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Impact of some Plant Growth Promoting Bacteria (PGPB) on Potato Plants under Salt Stress

Manal F. Abdelall[1*], Shimaa El-Gamal[1], Habiba Atef[1] and Mohamed S. Tawfik[2]

[1]Department of Microbial Molecular Biology, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa Street, 12619, Giza, Egypt.

[2]The Laboratory of Oil Crops Biotechnology, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa Street, 12619, Giza, Egypt.

*E.mail: manalfm@gmail.com, ORCID: 0000-0002-5625-4830

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ABSTRACT

Potato (*Solanum tuberosum*) is globally considered the fourth most important crop for human food consumption. Therefore, increasing potato production is demanded, however, the high salinity in different areas limits reaching this aim. Hence, plant growth-promoting bacteria (PGPBs) may be the suitable solution for this situation as it might improve the growth and production of potatoes under salt stress. In this study eight bacterial isolates (*Bacillus tropicus* (WHS48), *Metabacillus iocasae* (WHS98), *Bacillus velezensis* (WHS114), *Bacillus haynesii* (WHW37), *Bacillus thuringiensis* (WHS259), *Bacillus paramycoides* (WHS267), *Halomonas salifodinae* (WHS325), and *Bacillus licheniformis* (WHS343) were isolated, from the soil and water of Wadi El-Natrun, Egypt; characterized and identified as PGPBs. The impact of these isolates on potato tuber production was studied. Generally, the tested bacterial isolates have improved tuber production, catalase and peroxidase activity.

INTRODUCTION

Potato (*Solanum tuberosum*) stands out globally as a significant non-grain crop due to its rich nutritional profile, abundance in many vitamins (B1, B6, and C) and minerals (potassium, manganese, phosphorus, and copper) (Camire *et al.*, 2009 and McGill *et al.*, 2013 and Vander Donckt & Chan, 2019). On one hand, Egypt relies heavily on potato cultivation for local consumption and exportation to Europe, with a recorded production of 5.2 million tons in 2020 (FAO, 2022). On the other hand, the global endeavor to increase potato production faces considerable challenges, particularly from salinity-related issues.

Salinity poses a substantial threat to agricultural productivity worldwide. Projections suggest that by 2050, approximately 50% of arable land might encounter salinity concerns (Kumar and Sharma, 2020). Egypt, primarily reliant on Nile River irrigation, confronts escalating soil salinization due to intensive cultivation practices and inadequate drainage systems post the modifications in irrigation methods since the late 1960s, notably with the construction of the Aswan High Dam (Kalkhan *et al.*, 2000).

Salinity predominantly affects various regions in Egypt, particularly the northern and central parts of the Nile Delta, Wadi El-Natrun, Tal El-Kebeer, Oases in the eastern regions, and the Fayoum province. Approximately, 0.9 million hectares of cultivated areas grapple with salinity issues, notably impacting 60% of the northern Delta, 20% of the southern Delta and Middle Egypt, and 25% of Upper Egypt regions (Amer *et al.*, 2017; Hammam & Mohamed, 2018).

Microorganisms within saline environments have evolved mechanisms, halophilic microorganisms, for instance, possess stable enzymes, maintaining their catalytic properties, presenting advantages over their non-halophilic counterparts (Moreno *et al.,* 2013). Some studies propose that the characteristics of halophytic plants may be influenced by halophytic microorganisms residing within plant tissues or root zones (Bazihizina *et al.,* 2012). A more direct way is through the usage of PGPB and endophytes to mitigate salinity stress in plants. These approaches have provided some potential in the way to enhance plant resilience to salinity, as well as enable to use of low-quality or seawater-mixed irrigation systems (Vejan *et al.,* 2016). In the present study, potato and eight bacterial isolates were assessed for their intercalated impact as PGP rhizobacteria producing indole-3-acetic acid (IAA), siderophore, hydrogen cyanide (HCN), and phosphate solubilization under salt stress conditions.

MATERIALS AND METHODS

Bacterial Isolates:

Halo-tolerant bacteria were isolated from soil and water samples collected from Wadi El-Natrun using LB solid media supplemented with 5% NaCl. Approximately 345 bacterial colonies were purified using the repeated plate-streaks method and stored at −80°C as glycerol stocks (unpublished data). The purified colonies were then exposed to various concentrations of NaCl to identify the isolates with the highest halophilic features to be used in the current study.

Physical Characterization of Bacterial Growth on Different pH and NaCl Levels:

Purified isolates were inoculated in LB broth medium (pH 7 or 9), supplemented with or without different levels of NaCl (10, 15, or 20 %). A single colony of each sample was inoculated in LB broth medium and the cultures were incubated for up to 7 days at (35 °C). LB solid medium supplemented with 5 % NaCl was used as a first step to maximize the recovery of halophilic bacteria.

Identification of Bacterial Isolates:

Bacterial strain identification involves the characterization of biochemical properties as outlined in Bergey's manual of determinative bacteriology (Bergey *et al.,* 1994). The process comprised two main aspects: the determination of Gram-negative/positive status and an array of biochemical property tests.

1. Potassium Hydroxide (KOH) Test:

A potassium hydroxide (KOH) test was conducted using a single purified colony from each isolate that was mixed with two drops of KOH (3%) solution on top of a glass slide. Afterwards, continuous circular stirring was performed for 5-10 seconds using a needle. If a mucus layer was formed, the sample was considered gram-negative, otherwise, it was positive (Abegaz, 2007).

2. Biochemical Properties Test:

Different biochemical and enzymatic assays were used to fully unveil the biochemical properties of the isolates such as citrate utilization, catalase, urease, and lactose fermentation test (Smibert, 1994).

3. Molecular Characterization16S rRNA Gene Sequencing:

The bacterial DNA was isolated (Sambrook *et al.,* 2009); and the 16S rRNA gene was amplified using the primers 27F (5′-AGAGTTTGATCM

TGGCTCAG-3′) and 1492R (5′-GGTTA CCTTGTTACGACTT-3′) using the PCR. The following PCR conditions were used: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 45 sec, and elongation at 72 °C for 1.5 min. The final extension was set at 72 °C for 10 min. The PCR product was purified using the QIAquick Kit (Qiagen, USA), and samples were sequenced using the 3500 Genetic Analyzer (Applied Biosystems). All sequence reads were analyzed using the basic local alignment search tool (BLAST) against the published sequences in the public GenBank nucleotide database of the National Center for Biotechnology Information (NCBI).

