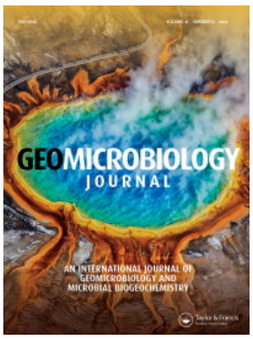

Geomicrobiology Journal

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ugmb20

# Isolation and Characterization of Plant Growth Promoting and Metal Resistant Archaea Associated with the Rhizosphere of *Salsola stocksii* and *Atriplex amnicola*

**Salma Mukhtar, Hijab Zahra Shah, Samina Mehnaz & Kauser Abdulla Malik**

**To cite this article:** Salma Mukhtar, Hijab Zahra Shah, Samina Mehnaz & Kauser Abdulla Malik (27 Jul 2024): Isolation and Characterization of Plant Growth Promoting and Metal Resistant Archaea Associated with the Rhizosphere of *Salsola stocksii* and *Atriplex amnicola*, Geomicrobiology Journal, DOI: 10.1080/01490451.2024.2381008

**To link to this article:** https://doi.org/10.1080/01490451.2024.2381008

View supplementary material

Published online: 27 Jul 2024.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ugmb20

GEOMICROBIOLOGY JOURNAL
https://doi.org/10.1080/01490451.2024.2381008

Taylor & Francis
Taylor & Francis Group

Check for updates

# Isolation and Characterization of Plant Growth Promoting and Metal Resistant Archaea Associated with the Rhizosphere of *Salsola stocksii* and *Atriplex amnicola*

Salma Mukhtar, Hijab Zahra Shah, Samina Mehnaz, and Kauser Abdulla Malik

School of Life Sciences, Forman Christian College, (A Chartered University), Lahore, Punjab, Pakistan

**ABSTRACT**

Halophilic microorganisms play a crucial role in plant health and growth in salinity affected lands. The objective of this study was to evaluate the haloarchaeal diversity from the rhizosphere of halophytes and plant growth promoting abilities of these strains. The whole genome sequences of two haloarchaeal strains, *Halorubrum lacusprofundi* HL1RP11 and *Halobacterium noricense* NRS2HaP9, were analyzed, and genes related to plant growth promoting traits were identified. Phylogenetic analysis showed that archaeal strains of *Halococcus, Halorubrum, Halobacterium* and *Natrinema* were dominant in the rhizosphere of halophytes. More than 60% of the strains were positive for phosphate solubilization and IAA production. About 33% were siderophore producers. More than 40% of haloarchaeal strains showed the heavy metal resistance for Nickel, Cadmium, Chromium and Zinc at a concentration of 5 mM. Genes involved in plant growth promotion were identified through annotation. Gene clusters related to secondary metabolites including phenazine, siderophore production and terpene were also identified in this study. Our results suggested that these haloarchaeal strains can be used as an eco-friendly biofertilizer to improve growth and productivity in hypersaline environment.

**ARTICLE HISTORY**
Received 29 February 2024
Accepted 12 July 2024

**KEYWORDS**
Halophilic archaea; plant growth promoting traits; osmoregulation; halophytes; *Halorubrum*; *Halobacterium*

## Introduction

Soil salinity is one of the most adverse environmental factors that limit the growth and productivity of various economically important crops. It covers over 6% of the land worldwide (Kijne 2006; Lekakis et al. 2015; Kumar and Sharma 2020). Salt stress affects the agricultural land in many ways which include ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress on phosphorous uptake from soil (Lekakis et al. 2015; Mukhtar et al. 2020b). It reduces crop production and has adverse effects on germination due to toxicity and change in enzymatic activities (Kibria and Hoque 2019; Mukhtar et al. 2018). Halophytes are plants that grow well in saline soil and water, such as para grass, *Salsola stocksii, Kochia indica* and *Atriplex amnicola* (Ahmad et al. 2007). Therefore, these offer a better alternative where conventional crops cannot be raised, and drainage is too expensive. These plants play a significant role to the developing world's supply of food, fiber, fuel and fodder (Dagla and Shekhawat 2005).

Halophiles are microorganisms that grow in environments with high salt concentrations. Halophiles are usually divided into categories; slight (1–3%); moderate (3–15%); and extreme (15–30%) (Oren 2012; DasSarma et al. 2010). Halophytes rhizosphere harbors a great diversity of halophilic bacterial, archaeal and fungal halophiles. The diverse microbial communities associated with halophyte rhizospheres help these plants to cope with high salinity and drought stress (Shrivastava and Kumar 2015; Etesami and Beattie 2018; Mukhtar et al. 2018). Microorganisms residing in the rhizosphere play a crucial role in plant fitness and productivity, especially under extreme conditions (Berendsen et al. 2012; Qu et al. 2020). Studies on rhizosphere microbiomes have revealed their influence on chemical exudates responsible for the production and secretion of signaling molecules by both microbes and plants (Hartmann et al. 2021; Yadav et al. 2015; Gaba et al. 2017; Reang et al. 2022).

Halophilic archaea grow in environments with salt concentrations varying from 15 to 30% NaCl (DasSarma et al. 2010; DasSarma and DasSarma 2015). They can survive at high salt concentrations reaching up to 5 M NaCl (Oren 2012). Halophilic archaea differ from halophilic bacteria because they usually use 'salt in' strategy for their survival in all high salt concentrations in which they maintain their internal environment by the accumulation of high concentration KCl ions (Oren 2012; DasSarma et al. 2010) while halophilic bacteria use accumulation of osmolytes for their survival at high salt concentrations. Some halophiles including *Halorhodospira* and *Halorubrum* can also use 'compatible solutes' such as trehalose, ectoine, betaine, and proline can

**CONTACT** Kauser Abdulla Malik kausermalik@fccollege.edu.pk School of Life Sciences, Forman Christian College, (A Chartered University), Ferozepur Road, Lahore, Punjab 54600, Pakistan.
Supplemental data for this article can be accessed online at https://doi.org/10.1080/01490451.2024.2381008.
© 2024 Informa UK Limited, trading as Taylor & Francis Group

be accumulated to maintain the cell's internal osmotic pressure (Oren 2012; Mukhtar et al. 2020a).

Plant growth promoting (PGP) microbes living in the rhizosphere of halophytes play an important role in plant health and soil fertility under salinity stress conditions. They enhance plant growth and increase grain yield of various crops such as wheat, corn, rice, sugarcane and legumes by solubilization of minerals (P, K, Zn), production of compounds such as indole acetic acid (phytohormone), siderophores, HCN, and breaking down of complex organic materials for the easy uptake by plants (Patel et al. 2016; Mukhtar et al. 2020a). Only a few studies reported the isolation and characterization of halophilic archaeal genera including *Halobacterium, Natrinema, Haloferax, Natrococcus* and *Haloarcula* from the rhizosphere of halophytes such as *Abutilon, Dicanthium, Sporobolous* and *Sueada* (Yadav et al. 2015; Kumar and Tiwari 2017; Smith-Moore and Grunden 2018). Data on phosphate solubilization, siderophore and IAA (indole acetic acid) production is available from methanogens, haloarchaea and thermococci (Dave et al. 2006; Trivedi et al. 2019; Patel et al. 2021). Halophilic archaeal genera including *Natrinema, Halobacterium* and *Halococcus* have been reported for P-solubilization and production of organic acids such as citric, succinic, oxalic, lactic, acetic and isovaleric acids (Yadav et al. 2015; Gaba et al. 2017). Some archaeal groups play an important role in nitrogen cycle. A number of previous studies have reported the ammonia oxidizing methanogens (Herrmann et al. 2008; Trivedi et al. 2019). Halophilic archaea isolated from hypersaline polluted environments also have the ability to tolerate heavy metal resistance for nickel, cadmium, uranium, chromium and zinc (Das et al. 2014; Voica et al. 2016; Moopantakath et al. 2023).

