selective pressures—bile acids, digestive enzymes, mucosal immune factors—begin to exert stronger filtering effects. Precision probiotic strategies must therefore specify not only the target species but the target life stage, since a probiotic strain and delivery format appropriate for post-larval shrimp may be entirely unsuitable, or even harmful, when applied to broodstock or juveniles reared in a different system.

2.3 Poikilothermy and environmental coupling

Because aquatic animals are ectothermic, their metabolic rate, digestive transit time, and immune function are tightly coupled to ambient water temperature, and this coupling extends to the microbiota. Gut transit time in fish can vary several-fold across the physiological temperature range of a species, altering the residence time available for probiotic colonization and metabolic activity. Immune competence in ectotherms is also temperature-dependent, with many species showing reduced adaptive immune function at the lower end of their thermal tolerance range, a period during which innate defenses—including those potentiated by probiotics—assume disproportionate importance. Any precision probiotic formulation must therefore be validated across the seasonal

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or system-specific temperature range the target species will actually experience, rather than under a single laboratory condition.

2.4 Mucosal surfaces as the primary interface

Unlike terrestrial animals, aquatic species present extensive mucosal surfaces—skin, gill, and gut epithelium—directly to the microbial environment, and each of these surfaces hosts a distinct, if compositionally related, microbial community. The skin mucus of fish contains antimicrobial peptides, lysozyme, and immunoglobulins that shape which bacteria can colonize, while the gill epithelium, given its role in respiration and osmoregulation, is particularly sensitive to microbial disruption. Precision probiotic design increasingly considers all three mucosal compartments rather than the gut alone, since a strain that improves gut health without addressing gill or skin colonization by opportunistic pathogens may deliver only partial protection against the polymicrobial disease pressure typical of intensive culture systems [8], [16].

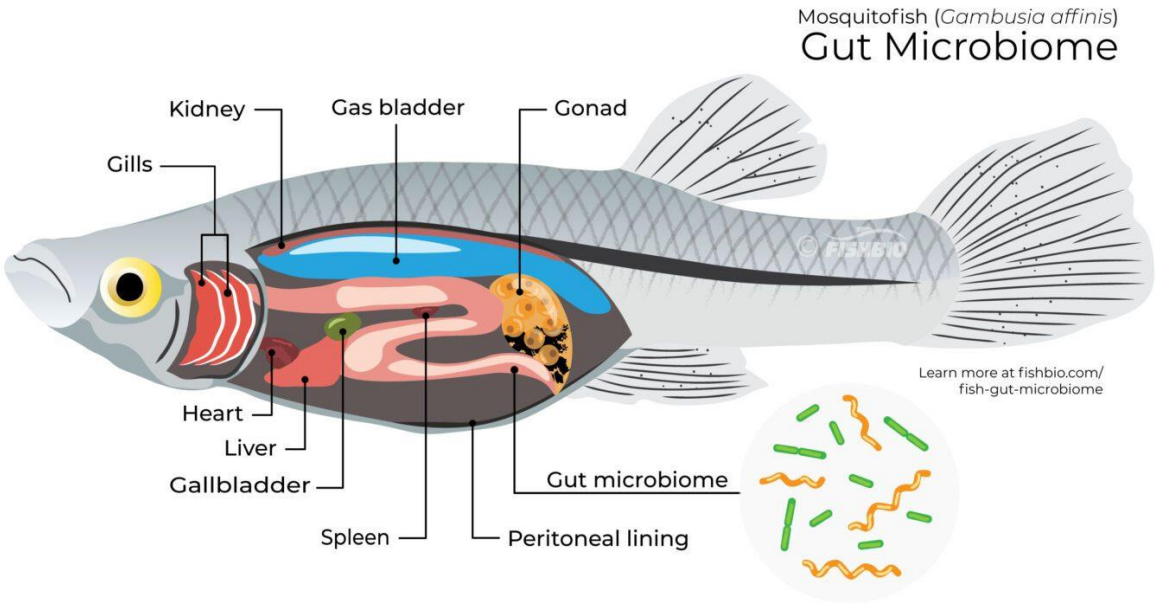

Figure 2. Anatomical location of the gut and its resident microbiome in a representative finfish host (mosquitofish, Gambusia affinis). Source: FISHBIO.

FROM EMPIRICAL TO PRECISION PROBIOTICS: A PARADIGM SHIFT

The first generation of commercial aquaculture probiotics emerged largely by analogy with human and terrestrial animal nutrition, and strain selection criteria reflected that origin: ease of large-scale fermentation, spore-forming capacity for shelf stability (favoring *Bacillus* species), or prior regulatory acceptance in other animal production contexts (favoring lactic acid bacteria). These criteria are commercially rational but biologically arbitrary with respect to any given aquatic host, and the resulting products have been tested and marketed across taxonomically and ecologically disparate species—salmon, shrimp, tilapia, carp often with only modest adaptation of dose or delivery format [9].

The precision paradigm inverts this sequence. Strain selection begins with characterization of the target host system: culture-independent sequencing of the gut, skin, and gill microbiota across healthy and diseased populations to identify taxa associated with favorable outcomes; identification of the principal pathogens and the ecological niches they occupy; and profiling of host immune gene expression under baseline and challenge conditions. Candidate probionts are then drawn preferentially from the native or ecologically compatible microbiota of the target species, on the reasoning that autochthonous strains are more likely to possess the adhesion factors, bile and gastric tolerance, and immune-signaling compatibility required for stable colonization. Where a native strain with the desired functional profile cannot be found, genome editing or directed evolution may be used to confer a specific trait enhanced bacteriocin production, a particular adhesin, or enhanced thermal tolerance onto a well-characterized chassis organism, again matched to the host's physiological range [4].

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This is not merely a semantic distinction. Comparative trials in several species have shown that host-derived probiotic candidates outperform allochthonous strains of the same genus in colonization persistence and in disease-challenge protection, even when in vitro antagonism assays against a pathogen show similar potency for both strains. The explanation generally offered is that colonization and persistence—not simple antagonism in a culture dish—are rate-limiting for in vivo efficacy, and that colonization success depends on host-specific adhesion and immune tolerance mechanisms that a strain isolated from an unrelated species is unlikely to possess by chance.

