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A Comparative Analysis of Biofloc-Producing *Klebsiella pneumoniae* Isolated from Various Sources

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Abstract

Biofloc technology is an innovative approach in aquaculture and wastewater treatment that leverages the flocculating abilities of microbial communities to improve water quality and enhance productivity. This study aimed to isolate and characterize biofloc-producing bacteria from shrimp pond environments in Visakhapatnam. Four distinct shrimp pond locations were selected, and water samples were incubated in an Imhoff cone to promote biofloc formation under laboratory conditions. Bacterial strains were isolated and identified based on their biochemical profiles and biofloc production capabilities. The results showed that biofloc production began at 48 h, and BMV-2, KBF-111, KDK-1, and BGP-6 reached 30 ml/L at 72 h. Biochemical tests and molecular analysis showed a high similarity to *Klebsiella pneumonia*. This study underscores the potential of these bacterial strains for aquaculture treatment, highlighting their optimal biofloc production times and diverse metabolic capabilities. These findings support the application of biofloc technology in sustainable aquaculture practices and environmental management.

Introduction

Biofloc technology is an emerging method for aquaculture and wastewater treatment that harnesses the potential of microbial communities to improve water quality and enhance productivity. The study of biofloc-producing bacteria encompasses a broad range of microorganisms, with potential applications in aquaculture and environmental management. Among these, *Staphylococcus sp*. has been identified as a significant bioflocculant-producing bacterium, achieving a high protein concentration in *Penaeus vannamei* ponds in Malaysia, which highlights its potential for extracellular polymeric substance production (Kasan et al., 2016). Bacillus species found in marine habitats have shown the capacity to make bioflocculants that exhibit strong flocculating activity. This indicates that these bioflocculants could be valuable in various industries (Ugbenyen & Okoh, 2013).

Further research has expanded the list of potential bioflocculant-producing bacteria, including *Bacillus cereus*, *Bacillus pumilus, Nitratireductor aquimarinus,* and *Pseudoalteromonas sp*., which have been recognized for their high potential to enhance bioflocculation in aquaculture wastewater treatment (Che Hashim et al., 2018). *B. subtilis* demonstrated a remarkable flocculation rate of 98.20% when applied to algal-polluted water, indicating its efficacy for industrial and environmental remediation purposes (Jayaprakash et al., 2023).

Marine bacterial strains, such as *Bacillus sp.* from Algoa Bay in South Africa, have been shown to produce polysaccharide-based bioflocculants with high flocculation activity, offering a sustainable alternative to conventional flocculants for water treatment (Ntozonke et al., 2017). Additionally, the process of selecting bioflocculant and cellulase-producing bacterial strains has highlighted the significance of using cost-effective

carbon sources, such as sugarcane bagasse, in biofloc culture systems to enhance the creation of biofloc and increase the growth of prawns. (Wu et al., 2022).

Proteus mirabilis and other bacterial isolates, such as *Aeromonas simiae* and *Exiguobacterium profundum*, have high flocculating activity values; additionally, this emphasises the wide range of uses for bioflocculant-producing bacteria, such as their use in aquaculture for biofloc production and in wastewater treatment (Nkosi et al., 2021; Al Khafaji et al., 2023). The inventive utilization of bioflocculants from microorganisms such as *Citrobacter sp*., which uses waste sludge digestion liquor, presents innovative strategies for environmental management (Fujita et al., 2001).

The objective of this study is to isolate, screen, and identify biofloc producing bacteria from various sources. A comparative analysis was conducted to compare the biofloc-producing bacteria isolated from different sources. It also checks the potential of biofloc production under laboratory conditions to improve aquaculture productivity and offers sustainable alternatives to conventional approaches.