The present work endeavors to identify culturable halophilic archaeal diversity isolated from the rhizosphere of halophytes (*S. stocksii* and *A. amnicola*) and non-rhizosphere soil samples collected from Khewra Salt Mines, Pakistan. We selected *S. stocksii* and *A. Amnicola* for this study because these plants have better salt tolerant ability as compared to other plants. These plants can be used as fodder crops and also for biofuel production. This study is the first report on the characterization of plant growth promoting halophilic archaeal strains with their ability to solubilize phosphate, production of indole acetic acid, siderophores, exopolysaccharides and heavy metal resistance. We also identified genes and operons involved in plant growth promotion, secondary metabolism, environmental adaptation, glycerol metabolism and membrane transportation from the genomes of *Halorubrum lacusprofundi* HL1RP11 and *Halobacterium noricense* NRS2HaP9.

## Material and methods

### Sampling of soil

Khewra Salt Mine is the world second largest salt mine, located near Pind Dadan Khan Tehsil of Jhelum District, Punjab, Pakistan. It has $Na^+$ and $Cl^-$ dominating ions and the pH is near neutral to slightly alkaline. The sampling area was selected according to land use and vegetation cover. Four locations were chosen with the following geographical details; site 1 was located at 32°35'N, 73°10'E, site 2 was located at 32°38'N, 73°02'E, site 3 was located at 32°38'N, 73°02'E and site 3 was located at 32°38'N, 73°02'E. Vegetation of this area is classified as sub-tropical dry evergreen forest. Rhizospheric soil samples were collected by gently removing the plants and obtaining the soil attached to the roots. For non-rhizospheric saline soil samples, the upper 8-10 cm of mineral soil was collected. At each site, soil samples of approximately 500 g each from four different locations were collected in black sterile polythene bags. These samples were stored at 4 °C for further analysis.

### Isolation of halophilic archaea from the rhizospheric and non-rhizospheric soils of Salsola stocksii and Atriplex amnicola

Modified growth medium (MGM) with NaCl 250 g/L, casamino acids 7.5 g/L, peptone 5 g/L, yeast extract 2 g/L, KCl 30 g/L, trisodium citrate 3 g/L, $MgSO_4$ 24 g/L, pH 7.2; was used for the isolation and purification of archaea from rhizospheric and non-rhizospheric soils of *Salsola stocksii* and *Atriplex amnicola* (Kauri et al. 1990). These soil samples were collected from Khewra Salt Mine, Pakistan. For isolation of individual archaeal strains, the soil sample was mixed thoroughly, sieved and then one gram of the representative soil sample was used for further analysis. All samples were serially diluted up to $10^{-6}$ and 100 µL of each dilution from $10^{-3}$ to $10^{-6}$ were spread on MGM plates for counting colony forming units (CFU) per gram of soil (dry weight). Plates were incubated at 37 °C until the appearance of archaeal colonies. Single, well-isolated colonies were selected, grown in MGM broth for 4 days to get OD600 around 1, and preserved these archaeal cultures in 33% glycerol at −80 °C for subsequent characterization.

### Isolation of genomic DNA and amplification of 16S rRNA gene

Genomic DNA of halophilic archaeal strains from the rhizospheric and non-rhizospheric soils was isolated by CTAB method (Winnepenninckx et al. 1993). The quality and quantity of extracted DNA was observed by agarose gel electrophoresis in TAE buffer (Tris base, 242 g/L; glacial acetic acid, 57 ml/L; 0.5 M EDTA, 100 ml/L) and the Nanodrop, respectively.

For PCR amplification of 16S rRNA gene, universal forward primer HA1 (5′-ATTCCGGTTGA TCCTGCCGGAGGTC-3′), and reverse primer HA1465 (5′-GATCCAGCCGCAGATTCCCC-3′), for prokaryotes were used (Yildiz et al. 2012). Denaturation temperature was 95 °C for 5 min followed by 35 rounds of 94 °C for 60 sec, 55 °C for 50 sec and 72 °C for 90 sec and final extension at 72 °C for 10 min. A reaction mixture of 25 µL was prepared by using Taq buffer 2.5 µL (10X), $MgCl_2$ 3 µL (25 mM), Taq polymerase 1 µL, dNTPs 2 µL (2.5 mM), 2 µL of forward and reverse primers (10 pmol) and the template DNA 2 µL (>50ng/µL). PCR products were purified by using gel extraction kit (Fermantas USA). Agarose gel (1%) method and NanoDrop equipment were used to determine the quality and quantity of

the sample, respectively. Purified PCR products were sequenced by using forward and reverse primers (Eurofins, Germany).

### Sequencing and phylogenetic analysis

Amplified gene of 16S rRNA was sequenced to identify the archaeal genera and species. The sequences were submitted to NCBI. Based on results obtained by BLAST sequence, A phylogenetic tree was constructed by neighbor-joining method with the help of software MEGA 10 (Kumar et al. 2018). Test of phylogeny was done by using bootstrap method (1000 replicates). The 16S rRNA sequences of bacterial strains were deposited in the GenBank with accession numbers LT634693- LT634709.

### Screening of archaeal strains for salt, pH and temperature tolerance ability

Archaeal strains were grown in the presence of different salt concentrations (2.0-5.0M NaCl), pH ranges 4-12 and temperature range 4-56 °C by using MGM broth medium. About 50 mL of each strain was cultured in 250 ml flask with continuous rotatory agitation at 150 rpm for 72 h. During incubation, archaeal growth was measured at different time intervals (3, 6, 12, 24, 48 and 72 h) by observing their OD at 600 nm. Control culture was used for each strain with optimum growth conditions. We calculated the percentage of strains as positive results with more than 50% growth as compared to control strain and negative results with less than 50% of control strain (Beal et al. 2020).

## Assays for plant growth promotion

### Phosphate solubilization Assay

Pikovskaya phosphate medium (PVK) with the following composition (g/L): 10.0 glucose, 1.0 yeast extract, 5.0 tri-calcium phosphate (TCP), 200.0 NaCl, 5.0 KCl, 31 $MgCl_2.6H_2O$, 35.0 $MgSO_4.7H_2O$, 0.5 $CaCl_2.2H_2O$ and 0.5 $KH_2PO_4$ (pH 7.2) was used to test the phosphate solubilizing ability of haloarchaea (Pikovskaya 1948). For inoculation onto plates, the archaeal strains were grown in a liquid medium until stationary phase at which time the cells were harvested by centrifugation (8000xg, 10 min) and the pellets washed with sterile water (SW) three times to remove any traces of the medium. The cell pellets were then diluted to OD600 = 0.2 in SW. Ten µL droplets were spotted onto the plates and allowed to air dry. then incubated upside down at 37 °C for 5 days and the size of the clearing zone around the colony was measured as an indication of positive activity.

Quantitative analysis of P-solubilization of archaeal strains was done by molybdate blue color method (Watanabe and Olsen 1965). Available phosphate was calculated after 7 and 14 days. Cell-free supernatants were used for the quantification of P-solubilization. After recording pH of cell-free supernatants, they were filtered through 0.2 µm sterile filters (Orange Scientific GyroDisc CA-PC, Belgium) to remove any residues. Solubilized phosphates (primary and secondary orthophosphate) were measured by spectrophotometer (Camspec M350-Double Beam UV-Visible Spectrophotometer, UK) at 882 nm and values were calculated by using a standard curve ($KH_2PO_4$ using 2, 4, 6, 8, 10, 12 ppm solutions).