4. Plant Growth Promoting (PGP) Tests:

The PGP traits were tested in the bacterial isolates using assays for the solubilization of phosphate and the production of indole acetic acid (IAA), hydrogen cyanide (HCN), and siderophore.

a. Phosphate Solubilization Assay:

To identify phosphate-solubilizing characteristics in the isolated bacteria, the procedure of Nautiyal (1999) was followed. In brief, 2 mL of overnight-grown culture was spotted on Pikovskaya plates (each liter consisting of 10.0 g glucose, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.5 g yeast extract, 0.0001 g $FeSO_4 \cdot 7H_2O$, 0.0001 g $MnSO_4 \cdot H_2O$, 15 g agar, 5.0 g $Ca_3(PO_4)_2$, and 0.1 g bromocresol purple), adjusted to pH 7 (Pikovskaya 1948). The plates were incubated at 28 ± 2°C for 7 days. Bacterial isolates showing a clear halo-zone within 7 days around bacterial colonies were considered positive isolates.

b. Production of Indole Acetic Acid (IAA)

Quantification of IAA in the inoculated tubes of the various isolates was conducted following the method of Brick *et al.* (1991). Briefly, bacterial isolates were cultured in the presence of tryptophan (1 g/L) (IAA precursor), for 24 hours. Subsequently, the Salkowsky reagent was added to the culture supernatant, leading to the development of a dark-red color, indicating positive indole production. The spectrophotometric measurement was taken at ≈540 nm. The obtained values were calibrated against an IAA solution (0-100 µg / mL).

c. Hydrogen cyanide (HCN) production:

HCN production was assessed following the method of Miller and Higgins (1970). Bacterial cultures grown for 24 hours in LB broth were inoculated onto King's medium supplemented with 0.4% glycine in test tubes. The presence of HCN was indicated by a change in the color of picric acid-soaked filter papers to reddish brown.

d. Siderophore Production Assay:

Siderophore production was evaluated using the blue agar plates method of Schwyn and Neiland (1987). Incubation of cultures on chrome azurole S (CAS) plates at 28°C for four days where halo zones around bacterial colonies were formed.

5. Greenhouse Experiment:

Potato tubers of the Spunta variety were kindly provided by Dr. Gihan Hosni (AGERI). Out of 350 screened bacterial isolates, eight bacterial isolates (WHS48, WHS98, WHS114, WHW37, WHS259, WHS267, WHS325, and WHS343) were selected based on biochemical surveys. Bacterial isolates were examined separately in the greenhouse to study their effect on promoting the growth of potato plants under salt stress. Bacterial treatments were conducted along with two controls: no bacterial application and foliage applications of a 1.0 mg/L IAA solution.

The tubers were stored in paper bags under dry conditions for up to a month to allow the different buds to develop. Each tuber was divided into 2-3 pieces (each piece should have at least one growing bud). Soil mixture (1 peat moss: 2 perlites:

1 sand) was prepared and was used to fill 25 cm-wide pots. In total, 180 pots were prepared.

The pots were divided into 3 groups (blocks/replicates), each consisting of 60 pots, and within each group, the pots were divided into 6 pots X 10 rows. Each row was assigned randomly to one of the following symbols (1, 2, 3, 4, 5, 6, 7, 8, C, and I) which represented the following (WHS48, WHS98, WHS114, WHW37, WHS259, WHS267, WHS325, and WHS343), non-treated with normal fertilizer (C), and IAA treated with fertilizer (I); respectively). Each row was split into 2 halves, one for irrigation with 100mM NaCl, and the other half irrigated with water. For the first five weeks, all plants were irrigated and fertilized normally (2 times a week).

Bacterial Isolates Preparation:

A week before starting the treatment (4 weeks post plantation), 40µl of each bacterial isolate was inoculated in 400 ml LB flasks. Each isolate was labeled and (WHS48, WHS98, WHS114, WHW37, WHS259, WHS267, WHS325, and WHS343) incubated at 28ºC for four days.

Bacterial Treatment and Salinity Treatment:

The different bacterial treatments were applied to the experiment by placing 20 ml (10^8 CFU/ml) into each corresponding pot. Three days post bacterial inoculation; pots were irrigated using 500 ml of water or 100 mM of NaCl solution according to their assigned treatment. Irrigation was applied once or twice a week. Leaf samples were collected from potato plants after four weeks of treatment applications to analyze peroxidase and catalase enzymes.

Harvesting:

The experiment was terminated after 3 months. Irrigation was withdrawn for 2-3 weeks before harvesting. Tubers resulting from each pot were collected in separate paper bags.

Agronomic Traits Measurements:

The growth of potato plants was measured after one month from bacterial application using the following records:

a. **Potato yield:** Total tuber number and average tuber weight (g/tuber) were collected one week after harvesting Gomaa, (2014).
b. **Peroxidase quantification** in potato leaves followed the method of Spychalla and Desborough (1990).
c. **Catalase activity** in potato leaves was measured following the method of Spychalla and Desborough (1990).

Statistical Analysis:

All experiments were performed in quadrates. EXCEL was used for statistical analysis. ANOVA was performed to provide evidence to reject or accept the null hypothesis with a cut-off value for the level of significance $p \leq 0.05$. All data are presented as arithmetic means.

RESULTS

Physiological and Biochemical Tests of Isolated Bacteria:

Among these isolates, were assessed for their growth capabilities in LB medium supplemented with either 5% or 10% (w/v) NaCl at pH 7.0 or 9. Five isolates exhibited growth at pH 7, while three (WHS48, WHW37, and WHS267) demonstrated resilience to an alkaline pH (pH 9), classifying them as alkaliphilic isolates. All isolates demonstrated the ability to grow under salt-stress conditions. Six out of the eight isolates were tolerant to 10% NaCl: WHS48, WHS98, WHW37, WHS259, WHS267, and WHS325, whereas WHS114 and WHS343 exhibited growth below 5% of NaCl (Table 1). These isolates were selected based on their biochemical features, particularly their tolerance to NaCl, indicative of their physiological characteristics as halophiles.

