

From Spore Biology to System Performance: Strain-Resolved Mechanisms, Biosafety and Translational Readiness of Bacillus Probiotics in Aquaculture

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Systematic Review

Keywords: Bacillus probiotics, aquaculture, antimicrobial resistance, biosafety, One Health, translational readiness

Posted Date: July 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10427121/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Probiotics and Antimicrobial Proteins on October 17th, 2026. See the published version at <https://doi.org/10.1007/s12602-026-11250-0>.

Abstract

Bacillus-based probiotics are widely promoted as antibiotic-sparing interventions in aquaculture, yet translation from controlled trials to commercial production remains constrained by strain heterogeneity, inconsistent dosing, incomplete biosafety assessment and frequent inference of mechanisms from non-specific biomarkers. This scoping review reorganizes the evidence reported for 67 empirical studies published during 2015–2025 into a strain–host–route–system framework. Across finfish and aquatic invertebrates, *Bacillus* supplementation was commonly associated with improved growth, feed utilization, digestive-enzyme activity, mucosal or systemic innate immune indices, challenge survival and, in water-applied or biofloc settings, nitrogenous-waste control. The evidence nevertheless does not support a universal optimal dose: reported regimens span colony-forming units per gram or kilogram of feed, colony-forming units per millilitre of culture water and mass-based commercial formulations, and efficacy varies with strain identity, host developmental stage, delivery route, diet, salinity, temperature and challenge design. Mechanistic support is strongest for pathogen antagonism, enzyme provision, barrier modulation and environmental bioremediation, whereas claims concerning stable colonization, antiviral action and antimicrobial-resistance mitigation are often indirect. *Bacillus* should therefore be evaluated at strain rather than genus level, with whole-genome screening for virulence determinants, toxins, transferable resistance genes and mobile elements. We propose a translational-readiness framework integrating identity, potency, formulation stability, host compatibility, environmental persistence, biosafety and farm-scale validation. This reframing positions *Bacillus* probiotics as promising but conditional tools for antibiotic stewardship and One Health-aligned aquaculture.

1 Introduction

Aquaculture supplies an increasing share of aquatic foods and contributes materially to global protein availability [1]. Intensification, however, concentrates susceptible hosts, organic matter and opportunistic microorganisms, making disease prevention a central production constraint. Antibiotics can be necessary for diagnosed bacterial disease, but routine or poorly targeted use creates selection pressure, disturbs host-associated microbial communities and releases residues and resistant bacteria into connected environments. Antibiotic stewardship therefore requires preventive measures that improve host resilience without substituting one unqualified biological input for another.

Probiotics are live microorganisms that, when administered in adequate amounts, confer a benefit on the host. In aquaculture, they may be delivered in feed, live food, rearing water, biofloc or encapsulated carriers. Earlier syntheses established that probiotic performance depends on host species, microbial strain, dose, route, diet and culture conditions [2]. *Bacillus* has attracted particular interest because endospores can tolerate feed manufacture and storage, while vegetative cells may produce extracellular enzymes, bacteriocins, lipopeptides and quorum-interfering metabolites [3, 4]. As summarized in Fig. 1, *Bacillus* probiotics can support growth, nutrient utilization, digestive-enzyme activity, immune function, disease resistance, microbiome modulation and water quality in diverse aquaculture species.

The field is no longer short of broad narrative reviews. Reviews published in 2019 and 2021 summarized *Bacillus* applications and the diverse functions attributed particularly to *B. subtilis* [5, 6]; a 2025 review again addressed recent *Bacillus*, synbiotic, encapsulated, paraprobiotic and postbiotic applications in sustainable aquaculture [7]. A new review must therefore do more than catalogue beneficial outcomes. It must distinguish strain-level evidence from genus-level generalization, direct mechanism from biomarker association, laboratory efficacy from farm readiness, and antibiotic-sparing potential from demonstrated antimicrobial-resistance (AMR) risk reduction.

This review addresses that need by reorganizing the reported 2015–2025 evidence into a strain–host–route–system matrix. It has four objectives: (i) to evaluate the mechanistic basis of pathogen control, digestive support, immune modulation and environmental bioremediation; (ii) to explain heterogeneity in growth, health and challenge outcomes; (iii) to examine biosafety, AMR and One Health claims using a strain-qualified perspective; and (iv) to propose minimum evidence requirements for translation from experimental trials to reproducible commercial use.

2 Review methods

2.1 Search strategy and eligibility

The source manuscript described a scoping-review search of Scopus, Web of Science and Google Scholar using combinations of *Bacillus* with aquaculture, host-group and outcome terms. The review aimed to identify key mechanisms, growth and immune outcomes, environmental impacts and knowledge gaps regarding *Bacillus* probiotic applications in fish and shellfish production. Eligible records were English-language, peer-reviewed empirical studies published from 2015 through 2025 that administered a *Bacillus* strain or *Bacillus*-containing product to cultured finfish or aquatic invertebrates and reported at least one growth, feed-utilization, immune, disease-resistance, digestive, microbiome or water-quality outcome. Reviews, conference abstracts, opinion articles and thematically irrelevant records were excluded. Pre-2015 studies and reviews were used only as contextual references and should not be counted in the eligible evidence set.

2.2 Study selection and data charting

The documented flow identified 312 records, removed 64 duplicates, screened 248 titles and abstracts, assessed 116 full texts and included 67 studies. Two reviewers reportedly conducted screening and data extraction independently, with disagreements resolved by discussion. For the revised synthesis, study information is organized by probiotic identity and strain, host species and life stage, culture system, administration route, viable dose and unit, duration, comparator, primary production outcomes, immune or molecular endpoints, pathogen-challenge design, microbiome or water-quality measures and reported safety observations. The study-selection process is summarized in Fig. 2.

2.3 Evidence synthesis and limitations

Because hosts, strains, dose units, routes, trial durations, challenge organisms and outcome definitions were highly heterogeneous, the evidence was synthesized narratively rather than pooled statistically. Mechanistic claims were classified as direct when supported by isolate-level antagonism, metabolite, genomic, transcriptomic, proteomic or metabolomic evidence; as intermediate when supported by host-pathway or microbiome changes; and as associative when inferred from growth, enzyme, antioxidant or survival endpoints alone. This hierarchy is essential because an increase in lysozyme, superoxide dismutase or survival does not by itself establish the causal mechanism.

The review is limited by English-language restriction, incomplete coverage of regional databases, likely publication bias toward positive trials, inconsistent reporting of strain identity and viable counts, and absence of a formal risk-of-bias appraisal in the source review. Google Scholar improves recall but reduces reproducibility unless the search date, result depth and deduplication rules are reported. These limitations require calibrated conclusions: the synthesis identifies recurring patterns and translational bottlenecks but does not estimate a universal effect size.

3 Evidence base and strain landscape

Most included experiments evaluated *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens* or closely related taxa, with additional evidence for *B. velezensis*, *B. pumilus*, *B. safensis*, *B. aerophilus* and other strains. Studies covered tilapia, carp, eel, grouper, salmon, pompano, sturgeon and catfish, as well as penaeid shrimp, freshwater prawn, abalone, oyster, scallop and sea cucumber. This breadth demonstrates biological plausibility across production groups, but it also conceals important taxonomic, physiological and immunological differences. For example, finfish possess innate and adaptive immune compartments, whereas molluscs and crustaceans rely primarily on innate cellular and humoral defence; “adaptive immunity” should not be used uncritically for these invertebrates.

Strain identity is the correct unit of inference. Host-derived *B. velezensis* LF01 improved growth and resistance to streptococcosis in Nile tilapia [8], while host-associated *Bacillus* combinations altered mucosal immunity and gut health in the same host [9]. *B. subtilis* HAINUP40 enhanced digestive and immune indices and challenge survival in tilapia [10]; *B. pumilus* affected non-specific immunity and resistance in golden pompano [11]; and *B. subtilis* 6-3-1 improved growth and disease resistance in hybrid grouper [12]. These findings cannot be transferred automatically to other strains bearing the same species name.

The principal evidence domains and the strength of inference supported by each are summarized in Table 1.

Table 1
Evidence domains and the level of inference supported by each domain

Evidence domain	Typical measurements	Strong inference	Principal limitation
Production	Weight gain, specific growth rate, feed-conversion ratio, survival	Practical performance under the tested conditions	Does not identify mechanism; vulnerable to diet, density and husbandry confounding
Host physiology	Digestive enzymes, antioxidant enzymes, serum or mucus indices, histology	Pathway-consistent host response	Biomarkers may be non-specific and transient
Pathogen control	Minimum inhibitory concentration, bacteriocin/lipopeptide assays, quorum sensing or biofilm assays	Direct antagonism or interference	In vitro activity may not occur in the host or culture system
Molecular response	Targeted gene expression, transcriptomics, proteomics, metabolomics	Mechanistic pathway activation	Association remains possible without perturbation or rescue experiments
Microbiome	Amplicon sequencing, metagenomics, quantitative culture	Community restructuring and possible colonization	Relative abundance alone does not prove persistence or function
System performance	Ammonia, nitrite, nitrate, dissolved oxygen, biofloc properties	Environmental bioremediation under the tested system	Effects depend strongly on carbon input, aeration, exchange and background microbiota
Biosafety	Whole-genome sequence, toxin and virulence screening, AMR genes, mobile elements, hemolysis	Strain qualification for intended use	Rarely reported comprehensively in efficacy trials

4 Mechanistic frameworks

4.1 Direct pathogen suppression and ecological exclusion

Bacillus isolates may suppress pathogens through bacteriocins, lipopeptides, organic acids, lytic enzymes, siderophore-mediated competition, adhesion-site competition, biofilm interference and quorum-quenching activity. Fish-gut *Bacillus* isolates with antimicrobial activity against aquaculture pathogens provide direct evidence that antagonistic metabolites can be a strain-selection trait [13]. However, agar inhibition and cell-free-supernatant assays should be treated as screening tools rather than proof of in vivo protection. Concentrations achieved in feed, gut mucus, pond water or sediment may differ substantially from those used in vitro.

Challenge studies offer stronger functional evidence when the challenge organism, dose, route and post-challenge observation period are reported. Dietary *Bacillus* reduced mortality or improved relative survival after bacterial challenge in tilapia, rohu, eel, grouper and other fish [14–17]. In shrimp, studies reported improved resistance to *Vibrio* species and, in some cases, lower viral loads [18–20]. Yet challenge models frequently use acute, high-dose exposure and may overestimate protection relative to chronic farm exposure. A convincing mechanism requires evidence linking the administered strain or its product to pathogen reduction, host pathway change and the observed survival outcome.

