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INVESTIGATION ON THE OCCURRENCE, SALINITY TOLERANCE, AND NON - VIRULENCE CHARACTERISTICS OF BACTERIAL STRAINS OBTAINED FROM WATER AND SEDIMENT SAMPLES OF THE MAYAMI SALINE ENVIRONMENT

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Abstract

Halotolerant bacteria are increasingly recognized for their potential in biotechnological applications, particularly in saline wastewater treatment. This study investigated the occurrence, salinity tolerance, and virulence characteristics of bacterial isolates obtained from salty water and sediment samples of the Mayami saline environment. Standard plate count techniques revealed high bacterial loads ranging from 1.80×10^6 to 3.30×10^6 CFU/g, indicating that the environment supports abundant halotolerant microbial populations. Salinity tolerance assays showed variable adaptability among isolates, with strain MS5 demonstrating the highest tolerance (2 – 10 % NaCl), while others exhibited limited growth at elevated salinity. Virulence screening using blood agar, Indian ink, and Coomassie brilliant blue assays confirmed that selected isolates (MS4 and MS5) were non-virulent, apathogenic, and non-haemolytic. These findings suggest that the isolates, particularly MS5, are promising candidates for safe application in saline wastewater bioremediation. The study highlights the ecological and biotechnological relevance of halotolerant bacteria in hypersaline environments.

Keywords: Apathogenic, Biotechnological relevance, non-haemolytic, non-virulent, Salinity tolerance

INTRODUCTION

Hypersaline environments such as saline rivers, lakes, and sediments are known to harbor diverse communities of halotolerant and halophilic microorganisms capable of surviving under high osmotic stress. These microorganisms have evolved adaptive mechanisms, including osmolyte accumulation and ion transport regulation, which enable them to thrive in extreme saline conditions (Oren, 2013; Uba *et al.*, 2020a; 2020b; Alisa *et al.*, 2020; Anukam *et al.*, 2020a; 2020b; Egurefa *et al.* 2020a; 2020b; Umeh *et al.,* 2020; 2021; Uba e*t al.,* 2021a; 2021b; Okolo *et al.*, 2025; Okpalaunegbu *et al.*, 2025; Obiefuna *et al.* 2025). Such adaptations make them valuable in environmental and industrial processes, particularly in the treatment of saline and hypersaline wastewater where conventional microorganisms often fail (Nkamigbo *et al.* 2020a; 2020b; Njoku *et al.* 2019a; 2019b).

Sevral studies have emphasized the role of halotolerant bacteria in the biodegradation of organic pollutants, including the reduction of chemical oxygen demand (COD) in saline effluents (Anichebe *et al.*, 2019; Uba, 2019a; 2019b; 2019c; Uba *et al.*,;Uba *et al.*, 2019b; 2019c; Nemec *et al.*, 2020; Nwigwe *et al.* 2022, Nwigwe *et al.* 2023, Ifediegwu *et al.* 2023a; 2023b; Ifediegwu *et al.* 2024a, 2024b; 2024c; Nnaka *et al.*, 2024;Uba *et al.* 2024; Mere *et al.* 2025; Enemchukwu *et al.* 2026a; 2026b). However, the selection of suitable bacterial strains for such applications requires not only high salt tolerance but also confirmation of non-pathogenicity to ensure environmental and public health safety (Uba *et al.* 2016; Uba *et al.*, 2017; Uba *et al.* 2018a; 2018b; 2018c; 2019d; 2019e; Cappuccino & Sherman, 2021;Ofunwa *et al.* 2024; Ezeamama *et al.*, 2025a; 2025b).

In this study, halotolerant bacteria were isolated from water and sediment samples of the Mayami salty river. The bacterial load of the samples was quantified, revealing high colony counts (10^6 CFU/g), which is consistent with previous reports that saline ecosystems support dense microbial populations. The isolates were further evaluated for their salinity tolerance across increasing NaCl concentrations (2 – 10 %), as well as for virulence-associated traits using standard microbiological assays, including haemolysis on blood agar, capsule detection via Indian ink, and protein-binding characteristics using

Coomassie brilliant blue medium. The aim of this study was to identify halotolerant bacterial strains with high salt tolerance and low virulence potential, suitable for potential application in saline wastewater bioremediation.

