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BIOTECHNOLOGICAL POTENTIAL AND PHYSIOLOGICAL ADAPTATION OF POLY-EXTREMOPHILIC *BACILLUS* STRAINS ISOLATED FROM THE HYPERSALINE SEBKHAS OF ADRAR, ALGERIA

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ABSTRACT

The hypersaline and alkaline sebkhas of Adrar (Algeria) constitute extreme ecosystems that have remained largely unexplored. The objective was to isolate and to characterize various *Bacillus* strains extracted from these specific environments. Two poly-extremophilic isolates, *Bacillus tequilensis* (KM9) and *Bacillus paralicheniformis* (KM2), were identified through the analysis of 16S rRNA phylogeny. Physiological testing demonstrated robust adaptations: Isolate KM9 exhibited a broad salinity range (ranging from 2 to 17.0% w/v NaCl, S_{opt} 6%) and strict alkaliphily ($pH_{optimum}$ 9.5, with growth observed up to pH 11.0). In contrast, Isolate KM2 exhibited higher thermal tolerance ($T_{maximum}$ 50°C, $T_{optimum}$ 40°C), while maintaining growth up to 20% NaCl and pH 10.5. Functional profiling via API ZYM (Analytical Profile Index ZYM) revealed Isolate KM9's N-acetyl-β-glucosaminidase activity, whereas Isolate KM2 demonstrated a broad hydrolytic spectrum (including proteases and glycosidases) and potent antimicrobial efficacy against *Staphylococcus aureus* (25 mm), *Pseudomonas aeruginosa* (20 mm), as well as showing 50% inhibition of *Fusarium oxysporum* and *Aspergillus flavus*. These findings underscore the Adrar sebkhas as untapped reservoirs for diverse *Bacillus* strains. Their ability to remain active under simultaneous thermal, saline, and alkaline stress underscores their potential as sources of robust extremozymes and biocontrol agents for industrial biotechnology.

Keywords: *Bacillus paralicheniformis*, halotolerance, adrar sebkha. extremozymes, biocontrol

INTRODUCTION

Extreme environments, defined by elevated salinity, extreme pH levels, and significant thermal variations, constitute distinctive ecological niches that challenge the limits of microbial existence. Notably, hypersaline and alkaline ecosystems, exemplified by sebkhas (salt flats), are particularly intriguing due to the dual selective pressures they impose on microbial physiology. Microorganisms thriving in these habitats, referred to as poly-extremophiles, have developed advanced molecular and physiological strategies to sustain osmotic equilibrium and cytoplasmic homeostasis in the face of combined stress conditions (**Martínez *et al.*, 2023**).

The sebkhas of the Adrar region in Algeria are among the most challenging and under-explored extreme environments in the Sahara. Despite their high conductivity and elevated alkaline pH, these sites remain poorly documented in terms of microbial diversity. While previous studies have explored global alkaliphilic genomics (**Lebre and Cowan, 2019**), the exact taxonomic and functional distribution of the *Bacillus* genus within the Adrar sebkhas is yet to be determined. To our understanding, this research constitutes the first report detailing the isolation and characterization of poly-extremophilic *Bacillus* strains from this particular geographical locale.

Bacillus species, commonly found in soil, have been thoroughly studied in large-scale industrial fermentation processes (**Fiechter, 1992; Zukowski, 1992**). This genus is essential for the production of industrial enzymes, in the fields of medicine and agriculture as a biological control agent and is instrumental in the breakdown of organic matter, generation of biogas, and nitrogen fixation, thus enhancing plant development (**Mackie *et al.*, 1996;Yilmaz *et al.*, 2006 ; Wafula *et al.*, 2014**).

The ability of members of the genus *Bacillus* genera to manufacture a variety of secondary metabolites, as well as to form robust endospores, is well known. The exceptional genetic adaptability of halotolerant *Bacillus* species, including *Alkalihalobacillus okhensis*, in adjusting to halo-alkaline environments has been demonstrated by recent studies (**Krishna *et al.*, 2021**). Furthermore, because of their strong proteolytic and keratinolytic capabilities, many strains of *Bacillus paralicheniformis* have shown considerable promise in industrial biotechnology (**Aktayeva and Khassenov, 2024**). Basic microbiology and applied biotechnology both depend on an understanding of how these bacteria control their metabolism under salt stress (**Yin *et al.*, 2015**).