A second defining feature of the precision paradigm is its emphasis on mechanistic validation prior to field deployment. Rather than screening candidate strains directly for growth or survival benefit in a full-scale trial—a slow, expensive, and mechanistically uninformative endpoint—precision probiotic development typically proceeds through a tiered validation pipeline: in vitro assays of adhesion, antagonism, and bile/acid tolerance; ex vivo or organoid-based assays of immune stimulation; controlled challenge trials in a defined pathogen model; and only then, farm-scale trials under commercial conditions. This tiered approach allows researchers to discard non-performing candidates early and to generate a mechanistic hypothesis for why a successful candidate works, which in turn supports rational optimization of dose, delivery timing, and combination with complementary strains or prebiotic substrates.

MECHANISMS OF PROBIOTIC ACTION IN AQUATIC HOSTS

Understanding species-specific probiotic design requires a clear account of the mechanisms through which candidate microorganisms are believed to confer benefit. These mechanisms are not mutually exclusive, and a well-designed precision probiotic often engages several simultaneously [10].

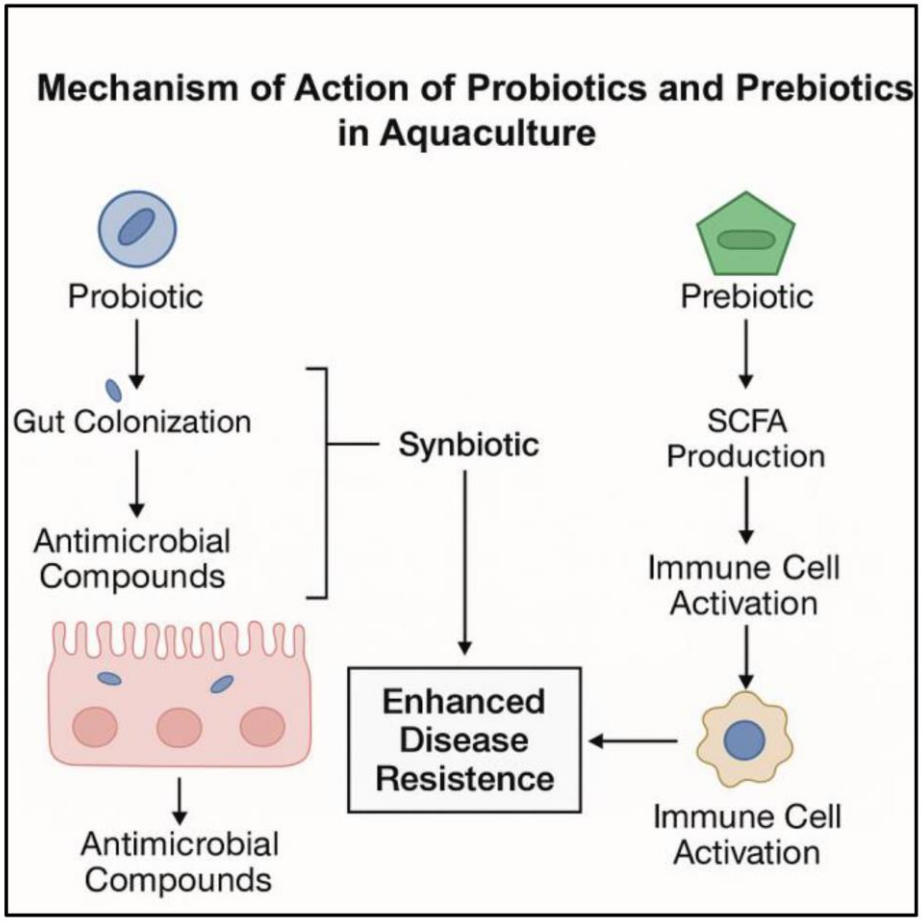

Figure 3. Overview of probiotic and prebiotic mechanisms of action in aquaculture, converging on enhanced disease resistance through gut colonization, antimicrobial compound production, SCFA production, and immune cell activation.

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4.1 Competitive exclusion and niche occupation

Probiotic bacteria can limit pathogen establishment by occupying adhesion sites on the gut or skin mucosa, by consuming shared nutrients before a pathogen can access them, and by altering local pH or redox conditions in ways that disfavor pathogen growth. This mechanism is particularly relevant against opportunistic pathogens such as *Vibrio* and *Aeromonas* species, which often exploit ecological disturbance—overfeeding, temperature stress, poor water quality—to expand from a minor community member into a dominant, disease-causing population. A probiotic strain that stably occupies the relevant niche under baseline conditions can blunt this opportunistic expansion, functioning as a form of ecological insurance rather than a direct antimicrobial agent [15].

4.2 Production of antimicrobial and inhibitory metabolites

Many candidate probionts produce bacteriocins, organic acids, hydrogen peroxide, or other secondary metabolites with direct antibacterial activity against key aquaculture pathogens. The precision approach places particular emphasis on confirming that such compounds are produced at physiologically relevant concentrations within the host gut environment—a condition frequently not met when in vitro antagonism, observed in nutrient-rich culture broth, is used as the sole selection criterion. Metabolomic profiling of gut contents following probiotic administration is increasingly used to confirm in situ production of candidate inhibitory metabolites, closing an important gap between laboratory screening and biological relevance [17].

4.3 Modulation of digestive and metabolic function

Probiotic strains frequently contribute exogenous digestive enzymes—proteases, lipases, amylases, and cellulolytic or chitinolytic enzymes—that supplement the host's endogenous digestive capacity, an effect of particular relevance in species or life stages where endogenous enzyme production is limited, such as larval fish or herbivorous and omnivorous species fed diets containing plant-based ingredients with recalcitrant carbohydrate fractions. Certain probiotic taxa also produce short-chain fatty acids through fermentation of otherwise indigestible dietary fiber, providing an energy source to enterocytes and contributing to gut epithelial integrity, a mechanism well described in mammalian systems and increasingly documented in fish.