### Indole acetic acid (IAA) production assay

For assessment of IAA, archaeal cultures were inoculated in 100 mL sterile MGM broth containing 0.1% L-tryptophan and the flasks were kept in shaking incubator at 120 rpm, 37 °C for 7 days in dark. The culture medium was transferred to sterile falcon tubes and centrifuged at 10,000 rpm for 15-20 minutes. The supernatant was transferred to a flask and pH was adjusted at 2.8 using hydrochloric acid. Later, it was extracted twice with equal volumes of ethyl acetate. The clear solution was transferred to a beaker and anhydrous sodium sulfate was added to absorb left over moisture. The clear solution was evaporated until dry using rotary evaporator and was resuspended in 1 mL methanol. These samples were analyzed by high-performance liquid chromatography (HPLC; Waters; e2995, separations module) with 299h photodiode-array (PDA) detector using a Nucleosil C18 column (4.6×250 mm, 3 µM; Macherey-Nagel, Germany). The mobile phase was a mixture of methanol/acetic acid/water (30:1:70, v/v/v) and the flow rate was adjusted at 1.2 mL/min. Pure indole-3- acetic acid (Sigma) was used to prepare standard solutions and IAA production was quantified.

### Siderophore productions assay

CAS (chrome azurol S) agar medium devoid of nutrients was used as an indicator of siderophore production. The components needed for a liter of the overlay medium were: 0.5 M MOPS buffer, 10 g $MgSO_4.7H_2O$, 1 g $CaCl_2.2H_2O$, 50 mL of Solution I (CAS) (0.065 g in 50 mL $H_2O$), 10 mL of Solution II (0.135 g $FeCl_3.2H_2O$ in 500 mL $H_2O$), 40 mL of Solution III (0.0729 g CTAB in 40 mL $H_2O$) and 9 g Bacto-Agar (Pérez-Miranda et al. 2007). Ten mL of the overlay medium was spread on culture plates of selected strains grown at 37 °C for 4 days on solid MGM medium. After a maximum period of 15 min, a color change in the blue medium was considered positive for siderophore production. The experiment was repeated three times.

### Analysis of heavy metal resistance

A total of 24 archaeal strains from the rhizosphere of halophytes were tested for resistance of Nickel (Ni), Cadmium (Cd), Chromium (Cr) and Zinc (Zn). We used chloride salts of all these metals. About 1, 2.5, 5.0, and 10 mM of each metal was used to analyze the resistance in the archaeal strains isolated using MGM agar plates supplemented with Ni, Cd, Cr and Zn. For inoculation, 3 day old cultures on agar plates were used in the four-sector quadrant streaking technique with a platinum loop. These strains were grown at 37 °C for six days and the minimum inhibitory concentration was measured when strains failed to grow on the respective plates. The strains which did not show any growth after six days of incubation were considered negative. Four replicates of each strain were used for each metal concentration.

### DNA isolation, genome sequencing and assembly of Halorubrum lacusprofundi HL1RP11 and Halobacterium noricense NRS2HaP9

Based on the results of plant growth-promoting traits, two archaeal strains *Halorubrum lacusprofundi* HL1RP11 and *Halobacterium noricense* NRS2HaP9 were selected for whole genome sequence analysis and identification of PGP-related genes. These strains were grown into 100 mL MGM broth overnight at 37 °C with 125 rpm. The Genomic DNA was isolated using Genomic DNA isolation kit (Thermo Scientific GeneJET, USA). Genome sequencing of these haloarchaeal strains was performed with the Illumina HiSeq2000® sequencing platform. For the current study, 5 µg of genomic DNA was extracted from each archaeal strain and was prepared for genome sequencing using the Illumina HiSeq2000® library preparation kit (Illumina, Inc.), following the manufacturer's instructions. The sequencing data was then assembled into complete contigs with SPAdes assembler Version 3.13.0 (Bankevich et al. 2012). The contigs were arranged against the genomes of *Halorubrum lacusprofundi* ATCC 49239 and *Halobacterium noricense* A1 by using Mauve (Darling et al. 2010).

### Genome annotation of Halorubrum lacusprofundi HL1RP11 and Halobacterium noricense NRS2HaP9

The de novo gene prediction was performed by using GeneMarks and CLC genomics workbench (Besemer et al. 2001). The functional classification was conducted through COG (corresponding cluster of orthologous groups of protein) analysis. The gene function was annotated by BLAST against Kyoto Encyclopedia of Genes and Genomes database KEGG pathway (Kanehisa et al. 2006). KEGG Orthology Based Annotation System (KOBAS 2.0) was used for the functional analysis of genes. To predict genes and operons involved in secondary metabolism and antibiotic resistance antiSMASH 4.0 software version 3 was used (Blin et al. 2017). The whole genome sequences of strains HL1RP11 and NRS2HaP9 were deposited in the GenBank database under the accession number JAJNEG000000000 and JAJSOI000000000.

## Results

### Isolation and identification of halophilic archaeal strains

A total of 24 halophilic archaeal isolates were characterized and identified from the rhizospheric and non-rhizospheric soil samples of *S. stocksii* and *A. amnicola* (Table 1; Figure 1). These isolates were identified based on 16S rRNA gene analysis. Four strains showed more than 99% homology with *Halobacterium* spp., four strains were related to *Halomicrobium* spp., three strains were identified as *Halococcus* spp., three strains were related to *Natrinema* spp., two strains NRS3HaP17 and LK4HAP18 were belonging to *Natrialba* spp., one strain HL1RS17 was identified as *Halolamina sediminis*, one strain AT3RS21 was identified as *Haloferax denitrificans* and one strain AT4RS18 was identified as *Halalkalicoccus jeotgali* (Table 1; Figure 1).

Phylogenetic analysis showed that haloarchaeal strains identified in this study belonged to 12 different genera. These strains were grouped into two main clades; one clade with 9 genera including *Halobacterium, Halomicrobium, Halococcus, Natrinema, Halalkalicoccus, Natrialba, Halolamina, Natrococcus* and *Halorubrum* and other clade with 3 genera including *Haloferax, Halolamina,* and *Halonotius. Halobacterium,* and *Halomicrobium* were two most dominant genera identified in the rhizosphere of *S. stocksii* and *A. Amnicola.* Archaeal strains from each genus were grouped into a separate clade (Figure 1).