4.2 Digestive and metabolic support

Extracellular proteases, amylases, lipases and other enzymes can increase the digestibility of plant-rich feeds, release otherwise inaccessible nutrients or modify intestinal substrates. In several fish and shrimp studies, *Bacillus* supplementation increased digestive-enzyme activity alongside growth or feed efficiency [10, 21, 22]. These paired outcomes are biologically coherent, but causation is not established unless enzyme production by the administered strain is quantified under gastrointestinal or culture conditions. Host enzymes, altered feed intake and shifts in resident microbiota can produce similar measurements.

Metabolomic evidence provides a more specific bridge between microbial exposure and host physiology. *B. subtilis* E20 altered glutamine metabolism and the hexosamine biosynthetic pathway in white shrimp, linking supplementation with immune-metabolic signalling [23]. Such studies are valuable because they move beyond descriptive enzyme panels; nevertheless, pathway activation should be validated across independent strains and conditions before it is treated as a general property of *Bacillus*.

4.3 Barrier, antioxidant and immune modulation

Recurring fish responses include increased lysozyme, complement, phagocytic activity, respiratory burst, immunoglobulin M, antioxidant enzymes and expression of cytokine, heat-shock, defensin or toll-like receptor genes. *Bacillus* supplementation also improved villus architecture, goblet-cell abundance and mucosal indices in some tilapia studies [9, 24]. These findings support a barrier- and innate-readiness model in which microbial-associated molecular patterns and metabolites prime local defence while improved digestion reduces metabolic stress.

Examples span viable *B. pumilus* in laboratory and farm tilapia trials [25], *B. subtilis* strains in sturgeon and red sea bream [26, 27], *B. velezensis* in goldfish and tilapia [8, 28], and *Bacillus* mixtures in Nile tilapia [29]. The direction of response is often consistent, but baseline immune status, sampling time and assay methodology differ. Cytokine upregulation is not uniformly beneficial: magnitude, duration and balance between pro- and anti-inflammatory signalling matter.

For crustaceans and molluscs, appropriate endpoints include hemocyte counts, phenoloxidase, antimicrobial peptides, lectins, mucins, respiratory burst and cellular immune activity. Probiotic pre-exposure modified immune signalling in oyster larvae [30], while shrimp and prawn studies reported changes in phenoloxidase-related genes, antioxidant capacity and challenge resistance [31–33]. These should be described as innate or trained immune-like responses rather than classical adaptive immunity.

The major functional features and immunomodulatory pathways attributed to *Bacillus* probiotics are summarized in Figs. 3 and 4.

4.4 Microbiome modulation and environmental bioremediation

Bacillus supplementation is frequently associated with higher *Bacillus* abundance, reduced *Vibrio* or *Aeromonas* abundance and altered community diversity. Host-derived strains may be more likely to interact productively with the resident microbiota, but detection after supplementation can reflect repeated intake rather than stable colonization. Demonstration of colonization requires strain-resolved tracking, viable recovery after withdrawal and evidence that the strain remains metabolically active. Amplicon relative abundance alone is insufficient.

Water-applied *Bacillus* can affect organic-matter turnover and nitrogen cycling, particularly in biofloc or low-exchange systems. Studies in shrimp reported improvements in ammonia, nitrite, dissolved oxygen or biofloc performance, although effects varied by strain and application strategy [34–36]. A commercial compound also declined over time and produced limited effects under the evaluated conditions, illustrating that label claims do not guarantee viable delivery or ecological persistence [37]. Environmental performance should therefore be evaluated against aeration, carbon-to-nitrogen management, exchange rate and baseline microbial activity.

Table 2 links the proposed mechanisms to representative evidence, expected outcomes and the minimum confirmation needed for causal interpretation.

Table 2
Mechanism-to-evidence map for *Bacillus* probiotics in aquaculture

Proposed mechanism	Representative evidence	Expected outcome	Minimum confirmation	Confidence in current literature
Antagonistic metabolites	Isolate inhibition, cell-free supernatant, bacteriocin/lipopeptide characterization	Lower pathogen load and challenge mortality	Identify active compound; measure exposure at target site; link pathogen reduction to outcome	Moderate for selected strains; low for genus-wide claims
Quorum sensing/biofilm interference	Signal degradation or reduced biofilm/virulence expression	Reduced pathogen virulence without broad killing	Chemical or reporter assay plus in vivo virulence endpoint	Emerging and strain-specific
Digestive enzyme provision	Strain enzyme assays and higher host digestive activity	Improved nutrient utilization and feed conversion	Demonstrate enzyme activity under feed/gut conditions and distinguish microbial from host enzyme	Moderate association; limited causal proof
Barrier and immune modulation	Histology, mucus indices, immune enzymes, gene expression	Improved readiness and challenge survival	Time-resolved pathway evidence linked to barrier integrity and pathogen clearance	Moderate, but biomarkers are heterogeneous
Microbiome restructuring	Reduced pathogens, increased beneficial taxa, functional genes	Colonization resistance and metabolic support	Strain-resolved tracking, absolute abundance, function and post-withdrawal persistence	Low to moderate
Bioremediation	Reduced ammonia/nitrite, improved biofloc and oxygen conditions	Better environmental stability and survival	Controlled system mass balance and microbial functional analysis	Moderate in biofloc/low-exchange systems

5 Host outcomes and sources of heterogeneity

5.1 Finfish

Finfish trials most consistently report improvements in growth, feed utilization, digestive activity, innate immune indices, antioxidant capacity and survival after bacterial challenge. Positive examples include *B. licheniformis* in Nile tilapia [15], *B. subtilis* in Japanese eel and hybrid grouper [12, 16], *B. velezensis*-based interventions in tilapia and Atlantic salmon compound formulations [8, 38], and *B. amyloliquefaciens* or related strains in tilapia and catfish [39, 40]. The consistency of direction supports utility, but effect magnitude cannot be compared directly because doses, units, basal diets, temperatures, stocking densities and endpoints differ.

The original draft proposed a broadly optimal range of 10^7 – 10^9 CFU/g. That conclusion is not defensible across the evidence base. Some fish studies used lower or higher viable counts, commercial mass-based inclusion rates or mixed products; several expressed dose per kilogram rather than per gram of feed. Moreover, the highest immune response did not always coincide with the best growth response. Dose optimization must therefore be strain-, host-, route- and formulation-specific and should include viable counts at feeding, not merely counts at manufacture.

Mucosal outcomes deserve greater weight than serum panels because the gut, skin and gill are primary interfaces for dietary or waterborne probiotics. Tilapia studies reporting intestinal morphology, mucus enzymes or mucosal gene expression provide a more plausible bridge to pathogen exclusion than systemic biomarkers alone [9, 14, 41]. Longitudinal sampling before, during and after supplementation is needed to determine whether benefits persist or require continuous administration.

Representative finfish growth and immunological findings are summarized in Tables 3 and 4, while the principal protective pathways are illustrated in Fig. 5.

Table 3
Effects of *Bacillus* probiotic strains on growth performance in cultured finfish

Fish Species	<i>Bacillus</i> Strain(s)	Dose/Formulation	Duration	Growth Outcomes Summary	Reference
Nile tilapia (<i>Oreochromis niloticus</i>)	<i>B. subtilis</i> & <i>B. licheniformis</i> (BS)	0, 3, 5, 7, 10 g/kg in basal diet	4 weeks	All BS groups showed increased weight gain, SGR and improved FCR; highest effects at 10 g/kg (BS10)	Abarike et al. (2018)
Striped catfish (<i>Pangasianodon hypophthalmus</i>)	<i>B. amyloliquefaciens</i> 54A & <i>B. pumilus</i> 47B (mixed)	1×10 ⁸ , 3×10 ⁸ , 5×10 ⁸ CFU/g feed	90 days	Highest dose (5×10 ⁸ CFU/g) increased average weight gain significantly; no significant change in SGR or FCR	Thy et al. (2017)
Nile tilapia	<i>B. subtilis</i> HAINUP40	1×10 ⁸ CFU/g feed	8 weeks	Significant increase in final body weight, percent weight gain and SGR; improved feed conversion ratio	Liu et al. (2017)
Goldfish (<i>Carassius auratus</i>)	<i>B. velezensis</i> JW	0, 1×10 ⁷ , 1×10 ⁹ CFU/g feed	4 weeks	Growth not significantly affected across groups	Yi et al. (2018)
Pla-mong catfish (<i>Pangasius bocourti</i>)	<i>B. aerius</i> strain B81e	0, 1×10 ⁷ CFU/g feed	60 days	Significantly improved weight gain, specific growth rate and feed utilization efficiency	Meidong et al. (2018)
Red sea bream (<i>Pagrus major</i>)	<i>B. subtilis</i>	0, 1×10 ⁴ , 1×10 ⁶ , 1×10 ⁸ , 1×10 ¹⁰ CFU/kg	60 days	Significant improvement in final body weight, weight gain and SGR at 1×10 ⁸ and 1×10 ¹⁰ CFU/kg doses	Zaineldin et al. (2018)
Golden pompano (<i>Trachinotus ovatus</i>)	<i>B. pumilus</i> A97	1×10 ⁸ CFU/g diet	56 days	Significantly improved weight gain, SGR and feed efficiency	Liu et al. (2020)
Dabry's sturgeon (<i>Acipenser dabryanus</i>)	<i>B. subtilis</i> BStH-5 & BStH-19	2×10 ⁸ CFU/g diet	8 weeks	No significant growth improvement detected	Di et al. (2019)
Nile tilapia	<i>B. licheniformis</i> (AlCare®)	0–0.1% feed (≥ 4.4×10 ⁶ CFU/g feed)	10 weeks	Significant increases in weight gain, final body weight, and SGR at doses ≥ 0.04%	Han et al. (2015)
<i>Oplegnathus fasciatus</i> (parrot fish)	<i>B. subtilis</i> E20	0, 10 ⁸ , 10 ⁹ , 10 ¹⁰ CFU/kg feed	56 days	Growth improved only at 10 ⁸ CFU/kg; higher doses suppressed growth	Liu et al. (2018)
Nile tilapia	<i>B. velezensis</i> LF01	1×10 ⁹ CFU/g feed	8 weeks	Significant improvement in weight gain and SGR with probiotic feeding	Zhang et al. (2019)
Atlantic salmon (<i>Salmo salar</i> L.)	<i>B. velezensis</i> V4 + <i>Rhodotorula mucilaginosa</i>	Various CFU doses	62 days	Significant improvement in growth performance and feed utilization	Wang et al. (2019)
Mozambique tilapia (<i>O. mossambicus</i>)	<i>B. licheniformis</i> Dahb1	10 ⁵ & 10 ⁷ CFU/g diet	4 weeks	Significantly improved final weight, SGR and FCR at both doses (p < 0.05)	Gobi et al. (2018)
Nile tilapia (<i>O. niloticus</i>)	<i>B. mojavensis</i> B191, <i>B. subtilis</i> MRS11	10 ⁶ & 10 ⁸ CFU/g feed	60 days	All probiotic groups showed significant growth improvement; highest in <i>B. subtilis</i> 10 ⁸ CFU/g group	Büyükdeveci et al. (2023)
Nile tilapia (<i>O. niloticus</i>)	Sanolife PRO-F (<i>Bacillus</i> spp.)	0.1 & 0.2 g/kg diet	10 weeks	Significant improvement in growth and FCR at both doses compared to control	Elsabagh et al. (2018)
Larval common snook (<i>Centropomus undecimalis</i>)	Mixed <i>B. licheniformis</i> & <i>B. amyloliquefaciens</i>	Probiotic in water + live feed	Larval rearing period	No direct growth enhancement; improved survival and proposed GIT development acceleration	Tarnecki et al. (2019)
Japanese eel (<i>Anguilla japonica</i>)	<i>B. subtilis</i> WB60	10 ⁶ , 10 ⁷ , 10 ⁸ CFU/g diet	6 weeks	WG, FE, PER significantly higher at 10 ⁷ & 10 ⁸ CFU/g; healthiest gut morphology at 10 ⁸ CFU/g	Lee et al. (2017)
Nile tilapia (<i>O. niloticus</i>)	<i>B. sp.</i> KUAQ1 & KUAQ2	**	8 weeks	No significant effects on growth parameters (weight, SGR, FCR)	Sookchaiyaporn et al. (2020)
Hybrid grouper (<i>E. fuscoguttatus</i> ♂ × <i>E. lanceolatus</i> ♀)	<i>B. subtilis</i> 6-3-1	10 ⁶ , 10 ⁷ , 10 ⁸ CFU/g diet	8 weeks	All doses promoted growth; 10 ⁶ & 10 ⁸ CFU/g significantly improved WGR and SGR at week 8	Liao et al. (2021)
Nile tilapia (<i>O. niloticus</i>)	<i>B. subtilis</i> , <i>B. megaterium</i> , <i>B. licheniformis</i>	1, 3, 5 g/kg diet (1×10 ⁶ CFU/g)	120 days	3 g/kg diet significantly improved weight gain, ADG, SGR and FCR (p < 0.05)	Van Doan et al. (2023)
Nile tilapia (<i>O. niloticus</i>)	<i>B. safensis</i> NPUST1	10 ⁵ , 10 ⁶ , 10 ⁷ CFU/g diet	8 weeks	Significant increases in weight gain, feed efficiency and SGR at 10 ⁶ & 10 ⁷ CFU/g	Wu et al. (2021)