MATERIALS AND METHODS

Sample Description

Saline was obtained from Mayami brackish water, pond water, salt pans, and saline lake waters located in Nembe, Bayelsa State, South–South Nigeria. These environments, characterized by high salinity levels and organic pollutant content, are known to support diverse halophilic microorganisms adapted to saline conditions.

Sample Collection

Approximately 3,000 mL of surface water was collected in sterile 4 L plastic containers and transported to the laboratory within 24 hours for microbial isolation. Immediately after collection, salinity, temperature, and pH of the surface water were determined (Uba and Chukwura, 2016; Huan, 2017; Uba *et al.,* 2020h).

Sample Dilution and Isolation

Samples were serially diluted up to 10^{-4} and inoculated onto saline nutrient agar plates containing 5% NaCl to enrich halophilic organisms. The plates were incubated at 37 °C for 2 – 5 days. Colonies showing growth will be documented in CFU/mL and purified by repeated streaking until pure cultures are obtained (Sivaprakasam *et al.*, 2008; Okafor *et al.* 2021a; 2021b; Oghonim *et al.,* 2026; Iheukwumere *et al.*, 2012a; 2012b; Mundi *et al.*, 2013; 2014).

Screening for Potent Salt Tolerance

Five morphologically distinct colonies with good surface growth on saline nutrient agar were selected and subjected to salt tolerance screening. Using basal mineral salt (BMS) agar containing NaCl concentrations of 2%, 4%, 6%, 8 % and 10 %, growth were monitored to evaluate the tolerance range and adaptation time of the halophilic isolates (Huan, 2017; Okoye *et al.* 2014; Okoye *et al.* 2016a; 2016b).

Identification of Halotolerant Bacterial Strains

The isolates were subjected to classical identification based on microscopic morphology and biochemical tests after which they were applied for wastewater treatment (Uba *et al.* 2020c; 2020d; 2020e; 2020f; 2020g; 2020h; Dokubo *et al.*, 2024)

Virulence and Toxicity Testing of Test Strains

Indian ink inclusion broth test

Following method of Behera *et al.* (2013), Okeke *et al.* (2025a) and (2025b), 50 µL of 24 h bacterial cultures was inoculated into tryptic soy broth (TSB) containing 10% Indian ink (0.45 µm filtered). Cultures was incubated at 37 °C for 10–12 h, and degradation of ink indicates capsular virulence. To check for A-layer protein virulence factors, positive cultures was streaked on Coomassie Brilliant Blue (CBB) agar (0.01% in TSB agar) and incubated anaerobically at 37 °C for 24 h. Blue colonies were considered pathogenic, while white colonies were classified as non-pathogenic.

Haemolytic test

Blood agar plates were streaked with bacterial isolates and incubated at 37 °C for 24 h. Hemolysis were observed as: α-hemolysis (greenish zone around colonies), β-hemolysis (clear, sharp zone around colonies indicating complete lysis), and γ-hemolysis (no change in agar, indicating no lysis). The test was carried out using 5% citrated human blood agar as described by Niederstebruch *et al.* (2017), Anameze *et al.*, (2023) and Umezulora *et al.* (2026).

RESULTS AND DISCUSSION

Table 1 presents the halotolerant bacterial counts obtained from different salty water and sediment samples from the Mayami environment. The results showed appreciable microbial populations across both water and sediment samples, with mean counts ranging from 1.80 × 10^6 to 3.30 × 10^6 CFU/g. Mayami salty river sample A recorded the highest mean bacterial count, indicating a favorable environment for halotolerant bacteria, possibly due to higher nutrient availability and optimal salinity conditions. The sediment samples also supported substantial microbial growth, reflecting their role as reservoirs for salt-tolerant microorganisms. Overall, the table demonstrated that the study area harbours a rich population of halotolerant bacteria, justifying their selection for further physiological and molecular analyses. This observation is consistent with several previous reports that saline and hypersaline habitats support diverse and abundant microbial communities capable of adapting to osmotic stress through physiological and metabolic mechanisms (Oren, 2011; Ventosa *et al*., 2015; Dokubo *et al*., 2022a; 2022b; Anidu *et al*., 2023; Obiefoka *et al*., 2023; Ubani *et al*., 2024a; 2024b; 2025; Ekwenze *et al*., 2025; Ele *et al*., 2025; Uba and Okonkwo *et al*. 2025; Ubajekwe *et al*., 2025; Uba *et al*., 2025; Okwonkwo *et al.* 2026; Uba *et al.,* 2026a; 2026b; 2026c).

