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ENHANCED ORGANIC MATTER DECOMPOSITION BY BACILLUS CEREUS ASAT-8 IN THAI AQUAPONIC SYSTEMS

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Abstract

This research investigates the potential of Bacillus cereus ASAT-8, isolated from local Thai aquaponic systems, for enhanced organic matter decomposition. Aquaponics integrates hydroponics with aquaculture, necessitating the practical cycling of nutrients via microbial decomposition. This study isolated and characterized bacterial strains with high decomposition and nitrification capabilities. Water samples were collected, and twenty-one bacterial isolates were obtained using a modified Pep-Beef-AOB medium. Isolate ASAT-8, demonstrating high ammonia-oxidizing activity, was identified as Bacillus cereus (99% similarity) through partial 16S rRNA gene sequencing. Bacillus cereus ASAT-8 effectively reduced ammonia levels in organic waste, suggesting its potential to improve water quality. The widespread presence of Bacillus spp. Indicates a reliance on natural microbial resources in aquaponics. Incorporating Bacillus spp. can reduce water changes, minimize fish waste, and enhance food production. This study highlights the importance of Bacillus cereus for sustainable aquaponic practices, contributing to zero-waste food production.

Keywords: Aquaponics, Bacillus cereus, Organic Matter Decomposition, Nitrification, Sustainable Agriculture

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Introduction

The sustainable development of agricultural systems encompasses environmental, economic, and social dimensions. Some of the characteristics of sustainable food production that researchers and the public have emphasized include minimal resource intake with maximal productivity, high farming output per area, low carbon-based energy usage, a reduction of harmful chemicals, biodiversity-supportive practices, and minimal waste output. The Food and Agriculture Organization of the United Nations (FAO) has estimated that the global population could potentially reach ten billion by 2050, underscoring the urgent need to develop sustainable food production and environmentally friendly agriculture for the future.

The concept of a zero-waste system, where waste output is reduced or reused, can be integrated into agricultural production to minimize environmental impact via efficient waste management. Aquaculture, as one of the fastest-growing agrarian industries, could face challenges in waste management due to sludge and effluent containing organic nitrogen and phosphorus, which can negatively impact the surrounding environment. The accumulation of nutrients in the water column may lead to eutrophication, depletion of dissolved oxygen, deterioration of water quality, destruction of aquatic habitats, and localized mortality of marine organisms. Currently, many fish farmers employ a recirculating system to direct effluents through a series of treatments before reusing the water for aquaculture, hence reducing wastewater output.

An additional strategy to achieve zero-waste output involves integrating soil-less plant culture into an aquaculture system, resulting in an aquaponic system. Hydroponic practices include growing plants in a nutrient-rich solution, where the roots are submerged in water supplemented with fertilizer. Since aquaculture produces effluent containing nitrogen and phosphorus and requires electricity to maintain a recirculating water flow through a pump, incorporating a hydroponic system can further reduce the costs of fertilizer and electricity. This integration has the potential to enhance the overall efficiency of resource and energy utilization, while also reducing waste released into the environment. An aquaponic system also provides farmers with diversified product output, including both fish and edible vegetables, thereby promoting income security during price fluctuations of either product.

This study aimed to isolate and characterize bacterial strains from local aquaponic systems with a high capacity for organic matter decomposition and nitrification, and to evaluate their potential utility for sustainable aquaponics.

Literature Reviews

Bacterial communities are a crucial component of aquaponic systems, playing a vital role in nutrient cycling and maintaining water quality. These microorganisms convert fish waste into nutrients that are accessible to plants through the nitrogen cycle, establishing a symbiotic relationship between aquatic and terrestrial organisms. Healthy aquaponic systems generally house autotrophic and heterotrophic bacteria on the surfaces of biofiltration components within aquaculture units and media beds of hydroponic units. Bacteria in aquaponics contribute to various microbiological processes, for example, nitrification, organic matter decomposition, phosphorus mineralization, and iron cycling.