Fish Species	Bacillus Strain(s)	Dose/Formulation	Duration	Growth Outcomes Summary	Reference
Nile tilapia (O. niloticus)	Ecobiol (B. amyloliquefaciens), Sanolife PRO-F FMC	Various doses	60 days	Sanolife PRO-F FMC 0.002% significantly enhanced growth (p < 0.05).	Ghalwash et al. (2022)
Nile tilapia (O. niloticus)	B. coagulans (Technospore®)	0.2, 0.4, 0.8 g/kg diet	Not specified	Significant growth and FCR improvement at all doses; 0.4 g/kg most effective.	Omar et al. (2024)

Table 4
Summary of the effects of *Bacillus* probiotics on immunological parameters in finfish

Species	Probiotic & Dose	Trial Duration	Immune Parameters Evaluated	Key Immunological Findings	Reference
Nile tilapia	<i>B. pumilus</i> AQAHBS01 (10 ⁷ –10 ⁹ CFU/kg)	**	Phagocytic activity, superoxide anion, survival post <i>S. agalactiae</i>	Improved phagocytosis, superoxide, resistance; high dose effective in field under stress	Srisapoom & Areechon, 2017
Golden pompano	<i>B. pumilus</i> A97 (10 ⁸ CFU/g)	56 days	TLR8, TLR9 expression, non-specific immunity	Upregulated TLRs, enhanced immunity, higher survival post <i>V. ponticus</i>	Liu et al., 2020
Dabry's sturgeon	<i>B. subtilis</i> BStH-5, BStH-19 (2×10 ⁸ CFU/g)	8 weeks	Antioxidant capacity, SOD, IgM, lysozyme	Enhanced immunity, antioxidant status, survival post <i>A. hydrophila</i>	Di et al., 2019
Nile tilapia	<i>B. licheniformis</i> AlCare (≥ 4.4×10 ⁶ CFU/g)	10 weeks	Serum C3, lysozyme, SOD	Increased C3, lysozyme, disease resistance, maintained gut integrity	Han et al., 2015
Parrot fish	<i>B. subtilis</i> E20 (10 ⁸ –10 ¹⁰ CFU/kg)	56 days	Lysozyme, phagocytosis, respiratory burst	High dose improved immunity & resistance, low dose best for growth	Liu et al., 2018
Nile tilapia	<i>B. velezensis</i> LF01 (10 ⁹ CFU/g)	8 weeks	Lysozyme, SOD, immune gene expression	Improved immunity, reduced gut pathogens, higher survival	Zhang et al., 2019
Atlantic salmon	<i>B. velezensis</i> V4 + <i>R. mucilaginosa</i>	62 days	Non-specific immune response	Improved immunity, antioxidant capacity, disease resistance	Wang et al., 2019
Mozambique tilapia	<i>B. licheniformis</i> Dahb1 (10 ⁵ –10 ⁷ CFU/g)	4 weeks	TP, ALP, lysozyme, MPO, SOD	Improved immune and antioxidant status, enhanced resistance	Gobi et al., 2018
Nile tilapia	<i>B. mojavensis</i> B191, <i>B. subtilis</i> MRS11 (10 ⁶ –10 ⁸ CFU/g)	60 days	Immune gene expression (HSP70, IL-1β, etc.)	Enhanced immunity, gut health, resistance post <i>S. iniae</i>	Büyükdeveci et al., 2023
Rohu	<i>B. subtilis</i> KADR1 (10 ⁶ –10 ¹⁰ CFU/g)	4 weeks	Lysozyme, phagocytosis, SOD, IgM	Enhanced immunity, highest protection at 10 ⁸ CFU/g	Ramesh & Souissi, 2018
Nile tilapia	Sanolife PRO-F (<i>Bacillus</i> spp.) (0.1–0.2 g/kg)	10 weeks	Blood profiles, gut morphology	Improved immunity, stress response, gut health	Elsabagh et al., 2018
Larval snook	Mixed <i>Bacillus</i> spp.	Larval phase	Innate immune enzymes, survival	Improved survival, potential immune enhancement	Tarnecki et al., 2019
Japanese eel	<i>B. subtilis</i> WB60 (10 ⁶ –10 ⁸ CFU/g)	6 weeks	Lysozyme, SOD, MPO	Enhanced immunity, gut health, disease resistance	Lee et al., 2017
Hybrid grouper	<i>B. subtilis</i> 6-3-1 (10 ⁶ –10 ⁸ CFU/g)	8 weeks	Lysozyme, T-AOC, SOD, immune gene expression	Improved immunity, gut health, resistance to <i>V. harveyi</i>	Liao et al., 2021
Nile tilapia	<i>Bacillus</i> spp. KUAQ1, KUAQ2	8 weeks	Lysozyme, phagocytosis, respiratory burst	Improved immune parameters, no growth improvement	Sookchaiyaporn et al., 2020
Nile tilapia (<i>Oreochromis niloticus</i>)	<i>Bacillus subtilis</i> and <i>Bacillus licheniformis</i> (0, 3, 5, 7, 10 g/kg)	4 weeks	Lysozyme, protease, anti-protease, superoxide dismutase, catalase, myeloperoxidase activities (serum and skin mucus), Immunoglobulin M (IgM), immune-related gene expression (C-lysozyme, HSP70, β-defensin, TGF-β, Smad3)	Enhanced immune enzyme activities and gene expression in all BS groups; highest effects at 10 g/kg. Disease resistance improved; mortality reduced from 80% (control) to 20% (BS10)	Abarike et al. (2018)
Nile tilapia (<i>Oreochromis niloticus</i>)	<i>Bacillus amyloliquefaciens</i> (1×10 ⁴ and 1×10 ⁶ CFU/g feed)	30 days	Serum bactericidal activity, lysozyme activity, nitric oxide, phagocytic activity, gene expression (IL-1, TNF-α)	Immune parameters improved with probiotic dose and time; higher expression of IL-1 and TNF-α with higher dose; enhanced disease resistance to <i>Yersinia ruckeri</i> and <i>Clostridium perfringens</i>	Selim & Reda (2015)
Striped catfish (<i>Pangasianodon hypophthalmus</i>)	Mixed <i>B. amyloliquefaciens</i> 54A and <i>B. pumilus</i> 47B (1×10 ⁸ , 3×10 ⁸ , 5×10 ⁸ CFU/g)	90 days	Phagocytic activity, respiratory burst, lysozyme activity	Increased immune activities at highest dose (5×10 ⁸ CFU/g); enhanced disease resistance to <i>Edwardsiella ictaluri</i> improved stress tolerance; probiotics colonized intestine	Thy et al. (2017)
Nile tilapia (<i>Oreochromis niloticus</i>)	<i>Bacillus subtilis</i> HAINUP40 (1×10 ⁸ CFU/g)	8 weeks (+ 2-week challenge)	Total antioxidant capacity (T-AOC), serum superoxide dismutase (SOD), respiratory burst, serum lysozyme activity,	Significant increases in antioxidant enzymes, immune responses, digestive enzyme activities; 52.94% relative	Liu et al. (2017)

