

Role of *Bacillus* Probiotics in Improving *Nile Tilapia* Health, Water Quality, and Lettuce Growth in Recirculating Aquaponic Systems

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Abstract: Aquaponics is an integrated food-production system that combines aquaculture with hydroponic plant cultivation through water recirculation. In this system, nutrients originating from fish production can be transformed by microbial processes and subsequently utilized by plants, while plant uptake contributes to nutrient removal from the circulating water [1,2]. *Nile Tilapia* (*Oreochromis niloticus*) is widely used in aquaponic production because of its suitability for intensive culture and integration with vegetable crops [1]. Microorganisms are essential to the functioning of recirculating aquaponic systems because microbial communities participate in nutrient transformation and occur across different system compartments, including fish-associated environments, water, biofilters, and plant-root zone [2,3]. Probiotic microorganisms, particularly *Bacillus* spp., have received considerable attention in aquaculture because selected strains can influence gut microbial communities, digestive processes, immune responses, and resistance to certain bacterial infections [4,5]. The potential application of *Bacillus* probiotics in aquaponics is particularly relevant because their effects may extend beyond fish health to interactions with environmental and plant-associated microbial communities. Studies in *Nile Tilapia* have reported beneficial responses following *Bacillus* supplementation, although outcomes vary according to strain, dose, administration route, and environmental conditions [4–6]. Selected *Bacillus* strains have also been investigated for their interactions with plant roots and their potential contribution to plant growth [7]. However, evidence directly connecting *Bacillus* supplementation with simultaneous improvements in *Nile Tilapia* health, water-quality microbiology, and lettuce growth in recirculating aquaponic systems remains limited [8]. In addition, the shared water pathway creates important microbiological food-safety considerations because microorganisms associated with fish and water may potentially reach edible plant tissues [8].

This review evaluates the role of *Bacillus* probiotics in recirculating aquaponic systems, focusing on *Nile Tilapia* health, water-quality microbiology, lettuce growth, fish–microbe–plant interactions, food-safety considerations, current challenges, and future research opportunities.

Keywords: *Bacillus*; probiotics; *Nile Tilapia*; *Oreochromis niloticus*; aquaponics; recirculating aquaponic systems; water microbiology; lettuce; fish health; plant–microbe interactions.

1. Introduction

Aquaponics integrates fish production with soilless plant cultivation by recirculating water between aquaculture and plant-production units. The system enables nutrients generated during fish culture to be processed and reused for plant production, creating a biological connection between fish, microorganisms, water, and plants [1,2].

Fish feed represents a major nutrient input into an aquaponic system. Nutrients that are not incorporated into fish biomass enter the system through metabolic excretion and solid wastes. Microbial activity then contributes to the transformation of organic and nitrogen-containing compounds, facilitating nutrient availability for plants and supporting water-quality management [2,3].

Nile Tilapia (*Oreochromis niloticus*) is an important species in aquaponic research and production. Its adaptability to culture conditions and its compatibility with vegetable production have made tilapia a frequently studied fish in integrated aquaponic systems [1,8]. Lettuce (*Lactuca sativa*) is also commonly investigated as an aquaponic crop because it can be cultivated using nutrient-containing recirculating water and has a relatively short production cycle [8].

The microbiology of an aquaponic system is complex because microbial communities occur in multiple interconnected compartments. Fish-associated microbiota, circulating water, biofilters, system surfaces, and plant-root environments can contain distinct microbial communities that contribute to nutrient transformation and other biological processes [3]. Consequently, changes in microbial communities in one compartment may influence processes occurring elsewhere in the recirculating system.

Probiotics have been investigated as biological tools for improving aquaculture production and reducing dependence on antimicrobial treatments. Among the microorganisms studied as probiotics, *Bacillus* spp. is particularly important because selected strains can survive under different environmental conditions and may produce enzymes, antimicrobial substances, and other bioactive compounds [4,5].

In *Nile Tilapia*, dietary supplementation with selected *Bacillus* strains has been associated with changes in growth performance, feed utilization, gut microbial composition, immune responses, and resistance to selected pathogens [4–6]. The mechanisms proposed for these effects include modification of intestinal microbial communities, competition with undesirable microorganisms, enhancement of digestive activity, and stimulation of host immune responses [4,5]. However, probiotic effects are strain-specific and can vary according to bacterial concentration, delivery method, fish developmental stage, diet, and environmental conditions [4–6].

The potential importance of *Bacillus* in aquaponics extends beyond the fish gastrointestinal tract. Some *Bacillus* strains can interact with plant roots and influence root-associated microbial communities and plant development [7]. This raises the possibility that probiotic application could affect several components of an integrated aquaponic system rather than producing effects only in fish.

The fish–microbe–plant relationship is therefore central to understanding probiotic activity in aquaponics. Fish release nutrients and organic materials, microorganisms transform these compounds, and plants remove and utilize a proportion of the resulting nutrients [2,3]. A microbial intervention such as *Bacillus* supplementation may consequently influence different stages of this interconnected pathway.

Food safety is another important consideration when probiotics are applied in fish–vegetable aquaponic systems. Continuous water circulation creates a potential pathway through which microorganisms associated with fish, faecal material, or the aquatic environment may reach plant-production areas. A recent systematic review identified Enterobacteriaceae, coliforms, and Shiga toxin-producing *Escherichia coli* among

microorganisms associated with microbiological contamination of lettuce in tilapia-based aquaponic systems [8].

Despite the growing literature on *Bacillus* probiotics in aquaculture and on plant-associated *Bacillus*, relatively limited research has examined their effects across the complete *Nile Tilapia*–water–lettuce system. The available evidence also indicates that the effectiveness of *Bacillus* in controlling foodborne microorganisms in aquaponic systems requires further investigation [8].

Therefore, this review focuses on the role of *Bacillus* probiotics in improving *Nile Tilapia* health, influencing water-quality microbiology, and supporting lettuce growth in recirculating aquaponic systems. It also examines the interactions among fish, microorganisms, and plants, evaluates food-safety considerations, and identifies major research gaps and future directions for developing reliable probiotic strategies in aquaponic production.