Materials and Method

Sites

The experiment was conducted across four distinct locations in Visakhapatnam, Andhra Pradesh, India, which is known for its abundant shrimp pond water. These sites included Konisa, Vizianagaram (18.2908° N, 83.2947° E), Kondakarla Lake (17.60492° N, 83.00072° E), Bhagporaum (18.0320° N, 83.4996° E), and Bhimavaram (16.5449° N, 81.5212° E). Each area was chosen to provide a diverse range of environmental conditions and water characteristics, ensuring a comprehensive assessment of the diversity and effectiveness of biofloc-producing bacteria in various aquatic settings.

Collection of Samples

Two liters of water were collected in pre-sterilized plastic containers. The containers were rinsed before water collection to ensure cleanliness and accuracy. After sealing, the samples were transported to the laboratory before 10:00 AM. Each sample was labelled appropriately to ensure correct identification and traceability.

Water Incubation for the Production of Biofloc

One liter of water was incubated in a pre-sterilized Imhoff cone using an aquarium air pump. Incubation was performed in complete darkness at 28°C. To facilitate the growth and production of biofloc, 25 -30 ml of Biofloc Standard Medium (BSM) nutrient solution was added every 24 h. This process was repeated with the supplementing of 25-30 ml BSM until the volume of the flocs exceeded 5 ml. Once this threshold was reached, flocs were collected for bacterial isolation (Saeedi & Chapara, 2024).

Isolation of Bacteria

To isolate bacteria, 10 ml of the incubated biofloc sample was collected in 15 ml centrifuge tubes, and the sample was centrifuged at 3000 rpm for 15 minutes. The liquid portion was discarded, and the solid portion was mixed with an equal volume of sterilized saline water and vigorously agitated. Subsequently, the mixture underwent another round of centrifugation at 3000 rpm for 15 minutes, after which the liquid portion above the sediment was removed and discarded. Ten milliliters of saline was added to the resulting pellet, and the solution was labeled as a stock for bacterial isolation. Bacteria-producing biofloc was obtained through serial dilution and plating. The sample was serially diluted, with each dilution increasing its volume by a factor of ten. The diluted samples were then plated onto nutrient agar medium containing 0.5-3% NaCl and TCBS agar. The dilutions used were 10^{-4}, 10^{-5}, and 10^{-6}. After incubation at 37°C for 24 hours, colonies were selected based on variations in their visual characteristics, such as their look, size, and color (Aneja, 2005). After enrichment, samples were streaked onto Nutrient Agar plates for isolation of pure cultures, Moreover, other selective media, such as MacConkey agar, which inhibits the growth of non-target bacteria and allows *K. pneumoniae* colonies to be easily identified, were also used.

Screening of Biofloc-producing Bacteria

Preparation of Inoculum

The bacterial cultures derived from the initial generation were assessed for their capacity to generate bioflocs. The growth of microorganisms was accelerated on a nutrient agar plate in a short period of time. A loopful of 15 to 18-hour-old culture was introduced to 10-15 ml of nutrient broth containing 0.5-3% sodium chloride. The culture was agitated until the turbidity reached a range of 0.5 nm to 0.7 nm at 600 nm, indicating the existence of bacterial growth and activity. Afterwards, the active bacteria were used as an inoculum to test for biofloc formation.

Screening

Each bacterium was examined separately for biofloc production. The isolates were examined in 1ppt Biofloc Standard Medium Water (BSMW) using Imhoff cones, with the addition of 25-30 ml BSM every 24 hours (Saeedi and Chapara, 2024). The isolates that showed positive biofloc production were then analyzed using biochemical and molecular methods for identification.

Morphostructure Observation of Biofloc

The morphostructure of biofloc was observed under an HD Digital LCD biological microscope (Model: AS3116) at 4x, 10, 40, and 100x magnification.

Bacterial Identification

After isolation and screening, the next crucial step was to identify biofloc-producing bacteria to confirm their species accurately.

Morphological Analysis

Initial identification based on colony morphology was performed. *K. pneumoniae* colonies are small, mucoid, and shiny.

Biochemical Tests

A series of biochemical tests, including the IMViC series (Indole, Methyl Red, Voges-Proskauer, and Citrate tests) (McDevitt, 2009), urease test, and sugar fermentation tests (Reiner, 2012) were conducted.