Species	Probiotic & Dose	Trial Duration	Immune Parameters Evaluated	Key Immunological Findings	Reference
			digestive enzymes (protease, amylase)	survival after <i>Streptococcus agalactiae</i> challenge	
Goldfish (<i>Carassius auratus</i>)	<i>Bacillus velezensis</i> JW (0, 1×10 ⁷ , 1×10 ⁹ CFU/g)	4 weeks	Acid phosphatase (ACP), alkaline phosphatase (AKP), glutathione peroxidase (GSH-PX), immune gene expression (IFN-γ, TNF-α, IL-1, IL-4, IL-10, IL-12)	Increased enzyme activities and immune gene expression; improved survival after <i>Aeromonas hydrophila</i> infection; broad antimicrobial activity	Yi et al. (2018)
Pla-mong catfish (<i>Pangasius bocourti</i>)	<i>Bacillus aerius</i> B81e (0, 1×10 ⁷ CFU/g)	60 days (+ 14-day challenge)	Serum lysozyme activity, bactericidal activity, alternative complement, phagocytic activity, respiratory burst activity	Significantly enhanced innate immune responses and survival against <i>A. hydrophila</i> ; probiotic showed strong adhesion and antibacterial properties	Meidong et al. (2018)
Red sea bream (<i>Pagrus major</i>)	<i>Bacillus subtilis</i> (0, 1×10 ⁴ , 1×10 ⁶ , 1×10 ⁸ , 1×10 ¹⁰ CFU/kg diet)	60 days	Serum and mucus bactericidal activity, lysozyme, peroxidase, catalase activity, nitro blue tetrazolium (NBT)	Improved immune enzyme activities and oxidative stress tolerance especially at 1×10 ⁸ and 1×10 ¹⁰ CFU/kg doses	Zaineldin et al. (2018)
Nile tilapia (<i>Oreochromis niloticus</i>)	<i>B. velezensis</i> TPS3N, <i>B. subtilis</i> TPS4, <i>B. amyloliquefaciens</i> TPS17, and their combination	8 weeks	Nitric oxide (NO), Immunoglobulin M (IgM), lysozyme (LZM), alkaline phosphatase (AKP), catalase (CAT), superoxide dismutase (SOD), lipase activity, intestinal morphology, microbiome composition	Enhanced mucosal immunity, antioxidant enzymes, digestive enzyme activity, improved gut morphology, modulated microbiota, and reduced mortality after <i>Aeromonas hydrophila</i> challenge; combination treatment most effective	Kuebutornye et al. (2020)

5.2 Aquatic invertebrates

In shrimp, *Bacillus* has been delivered in formulated diets, enriched live feed, rearing water and biofloc. Studies report improved growth, feed utilization, antioxidant status, innate defence, microbiome composition and resistance to *Vibrio*, fungal or viral challenges [22, 42, 43]. Larval-feed enrichment with *Bacillus* improved growth and survival in whiteleg shrimp hatchery stages [44], whereas biofloc augmentation produced system-level gains when the introduced strain interacted productively with the carbon-rich microbial community [36].

Evidence also extends to freshwater prawn, abalone, scallop, oyster and sea cucumber. *B. coagulans* improved production and immune outcomes in freshwater prawn [45]; *B. amyloliquefaciens* or *B. licheniformis* improved growth, immune indices or challenge resistance in abalone [46, 47]; *Bacillus*-containing treatments improved scallop early development [48]; and *B. cereus* EN25 affected growth and resistance in sea cucumber [49]. The presence of *B. cereus*-group strains in efficacy studies underscores why safety cannot be inferred from a probiotic label or a beneficial phenotype.

Developmental stage changes both opportunity and risk. Larvae have immature barriers, rapidly changing microbiota and limited control over microbial exposure. A strain that improves settlement or early survival may not remain effective in juveniles, while high water-phase counts can alter system ecology. Hatchery studies should report background microbial loads, water renewal, live-feed microbiology and post-withdrawal survival, rather than treating larval response as directly transferable to grow-out systems.

Representative immunological findings for shrimp and other aquatic invertebrates are summarized in Table 5.

Table 5
Immunomodulatory effects of *Bacillus* probiotics in shrimp and other aquatic invertebrates

Species	<i>Bacillus</i> Species & Dose	Duration	Key Immunological Parameters & Findings	Reference
<i>Litopenaeus vannamei</i>	<i>B. subtilis</i> (BS7: 10 ⁷ CFU/g; BS8: 10 ⁸ CFU/g) + others (PP8, LL8)	8 weeks	↑ Lysozyme activity, ↑ SOD, ↑ immune gene expression (BS8, PP8, LL8), improved gut histology, enhanced disease resistance	Won et al. (2020)
<i>Litopenaeus vannamei</i>	<i>B. subtilis</i> AQAHBS001 (feed: 10 ⁷ and 10 ⁹ CFU/kg)	5 weeks	↑ Phagocytic activity, clearance efficiency, ↑ immune gene expression (proPO, lysozyme, ALF), improved gut morphology, enhanced resistance to VPAHPND	Kewcharoen & Srisapoom (2019)
<i>Macrobrachium rosenbergii</i>	<i>B. coagulans</i> (10 ⁵ , 10 ⁷ , 10 ⁹ CFU/g diet)	60 days	↑ Lysozyme, respiratory burst activity, ↑ digestive enzymes (protease, amylase, lipase), improved disease resistance against <i>Vibrio harveyi</i>	Gupta et al. (2016)
<i>Litopenaeus vannamei</i>	<i>B. aryabhattai</i> TBRC8450 (dietary)	6 weeks	↑ Immune gene expression (C-type lectin, penaeidin-3, HSP60), ↑ phenoloxidase activity, ↑ antioxidant capacity, reduced <i>Vibrio</i> abundance, improved survival post-challenge	Tepaamorndech et al. (2019)
<i>Litopenaeus vannamei</i>	Mix: <i>B. licheniformis</i> MAT32, <i>B. subtilis</i> MAT43, GATB1 (1–6×10 ⁶ CFU/g)	8 weeks	↑ proPO, LvToll1, SOD gene expression, ↓ viral prevalence (WSSV, IHNV), improved immune gene expression	Sánchez-Ortiz et al. (2016)
<i>Litopenaeus vannamei</i>	<i>B. subtilis</i> + <i>B. licheniformis</i> (1×10 ⁴ & 1×10 ⁸ CFU/g feed)	60 days	↑ Lysozyme activity, ↑ hemocyte count, ↑ total protein, ↓ serum glucose and cortisol, improved immune parameters	Sadat Hoseini Madani et al. (2018)
<i>Litopenaeus vannamei</i>	<i>B. subtilis</i> + <i>B. licheniformis</i> (water application, 0.01–0.03 g/m ³)	56 days	↑ Lysozyme, proPO, respiratory burst, hemocyte count, antioxidant enzymes (SOD, catalase, GPx), improved disease resistance to <i>Fusarium</i>	Monier et al. (2023)
<i>Litopenaeus vannamei</i>	<i>B. coagulans</i> ATCC 7050 (1×10 ⁶ –1×10 ⁸ CFU/g feed)	56 days	↑ Lysozyme, acid phosphatase, SOD, glutathione peroxidase, ↓ malondialdehyde, improved intestinal morphology, ↑ disease resistance to <i>Vibrio parahaemolyticus</i>	Amoah et al. (2019)
Abalone (<i>Haliotis discus hannai</i>)	<i>Bacillus licheniformis</i> , sprayed at 10 ³ –10 ⁷ CFU/MI	8-week culture + 2-week <i>Vibrio</i> challenge	↑ total blood lymphocytes, acid phosphatase, phagocytic activity, myeloperoxidase, CAT, SOD, nitric oxide, O ₂ ⁻ respiratory burst; ↑ HSP70, CAT, thioredoxin peroxidase, Mn-SOD gene expression; ↓ mortality post-challenge	Gao et al. (2018)
White shrimp (<i>Litopenaeus vannamei</i>) larvae	<i>Bacillus subtilis</i> IS02, 0–10 ⁸ CFU/mL in water	21 days	↑ prophenoloxidase (proPO), peroxinectin (PE) gene expression; improved survival, larval development, immune response; ↓ <i>Vibrio</i> and total bacterial counts	Mirbakhsh et al. (2021)
Whiteleg shrimp (<i>L. vannamei</i>)	Mixed <i>Bacillus</i> spp., freeze-dried (FB) or microencapsulated (MB)	30 days + 10-day challenge	Improved survival and disease resistance; enhanced immune response post- <i>Vibrio harveyi</i> infection; ↑ <i>Bacillus</i> colonization in gut and water	Nimrat et al. (2020)
Whiteleg shrimp juveniles (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> -fermented rice bran (FRB) feed	12 weeks	↑ lysozyme (LYZ), phenol oxidase (PO); improved immune biomarkers, growth, antioxidant enzymes (SOD, CAT); ↓ oxidative stress markers	Abdel-Tawwab et al. (2022)
Whiteleg shrimp (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> CM3.1, water supplementation 10 ² –10 ⁴ CFU/mL	**	Improved water quality; ↑ survival rate and specific growth rate; probiotic showed strong enzyme activity enhancing immunity	Truong et al. (2021)
Whiteleg shrimp (<i>Penaeus vannamei</i>)	<i>Bacillus subtilis</i> BSXE-1601, diet (10 ⁹ CFU/kg) or water (10 ⁷ CFU/L)	42 days	↑ serum immune enzyme activities (acid phosphatase, lysozyme, SOD, phenoloxidase), ↑ immune gene expression (proPO, LZM, SOD, HSP70, etc.); improved disease resistance	Luo et al. (2024)
Whiteleg shrimp (<i>L. vannamei</i>) juveniles	<i>B. subtilis</i> NT9 and <i>B. cereus</i> YB3 in biofloc	7-day biofloc + 63-day culture	↑ survival and specific growth rate; <i>B. subtilis</i> NT9 improved shrimp productivity and health under low salinity conditions	Huang et al. (2023)
Giant freshwater prawn (<i>Macrobrachium rosenbergii</i>)	<i>B. subtilis</i> and Effective Microorganisms (EM) in biofloc system	**	↑ plasma SOD, lysozyme, acid phosphatase; improved nonspecific immunity; ↓ plasma malondialdehyde; enhanced intestinal enzyme activity	Qiu et al. (2021)
Whiteleg shrimp (<i>L. vannamei</i>)	<i>Bacillus</i> PC465, dietary supplementation 10 ⁷ –10 ⁹ CFU/g feed	30 days + 20-day WSSV challenge	↑ immune gene expression (proPO, peroxinectin, hemocyanin, etc.); ↑ digestive enzyme activity; reduced viral load; enhanced survival and immunity	Chai et al. (2016)
Whiteleg shrimp (<i>Penaeus vannamei</i>)	BG4 probiotic (mix of <i>Bacillus</i> spp.) in water	7 days	Evaluated water microbiota and survival; no significant shrimp survival difference; probiotic declined over time, indicating need for quality control	Hu et al. (2023)
Giant freshwater prawn (<i>Macrobrachium rosenbergii</i>)	<i>Bacillus cereus</i> (quorum sensing degrader) 10 ⁴ CFU/g diet	28 days + pathogen challenge	↑ SOD activity; improved hepatopancreatic histopathology post- <i>Aeromonas hydrophila</i> challenge; no significant survival difference	Wee et al. (2018)