Table 1: Halotolerant bacterial count obtained from different salty water and sediment samples

Sample	10^{-2} Dilution	10^{-4} Dilution	Mean count (CFU/g)
Mayami salty river A	55	65	3.30 X 10^6
Mayami salty river B	38	45	2.30 X 10^6
Mayami sediment A	41	51	2.60 X 10^6
Mayami sediment B	25	35	1.80 X 10^6

Table 2 illustrates the varying salinity tolerance of the halotolerant bacterial isolates across increasing sodium chloride (NaCl) concentrations (2 – 10 %). All isolates exhibited growth at 2 % NaCl, indicating that they are at least mildly halotolerant. However, as salinity increased, growth progressively declined for most strains, reflecting differences in osmoadaptation capacity. At 4 % NaCl, several isolates (MS1, MS2, MS4, MS5, MG6, and MG9) still maintained growth, suggesting moderate halotolerance. Beyond this level, only a few strains sustained growth: MS4 tolerated up to 6 % NaCl, while MS5 demonstrated the highest tolerance by growing across all tested concentrations (2 – 10 %). This identifies MS5 as a highly halotolerant strain, possibly possessing efficient osmoprotective mechanisms such as compatible solute accumulation or ion regulation. In contrast, isolates such as MS3, MG7, and MG8 showed limited tolerance, with growth restricted mainly to 2 % NaCl, indicating low halotolerance. Overall, the results highlight significant variability among the isolates, with MS5 being the most robust candidate for applications in high-salinity environments, such as saline wastewater treatment, where strong salt tolerance is essential. Furthermore, this highlighted both their physiological resilience and potential use in remediating hypersaline industrial effluents such as those from tanneries, oil drilling, and desalination plants (Dokubo and Uba, 2023; Uba and Obiefuna, 2023; Okafor *et al*., 2023). Ventosa *et al.* (2015) attributed this adaptability to the organisms' use of salt-in and compatible solute strategies, which enable osmoregulation and metabolic activity under extreme salinity.

Table 2: Salinity tolerant abilities of the different halotolerant bacterial strains

Salinity level Test strain	Sodium chloride concentration (%)				
	2 %	4 %	6 %	8 %	10 %
MS1	+	+	-	-	-
MS2	+	+	-	-	-
MS3	+	-	-	-	-
MS4	+	+	+	-	-
MS5	+	+	+	+	+
MG6	+	+	-	-	-
MG7	+	-	-	-	-
MG8	+	-	-	-	_
MG9	+	+	-	-	-

Key: + = Positive (Showing growth); - = Negative (Showing no growth)

Halophilic bacteria were effectively isolated from hypersaline sites in Bayelsa State, Nigeria, and subsequently assessed for their ability to tolerate high salinity and facilitate desalination. Two distinct isolates, differentiated by their morphological characteristics, exhibited varying degrees of salt tolerance, supporting earlier findings that microorganisms can survive and adapt in highly saline habitats such as salt pans, saline lakes, and brackish water bodies (Oren, 2013; Okoye *et al.* 2020a; 2020b; 2020c). The biochemical characterization presented in Table 3 indicated that *Acinetobacter calcoaceticus* MS4 is oxidase-negative and non-motile, features that clearly separate it from most *Pseudomonas* species. In contrast, *Pseudomonas* *extremaustralis* MS5 demonstrated oxidase positivity, motility, and a higher level of halotolerance, suggesting its suitability for application in saline wastewater treatment systems. Both isolates were identified as non-fermentative organisms, aligning with their established role in the aerobic breakdown of organic pollutants, particularly in chemical oxygen demand (COD) reduction as reported by several researchers (Nemec *et al*. 2020; Cappuccino and Sherman, 2021; Alfred *et al*. 2023; 2025; Idu *et al*., 2026a; 2026b; Ibo *et al*. 2020; Ibe *et al*. 2023, Chukwura *et al*. 2025; Uba and Udaba *et al*. 2026; Dokubo and Uba, 2026).