Table 1: Biochemical and physiological characterization.

Isolate code	Physiological and Biochemical tests						
	NaCl tolerance (%)	PH	3%KOH test	Catalase test	Citrate utilization	Lactose fermentation	Urease
WHS48	10%	**9**	-	+	-	-	+
WHS98	10%	**7**	-	+	-	+	-
WHS114	5%	**7**	-	+	+	+	+
WHW37	10%	**9**	-	+	+	-	-
WHS259	10%	**7**	-	+	+	-	-
WHS267	10%	**9**	-	+	+	+	+
WHS325	10%	**7**	+	+	+	-	+
WHS343	5%	**7**	-	+	-	-	-

Identification of Bacterial Isolates:

All isolates were identified as gram-positive, as determined by the KOH test, except for the WHS325 isolate which was characterized as gram-negative (Table 1). Additionally, all isolates exhibited the presence of the catalase enzyme. Most of the isolates demonstrated the capability to utilize citrate as the sole carbon source for energy, with the exception of WHS48, WHS98, and WHS343. Among these isolates, only WHS98, WHS114, and WHS267 were able to ferment lactose. Furthermore, specific isolates (WHS48, WHS114, WHS267, and WHS325) showed the presence of the urease enzyme and possessed the ability to hydrolyze urea (Table 1**).**

Molecular Sequence Analysis of 16S rRNA Gene:

The identification of all eight bacterial isolates was conducted through PCR targeting the 16S rRNA gene sequences. Subsequently, the acquired sequences underwent analysis against existing sequences within the NCBI GenBank nucleotide database (http://www.ncbi.nlm.nih.gov). Seven isolates belonged to the Bacillus species (WHS48 as *Bacillus tropicus*, WHS98 as *Metabacillus iocasae*, WHS114 as *Bacillus velezensis*, WHW37 as *Bacillus haynesii*, WHS259 as *Bacillus thuringiensis*, and WHS343 as *Bacillus licheniformis*), while the isolate WHS325 was identified as *Halomonas salifodinae*. The DNA sequences for all isolates were submitted to the NCBI database for documentation (Table 2). The construction of a phylogenetic tree depicting bacterial isolates was carried out utilizing the neighbor-joining method for evolutionary analysis in MEGAX. Sequence alignment was executed using CLUSTALW, while bootstrap values were computed from 1000 resampling iterations. Genetic distances were represented on scale bars within the constructed tree to illustrate the evolutionary relationships among the isolates based on their 16S nucleotide sequences (Fig. 1). The BLASTn analysis demonstrated that the majority of isolates tested in this investigation exhibited a high degree of kinship with all other database isolates. The 16S sequences displayed 100% homology with Bacillus sp. strains. In contrast, one isolate exhibited a closer relationship with Halomona strains.

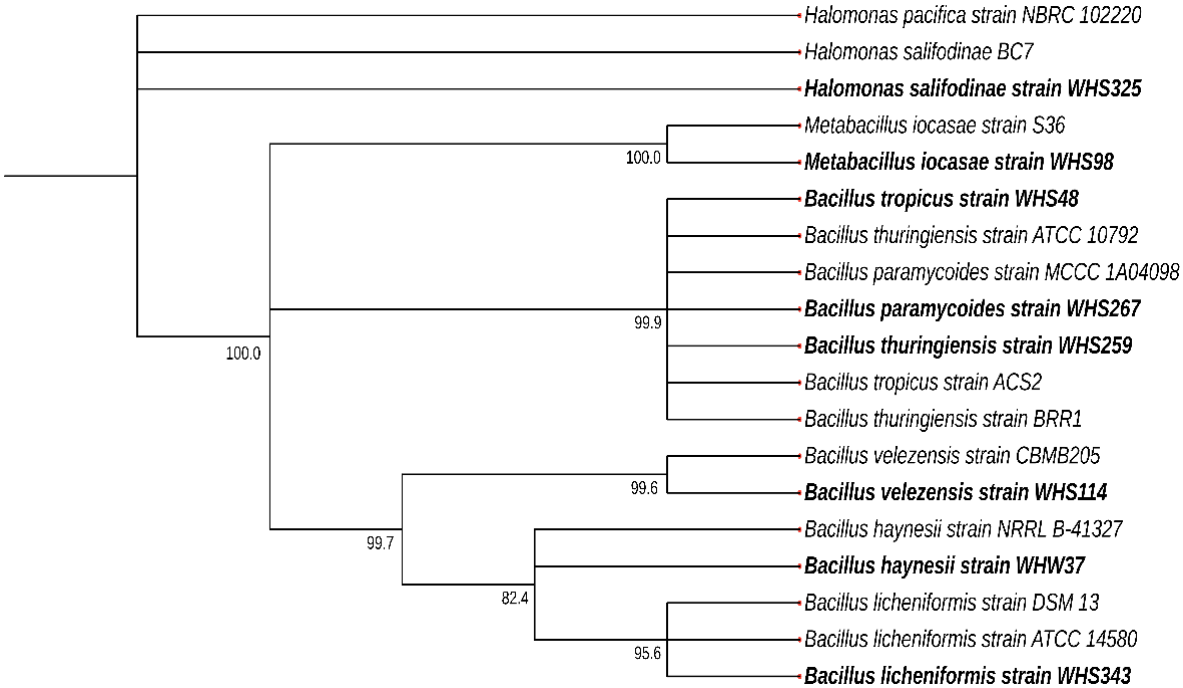

Fig. 1: Phylogenetic of bacterial isolates distribution based on 16S rDNA gene sequence from soil and water samples of El-Hamra Oasis, EL-Natrun Valley. The phylogenetic relationships were inferred using the neighbor-joining approach from the 16S rRNA gene, and an evolutionary analysis was performed in MEGA X. Alignment of the sequences was done with CLUSTALW, and bootstrap values were calculated from 1000 re-sampling, with genetic distances shown on scale bars.