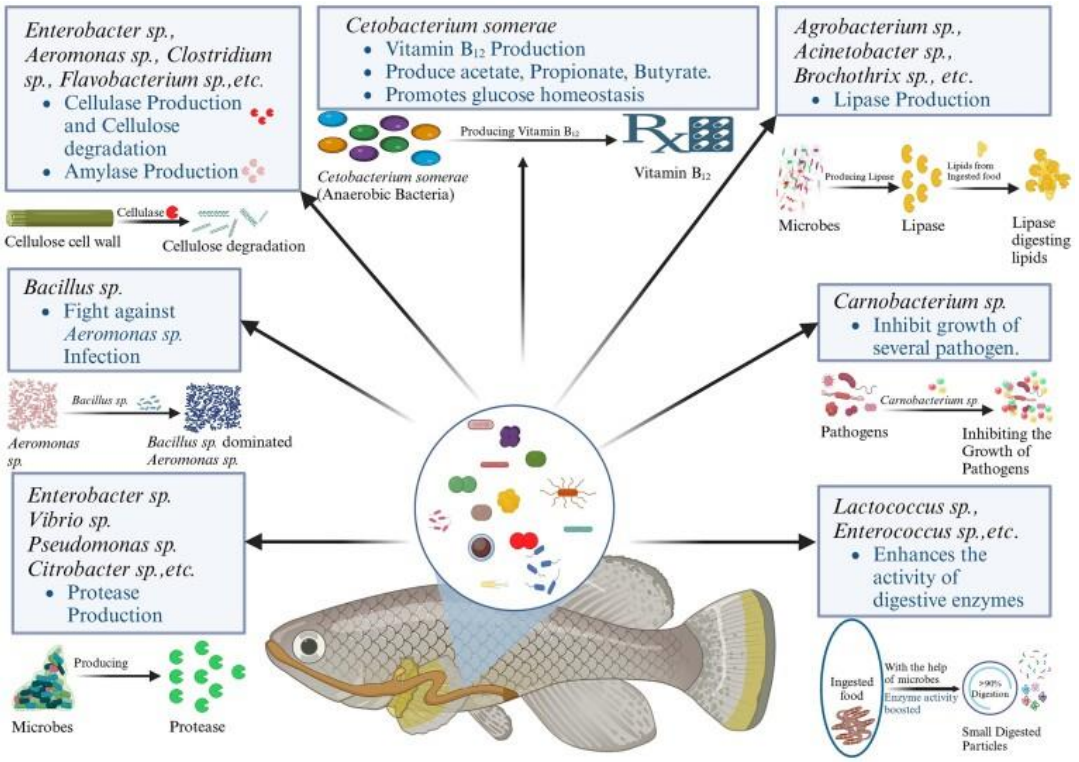

Figure 4. Examples of host-beneficial metabolic functions contributed by specific gut-associated taxa in fish, including cellulose/amylase degradation, vitamin B12 synthesis, lipase and protease production, pathogen inhibition, and digestive enzyme support.

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4.4 Immunomodulation

Probiotic administration has been repeatedly shown to upregulate innate immune parameters in aquatic hosts, including lysozyme activity, complement activity, phagocytic activity of macrophages, and expression of pro-inflammatory and antimicrobial peptide genes [7]. In many cases these effects are transient, peaking days to a few weeks after the initiation of supplementation and declining thereafter, a pattern consistent with a priming or trained-immunity model rather than sustained pharmacological stimulation. Precision probiotic design increasingly incorporates this temporal dynamic explicitly, timing probiotic administration to precede periods of anticipated stress or pathogen exposure—for example, prior to a known seasonal disease window or prior to a stressful handling event such as grading or transport—rather than administering probiotics continuously at a constant dose throughout the production cycle [13].

4.5 Water and sediment quality mediation

In pond and recirculating systems, certain probiotic bacteria, particularly nitrifying and denitrifying taxa and organic-matter-degrading *Bacillus* strains, are applied directly to the rearing water rather than the feed, with the goal of accelerating the breakdown of nitrogenous waste and organic sludge, thereby indirectly benefiting host health by improving water quality rather than acting directly on host physiology. This water-column probiotic strategy is mechanistically distinct from feed-based approaches and is generally evaluated using water chemistry endpoints—ammonia, nitrite, and organic load—alongside host performance metrics [19].

SPECIES-SPECIFIC CONSIDERATIONS

5.1 Salmonids

Salmonids—principally Atlantic salmon and rainbow trout—present a gut environment characterized by a relatively simple, stomach-containing digestive tract, carnivorous dietary physiology, and a marine or anadromous life history that exposes the animal to markedly different microbial environments across its life cycle. The freshwater-to-seawater transition (smoltification) is accompanied by a substantial restructuring of the gut microbiota and a period of heightened disease susceptibility, making this transition a priority target for precision probiotic intervention. Candidate probionts for salmonids have often been isolated from the salmonid gut itself or from cold-water marine environments, reflecting the need for strains that remain metabolically active at the relatively low temperatures characteristic of salmonid aquaculture. Documented benefits include reduced incidence of bacterial kidney disease and furunculosis-associated mortality, improved resistance to sea lice-associated secondary infection, and improved gut morphology—villus height and goblet cell density—under diets containing high inclusion levels of plant protein, a formulation shift the industry has pursued to reduce reliance on marine-derived feed ingredients.

5.2 Tilapia

Tilapia species are farmed across an exceptionally broad range of water quality and temperature conditions, from small-scale earthen ponds to large-scale recirculating systems, and are omnivorous with a digestive physiology well adapted to plant-based feed ingredients. This ecological flexibility is mirrored in a correspondingly variable native gut microbiota, which complicates the identification of a single "core" probiotic community and has led researchers toward system-specific rather than purely species-specific probiotic design for tilapia. *Bacillus* species remain the most extensively studied probiotic taxon in tilapia, valued for their spore-forming stability under the variable storage and application conditions typical of smallholder tilapia farming, and trials have documented improvements in growth performance, feed conversion, and survival following challenge with *Streptococcus agalactiae* and *Aeromonas hydrophila*, two of the most economically damaging tilapia pathogens [14].