**Table 1.** Identification of haloarchaeal strains based on 16S rRNA sequences.

| Isolate code | Source of isolation | Identification based on 16S rRNA gene | Sequence similarity (%) | 16S rRNA sequence length | Accession No. |
|---|---|---|---|---|---|
| HL1RS2 | *Salsola* rhizosphere | *Halomicrobium* sp. | 99.81 | 1465 | LT634693 |
| HL1RS17 | *Salsola* rhizosphere | *Halolamina sediminis* | 99.71 | 1423 | LT634694 |
| HL1RP1 | *Salsola* rhizosphere | *Natrinema gari* | 99.33 | 1345 | LT634695 |
| HL1RP11 | *Salsola* rhizosphere | *Halorubrum lacusprofundi* | 99.63 | 1455 | OL580737 |
| HL1HP3 | *Salsola* rhizosphere | *Natrinema* sp. | 99.40 | 1295 | LT634696 |
| HAL1RS5 | *Salsola* rhizosphere | *Halococcus* sp. | 100 | 1463 | OL580738 |
| HL2RS19 | *Salsola* rhizosphere | *Halonotius pteroides* | 99.53 | 1437 | LT634697 |
| AT1RS23 | *Atriplex* rhizosphere | *Natrinema ejinorense* | 99.45 | 1379 | LT634698 |
| AT2RS16 | *Atriplex* rhizosphere | *Halobacterium salinarum* | 99.61 | 1395 | LT221179 |
| AT3RS7 | *Atriplex* rhizosphere | *Halomicrobium mukohataei* | 99.25 | 1415 | LT634699 |
| AT3RS9 | *Atriplex* rhizosphere | *Halomicrobium mukohataei* | 99.63 | 1339 | OL580739 |
| AT3RS21 | *Atriplex* rhizosphere | *Haloferax denitrificans* | 99.71 | 1433 | LT634700 |
| AT4RS18 | *Atriplex* rhizosphere | *Halalkalicoccus jeotgali* | 99.37 | 1428 | LT634701 |
| NRS1HAP9 | Non-rhizospheric soils | *Halobacterium salinarum* | 99.29 | 1199 | LT221209 |
| NRS2HaP9 | Non-rhizospheric soils | *Halobacterium noricense* | 99.68 | 1359 | LT634702 |
| NRS2HAP14 | Non-rhizospheric soils | *Halococcus* sp. | 98.55 | 1135 | LT221216 |
| NRS3HaP17 | Non-rhizospheric soils | *Natrialba magadii* | 99.69 | 1457 | LT634703 |
| NRS3HaP19 | Non-rhizospheric soils | *Halobacterium* sp. | 99.89 | 1429 | LT634704 |
| NRS4HAP4 | Non-rhizospheric soils | *Natronococcus occultus* | 99.52 | 1181 | LT221261 |
| LK1HaP12 | Non-rhizospheric soils | *Halococcus* sp. | 99.19 | 1129 | LT634705 |
| LK2HaP14 | Non-rhizospheric soils | *Halomicrobium zhouii* | 99.17 | 1351 | LT634706 |
| LK2HaP16 | Non-rhizospheric soils | *Natronomonas moolapensis* | 99.27 | 1309 | LT634707 |
| LK3HaP15 | Non-rhizospheric soils | *Natronococcus jeotgali* | 99.87 | 1375 | LT634708 |
| LK4HaP18 | Non-rhizospheric soils | *Natrialba chahannaoensis* | 99.52 | 1381 | OL580740 |

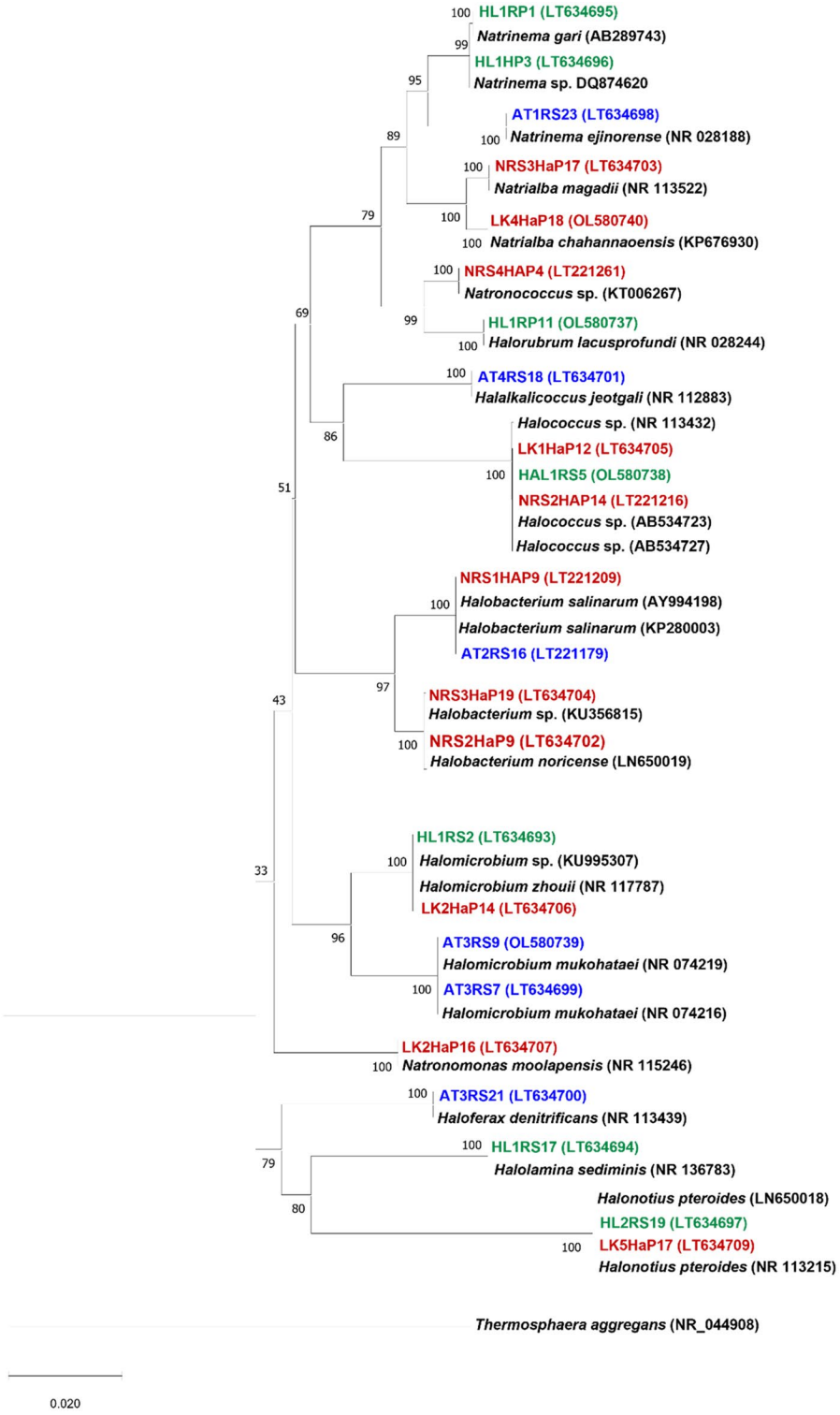


**Figure 1.** Phylogenetic tree based on 16S rRNA gene sequences of halophilic archaeal isolates from the rhizospheric and non-rhizospheric soils of halophytes (*salsola stocksii* and *atriplex amnicola*). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches.

## *Salt, pH, and temperature tolerance of halophilic archaea*

The halophilic archaeal cultures were grown on MGM agar. On incubation, translucent and opaque colonies with orange, red or pink pigmentation were observed. As shown in Figure 2A, haloarchaeal strains were extremely halophiles and only few strains were able to grow under 2 M of total salt but grew optimally at 2.5 – 4.0 M of salt (NaCl). These strains were able to grow at a wide range of temperature with 37-42 °C optimum temperature (Figure 2B). Mostly strains grew well at pH range from 6 to 8, however, some strains did grow at 55 °C and the pH of 9.5 (Figure 2C).

### Plant growth promoting potential of Halophilic archaea

Halophilic archaeal strains were screened for various plant growth promoting (PGP) abilities such as IAA production, P-solubilization, and siderophore production. Most of the strains showed more than two PGP traits (Table 2; Figure S1). Twenty-one strains showed P-solubilization activity with a range from 8.15 to 87.77 µg/mL. *Natrinema gari* strain HL1RP1 had the maximum P-solubilization activity (87.77 µg/mL). Fifteen strains showed production of IAA with a range from 2.11 to 63.42 µg/mL. Only 8 strains showed positive results for siderophore production assay (Table 2; Figure S1).