Because of the intense selection pressures imposed by these particular niches, microbial occupants are forced to develop distinct genetic and metabolic adaptations. Specifically, the Algerian Sahara's sebkhas, especially those in the Adrar region (such as Ouled Mahmoud and Tililane). The natural selection of microorganisms that can produce stable and functional molecules under conditions that normally inactivate conventional compounds, a crucial requirement for commercial application, is their main area of interest for biotechnology (**Singh, 2012**).

In the present study, we describe the isolation of two poly-extremophilic strains, identified as *Bacillus tequilensis* (Isolate KM9) and *Bacillus paralicheniformis* (Isolate KM2), from the Adrar sebkhas. Our objectives were to evaluate their physiological growth limits across gradients of temperature, pH, and salinity (NaCl).To characterize their enzymatic profiles using the API ZYM system. In addition, to assess their antimicrobial and antifungal potential against clinically and agriculturally relevant pathogens. This work identifies novel biological resources by determining the physiological limits and functional capacities of these isolates.

MATERIALS AND METHODS

Collection and Preparation of Soil Samples

The soils were collected from various locations in the Adrar province of Algeria, specifically from two distinct sebkhas: Ouled Mahmoud and Tililane. These samples, obtained at random, were stored in sterile, dry polyethylene sacs and transferred directly to the laboratory for analysis. The geographical coordinates for the sebkha of Ouled Mahmoud are approximately 28° 35′ 49″ N, 0° 02′ 49″ E, while Tililane is located at 27° 54′ 05″ N and 0° 14′ 28″ E, according to Google

Earth (2025)[1]. Three surface horizon samples from each sebkha were collected using a sterile spatula, from a depth of 0-20 cm.

Physico-Chemical Soil Analysis

The moisture percentage (H%) was determined by drying 5 g of soil from each sample in an oven at 105°C until a constant weight was reached, following the method described by **Lee and Hwang (2002)**. The moisture percentage was calculated using the following formula:

$$H\% = \frac{(W_H - W_S)}{W_S} \times 100$$

Where W_H the wet weight and W_S represents the dry weight. Additionally, the pH of each soil sample was measured using a calibrated pH meter in a soil-to-distilled water suspension ratio of 2:5 g/mL. Electrical conductivity (EC) was obtained using a conductivity meter as described by **Thirion (2014)**.

Isolation of *Bacillus* Strains and Biochemical Tests

To improve the selectivity of the *Bacillus* isolation medium, a thermal pre-treatment of 10 min at 80°C was applied to promote the survival of bacterial spores while eliminating vegetative forms. Soil samples underwent serial dilutions from 10^{-1} to 10^{-4} in sterile distilled water, followed by plating on plate count agar (PCA). The medium consisted of (per liter of distilled water): 5.0 g enzymatic digest of casein (Tryptone), 2.5 g yeast extract, 1.0 g glucose (Dextrose), and 15.0 g agar, which was incubated at 30°C for 24 to 48 h. Pure cultures were then obtained through subculturing on the same medium (**Chilcott and Wigley, 1993**). Biochemical characterization was performed using standard microbiological protocols. Catalase activity was determined by the appearance of bubbles in 3% (v/v) H2O2. Oxidase activity was tested using Oxidase discs. Hemolysis was assessed on Blood Agar (5% sheep blood) after 24h at 30°C. Methyl Red (MR) and Voges-Proskauer (VP) tests, Citrate utilization, Nitrate reduction, and Starch hydrolysis were carried out according to the methods described in Bergey's Manual of Systematic Bacteriology (**Goodfellow *et al.*, 2012**).