Molecular Identification

DNA was extracted from KBF-111, KDK-01, BMV-02, and BGP-06 strains. When evaluating the quality of the DNA, a 1.0% agarose gel revealed a single band of high molecular weight DNA. 16SrRNA-F and 16SrRNA-R primers were used to amplify a fragment of the 16S rRNA gene. A single 1500 bp distinct PCR amplicon band was seen when resolved on an agarose gel. Using BDT v3, the PCR amplicon's forward and reverse DNA sequencing reactions were performed using 16SrRNA-F and 16SrRNA-R primers. An ABI 3730xl genetic analyzer and a one-cycle sequencing kit were used. The NCBI GenBank "nr" database and the 16S rRNA gene sequence, BLAST, were performed. The multiple alignment software programme Clustal W was used to pick and align the first 10 sequences based on the maximum identity score. Utilizing MEGA 10, a distance matrix and a phylogenetic tree were created.

Monitoring and Optimization

To ensure optimal conditions for biofloc formation, water quality parameters and biofloc characteristics (e.g., floc size, microbial composition, and aeration) were regularly monitored.

Results

Four bacterial strains isolated from different sources produced bioflocs and exhibited similar biofloc morphologies. All strains formed white micro-bioflocs. Microscopic observations of biofloc were conducted under a biological microscope at magnifications of 4x, 10x, 40x, and 100x. At 4x magnification, the overall structure and distribution of biofloc aggregates were visible, providing a broad view of their formation. At 10x magnification, more detailed clusters of microorganisms within the biofloc were observed. At 40x magnification, the individual bacterial cells and extracellular polymeric substances (EPS) binding them together became more distinct. Finally, at 100x magnification, the microbial community's intricate details and interactions within the biofloc matrix were visible, allowing us to identify the type of bacteria and its purity for in-depth analysis of the biofloc composition and structure.

Table 1 provides a comprehensive biochemical profile of four strains isolated from different sources, coded as KBF-111, KDK-01, BMV-01, and BGP-6. Each strain was subjected to a series of biochemical tests to determine its growth characteristics, metabolic activities, and enzymatic capabilities.

The biochemical characterization of four isolates (KBF-111, KDK-01, BMV-02, BGP-06) reveals several consistent and significant insights into their metabolic and enzymatic capabilities. Morphologically, all isolates exhibit similar traits, presenting tiny, mucoid, and glistening colonies and demonstrating Gram-negative small rod-shaped cells. None of the isolates form endospores or exhibit motility, aligning with typical *Klebsiella pneumoniae* characteristics.

Biochemically, all isolates tested positive for KOH and catalase tests, indicating their capability to break down hydrogen peroxide. As expected for *Klebsiella pneumoniae*, they tested negative for the oxidase test, confirming the absence of cytochrome oxidase. In the IMViC tests, all isolates were negative for indole production, and the methyl red test suggested they did not produce indole or perform mixed acid fermentation. Conversely, they were positive for the VP test, indicating acetoin production from glucose fermentation, and they also utilized citrate as a sole carbon source.

The isolates demonstrated robust growth at 42°C, reflecting their thermotolerance, but did not survive an 80°C heat shock, consistent with *Klebsiella pneumoniae's* non-heat-resistant nature. They showed strong growth in 0% NaCl, moderate growth in 6% NaCl, weak growth in 8% NaCl, and no growth in 10% NaCl, indicating moderate halotolerance.

In carbohydrate fermentation tests, all isolates fermented glucose, fructose, cellulose, sucrose, trehalose, cellobiose, lactose, and salicin, but not arabinose, showcasing their metabolic versatility. For amino acid decarboxylase activity, all isolates were positive for lysine decarboxylase but negative for arginine and ornithine decarboxylases, suggesting a specific decarboxylation profile useful for identification.

The isolates did not grow on TCBS agar, confirming they are not Vibrio species, and produced light red colonies on Congo red agar, indicating the production of certain extracellular polymers. On MacConkey agar, they formed white yellowish colonies, typical for non-lactose fermenting Klebsiella species. None exhibited

hemolysis on blood agar, indicating they do not lyse red blood cells.

