Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

ISOLATION AND IDENTIFICATION OF NEW HALOTOLERANT BACTERIAL STRAINS FROM SALTY SOIL

Ahmed Shihab Salman[1], Elham Ismael AL-Shamary*[2]
[1]Ministry of Water Resources, Iraq
[2]Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq

ABSTRACT

This study was aimed to isolate a halotolerant bacterial strains, four samples were collected from four sources (salty soil) during February 2023. Five bacterial isolates were obtained using Modified Nutrient Agar (MNA) by raising the concentration of sodium chloride to 5% as a medium for isolation. Bacterial isolates were initially diagnosed based on culture and morphological characteristics. The obtained isolates showed varying sensitivity to five types of antibiotics, including ampicillin, azithromycin, amoxicillin, cefexin, and gentamycin. The isolates also showed varying ability to grow in a temperature range from 10 to 50 °C. The results indicated that the isolates were able to grow differently in different concentrations of sodium chloride, which included (5, 10, 15, 20, 25 and 30%), two isolates showed their ability to grow at a concentration of 30% of sodium chloride. These two isolates were subjected to molecular diagnosis based on the sequences of the 16s rRNA gene, and the result showed that they are two new, previously undiagnosed strains that were registered at the National Center for Biotechnology Information (NCBI), which are *Salinicoccus sp. strain Salman and Oceanobacillus sp. Mostafa*.

Key words: **antibiotic sensitivity, halophilic bacteria, high salinity, molecular diagnosis.**

Received: 15/8/2023, Accepted: 20/12/2023, Published: 30/5/2026

INTRODUCTION

The microorganisms that have the capability to survive under extreme environments such as (pH), temperature, salinity, pressure, are termed as extremophiles. There are many types of extremophiles corresponding to different environmental conditions (Fierer et al., 2005). There are a wide ranges of extremophiles, each comparing to several conditions in which microorganisms have inhabited. These microorganisms produce many bioactive compounds such as enzymes and antibiotics (Mesa-Marin et al., 2019). Extremophiles classified into different categories depending on the different conditions of their habitat. Among many extremophiles, halophiles are found in an environment with high concentration of salt. By preserving a balance between the inside and outside of the cell Halophilic microorganism can withstand extremely high salt concentrations and resist osmotic stress (Oren, 2010). Halophiles are increasingly significant in biotechnological applications because of this characteristic. Based on their tolerance to salt halophilic are categorized as extreme (20-25%), moderate (15-20%), and slight (5-10%) (Yadav et al., 2021). According to reports organic nitrogen compounds and pollutants which can be found in food, fertilizers, poisons, organic materials and explosives can be degraded by moderately halophilic bacteria (Yoo et al., 2023), Furthermore they may be appropriate organisms for the bioremediation of hypersaline environments because they have distinct metabolisms and can withstand high salinities (Al Zamzami et al., 2025). In

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

hypersaline ecosystem, the additive effect of high concentrations heavy metals strongly influences microorganisms living there. So, microorganisms thrive in such environments must be adapted both of high salinity and heavy metals (saibi et al., 2022).The external osmolarity is a limiting physical factor that determines the ability of organisms to survive in a given habitat. Bacterial and plant cellular responses to high osmolarity are remarkably similar or closely parallels in the mechanisms because both organisms accumulate the same set of cytoplasmic solutes under high salinity (Arayes et al., 2021). It is estimated that there are 8 billion people on the planet by 2050, this is expected to increase roughly 10 billion people worldwide, the demand for food product increased as a result of the sharp increase in global population (Youn and Seo, 2022).In addition to being used for human needs, water is a vital resource for all living things and is employed in industrial and agricultural projects (Puspaingrum and Titab, 2020). The world population growth and technological advancement have been recognized as major factors in the search for improved supplies and a reduction in surface and ground-water pollution, due to these issues researchers are looking into alternative purification technique such as brackish water desalination, which uses salt-water, additionally the removal of dissolved solids generally referred to desalination (Andharia et al., 2025). This study aimed to isolate and identificate bacterial strains from high salinity Iraqi soils and study some of their characteristics such as their tolerance to high salt concentrations, their growth at different temperatures, and their sensitivity to some antibiotics as a prelude for later use in the treatment of drain water with high salt concentrations and converting it into suitable water for irrigation in the current water scarcity in Iraq.