2. Recirculating Aquaponic Systems

Recirculating aquaponic systems integrate fish production with hydroponic plant cultivation by continuously circulating water between the fish-rearing and plant-production components. This configuration allows nutrients originating from aquaculture to be recovered and utilized for plant production while supporting repeated use of the circulating water [1,2].

A typical recirculating aquaponic system contains a fish-culture unit, solids-removal components, biological filtration, plant-growing units, water pumps, and aeration equipment. These components operate together to maintain suitable conditions for fish, microorganisms, and plants [2,3]. The major components and nutrient-flow relationships within a recirculating aquaponic system are illustrated in Figure 1.

Figure 1. Schematic representation of a recirculating aquaponic system showing *Nile tilapia* culture, microbial biofiltration, lettuce production, nutrient transformation, and water recirculation.

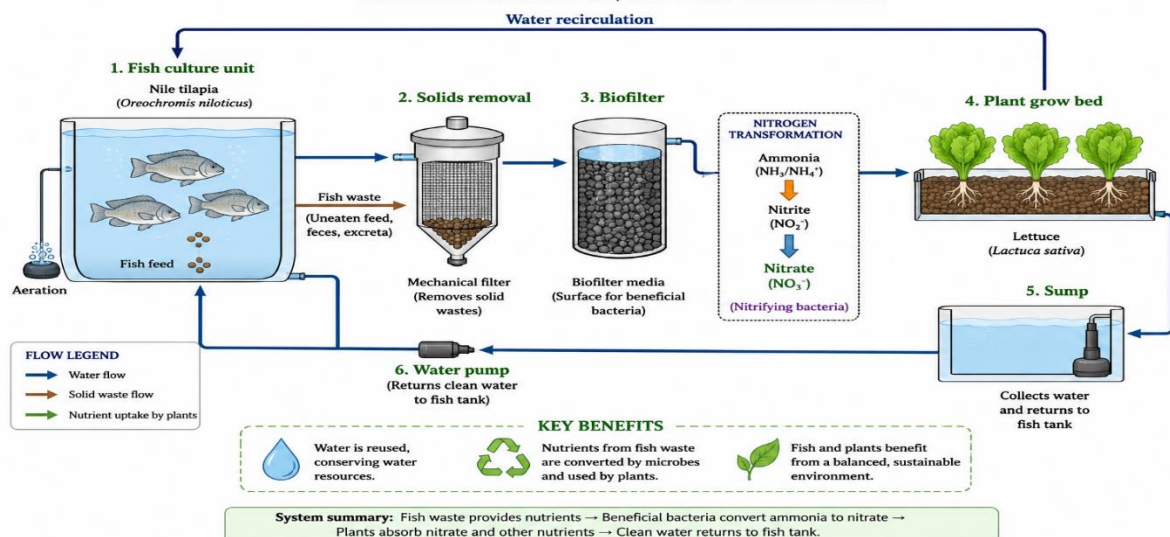


Figure 1. Schematic representation of a recirculating aquaponic system showing *Nile Tilapia* culture, microbial biofiltration, lettuce production, nutrient transformation, and water recirculation.

As illustrated in Figure 1, water from the *Nile Tilapia* culture unit passes through solids-removal and biological filtration stages before reaching the lettuce-growing unit. Fish-derived wastes provide organic and nitrogenous substrates for microbial transformation, while nitrifying microorganisms contribute to the conversion of ammonia to nitrite and nitrate. The nutrient-containing water can then reach the plant-growing unit, where lettuce takes up available nutrients. Treated water is subsequently collected and returned to the fish-culture unit, maintaining the recirculating nature of the system [2–4].

Fish feed represents a major nutrient input into aquaponic systems. A proportion of the nutrients supplied through feed is incorporated into fish biomass, whereas other materials enter the water through metabolic excretion, faecal material, and uneaten feed. These materials contribute organic matter and nitrogenous compounds that undergo microbial transformation within the system [2,3].

Biological filtration is particularly important for nitrogen cycling. Ammonia released from fish metabolism is transformed by nitrifying microorganisms, first into nitrite and subsequently into nitrate. Nitrate can then be taken up by plants and used as a nitrogen source for growth [3,4]. The efficiency of this process depends on environmental conditions such as pH, temperature, and dissolved oxygen [3].

The microbial community of a recirculating aquaponic system is not restricted to the biofilter. Microorganisms can occur in the fish gastrointestinal tract, culture water, biofilter, system surfaces, and plant-root environment. These microbial communities contribute to nutrient transformation and interact with fish and plants within the integrated system [3,5].

Plant roots provide an additional microbial habitat. Root-associated microorganisms can participate in nutrient transformations and interact with plants, potentially influencing nutrient availability and plant development [5,6]. Therefore, the plant-growing unit represents an important biological interface between circulating water, microorganisms, and plant tissues.

System performance is influenced by several environmental and management factors, including temperature, pH, dissolved oxygen, nutrient concentration, stocking density, feeding rate, and microbial activity [2,3]. Changes in these factors can affect fish health, microbial processes, nutrient availability, and plant growth. Maintaining appropriate environmental conditions is therefore essential for stable operation of the integrated system.

Lettuce is frequently investigated in aquaponic production because it can be cultivated under hydroponic conditions and can utilize nutrients present in aquaculture-derived water [7]. Studies involving *Nile Tilapia* and lettuce have demonstrated that nutrient availability and system configuration can influence both fish and plant performance [7].

The interconnected nature of recirculating aquaponics means that changes in one component can influence the others. For example, changes in fish feeding or stocking density can modify nutrient loading and consequently affect microbial activity and plant nutrient availability [2,3]. Similarly, plant nutrient uptake can alter nutrient concentrations in the circulating water.

For this reason, microbial management is an important consideration in aquaponic production. Beneficial microorganisms may contribute to nutrient transformation, organic matter degradation, microbial interactions, and the biological stability of the system [5,6]. The deliberate introduction of selected probiotic microorganisms, including *Bacillus* spp., therefore represents a potential approach for influencing microbial conditions within integrated aquaponic systems.