(PGPB) Tests/Characterization of The Bacterial Isolates:

Various tests were employed for the screening of plant growth-promoting traits in isolated bacteria; including Indole Acetic Acid (IAA) production, siderophore production, phosphate-solubilizing characteristics, and hydrogen cyanide (HCN) production (Table 2). Among the six bacterial isolates *Bacillus tropicus* (WHS48), *Metabacillus iocasae* (WHS98), *Bacillus velezensis* (WHS114), *Bacillus thuringiensis* (WHS259), *Bacillus paramycoides* (WHS267), and *Bacillus haynesii* (WHW37), the capacity to produce HCN was observed. Siderophore production was evident in six bacterial isolates: *Bacillus tropicus* (WHS48), *Metabacillus iocasae* (WHS98), *Bacillus thuringiensis* (WHS259), *Bacillus paramycoides* (WHS267), *Halomonas salifodinae* (WHS325), and *Bacillus haynesii* (WHW37). Furthermore, all eight bacterial isolates exhibited IAA-producing capabilities; with *Bacillus tropicus* (WHS48) being the highest producer at 40 µg/ml, and *Bacillus licheniformis* (WHS343) exhibiting the lowest IAA production at 6.4%. Additionally, five isolates *Bacillus tropicus* (WHS48), *Bacillus velezensis* (WHS114), *Bacillus haynesii* (WHW37), *Bacillus thuringiensis* (WHS259), and *Halomonas salifodinae* (WHS325) demonstrated the ability to solubilize phosphate (Table 2).

Table 2. Quantification of Plant Growth Promoting (PGPB) characterization Assay for bacterial isolates.

No.	Isolates code	Accession No.	Isolate species	PGPB properties			
				IAA (µg/ml)	Sidrophore production	HCN production	Phosphate solublization
1	WHS48	OQ534093	*Bacillus tropicus*	40.5	+	+	-
2	WHS98	OQ534283	*Metabacillus iocasae*	29.8	+	+	+
3	WHS114	OQ534094	*Bacillus velezensis*	12.1	-	+	+
4	WHW37	OQ534095	*Bacillus haynesii*	17.7	+	+	+
5	WHS259	OQ534096	*Bacillus thuringiensis*	21.8	+	+	+
6	WHS267	OQ534097	*Bacillus paramycoides*	7.4	+	+	-
7	WHS325	OQ534098	*Halomonas salifodinae*	13.9	+	-	+
8	WHS343	OQ534099	*Bacillus licheniformis*	6.4	-	-	-

Influence of Bacterial Strains on Potato Plants:

- Impact On Potato Yield:

Potato plants growing in a greenhouse exposed to salinity stress (0, and 100 mM NaCl) with or without the application of bacterial isolates. The application of eight bacterial isolates significantly enhanced the growth and yield of potato tubers enhanced the growth and yield of potato tubers under salinity stress. Among the tested isolates, *Halomonas salifodinae* (WHS325) (No.7) exhibited the most pronounced effect, inducing an approximate 90 g increase in potato tuber yield (average tuber weight g/tuber) compared to the non-inoculated control and other isolates. This was followed by *Bacillus velezensis* (WHS114) (No.3) and *Bacillus haynesii* (WHW37) (No.4), which promoted potato tuber yield by approximately 80 g. These findings demonstrate the potential of these bacterial isolates as effective biofertilizers for enhancing potato production under saline conditions (Fig. 2). Additionally, the combined application of bacterial isolates resulted in a substantial increase in potato tuber yield, approximately 80 g higher than the non-inoculated controls (Fig. 3).

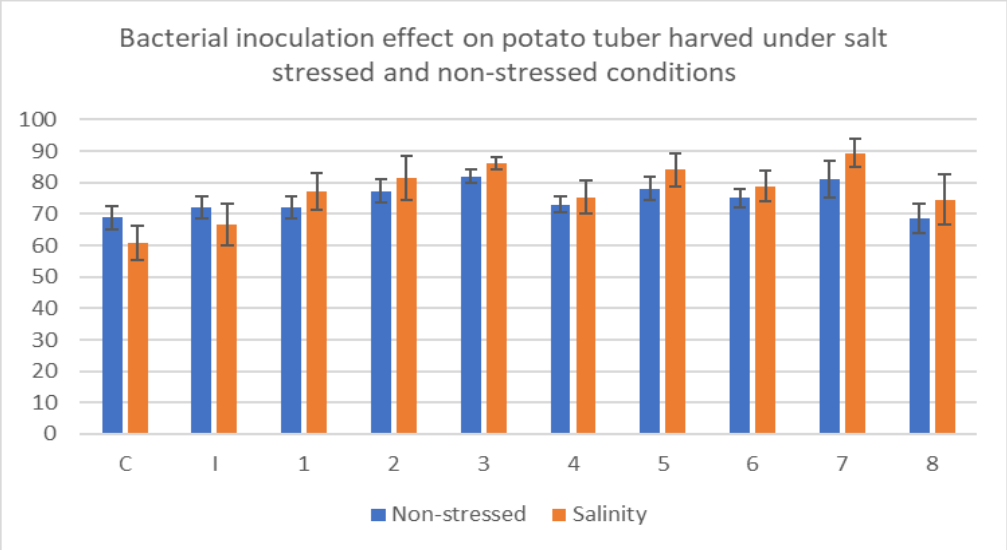

Fig. 2: Effect of Individual bacterial isolates on potato tuber yield under salt and non-salt conditions. (C) Control non-treated with normal fertilizer, (I) IAA treated, (from 1 to 8) bacterial isolates.