Phenotypic Characterization and Extreme Tolerance Tests (Adaptation)

The isolated bacterial strains KM9 and KM2 were subjected to detailed phenotypic characterization through morphological, physiological, and biochemical analyses, following protocols established for new isolates with slight modifications (**Benine *et al.*, 2024**). Initial morphological analysis involved microscopic examination of cell shape (cocci, bacilli) and structure, including Gram-staining. Physiological tests encompassed catalase activity and the ability to grow across various extreme conditions as outlined in Bergey's Manual of Determinative Bacteriology (**Goodfellow *et al.*, 2012**). Halotolerance was determined by culturing isolates in Luria-Bertani (LB) supplemented with increasing NaCl concentrations, typically ranging from 0% up to 25% (w/v), pH tolerance (alkaliphily) was assessed by adjusting the medium across a range of pH 7.0 to pH 11.0 to determine the maximal pH tolerated (pH_{max}) and the optimal pH (pH_{opt}). Temperature tolerance was determined across a wide thermal range, including psychrophilic (4°C), mesophilic (25°C, 37°C), and thermotolerant (45°C to 60°C) temperatures, to define the minimal (T_{min}), optimal (T_{opt}), and maximal (T_{max}) growth temperatures. Growth was quantified by measuring Optical Density (OD) at 600 nm or by CFU counts after 48 to 72 h of incubation (**Benine *et al.*, 2024**).

Screening of Antibacterial Activity of Isolated Strains

Testing Microbial Pathogens

Test bacteria pathogen for the experiment included 10 strains: four Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 49452, *and Enterococcus hirae* ATCC 10541) and six Gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 25837, *Klebsiella pneumoniae* ATCC 52145, *Citrobacter freundii* ATCC 5732, *Salmonella enterica* ATCC 5630, and *Proteus mirabilis* ATCC 783CI), plus one yeast strain (*Candida albicans* ATCC 10231). Furthermore, a phytopathogenic fungus (*Fusarium oxysporum*) and the aflatoxigenic fungus *Aspergillus flavus* were included.

Cross Streak Method

Luria-Bertani (LB) agar plates were inoculated with *Bacillus* species, incubated at 37 °C for 48 h, and then seeded with indicator by a single streak at a 90° angle to the *Bacillus* species. The microbial interactions were assessed by measuring the inhibition distances formed around the *Bacillus* strains (**Madigan *et al.*, 1997**).

Disc Diffusion Method

Disc diffusion method for testing antimicrobial susceptibility was conducted according to the standards of **Bauer *et al.* (1966)** to evaluate the antibacterial activity of *Bacillus* strains. A bacterial culture was standardized to a 0.5 McFarland. Discs impregnated with *bacillus* strains were placed on Mueller-Hinton agar and incubated at 37°C for 18 to 24 h. After incubation, the inhibition zones were measured in mm.

Antifungal Activity

The *in vitro* antagonistic assay was conducted using a dual culture assay on (Potato Dextrose Agar) PDA medium, based on modifications by **Xu and Kim (2014)**. An agar plug of each fungus from a 7 days culture was placed on PDA plates, with bacterial colonies streaked 3 cm around it. After 6 days of incubation at 30 °C in darkness, the inhibition zone width was measured, with control plates containing only fungi. The antifungal activity was calculated using the formula:

$$Inhibition\ rate\ (\%) = \frac{(C_d - T_d)}{C_d} \times 100\%$$

, where C_d is the mycelial growth in the control, and T_d is in the treatment.

API ZYM Assay Procedure: Enzymatic Activity

The API ZYM system is a semiquantitative micromethod for detecting enzymatic activities in various specimens, described by **Buissiere *et al.* (1967)**. Each strip contains 19 test enzymes, to which a 0.05 mL cell suspension is added. The strips are incubated in darkness at 30°C for 5 h. Following incubation, specific reagents are added, and nonspecific yellowing is eliminated by exposure to bright daylight before reading the color reactions against the API ZYM color chart.