### Heavy metal resistance profile of bacterial strain

More than 90% of archaeal strains showed heavy metal tolerance for Cd, Ni, Cr and Zn at a concentration of 1 mM while 71-85% archaeal strains showed heavy metal tolerance at a concentration of 2.5 mM, 51-67% rhizospheric archaeal strains showed heavy metal tolerance at a concentration of 5.0 mM while 31-37% non-rhizospheric archaeal strains showed heavy metal tolerance at a concentration of 5.0 mM, and only a few archaeal strains especially *Atriplex* associated strains (31%) showed tolerance at a concentration of 10 mM for chromium and Zinc (Figure 3).

### General features of genomes of Halorubrum lacusprofundi HL1RP11 and Halobacterium noricense NRS2-HaP9

The size of genomes of *Halorubrum* HL1RP11 and *Halobacterium* NRS2-HaP9 was 3,550,491 and 3,037,489 bp, respectively (Figure S1). In the genome of *Halorubrum* HL1RP11 strain, a total of 3517 genes were predicted with 3,431 protein coding sequences (CDs) and RNA related genes were 55 (Table S1). In the genome of *Halobacterium* NRS2-HaP9 strain, a total of 3228 genes were predicted with 3155 protein coding sequences (CDs) and RNA related genes were 51 (Table S1). Plasmid sequence was not identified in both strains.

### Functional Annotation of Halorubrum lacusprofundi HL1RP11 and Halobacterium noricense NRS2-HaP9 genomes

Most of the unique genes were predicted to code hypothetical proteins in both genomes. In the genome of *Halorubrum lacusprofundi* HL1RP11, out of 3,431 proteins, 1354 (49.7%) were assigned to COG functional categories while in case of *Halobacterium noricense* NRS2-HaP9 genome, out of 3155 proteins, 1419 (44%) were assigned to COG functional categories (Figure 4). The functional analysis of these genes

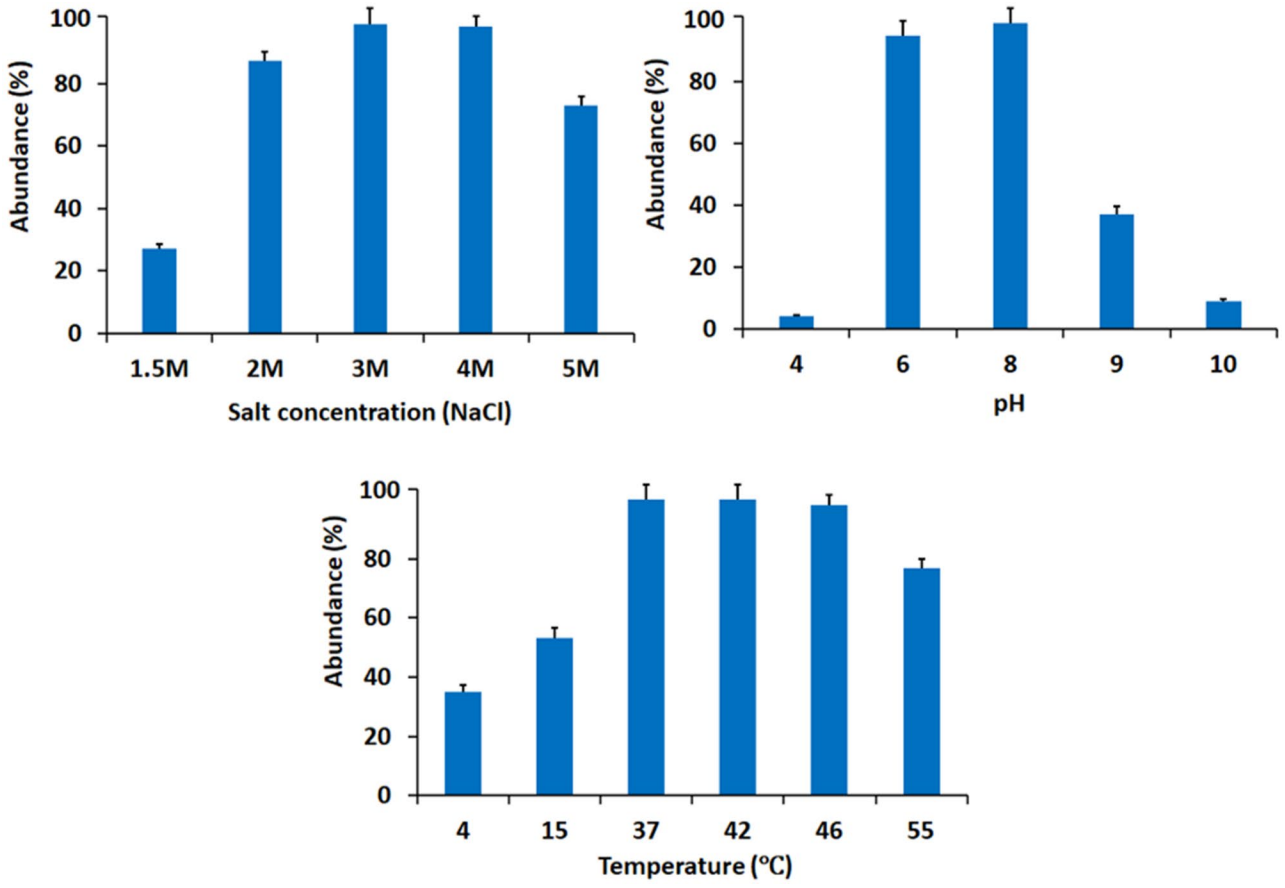


**Figure 2.** Phenotypic characterization, salt, pH, and temperature tolerance of halophilic archaeal strains.

**Table 2.** Determination of plant growth promoting traits of haloarchaeal strains.

| Archaeal strains | Strain Name | Phosphate solubilization (µg/mL) | IAA production (µg/mL) | Siderophore production |
|---|---|---|---|---|
| HL1RS2 | *Halomicrobium* sp. | 33.42$^{b}$ | 2.11$^{a}$ | – |
| HL1RS17 | *Halolamina sediminis* | 53.65$^{c}$ | 39.54$^{de}$ | – |
| HL1RP1 | *Natrinema gari* | 87.77$^{e}$ | 15.84$^{b}$ | + |
| HL1RP11 | *Halorubrum lacusprofundi* | 55.92$^{c}$ | 63.42$^{f}$ | ++ |
| HL1HP3 | *Natrinema* sp. | 47.24$^{c}$ | 23.57$^{c}$ | – |
| HAL1RS5 | *Halococcus* sp. | 49.91$^{c}$ | 35.89$^{d}$ | ++ |
| HL2RS19 | *Halonotius pteroides* | 17.71$^{a}$ | 0.00 | – |
| AT1RS23 | *Natrinema ejinorense* | 75.46$^{d}$ | 31.19$^{d}$ | – |
| AT2RS16 | *Halobacterium salinarum* | 15.11$^{a}$ | 23.23$^{c}$ | ++ |
| AT3RS7 | *Halomicrobium mukohataei* | 65.17$^{cd}$ | 0.00 | + |
| AT3RS9 | *Halomicrobium mukohataei* | 39.23$^{bc}$ | 0.00 | ++ |
| AT3RS21 | *Haloferax denitrificans* | 12.16$^{a}$ | 0.00 | – |
| AT4RS18 | *Halalkalicoccus jeotgali* | 51.41$^{c}$ | 0.00 | – |
| NRS1HAP9 | *Halobacterium salinarum* | 47.84$^{c}$ | 31.43$^{d}$ | – |
| NRS2HaP9 | *Halobacterium noricense* | 53.41$^{c}$ | 47.54$^{e}$ | +++ |
| NRS2HAP14 | *Halococcus* sp. | 41.7$^{bc}$ | 0.00 | ++ |
| NRS3HaP17 | *Natrialba magadii* | 37.55$^{bc}$ | 27.98$^{cd}$ | + |
| NRS3HaP19 | *Halobacterium* sp. | 54.74$^{c}$ | 35.64$^{d}$ | – |
| NRS4HAP4 | *Natronococcus occultus* | 19.45$^{a}$ | 0.00 | ++ |
| LK1HaP12 | *Halococcus* sp. | 0.00 | 0.00 | +++ |
| LK2HaP14 | *Halomicrobium zhouii* | 0.00 | 19.98$^{bc}$ | +++ |
| LK2HaP16 | *Natronomonas moolapensis* | 39.96$^{bc}$ | 0.00 | + |
| LK3HaP15 | *Natronococcus jeotgali* | 0.00 | 15.31$^{b}$ | ++ |
| LK4HaP18 | *Natrialba chahannaoensis* | 71.55$^{d}$ | 6.98$^{a}$ | – |