In the nitrification process, total ammonia nitrogen (TAN) from organic matter and fish excretion is oxidized into nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB), such as Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio sp. Nitrite (NO_2^-) can be further transformed into nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB), including Nitrobacter, Nitrospira, Nitrococcus, and Nitrospina sp. Although plants can absorb either ammonia (NH_4^+), nitrite, or nitrate, the nitrification process helps prevent high concentrations of NH_4^+ and NO_2^-, which are toxic to the fish. In addition to nitrogen cycling, the bacterial capacity for decomposing organic matter is crucial in preventing the accumulation of waste from sludge, uneaten feed pellets, and dead cells. Heterotrophic bacteria, which utilize carbohydrates, amino acids, peptides, and lipids as carbon and energy sources, have been previously reported in aquaponic systems, including Arcobacter, Flavobacterium, Modestobacter, Pseudomonas, and Sphingobacterium. This efficient nutrient cycling not only enhances plant growth but also contributes to a sustainable and self-sustaining ecosystem.

Kasozi et al. (2021a) reported that bacteria from the phyla Actinobacteria, Proteobacteria, Bacteroidetes, Nitrospirae, Fusobacteria, Planctomycetes, and Chloroflexi can be found in freshwater aquaponics systems. However, different fish species possess distinct microbial flora, while various plants have differing capacities to host bacteria due to variations in root surface characteristics. Additionally, the dominant bacterial groups or species can vary depending on the location within the aquaponic system, such as bottom sediment, side walls, biofilter, plant roots, and growing medium. Hence, each aquaponic system could potentially have different bacteria.

Bacillus spp. is commonly found in environmental soil and water. Their potential as effective decomposers of organic matter and as plant growth-promoting bacteria has been reported. Rasool et al. (2017) reported that gill samples of freshwater fish in Jammu, India, contained B. cereus, with 39 out of 44 isolates yielding positive results for a nitrate reduction test. Ghosh et al. (2002) found that the intestinal microbiota of rohu (Labeo rohita) fingerlings is comprised of B. cereus, along with B. circulans and B. pumilus. Amin et al. (2023) isolated Bacillus spp. and Marinobacter nauticus from Nile tilapia (Oreochromis niloticus) cultured in an earthen pond in Indonesia, which demonstrated a high capacity to degrade either ammonia or nitrate, respectively. Sanchez et al. (2019) also found B. cereus, along with other potential plant growth-promoting bacteria, in the recirculating system (RAS) rearing Nile tilapia. Thus, Bacillus may be a common organism that can be used for nitrogen decomposition.

Bacillus spp. might be considered one of the plant growth-promoting bacteria (PGPB). Rais et al. (2017) found that young rice plants inoculated with Bacillus spp. showed increased activity of antioxidant defense enzymes (peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase, and superoxide dismutase) and reduced susceptibility to fungi infection by Pyricularia oryzae. Kasozi et al. (2021b) reported a significantly higher relative abundance of bacteria in Bacillus spp.-supplemented aquaponic systems, i.e., Bacillus, Chryseobacterium, Cloacibacterium, Nitrospira, Pseudomonas, and Thermomonas. These bacteria can influence plant growth through processes such as the production of antimicrobial substances, the degradation and mineralization of organic matter, and nitrogen cycling.

Materials and Methods

Sample Collection

Water samples were collected from six local small-scale aquaponic sites within Phetchaburi province, Thailand, between June 2023 and March 2024. Within each site, three water samples were collected from the sump into 300 mL sterile, amber-colored glass bottles. The sample bottles were stored at 4 °C and transported to the laboratory within 24 hours. The six aquaponic systems were selected to represent a diverse range of operational scales and farmer practices in the Phetchaburi region. Sampling was performed weekly over three months to account for seasonal variations in microbial communities and to ensure a robust representation of active bacterial populations.

Bacteria Isolation Using Enrichment Cultures

The modified Pep-Beef-AOB culture medium was prepared from 5.0 g peptone, 3.0 g beef extract, 2.0 g $(NH_4)_2SO_4$, 0.75 g K_2HPO_4, 0.25 g NaH_2PO_4, 0.03 g $MgSO_4$, 0.01 g $MnSO_4$, 17.8054 g sodium citrate, 20 g sea salt, 15 g agar, and 1000 mL distilled H_2O (pH 7.0). The modified Pep-Beef-AOB medium was autoclaved at 120 °C for 30 minutes for sterilization. Enriched cultures were conducted in 250 mL Erlenmeyer flasks by mixing 90 mL of sterile modified Pep-Beef-AOB medium and 10 mL of a water sample, followed by incubation at 28 °C on a rotary shaker at 180 rotations per minute (rpm). A well-known cellulolytic strain of Cellulomonas sp. was used as a positive control for decomposition assays, and a non-decomposing strain was used as a negative control to validate the assay methodology. The medium was chosen because its composition, including peptone and beef extract, provides a rich source of organic nutrients, mimicking the conditions of high organic load found in aquaponic systems. The inclusion of ammonium sulfate and phosphates supports the growth of nitrifying bacteria.