Genomic DNA Isolation and PCR Analyses

The study describes the procedure for DNA extraction and amplification of the 16S rRNA gene from two *Bacillus* isolates. The isolates were incubated at 30°C for 24 hours and subsequently centrifuged. The bacterial pellets were suspended in a TE buffer and treated with SDS and proteinase K at 55°C. DNA was extracted using a phenol/chloroform method, followed by precipitation with sodium acetate and isopropanol. The DNA samples were washed and suspended with TE buffer containing RNase, then stored at -20°C for PCR analysis (**Sambrook *et al.*, 1989**). The 16S rRNA gene was amplified using the universal primers 27F and 1492R under specified PCR conditions, followed by storage of reaction mixtures at 4°C until electrophoresis was performed. Gel bands were stained and visualized, and the resulting amplicons were purified and sequenced using the Sanger method. The sequences were deposited in GenBank with specific accession numbers for each *Bacillus* spp. The phylogenetic analysis involved aligning related sequences with Clustal W software and constructing phylogenetic trees using MEGA version 12 with the Neighbor-joining method and bootstrap analysis.

RESULTS AND DISCUSSION

The purpose of this study was to isolate and characterize poly-extremophilic *Bacillus* strains from the sebkhas of Adrar (Algeria), to evaluate their physiological adaptations and functional potential highlighting their value for biotechnological applications.

Physico-Chemical Characteristics of Saharan Soil Samples

The analysis of soil samples from Ouled Mahmoud and Tililane sebkhas revealed extreme environmental conditions. As shown in **Table 1**, the moisture content was low (0.17% and 5.83%.). The pH values indicated a highly alkaline environment, reaching 9.0 in Tililane and 8.4 in Ouled Mahmoud. Electrical conductivity (EC) measurements confirmed hypersaline conditions, with values exceeding 39.43 mS/cm. These results justify the presence of specialized microorganisms adapted to simultaneous saline and alkaline stress.

Table 1 illustrates the environmental conditions of the two primary sampling sites: the Sebkha of Ouled Mahmoud and the Sebkha of Tililane. The analysis focuses on three key stress factors: electrical conductivity (EC) as an indicator of salinity, moisture content reflecting the aridity of the habitat, and pH levels confirming the alkaline nature of the soil. These parameters define the ecological niche from which the *Bacillus* strains were isolated and highlight the extreme conditions of the Adrar region.

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[1] ***Google Earth** (2025). Geographic data and location of isolation sites (Tililane and Ouled Mahmoud) [online]. Website: https://earth.google.com/web/ [Accessed 26 November 2025].

Table 1 Physico-chemical properties of the two sampled sites: the Sebkha of Ouled Mahmoud and the Sebkha of Tililane, focusing on electrical conductivity (EC), moisture content, and pH.

Region	Sebkha of Ouled Mahmoud	Sebkha of Tililane
pH value	8.4	9.0
Electrical conductivity (mS/cm)	17.20	39.43
Moisture %	5.83	0.17

Isolation, Selection, and Characterization of Halophilic Bacillus Strains

A total of 11 *Bacillus* strains were isolated from the two sampling sites, exhibiting diverse cultural characteristics. Among these, two specific isolates were selected based on their distinct cell morphology and robust enzymatic profiles. Biochemical characterization of the isolates KM9 and KM2 (detailed in Table 2).

This table summarizes the phenotypic identity of the strains isolated from the halalkaline sebkhas. Morphological assessment includes colonial characteristics (diameter, margin, and elevation) and microscopic features (Gram reaction and endospore formation). The biochemical profile details the enzymatic activities and metabolic capabilities, including catalase, oxidase, and specific substrate utilization tests, which serve as the primary criteria for the taxonomic assignment of the isolates to the genus *Bacillus*.

Table 2 Characterization of the isolates based on morphology and different biochemical tests.