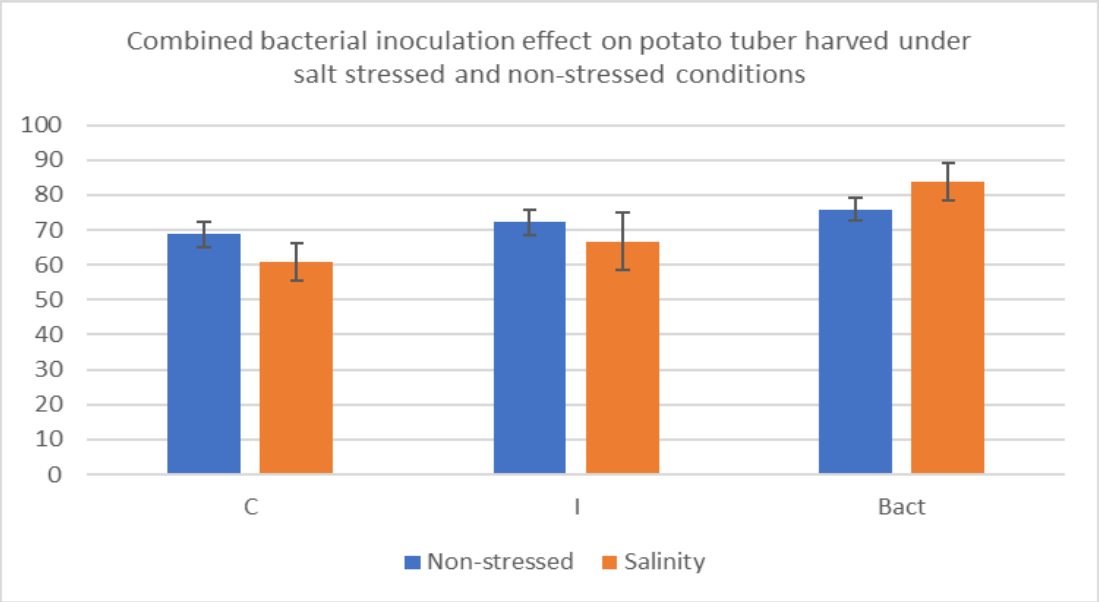

Fig. 3: Effect of combined bacterial isolates on potato tuber yield under salt and non-salt conditions. (C) Control non-treated with normal fertilizer, (I) IAA treated, (Bact.) combined bacterial isolates.

- Impact on Some Cellular Antioxidant Enzymes:

To cope with salinity stress, plants have evolved various adaptive mechanisms, including the synthesis of antioxidant enzymes such as peroxidase and catalase. These enzymes play a crucial role in scavenging reactive oxygen species (ROS) generated under salt-stress conditions, thereby protecting cellular components from oxidative damage.

In this study, inoculation with eight bacterial isolates was investigated for its effect on catalase and peroxidase activity in potato leaves under salinity stress, catalase and peroxidase activity were measured in potato leaves exposed to different salinity levels, with and without bacterial inoculation.

- Catalase activity:

Four weeks post-treatment, catalase enzyme activity in potato leaves was significantly affected with the bacterial treatment of seven isolates (WHS48, WHS98, WHS114, WHW37, WHS259, WHS267, and WHS343). Notably, the *Halomonas salifodinae* (WHS325) isolate did not exert any noticeable effect on catalase activity. Among the effective isolates, *Bacillus thuringiensis* (WHS259), *Bacillus haynesii* (WHW37), *Metabacillus iocasae* (WHS98) and *Bacillus paramycoides* (WHS267) induced remarkable increases in catalase activity, reaching 11.8, 11.0, 10.5, and 10.4 units, respectively under salt stress (Fig. 4).

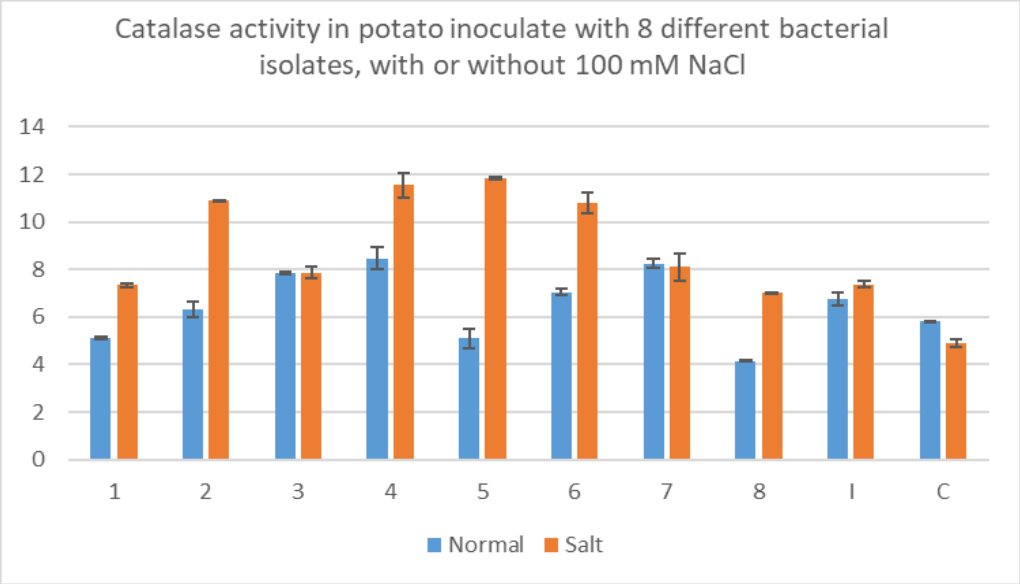

Fig. 4: Showed effect of bacterial isolates on catalase activity of potato leaves inoculated with bacterial isolates under salt and non-salt conditions. (C) Control non-treated with normal fertilizer, (I) IAA treated, (from 1 to 8) bacterial isolates.

- Peroxidase Activity:

Additionally, inoculation with eight bacterial isolates significantly impacted peroxidase enzyme activity in potato leaves after four weeks of treatment. Interestingly, the *Bacillus thuringiensis* (WHS259) isolates exhibited a slight decrease in peroxidase activity (approximately 5.0 units), while the remaining bacterial isolates induced notable enhancements in peroxidase activity. *Metabacillus iocasae* (WHS98), *Bacillus paramycoides* (WHS267), and *Bacillus haynesii* (WHW37) isolates demonstrated remarkable increases in peroxidase activity, reaching 4.8, 4.7, and 4.0 units, respectively, compared to other isolates and non-inoculated controls under salt stress conditions (Fig. 5).