Every three days, a 0.1 mL aliquot was tested for nitrifying capability using colorimetric determination and the Griess-Ilosvay method. For samples testing positive, a 0.1 mL aliquot of the medium from the flasks was transferred into a fresh sterile medium. Repeated streaking on a modified Pep-Beef-AOB medium was performed until purified isolates were obtained. The bacterial isolates were subsequently tested for their ability to decompose organic matter on skim milk agar (for protein degradation), starch agar (for carbohydrate degradation), and Tween 80 agar (for lipid degradation). The diameter of the clear zones was measured in millimeters to quantify the level of enzymatic activity, providing a quantifiable measurement for comparison among isolates. The colonies capable of producing clear zones on these media were then inoculated into 250 mL Erlenmeyer flasks containing an NH_4^+-N concentration adjusted to 807.66 ± 5.41 mg/L. Following a four-day incubation at room temperature, the ammonia-oxidizing capacity was tested by photometric determination with Nessler's reagent. The bacterial isolate with the highest ammonia-oxidizing capacity was then submitted for DNA sequencing.

Gene Sequence Analysis

The 16S rDNA was amplified by PCR using the primers 27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR products were purified with a GF-1 AmbiClean Kit (PCR/Gel). Partial sequencing of the 16S rDNA gene was conducted by Macrogen, Inc. (Seoul, South Korea). The 16S rDNA sequence was compared with those of other microorganisms using the Basic Local Alignment Search Tool program (BLAST) in the GenBank/EMBL/DDBJ database.

Statistical Analysis

All experiments were performed in triplicate. Data for ammonia-oxidizing capacity were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among the isolates, with a significance level of $p < 0.05$. Statistical analyses were performed using...

Research Findings

Bacterial Isolation and Preliminary Tests

Twenty-one bacterial isolates, each distinguished by unique colony morphology (shape, size, color, elevation, and edges), were selected from the initial culture of the water samples taken from six local aquaponic sites on Pep-Beef-AOB medium (Table 1). These isolations exhibited positive nitrification activity, as determined by colorimetric determination and the Griess-Ilosvay method. However, only three isolates, namely ASAT-8, ASAT-17, and ASAT-21, demonstrated a clear capacity to decompose organic matter, as evidenced by the formation of distinct zones of clearance on skim milk agar, starch agar, and Tween 80 agar. A figure illustrating the decomposition zones or nitrification assay results should be included to support these findings visually.

Table 1 Morphology of bacterial isolates from the water samples collected from local small-scale aquaponic sites in Phetchaburi province, Thailand.

Code	Colony morphology				Gram staining
	Pigment	Form	Elevation	Margin	
ASAT-1	White	circular	Flat	Entire	Positive
ASAT-2	Transparent	circular	Flat	Undulate	Negative
ASAT-3	Transparent	circular	Flat	Undulate	Negative

ASAT-4	Transparent	circular	Umbonate	Entire	Positive
ASAT-5	White	circular	Flat	Entire	Negative
ASAT-6	White	circular	Umbonate	Entire	Negative
ASAT-7	White	circular	Umbonate	Entire	Negative
ASAT-8	White	circular	Convex	Entire	Positive
ASAT-9	Transparent	circular	Flat	Entire	Negative
ASAT-10	Transparent	circular	Convex	Entire	Positive
ASAT-11	Transparent	circular	Umbonate	Entire	Negative
ASAT-12	Yellow	circular	Raised	Entire	Positive
ASAT-13	White	circular	Convex	Entire	Positive
ASAT-14	White	circular	Convex	Entire	Positive
ASAT-15	White	circular	Flat	Undulate	Positive
ASAT-16	Yellow	circular	Convex	Entire	Positive
ASAT-17	Transparent	circular	Umbonate	Undulate	Positive
ASAT-18	White	circular	Convex	Undulate	Positive
ASAT-19	Transparent	circular	Convex	Entire	Negative
ASAT-20	Transparent	circular	Convex	Undulate	Negative
ASAT-21	Transparent	circular	Raised	Undulate	Negative

