Novel Probiotic Bacillus velezensis PED-003 as an Effective Biological Control Agent against Fish Pathogenic Bacteria

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Abstract

The rapid expansion of aquaculture has been accompanied by an increased incidence of bacterial diseases, resulting in substantial economic losses and reduced productivity. The excessive use of antibiotics for disease control has contributed to the emergence of antimicrobial resistance, environmental contamination, and residual toxicity in aquatic products. Therefore, the identification of effective probiotic bacteria with antimicrobial properties has become an important strategy for sustainable aquaculture. The present study evaluated the antibacterial activity of Bacillus velezensis PED-003 against selected fish pathogenic bacteria, including Aeromonas hydrophila, Vibrio parahaemolyticus, Pseudomonas aeruginosa, Edwardsiella tarda, Streptococcus iniae, and Staphylococcus aureus. Antibacterial efficacy was assessed using agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays.

The results demonstrated that B. velezensis PED-003 exhibited broad-spectrum antibacterial activity against all tested pathogens. The highest inhibition zone was recorded against A. hydrophila (22.4 ± 0.5 mm), followed by S. aureus (20.3 ± 0.6 mm), V. parahaemolyticus (19.8 ± 0.4 mm), and S. iniae (18.7 ± 0.4 mm). Lower but significant inhibition was observed against P. aeruginosa (17.6 ± 0.3 mm) and E. tarda (15.9 ± 0.5 mm). MIC analysis revealed the strongest inhibitory activity against A. hydrophila and S. aureus, with complete growth inhibition at 1.25 mg/ml. Similarly, the lowest MBC values (2.50 mg/ml) were recorded for these pathogens, indicating potent bactericidal activity. The close relationship between MIC and MBC values suggested that the antimicrobial metabolites produced by B. velezensis PED-003 exerted both inhibitory and lethal effects on bacterial pathogens.

The strong antagonistic activity observed in this study may be attributed to the production of bioactive compounds such as bacteriocins, lipopeptides, antimicrobial peptides, and other extracellular metabolites. These findings demonstrate the potential of Bacillus velezensis PED-003 as an effective probiotic and biological

control agent for disease prevention in aquaculture. The application of this strain may reduce dependence on antibiotics and contribute to sustainable fish health management. Further investigations involving metabolite characterization and in vivo challenge studies are recommended to validate its commercial application in aquaculture systems.

Keywords: Bacillus velezensis PED-003, Probiotics, Sustainable aquaculture, Antimicrobial metabolites.

1. Introduction

Aquaculture has emerged as one of the fastest-growing food production sectors worldwide, contributing significantly to global food security, nutritional requirements, and economic development. The rapid expansion of aquaculture practices has been accompanied by an increased incidence of infectious diseases, particularly those caused by bacterial pathogens. Disease outbreaks caused by bacteria such as Aeromonas hydrophila, Vibrio parahaemolyticus, Edwardsiella tarda, Pseudomonas aeruginosa, and Streptococcus iniae have become major constraints to sustainable fish production, resulting in severe economic losses and reduced productivity. These pathogens are capable of causing mass mortalities, impaired growth, tissue damage, and compromised immune responses in cultured fish species, thereby threatening the sustainability of the aquaculture industry (Austin and Austin, 2016; Defoirdt et al., 2011).

Traditionally, antibiotics have been extensively used to control bacterial infections in aquaculture systems. Although antibiotics are effective in reducing disease outbreaks, their indiscriminate and prolonged use has led to the emergence of antibiotic-resistant bacterial strains, accumulation of drug residues in aquatic products, environmental contamination, and disruption of beneficial microbial communities. The increasing prevalence of antimicrobial resistance has become a major public health concern, prompting researchers and regulatory agencies to seek environmentally friendly and sustainable alternatives to conventional antibiotic therapies (Cabello et al., 2013; Romero et al., 2012). Consequently, the application of probiotics has gained considerable attention as an effective strategy for disease prevention and health management in aquaculture.

Probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts. In aquaculture, probiotic bacteria improve water quality, enhance nutrient utilization, stimulate immune responses, and inhibit the growth of pathogenic microorganisms through competitive exclusion and the production of antimicrobial substances. Among the various probiotic genera, Bacillus species have received particular interest because of their ability to form endospores, survive under adverse environmental conditions, and produce a wide range of bioactive compounds with antimicrobial properties (Ringø et al., 2018). Their stability during feed processing and storage further enhances their suitability for aquaculture applications.

Among the members of the genus Bacillus, Bacillus velezensis has recently emerged as a promising probiotic and biological control agent. This species is well known for its ability to synthesize a diverse array of antimicrobial metabolites, including bacteriocins, lipopeptides,

polyketides, surfactins, fengycins, and iturins. These compounds exhibit broad-spectrum antibacterial activity against numerous Gram-positive and Gram-negative pathogens by disrupting cell membrane integrity, inhibiting essential metabolic pathways, and interfering with pathogen colonization. Furthermore, B. velezensis has been reported to enhance host immunity, improve gut microbial balance, and promote growth performance in various aquatic organisms (Rabbee et al., 2019; Cao et al., 2018).

The antagonistic activity of probiotic bacteria against fish pathogens is one of the most important criteria for evaluating their potential application in disease control. Antimicrobial metabolites secreted by probiotic microorganisms can suppress pathogenic bacteria through multiple mechanisms, including competition for nutrients and adhesion sites, production of organic acids, secretion of extracellular enzymes, and synthesis of potent antimicrobial peptides. Such antagonistic interactions help maintain microbial homeostasis within the aquatic environment and reduce the incidence of infectious diseases. Therefore, screening and characterization of probiotic isolates with strong antibacterial activity are essential for developing effective biological alternatives to antibiotics in aquaculture systems (Hai, 2015).

Evaluation of antibacterial activity using agar well diffusion assays provides preliminary evidence of the inhibitory potential of probiotic metabolites against target pathogens. However, quantitative determination of antimicrobial efficacy requires the assessment of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC represents the lowest concentration of an antimicrobial agent capable of preventing visible bacterial growth, whereas MBC indicates the minimum concentration required to kill bacterial cells completely. Together, these parameters provide comprehensive information regarding the inhibitory and bactericidal properties of probiotic metabolites and facilitate comparison among different bacterial pathogens (Wiegand et al., 2008).

Several studies have demonstrated the effectiveness of Bacillus probiotics in controlling bacterial diseases in aquaculture. The production of antimicrobial compounds by Bacillus species has been associated with significant reductions in pathogen load and improved survival of cultured fish and shellfish. Moreover, the use of probiotic bacteria has been shown to reduce dependence on antibiotics while promoting sustainable aquaculture practices. Nevertheless, the antibacterial efficacy of newly isolated strains must be thoroughly investigated before their application as commercial probiotics or biological control agents.

In this context, the present study was undertaken to evaluate the antibacterial potential of Bacillus velezensis PED-003 against selected fish pathogenic bacteria. The study assessed the antagonistic activity of the isolate through agar well diffusion assays and further determined its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against major fish pathogens. The findings of this investigation are expected to provide valuable insights into the potential application of Bacillus velezensis PED-003 as a probiotic and eco-friendly biological control agent for disease prevention and sustainable health management in aquaculture.

2.Materials and Methods

2. 1 Preparation of Bacillus velezensis PED-003 Cell-Free Supernatant

A pure culture of Bacillus velezensis PED-003 was inoculated into sterile nutrient broth and incubated at 30 ± 2°C for 24–48 h under shaking conditions (150 rpm). Following incubation, the culture was centrifuged at 10,000 rpm for 15 min at 4°C to remove bacterial cells. The resulting supernatant was filtered through a 0.22 µm membrane filter to obtain a sterile cell-free supernatant (CFS), which was subsequently used for antibacterial assays, including agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) studies.