Understanding the physical and microbial organization of a recirculating aquaponic system provides the foundation for evaluating the role of *Bacillus* probiotics. Their effects should be considered in relation to the interconnected fish, water, biofilter, and plant-root environments rather than as an isolated effect on fish alone.

3. Microbial Communities and Nutrient Cycling in Aquaponic Systems

Microorganisms are fundamental to the functioning of recirculating aquaponic systems because they mediate the transformation of organic matter and inorganic nutrients within the circulating water [3,5]. Microbial communities are distributed throughout different components of the system, including the biofilter, culture water, fish gastrointestinal tract, system surfaces, and plant-root environment [3].

One of the most important microbial processes in aquaponics is the transformation of nitrogenous compounds. Fish excrete ammonia as a major nitrogenous waste product, and microbial communities associated with biological filtration convert ammonia into nitrite and subsequently nitrate [3,4]. This transformation is important because excessive accumulation of ammonia and nitrite can negatively affect cultured fish, whereas nitrate can be taken up by plants as a nitrogen source [3,4].

Nitrifying microorganisms are therefore essential for maintaining biological stability in recirculating systems. The conversion of ammonia to nitrite and nitrate occurs through sequential microbial processes involving ammonia-oxidizing and nitrite-oxidizing microorganisms [4]. The efficiency of these processes is influenced by environmental conditions such as temperature, pH, dissolved oxygen, and the availability of suitable microbial surfaces [4].

In addition to nitrogen transformation, heterotrophic microorganisms contribute to the degradation of organic materials entering the system through feral matter, uneaten feed, and other organic residues [3,5]. The breakdown of these materials can release nutrients and influence the availability of carbon and nitrogen compounds within the system. Maintaining an appropriate balance between organic loading and microbial activity is therefore important for stable aquaponic operation.

The microbial composition of aquaponic systems can vary considerably among different compartments. Biofilters provide surfaces that support dense microbial communities, while fish gastrointestinal environments contain microorganisms associated with digestion and host physiology [3]. Similarly, plant roots provide surfaces for colonization by microorganisms from the surrounding water and root environment [6].

Plant-associated microorganisms may contribute to nutrient availability and influence plant development through interactions occurring around the root system [6,7]. These microorganisms can potentially modify nutrient transformations and create localized conditions that affect plant nutrient uptake. Therefore, the plant-root environment represents an important microbial component of aquaponic systems.

Microbial communities may also contribute to biological competition against undesirable microorganisms. Beneficial bacterial populations can occupy available niches and interact with other microorganisms through competition for nutrients or production of antimicrobial compounds [5]. Such interactions are relevant to aquaponic systems because maintaining a stable microbial community may contribute to overall biological stability.

However, microbial communities in aquaponics are dynamic and can change in response to environmental conditions and system management. Variations in feeding rate, fish stocking density, temperature, pH, dissolved oxygen, nutrient loading, and hydraulic conditions can influence microbial composition and activity [3,4]. Consequently, microbial interventions should be evaluated in relation to the complete system rather than as isolated treatments.

The introduction of probiotic bacteria represents one possible approach for modifying microbial conditions within aquaponic systems. Among probiotic candidates, *Bacillus* spp. is of particular interest because selected strains possess characteristics that may support digestive activity, microbial competition, antimicrobial activity, and host immune modulation [5,9].

Importantly, *Bacillus* probiotics should not be considered replacements for the established nitrifying community of an aquaponic biofilter. Their potential role is better understood as an additional microbial influence that may interact with existing microbial populations and environmental processes [4,5]. Understanding these interactions is essential when evaluating the potential effects of *Bacillus* supplementation on water quality, fish health, and plant performance.

Overall, nutrient cycling in aquaponics depends on interactions among fish-derived wastes, microbial communities, biofiltration processes, and plant uptake. This interconnected microbial network provides the biological foundation for examining how targeted probiotic supplementation with *Bacillus* may influence the performance of recirculating aquaponic systems.

4. *Bacillus* Probiotics in Nile Tilapia Health

Bacillus spp. is among the bacterial groups widely investigated as probiotics in aquaculture because selected strains can survive under different environmental conditions and produce biologically active compounds, including extracellular enzymes and antimicrobial substances [5,9]. Their use in fish production has been associated with effects on growth, digestion, intestinal microbial communities, immune responses, and resistance to selected pathogens [5,9].

In Nile Tilapia (*Oreochromis niloticus*), dietary supplementation with selected *Bacillus* strains has been investigated as a strategy for improving growth performance and physiological health. The effects reported in experimental studies include changes in weight gain, feed utilization, digestive enzyme activity, and other indicators of nutritional performance [10,11].

One proposed mechanism is the production of extracellular digestive enzymes by probiotic bacteria. Enzymes produced by *Bacillus* may contribute to the breakdown of nutrients and can potentially improve the utilization of dietary components [5,9]. Improved nutrient utilization may support fish growth and reduce the proportion of undigested material entering the culture environment.

The intestinal microbiota is another important target of probiotic activity. Dietary *Bacillus* supplementation can modify the composition of gastrointestinal bacterial communities and may contribute to the establishment of a more favourable microbial environment [5,10]. Competition for nutrients and attachment sites may also limit the opportunity for some undesirable microorganisms to establish within the gastrointestinal tract [5].

Bacillus probiotics have also been associated with modulation of immune responses in Nile Tilapia. Experimental studies have reported changes in immune-related parameters following dietary supplementation with selected *Bacillus* strains [10,11]. These responses may involve stimulation of components of the innate immune system, which represents an important part of the fish's early defence against infectious agents.

Another proposed probiotic mechanism is the production of antimicrobial compounds. Some *Bacillus* strains produce bacteriocins, lipopeptides, and other bioactive substances that can inhibit selected microorganisms [5,9]. Such antimicrobial activity may contribute to microbial competition within the intestinal environment and may help explain some of the observed effects on disease resistance.