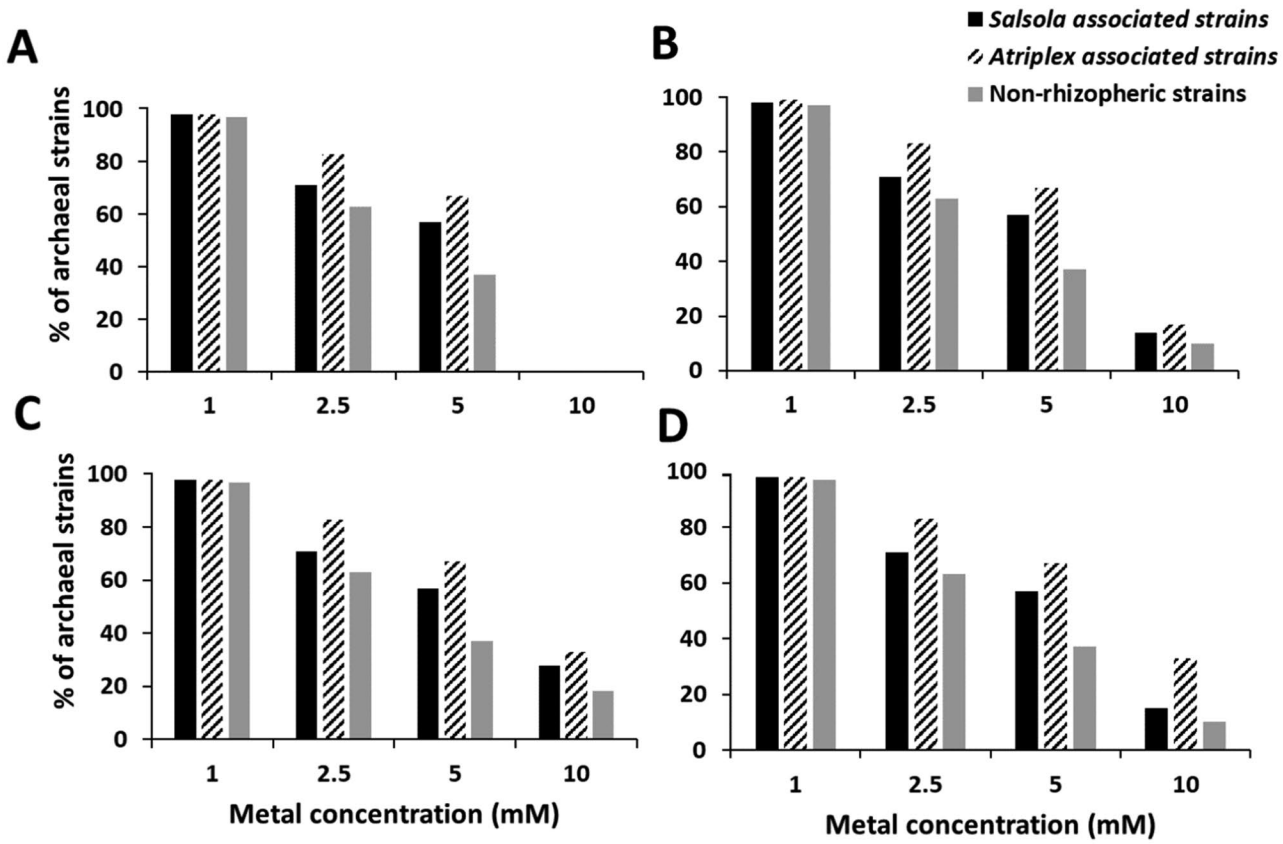


**Figure 3.** Resistance profile of cadmium (A), nickel (B), chromium (C) and Zinc (D) of archaeal strains isolated from the rhizosphere and non-rhizosperic soils of halophytes at various concentrations (i.e., 2, 5, and 10 mM).

using KEGG pathway database showed that they have an important role in various metabolic pathways including plant growth promotion, environmental adaptation, bioremediation of different toxic compounds, heavy metals, and other abiotic stresses. The functional analysis of CDSs showed that they could be classified into 19 general COG categories including the metabolism of carbohydrates, amino acids, lipids, transcription, energy, cofactors and vitamins, inorganic ions, signal transduction and cellular processes, glycan biosynthesis and metabolism, nucleotide metabolism, secondary metabolites, Iron acquisition and metabolism and xenobiotics biodegradation (Figure 4).