2. 2 Agar Well Diffusion Assay

The antibacterial activity of Bacillus velezensis PED-003 was evaluated against selected fish pathogenic bacteria, namely Aeromonas hydrophila, Vibrio parahaemolyticus, Pseudomonas aeruginosa, Edwardsiella tarda, Streptococcus iniae, and Staphylococcus aureus, using the agar well diffusion method. Fresh cultures of each pathogen were adjusted to approximately 1 × 10^8 CFU/ml and uniformly spread onto Mueller–Hinton agar plates using sterile cotton swabs. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Subsequently, 100 µl of the sterile cell-free supernatant was dispensed into each well. Plates were incubated at 37°C for 24 h, and the antibacterial activity was assessed by measuring the diameter of the clear inhibition zones surrounding each well. All experiments were performed in triplicate, and the results were expressed as mean inhibition zone diameter (mm) ± standard deviation.

2. 3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the antimicrobial metabolites produced by Bacillus velezensis PED-003 was determined using the broth microdilution method. Serial two-fold dilutions of the cell-free supernatant were prepared in sterile Mueller–Hinton broth to obtain different concentrations. Each dilution was inoculated with an equal volume of standardized bacterial suspension (approximately 10^6 CFU/ml). Positive and negative controls were included in each experiment. The inoculated tubes were incubated at 37°C for 24 h. Following incubation, bacterial growth was assessed by observing turbidity. The lowest concentration that completely inhibited visible bacterial growth was recorded as the MIC value. All assays were conducted in triplicate to ensure reproducibility.

2. 4 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined following the MIC assay. Aliquots (100 µl) from tubes showing no visible bacterial growth were aseptically streaked onto fresh nutrient agar plates and incubated at 37°C for 24 h. After incubation, the plates were examined for bacterial colony formation. The lowest concentration of the antimicrobial metabolite that completely prevented the growth of bacterial colonies was recorded as the MBC value. The experiment was performed in triplicate, and the mean values were calculated. The MBC assay enabled differentiation between bacteriostatic and bactericidal effects of the probiotic metabolites.

2. 5 Comparative Evaluation of Antimicrobial Efficacy

The antimicrobial efficacy of Bacillus velezensis PED-003 was comparatively assessed using inhibition zone measurements obtained from agar well diffusion and disc diffusion assays together with MIC and MBC values. The susceptibility of each fish pathogen was categorized based on the magnitude of inhibition zones and the concentration required for complete inhibition and bacterial killing. Lower MIC and MBC values coupled with larger inhibition zones were considered indicative of higher antimicrobial potency. This comprehensive evaluation provided a reliable assessment of the probiotic strain's antagonistic activity against major fish pathogenic bacteria and its potential application as a biological control agent in aquaculture.

Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software (Version 25.0). Differences among treatments were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Antibacterial Activity of Bacillus velezensis PED-003 Against Fish Pathogenic Bacteria

The antibacterial potential of Bacillus velezensis PED-003 was assessed against six important fish pathogenic bacteria using the agar well diffusion method. The results revealed that the probiotic isolate exhibited broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens (Table 1). Distinct zones of inhibition were observed around the wells containing the cell-free supernatant, indicating the production of extracellular antimicrobial metabolites capable of suppressing pathogen growth.

Among the tested pathogens, Aeromonas hydrophila showed the highest susceptibility, exhibiting an inhibition zone of 22.4 ± 0.5 mm. This was followed by Staphylococcus aureus (20.3 ± 0.6 mm), Vibrio parahaemolyticus (19.8 ± 0.4 mm), and Streptococcus iniae (18.7 ± 0.4 mm), all of which demonstrated strong sensitivity to the probiotic metabolites. Comparatively lower inhibition zones were recorded against Pseudomonas aeruginosa (17.6 ± 0.3 mm) and Edwardsiella tarda (15.9 ± 0.5 mm), although the observed activity remained significant. The formation of clear inhibition zones confirmed the antagonistic nature of B. velezensis PED-003 and its ability to inhibit a wide range of fish pathogenic bacteria.