Studies using combinations of *Bacillus subtilis* and *Bacillus licheniformis* have reported beneficial effects on growth, immune responses, and disease resistance in Nile Tilapia [10]. These findings support the potential value of *Bacillus*-based probiotic preparations, although the response depends on the bacterial strains and experimental conditions used.

The effectiveness of probiotics is not uniform across all *Bacillus* strains. Differences in strain characteristics, probiotic concentration, administration route, feeding period, fish age, diet composition, and environmental conditions can influence the outcome of supplementation [5,9]. Therefore, results obtained from one probiotic preparation should not automatically be generalized to other *Bacillus* strains.

An important advantage of *Bacillus* is its ability to form endospores, which can provide greater stability during processing and storage compared with many non-spore-forming probiotic bacteria [5,9]. This characteristic has contributed to the interest in *Bacillus* as a practical probiotic candidate for aquaculture feeds.

Overall, available evidence indicates that selected *Bacillus* probiotics can influence several aspects of *Nile Tilapia* health, particularly growth performance, digestive processes, intestinal microbial communities, and immune responses [5,10,11]. However, these effects should be evaluated on a strain-specific basis, and further research is required to determine how dietary probiotic effects interact with the wider microbial and environmental conditions of recirculating aquaponic systems.

5. Mechanisms of Action of *Bacillus* Probiotics

Bacillus probiotics can influence fish health through several interconnected mechanisms, including modulation of intestinal microbial communities, production of digestive enzymes and antimicrobial compounds, competition with undesirable microorganisms, and stimulation of host immune responses [5,9,10]. The relative contribution of these mechanisms depends on the bacterial strain and the conditions under which the probiotic is administered.

5.1 Modulation of the Intestinal Microbiota

One important mechanism of *Bacillus* probiotics is their ability to modify the microbial environment of the fish gastrointestinal tract. After administration, selected *Bacillus* strains may interact with resident microorganisms and influence the relative abundance of different bacterial groups [5,10]. Such changes can contribute to a more favourable intestinal microbial environment and may reduce opportunities for some undesirable bacteria to establish.

Competition for nutrients and available attachment sites is another possible mechanism through which probiotic bacteria can influence intestinal microbial communities [5]. By occupying ecological niches and utilizing available nutrients, beneficial bacteria may limit the resources accessible to competing microorganisms.

5.2 Production of Digestive Enzymes

Several *Bacillus* strains are capable of producing extracellular enzymes, including proteases, amylases, and lipases [5]. These enzymes can contribute to the breakdown of dietary proteins, carbohydrates, and lipids and may therefore support digestive processes in fish.

Improved digestion and nutrient utilization can contribute to better feed utilization and growth performance. However, the magnitude of these effects depends on the characteristics of the probiotic strain and the diet provided to the fish [5,10].

5.3 Production of Antimicrobial Compounds

Selected *Bacillus* strains can produce biologically active substances that inhibit certain competing microorganisms. These include antimicrobial peptides and other secondary metabolites that may contribute to the suppression of susceptible bacteria [5,10]. This property is particularly relevant in aquaculture because competition between beneficial and potentially harmful microorganisms can influence the microbial stability of the culture environment.

However, antimicrobial activity observed under laboratory conditions should not automatically be interpreted as effective pathogen control in an aquaponic system. The actual outcome can depend on bacterial strain, concentration, environmental conditions, and interactions with the existing microbial community.

5.4 Competitive Exclusion

Probiotic *Bacillus* populations may also contribute to competitive exclusion by competing with undesirable bacteria for nutrients and ecological niches [5,10]. This mechanism may reduce the ability of some opportunistic microorganisms to establish or proliferate within the gastrointestinal environment.

Competitive exclusion is particularly relevant to integrated aquaponic systems because fish, water, biofilter, and plant-root microbial communities are interconnected. Consequently, changes in the microbial community associated with fish may potentially influence the wider microbial environment of the system.

5.5 Modulation of Host Immune Responses

Another important mechanism involves interaction between probiotic bacteria and the host immune system. Studies in *Nile Tilapia* indicate that selected *Bacillus* strains can influence immune-related responses, including cellular and physiological indicators of innate immunity [5,10]. Such modulation may improve the ability of fish to respond to environmental and microbial challenges.

The immune effects of probiotics should nevertheless be interpreted as strain-specific rather than as a universal characteristic of all *Bacillus* species. Differences in bacterial strain, dosage, feeding period, and host condition can influence the magnitude of the response [5,10].

5.6 Potential Interaction with the Aquaponic Microbiome

In an aquaponic system, the potential effects of *Bacillus* may extend beyond the fish gastrointestinal tract. A study using a commercial mixture of *B. subtilis* and *B. licheniformis* in a tilapia–lettuce aquaponic system reported changes in water nutrient concentrations, lettuce growth, and bacterial communities associated with lettuce roots [11]. These findings suggest that probiotic supplementation can interact with the broader microbial environment of an integrated fish–plant system.

However, the available evidence does not establish that *Bacillus* supplementation will consistently improve every component of an aquaponic system. More studies are required to determine how probiotic strains interact with established biofilter communities, water microbiota, fish-associated microorganisms, and plant-root communities under different operating conditions [8,11].

6. Role of *Bacillus* Probiotics in Water-Quality Microbiology

Bacillus probiotics may influence the microbial and physicochemical characteristics of aquaculture water through their interactions with organic matter, nutrients, and resident microbial communities [5,9]. In recirculating aquaponic systems, these interactions are particularly relevant because water continuously moves between the fish-rearing, filtration, and plant-production units.

6.1 Degradation of Organic Matter

Organic materials entering aquaponic systems include uneaten feed, faecal matter, and other metabolic wastes. Heterotrophic microorganisms contribute to the decomposition of these materials and thereby influence the availability of dissolved and particulate organic compounds [3,5]. Selected *Bacillus* strains produce extracellular enzymes that can contribute to the breakdown of complex organic substrates [5,9].