MATERIALS AND METHODS

Isolation sources : Saline soil samples were collected from different regions of central and southern Iraq on February 2023. The samples were taken from the surface down to a depth of 5 cm. approximately 500gr. per sample. They were placed in sterile plastic boxes and transported to the laboratory

Culture media: Modified nutrient agar (M NA): The MNA media was prepared as described by the manufacturing company. The media was autoclaved at 121℃ and 15 pound/inch² for 15 min. The prepared media was modified by adding 5% NaCl.

Modified nutrient broth (M N B): The MNB media was prepared as described by the manufacturing company. The prepared media was modified by adding 5% NaCl.

Isolation and purification: Decimal dilutions of the soil samples were prepared and the MNA media was inoculated with 1 ml of all the decimal dilutions by pouring plates in sterile atmospheres, after that the media was left until solidification and placed to the incubator at 37 °C for 48 hours (Sedrah and Al-Shamary, 2021). The purification process of isolates was carried out by sequential transferring into modified N.A. by streaking method and the plates were incubated at 37℃ for 48hrs. After that, the one colony of each isolates were transferred to the solid slant medium, and kept at 4 °C for using it in subsequent experiments (Salman and Asmaa, 2019).

Cultural and morphological identification of bacterial isolates: Morphological and Microscopical Characterization of the bacterial isolates were detected by observing the shape, size and color, texture and edge shape of the bacterial colonies, and by microscopic examination of bacterial cells after staining with gram stain and spore stain with malachite green for bacterial cells (Al-Musawi and Elham, 2021).

Halotolerant test: In order to identify the halotolerant isolates and rank them based on their level of tolerance to deferent concentrations of NaCl, the five isolates were cultivated on N.A media that contained (10, 15, 20, 25, and 30%) NaCl and incubated at 37°C for 48hrs .and the results was observed to detect the Halotolerant isolates.

Antibiotic sensitivity test: Bacterial cultures were prepared using modified NA media using the streaking method and then adding antibiotic discs at sterile atmospheres, five types of antibiotics were used (Ampicillin,

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

Azithromycin, Amoxicillin, Cefexin, and Gentamycin) the plates were incubated at 37℃ for 24 hours, then the results of the sensitivity of the bacterial isolates to the antibiotics were observed and recorded (Matuschek *et al.,* 2014).

Ability to grow at different temperatures test: The selected bacterial isolates cultured on modified N.A. media and incubated at (10, 20, 30, 40 and 50℃) for 48 hours, to examine their ability of growth in different range of temperatures, the results were observed and recorded after the end of the incubation period for each isolate.

Molecular identification: Analysis on sequences and confirmation of microorganism's data using rRNA database of (NCBI) after amplification of ribosomal RNA in bacteria. Every procedure such as the extraction of bacterial DNA, PCR amplification, gene sequencing, and assembly. For Bacteria, PCR on 16S rRNA using 27F and 1492R primers, (Table 1) and yielding of 1,300bp or more sequencing data (Majeed and Al-shamary, 2020).

Primers

Table 1. Primers used in identification experiment

Primer Name	Seq.	Annealing Temp.℃	Product Size(bp)
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500
1492R	5`-TACGGTTACCTTGTTACGACTT-3`	60	1500

The Macrogen Company/ Korea provided these primers in a lyophilized form. Nuclease-free water was used to dissolve lyophilized primers, resulting in a stock solution at a final concentration of 100 pmol/µl. In order to create a workable primer solution with 10pmol/µl., 10 µl of primer stock solution which was kept at (-20 ℃) mixed with 90µl of nuclease-free water.

DNA Extraction: Genomic DNA was extracted from two bacterial isolates according to the protocol of (ABIO pure).

Quantitation of DNA: Quants Fluorometer was used to detect the concentration of extracted DNA in order to detect the quality of samples for downstream applications. For 1 µl of DNA, 200µl of diluted Quantifluor Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected.

Polymerase chain reaction (PCR):

Table 2. PCR master mixture conditions

Compounds in the master mix	Vol.(µl)
Master mix	12.5
10 P mole Forward primer	1
10 P mole Reverse primer	1
Nuclease free water	8.5
DNA extract	2
Total volume5	25

The master mixture, (Table 2) was mixed for few seconds using vortex. The tube was placed in PCR thermo cycler. The device was programmed according to (Table 3), and the amplification was taken place to amplify the extracted DNA.by the end of the reaction time.

Table 3. PCR Program

Steps	℃	M:S	Cycle
Initial Den.	95	05:00	1
Denaturation	95	00:30	
Annealing	60	00:30	30
Extension	72	01:00	
Final Ext.	72	07:00	1
Hold	10	10:00	

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to verify the existence of amplification following PCR amplification. Regarding the criteria for extracted DNA, PCR was entirely reliable.