Table 1. Antibacterial Activity of Bacillus velezensis PED-003 Against Fish Pathogenic Bacteria by Agar Well Diffusion Method

S. No	Test Pathogen	Gram Reaction	Source of Isolation	Zone of Inhibition (mm)	Antibacterial Activity
1	Aeromonas	Gram-	Diseased	22.4 ± 0.5	Strong

	hydrophila	negative	freshwater fish		
2	Vibrio parahaemolyticus	Gram-negative	Diseased marine fish	19.8 ± 0.4	Strong
3	Pseudomonas aeruginosa	Gram-negative	Fish skin lesions	17.6 ± 0.3	Moderate
4	Edwardsiella tarda	Gram-negative	Fish intestine	15.9 ± 0.5	Moderate
5	Streptococcus iniae	Gram-positive	Diseased fish tissue	18.7 ± 0.4	Strong
6	Staphylococcus aureus	Gram-positive	Clinical reference strain	20.3 ± 0.6	Strong

3.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration assay was conducted to determine the lowest concentration of antimicrobial metabolites required to inhibit visible bacterial growth. The results demonstrated considerable variation in pathogen susceptibility (Table 2). The lowest MIC value (1.25 mg/ml) was observed against A. hydrophila and S. aureus, indicating high sensitivity to the metabolites produced by B. velezensis PED-003.

Moderate MIC values of 2.50 mg/ml were recorded for V. parahaemolyticus and S. iniae, while P. aeruginosa and E. tarda required a higher concentration of 5.00 mg/ml for complete growth inhibition. The absence of turbidity in the broth cultures at MIC concentrations confirmed effective suppression of bacterial proliferation. These findings indicate that the probiotic metabolites possess strong inhibitory activity against major aquaculture pathogens.

Table 2. Minimum Inhibitory Concentration (MIC) of Bacillus velezensis PED-003 Against Fish Pathogenic Bacteria

S. No	Test Pathogen	MIC (mg/ml)	Growth Inhibition	Antimicrobial Activity
1	Aeromonas hydrophila	1.25	Complete inhibition	Highly susceptible
2	Vibrio parahaemolyticus	2.50	Complete inhibition	Susceptible
3	Pseudomonas aeruginosa	5.00	Complete inhibition	Moderately susceptible
4	Edwardsiella tarda	5.00	Complete inhibition	Moderately susceptible
5	Streptococcus	2.50	Complete	Susceptible

	iniae		inhibition	
6	Staphylococcus aureus	1.25	Complete inhibition	Highly susceptible

3.3 Minimum Bactericidal Concentration (MBC)

The bactericidal efficacy of B. velezensis PED-003 was further evaluated through MBC analysis. The results revealed that complete bacterial elimination occurred at concentrations ranging from 2.50 to 10.00 mg/ml (Table 3). The lowest MBC values were observed for A. hydrophila and S. aureus (2.50 mg/ml), indicating strong bactericidal activity against these pathogens.

Similarly, complete killing of V. parahaemolyticus and S. iniae was achieved at 5.00 mg/ml, whereas P. aeruginosa and E. tarda required a higher concentration of 10.00 mg/ml. No bacterial colonies were observed on nutrient agar plates inoculated from MBC tubes, confirming the lethal action of the antimicrobial metabolites. The close relationship between MIC and MBC values suggests that the metabolites produced by B. velezensis PED-003 exert predominantly bactericidal effects rather than merely inhibiting bacterial growth.

Table 3. Minimum Bactericidal Concentration (MBC) of Bacillus velezensis PED-003 Against Fish Pathogenic Bacteria

S. No	Test Pathogen	MIC (mg/ml)	MBC (mg/ml)	Bactericidal Effect
1	Aeromonas hydrophila	1.25	2.50	Strongly bactericidal
2	Vibrio parahaemolyticus	2.50	5.00	Bactericidal
3	Pseudomonas aeruginosa	5.00	10.00	Moderately bactericidal
4	Edwardsiella tarda	5.00	10.00	Moderately bactericidal
5	Streptococcus iniae	2.50	5.00	Bactericidal
6	Staphylococcus aureus	1.25	2.50	Strongly bactericidal

3.4 Comparative Antimicrobial Efficacy

A comparative evaluation of antimicrobial efficacy based on inhibition zone diameter, MIC, and MBC values demonstrated consistent susceptibility patterns among the tested pathogens (Table 4). Aeromonas hydrophila exhibited the highest susceptibility, characterized by the largest inhibition zones and the lowest MIC and MBC values. Likewise, S. aureus showed

high sensitivity to the probiotic metabolites. In contrast, P. aeruginosa and E. tarda displayed comparatively lower susceptibility, as reflected by smaller inhibition zones and higher MIC and MBC values.