Regarding enzymatic activities, all isolates were negative for starch and gelatin hydrolysis, indicating the absence of amylase and gelatinase, respectively. However, they were positive for esculin hydrolysis and nitrate reduction, demonstrating their ability to hydrolyze esculin and reduce nitrate to nitrite, which is consistent with *Klebsiella pneumoniae* characteristics. This comprehensive biochemical profile underscores these isolates' metabolic flexibility and potential application in environments requiring specific biochemical capabilities.

Production of Biofloc

Individual bacterial isolates were tested for biofloc production, and each demonstrated similar levels of biofloc formation (Figure. 1).

The graph shows the biofloc volume produced by different bacterial strains over time, measured in milliliters per liter (ml/L). At 0 h, no biofloc production was observed in any strain. After 24 h, no production was still observed. However, between 24-48 h, the strains began to show biofloc production; KBF-111 and BGP-6 each produced 15 ml/L, KDK-1 produced 18 ml/L, and BMV-2 produced the highest amount at 20 ml/L. At 72 h, biofloc production increased significantly, with KBF-111, KDK-1, and BGP-6 each producing 30 ml/L, while BMV-2 produced 25 ml/L. Finally, at 90 h, the biofloc volume for KBF-111 remained constant at 30 ml/L, for KDK-1 slightly decreased to 25 ml/L, for BMV-2 remained steady at 25 ml/L, and that for BGP-6 decreased to 25 ml/L. These data indicated that all strains peaked production between 48 and 72 h, with slight variations in the final volumes at 90 h.

Molecular Result

The molecular results obtained in the study are given in Table 2 and Figure 2.

Table 1. Biochemical test of strains isolated from different sources

	Parameter	KBF-111	KDK-01	BMV-02	BGP-06
Morphological observation	Colony	tiny, mucoid, glistening	tiny, mucoid, glistening	tiny, mucoid, glistening	tiny, mucoid, glistening
	Gram's reaction	-	-	-	-
	Cell shape	Small rod	Small rod	Small rod	Small rod
	Endospore forming ability	Absent	Absent	Absent	Absent
	Motility	-	-	-	-
Biochemical Test	KOH	+	+	+	+
	Catalase	+	+	+	+
	Oxidase	-	-	-	-
IMViC test	Indole	-	-	-	-
	MR	-	-	-	-
	VP	+	+	+	+
	Citrate Utilization	+	+	+	+
Salt and Temperature tolerance	42°C	+++	+++	+++	+++
	80°C heat shock	-	-	-	-
	0% NaCl Agar	+++	+++	+++	+++
	6% NaCl Agar	++	++	++	++
	8% NaCl Agar	+	+	+	+
	10% NaCl Agar	-	-	-	-
Carbohydrate fermentation test	Arabinose	-	-	-	-
	Cellobiose	+	+	+	+
	Cellulose	+	+	+	+
	Fructose	+	+	+	+
	Glucose	+	+	+	+
	Lactose	+	+	+	+
	Salicin	+	+	+	+
	Sucrose	+	+	+	+
	Trehalose	+	+	+	+
Amino acid decarboxylase test	Arginine	-	-	-	-
	Lysine	+	+	+	+
	Ornithine	-	-	-	-
Selective media growth	TCBS	-	-	-	-
	Congo red Agar	Light red	Light red	Light red	Light red
	MacConkey agar	White yellowish	White yellowish	White yellowish	White yellowish
	Blood hemolysin Agar	-	-	-	-
Enzymatic activities	Esculin	+	+	+	+
	Gelatine (Agar)	-	-	-	-
	Nitrate reduction	+	+	+	+
	Starch (Agar)	-	-	-	-

+++ indicates strong positive growth, ++ medium growth, and + indicates weak growth. This is only applicable to salt tolerance tests, and the remaining single + indicates positive results.