5.3 Common carp and other cyprinids

Carp species, farmed predominantly in freshwater pond systems across Asia and parts of Europe, lack a true stomach and rely heavily on microbial fermentation in the intestine for digestion of plant-derived feed components, a physiological feature that increases the functional importance of the resident gut microbiota relative to species with a more acid-dominated digestive strategy. Probiotic research in carp has accordingly emphasized fermentative and cellulolytic taxa capable of enhancing the breakdown of plant polysaccharides, alongside more conventional immunostimulatory strains, with documented benefits in growth performance and resistance to *Aeromonas* and *Edwardsiella* infections, both common in pond-based carp culture.

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5.4 Penaeid shrimp

Shrimp aquaculture presents a distinct set of challenges for precision probiotic design because crustaceans possess an innate immune system only, lacking the adaptive immune components antibodies, immunological memory—that underlie much of vertebrate probiotic immunomodulation research. Probiotic strategies in shrimp therefore rely more heavily on competitive exclusion, direct antagonism against *Vibrio* pathogens (the dominant bacterial disease threat in shrimp culture), and stimulation of innate immune parameters such as prophenoloxidase activity, hemocyte count, and antimicrobial peptide expression. Because shrimp molt periodically, shedding and regenerating the cuticle and, to some extent, the foregut and hindgut lining, probiotic colonization dynamics in shrimp differ substantially from those in finfish, and precision approaches increasingly account for molt-cycle timing when scheduling probiotic administration. Water-column probiotic application is particularly important in shrimp culture given the extensive interface between the animal and pond water, and successful precision formulations frequently combine a feed-based probiotic component with a water-treatment component targeting *Vibrio* suppression and organic matter management [5].

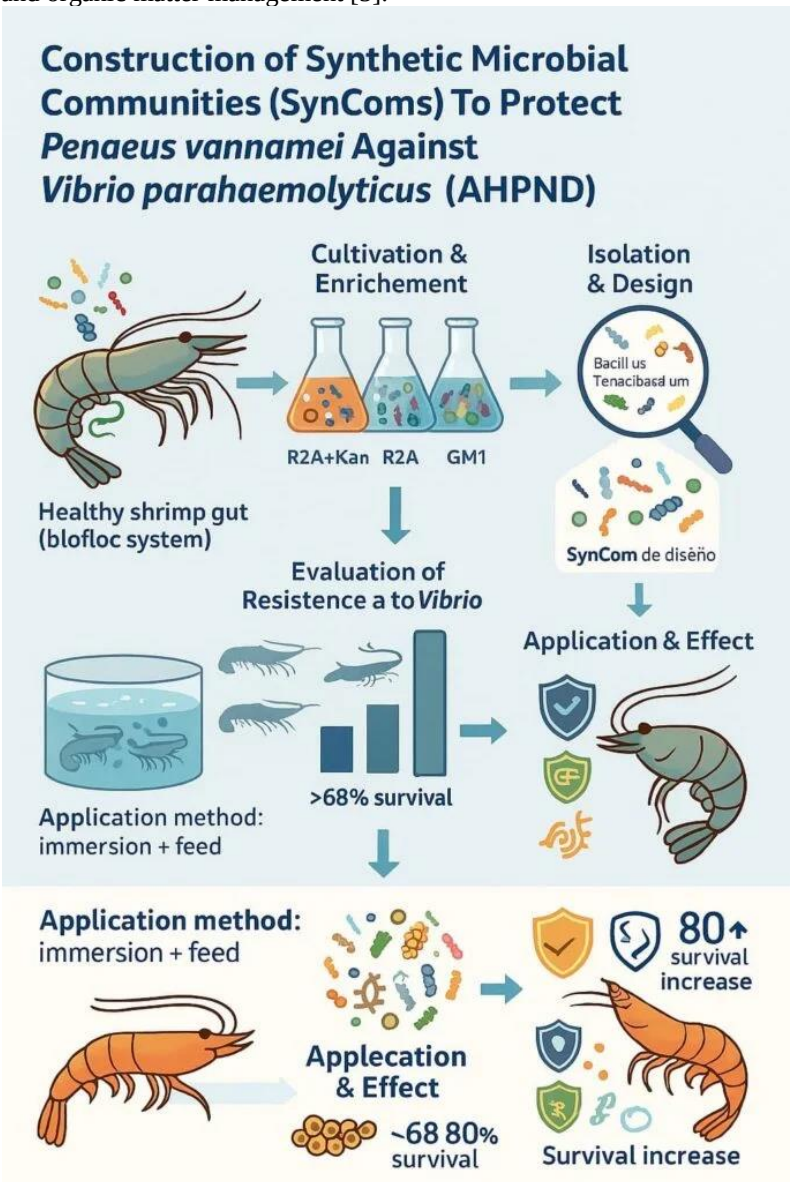

Figure 5. Workflow for constructing a synthetic microbial community (SynCom) to protect Penaeus vannamei against Vibrio parahaemolyticus (AHPND), from cultivation and isolation through application and survival outcome.

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5.5 Bivalve mollusks

Bivalve mollusks such as oysters and mussels are filter feeders that acquire the overwhelming majority of their associated microbiota directly from the surrounding water column, with limited capacity to selectively exclude ingested microorganisms prior to filtration. This biology fundamentally shapes probiotic strategy: because bivalves cannot be fed a formulated probiotic diet in the conventional sense, probiotic delivery in bivalve aquaculture is typically achieved either by dosing rearing or hatchery water directly, or by bioencapsulating candidate probionts within microalgal feed, since microalgae constitute the natural food source for filter-feeding bivalves at the larval and juvenile stages. Precision probiotic research in bivalves has concentrated heavily on hatchery-stage interventions, where mass mortality events associated with vibriosis remain a major economic constraint, and where the very high surface-area-to-volume ratio of larval stages makes water-column microbial management especially consequential [12].