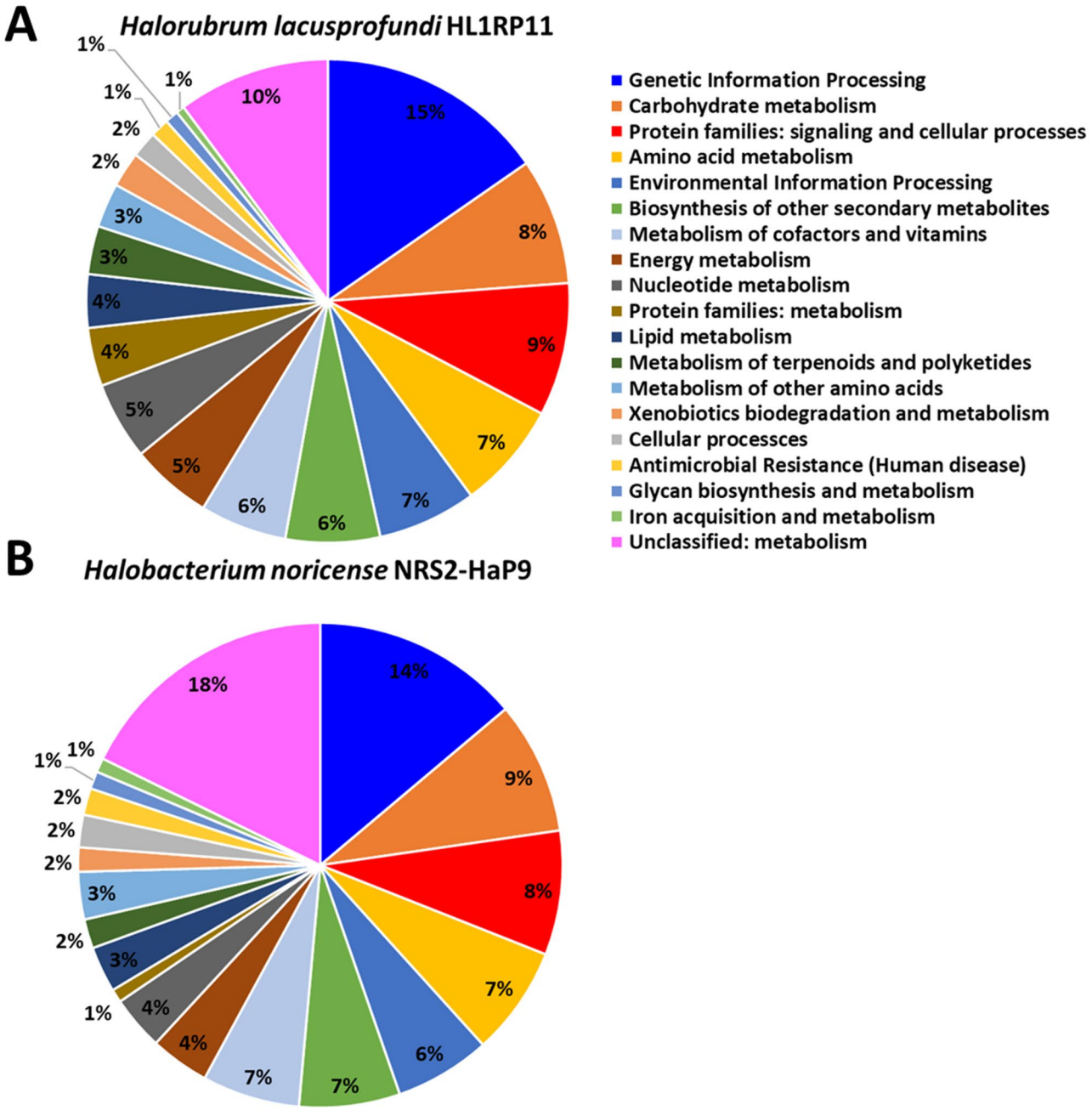


**Figure 4.** Functional analysis of Halorubrum lacusprofundi HL1RP11 (A) and Halobacterium noricense NRS2-HaP9 (B) encoded genes by using KEGG metabolic pathways.

### *Prediction of genes related to plant growth promotion*

The functional analysis of *Halorubrum* HL1RP11 and NRS2HaP9 genomes identified different genes involved in plant growth promotion of halophytes. The presence of genes involved in indole acetic acid production through tryptophan biosynthesis such as *trpA, trpB, trpC, trpD, trpE, trpG, tyrA2* and *pheA2* was confirmed by genome analysis of both HL1RP11 and NRS2HaP9 strains (Table S2; Figures 5, 6A,B). Forty seven genes in HL1RP11 and 53 genes in NRS2HaP9 genomes involved in P-solubilization by using the PQQ-dependent alcohol dehydrogenase and phosphatase production pathways including *PstA, PstB, gcd, pqq_1, PiT* and *phoU* were predicted. Eleven genes including *afuB, fbpB, afuA, fbpA, menF, ABC.FEV.S, ABC.FEV.P, feoA, FRD* and *adhB-1* involved in iron metabolism and siderophore production were identified in the genomes of both haloarchaeal strains (Table S2; Figures 5, 6A,B). Fifteen genes involved in the production of exopolysaccharides such as pgdA, dgoD, EOI, rhaM and uxuA were also predicted in this study (Table S2; Figures 5, 6A,B). When PGP genes from the genomes of HL1RP11 and NRS2HaP9 were compared with their reference strains, there was a difference in the number of genes especially siderophore production, and phosphate solubilization related genes (Figure 6A,B).

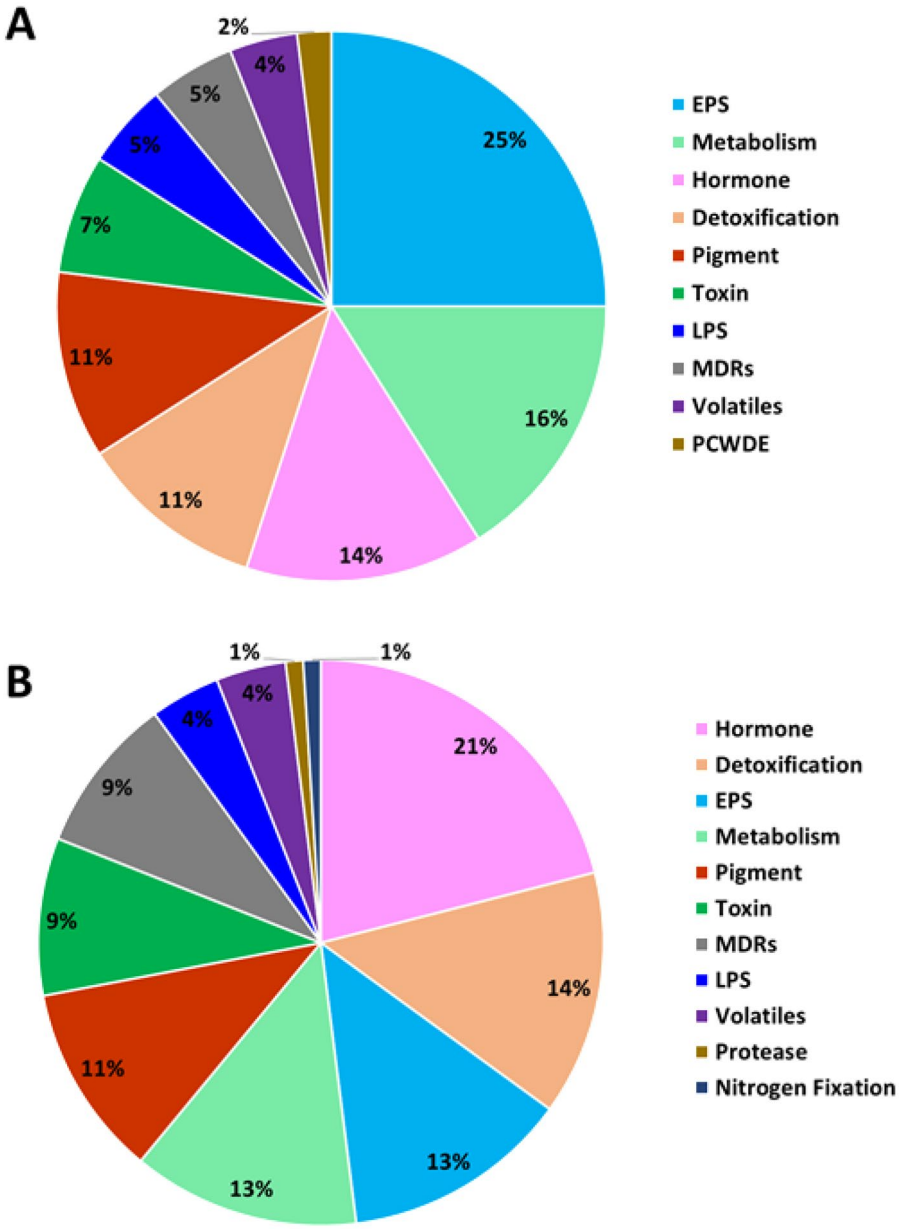


**Figure 5.** Identification of genes related to PGP traits in the genomes of *Halorubrum lacusprofundi* HL1RP11 (A) and *Halobacterium noricense* NRS2-HaP9 (B). EPS; exopolysaccharides; LPS: lipopolysaccharides; PCWDE: plant cell wall degrading enzymes.