A strong inverse relationship was observed between inhibition zone diameter and MIC/MBC values, indicating that pathogens with larger inhibition zones required lower concentrations of antimicrobial metabolites for growth inhibition and bacterial killing. This relationship confirms the reliability of the antibacterial assays and highlights the effectiveness of B. velezensis PED-003 against diverse bacterial pathogens.

Overall, the results clearly demonstrate that Bacillus velezensis PED-003 possesses potent antibacterial and bactericidal activities against major fish pathogenic bacteria. The strongest activity was observed against Aeromonas hydrophila and Staphylococcus aureus, while effective inhibition was also achieved against Vibrio parahaemolyticus, Streptococcus iniae, Pseudomonas aeruginosa, and Edwardsiella tarda. These findings suggest that B. velezensis PED-003 has considerable potential as a probiotic and biological control agent for disease prevention and sustainable health management in aquaculture systems.

Table 4. Comparative Antimicrobial Efficacy of Bacillus velezensis PED-003 Against Fish Pathogenic Bacteria

S. No	Test Pathogen	Agar Well Diffusion Zone (mm)	Disc Diffusion Zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Susceptibility Level
1	Aeromonas hydrophila	22.4 ± 0.5	24.6 ± 0.7	1.25	2.50	Highly Susceptible
2	Vibrio parahaemolyticus	19.8 ± 0.4	21.8 ± 0.5	2.50	5.00	Susceptible
3	Pseudomonas aeruginosa	17.6 ± 0.3	19.2 ± 0.4	5.00	10.00	Moderately Susceptible
4	Edwardsiella tarda	15.9 ± 0.5	17.5 ± 0.6	5.00	10.00	Moderately Susceptible
5	Streptococcus iniae	18.7 ± 0.4	20.4 ± 0.5	2.50	5.00	Susceptible
6	Staphylococcus aureus	20.3 ± 0.6	23.1 ± 0.8	1.25	2.50	Highly Susceptible

4. Discussion

The increasing occurrence of bacterial diseases in aquaculture has created an urgent need for sustainable alternatives to antibiotics. In the present study, Bacillus velezensis PED-003

demonstrated remarkable antibacterial activity against several important fish pathogenic bacteria, including Aeromonas hydrophila, Vibrio parahaemolyticus, Pseudomonas aeruginosa, Edwardsiella tarda, Streptococcus iniae, and Staphylococcus aureus. The observed broad-spectrum antagonistic activity suggests that this probiotic isolate produces potent extracellular antimicrobial compounds capable of suppressing the growth of both Gram-positive and Gram-negative bacteria. These findings are consistent with previous reports indicating that Bacillus species are effective biological control agents due to their ability to synthesize diverse antimicrobial metabolites such as bacteriocins, lipopeptides, cyclic peptides, and organic acids (Rabbee et al., 2019; Caulier et al., 2019).

The agar well diffusion assay revealed that A. hydrophila was the most susceptible pathogen, exhibiting the largest inhibition zone among all tested bacteria. Aeromonas hydrophila is considered one of the most destructive bacterial pathogens in freshwater aquaculture and is responsible for motile aeromonad septicemia, causing significant economic losses worldwide (Austin and Austin, 2016). The strong inhibitory activity observed against this pathogen suggests that B. velezensis PED-003 may serve as an effective biological control agent in preventing aeromoniasis outbreaks. Similar findings were reported by Zokaeifar et al. (2014), who demonstrated that probiotic Bacillus strains effectively inhibited Aeromonas species through the production of antimicrobial substances and competitive exclusion mechanisms.

The substantial inhibition observed against Vibrio parahaemolyticus is also of considerable importance. Species belonging to the genus Vibrio are among the most problematic pathogens in marine aquaculture systems, causing vibriosis in fish, shrimp, and other aquatic organisms. The ability of B. velezensis PED-003 to inhibit V. parahaemolyticus suggests that the isolate produces metabolites capable of interfering with the growth and survival of Vibrio species. Previous studies have demonstrated that lipopeptides such as surfactin, fengycin, and iturin produced by Bacillus species possess strong anti-Vibrio activity and can significantly reduce disease incidence in cultured aquatic animals (Kuebutornye et al., 2019; Pérez-Sánchez et al., 2014).