5.6 Cross-cutting implications

Considered together, these species-specific profiles demonstrate that no single probiotic strain, dose, or delivery format can be expected to generalize across aquaculture taxa, and that the biological features distinguishing these groups digestive anatomy, immune system architecture, life history, and feeding mode map directly onto specific requirements for probiotic strain selection, delivery route, and administration timing. This is the empirical foundation on which the precision probiotic paradigm rests: the substantial documented variance in probiotic efficacy across species is not primarily a matter of strain quality but of host-strain mismatch, and closing that mismatch requires species- and even system-specific formulation rather than a generic product line.

A further cross-cutting consideration concerns broodstock and reproductive-stage probiotic application, an area that has received comparatively little attention relative to grow-out stage supplementation but that carries disproportionate downstream significance, since the maternal and, in some species, paternal microbiota can influence the initial microbial seeding of eggs and larvae. Probiotic supplementation of broodstock diets in several finfish and crustacean species has been associated with improved egg quality, hatching rate, and larval robustness, suggesting that precision strategies extended to the broodstock stage may offer a more efficient point of intervention than repeated supplementation across every subsequent life stage. Similarly, polyculture and integrated multi-trophic aquaculture systems, in which two or more species are reared together to exploit complementary ecological niches, introduce an additional layer of complexity for precision probiotic design, since a strain or consortium optimized for one co-cultured species must also be evaluated for its effects, whether neutral, beneficial, or antagonistic, on the other species sharing the same water body.

TECHNOLOGIES ENABLING PRECISION DESIGN

6.1 High-throughput sequencing and metagenomics

Culture-independent sequencing of the 16S rRNA gene, and increasingly shotgun metagenomic sequencing, has transformed the characterization of aquaculture microbiomes, revealing that the majority of microbial taxa present in the gut, skin, and water column of most aquaculture species had never been isolated in culture and were therefore invisible to earlier, culture-dependent probiotic screening programs. Metagenomic sequencing additionally provides functional gene content, allowing researchers to identify taxa carrying genes for specific metabolic or antagonistic functions of interest even before those organisms have been isolated, effectively inverting the traditional screening workflow from "isolate then characterize" to "identify function in the metagenome, then isolate the responsible organism."

6.2 Culturomics

Because probiotic products require a living, culturable microorganism, the expansion of culturable diversity through culturomics—the systematic application of varied media formulations, atmospheric conditions, and enrichment strategies to recover previously unculturable taxa—has been an essential complement to sequencing-based discovery. Culturomic pipelines applied to fish and shrimp gut samples have recovered numerous novel isolates from genera not previously associated with aquaculture probiotics, substantially expanding the pool of candidate strains available for host-matched screening.

6.3 Metabolomics and transcriptomics

Metabolomic profiling of host tissue and gut contents, alongside host transcriptomic profiling, allows researchers to move beyond correlational taxonomic associations toward mechanistic hypotheses linking specific probiotic strains to specific host outcomes. For example, transcriptomic comparison of probiotic-supplemented and control

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animals can reveal whether an observed growth benefit is mediated primarily through upregulation of digestive enzyme genes, through modulation of the growth hormone/insulin-like growth factor axis, or through suppression of inflammatory signaling that would otherwise divert metabolic resources away from growth.

6.4 Genome editing and synthetic biology

Where a native or naturally occurring strain cannot be identified that combines all desired functional traits, genome-editing tools, most notably CRISPR-based approaches, offer the possibility of engineering a well-characterized chassis organism—commonly a *Bacillus subtilis* or *Lactiplantibacillus plantarum* strain with an established safety record—to express a specific adhesin, bacteriocin, or immunostimulatory molecule matched to the target host. This approach remains at an earlier stage of commercial development than natural-strain selection, both because of the additional regulatory scrutiny applied to genetically modified organisms in food-producing animal systems and because of unresolved ecological questions concerning the fate of engineered strains released, deliberately or through farm effluent, into open water bodies.

6.5 Machine learning and computational strain prioritization

The accumulation of multi-omic datasets linking microbial genomic and metabolic features to host outcomes has created the conditions for machine learning approaches to strain prioritization, in which classifiers trained on existing efficacy data are used to rank untested candidate strains by predicted probability of success before committing to in vivo trials. While still an emerging application, this computational layer has the potential to substantially compress the discovery timeline for precision probiotics, particularly as training datasets grow through collaborative data-sharing initiatives across research groups and companies.