Evolutionary Analysis by Maximum Likelihood Method

The evolutionary history was inferred using the Maximum Likelihood approach and the Tamura-Nei model (Kimura, 1980). The tree showcasing the maximum log likelihood (-1881.00) is illustrated. The initial trees for the heuristic search were automatically generated by applying the Neighbor-Join and BioNJ algorithms on a matrix of pairwise distances assessed using the Tamura-Nei model. The topology with the highest log likelihood value was selected. This analysis included a total of 11 nucleotide sequences. The codon positions considered in the analysis were the first, second, and third positions, as well as the noncoding regions. The complete dataset comprised 1226 locations. The MEGA11 software (Kumar et al., 2018) was utilized for conducting evolutionary studies.

Discussion

Biofloc technology is an eco-friendly and cost-effective approach that harnesses the inherent flocculating properties of microbial communities for aquaculture and wastewater treatment. The present research concentrated on the isolation and analysis of biofloc-forming bacteria from shrimp pond ecosystems in Visakhapatnam, Andhra Pradesh, India, aiming to delve into their biofloc production potential and biochemical profile.

Biochemical characterization of the isolated strains (KBF-111, KDK-01, BMV-02, and BGP-6) demonstrated their robust metabolic and enzymatic capabilities. All strains exhibited strong growth at 42°C in low-salinity environments, with moderate growth at 6% NaCl and weak growth at 8% NaCl, indicating thermotolerance and halotolerance. None of the strains showed hemolytic activity or similar growth in colony morphology on MacConkey agar. They were consistently positive for citrate utilization, esculin hydrolysis, urease activity, and the Voges-Proskauer test and could ferment glucose, cellobiose, trehalose, salicin, and xylose, indicating their potential for nutrient recycling and bioremediation. Negative results for starch and gelatin hydrolysis, arginine dihydrolase activity, indole production, and methyl red test highlighted a shared metabolic profile suitable for biofloc technology applications.

The biofloc production dynamics observed in this study provided valuable insights into the temporal growth patterns of various bacterial strains. Initially, no biofloc formation was detected across any strain at 0 and 24 h. However, after 48 h, the strains exhibited significant biofloc production. At 72 h, biofloc production peaked for KBF-111, KDK-1, and BGP-6 at 30 ml/L, whereas BMV-2 reached 25 ml/L. The peak production phase indicates the optimal period for biofloc harvesting for practical applications. Interestingly, at 90 h, the biofloc volume for KBF-111 remained constant at 30 ml/L, while KDK-1 and BGP-6 showed a slight decline to 25 ml/L, suggesting possible stabilization or partial disintegration of the bioflocs over time. BMV-2 maintained a steady production at 25 ml/L, highlighting its potential for consistent biofloc production over an extended period. Overall, they showed highly similar biofloc production. Research conducted by Saeedi and Chapara (2024) demonstrated that bioflocs were generated within 24-hour, with its peak occurring at 48 h and then declining. This finding indicates a divergence from the previous findings (Table 3.).