Preparation of agarose

• Amount of100 ml of 1X TAE was taken in a flask.
• Agarose1.5 gm. (for 1.5%) was added to the buffer.
• The solution was heated to boiling (using Microwave) until all the gel particles were dissolved.
• Ethidium Bromide 1µl of (10mg/ml) was added to the Agarose.
• The Agarose was stirred in order to get mixed and to avoid bubbles
• The solution was left to cool down at 50-60°C.

Casting of the horizontal agarose gel

After sealing the tow edges of the gel-tray with cellophane tape, the Agarose solution was poured in, and it was allowed to solidify for 30 minutes at room temperature .the gel was put in the gel-tray after the comb was carefully taken out 1XTAE- electrophoreses buffer was added to the tray until it covered the gel surface by 3-5mm .

DNA loading: PCR products were loaded directly. For PCR product, 5µl was directly loaded to well. Electrical power was turned on at 100v/m Amp for 60min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system.

Standard Sequencing: PCR product were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogene Corporation – Korea. The results were compared with the available information with NCBI website using BLAST Nucleotides software to identify the chosen strains.

RESULTS AND DISCUSSION

Isolation: The process of microorganism's isolation is the cornerstone for obtaining pure isolates for conducting physiological and biochemical tests (Mohamed and Al-shamary, 2022). Five bacterial isolates were obtained from five saline soil isolate sources from central and southern Iraq (A1, B1, C1, D1, E1) as showed in (Table 4).Saline soil were chosen as a selective pressure environment on the bacterial isolates to be obtained. The obligate halophilic microorganisms are unable to survive in the environments without high salt concentration due to their macromolecular modifications in the primary and secondary structure of enzymes such as enzyme synthesis becomes dependent on the presence of high salt concentrations. At the same time, there are organisms that live in salty environments, but their enzymes are sensitive to salinity. These organisms depend on increasing the concentration of compatible solvents inside the cell to face the high external osmotic pressure, if this is possible (Neagu and Stancu, 2025). Solvents protect enzymes from being inhibited by salts by providing them with some aggregates. Some sources indicate that among the adaptations that bacteria use to salinity is that their cell walls do not contain Muramic acid, and instead there are complex polymers containing sulfur, which makes them resistant to antibiotics, as noted. The proteins and enzymes present in their cell membranes contain negative amino acids, so they need high salt concentrations to stabilize the weak hydrophobic bonds, which are unstable in the presence of water alone. These proteins and enzymes suffer morphological changes when the salts are removed, which leads to the loss of their function (Adhvaryu *et al.,* 2025). These changes include: Unraveling their structural folds, it was also observed that the fatty components in the membranes of salt-loving bacteria do not contain phosphate linked to the glycerol as is usual with an ester bond, but rather contain long hydrocarbon chains of Isoprenoids linked to the glycerol with ether bonds, which are more stable in high salt concentrations. It was also observed that a modification occurred. In interactions between proteins and fats, a more stable modification occurs at high salt concentrations. It has also been noted that potassium ions are very important for linking the 30S, 50S, and then 70S units, thus carrying out the translation process correctly and binding proteins. (Kapadia *et al.,* 2022).

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

Table 4. Bacterial Isolation sources and codes

Isolate	Source
A1	South of Babylon
B1	Al-Qadisiah
C1	Al-Qadisiah
D1	Dhi Qar
E1	Dhi Qar

Cultural identification

The growing bacterial colonies on the modified medium (N.A+ 5%NaCl) after 48h. Of incubation at 37 °C showed that four out of five isolates had sticky, creamy-white colonies with a dense growth center with branched ends at the edges of the colonies. One isolate was light orange in color with a sticky texture and homogeneous ends (Table 5).

Morphological identification

Gram staining showed that one isolate had spherical cells (cocci) positive for Gram stain, while the remaining four isolates had rod cells (bacilli) positive for Gram stain (Figure1) and after using spore staining the bacillus sp. isolates showed that are spore formers (Figure 2).