Species	Bacillus Species & Dose	Duration	Key Immunological Parameters & Findings	Reference
White shrimp (<i>Litopenaeus vannamei</i>)	<i>Bacillus subtilis</i> E20, 10 ⁹ CFU/kg diet	8 weeks	Upregulated immune-related genes and antioxidant enzymes; enhanced immune activation pathways; increased HSP70 and protein glycosylation	Chien et al. (2020)
White shrimp (<i>Penaeus vannamei</i>)	<i>Bacillus tequilensis</i> AP BFT3 in biofloc system and clear water	**	Upregulated immune-related genes; enhanced growth and survival; increased protective proteins by proteomics analysis	Panigrahi et al. (2022)
Pacific white shrimp larvae (<i>L. vannamei</i>)	<i>B. licheniformis</i> + <i>B. subtilis</i> enriched in live feed (Artemia, rotifers)	Until PL10 larval stage	Enhanced larval growth and survival rates with probiotic-enriched live feed	Jamali et al. (2015)
Pacific abalone (<i>Haliotis discus hannai</i>)	<i>Bacillus amyloliquefaciens</i> , 10 ³ –10 ⁷ CFU/g feed	8-week culture + 2-week <i>Vibrio</i> challenge	↑ blood lymphocytes, acid phosphatase, SOD, catalase; ↑ antioxidant capacity and immune gene expression; ↓ mortality after <i>Vibrio</i> challenge	Xiaolong et al. (2019)
Eastern oyster larvae (<i>Crassostrea virginica</i>)	<i>Bacillus pumilus</i> RI06–95, 5 × 10 ⁴ CFU/mL	6–24 h probiotic exposure + up to 15 days	Activation of immune signaling pathways and effector genes; reduction of oxidative stress; enhanced larval immune responses	Modak & Gomez-Chiarri (2020)
Pacific white shrimp fingerlings (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> dietary 0–1.5 g/kg feed	56 days	↑ lysozyme, phenoloxidase activities, survival, growth, feed utilization; improved antioxidant parameters	Eissa et al. (2022)
Pacific white shrimp (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> (water addition, feed addition)	**	Hepatopancreas catalase (CAT) and total antioxidant capacity (T-AOC) varied by delivery method, SOD and acid phosphatase (ACP) activities independent of delivery, Increased intestinal pepsin activity, Non-specific immune enzyme activities improved	He et al. (2023)
Catarina scallop (<i>Argopecten ventricosus</i>)	Mixed <i>Bacillus</i> and <i>Lactobacillus</i> strains	9–21 days	Increased SOD enzyme activity, enhanced disease resistance to <i>Vibrio alginolyticus</i> , probiotic effects strain- and stage-specific, improving immune resistance	Abasolo-Pacheco et al. (2017)
Freshwater prawn (<i>M. rosenbergii</i>)	<i>Bacillus vireti</i> 01 in diet	2 weeks	Elevated antioxidant enzyme activities: SOD, catalase, glutathione (GSH), improved immune response reducing mortality under <i>Pseudomonas aeruginosa</i> infection, reduced histopathological tissue damage	Vidhya Hindu et al. (2018)
Freshwater prawn (<i>M. nipponense</i>)	<i>Bacillus velezensis</i> CPA1–1 (immersion)	20 days	Increased immune enzyme activities: ACP, alkaline phosphatase (AKP), SOD, Upregulated immune-related genes (Crustin, Dorsal, Hemocyanin, p38, Prophenoloxidase, Stat), higher survival after <i>Vibrio cholerae</i> challenge	Zhu et al. (2021)
Giant tiger shrimp (<i>Penaeus monodon</i>)	PHB-accumulating <i>Bacillus</i> sp. JL47	15 days	Upregulation of immune genes prophenoloxidase (proPO), transglutaminase (TGase) pre- and post-challenge, Hsp70 increased post-challenge, Enhanced innate immune response indicating immunostimulatory potential	Laranja et al. (2017)
White shrimp (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> fermented Caulerpa byproduct	56 days	Upregulation of immune-related genes in hepatopancreas and hemocytes, increased phenoloxidase activity in vitro, enhanced ROS production, improved survival post- <i>Vibrio parahaemolyticus</i> challenge	Lee et al. (2023)
White shrimp (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> E20 10 ⁹ CFU/kg feed	56 days	Improved immune enzyme activities, enhanced feed utilization linked to immune status, increased digestive enzyme activities aiding immune function	Tsai et al. (2019)
White shrimp (<i>L. vannamei</i>)	<i>Bacillus licheniformis</i> BCR 4 – 3 (feed/water)	**	Significant upregulation of innate immune genes: lysozyme, penaeidin4, crustin, superoxide dismutase, improved survival against <i>Vibrio parahaemolyticus</i> - AHPND challenge, immune activation via antimicrobial pathways	García-Medel et al. (2020)
Pacific white shrimp (<i>L. vannamei</i>)	<i>Bacillus licheniformis</i> 10 ⁸ CFU/g feed	35 days + nitrite stress	Elevated antioxidant enzymes: glutathione peroxidase, peroxidase, total antioxidant capacity, SOD, enhanced digestive enzyme and metabolic enzyme activities supporting immunity, improved gut microbiota diversity correlated with immune health, higher survival under nitrite stress	Fan et al. (2021)
Pacific white shrimp (<i>L. vannamei</i>)	<i>Bacillus subtilis</i> and <i>Bacillus circulans</i> diet supplementation	60 days	Upregulation of immune genes proPO, LGBP, HEM, improved survival after <i>Vibrio parahaemolyticus</i> challenge, increased gut <i>Bacillus</i> abundance associated with immune enhancement, overall improved immune status.	Vogeley et al. (2019)
Pacific white shrimp (<i>L. vannamei</i>)	<i>Bacillus velezensis</i> BV007 (varied doses)	8 weeks + 2-week challenge	Increased respiratory burst, SOD, CAT, AKP activities, enhanced immune gene expression post <i>Vibrio parahaemolyticus</i> challenge, modulation of gut microbiota with increased beneficial <i>Bacillus</i> and reduced pathogens, – 1×10 ⁷ CFU/g recommended for optimal immune stimulation	Chen et al. (2021)

6 Determinants of efficacy and reproducibility

The main causes of between-study variation can be organized into seven interacting determinants: strain identity, host compatibility, viable dose, delivery route, formulation stability, production environment and outcome design. Reporting only the *Bacillus* species and nominal inclusion rate leaves several of these determinants unresolved.

The minimum reporting information required to improve efficacy assessment and reproducibility is presented in Table 6.

Table 6
Minimum reporting framework for efficacy and reproducibility

Domain	Minimum information	Why it matters	Recommended verification
Identity	Current taxonomic name, strain code, accession/depository and whole-genome accession	Species-level labels can include functionally and toxicologically distinct strains	Whole-genome-based identity and phylogenomic placement
Potency	Viable count, unit, analytical method, batch and count at administration	Nominal manufacturer values may not equal delivered dose	Plate count or validated molecular viability assay at start and end of storage/trial
Route/formulation	Feed coating, incorporation, encapsulation, water addition or live-feed enrichment; carrier and processing	Heat, moisture, pelleting and water exposure affect viability and release	Stability, leaching, germination and release testing
Host/context	Species, life stage, health status, stocking density, diet, temperature, salinity and system	Compatibility and environmental performance are context dependent	Factorial or multi-site validation
Comparator	Unsupplemented control, carrier control and, where ethical, standard management comparator	Separates probiotic effect from carrier, diet or handling effects	Randomization, allocation concealment and blinded outcome assessment where feasible
Outcomes	Prespecified primary outcome, standardized growth calculations, challenge details and mortality window	Selective reporting inflates apparent efficacy	Protocol registration and complete outcome reporting
Persistence	Strain-resolved abundance during use and after withdrawal	Repeated detection may reflect intake rather than colonization	Culture/WGS or strain-specific qPCR with absolute quantification
Economics	Product cost, feed-processing effect, application labour and benefit per production unit	Biological efficacy may not be commercially viable	Farm-scale cost-effectiveness and sensitivity analysis

6.1 Strain–host compatibility

Host-derived candidates are often prioritized because they may tolerate local bile, mucus, salinity and temperature conditions and interact with the resident microbiota. This is a rational starting point, not proof of superiority. Comparative trials should test host-derived and non-host-derived strains under the same diet and system, and should distinguish transient passage from persistent functional engagement.

6.2 Dose, unit and administration route

Dose–response interpretation is currently weakened by mixed units. CFU/g feed, CFU/kg feed, CFU/mL water and g/kg of commercial product are not interchangeable without viable-count and exposure information. Water addition creates a system exposure affecting host and environment; dietary addition creates a pulsed oral exposure; live-feed enrichment introduces a third trophic carrier. Future studies should report delivered viable cells per animal biomass or per system volume where possible and verify viable counts after feed processing and water contact.

6.3 Formulation and shelf stability

Spore formation improves robustness but does not eliminate losses during storage, pelleting, coating, transport or prolonged immersion. Microencapsulation has improved stability and performance in postlarval shrimp [43], while fermented ingredients and synbiotic combinations can alter both dose delivery and substrate availability [50, 51]. Formulation trials should separate the effects of the organism, carrier and fermentation products.

6.4 Environment and husbandry

Temperature, salinity, dissolved oxygen, pH, organic loading, water exchange and carbon management determine whether spores germinate, cells persist and metabolites accumulate. They also affect the host and pathogen directly. Multi-environment trials and reporting of background microbial communities are therefore necessary before a strain can be described as broadly effective or climate-resilient.

7 Biosafety, AMR and One Health

The original manuscript repeatedly contrasted probiotics as inherently safe with antibiotics as inherently harmful. That binary framing is scientifically and regulatorily inadequate. Antibiotics are legitimate therapeutic tools when prescribed and used responsibly; probiotics are biological products whose benefit and risk are strain- and context-dependent. Their role is principally preventive and antibiotic-sparing, not a universal replacement for diagnosis, vaccination, biosecurity or indicated treatment.

Bacillus can support antibiotic stewardship through pathogen exclusion, improved host resilience and better system conditions. Reviews of probiotics and bacteriocins describe this potential [52], and selected fish-gut isolates produce natural antimicrobial compounds [13]. However, evidence that commercial *Bacillus* use reduces the selection or dissemination of resistance genes in farms remains limited. A probiotic strain may itself contain intrinsic or acquired resistance genes; the key questions are whether these genes are clinically relevant, mobile and transferable under intended-use conditions.

Safety qualification should include whole-genome sequencing, verified taxonomic identity, screening for virulence and toxin genes, phenotypic antimicrobial susceptibility, assessment of mobile genetic elements, hemolysis and cytotoxicity, and evaluation of undesirable metabolite production. Particular caution is required for strains assigned to the *B. cereus* group. Historical efficacy studies that used *B. cereus* should not be interpreted as evidence that all members or preparations are suitable for feed or water application.

Environmental risk assessment is also necessary. Repeated high-density application may alter pond, biofloc or sediment communities; persistence beyond the production cycle could affect non-target organisms; and plasmids or other mobile elements could move through microbial networks. At the same time, reduced organic loading, ammonia or pathogen abundance may provide environmental benefits. A One Health claim is justified only when animal-health benefit, environmental fate and AMR safety are assessed together rather than inferred from the absence of antibiotic treatment.

