
# Application Mechanism, Current Status, and Prospects of the Functional Additive *Bacillus cereus* in Aquaculture

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**Abstract**
With the rapid intensification and scaling up of aquaculture, issues such as water quality deterioration, pathogen proliferation, compromised immunity of aquatic animals, and frequent disease outbreaks caused by high-density farming have become increasingly prominent. Traditional antibiotic-based prevention and control approaches pose ecological and food safety risks, including drug residues, microbial imbalance, and excessive antibiotic resistance, making them inadequate for meeting the demands of green, antibiotic-free aquaculture. *Bacillus velezensis*, a novel functional probiotic characterized by strong stress resistance, excellent colonization ability, and comprehensive probiotic functions, achieves multiple benefits-including water quality regulation, disease prevention, and growth enhancement-through various mechanisms such as secreting antibacterial compounds, modulating aquatic microecology, improving intestinal structure, and boosting non-specific immunity. It serves as a core functional additive for antibiotic-free aquaculture. This article systematically elucidates the biological properties and probiotic mechanisms of *Bacillus velezensis*, focusing on its applications in purifying aquaculture water, promoting aquatic animal growth, enhancing immune resistance, and optimizing intestinal microbiota composition. It also identifies current technical challenges and limitations in strain application, industrial production, and practical use, while proposing targeted optimization strategies and future development directions to provide theoretical and technical support for the large-scale, standardized application of *Bacillus velezensis* in sustainable aquaculture.

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**Keywords:** Functional additives, *Bacillus cereus*, Aquaculture, Microbial ecological regulation, Antibiotic-free farming

## 1. Introduction

Aquaculture serves as the cornerstone of China's modern agricultural sector. In recent years, China has consistently ranked first globally in aquaculture production, with intensive and industrialized farming models becoming the industry standard. However, challenges such as high-density farming practices, accumulation of feed residues and feces, and external water pollution have led to excessive levels of ammonia nitrogen and nitrite in aquaculture waters, rampant proliferation of pathogenic bacteria, intensified stress responses in aquatic organisms, diminished disease resistance, and frequent outbreaks of bacterial diseases-all of which severely hinder the improvement of aquaculture quality, efficiency, and sustainable development. For decades, the reliance on antibiotics and chemical disinfectants for disease control has temporarily reduced incidence rates but readily induces antibiotic resistance in pathogens, resulting in drug residues in water bodies and aquatic products, as well as disruption of the aquatic microecosystem. These practices violate food safety and ecological protection requirements. Consequently, the development and application of green, safe, and residue-free functional aquaculture additives have emerged as the pivotal breakthrough for the industry's transformation. As a novel green functional feed and aquatic additive, probiotics have emerged as a core alternative to antibiotics due to their advantages of being non-toxic, residue-free, environmentally friendly, and multifunctional. *Bacillus beilaiensis*, a Gram-positive beneficial strain within the Bacillus genus, exhibits unique biological advantages over conventional aquatic probiotics such as lactic acid bacteria and yeast, including resistance to extreme temperatures and pH conditions, high stress tolerance, superior survival capacity, high colonization efficiency, and the ability to form endogenous spores, enabling stable probiotic effects in complex aquaculture environments. Additionally, this strain secretes various antimicrobial metabolites such as lipopeptides and polyketides, demonstrating significant antagonistic activity against common pathogenic bacteria in aquaculture. It offers multiple benefits including water purification, growth promotion, immune enhancement, and microbial community regulation, and has been widely applied in the production of fish, shrimp, crabs, and other aquatic species. This article provides a comprehensive review of the probiotic mechanisms of *Bacillus beilaiensis*, its practical applications in aquaculture, analysis of existing implementation challenges, and prospects for future development, offering insights for its industrial promotion and precision-based application.