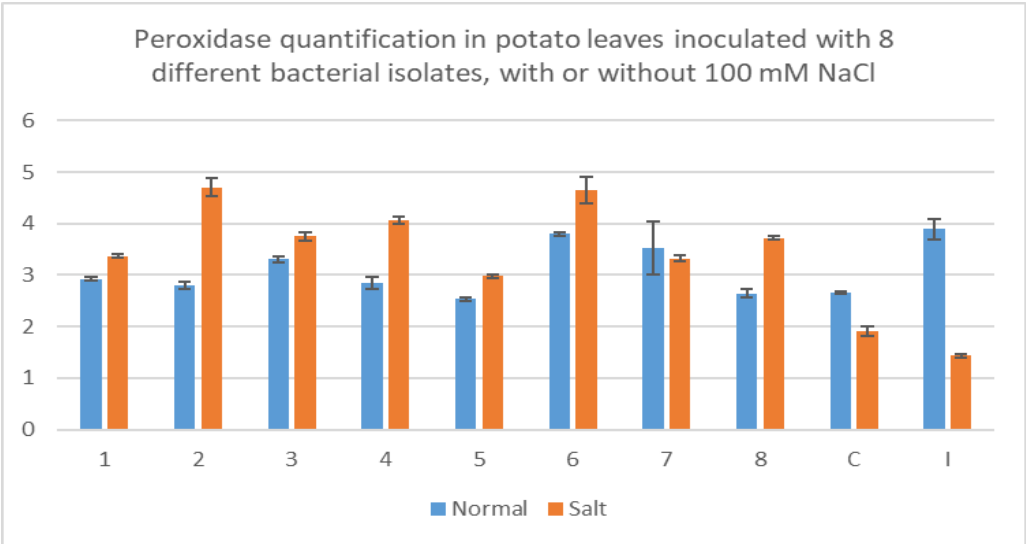

Fig. 5: Showed effect of bacterial isolates on peroxidase quantification of potato leaves inoculated with bacterial isolates under salt and non-salt conditions. (C) Control non-treated with normal fertilizer, (I) IAA treated, (from 1 to 8) bacterial isolates.

DISCUSSION

In agricultural practices, high salinity remains a considerable challenge, impacting crop productivity. However, specific microorganisms have demonstrated adaptation to thrive in such adverse conditions (Flowers *et al.,* 2010). Notably, halophilic microorganisms possess stable enzymes, providing them with a unique advantage in extremely saline environments (Moreno *et al.,* 2013). Additionally, there's a growing understanding that halophytic plants might exhibit characteristics influenced by microorganisms residing within their tissues or root-growing zones (Bazihizina *et al.*, 2012).

The present study sought to characterize halophilic and alkaliphilic bacterial isolates from hypersaline alkaline soda lakes in the Wadi El Natrun region of Egypt. Eight isolates were obtained from the lake water and soil samples, and their growth capabilities under various salt stress and pH conditions were assessed.

Out of the eight isolates, five exhibited growth at pH 7 and three (*Bacillus tropicus* (WHS48), *Bacillus haynesii* (WHW37), and *Bacillus paramycoides* (WHS267) demonstrated resilience to an alkaline pH (pH 9), classifying them as alkaliphilic isolates. This finding is consistent with previous studies that have reported the presence of alkaliphilic bacteria in soda lakes (Oren, 2010 and Coahuila & Pozzuoli, 2015). The ability of these isolates to thrive in alkaline environments is likely due to adaptations in their cellular processes and enzyme activity that allow them to maintain homeostasis under high pH conditions (Yoshimune, 2010). Additionally, all isolates demonstrated the ability to grow under salt-stress conditions. *Bacillus tropicus* (WHS48), *Metabacillus iocasae* (WHS98), *Bacillus haynesii* (WHW37), *Bacillus thuringiensis* (WHS259), *Bacillus paramycoides* (WHS267), *Halomonas salifodinae* (WHS325) were tolerant to 10% NaCl, whereas *Bacillus velezensis* (WHS114) and *Bacillus licheniformis* (WHS343) exhibited growth below 5% of NaCl. These findings suggest that the isolates possess adaptations that enable them to cope with high salt concentrations. Halophilic bacteria typically employ strategies such as osmoregulation, the accumulation of compatible solutes, and the biosynthesis of halophilic enzymes to maintain cellular integrity and function in high-salinity environments (Oren, 2010). Moreover, the diverse biochemical characteristics exhibited by these isolates, including their ability to utilize citrate as the sole carbon source for energy, hydrolyze urea, and ferment lactose, suggest their potential for various biotechnological applications (Oren, 2010).

Molecular identification using 16S rRNA gene sequencing revealed that seven isolates belonged to the Bacillus species (*Bacillus tropicus* (WHS48), *Metabacillus iocasae* (WHS98), *Bacillus velezensis* (WHS114), *Bacillus haynesii* (WHW37), *Bacillus thuringiensis* (WHS259), and *Bacillus licheniformis* (WHS343)), while the isolate WHS325 was identified as *Halomonas salifodinae*. The presence of Bacillus species in soda lakes is not surprising, as members of this genus are known for their versatility and ability to thrive in diverse environments, including extreme conditions (Yadav *et al.,* 2015). *Halomonas salifodinae*, on the other hand, is a halophilic bacterium commonly found in saline environments (Hu, 2022).

Recent strategies involving plant growth-promoting bacteria (PGPB) have emerged to mitigate salinity stress in plants. In this study, eight bacterial isolates isolated from Wadi El Natrun were identified and characterized for their PGPB traits, such as phosphate solubilization, indole acetic acid (IAA) production, hydrogen cyanide (HCN) production, and siderophore production.