Table 5. Cultural identification characteristics of bacterial isolations

Isolate	Shape	color	Colony	Gram stain	Spore forming
A1	cocci	Light orange	sticky texture and homogeneous ends.	+	-
B1	bacilli	Creamy-white	Sticky with a dense growth center with branched ends at the edges of the colonies.	+	+
C1	bacilli	=	=	+	+
D1	bacilli	=	=	+	+
E1	bacilli	=	=	+	+

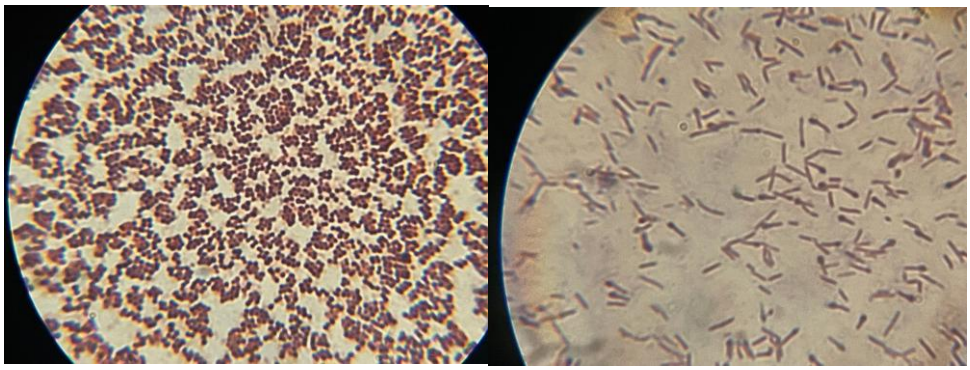

Figure 1. Morphological identification of cocci and bacilli bacterial isolates.

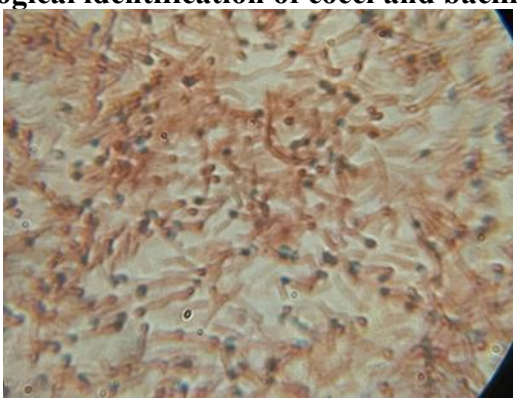

Figure 2. Spore forming bacteria

The ability to grow at different temperatures: All the five bacterial isolates showed varying ability to grow in a range of temperatures (Table 6), as the best growth for all isolates was within the range of 30-40 °C, while isolates A1 and B1 were able to grow at a higher rate, reaching 50 °C. There is a relationship between the high salinity and the

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

growth temperature of Halophilic and halotolerant bacteria (Fitri *et al.,* 2022).The growth temperature and the optimal growth Temperature. Are affected by the NaCl concentration in the growth media (Tourova *et al.,* 2022), so the isolates A1 and B1wich are tolerant to high salt concentration showed a growth at wide range of Temperature (10 - 50°C). Water environments with high salt concentration are usually located in tropical areas with high evaporation rates at high daytime temperatures, in addition to that salty water cooling faster during the night or winter season. The majority of halophilic and halotolerant microorganisms are mesophilic or slightly thermophilic (Teles *et al.,* 2022).

Table 6. The ability of bacterial isolates to grow at different temperatures

Isolate no.	10°C	20°C	30°C	40°C	50°C
A1	++	++	+++	++	+
B1	++	++	+++	++	+
C1	+	++	+++	++	-
D1	+	++	+++	++	-
E1	+	++	+++	++	-

*(+++) heavy growth, (++) moderate growth, (+) weak growth, (-) no growth

Antibiotic sensitivity

Through the results, it was found that the five bacterial isolates are sensitive to different levels towards the types of antibiotics used in the experiment (Table7), which means that the effect of antibiotics is variable according to various factors, the producing of enzymes such as β-lactamases, alterations of cell wall permeability and chromosomal mutations and activation of efflux pumps are few of the mechanism of drug resistance (Chawla *et al.,* 2022). Antibiotic resistance and multi-drug resistance are common in halophilic bacteria that have been isolated from their natural habitat as well as pathogenic bacteria, the bacteria can either transform by absorbing naked DNA from surrounding environment or they can transfer plasmid through conjugation (Shinde and Thomber, 2016). Antimicrobial resistance in bacteria is frequently linked to the presence of plasmids, and the majority of these extra chromosomal replicates carry drugs resistance genes, mega- plasmids and plasmids have previously been reported in halophiles (Perez *et al.,* 2021) .It has previously been documented that plasmids are presents in halophile isolates from tannery wastes (Thompson and Gilmore, 2024). As the results summarized in (Table 7), all the isolates in general are sensitive to Ampicillin and Amoxicillin which are inhibits cell wall synthesis, while the other types of antibiotics have molecular level effects at the bacterial cell like inhibit DNA replication and protein 30s and 50s inhibition.