## 2. Biological Characteristics of Bacillus cereus

*Bacillus velezensis* is a Gram-positive aerobic bacterium belonging to the Bacillus genus, first named in 2005. It is widely distributed in natural environments such as water bodies, sediment, and the intestines of animals and plants, making it an excellent indigenous beneficial strain suitable for aquaculture ecosystems. The bacterial cells exhibit a regular short rod-shaped morphology, measuring 0.5–1.0 μm × 1.2–3.5 μm, and produce mesophilic dormant spores with a dense and highly stable structure, which serves as the core structural foundation for its adaptation to complex aquaculture environments. Compared to conventional aquatic probiotics such as lactic acid bacteria and yeast, this strain demonstrates outstanding biological advantages, including rapid growth rates, exceptional environmental stress resistance, superior colonization capabilities, and diverse metabolic functions. In terms of growth characteristics, *Bacillus cereus* exhibits an exceptionally broad environmental adaptability, with an optimal growth temperature ranging from 42–45° C. It tolerates extreme conditions such as temperatures as high as 55° C or low temperatures, aquatic hypoxia, and fluctuations in pH levels. The ideal growth pH range is 5.0–7.0, and it thrives in salinity environments of 1.0% to 6.0%, making it suitable for various aquaculture settings including freshwater, seawater, and high-salinity ponds. The strain follows a well-defined growth cycle: the initial 0-2 hours represent a lag phase; followed by a rapid logarithmic growth phase lasting 2-12 hours; and a stabilization phase thereafter. The microbial population concentration peaks around 22 hours, enabling efficient establishment of dominant bacterial communities in both aquatic environments and intestinal systems, thereby rapidly delivering probiotic benefits.In terms of stress resistance and survival characteristics, dormant spores can survive long-term away from nutrient substrates, tolerating harsh conditions such as high temperature, high humidity, desiccation, and mechanical processing. The survival rates of aquatic probiotic preparations during storage, transportation, and post-introduction into water systems are significantly higher than those of other probiotics, with minimal risk of inactivation. These strains exhibit outstanding colonization capabilities, adhering stably to aquaculture sediment, suspended particulates, and the intestinal mucosal surfaces of aquatic animals, maintaining microbial dominance over extended periods without being easily displaced by existing microbial communities. Additionally, the strains demonstrate exceptional biosafety-being non-pathogenic, non-endotoxin-producing, and free of antibiotic resistance genes. They exhibit excellent environmental compatibility, do not disrupt the microbial balance in aquaculture systems or intestinal microecologies, and fully comply with safety standards for green aquaculture additives. In terms of metabolic characteristics, this strain exhibits stable metabolic activity and multifunctional capabilities. Its genome is enriched with multiple gene clusters encoding secondary metabolites, enabling sustained synthesis of antimicrobial compounds such as lipopeptides, polyketides, and peptides. Additionally, it efficiently secretes digestive enzymes including amylase, protease, and lipase, while also producing bioactive nutrients like amino acids, vitamins, and growth-promoting factors. This strain possesses comprehensive metabolic functions encompassing antibacterial and pathogen-inhibitory effects, water purification, growth enhancement, and immune regulation, making it a functional aquatic probiotic that integrates multiple beneficial properties.

## 3. Specific Application Effects of *Bacillus cereus* in Aquaculture

### 3.1. Water Quality Regulation and Improvement of Aquaculture Ecological Environment

Extensive aquaculture trials have demonstrated that regular application of *Bacillus cereus* preparations in fish and shrimp farming waters rapidly degrades toxic and harmful substances, stabilizing water quality parameters. Compared to traditional chemical bottom treatment products, this probiotic formulation offers sustained water purification effects without secondary pollution. Studies confirm that administering an appropriate amount of *Bacillus cereus* fermentation product in Pacific white shrimp farming systems significantly reduces ammonia nitrogen and nitrite concentrations, inhibits the accumulation of harmful substances in sediment, improves water transparency and dissolved oxygen levels, and effectively addresses challenges such as water turbidity, substrate degradation, and odor formation during the mid-to-late stages of high-density farming. Additionally, the strain remodels the microbial community structure, enhances microbial diversity and stability, suppresses harmful algal blooms, mitigates aquaculture diseases caused by algal imbalance, and continuously optimizes the microecological environment for farming.

### 3.2. Promote the growth of aquatic animals and reduce breeding costs

When used as a functional feed additive in feed formulations, *Bacillus Balesii* significantly enhances the production performance of aquatic animals. Relevant aquaculture trials demonstrated that adding 0.3‰-0.4‰ of *Bacillus Balesii* solid fermentation product to Pacific white shrimp feed, followed by continuous feeding for four weeks, markedly increased digestive enzyme activity, improved feed conversion efficiency, elevated weight gain and survival rates, and effectively reduced the feed conversion ratio. The application of this strain in freshwater fish, marine fish, and crab farming also exhibited excellent growth-promoting effects, effectively shortening cultivation cycles, improving product quality, reducing feed waste, lowering production costs, and enhancing overall economic returns.