The degradation of organic matter may reduce the accumulation of biodegradable waste and contribute to microbial processing within the system. However, the extent of this effect depends on the probiotic strain, its abundance, organic loading, and environmental conditions.

6.2 Influence on Nitrogenous Compounds

Nitrogen transformation is a central process in recirculating aquaponic systems. Fish metabolism releases ammonia into the culture water, which is subsequently converted through microbial nitrification into nitrite and nitrate [3,4]. Although *Bacillus* probiotics are not substitutes for established nitrifying microorganisms, their presence may interact with the wider microbial community involved in organic matter and nutrient processing.

The potential contribution of probiotic bacteria to water-quality management should therefore be considered as part of the overall microbial community rather than as an independent nitrification mechanism [4,5]. Maintaining a stable biofilter and suitable environmental conditions remains essential for effective nitrogen removal.

6.3 Microbial Competition and Water Microbiota

The addition of probiotic bacteria can modify microbial interactions within the aquatic environment. Selected *Bacillus* strains may compete with other microorganisms for nutrients and ecological niches and may produce substances with antimicrobial activity [5,9]. These interactions can potentially influence the composition and abundance of microorganisms present in the water.

In aquaponic systems, changes in water microbiota may have consequences for both fish and plant-associated microbial communities because water provides a continuous connection between different system compartments. However, the direction and magnitude of these changes are dependent on the probiotic strain and the existing microbial community.

6.4 Potential Effects on Water-Quality Parameters

Probiotic supplementation has been investigated as a biological approach for supporting water-quality management in aquaculture. Reported effects can include changes in concentrations of nitrogenous compounds, organic matter, and other water-quality indicators [9,11]. In a tilapia–lettuce aquaponic system, supplementation with a commercial *Bacillus* preparation was associated with changes in water nutrient concentrations and microbial communities [11].

Nevertheless, water-quality responses cannot be attributed exclusively to *Bacillus* supplementation because feeding rate, fish biomass, stocking density, biofilter performance, plant nutrient uptake, water exchange, and environmental conditions also influence water chemistry [2,3]. Therefore, controlled comparisons are required to distinguish probiotic effects from normal variation within recirculating systems.

6.5 Interaction with Biofilter Microorganisms

The biofilter is a critical microbial component of a recirculating aquaponic system because it supports microorganisms responsible for nitrogen transformation [3,4]. Introducing additional bacteria into the system may create interactions with established biofilter communities. Such interactions may involve competition, coexistence, or changes in the relative abundance of resident microorganisms.

Because nitrifying communities are essential for maintaining suitable ammonia and nitrite concentrations, probiotic applications should not disrupt established biological filtration processes. Further research is needed to determine how different *Bacillus* strains behave within mature aquaponic biofilters and whether their presence produces measurable improvements in system stability.

6.6 Relevance to Integrated Aquaponic Production

The potential influence of *Bacillus* on water microbiology is important because water serves as the connecting medium between fish, microorganisms, and plants. A probiotic intervention introduced through fish feed or directly into the system may therefore influence multiple microbial compartments [5,11].

However, evidence for consistent improvement of water quality through *Bacillus* supplementation in aquaponic systems remains limited. More strain-specific and controlled studies are required to establish appropriate probiotic concentrations, application methods, interactions with biofilter communities, and long-term effects on water quality.

7. Influence of *Bacillus* Probiotics on Lettuce Growth

Lettuce (*Lactuca sativa*) is one of the commonly cultivated vegetables in aquaponic systems because it can utilize nutrients present in recirculating water and can be grown under soilless conditions [7,8]. In an integrated *Nile Tilapia*–lettuce system, plant performance is influenced by nutrient availability, water chemistry, root-zone conditions, and interactions with microorganisms.

7.1 *Bacillus*–Plant Interactions

Selected *Bacillus* species can associate with plant roots and function as beneficial plant-associated microorganisms [6,7]. These bacteria may colonize the rhizosphere and interact with plants through mechanisms involving nutrient mobilization, production of growth-promoting substances, and modification of the local microbial environment.

Root-associated *Bacillus* populations may influence plant development by improving the availability of certain nutrients and by producing metabolites that affect plant physiological processes [6]. Such interactions are particularly relevant in aquaponics because plant roots are continuously exposed to microorganisms and dissolved nutrients present in the circulating water.

7.2 Nutrient Availability and Plant Growth

The growth of lettuce in aquaponic systems depends largely on the availability of essential nutrients in the circulating water. Fish-derived nutrients undergo microbial transformation before becoming available for plant uptake [2,3]. Consequently, microbial activity can indirectly influence plant nutrient acquisition.

The potential contribution of *Bacillus* to plant growth may involve interactions with nutrient cycling and root-associated microorganisms. However, the extent to which probiotic supplementation improves lettuce growth depends on the nutrient composition of the system and the characteristics of the bacterial strain used.

7.3 Growth-Promoting Activities

Some plant-associated *Bacillus* strains possess plant-growth-promoting properties. These may include production of phytohormone-like compounds, nutrient mobilization, and other activities that can support root development and plant growth [6,7].

Improved root development can potentially increase the surface area available for nutrient and water absorption. In an aquaponic system, a healthy root system may also provide a suitable habitat for beneficial microorganisms, creating interactions between plant physiology and the surrounding microbial community.

7.4 Evidence from *Tilapia*–Lettuce Aquaponic Systems

Evidence specifically linking *Bacillus* supplementation with lettuce growth in aquaponic systems is more limited than the evidence available for *Bacillus* probiotics in fish culture. A study using a commercial preparation containing *Bacillus subtilis* and *Bacillus licheniformis* in a *tilapia*–lettuce aquaponic system reported changes in lettuce growth together with changes in water nutrient concentrations and bacterial communities associated with lettuce roots [11].

These findings suggest that probiotic supplementation may influence the plant component of an integrated aquaponic system. However, changes in lettuce growth cannot necessarily be attributed solely to direct plant-growth-promoting activity because probiotic treatment may simultaneously alter fish-derived nutrient availability, water microbiology, and root-associated microbial communities.