Figure 6 contrasts the roles of *Bacillus* probiotics and antibiotics, while Tables 7 and 8 summarize One Health evidence and strain-level biosafety qualification requirements.

Table 7
Experimental and field studies evaluating *Bacillus* probiotics in aquaculture and their relevance to One Health

Reference	Aquatic Species	Culture system	Intervention	Target pathogens	Mode of Action/Mechanisms	Host Response	Microbiome and AMR Impact	Relevance to One Health
Santos et al. (2021)	European seabass, gilthead seabream, white seabream	Marine aquaculture (gut-derived isolates)	<i>Bacillus</i> sp. (3 lead candidates) vs. Antibiotic-free	<i>Aeromonas hydrophila</i> , <i>Vibrio anguillarum</i> , etc.	<i>Bacillus</i> : NACs, anti-biofilm, anti-QS; Antibiotic: bactericidal	Not assessed (in vitro); inferred pathogen control	<i>Bacillus</i> : minimal ARG selection, potential microbiome stabilization; Antibiotics: high ARG selection risk	Ba ar su He en AM
Monier et al. (2023)	Whiteleg shrimp (<i>Litopenaeus vannamei</i>)	Concrete ponds (1 m ³), 240 shrimp, 4 treatments	<i>Bacillus</i> probiotic SANOLIFE®PRO-W (B. subtilis + B. licheniformis), 0.01–0.03 g/m ³ ; Control: 0 g/m ³	<i>Fusarium solani</i>	Water-applied probiotics improved water quality (↑ DO, ↓ NH ₃ , NO ₂ ⁻), enhanced digestive enzymes (chymotrypsin, trypsin, protease, lipase, amylase), and antioxidant defenses (SOD, CAT, GPx)	Growth performance ↑ with probiotic dose; survival improved (mortality 35% in T3 vs 100% in control); innate immunity enhanced (lysozyme, prophenoloxidase, respiratory burst, total hemocyte count)	Not assessed	Re ar an ap AM sh ar Or pr
Zokaeifar et al. (2014)	Whiteleg shrimp (<i>Litopenaeus vannamei</i>)	Rearing water (8-week experiment), vegetative cells in water	<i>Bacillus subtilis</i> strains L10 + G1, 10 ⁵ CFU/ml (BM5) and 10 ⁸ CFU/ml (BM8); Control: no probiotic	<i>Vibrio harveyi</i>	Water-applied probiotics improved water quality (↓ ammonia, nitrite, nitrate) and enhanced digestive enzyme activity	Growth performance ↑ (final weight, weight gain, SGR, FCR) in BM5 & BM8; survival ↑ (BM8 63.3%, BM5 50% vs 20% control); immune gene expression ↑ (proPO, PE, LGBP, SP) in BM5	Not assessed	Im qu re ar ap AM su He
Sandoval et al. (2016)	Salmonids (farm-raised, Chile)	Aquaculture tanks / farmed salmon	Antibiotics: florfenicol and oxytetracycline	<i>Piscirickettsia salmonis</i> (strains LF-89 and AUSTRAL-005)	Antibiotics: bacteriostatic/bactericidal activity; florfenicol exposure induces efflux pump activation (acrAB) and modulates RND gene expression	Not assessed (study focused on bacterial resistance, not host outcomes)	Florfenicol exposure increased efflux pump activity, suggesting enhanced antibiotic resistance potential	Re us m P. en re ar cc ris Or
	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Farmed freshwater aquaculture (France, 2012–2021)	Antibiotics: florfenicol, oxytetracycline, doxycycline, sulfadiazine + trimethoprim, oxolinic acid, flumequine, enrofloxacin	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i>	Antibiotics: bacteriostatic/bactericidal activity; MICs measured to determine susceptibility; cross-resistance observed in quinolones	Not assessed (focus on bacterial susceptibility, not host outcomes)	3% of strains classified as non-wild-type (NWT) for all antibiotics; cross-resistance and rising MICs for tetracyclines indicate AMR emergence	Hi ep cu cli br ro us to ac en di: su He

Table 8
Strain-level biosafety and One Health qualification checklist

Assessment	Minimum test	Decision question	Reporting requirement
Identity and purity	Whole-genome sequence; culture purity; deposited strain	Is the product the claimed strain and free of contamination?	Accession numbers, assembly quality and batch quality-control method
Virulence/toxicity	Virulence, toxin and biogenic-metabolite gene screening; cytotoxicity where relevant	Could the strain or its products damage the host or consumer?	Gene database/version, thresholds and confirmatory phenotype
AMR	Phenotypic susceptibility plus genomic detection of resistance determinants and mobility	Are clinically relevant genes present and potentially transferable?	Antimicrobial panel, interpretive criteria, genomic context and mobile elements
Host safety	Tolerance, histopathology, blood/hemolymph indices and dose margin	Is the intended dose safe across life stages and stressed animals?	Adverse events, no-observed-adverse-effect level and withdrawal observations
Environmental fate	Persistence in water/sediment, non-target effects and post-application decline	Does the strain accumulate or restructure the system undesirably?	Absolute counts, strain-specific tracking and post-cycle monitoring
Food-chain relevance	Residue/persistence on edible tissues where applicable and processing survival	Could exposure extend to workers or consumers?	Sampling point, detection limit and risk interpretation
Benefit–risk integration	Production benefit, antibiotic-use change, ecological effects and cost	Does net benefit justify routine use?	Predefined decision criteria and farm-scale validation

8 Translational readiness and research priorities

A useful probiotic must progress through linked stages: candidate discovery, genomic and phenotypic safety qualification, mechanism-supported efficacy, formulation and shelf-life validation, controlled host trials, farm-scale replication, environmental assessment and post-market quality control. Many current studies stop after a single controlled feeding trial. This creates a large evidence gap between statistically significant biomarkers and a product that performs reproducibly across farms.

8.1 Mechanism-informed strain selection

Candidate selection should start from the production problem. A strain intended for enteric pathogen exclusion should be screened for mucus interaction, relevant antagonistic products and barrier effects; a biofloc inoculant should be selected for carbon utilization, nitrogen transformation and ecological compatibility; a digestive adjunct should demonstrate enzyme activity under feed-processing and gastrointestinal conditions. Selecting one strain because it performs many unrelated *in vitro* assays risks producing a generalist label without a validated use case.

8.2 Multi-omics with causal validation

Transcriptomics, proteomics, metabolomics and metagenomics can identify host and microbial pathways altered by supplementation. Proteomic changes in probiotic-augmented biofloc and metabolomic changes in shrimp illustrate the value of system-level measurement [23, 36]. Multi-omics should not be used as a decorative endpoint: hypotheses generated from these data require targeted validation, ideally through metabolite supplementation, gene-expression perturbation, strain mutants or fractionated cell products.

8.3 Comparative and multi-site trials

Future efficacy studies should compare at least two qualified strains or formulations under identical conditions, prespecify a primary production endpoint and include sufficient replication at tank or pond not individual-animal level. Multi-site trials should span relevant salinity, temperature and management regimes. Reporting should include null and adverse findings, because the present literature is likely enriched for positive results.

8.4 Integration rather than substitution

The most credible path to reduced antibiotic dependence is an integrated health programme combining certified seed or stock, vaccination where available, nutrition, water-quality management, biosecurity, diagnostics and qualified microbial interventions. Probiotics may complement prebiotics, fermented feeds, immunostimulants, phage strategies and biofloc systems, but combination products require factorial testing to identify synergy, redundancy or antagonism. A positive combination cannot be attributed automatically to *Bacillus*.

8.5 Data standards and regulatory alignment

Study-level data should be deposited in reusable form, including strain accession, dose verification, trial unit, environment, outcome definitions and adverse observations. Regulatory dossiers should be updated when taxonomy changes and should evaluate the actual production strain rather than relying on species-level histories of use. Consistent data standards would enable future meta-analysis and reduce repeated small trials that cannot be compared.

9 Conclusions

Evidence accumulated during 2015–2025 supports *Bacillus* probiotics as promising tools for improving growth, feed use, innate defence, barrier function, challenge resilience and environmental management in aquaculture. The strongest conclusion is not that *Bacillus* universally replaces antibiotics or that a

single dose range is optimal. Rather, selected strains can produce useful outcomes when identity, viable exposure, host compatibility, formulation and production environment are aligned.

The next phase of the field should move from species-level benefit catalogues to strain-resolved, mechanism-supported and safety-qualified interventions. Whole-genome biosafety, standardized dose reporting, direct mechanism tests, post-withdrawal tracking, multi-site farm validation and environmental fate assessment are minimum requirements for credible One Health claims. Under this framework, *Bacillus* can contribute to antibiotic stewardship as one component of integrated disease prevention, while avoiding the equally problematic assumption that all products labelled probiotic are inherently safe or effective.

Declarations

Funding

The authors reported that this research received no external funding.

Competing Interests

The authors declare no competing interests.

Author Contributions

Samuel Ayeh Osei: Conceptualization, data curation, original draft writing, and literature investigation

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Christian Larbi Ayisi: Conceptualization, supervision and writing—review and editing.

Gabriel Arome Ataguba: Review

Sahr Lamin Sumana: Conceptualization and review

Data Availability

No new experimental data were generated. The study-selection log and study-level data-charting matrix should be made available as supplementary information or in a public repository.

Ethics Approval

Not applicable. This article reviews published studies and involved no new experiments with humans or animals.