### 3.3. Enhance disease resistance and reduce disease occurrence

The dual biological antagonistic and immunomodulatory effects of *Bacillus cereus* significantly reduce the incidence of bacterial diseases in aquatic organisms. For mainstream farmed species such as tilapia and shrimp, this strain exhibits potent antagonistic activity against prevalent pathogens including *Aeromonas hydrophila* and *Vibrio parahaemolyticus*, effectively preventing common diseases such as bacterial gill rot, enteritis, septicemia, and black fin disease. Additionally, by enhancing non-specific immune capacity and antioxidant levels, it strengthens the stress and infection resistance of aquatic animals, substantially lowering disease recurrence rates and reducing the frequency and dosage of antibiotic and disinfectant usage during farming operations. This contributes to achieving antibiotic-free, sustainable, and healthy aquaculture practices, ensuring the safety and quality of aquatic products.

### 3.4. Optimize intestinal structure to ensure bodily health

Long-term feeding of functional feed supplemented with *Bacillus cereus* can sustainably optimize the intestinal morphology and microbial community structure of aquatic animals. This strain significantly increases the height of intestinal villi, reduces crypt depth, thickens the intestinal mucosal muscular layer, and enhances intestinal barrier function, effectively preventing intestinal damage and inflammation. Additionally, it markedly enriches beneficial intestinal microbiota, inhibits the proliferation of harmful bacteria, and maintains intestinal microecological balance, thereby addressing common issues in intensive aquaculture such as anorexia, dyspepsia, intestinal congestion, and diarrhea. These effects fundamentally improve the health status of aquatic animals and reduce the incidence of suboptimal health conditions.

## 4. Current Issues in the Application Process

### 4.1. Inaccurate strain compatibility and application protocols

Currently, the application of *Bacillus cereus* in aquaculture predominantly follows a general application protocol, without differentiated application standards tailored to specific species, farming systems, growth stages, or water quality conditions. Significant variations exist among aquatic organisms in terms of intestinal structure, habitat requirements, and microbial community needs; strains exhibit differing tolerance levels and requirements during juvenile and adult stages; and water environments vary markedly between pond farming, industrial recirculating water systems, and elevated tank farming. The standardized dosages and application frequencies fail to maximize the probiotic benefits of these strains, often leading to either excessive application resulting in microbial overgrowth or insufficient application yielding suboptimal outcomes, thereby limiting their practical effectiveness.

### 4.2. The quality of industrialized products varies significantly

With the continuous expansion of market demand, a wide variety of *Bacillus* licheniformis-related products are available on the market, yet their quality varies significantly. Some manufacturers employ outdated production processes, resulting in low fermentation activity of the strains, insufficient spore formation rates, and elevated levels of contaminating bacteria. This leads to poor survival rates, weak colonization capabilities, and short efficacy durations after product application in water. Additionally, certain products exhibit issues such as inaccurate labeling of viable bacterial counts and poor stability, making them prone to inactivation under high-temperature and high-humidity storage conditions. Consequently, their field application performance is inconsistent, severely impacting the industrial promotion of these strains and the industry's reputation.

### 4.3. The research on the mechanism of action of bacterial strains is not sufficiently in-depth or systematic

Current research primarily focuses on verifying the field application efficacy of *Bacillus Balesii*, while studies on its underlying mechanisms remain insufficient. Existing investigations predominantly concentrate on superficial effects such as antibacterial activity, water purification, and growth promotion. However, fundamental mechanistic studies-including the specific target sites of bacterial metabolites, molecular pathways regulating aquatic animal immunity and metabolism, interaction mechanisms between strains and aquatic microbiota/host gut microbiota, as well as gene expression regulatory patterns under diverse environmental conditions-are still inadequate. This gap hinders the precise and intelligent application of these strains and the iterative upgrading of related products.