7.5 Factors Affecting the Response of Lettuce

The response of lettuce to microbial supplementation can vary according to several factors, including bacterial strain, inoculum concentration, nutrient availability, water quality, temperature, pH, light conditions, and the composition of the existing root-associated microbial community [6,7].

Therefore, the presence of *Bacillus* in an aquaponic system does not guarantee improved plant growth under all conditions. Further controlled studies are needed to determine which *Bacillus* strains provide consistent benefits to lettuce and how these effects are influenced by the characteristics of the fish-production and plant-production components.

Overall, *Bacillus* probiotics have potential to influence lettuce performance through plant-microbe interactions and indirect effects associated with nutrient and microbial processes. Nevertheless, evidence from complete recirculating aquaponic systems remains comparatively limited, highlighting the need for further investigation of the fish-microbe-plant relationship.

8. Fish-Microbe-Plant Interactions in Recirculating Aquaponic Systems

Recirculating aquaponic systems function through continuous interactions among fish, microorganisms, water, and plants. Fish provide the major organic and nutrient inputs, microorganisms transform these materials, and plants utilize a proportion of the nutrients released into the circulating water [2,3]. This interconnected relationship forms the biological basis of nutrient recycling in aquaponics.

8.1 Interaction Between Fish and Microorganisms

The gastrointestinal tract and external surfaces of *Nile Tilapia* contain diverse microbial communities that can influence digestion, nutrient utilization, and host health [5]. Environmental microorganisms are also continuously exchanged between the fish and surrounding water. Consequently, changes in fish-associated microbiota may influence the microbial composition of the wider aquaponic environment.

The addition of probiotic *Bacillus* strains may modify these interactions by introducing beneficial bacterial populations into the fish gastrointestinal environment [5,9]. Their potential effects include modulation of intestinal microbiota, production of digestive enzymes, microbial competition, and stimulation of host immune responses.

8.2 Interaction Between Microorganisms and Water

Water acts as the connecting medium through which microorganisms and dissolved nutrients move between different components of a recirculating aquaponic system. Microbial processes occurring in the water and biofilter contribute to the transformation of fish-derived wastes and influence the chemical conditions experienced by both fish and plants [3,4].

The microbial community in the circulating water is influenced by factors such as feed input, fish biomass, temperature, dissolved oxygen, pH, and filtration efficiency [3]. Probiotic supplementation may introduce additional microbial interactions, but the resulting changes depend on the characteristics of the probiotic strain and the resident microbial community.

8.3 Interaction Between Water Microbiota and Plant Roots

Plant roots are continuously exposed to the circulating aquaponic water and therefore interact with microorganisms present in the system. The root-associated microbial community can influence nutrient availability and may participate in processes that affect plant development [6,7].

The rhizosphere can also provide a suitable environment for colonization by beneficial bacteria. Selected *Bacillus* strains may interact with plant roots through nutrient mobilization, production of biologically active compounds, and modification of the local microbial environment [6].

8.4 Integrated Role of *Bacillus*

The potential value of *Bacillus* probiotics in aquaponics lies in their ability to interact with more than one biological component. In *Nile Tilapia*, selected strains may influence intestinal microbial communities and immune responses, while their presence in the wider system may interact with water and plant-root microbiota [5,11].

However, the presence of *Bacillus* in one component does not necessarily result in identical effects throughout the system. The fish gastrointestinal tract, water column, biofilter, and plant-root environment have different ecological conditions and microbial communities. Therefore, probiotic effects should be evaluated across these interconnected compartments rather than using a single indicator of system performance.

8.5 Implications for System Stability

A balanced interaction among fish, microorganisms, and plants is essential for maintaining stable aquaponic operation. Excessive organic loading can increase microbial oxygen demand, while insufficient microbial activity can interfere with nutrient transformation. Similarly, changes in plant nutrient uptake can alter nutrient concentrations available within the circulating water [2,3].

The introduction of *Bacillus* probiotics may provide an additional biological component within this network. Nevertheless, probiotic supplementation should complement rather than replace appropriate feeding practices, biofiltration, aeration, and water-quality management.

8.6 Conceptual Significance

The fish–microbe–plant relationship demonstrates why probiotic interventions in aquaponics should be considered as system-level approaches. A change in fish-associated microbiota may influence waste processing, while changes in microbial activity can affect nutrient availability to plants. In turn, plant nutrient uptake can influence the composition of the circulating water [2,3,6].

Thus, understanding these interconnected interactions is important for determining whether *Bacillus* probiotics can provide simultaneous benefits to *Nile Tilapia* health, water-quality management, and lettuce production. More integrated studies are required to establish the relationships among probiotic dose, microbial community structure, nutrient cycling, and plant performance under practical recirculating aquaponic conditions.

9. Food Safety and Microbiological Considerations

Food safety is an important consideration in aquaponic systems because fish culture and vegetable production are connected through a shared recirculating water system. Microorganisms originating from fish, feed, faecal material, or the surrounding environment may therefore move through different components of the system and potentially reach plant-production areas [8,12].

9.1 Microbiological Contamination of Aquaponic Systems

Aquaponic systems can contain diverse microorganisms originating from fish, water, biofilters, plant roots, equipment, and the surrounding environment. While many of these microorganisms are harmless or beneficial, some may include opportunistic or foodborne microorganisms that are relevant to the safety of edible crops [8,12].

Lettuce is particularly important from a food-safety perspective because it is commonly consumed fresh or with minimal processing. Consequently, contamination occurring during cultivation, harvesting, handling, or post-harvest processing may remain relevant to the final product.

9.2 Fish-Derived Microorganisms and Lettuce

Fish and their associated wastes can contribute microorganisms to the aquaponic water. Faecal material and other organic inputs may introduce bacteria into the circulating water, creating a potential pathway for microbial transfer toward plant-growing units [8,12].