Characteristic	KM9	KM2
Gram stain	Gram-positive	Gram-positive
Colony Shape	Round	Round
Elevation	Convex	Convex
Margin	Entire (Even)	Entire (Even)
Texture	Smooth	Smooth
Spore	Spore-former	Spore-former
Motility	Motile	Motile
Colony Diameter (mm)	<1 mm	<1 mm
Hemolysis	+	+
Catalase	+	+
Oxidase	+	+
MR (Methyl Red)	+	−
VP (Voges-Proskauer)	−	+
Citrate	+	+
Starch Hydrolysis	+	+
Nitrate Reduction	+	+
Acid from Glucose	+	+
Acid from Mannitol	−	+
Acid from Arabinose	−	−
Presumptive Identification	*Bacillus tequilensis*	*Bacillus paralicheniformis*

(+): positive; (-): negative

Adaptation Limits of *Bacillus* Strains

The initial physiological characterization of the isolated *Bacillus* strains confirmed their robust adaptation to extreme conditions, justifying their classification as poly-extremotolerant. The results of tolerance tests, including growth under varying pH, salinity (NaCl), and temperature, are summarized in **Table 3**. This table presents the minimal, optimal, and maximal growth conditions for Strain KM9 and Strain KM2, confirming their adaptation to extreme environments. Strain KM9 demonstrated strict alkaliphily, with optimal growth at pH 9.5 and the ability to survive up to pH 11.0. In contrast, Strain KM2 exhibited superior thermal tolerance, maintaining functional growth at 50°C. Both strains displayed high halotolerance; Strain KM2 remained active in media containing up to 20% NaCl, while Strain KM9 reached its limit at 17% NaCl.

This section summarizes their physiological properties by defining the minimum, optimum, and maximum growth thresholds for salinity (NaCl %), alkalinity (pH), and temperature. These parameters provide insight into the survival strategies and adaptive mechanisms employed by these strains to thrive in the extreme conditions of the Saharan halalkaline sebkhas.

Table 3 Phenotypic Characterization and Adaptation Limits of *Bacillus* Strains Isolated from Halalkaline Sebkhas.

Adaptation Characteristic	Measured Parameter	KM9 strain	KM2 strain
Thermal Tolerance	Minimum Growth Temperature (T_{min})	25°C	25°C
	Optimal Growth Temperature (T_{opt})	37°C	40°C
	Maximum Growth Temperature (T_{max})	45°C	50°C
Halotolerance	Minimum NaCl Concentration for Growth (S_{min})	2% (w/v)	3% (w/v)
	Optimal NaCl Concentration for Growth (S_{opt})	6% (w/v)	7% (w/v)
	Maximum NaCl Concentration for Growth (S_{max})	17% (w/v)	20% (w/v)
Alkaliphily	Minimum pH for Growth (pH_{min})	7.0	6.5
	Optimal pH for Growth (pH_{opt})	9.5	9.0
	Maximum pH for Growth (pH_{max})	11.0	10.5

While many extremophilic *Bacillus* strains previously described are commonly isolated from standard soil or marine environments, strains KM2 and KM9 are distinguished by their origin in the Saharan sebkhas of Adrar. These ecosystems are defined by 'poly-extreme' stressors, including hyper-aridity, extreme thermal fluctuations, and combined halo-alkaline conditions. The ability of our isolates to maintain high physiological activity under these coupled stresses suggests a specialized adaptive evolution. Our Saharan isolates demonstrate a broader tolerance range, marking them as unique bio-resources for studying stress-resistance mechanisms.

Characterization of Antimicrobial Producing Isolates

Among the 11 isolates studied, strain KM9 and strain KM2 demonstrated significant antibacterial and antifungal activity, as shown in Table 4 and figure 1. Notably, strain KM2 exhibited the highest activity, with a 25 mm inhibition zone against *Staphylococcus aureus* ATCC 25923.

Table 4 Antimicrobial activity of *Bacillus* strains against the test microbial pathogen at 24 h for bacteria and 5 days for fungus. The table presents the diameters of inhibition zones (mm) obtained using the agar well diffusion method against Gram-positive and Gram-negative bacteria, against *Candida albicans*, phytopathogenic and mycotoxigenic fungi.