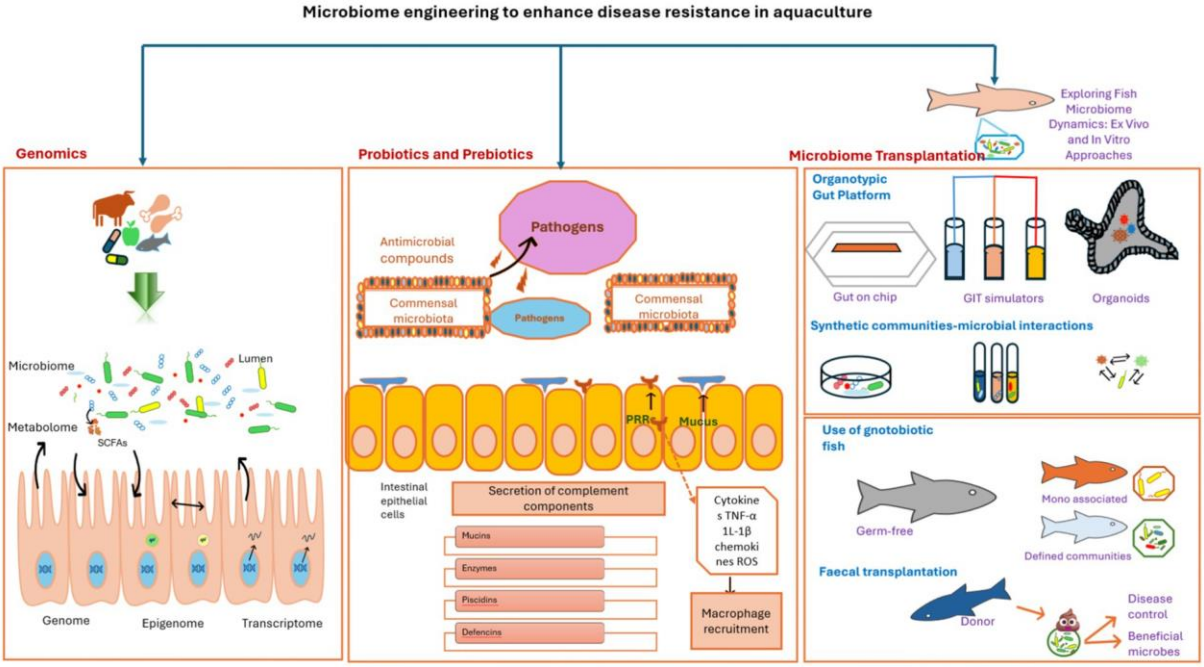

Figure 6. Technological toolkit for microbiome engineering in aquaculture disease resistance, spanning host genomics, probiotic/prebiotic modulation of the gut epithelium, and ex vivo/in vitro microbiome transplantation platforms.

DELIVERY SYSTEMS AND FORMULATION STRATEGIES

Even a mechanistically well-validated probiotic strain delivers no benefit if it does not survive feed processing, storage, and gastric transit in sufficient viable numbers to reach its site of action. Formulation science is therefore inseparable from precision probiotic design. Spore-forming genera such as *Bacillus* have been favored commercially in large part because their spores tolerate the heat and pressure of feed pelleting and extrusion far better than vegetative cells of most lactic acid bacteria, which typically require post-pelleting application by top-coating with oil-based carriers, or microencapsulation, to survive processing. Microencapsulation technologies, including alginate and chitosan-based matrices, have been developed specifically to protect non-spore-forming

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probiotic strains through both feed processing and the low-pH environment of the stomach in gastric species, releasing the encapsulated organism preferentially in the intestine.

For species and life stages that cannot be fed a formulated diet—larval fish and crustaceans reliant on live feed, and filter-feeding bivalve larvae—bioencapsulation within live feed organisms such as rotifers and Artemia, or within cultured microalgae, provides an alternative delivery route, exploiting the natural feeding behavior of the target host to introduce the probiotic organism. Water-column application, whether through direct dosing of pond or tank water or through slow-release formulations designed to maintain a target probiotic concentration over an extended period, constitutes a third delivery route of particular importance for shrimp and bivalve systems, where the water itself constitutes a major route of microbial exposure.

Precision formulation additionally requires attention to dose-response relationships, which are frequently non-monotonic in probiotic applications: doses below a threshold may fail to achieve colonization, while excessive doses have in some cases been associated with immune overstimulation or competitive disruption of beneficial resident taxa. Establishing an appropriate dose therefore requires host- and strain-specific titration rather than reliance on a generic industry-standard dose expressed simply as colony-forming units per gram of feed.

Administration schedule constitutes a further formulation variable that precision approaches treat as a design parameter rather than a fixed default. Continuous, season-long probiotic inclusion in feed remains the most common commercial practice largely because it is operationally simple, but a growing body of evidence suggests that pulsed or cyclical administration—several weeks of supplementation followed by a withdrawal interval may sustain immunostimulatory benefit while reducing the risk of immune habituation and lowering overall input cost. Precision protocols increasingly specify administration windows timed to coincide with known periods of elevated risk, such as the weeks surrounding a seasonal temperature drop, a stocking density increase, or a scheduled vaccination, on the reasoning that the benefit of transient innate immune priming is greatest when it overlaps with an actual period of elevated pathogen exposure or physiological stress, rather than being diffused evenly across a production cycle that includes long stretches of comparatively low risk.

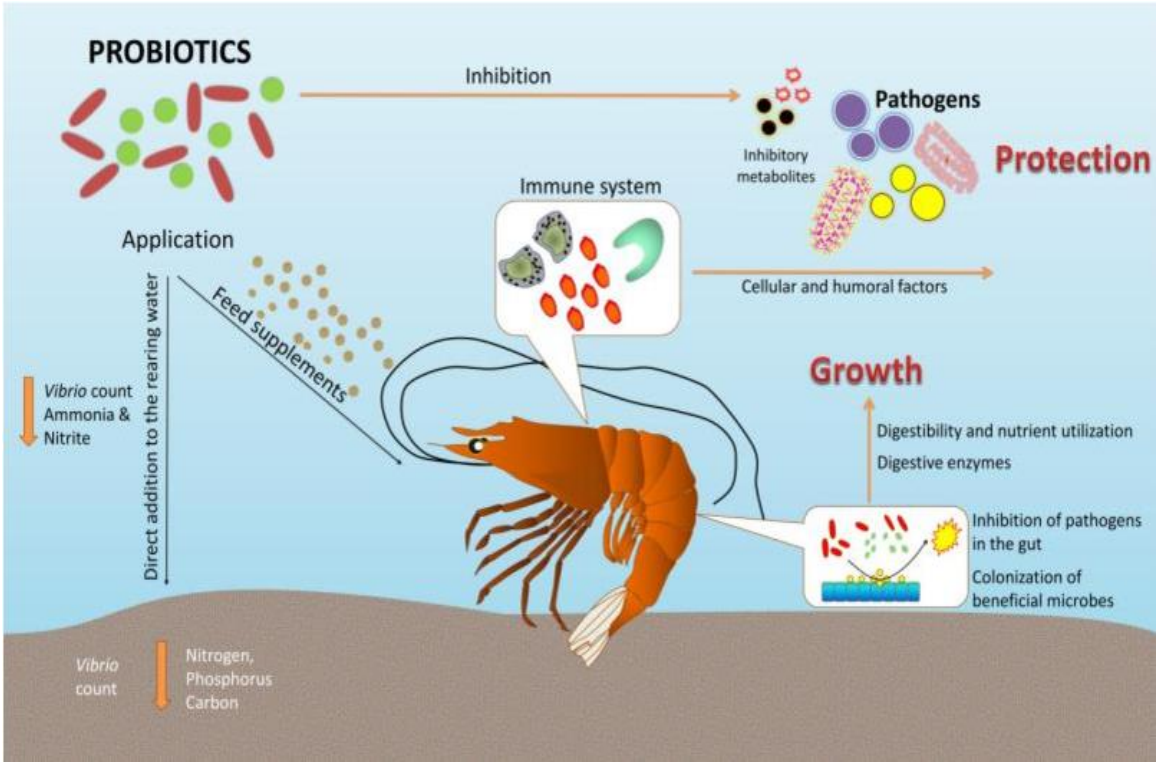

Figure 7. Feed- and water-based probiotic application routes in shrimp culture and their downstream effects on pathogen inhibition, immune activation, and growth performance.