All eight isolates in the current study exhibited IAA production, with *Bacillus tropicus* (WHS48) demonstrating the highest production at 40 µg/ml. IAA plays a critical role in plant growth processes such as root development, cell division, and nutrient uptake (Imperlini *et al.,* 2009). Most isolates produced siderophores, which are iron-chelating compounds that can enhance plant growth by making iron available to plants, especially in iron-deficient soils (Kloepper *et al.,* 1980). Five isolates demonstrated phosphate solubilization capabilities, enabling them to make phosphate available to plants in phosphate-deficient soils (Richardson, 2001). HCN production was observed in six isolates, suggesting their potential biocontrol activity against plant pathogens. These findings highlight the potential of these isolates to promote plant growth and mitigate salinity stress under saline conditions (Kashyap *et al.,* 2020). According to these results, the isolates may be able to stimulate plant growth and be applied as biopesticides or fertilizers

The impact of these eight isolates as PGPB on potato yield under salt stress was evaluated separately. The PGPB isolates significantly enhanced tuber production, aligning with findings from previous studies suggesting the improved productivity of beneficial microbes (Abbas *et al.,* 2014). Various reports have highlighted the potential of specific PGPB strains to enhance potato growth and yield under stress conditions, further supporting the efficacy of PGPB application in agriculture (Naqqash *et al.,* 2016 and Purwantisari *et al.,* 2019).

The dominance of Bacillus species in the rhizosphere of potato and sweet potato suggests their suitability as PGPB candidates (Costa-Santos *et al.,* 2021 and Gupta *et al.,* 2022). Notably, seven out of the eight isolates used in this study belonged to the Bacillus genus. *Halomonas salifodinae* (WHS325), *Bacillus velezensis* (WHS114) and *Bacillus haynesii* (WHW37) demonstrated a notable impact on potato yield (average tuber weight (g/tuber).

Plants have different strategies to overcome salinity stress and the osmotic stresses caused by salinity, one of these strategies is to produce different antioxidant enzymes such as peroxidase and catalase enzymes in order to reduce the cytoplasmic osmotic pressure to increase water absorption (Sarker & Oba 2020). Therefore, catalase and peroxidase activity were measured in potato leaves, to determine if it is affected by inoculum with the tested bacteria. Previous research on potato plants indicated an increase in enzymatic activities, particularly catalase (CAT) and peroxidase (POD), in response to stress conditions facilitated by PGPB (Batool *et al.,* 2020 and Bhat *et al.,* 2022). In this study, seven out of eight isolates increased POD activity, while six increased CAT activity in potato leaves under salinity stress. This observation suggests that at least six isolates potentially employed an antioxidant enzyme activity strategy to counteract salinity stress. However, the isolate *Halomonas salifodinae* (WHS325), despite promoting high potato yield, showed different responses in CAT and POD activities, indicating potential alternative mechanisms beyond antioxidant production to alleviate salinity stress.

CONCLUSION

Soil salinity is a major obstacle to crop production, but plant growth-promoting bacteria (PGPB) can help to overcome this challenge. PGPB is a diverse group of bacteria that live in the root zone of plants and promote plant growth through a variety of mechanisms, including nutrient solubilization, hormone production, and stress tolerance. In this study, some bacterial isolates were isolated from soil and water samples in Wadi El-Natrun, Egypt, and then were screened for their salt tolerance and PGPR activities. The most promising isolates were able to solubilize phosphate and produce the plant hormones hydrogen cyanide (HCN) and

indole acetic acid (IAA). These isolates were then molecularly characterized using 16S rRNA gene sequencing. The findings of this study suggest that PGPR isolated from Wadi El-Natrun, Egypt, has the potential to improve potato growth and yield in saline soils. Further research is needed to evaluate the effectiveness of these isolates under field conditions.

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REFERENCES

Abegaz, K. (2007). Isolation, characterization, and identification of lactic acid bacteria involved in traditional fermentation of border Ethiopian cereal beverages. *African Journal of Biotechnology*. 6 (12): 1469-1478

Amer, M.H.; Abd El Hafez, S.A. and Abd El Ghany, M.B. (2017). Water Saving in Irrigated Agriculture in Egypt. Book, LAP LAMBERT Academic Publishing.

Batool, T., Ali, S., Seleiman, M.F., Naveed, N.H., Ali, A., Ahmed, K., Abid, M., Rizwan, M., Shahid, M.R., Alotaibi, M. and Al-Ashkar, I. (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. *Scientific Reports*. 10(1), p.16975.

Bazihizina, N.; Barrett-Lennard, E. and Colmer, T. (2012). Plant responses to heterogeneous salinity: growth of the halophyte Atriplexnummularia is determined by the root-weighted mean salinity of the root zone. *Journal of Experimental Botany*. Volume 63 (18): 6347-6358.

Bergey, D.H.; Holt, J.G. and Krieg, P. (1994). Bergey's manual of determinative bacteriology. 1994. Williams and Wilkins, Baltimore, MD, USA.

Bhat, M.Y., Gul, M.Z. and Dar, J.S. (2022). Gene Expression and Role of Antioxidant Enzymes in Crop Plants Under Stress. In Antioxidant Defense in Plants: Molecular Basis of Regulation (pp. 31-56). Singapore: Springer Nature Singapore.

Brick, J.M.; Bostock, R.M. and Silverstone, S.E. (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and environmental Microbiology*. 57(2), pp.535-538.

Camire M.E.; Kubow, S.; Donnelly, D. E. (2009). Potatoes and human health.*Critical Reviews in Food Science and Nutrition.* 49: 823 – 40.

Coahuila, C. and Pozzuoli, C. (2015). Chapter Title Isolation and Screening of Halophilic Bacteria for Production of Hydrolytic Enzymes Copyright Year 2015 Copyright Holder Springer International Publishing Switzerland Author Family Name Delgado-García.

Costa-Santos, M.; Mariz-Ponte, N.; Dias, M.C.; Moura, L.; Marques, G. and Santos, C. (2021). Effect of Bacillus spp. and Brevibacillus sp. on the Photosynthesis and Redox Status of Solanum lycopersicum. *Horticulturae*. 7(2), p.24.

FAO. (2022). World Food and Agriculture. Statistical Yearbook 2022. Rome. https://doi.org/10. 4060/cc2211en

Flowers, T.J.; Galal, H.K. and Bromham, L. (2010). Evolution of halophytes: multiple origins of

salt tolerance in land plants. *Functional Plant Biology*. 37(7), pp.604-612.