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Figures

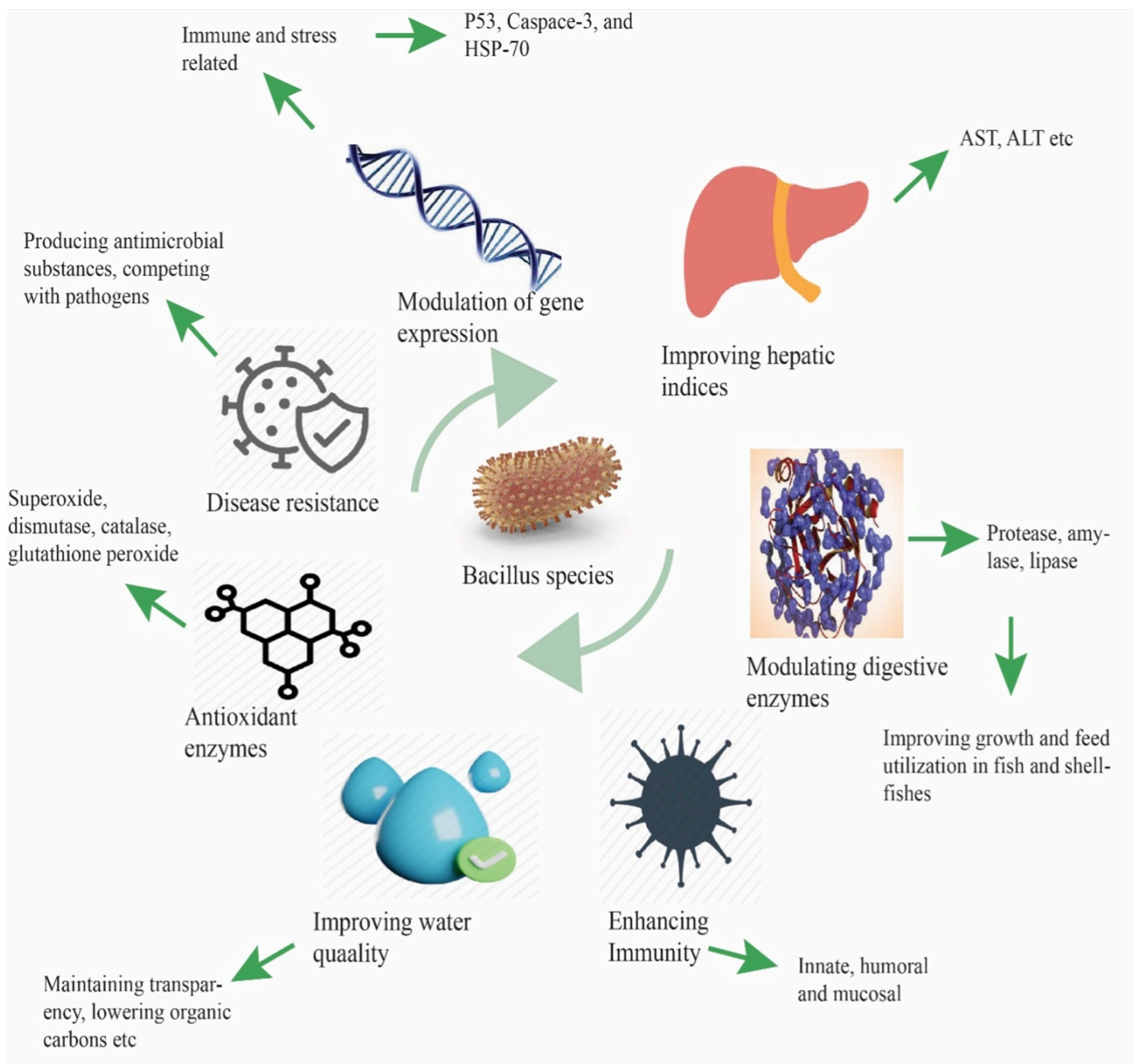


Figure 1

Probiotic Bacillus effects on the aquaculture environment, finfish and shellfish

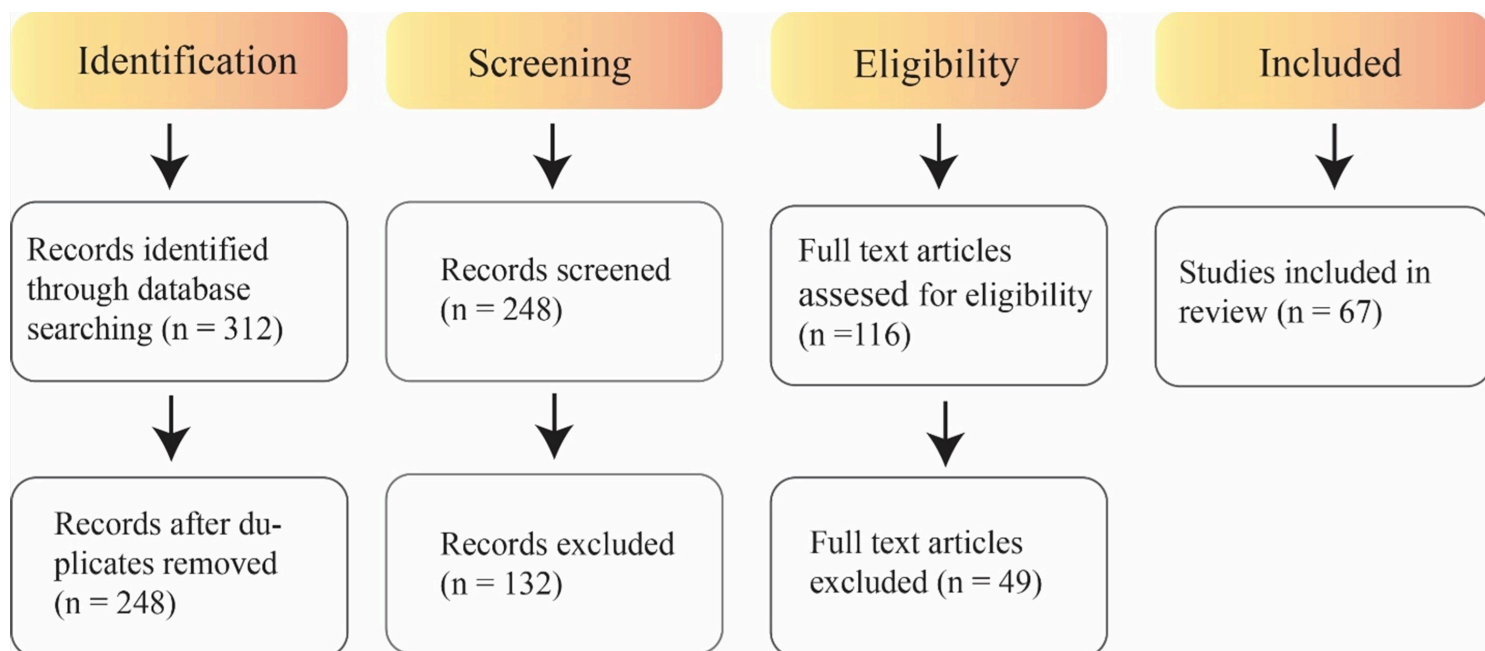


Figure 2

PRISMA flow diagram illustrating the study-selection process

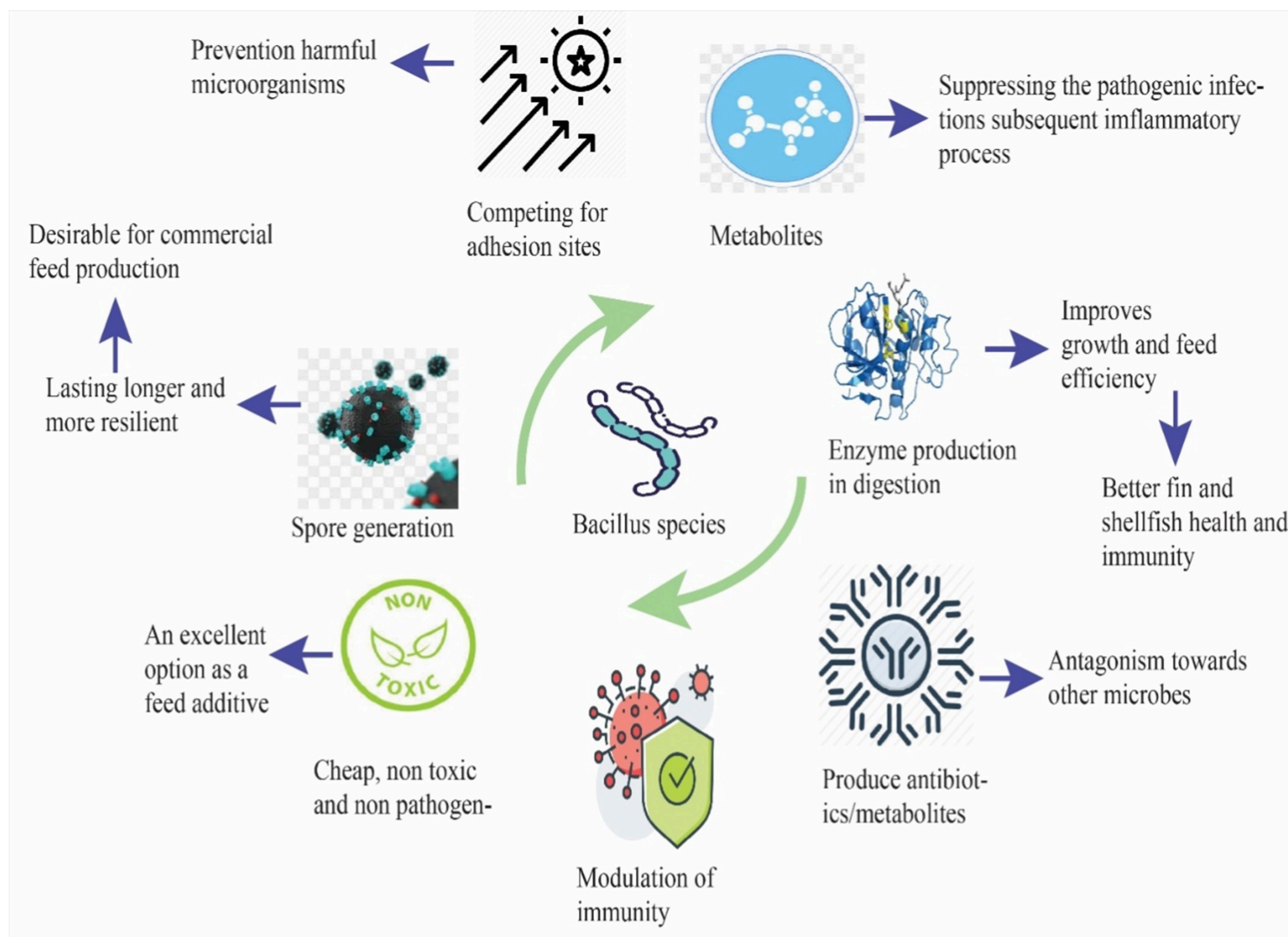


Figure 3

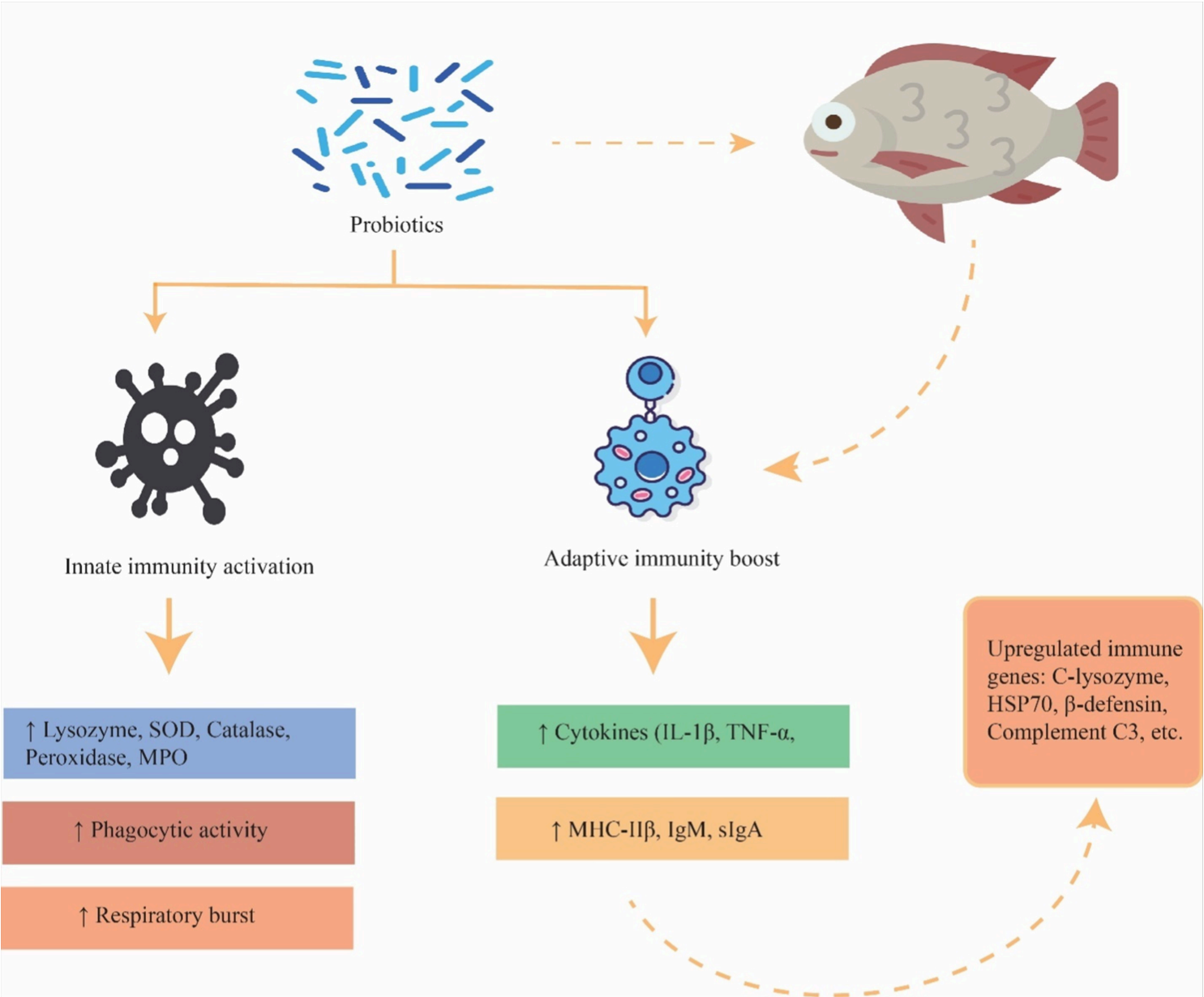


Figure 4