Table 7. Sensitivity of bacterial isolates to some types of antibiotics.

Isolate	Ampicillin	Azithromycin	Amoxicillin	Cefixime	Gentamicin
A1	+++	+	++	-	+
B1	+++	+	++	-	+
C1	+++	+	++	-	+
D1	++	+	++	-	+
E1	++	+	++	-	+

*(+++) very sensitive, (++) moderate sensitivity, (+) weak sensitivity, (-) not sensitive

Halotolerant: All five isolates showed their ability to grow intensively in the modified nutrient agar media containing concentrations of sodium chloride between 5-20%, all isolates were not able to grow on media free of sodium chloride, isolates A1 and B1 showed their ability to grow in concentrations that reached 30% of Sodium chloride (Table 8). Microorganisms that classified as halophilic need NaCl concentration reach to 12% to grow well (Vreeland, 2020). The main mechanism of salt tolerant operates by internal retention of balancing solute (K+) equal to external NaCl concentration. The second mechanism involves protein with acidic and nonpolar amino acids, the protein needs high salt

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

concentration to balance its charge for its optimal activity (Olaleye *et al.,* 2025).

Table 8. Effect of different NaCl concentrations on the growth of bacterial isolates

Isolate	NaCl %						
	0	5	10	15	20	25	30
A1	-	++	+++	+++	+++	++	+
B1	-	++	+++	+++	+++	++	+
C1	-	++	+++	+++	++	+	-
D1	-	++	+++	+++	++	+	-
E1	-	++	+++	+++	++	+	-

*(+++) heavy growth, (++) moderate growth, (+) weak growth, (-) no growth

Molecular Identification

Genetic diagnosis technology is considered one of the latest technologies used in recent years, especially in the field of diagnosis and genetic modifications to benefit from microorganisms in many fields (Auda and Khalifa, 2019). Isolates A1 and B1 were selected from the previous experiment, as they showed tolerance to the highest concentrations of sodium chloride and were diagnosed genetically. The results of DNA extraction (Figure 3**),** were send to Macrogen/ Korea, detection of nitrogenous base sequences (Figure4), and matching them with the NCBI International Gene Bank database showed that the two strains are new, named and registered.in the NCBI World GenBank database under the code (OQ825941.1) and (OQ825942.1) *Salinicoccus sp. Strain Salman* and *Oceanobacillus sp. Mostafa***.**

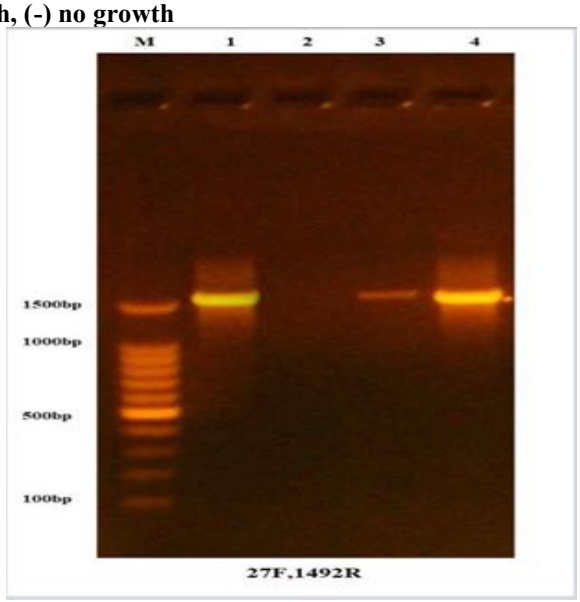

Figure 3. Results of the amplification of 16s RNA gene of Unknown bacterial species were fractionated on 1.5% Agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-4 resemble 1500bp PCR products

Sample 1:

TACATGCAAGTCGAACGCGCGGATCAGGAGCTTGCTCCTGTGACGCGAGTGGCGGACGGGTGAGTAACA
CGTAGGCAACCTGCCCATCAGA
CTGGGATAACCACGGGAAACCGTGGCTAATACCGGATAATCCTTTTCCACACAGGTGGGAAAGTTGAAAG
GCGGTCTTTTGGCTGTCACTG
ATGGATGGGCCTGCGGCGCATTATCTGGTTGGTGGGGTAACGGCCCACCAAGGCGACGATGCGTACCCGA
CCTGAGAGGGTGATCGGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGT
GAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTCAGGGAAGAACGCCGACGGGAGTAACTGCCCG
TCGGGTGACGGTACCTGACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTG
GGCGTAAAGCGCGCGTAGGCG
GTTCGTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGCGGACTTGAGT
GCAGAAGAGGAGAGTGGAATT
CCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGC
AACTGACGCTGAGGTGCGAAAG
CGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGG
GTTTCCGCCCCTTAGTGCTGC
AGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTCTGACCACCCTGGAGACAGG
GTTTCCCTTCGGGGCAGAGTG

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTATCATTAGTTGCCAGCAT
TCAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA
TGCCCCTTATGATTTGGGCTAC
ACACGTGCTACAATGGACAGGTTACAAAGGGCAGCTAAGCCGCGAGGCCAAGCGAATCCCATAAAACTG
TTCTCAGTTCGGATTGGAGTCT
GCAACTCGACTCCATGAAGCTGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCG
GGTCTTGTACACACCGCCCGT
CACACCACGAAAGTCGGTAACACCTGAAGCCGGTGGGCCAACCTCTTGGAGGCAGCC

Sample 2:

GGGCCCATTAACTTATTTGGTAAGGTAATGGCTCACCCAAGGCAACGATGCGTAACCGACCTGAGAGGGT
GATCGGCCCACCCTGGGACTG
AGACAGGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGACGAAAGTCTGACGGAG
CAACGCCGCGTGAGTGATGAAG
GTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTATAGTAACTGTTAGCACCTTGACGGT
ACCTAACCAGAAAGCACCGGC
TAACTACGTGCCAGCATCCGCGGTAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCG
CGCGTAGGCGGTCCTTTAAGT
CTGATGTGAAAGCCCACGGCTTAGCCGTGGAGGGTCATTGGAAACTGGAGGACTTGAGTGCAGAAGAGG
AGAGTGGAATTCCACGTGTAGC
GGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG
AGGTGAGAAAGCGTGGGTAGCG
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGGGGTTTCCGCCCC
TTAGTGCTGAAGTTAACGCAT
TAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAAGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATT
CGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGATCGCTCTAGAGATAGAGTTTCCCCTTC
GGGGGCAGAGTGACAGGTGGT
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTA
GTTGCCAGCATTAAGTTGGG
CACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT
GACCTGGGCTACACACGTGCT
ACAATGGATGGAACAAAGGGCAGCGAAGCCGCGAGGCCAAGCAAATCCCATAAAACCATTCTCAGTTCG
GATTGTAGGCTGCAACTCGCCT
ACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACA
CACCGCCCGTCACACCACGAG AGTTGGTAACACCCGAAGTCGTTGAGGTAACC

Figure 4. FASTA Sequence of the new 2 bacterial strains

CONCLUSION

The research results showed the possibility of obtaining tolerant and halophilic isolates from extreme environments with high salt concentrations, which are the best for the isolation process, as they exert selective pressure to filter out microorganisms with the desired trait. In addition, these genetically identified bacterial strains in this study have different sensitivities to some types of antibiotics, which confirms their adaptation and the modifications that enabled them to grow and multiply in environments with high salt concentrations.

JOURNAL DECLARATION

The Second author (**Elham Ismael AL-Shamary**) serves as an editor for Iraqi Journal of Agricultural Sciences but was not involved in the peer review process of this manuscript beyond their role as an author. The authors declare no other conflict of interest.

ACKNOWLEDGEMENT

The authors wish to express their thanks and appreciation to the University Of Baghdad\ College Of Agricultural Engineering Sciences for providing the necessary facilities to this work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required

permissions for re-publication, which are included with the manuscript.
Author/s signature on Ethical Approval Statement.
Ethical Clearance and Animal welfare
Funds: No funds were obtained for this research.