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ILLUSTRATIVE CASE EXAMPLES

Several documented research programs illustrate the practical application of precision probiotic principles. In hybrid grouper aquaculture, comparative trials of multiple *Bacillus* strains isolated specifically from the grouper gastrointestinal tract identified pronounced strain-level, rather than merely species-level, differences in growth promotion, metabolic health parameters, and resistance to challenge with *Vibrio harveyi* [11], a finding that underscores why probiotic products defined only to the bacterial species level provide an inadequate basis for predicting field performance. In Atlantic salmon, probiotic strains isolated from the salmonid gut and selected specifically for activity against *Aeromonas salmonicida* and for tolerance of the low temperatures characteristic of salmon farming have demonstrated more consistent colonization and disease protection than terrestrially derived lactic acid bacteria applied to the same host. In shrimp culture, host-derived and environmentally isolated candidate probionts screened specifically for antagonism against acute hepatopancreatic necrosis disease-associated *Vibrio parahaemolyticus* strains, combined with water-column management protocols, have been associated with reduced mortality in commercial-scale trials in several Southeast Asian production systems, though the magnitude of protection has varied with pond management practice, underscoring the continued importance of system-level, and not only strain-level, precision.

Bivalve hatchery research provides a further illustration of species-specific delivery innovation: because oyster and mussel larvae cannot be fed a conventional probiotic-supplemented diet, researchers have developed protocols in which candidate probiotic bacteria are bioencapsulated within the microalgal feed used in hatchery rearing, with documented reductions in larval mortality attributable to vibriosis during the particularly vulnerable early planktonic life stages.

Collectively, these examples reinforce a consistent lesson: the magnitude and consistency of probiotic benefit increase markedly when strain selection, dose, delivery format, and administration timing are all matched explicitly to the biology of the target host and the conditions of the production system in which it is reared, relative to generic, cross-species probiotic application.

CHALLENGES, RISKS, AND BARRIERS TO ADOPTION

9.1 Strain stability and manufacturing scale-up

A probiotic strain validated at laboratory scale must retain both viability and functional activity through the transition to industrial-scale fermentation, formulation, and long-term storage under the variable temperature and humidity conditions typical of feed mills and farm storage facilities, a transition at which many otherwise promising candidates fail. Precision probiotics, by drawing more heavily on host-adapted or engineered strains that may not have the extensive industrial fermentation history of long-established commercial species, face a particular scale-up burden that must be addressed early in development rather than treated as a downstream manufacturing detail.

9.2 Ecological and biosafety considerations

The deliberate introduction of live microorganisms into open or semi-open aquaculture systems raises legitimate ecological questions, particularly for engineered strains, non-native isolates, or strains applied at high density in systems with direct hydrological connection to natural water bodies, such as net-pen salmon farming or coastal shrimp ponds with tidal exchange. Regulatory frameworks in most jurisdictions require some demonstration that a candidate probiotic strain does not carry transferable antimicrobial resistance genes, does not itself pose a pathogenic risk to the target species or to humans handling or consuming the product, and does not establish persistently in the wild environment in ways that could disrupt native microbial communities. These requirements are appropriately stringent but are not always matched by harmonized international testing standards, creating uncertainty for developers seeking to market a single formulation across multiple national markets.

9.3 Regulatory heterogeneity

Probiotic products for aquaculture are regulated inconsistently across jurisdictions, sometimes as feed additives, sometimes as veterinary biologics, and sometimes under frameworks not originally designed with living microbial products in mind. This heterogeneity is a particular obstacle for precision probiotics, which by design may involve host- or region-specific strains not previously subject to any regulatory review, and for which the additional mechanistic and safety data expected under a precision framework can lengthen and complicate the approval pathway relative to reformulation of an already-approved generic strain.

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9.4 Colonization resistance and inter-strain competition

The resident microbiota of a healthy host is not a passive substrate awaiting probiotic colonization but an actively competing ecological community, and colonization resistance the capacity of an established microbial community to resist invasion by an introduced organism can substantially blunt the efficacy of an otherwise well-matched probiotic candidate, particularly in older animals with a more developed and stable resident microbiota. Precision probiotic strategies have begun to address this challenge through combination approaches, pairing a probiotic strain with a matched prebiotic substrate that selectively favors its growth (a synbiotic approach), or through transient host microbiota disruption protocols intended to open an ecological niche prior to probiotic introduction, though the latter approach carries its own risks of destabilizing the resident community in unintended ways.