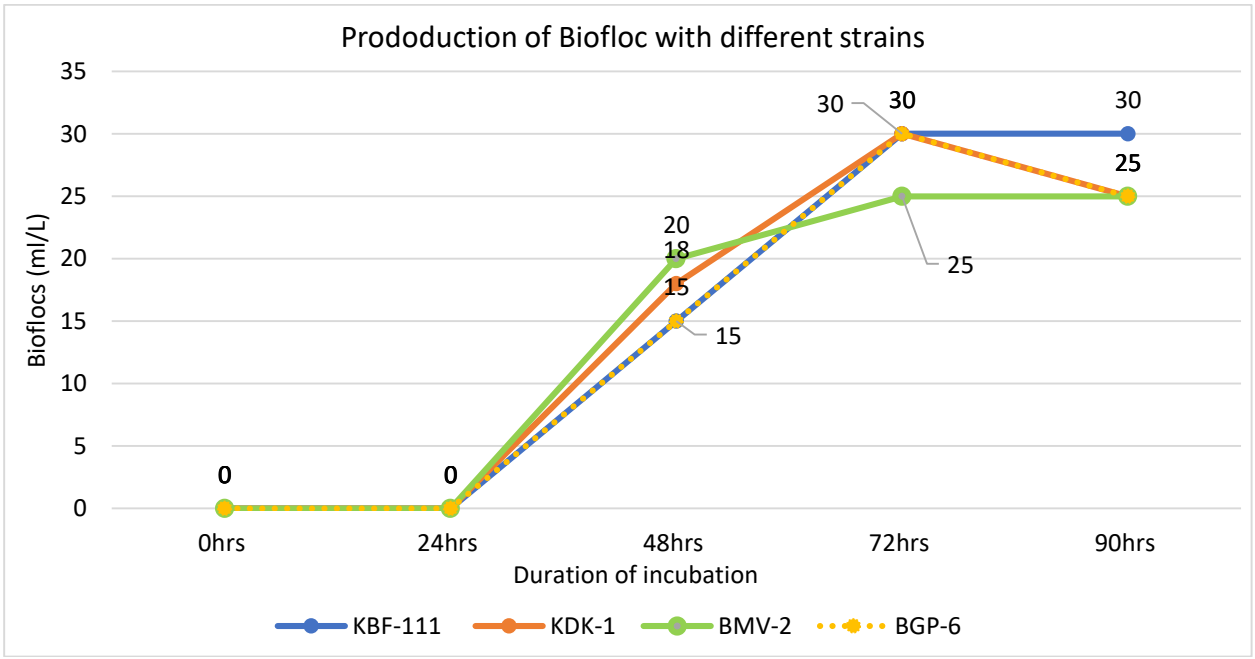

Figure 1. The graph shows biofloc production by four bacterial strains, with formation observed between 24 and 48 hours. Over a period of 90 hours, all strains produced comparable biofloc volumes, indicating similar production timelines and capacities.

Previous studies have identified several bacterial strains with significant biofloc production capabilities. Hashim et al. (2018) reported that *Bacillus cereus, Bacillus pumilus, Nitratireductor aquimarinus, and Pseudoalteromonas sp*. isolated from Pacific whiteleg shrimp culture ponds were effective in aquaculture wastewater treatment. Similarly, Sitorus et al. (2019) demonstrated that lactic acid bacteria, such as Bacillus and Streptococcus, could support aquaculture environments, particularly in red tilapia farming. These studies underscore the importance of specific bacterial strains for maintaining water quality and promoting sustainable aquaculture practices.

Furthermore, Wong et al. (2021) highlighted the role of *Moellerella wisconsensis, Achromobacter xylosoxidans, Burkholderia pseudomallei,* and *Bacillus s*p. in bioconversion processes within aquaponic systems, enhancing plant growth. Soedibya et al. (2023) explored the biofloc applications of *Vibrio navarensis, Staphylococcus gallinarum, Pseudoalteromonas ganghwensis,* and *Cytobacillus kochii* in vannamei shrimp ponds and demonstrated their efficacy in biofloc systems.

Wijayanti et al. (2020) investigated the use of fungi, yeast, and microalgae in brackishwater aquaculture ponds for water quality control and highlighted the potential of diverse microbial communities in aquaculture systems.

The isolation and identification of *K. pneumoniae* in this study add a new dimension to the application of biofloc technology in aquaculture. While the aforementioned studies focused on various bacterial species, none of them specifically addressed the biofloc production capabilities of *K. pneumoniae*. This study bridges this gap by demonstrating that *Klebsiella pneumoniae* can produce biofloc efficiently, thus offering a new bacterial candidate for biofloc systems.