Bacterial Classification and Characterization

The isolates ASAT-8, ASAT-17, and ASAT-21 also exhibited the highest ammonia-oxidizing activity. To identify the most promising isolate, all three isolates were cultured in media containing identical initial ammonia concentrations. After a four-day incubation at room temperature, isolate ASAT-8 consistently displayed the lowest residual ammonia levels. The actual ammonia concentrations and statistical comparisons are presented in a graph comparing residual ammonia levels for ASAT-8, -17, and -21, with error bars to illustrate consistency and variation. This isolation was subsequently subjected to DNA sequencing to determine its taxonomic identity. Based on partial 16S rRNA gene sequencing, ASAT-8 was definitively identified as Bacillus cereus, exhibiting a remarkable 99% similarity to the reference sequence.

Conclusion and Discussion

This study isolated and identified bacterial species capable of organic matter decomposition from local aquaponic systems operated by farmers in Phetchaburi, Thailand. Twenty-one isolates were capable of being enriched on Pep-Beef-AOB medium, exhibited positive nitrification activity, and decomposed organic matter. One isolate, ASAT-8, which showed high ammonia-oxidizing activity, was identified through partial 16S rRNA gene sequencing as Bacillus cereus with 99% similarity. Since Bacillus cereus is ubiquitous in environmental soil and water, the result might indicate the dependence of local farmers on natural resources. Bacillus spp. can act as effective decomposers of organic matter and plant growth-promoting bacteria, which could benefit aquaponic systems by maintaining water quality and promoting plant growth. The incorporation of Bacillus spp. may reduce the need for water changes, minimize the release of fish waste, increase productivity in aquaponics, and ultimately contribute to the advancement of a zero-waste food production system.

The finding that Bacillus cereus possesses dual functionality for both nitrification and organic matter decomposition is a significant contribution to the field of microbiology. While previous studies have noted its role as a plant growth promoter or a single-function degrader, our findings highlight its multifaceted role, suggesting its potential as a comprehensive bio-inoculant for aquaponics.

Bacillus cereus is recognized for its ability to produce spores and withstand harsh conditions, making it a robust candidate for commercial bio-inoculants. However, it is also essential to acknowledge its potential risks, such as certain strains causing foodborne illnesses. Therefore, further research is needed to ensure the safety of the specific ASAT-8 strain before recommending its widespread use. Acknowledging these potential risks and benefits provides a balanced discussion.

A limitation of this study is the reliance on partial 16S rRNA gene sequencing for bacterial identification. While this method provides a high level of confidence for genus-level identification, species-level misidentification can occur. We also acknowledge the study's sampling constraints, which are limited to a small number of local systems, potentially affecting the generalizability of our findings.

Future Work

This study provides a foundational understanding of the microbial roles in aquaponic systems in tropical regions, specifically identifying Bacillus cereus as a potential dual-purpose bio-inoculant capable of both nitrification and organic matter decomposition. Our findings advance the field by demonstrating the utility of native microbial strains for improving system performance and sustainability.

Based on these findings, we recommend conducting further research to trial B. cereus in various crop-fish combinations to assess its performance under different conditions. The development of a practical application protocol for farmers, such as an on-site culturing method, would facilitate the widespread use of this native strain to enhance the economic and environmental viability of small-scale aquaponic systems. We suggest that local farmers consider introducing the ASAT-8 strain by preparing a culture from a sterile stock and adding it directly to their biofilter or grow beds. Regular monitoring of water quality parameters, such as ammonia, nitrite, and nitrate, will help them gauge the strain's effectiveness and maintain a balanced microbial community.

We also suggest that future research should focus on the long-term effects of introducing a single bacterial strain on the overall structure and function of the microbial community. Studies could explore the optimal population density for the ASAT-8 strain, its persistence in the system, and its potential for scaling up for commercial production.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflicts of Interest: The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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