AUTHOR'S CONTRIBUTION STATEMENT

REFERENCES

Adhvaryu, S., Kiskova, J., Piknova, M., Malinicova, L., Beck, T., Buchtikova, I., & Pristas, P. (2025). The characterization of halophilic polyhydroxyalkanoate-producing bacteria from brine in Solivar near Prešov (Slovakia). World Journal of Microbiology and Biotechnology, 41(12), 505. https://doi.org/10.13005/bbra/1874

Al Zamzami, I. M., Yona, D., Faqih, A. R., & Kurniawan, A. (2025). Halophilic bacteria in biotechnology: A seven-decade scientometric analysis of global research trends, knowledge gaps, and emerging applications (1955–2024). Journal of Ecological Engineering, 26(10), 252-271 doi.org/10.12911/22998993/205826

Andharia, K., Katariya, D., & Kothari, R. (2025). Isolation and Characterization of Several Halophilic Bacteria from Marine Environments. Current Biotechnology. doi:10.2174/0122115501425807251017044247

Arayes, M. A., Mabrouk, M. E., Sabry, S. A., & Abdella, B. (2021). Diversity and characterization of culturable haloalkaliphilic bacteria from two distinct hypersaline lakes in northern Egypt. Biologia, 76(2), 751-761. https://doi.org/10.1007/s00792-016-0879-x

Chawla, M., Verma, J., Gupta, R., & Das, B. (2022). Antibiotic potentiators against multidrug-resistant bacteria: discovery, development, and clinical relevance. Frontiers in microbiology, 13, 887251. /doi.org/10.3389/fmicb.2022.88725

Fierer, N.; J. A. Jackson; R. Vilgalys; and R. B. Jackson. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology. 71(7): 4117-4120. doi: 10.1128/AEM.71.7.4117-4120.2005

Fitri, D. A., Asih, E. N. N., Kartika, A. G. D., Agustina, N., Fadholi, B., Dewi, K., & Efendy, M. (2022). Morphological characteristics of halophilic bacteria in traditional salt production. Journal of Marine Resources and Coastal Management, 3(1), 1-7. doi: https://doi.org/10.29080/mrcm.v3i01.1360

Kapadia, C., Patel, N., Rana, A., Vaidya, H., Alfarraj, S., Ansari, M. J., & Sayyed, R. Z. (2022). Evaluation of plant growth-promoting and salinity ameliorating potential of halophilic bacteria isolated from saline soil. Frontiers in plant science, 13, doi.org/10.3389/fpls.2022.946217

Matuschek, Erika, FJ. D. Brown, and Gunnar Kahlmeter. (2014). "Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories." Clinical microbiology and infection 20(4): 255-266. doi:10.1111/1469-0691.12373.

Mesa-Marín, J., E. Mateos-Naranjo, I. D. Rodríguez-Llorente, E. Pajuelo, & S. Redondo-Gómez, (2019). Synergic effects of rhizobacteria increasing use of halophytes in a changing world Halophytes and climate change: adaptive mechanisms and potential uses. Wallingford UK: 240-254. doi:10.1079/9781786394330.0240.

Neagu, S., & Stancu, M. M. (2025). Novel Halotolerant Bacteria from Saline Environments: Isolation and Biomolecule Production. BioTech, 14(2), 49. doi.org/10.3390/biotech14020049

Olaleye, A. C., Oyewusi, H. A., Akinyede, K. A., Oladipo, O. O., & Oyeyemi, B. F. (2025). Bacterial community structure and secondary metabolite insights from halophiles at Oniru Beach, Lagos. Archives of Microbiology, 207(11), 299. doi.org/10.1007/s10661-020-08888-5

Oren, A. (2010). Industrial and environmental applications of halophilic microorganisms. Environmental technology, 31(8-9): 825-834 doi: 10.1080/09593330903370026

Perez, M. F., Saona, L. A., Farías, M. E., Poehlein, A., Meinhardt, F., Daniel, R., & Dib, J. R. (2021). Assessment of the plasmidome of an extremophilic microbial community from the Diamante Lake, Argentina. Scientific

Reports, 11(1), 21459. doi.org/10.1038/s41598-021-00753-1

Puspaningrum, T. C., & H.S. Titah, (2020). The removal of salinity in a reed bed system using mangroves and bacteria in a continuous flow series reactor. Journal of Ecological Engineering, 21(6): 212-223. doi.org/10.12911/22998993/124075.

Saibi, A. N. E., Nas, F., Arab, M., Aissaoui, N., Boukeroui, Y., & Klouche-Khelil, N. (2022). Antimicrobial and enzymatic profiling of halophilic and halotolerant bacteria from a hypersaline lake 'The Great Sebkha of Oran, Northwestern Algeria'. Geomicrobiology Journal, 39(9), 816-831. doi.org/10.1080/01490451.2022.2079777.