Table 3: Biochemical profile of halotolerant bacterial strains

Biochemical test	*Acinetobacter calcoaceticus*	*Pseudomonas extremaustralis*
Gram reaction	Negative	Negative
Cell shape	Coccobacilli	Rod-shaped
Motility	Non-motile	Motile
Catalase test	Positive (+)	Positive (+)
Oxidase test	Negative (−)	Positive (+)
Indole production	Negative (−)	Negative (−)
Methyl red (MR)	Negative (−)	Negative (−)
Voges–Proskauer (VP)	Negative (−)	Negative (−)
Citrate utilization	Positive (+)	Positive (+)
Urease test	Negative (−)	Variable (±)
Nitrate reduction	Positive (+)	Positive (+)
Glucose fermentation	Non-fermentative	Non-fermentative
Lactose fermentation	Negative (−)	Negative (−)
Growth at high salinity	Moderate	High
Pigment production	None	May produce pigments
Gelatin hydrolysis	Negative (-)	Positive (+)
Starch hydrolysis	Negative (−)	Negative (−)
Growth at 42 °C	Negative (−)	Positive (+)

Table 4 presents the virulence-related characteristics of the halotolerant bacterial isolates (MS4 and MS5) using differential media. Both isolates showed negative results in the Indian ink (indicator ink) test, indicating absence of capsule formation, a key virulence factor in many pathogenic bacteria. This suggests that the isolates are likely non-virulent (Cheesbrough, 2020). In addition, growth on Coomassie brilliant blue–incorporated tryptic soy medium produced white colonies for both isolates, which were classified as apathogenic. This observation further supports the lack of pathogenic traits, as virulent strains often exhibit dye-binding properties associated with extracellular proteins and toxins (Forbes *et al*., 2021). Furthermore, both MS4 and MS5 exhibited gamma haemolysis on blood agar, indicating no haemolytic activity. The absence of red blood cell lysis confirms that the isolates do not produce haemolysins, which are important virulence determinants in many pathogenic microorganisms (Cappuccino & Sherman, 2021; Obiefuna *et al*., 2026; Afulukwe *et al.,* 2025; 2026). Overall, the consistent non-virulent and apathogenic profiles of MS4 and MS5 suggest that these halotolerant bacteria are environmentally safe and suitable for applications such as saline wastewater bioremediation, where non-pathogenicity is essential.

Table 4: Growth of the selected halotolerant bacterial strains on blood agar, Indian ink and Coomassie brilliant blue incorporated tryptic soy media

Isolate code	Indicator ink test	Coomassie brilliant blue test			Haemolytic response
	Growth	Classification	Growth	Classification	
MS4	-	Non- virulent	White colonies	Apathogenic	
MS5	-	Non- virulent	White colonies	Apathogenic	
MS4					Gamma haemolysis
MS5					Gamma haemolysis

Key: MS = Isolate code

CONCLUSION

The findings of this study demonstrate that the Mayami saline environment is a rich source of halotolerant bacteria with significant biotechnological potential. High bacterial counts across water and sediment samples confirm the adaptability and abundance of microorganisms in hypersaline conditions. Among the isolates, MS5 exhibited the highest salinity tolerance, growing across all tested NaCl concentrations (2 – 10 %), while MS4 showed moderate tolerance.Importantly, virulence assessment revealed that the selected isolates (MS4 and MS5) are non-virulent, as evidenced by negative capsule formation, absence of haemolysis (gamma haemolysis), and apathogenic colony characteristics. These properties indicate that the isolates are environmentally safe and suitable for practical applications. Overall, the combination of high salt tolerance and non-pathogenicity makes these halotolerant bacterial strains promising candidates for use in saline wastewater treatment and other biotechnological processes. Further studies focusing on their metabolic capabilities, particularly in pollutant degradation and COD reduction, are recommended to fully exploit their potential.

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