Immunomodulatory effects of Bacillus-based probiotics in finfish

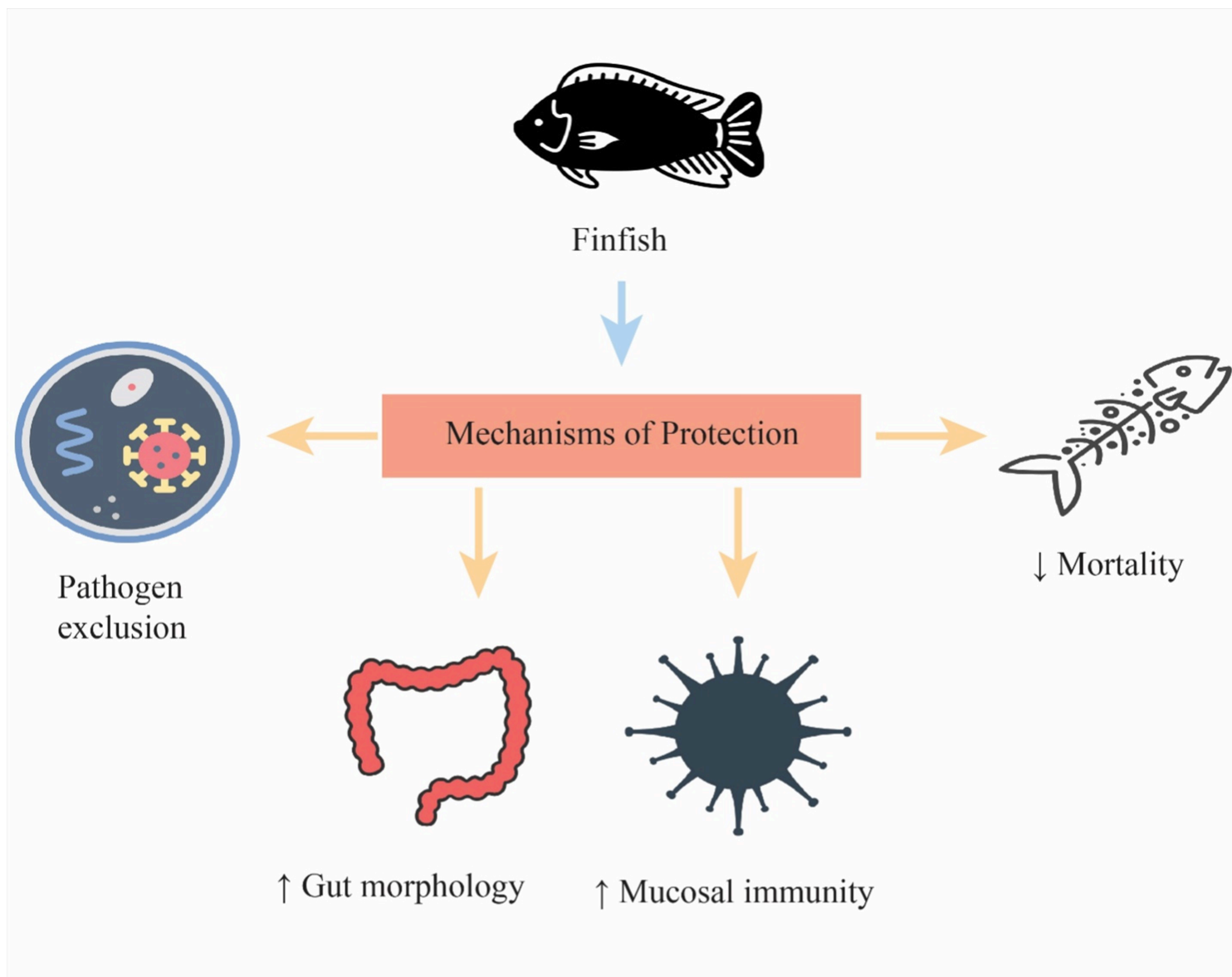


Figure 5

Protective roles of *Bacillus* probiotics in finfish

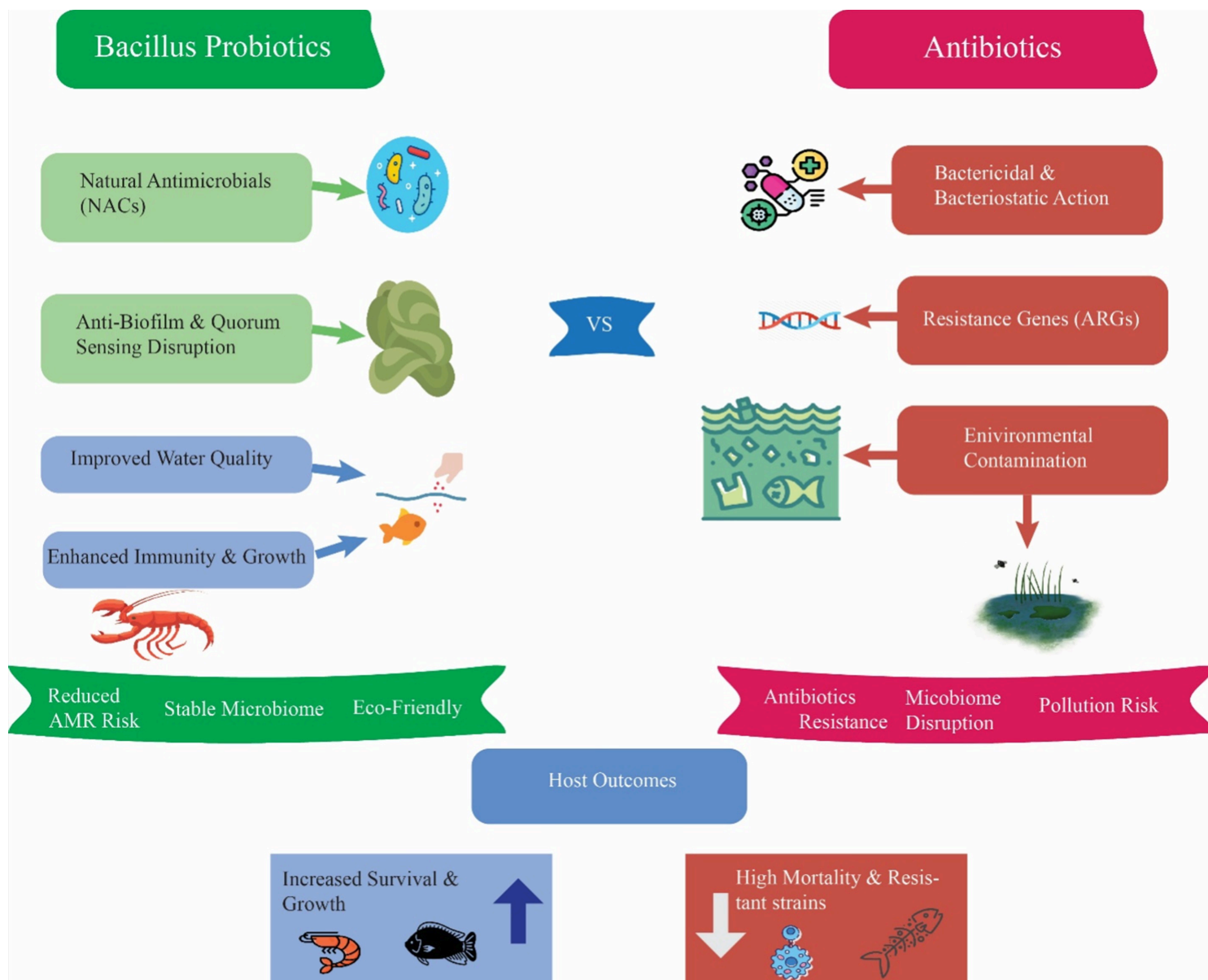


Figure 6

Comparative roles of *Bacillus* probiotics and antibiotics in aquaculture, including mechanisms, host outcomes and One Health implications

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