**4.4. The integrated application technology system is incomplete**

Currently, most aquaculture practices rely solely on the application of *Bacillus cereus* preparations, neglecting the importance of probiotic combination strategies and their synergistic interaction with farming management. The functionality of single bacterial strains is limited and struggles to adapt to the complex and dynamic aquaculture environment. Moreover, standardized protocols for the formulation ratios and synergistic mechanisms among *Bacillus cereus*, lactic acid bacteria, butyric acid bacteria, photosynthetic bacteria, and other beneficial strains remain underdeveloped. Additionally, there is insufficient integration between probiotic applications and supporting technologies such as water quality management, feeding practices, and disease prevention, resulting in underutilized potential for overall aquaculture efficiency enhancement.

## 5. Development Outlook

### 5.1. Conduct precise strain matching research and establish a standardized application system

Future efforts should focus on different aquaculture species, farming models, growth stages, and cultivation environments to conduct differentiated application trials of Bacillus licheniformis. This will clarify the optimal dosage, application frequency, and timing for various scenarios, establishing refined and standardized application protocols. Additionally, tailored application strategies should be developed for critical periods such as the larval stage, growth phase, disease peak periods, and water quality deterioration phases, ensuring precise alignment between bacterial strain functions and farming requirements. This approach maximizes the core roles of the strains in water quality regulation, growth promotion, and disease prevention.

### 5.2. Optimize industrial production processes to enhance product stability

Leveraging modern microbial fermentation technology, we have optimized the production processes for *Bacillus cereus* high-density fermentation, spore induction, and low-temperature drying, thereby enhancing the viable bacterial count, spore formation rate, and strain activity of the products. We have developed novel formulation products with excellent storage stability, strong stress resistance, and rapid activation upon water contact, addressing the limitations of traditional products such as poor stability, susceptibility to inactivation, and low colonization rates. Additionally, we have established comprehensive product quality testing standards and a traceability system to standardize industry practices, ensure consistent product quality in the market, and improve the reliability of industrial applications.

### 5.3. Deepen mechanism research to drive technological innovation and upgrading

By employing cutting-edge technologies such as multi-omics integrated analysis, molecular biology, and microbial ecology, this study provides an in-depth elucidation of the antibacterial molecular mechanisms, immune regulatory pathways, microbial community interaction mechanisms, and core principles of water purification associated with *Bacillus Balesii*. It identifies novel functional genes and active metabolites in the strain, optimizes strain performance through techniques such as strain mutagenesis and genetic modification, and cultivates highly active, highly colonizing, and multifunctional mutant strains. These advancements facilitate the transition of *Bacillus Berleii* application technologies from empirical practices to mechanistic, precise, and intelligent approaches.

### 5.4. Construction of a composite probiotic system to achieve synergistic effects

Conduct comprehensive research on the formulation of *Bacillus cereus* combined with various high-quality aquatic probiotics, including lactic acid bacteria, butyric acid bacteria, and photosynthetic bacteria. Optimize the strain ratios, elucidate the synergistic mechanisms among different strains, and develop multifunctional composite probiotic preparations to address the functional limitations of single-strain products. Simultaneously, establish an integrated green aquaculture technology system encompassing "probiotic regulation+ scientific feeding practices + water quality management + ecological maintenance," achieving deep integration of microecological control and farming management. This approach significantly enhances the green prevention and control standards in aquaculture, thereby promoting its high-quality and sustainable development.

## 6. Conclusion

*Bacillus cereus*, as an excellent functional aquatic probiotic additive, demonstrates superior performance through its strong stress resistance, high colonization rate, multifunctional properties, and safety without residual effects. It effectively addresses core challenges in intensive aquaculture-such as water quality deterioration, frequent disease outbreaks, and slow growth-via multiple mechanisms including biological antagonism, water purification, microbial community regulation, immune enhancement, and growth promotion. This bacterium serves as a pivotal functional product for replacing antibiotics and advancing antibiotic-free green aquaculture. However, current applications face limitations such as imprecise adaptation strategies, inconsistent product quality, insufficient in-depth mechanistic research, and incomplete integrated application systems. Future efforts should focus on leveraging biotechnological innovations to deepen mechanistic studies, optimize production processes, establish standardized application protocols, and develop comprehensive integrated application systems. These measures will continuously unlock the potential of *Bacillus cereus*, promoting its large-scale, standardized, and precision-based application in aquaculture, thereby providing critical technical support for the green transformation, quality improvement, efficiency enhancement, and water product quality assurance in China's aquaculture industry.

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