Shinde, V. D., & R. S. Thombre, (2016). Antibiotic resistance profiling of marine halophilic bacteria and haloarchaea. Journal of Applied Pharmaceutical Science, 6(10):132-137. doi: 10.7324/JAPS.2016.601018

Teles, E. A. P., Xavier, J. F., Arcênio, F. S., Amaya, R. L., Gonçalves, J. V. S., Rouws, L. F. M., & Coelho, I. S. (2024). Characterization and evaluation of potential halotolerant phosphate solubilizing bacteria from Salicornia fruticosa rhizosphere. Frontiers in Plant Science, 14, 1324056. doi.org/10.3389/fpls.2023.1324056

Thompson, T. P., & Gilmore, B. F. (2024). Exploring halophilic environments as a source of new antibiotics. Critical reviews in Microbiology, 50(3), 341-370. doi.org/10.1080/1040841X.2023.2197491

Tourova, T. P., Sokolova, D. S., Semenova, E. M., Ershov, A. P., Grouzdev, D. S., & Nazina, T. N. (2022). Genomic and physiological characterization of halophilic bacteria of the genera Halomonas and Marinobacter from petroleum reservoirs. Microbiology, 91(3), 235-248. doi.org/10.1007/s00792-010-0312-9

Vreeland, R. H. (2020). Taxonomy of halophilic bacteria. In The Biology of Halophilic Bacteria: 105-134. CRC Press doi: 10.1201/9781003069140.

Yadav, D., A. Singh, N. Mathur, A. Agarwal, & J. Sharma, (.2021). Isolation of halophilic bacteria and their screening for extracellular enzyme production. Journal of Scientific & Industrial Research, 80(7): 617-622. doi: 10.56042/jsir.v80i7.39611

Yoo, Y., Lee, H., Lee, J., Khim, J. S., & Kim, J. J. (2023). Insights into saline adaptation strategies through a novel halophilic bacterium isolated from solar saltern of Yellow sea. Frontiers in Marine Science, 10, 1229444. doi.org/10.3389/fmars.2023.1229444

Youn, H. Y., & Seo, K. H. (2022). Isolation and characterization of halophilic Kocuria salsicia strains from cheese brine. Food science of animal resources, 42(2), 252. doi: 10.5851/kosfa.2022.e1

Iraqi Journal of Agricultural Sciences –2026:57(5):1411-1421
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/npk28a23

عزل وتشخيص سلالات بكتيرية جديدة متحملة للملوحة العالية من الترب المالحة في العراق

احمد شهاب احمد سلمان[1]، الهام اسماعيل الشمري[2]

[1] وزارة الموارد المائية/ العراق

[2] قسم علوم الاغذية/كلية علوم الهندسة الزراعية/جامعة بغداد

المستخلص

جمعت خمسة عينات من خمسة مصادر (تربة مالحة) من مناطق مختلفة في وسط العراق وجنوبه خلال شهر شباط 2023 لغرض العزل، تم الحصول على خمسة عزلات بكتيرية باستعمال الوسط المغذي الصلب المحور Modified Nutrient Agar (MNA) وذلك برفع تركيز كلوريد الصوديوم الى 5% وسطا للعزل، شخصت العزلات البكتيرية بصورة اولية اعتمادا على الخواص المزرعية والمورفولوجية، اظهرت العزلات المستحصل عليها حساسية متفاوتة تجاه خمسة انواع من المضادات الحيوية شملت Gentamycin, Cefexin, Amoxicillin, Azithromycin, Ampicillin كما أظهرت العزلات قدرة متفاوتة على النمو في مدى حراري تراوح من 10 لغاية 50 درجة مئوية ,وتشير النتائج الى قابلية نمو متفاوتة للعزلات في تراكيز مختلفة من كلوريد الصوديوم شملت (5, 10, 15, 20, 25و 30%) واظهرت عزلتان قابليتهما على النمو بتركيز 30% من كلوريد الصوديوم, أخضعت هاتين العزلتين الى التشخيص الجزيئي بالاعتماد على تتابعات الجين 16S rRNA واظهرت النتيجة بانهما سلالتين جديدتين غير مشخصتين مسبقا تم تسجيلهما في المركز الوطني لمعلومات التقنيات الحيوية NCBI وهما Salinicoccus sp. Strain Salman و Oceanobacillus sp. Strain Mostafa.

الكلمات المفتاحية: الحساسية للمضادات الحيوية، البكتيريا المحبة للملوحة، الملوحة العالية، التشخيص الجزيئي.