Table 2. Bacterial identification by 16 sRNA sequences producing significant alignments

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Klebsiella pneumoniae strain DSM 30104	2209	2209	100%	0.0	99.18%	NR_117683.1
Klebsiella quasipneumoniae strain 01A030	2207	2207	100%	0.0	99.02%	NR_134062.1
Klebsiella quasipneumoniae strain 07A044	2204	2204	100%	0.0	99.02%	NR_134063.1
Klebsiella pneumoniae strain ATCC 11296	2200	2200	99%	0.0	99.02%	NR_119276.1
Klebsiella africana strain SB5857	2198	2198	100%	0.0	99.02%	NR_180233.1
Klebsiella pneumoniae strain NBRC 14940	2194	2194	100%	0.0	98.86%	NR_113702.1
Klebsiella pneumoniae strain R-70	2194	2194	99%	0.0	99.02%	NR_037084.1
Klebsiella pneumoniae strain JCM 1662	2194	2194	100%	0.0	98.86%	NR_113240.1
Klebsiella pneumoniae strain ATCC 13883	2193	2193	100%	0.0	98.94%	NR_114506.1
Klebsiella pneumoniae strain DSM 30104	2187	2187	100%	0.0	98.86%	NR_117686.1

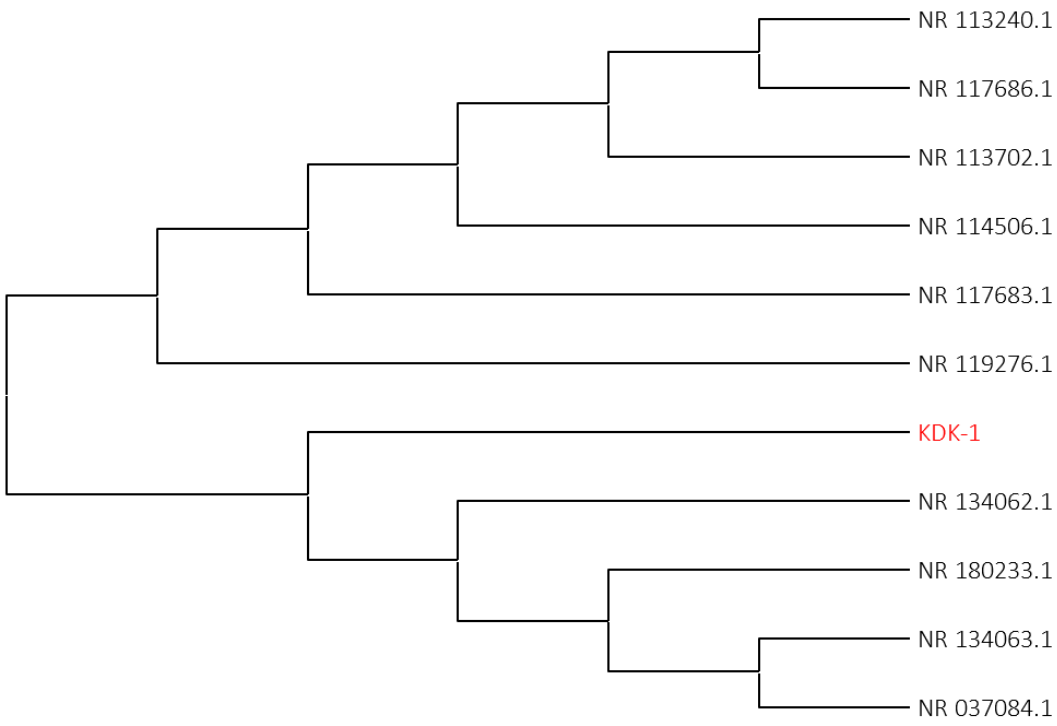

Figure 2. Phylogenetic tree showing evolutionary relationship with closely related strains.

K. pneumoniae's ability to form bioflocs suggests its potential utility in enhancing water quality and nutrient recycling in aquaculture systems. This finding is significant, as it opens new avenues for research and application, particularly in optimizing biofloc production and understanding the genetic and metabolic pathways involved.