A systematic review of microbiological hazards associated with aquaponically produced lettuce identified microorganisms including Enterobacteriaceae, coliforms, and Shiga toxin-producing *Escherichia coli* among organisms reported in tilapia-based aquaponic systems [12]. These findings emphasize the importance of monitoring microbial quality throughout the production system.

9.3 Role of Water Quality Management

Maintaining appropriate water-quality conditions is an important part of microbiological risk management. Effective solids removal, biological filtration, adequate aeration, appropriate stocking density, and good system hygiene can help maintain stable operating conditions [2,3].

Water-quality management alone, however, cannot guarantee microbiological safety. The quality of fish feed, source water, equipment hygiene, worker handling, harvesting practices, and post-harvest processing can also influence the microbiological quality of aquaponically produced vegetables.

9.4 Potential Role of *Bacillus* in Microbial Competition

The antimicrobial and competitive properties of selected *Bacillus* strains have generated interest in their potential use for controlling undesirable microorganisms [5,9]. Some strains can produce antimicrobial compounds or compete with other bacteria for nutrients and ecological niches.

However, the presence of probiotic *Bacillus* should not be interpreted as a guaranteed food-safety intervention. Laboratory inhibition of a target microorganism does not necessarily demonstrate effective pathogen control under practical aquaponic conditions. The effectiveness of a probiotic approach depends on strain characteristics, bacterial concentration, environmental conditions, and interactions with the resident microbial community.

9.5 Food-Safety Assessment of Lettuce

Microbiological assessment of lettuce can include monitoring for indicator organisms and relevant foodborne pathogens. Such assessments can help determine whether production and handling practices are maintaining acceptable microbiological quality [8,12].

Particular attention should be given to the relationship between water microbiology and plant-surface contamination. Because lettuce leaves may come into contact with irrigation water or splashes from the production environment, controlling microbial contamination at the system level is important.

9.6 Need for Integrated Risk Management

Food safety in aquaponics requires a combination of biological, environmental, and hygienic management practices. Probiotic supplementation may potentially contribute to microbial management, but it should be considered as one component of a broader food-safety strategy rather than a replacement for good aquaculture and food-handling practices [8,12].

Future studies should evaluate whether specific *Bacillus* strains can consistently reduce undesirable microorganisms in complete *Nile Tilapia*–lettuce aquaponic systems while maintaining fish health and plant productivity. Such research would help determine whether probiotic supplementation can provide measurable food-safety benefits in addition to its potential effects on fish and plant performance.

10. Research Gaps and Current Challenges

Although *Bacillus* probiotics have been extensively investigated in aquaculture, evidence regarding their combined effects on fish health, water microbiology, and vegetable production in recirculating aquaponic systems remains comparatively limited [8,11,12]. Most available studies have focused on individual components of aquaculture or plant production rather than evaluating the complete fish–microbe–plant system.

10.1 Limited Integrated Aquaponic Studies

A major research gap is the limited number of studies that simultaneously evaluate *Bacillus* supplementation in *Nile Tilapia*, water-quality microbiology, and lettuce performance. Studies conducted under aquaculture conditions cannot always be directly extrapolated to aquaponic systems because the presence of plants, biofilters, and continuous water circulation creates additional microbial interactions [3,8].

Integrated experiments are therefore needed to determine whether probiotic effects observed in fish are maintained when the same microorganisms are introduced into a complete aquaponic system.

10.2 Strain-Specific Effects

The term *Bacillus* probiotics represents a diverse group of bacterial strains with different physiological and functional properties. Their ability to produce enzymes, antimicrobial compounds, or host-interacting molecules can vary considerably between strains [5,9].

Therefore, future studies should identify and characterize individual probiotic strains rather than assuming that all *Bacillus* species will produce similar effects. Comparative evaluation of different strains could help identify candidates that are particularly suitable for *Nile Tilapia*–lettuce aquaponic systems.

10.3 Optimization of Probiotic Dosage

The effectiveness of probiotic supplementation can depend on bacterial concentration and duration of administration [5,9]. An insufficient dose may produce little measurable effect, whereas inappropriate application levels may not provide additional benefits.

Systematic dose-response studies are therefore required to determine suitable probiotic concentrations for different aquaponic operating conditions. The method of administration, such as dietary supplementation or direct application to the water, should also be evaluated separately.

10.4 Interaction with Established Microbial Communities

One of the important unanswered questions concerns the interaction between introduced *Bacillus* strains and resident microorganisms in the aquaponic system. The biofilter, water, fish gastrointestinal tract, and plant roots each contain established microbial communities [3,5].

Introducing an additional bacterial population may alter these communities through competition, coexistence, or other ecological interactions. High-throughput sequencing and microbial community analysis could help determine how probiotic supplementation changes the structure and function of these microbial populations.

10.5 Standardization of Experimental Conditions

Comparisons between probiotic studies are difficult because experimental conditions can differ in fish stocking density, feed composition, probiotic concentration, water temperature, pH, dissolved oxygen, plant species, and system design [5,8].

Standardized experimental protocols would improve the comparability and reproducibility of results. Reporting detailed information about probiotic strain identity, viability, dosage, administration period, and system conditions would also strengthen future research.

10.6 Long-Term and Practical Evaluation

Many probiotic studies are conducted over relatively short experimental periods. Long-term investigations are needed to determine whether observed improvements in fish health, water quality, or lettuce growth are maintained throughout repeated production cycles.

Practical studies should also consider the economic feasibility, stability of probiotic preparations, compatibility with commercial aquaponic operations, and consistency of outcomes under variable environmental conditions.

10.7 Food-Safety Research Gap

The potential food-safety role of *Bacillus* probiotics in aquaponic systems requires further investigation. Although selected *Bacillus* strains may exhibit antimicrobial activity, evidence demonstrating consistent reduction of foodborne microorganisms on aquaponically produced lettuce remains limited [8,12].

Future research should therefore combine probiotic evaluation with microbiological safety assessment of irrigation water, plant surfaces, and harvested lettuce. This would help determine whether probiotic supplementation can provide measurable food-safety benefits without compromising system performance.