Pathogens tests	KM9	KM2
	Antimicrobial activity (mm)	
Gram-Negative Bacteria		
Escherichia coli **ATCC8739**	12	15
Pseudomonas aeruginosa **ATCC25837**	20	-
Citrobacter freundii **ATCC5732**	-	14
Klebsiella pneumoniae **ATCC52145**	-	15
Salmonella enterica **ATCC5630**	14	20
Proteus mirabilis **ATCC783CI**	-	-
Gram-Negative Bacteria		
Enterococcus hirae **ATCC10541**	13	17
Staphylococcus aureus **ATCC25923**	22	25
Bacillus cereus **ATCC10876**	-	-
Enterococcus faecalis **ATCC49452**	20	-
Yeast		
Candida albicans **ATCC10231**	18	10
Microfungi	Antifungal activity %	
Fusarium oxysporum	33.33	38.88
Aspergillus flavus	44.44	50

The antimicrobial potential of KM9 and KM2 was characterized using a complementary approach that included the cross-streak method, disc diffusion, and dual-culture assays. These tests were performed using crude cultures and cell-free supernatants, confirming that the observed antagonism is linked to the secretion of extracellular bioactive metabolites. However, it is important to note that the limitations of these diffusion-based assays are qualitative screenings. The resulting inhibition zones are strictly dependent on the molecular weight and diffusion coefficients of the metabolites within the agar matrix.

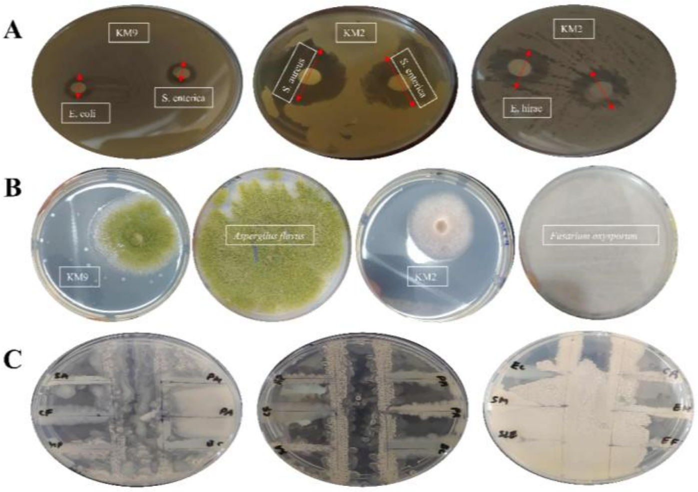

Figure 1 Antibacterial and antifungal activities of the selected *Bacillus* isolates. (A) Antibacterial activity against pathogenic bacteria was tested by the disc diffusion method. (B) Antifungal activity against phytopathogenic and mycotoxigenic fungi by the direct confrontation method. *B. paralicheniformis* exhibits strong inhibition of fungal growth. (C) Antibacterial activity against pathogenic bacteria tested by the cross-streak method.

Enzymatic activity of isolated strains

The *Bacillus* strains of our investigation have a considerable enzymatic capability, as shown in the Table 5. Significant hydrolytic capabilities were consistently identified among the strains, especially concerning peptidases (including Leucine arylamidase, Valine arylamidase, Cystine arylamidase, and Trypsin), indicating a remarkable ability for protein degradation. Additionally, highly active phosphatases (such as Alkaline phosphatase, Acid phosphatase, and Naphtol-AS-BI-phosphohydrolase) and crucial glycosidases (notably N-acetyl-β-glucosaminidase, α-galactosidase, and β-galactosidase) were noted. This extensive array of vigorous enzymatic functions substantiates the physiological flexibility of the strains for nutrient recycling in their extreme, high-pH surroundings and emphasizes their potential for biotechnological utility.

The API-ZYM gallery provides a rapid, semi-quantitative method for detecting 19 different extracellular hydrolytic enzymes. This profiling is essential for evaluating the biotechnological potential of the strains, particularly their ability to degrade complex organic substrates such as proteins, lipids, and carbohydrates. The enzymatic activities reflect the metabolic adaptation of the isolates to the organic matter available in the hypersaline and alkaline environments of the Adrar sebkhas.