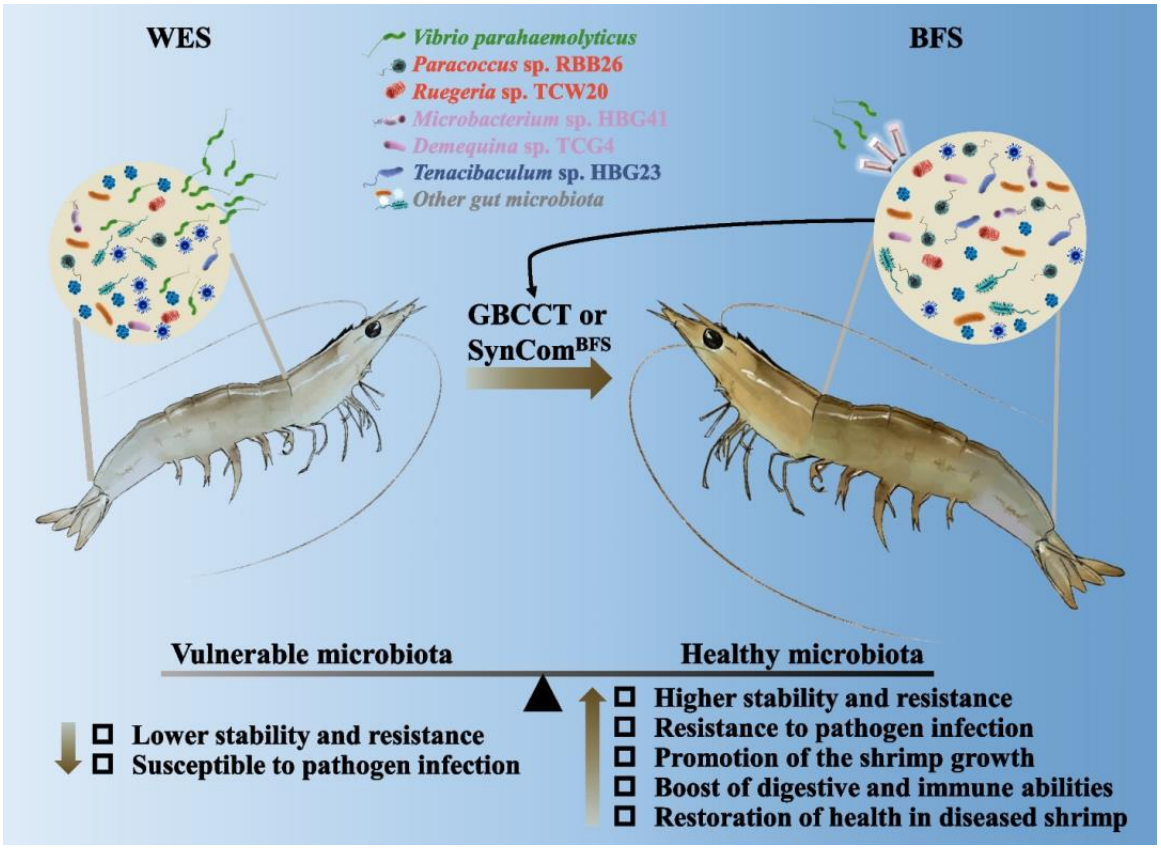

Figure 8. Shift from a vulnerable, water-exposed shrimp (WES) gut microbiota to a stable, healthy biofloc-system (BFS) microbiota following synthetic community (SynCom) application, illustrating colonization resistance to Vibrio parahaemolyticus.

9.5 Standardization of efficacy testing

The aquaculture probiotic literature has been repeatedly criticized for methodological heterogeneity across trials—variation in dose, duration, challenge model, and outcome measures—that complicates comparison across studies and slows the accumulation of a reliable evidence base. Precision probiotic development, with its emphasis on mechanistic validation, offers a partial remedy by generating a richer set of intermediate outcome measures beyond simple growth or survival endpoints, but the field would benefit substantially from harmonized reporting standards analogous to those developed for clinical probiotic trials in human medicine.

9.6 Economic and adoption barriers

Precision probiotic formulations, by virtue of the additional research investment required for host-specific strain selection and mechanistic validation, are likely to carry a higher development and, at least initially, retail cost than generic probiotic products, a consideration of particular importance in the smallholder-dominated production systems that account for a substantial share of global aquaculture output, especially in freshwater finfish and shrimp farming in Asia. Ensuring that the benefits of precision probiotic research reach smallholder farmers, rather than remaining confined to large, vertically integrated production systems capable of absorbing higher input costs,

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is likely to require deliberate investment in cost-reduction strategies, extension services, and potentially public or philanthropic subsidy of early-stage adoption.

FUTURE DIRECTIONS

Several research directions appear particularly likely to shape the next phase of precision probiotic development in aquaculture. First, expansion of reference multi-omic datasets across a wider range of commercially important species—many of which remain far less thoroughly characterized than salmon, tilapia, or major shrimp species—will be necessary to extend precision probiotic principles beyond the handful of taxa that have so far received the bulk of research attention. Second, integration of probiotics with complementary microbiome-engineering approaches, including prebiotics, postbiotics (non-viable microbial products or purified microbial metabolites), and synbiotic combinations, is likely to become increasingly central, both because such combinations can address colonization resistance and because postbiotic approaches sidestep some of the regulatory and stability challenges associated with live organisms. Third, continued development of AI-assisted strain prioritization, contingent on the growth of shared, standardized efficacy datasets across the research community, has the potential to substantially reduce the time and cost of bringing a validated precision probiotic to market. Fourth, closer integration of probiotic strategy with host genetic selection programs—breeding aquaculture species for traits that favor beneficial microbiota establishment, alongside selecting microbial strains matched to the host—represents a largely unexplored but potentially powerful complementary approach. Finally, sustained attention to regulatory harmonization, ideally through international coordination among food safety and veterinary authorities, will be essential to ensure that the pace of scientific advance in this field is matched by a coherent and predictable pathway to commercial deployment.

CONCLUSION

The shift from empirical, cross-species probiotic supplementation toward precision, host-matched microbial formulations reflects a broader maturation of microbiome science as it has been applied to aquaculture. The considerable variance that has characterized probiotic efficacy trials in aquatic species is increasingly understood not as an inherent limitation of the probiotic concept but as a predictable consequence of mismatch between generic microbial products and the specific physiological, immunological, and ecological context of the host species and production system in question. Advances in sequencing, culturomics, metabolomics, genome editing, and computational strain prioritization have, for the first time, made it feasible to design probiotic interventions around a mechanistic understanding of a specific host rather than around the historical convenience of a small number of well-studied bacterial genera. Realizing the full potential of this approach will require sustained investment in species-specific mechanistic research, in delivery and formulation technology adapted to the full diversity of aquaculture production systems, and in regulatory frameworks capable of evaluating host-adapted and engineered strains fairly and efficiently. Precision probiotics are unlikely to fully displace conventional disease management tools in aquaculture in the near term, but the trajectory of the underlying science suggests they will play an increasingly central role in an industry under sustained pressure to grow production while reducing both antimicrobial use and environmental impact.

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