10.8 Need for Multi-Parameter Evaluation

A major limitation of evaluating probiotics using only one outcome is that aquaponic systems are highly interconnected. An intervention that improves fish growth may not necessarily improve water quality or lettuce production.

Future studies should therefore simultaneously monitor **fish health, growth performance, gut microbiota, water-quality parameters, microbial community composition, nutrient removal, lettuce growth, and microbiological safety**. Such multi-parameter approaches would provide a more complete understanding of the role of *Bacillus* probiotics in integrated aquaponic production.

Taken together, these research gaps indicate that future work should move from isolated probiotic assessments toward integrated, strain-specific, and system-level investigations. This approach will be important for establishing reliable *Bacillus*-based strategies for recirculating *Nile Tilapia*–lettuce aquaponic systems.

11. Future Perspectives

The increasing interest in sustainable aquaculture and integrated food-production systems creates opportunities for further development of probiotic-based approaches in aquaponics. *Bacillus* probiotics have potential to contribute to fish health and microbial management, but their application in complete recirculating aquaponic systems requires further validation under controlled and practical conditions [5,8,11].

11.1 Development of Strain-Specific Probiotics

Future research should focus on identifying *Bacillus* strains with well-characterized probiotic properties and suitability for aquaponic conditions. Strain selection should consider survival, enzyme production, antimicrobial activity, compatibility with fish, and interactions with plant-associated microorganisms [5,9].

11.2 Integrated Microbiome Studies

Modern molecular approaches can provide a better understanding of how probiotic supplementation influences microbial communities throughout the aquaponic system. Metagenomic and sequencing-based approaches could be used to monitor changes in the fish gut, water, biofilter, and lettuce-root microbiomes [3,5].

Such studies may help identify whether introduced *Bacillus* strains become established within the system or primarily influence resident microbial communities through ecological interactions.

11.3 Optimization of Probiotic Application

Future studies should establish appropriate probiotic doses, administration routes, and supplementation periods for *Nile Tilapia*–lettuce aquaponic systems. Comparisons between dietary administration and direct water application may help determine the most effective and practical approach.

Probiotic formulations should also be evaluated for stability during storage and under different aquaponic environmental conditions. This is important for translating experimental findings into practical aquaponic production.

11.4 Development of Multi-Functional Probiotic Strategies

Future probiotic research could explore strains or combinations of microorganisms capable of providing multiple beneficial functions. These may include supporting fish health, contributing to organic matter processing, interacting with plant-root microbiota, and potentially limiting selected undesirable microorganisms [5,9].

However, claims of multiple benefits should be supported by experiments measuring each outcome independently rather than assuming that improvement in one component will automatically improve the entire system.

11.5 Application of Smart Monitoring Technologies

Integration of microbial management with real-time monitoring technologies could improve the stability of recirculating aquaponic systems. Continuous monitoring of parameters such as temperature, pH, dissolved oxygen, ammonia, nitrite, and nitrate can help identify changes in system conditions and support timely management decisions [2,3].

Combining environmental monitoring with microbial analysis may provide a more comprehensive approach for evaluating probiotic performance.

11.6 Food-Safety-Oriented Research

Future investigations should also determine whether selected *Bacillus* strains can contribute to reducing microbiological risks associated with aquaponically produced lettuce. Such studies should assess microbial quality of the water, plant surfaces, and harvested vegetables rather than relying only on laboratory antimicrobial assays [8,12].

The safety characteristics of the probiotic strain itself should also be carefully evaluated before practical application in food-production systems.

11.7 Commercial and Sustainable Applications

For successful commercial application, probiotic strategies should provide consistent benefits without increasing production complexity or cost substantially. Future research should therefore combine biological performance with economic assessment, resource-use efficiency, and long-term system stability.

A successful *Bacillus*-based approach could potentially support a more sustainable aquaponic production model by integrating fish health management with microbial and plant-production processes. Nevertheless, large-scale trials are required before widespread application can be recommended.

11.8 Future Research Direction

Overall, future research should move toward **strain-specific, dose-optimized, microbiome-informed, and system-level evaluation** of *Bacillus* probiotics. Experiments that simultaneously assess *Nile Tilapia* health, water microbiology, nutrient cycling, lettuce growth, and food safety would provide stronger evidence for determining the practical value of probiotics in recirculating aquaponic systems.

12. Conclusion

Bacillus probiotics represent a promising biological approach for supporting sustainable production in recirculating aquaponic systems. Evidence from aquaculture studies indicates that selected *Bacillus* strains can influence *Nile Tilapia* health through modulation of intestinal microbiota, production of digestive enzymes and antimicrobial compounds, and stimulation of immune responses [5,9,10].

In an aquaponic environment, the potential role of *Bacillus* extends beyond the fish gastrointestinal tract. Interactions with water-associated microorganisms, organic matter, biofilter communities, and plant-root microbiota may influence nutrient transformation and the overall microbial environment of the system [3,5,11]. Selected *Bacillus* strains may also contribute to plant–microbe interactions and potentially support lettuce growth, although direct evidence from complete tilapia–lettuce aquaponic systems remains limited [7,11].

The interconnected nature of fish, microorganisms, water, and plants means that probiotic effects should be evaluated at the system level rather than through fish or plant performance alone. At the same time, food safety must remain an important consideration because microorganisms present in aquaponic water may potentially reach edible plant tissues [8,12]. Therefore, probiotic supplementation should complement, rather than replace, appropriate biofiltration, water-quality management, hygiene, and good aquaculture practices.

Overall, current evidence supports the **potential** of *Bacillus* probiotics to contribute to *Nile Tilapia* health and microbial management in recirculating aquaponic systems, while their effects on water quality and lettuce production require further systematic investigation. Future research should focus on strain-specific selection, optimized dosage, microbiome analysis, long-term trials, and simultaneous evaluation of fish health, water microbiology, lettuce growth, and food safety. Such integrated approaches will be essential for determining the practical value of *Bacillus* probiotics in sustainable *Nile Tilapia*–lettuce aquaponic production.

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