Table 5 Extracellular enzymatic activity of the tested strains using API-ZYM gallery.

Enzyme	KM9	KM2
Alkaline phosphatase	+ (4)	- (0)
Esterase (C4)	+ (4)	- (0)
Esterase Lipase (C8)	+ (2)	+ (3)
Lipase (C14)	- (0)	+ (3)
Leucine arylamidase	- (0)	+ (5)
Valine arylamidase	- (0)	+ (5)
Cystine arylamidase	- (0)	+ (5)
Trypsin inhibitor	- (0)	+ (5)
α-Chymotrypsin	- (0)	- (0)
Acid phosphatase	- (0)	+ (4)
Naphthol-AS-BI-phosphohydrolase	+ (4)	+ (4)
α-galactosidase	- (0)	+ (4)
β-galactosidase	- (0)	+ (4)
β-glucuronidase	- (0)	+ (4)
α-glucosidase	- (0)	+ (2)
β-glucosidase	+ (3)	+ (4)
N-acetyl-β-glucosaminidase	+ (5)	+ (4)
α-mannosidase	- (0)	- (0)
α-fucisidase	- (0)	- (0)

(-): absence of enzyme; (+): enzyme production / the results were graded from 0 to 5 by comparison of the observed color with the color reaction chart. A value of 0 corresponded to a negative reaction and 5 to a reaction of maximal intensity.

Taxonomic identification and phylogenetic analysis

Two bacterial isolates were successfully identified using 16S rRNA gene sequencing. Analysis of the sequences showed that the strain KM9 was tentatively identified as *Bacillus tequilensis*, and the strain KM2 was tentatively identified as *Bacillus paralicheniformis*. with 99% similarity. The sequences were deposited in GenBank under accession numbers [PX495488 and PX495489]. Figure 2 presents the phylogenetic tree, illustrating the evolutionary relationship between these Saharan isolates and reference strains.

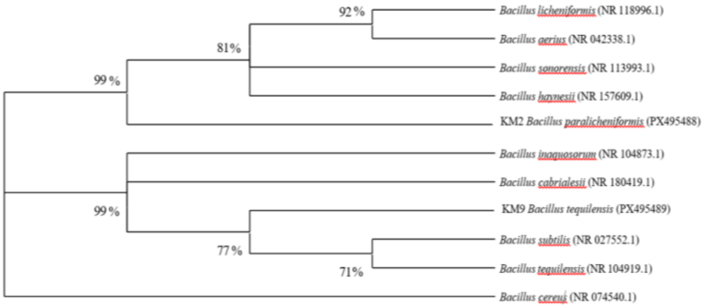

Figure 2 Phylogenetic tree showing the relationship of the isolated *Bacillus* strains (*Bacillus tequilensis* PX495489, *Bacillus paralicheniformis* PX495488) with closely related type strains based on 16S rRNA gene sequences. The tree was constructed using the Maximum Likelihood method, using the MEGA version 12.0 software, with *Bacillus cereus* utilized as the outgroup.

The physicochemical examination of the Tililane and Ouled Mahmoud sebkhas affirms their classification as poly-extreme ecosystems. With elevated electrical conductivity (>4 mS/cm) and alkaline pH levels, these soils impose a significant selective pressure, promoting microorganisms with specialized adaptive characteristics (**Chamekh *et al.*, 2019**). The capacity of our isolates to thrive in such conditions stems directly from their physiological adaptability, reflecting mechanisms noted in other hypersaline environments where low water activity (a_w) and high salinity govern microbial persistence (**Martínez *et al.*, 2023**). Both isolates were identified by 16S rRNA gene sequencing. These taxonomy designations were highly supported by the phylogenetic tree built using the Neighbor-Joining approach, which demonstrated a high bootstrap consensus with previously published sequences of known halo-alkalitolerant bacteria. Our isolates physiological ability to survive in the distinctive halo-alkaline sebkha environment of Adrar is further supported by their close relationship to known extremophiles, which justifies future research into their enzymatic and antibacterial potential.The clustering of our strains with established type strains supports their taxonomic classification within the *Bacillus* group.