The findings of this study underscore the significant potential of biofloc-producing bacteria in enhancing aquaculture productivity and wastewater treatment. The temporal dynamics of biofloc production indicate optimal harvest times, which are crucial for maximizing the efficiency of biofloc systems. Biochemical characterization provides a foundational understanding of the metabolic capabilities of these bacteria, facilitating their targeted application under different environmental conditions. For instance, the ability of these strains to grow under low-salinity conditions and their moderate halotolerance makes them suitable for various aquaculture settings, including inland shrimp farming, where salinity levels can fluctuate. Moreover, their diverse metabolic pathways and enzymatic activities can aid in the bioremediation of organic pollutants and recycling of nutrients, contributing to improved water quality and sustainable aquaculture practices.

Future research should explore the genetic mechanisms underlying *Klebsiella pneumoniae* biofloc production and its interactions within microbial communities. Additionally, optimizing culture conditions to maximize biofloc production and evaluating the impact of *Klebsiella pneumoniae* biofloc on aquaculture productivity and water quality is crucial. Comparative studies with other known biofloc-producing bacteria will also be valuable in positioning *Klebsiella pneumoniae* within the broader context of biofloc technology.

Conclusion

This research emphasizes the diverse functionalities of biofloc-producing bacteria that have been isolated from various sources. The isolation, identification, and production of biofloc using *K. pneumoniae* from diverse sources demonstrate a promising approach for the aquaculture industry. The findings indicate that *K. pneumoniae* produces significant amounts of bioflocs, and the floc size is appropriate and suspended in nature, which the cultured organism can readily utilize. Furthermore, this study contributes to the expanding understanding of biofloc-producing bacteria, highlighting *K. pneumoniae* as a promising candidate for biofloc production in aquaculture. Incorporating *K. pneumoniae* into biofloc systems can benefit aquaculture operations by enhancing water quality and nutrient management, ultimately promoting productivity and sustainability.

Limitation

Although the bacterial strains have shown exceptional biofloc-producing capabilities when isolated, their application in aquaculture is limited due to the controlled conditions under which these results were obtained in the laboratory. In addition, the effectiveness of these bacteria in real aquaculture environments may be impacted by several factors, such as changes in water quality, the presence of pathogens, and fluctuations in ambient conditions. Consequently, further research is necessary to confirm these findings in practical aquaculture settings and to assess the efficacy and dependability of the bacteria under a range of varying and fluctuating circumstances.

Ethical Statement

No ethical concerns arose during the course of this study.

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This research was not financially supported; it is a component of the doctoral dissertation of the first author.

Table 3. Beneficial Biofloc-producing bacteria and microorganisms isolated from different aquaculture sources

Isolated Bacteria/other Microorganism	Source	Application	Reference
Bacillus cereus, Bacillus pumilus, Nitratireductor aquimarinus, and *Pseudoalteromonas sp*.	Pacific white leg shrimp, *Litopenaeus vannamei* culture ponds	Aquaculture wastewater treatment	Che Hashim et al., 2018
Lactic acid bacteria (Bacillus, Streptococcus)	Red tilapia aquaculture	Supporting aquaculture environments	Sitorus et al., 2019
Moellerella wisconsensis, Achromobacter xylosoxidans, Burkholderia pseudomallei, Bacillus sp.	Aquaponics system	Bioconversion processes for plant growth	Wong et al., 2021
Vibrio Navarensis, Staphylococcus gallinarum, Pseudoalteromonas ganghwensis, Cytobacillus kochii	Vannamei shrimp ponds	Biofloc applications	Soedibya et al., 2023
Fungi, yeast, and microalgae	Brackishwater aquaculture ponds	Control water quality in aquaculture	Wijayanti et al., 2020
Bacillus cereus	Aquaculture Shrimp Pond	Biofloc Application	Saeedi & Chapara, 2024

Author Contribution

The first author developed the topic, collected and organized the data, conducted the investigation, wrote the initial draft, reviewed it, and revised the paper. The manuscript was supervised and edited by the second and third authors.

Conflict of Interest

The authors assert that they do not have any identifiable competing financial, non-financial, professional, or personal conflicts that may have impacted the research presented in this study.

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