The inhibition of Streptococcus iniae and Staphylococcus aureus observed in the present study further highlights the broad-spectrum antimicrobial properties of B. velezensis PED-003. Gram-positive bacteria are generally more susceptible to antimicrobial peptides because of differences in cell wall composition and membrane structure. The large inhibition zones and low MIC values observed against S. aureus indicate that the probiotic metabolites can effectively penetrate and disrupt bacterial cell membranes. Similar observations have been reported for several Bacillus-derived bacteriocins and lipopeptides, which exhibit strong activity against Gram-positive pathogens through membrane permeabilization and intracellular leakage mechanisms (Abriouel et al., 2011).

Although Pseudomonas aeruginosa and Edwardsiella tarda exhibited comparatively smaller inhibition zones and higher MIC and MBC values, the isolate was still able to suppress their growth effectively. These pathogens are known for their intrinsic resistance to many antimicrobial compounds due to the presence of efficient efflux pumps, biofilm-forming ability, and low membrane permeability (Breijyeh et al., 2020). The ability of B. velezensis PED-003 to inhibit these relatively resistant bacteria indicates the presence of potent

bioactive metabolites with multiple modes of action. Such broad-spectrum efficacy is a desirable characteristic for probiotics intended for aquaculture disease management.

The MIC results provided quantitative evidence regarding the antimicrobial potency of B. velezensis PED-003. The lowest MIC values recorded against A. hydrophila and S. aureus indicate high susceptibility of these pathogens to the probiotic metabolites. Lower MIC values generally reflect stronger antimicrobial activity because smaller concentrations are sufficient to inhibit bacterial growth. Similar low MIC values have been reported for antimicrobial compounds produced by Bacillus velezensis and related species against aquaculture pathogens (Chen et al., 2009). The effectiveness of the isolate at relatively low concentrations enhances its practical applicability as a probiotic supplement and biological control agent.

The MBC assay further confirmed the bactericidal nature of the antimicrobial compounds produced by B. velezensis PED-003. The close relationship between MIC and MBC values observed in this study suggests that the antimicrobial metabolites not only inhibit bacterial growth but also induce bacterial cell death. Such bactericidal activity is particularly advantageous in aquaculture because it reduces pathogen persistence in the culture environment and minimizes the possibility of recurrent infections. The bactericidal effects may result from membrane disruption, inhibition of protein synthesis, interference with DNA replication, and oxidative damage induced by antimicrobial metabolites (Ongena and Jacques, 2008).

The antimicrobial efficacy demonstrated by B. velezensis PED-003 may largely be attributed to its ability to synthesize a wide range of secondary metabolites. Members of the B. velezensis group are known producers of surfactins, fengycins, bacillomycins, difficidins, macrolactins, and bacilysin, all of which possess strong antibacterial activity against numerous pathogens (Caulier et al., 2019). These compounds can disrupt bacterial membranes, inhibit cell wall biosynthesis, and suppress essential metabolic pathways. In addition, probiotic bacteria may compete with pathogens for nutrients and colonization sites, thereby preventing pathogen establishment within the host and culture environment.

The findings of the present study support the growing body of evidence indicating that Bacillus probiotics can serve as sustainable alternatives to antibiotics in aquaculture. The use of probiotics offers several advantages, including reduced antimicrobial resistance, improved host immunity, enhanced water quality, and environmentally friendly disease management (Hai, 2015). The strong antibacterial and bactericidal activities exhibited by B. velezensis PED-003 against major fish pathogens suggest that this strain has considerable potential for application as a feed additive or water probiotic in aquaculture systems.

Overall, the results clearly demonstrate that Bacillus velezensis PED-003 possesses significant antagonistic activity against a broad spectrum of fish pathogenic bacteria. The combination of large inhibition zones, low MIC values, and effective bactericidal activity confirms its potential as a probiotic and biological control agent. Future studies should focus on purification and characterization of the antimicrobial metabolites, evaluation of immune-

stimulatory effects, and in vivo challenge experiments to further validate the efficacy of this promising probiotic strain under commercial aquaculture conditions.

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