Gomaa, S.S. (2014). Effect of planting dates and seed tuber sources on productivity of potato in siwa oasis. *Journal of Plant Production*. 5(12), pp.2001-2016.

Gupta, A.; Rai, S.; Bano, A.; Sharma, S.; Kumar, M.; Binsuwaidan, R.; Suhail Khan, M.; Upadhyay, T.K.; Alshammari, N.; Saeed, M. and Pathak, N. (2022). ACC deaminase produced by PGPR mitigates the adverse effect of osmotic and salinity stresses in *Pisum sativum* through modulating the antioxidants activities. *Plants*. 11(24), p.3419.

Hammam, A.A. and Mohamed, E.S. (2018). Mapping soil salinity in the East Nile Delta using several methodological approaches of salinity assessment. *The Egyptian Journal of Remote Sensing and Space Science*. 23(2), pp.125-131.

Hu, J., Yan, J., Wu, L., Bao, Y., Yu, D. and Li, J. (2022). Insight into halotolerance of a robust heterotrophic nitrifying and aerobic denitrifying bacterium *Halomonas salifodinae*. *Bioresource Technology*. 351, p.126925.

Imperlini, E., Bianco, C., Lonardo, E., Camerini, S., Cermola, M., Moschetti, G. and Defez, R., (2009). Effects of indole-3-acetic acid on *Sinorhizobium meliloti* survival and on symbiotic nitrogen fixation and stem dry weight production. *Applied microbiology and biotechnology*. 83, pp.727-738.

Kalkhan, M.A.; Stohlgren, T.J.; Chong, G.W.; Lisa, D. and Reich, R.M. (2000). A predictive spatial model of plant diversity: integration of remotely sensed data, GIS, and spatial statistics. In Eighth Biennial Remote Sensing Application Conference (RS 2000).

Kashyap, P.L., Solanki, M.K., Kushwaha, P., Kumar, S. and Srivastava, A.K., (2020). Biocontrol potential of salt-tolerant Trichoderma and Hypocrea isolates for the management of tomato root rot under saline environment. *Journal of Soil Science and Plant Nutrition*. 20, pp.160-176.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*. 286(5776), pp.885-886.

Kumar, P. and Sharma, P.K., (2020). Soil Salinity and Food Security in India. *Frontiers in Sustainable Food Systems*. 4: 533781. doi: 10.3389/fsufs.

McGill, C.; Kurilich, A. and Davignon, J. (2013). The role of potatoes and potato components in cardiometabolic health: a review. *Annals of Medicine*. 45(7):467-473.

Miller, R. and Higgins, V. J. (1970). Association of cyanide with infection of birdsfoot trefoil by Stemphylium loti. *Phytopathology*. 60, 104–110.

Moreno Amador, M.D.L.; Pérez Gómez, D.; García Gutiérrez, M.T. and Mellado Durán, M.E. (2013). Halophilic Bacteria as a source of novel hydrolytic enzymes. *Life*. 3, 38-51.

Naqqash, T.; Hameed, S.; Imran, A.; Hanif, M.K.; Majeed, A. and van Elsas, J.D. (2016). Differential Response of Potato Toward Inoculation with Taxonomically Diverse Plant Growth Promoting Rhizobacteria. *Frontiers in Plant Science*.7:144. doi: 10.3389/fpls. 2016.00144

Nautiyal, C. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*. 170, 265–270. doi: 10.1111/j.1574-6968. 1999.tb13383

Oren, A., (2010). Industrial and environmental applications of halophilic microorganisms. *Environmental technology*. 31(8-9), pp.825-834.

Pandey, S.; Fartyal, D.; Agarwal, A.; Shukla, T.; James, D.; Kaul, T.; Negi, Y.K.; Arora, S. and Reddy, M. K. (2017) Abiotic Stress Tolerance in Plants: Myriad Roles of Ascorbate Peroxidase. *Frontiers in Plant Science*.8:581. doi: 10.3389/fpls.2017.00581

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* ;17: 362–370.

Purwantisari, S.; Parman, S. and Budihardjo, K. (2019). The growth and the production of potato plant supplemented by plant growth promoting rhizobacteria (PGPR). IOP Conf. Series: *Journal of Physics*: Conf. Series 1217 (2019) 012144 IOP Publishing doi:10.1088/1742-6596/1217/1/012144

Richardson, A.E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology*. 28(9), pp.897-906.

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (2009). Molecular cloning: a laboratory manual by Joe Sambrook, 3rd edn. Cold Spring Harbor Laboratory Press, New York.

Sarker, U. and Oba, S. (2020) The Response of Salinity Stress-Induced A. tricolor to Growth, Anatomy, Physiology, Non-Enzymatic and Enzymatic Antioxidants. *Frontiers in Plant Science.* 11:559876. doi: 10.3389/fpls.2020.559876

Schwyn, B. and Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*. 160, 47–56.

Smibert, R.M. (1994). Phenotypic characterization. *Methods for general and molecular bacteriology*. pp.607-654.

Spychalla, J.P. and Desborough, S.L. (1990). Superoxide dismutase, catalase, and α-tocopherol content of stored potato tubers. *Plant Physiology*. 94(3), pp.1214-1218.

Vander Donckt, M. and Chan, P. (2019). The new FAO global database on agriculture investment and capital stock. Food and Agriculture Organization of the United Nations.

Vejan, P.; Abdullah, R.; Khadiran, T.; Ismail, S. and Nasrulhaq, A.B. (2016). Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability. *Molecules*, 21 (573): 1-17.

Yadav, A.N., Verma, P., Kumar, M., Pal, K.K., Dey, R., Gupta, A., Padaria, J.C., Gujar, G.T., Kumar, S., Suman, A. and Prasanna, R. (2015). Diversity and phylogenetic profiling of niche-specific Bacilli from extreme environments of India. *Annals of Microbiology*. 65(2), pp.611-629.

Yoshimune, I.Y.K.H.K. (2010). 2.3 Environmental Distribution and Taxonomic Diversity of Alkaliphiles. Extremophiles Handbook, p.55.