The growth characteristics of our isolates reveal unique approaches to managing halo-alkaline and thermal stress. Isolate KM9 exhibited a strict alkaliphilic profile with growth persisting up to pH 11.0 and an optimal salinity (S_{opt}) of 7.0% NaCl. This level of halotolerance is consistent with the metabolic adjustments described by **Yin *et al.* (2015)**, where *Bacillus* species utilize compatible solute accumulation and ion transporters to maintain osmotic equilibrium.

In comparison, Isolate II KM2 demonstrated exceptional thermal resilience, achieving a peak growth temperature (T_{max}) of 50°C. This thermo-halo-alkaline endurance is a defining characteristic of strong poly-extremophiles. Corresponding genomic modifications for halo-alkali resilience have been observed in related genera such as *Alkalihalobacillus*, where particular gene clusters support pH equilibrium (**Krishna *et al.*, 2021**). The endurance of these strains at temperatures reaching 50°C and salinities of 17–20% NaCl indicates the isolates possess significant halotolerance, characterized by their capacity to grow in extreme environments through compatible solute retention and effective ion transport (**Cárdenas *et al.*, 2021**). This adaptation is crucial, as organisms under osmotic strain frequently produce stable enzymes and distinctive metabolites, increasing the probability of discovering new bioproducts relative to mesophilic strains (**Tuteja *et al.*, 2022**). The API ZYM profiling, emphasizing the biotechnological value of these isolates revealed significant hydrolytic variation. Isolate KM2 (*B. paralicheniformis*) displayed a remarkably broad enzymatic spectrum (14 active enzymes), with intense proteolytic activity. These findings are consistent with previous research by **Aktayeva and Khassenov (2024)**, who described a strain of *B. paralicheniformis* with significant proteolytic and keratinolytic activity. Conversely, Isolate KM9 (*Bacillus tequilensis*) exhibited specific activity for N-acetyl-beta-glucosaminidase (NAGase), an enzyme necessary for the decomposition of chitin. The presence of alkaline phosphatase and esterase activities in these strains suggests a potential for further investigation into industrial applications where enzymes must remain functional under alkaline conditions (**Lebre and Cowan, 2019**).although further quantitative assays are needed to confirm their stability.

The antagonistic tests revealed that Isolate KM2 exhibits broad-spectrum antimicrobial activity, with notable inhibition zones against *S. aureus* (25 mm) and *P. aeruginosa* (20 mm). The targeted efficacy of Isolate KM9 towards certain pathogens indicates the synthesis of unique secondary metabolites, a characteristic feature of extremophilic *Bacillus* species utilized for biocontrol (**Khare *et al.*, 2024**). Moreover, the 50% growth suppression of *Aspergillus flavus* and *Fusarium oxysporum* indicates an interesting antifungal potential. These preliminary screening results justify future studies to characterize the specific bioactive molecules involved (**Praveen *et al.*, 2023**).

CONCLUSION

This research provides a detailed physiological and functional analysis of *Bacillus* strains from sebkhas in Adrar, Algeria. The findings indicate that these hypersaline and alkaline environments host distinct microbial communities capable of thriving under extreme conditions. Isolate KM9 (*B. tequilensis*) and Isolate KM2 (*B. paralicheniformis*) are identified as poly-extremotolerant with remarkable growth tolerance up to pH 11.0, 20% NaCl, and temperatures of 50°C. The biochemical profiling reveals various extremozymes and strong antimicrobial and antifungal properties, particularly of Isolate KM2 against *Staphylococcus aureus* and *Aspergillus flavus*, suggesting its potential for biocontrol and pharmaceutical applications. The study highlights the Adrar sebkhas as a valuable reservoir for resilient biocatalysts, emphasizing the importance of further research in whole-genome sequencing and the purification of specialized metabolites, which will be crucial to understanding the molecular foundation of this poly-extremotolerance and to facilitating the transition of these strains from laboratory study to industrial use.

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