### *Prediction of genes related to environmental adaptation, glycerol metabolism and membrane transport*

Genes potentially involved in environmental adaptation, glycerol metabolism and membrane transport have been identified in the genomes of halophilic archaea. In the genome of *Halorubrum lacusprofundi* HL1RP11, 37 genes for environmental adaptation such as *ACSL* and *fadD* involved in the production of long-chain acyl-CoA synthetase, *mnhB, mnhC, mnhD, mnhE* and *mnhF* encoded the H+_antiporter proteins, *glnA, glnB* and *glnC* encoded the glutamine synthase pathway, *argB, argC, argD, argE,* and *argF* involved in the proline metabolism, *nadA, nadB, nadC, nadE* and *nadM* involved in the vitamin_b3/niacin biosynthesis pathway were identified (Tables S3 and S4). Glycerol metabolism of *Halorubrum lacusprofundi* HL1RP11 included the carbohydrate phosphotransferase system (*pssA, gldA, ALDH, dhaK*), production of UDP-sulfoquinovose synthase (*SQD1*), glycerate 2-kinase and glucosyltransferase *(sqdB, gck, gckA, dgs, bgsA, araM, egsA*) and glycerol-3-phosphate dehydrogenase (*glpA, glpD, glpB, glpC, pgsA, PGS1* and *carS*). About 63 genes related to amino acids, sugar molecules, iron, phosphate, thiamin, biotin, zinc, sulfonate and arabinogalactan transportation and signal recognition proteins were also identified in the genome of *Halorubrum lacusprofundi* HL1RP11 (Tables S3 and S4).

Thirty five genes for environmental adaptation including the production of long-chain acyl-CoA synthetase, the H+antiporter proteins, glutamine synthase, proline metabolism and vitamin_b3/niacin biosynthesis pathway were also identified in the genome of *Halobacterium noricense* NRS2-HaP9 (Tables S3 and S4). Glycerol metabolism involved by using the production of UDP-sulfoquinovose synthase (*SQD1*), glycerate 2-kinase and glucosyltransferase

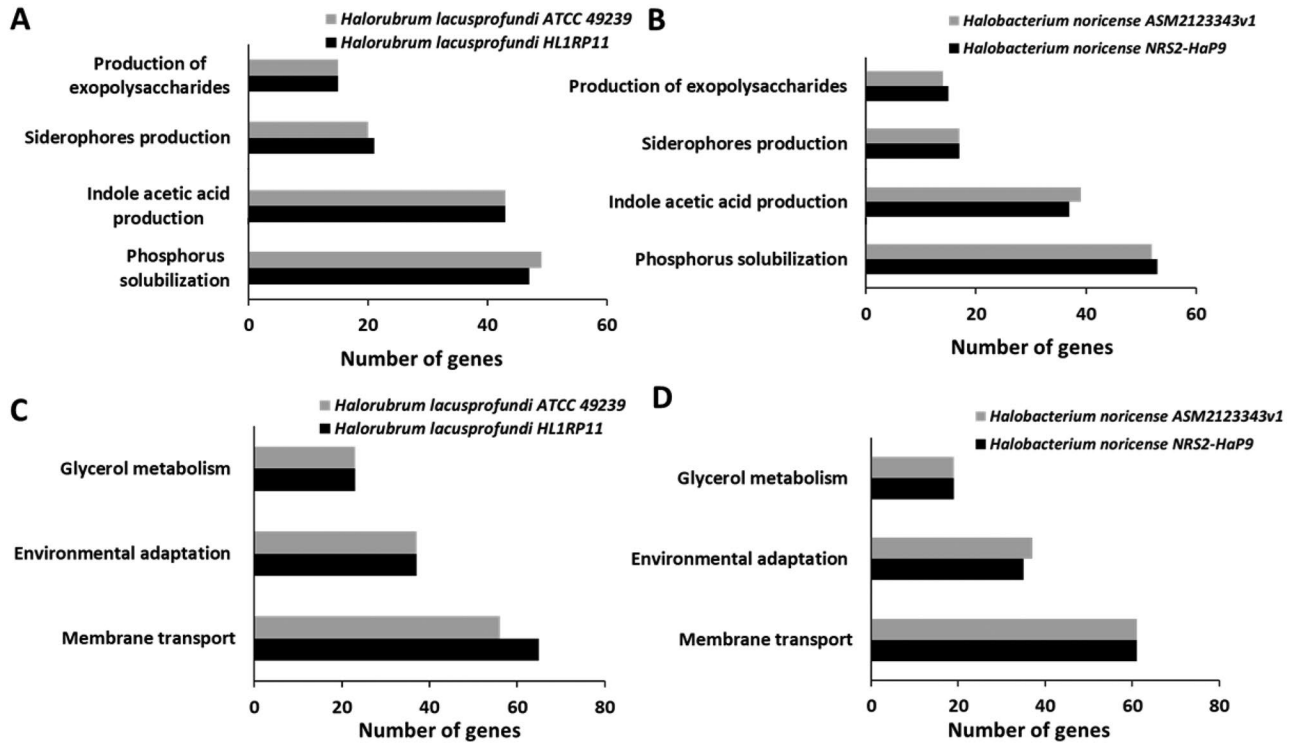


**Figure 6.** Gene ontology of PGP traits (A,B), membrane transportation, environmental adaptation, and glycerol metabolism (C,D) in the genomes of *Halorubrum lacusprofundi* HL1RP1 and *Halobacterium noricense* NRS2-HaP9 with the comparison of their reference strains.

*(sqdB, gck, gckA, dgs, bgsA, araM, egsA)* and glycerol-3-phosphate dehydrogenase (*glpA, glpD, glpB, glpC, pgsA, PGS1* and *carS*). About 44 genes related to amino acids, sugar molecules, iron, phosphate, thiamin, biotin, copper, molybdate, sulfonate, nucleotides and urea transportation and some signal recognition proteins were identified (Tables S3 and S4).

When genomes of HL1RP11 and NRS2HaP9 were compared with their reference strains, there was a difference in the number of genes related to environmental adaptation, glycerol metabolism and membrane transport (Figure 6C,D). The genes for carbohydrate phosphotransferase system (*dhaL* and *dhaM*), alpha-glucosyltransferase (*mgs* and *bgsB*) glycerol-3-phosphate 3-phosphatidyl transferase (*pgsA* and *GS1*), vitamin_b3/niacin biosynthesis (*nadA, nadB,* nadC) and phosphonate transport system substrate-binding protein (*phnD*) were missing in the genome of HL1RP11 and NRS2-HaP9 (Tables S3 and S4).

### *Prediction of genes related to heavy metal resistance*

Based on functional analysis of *Halorubrum lacusprofundi* HL1RP11, 159 genes related to heavy metal resistance including Nickel, Cadmium, Antimony, Arsenic, Iron, Chromium and Zinc were identified (Tables S5; Figure 7) while in the genome of *Halobacterium noricense* NRS2-HaP9, 171 genes related to heavy metal resistance were identified (Tables S5; Figure 7). When genomes of HL1RP11 and NRS2HaP9 were compared with their reference strains, we observed a clear difference in number of genes related to heavy metal resistance such as genes related to iron resistance through FoxABCD operon (*coxC* and *ctaE*), chromium resistance genes *dpsB* and zinc resistance gene *ZRT2, znuA, znuB* and *znuC* were not identified in the genomes of HL1RP11 and NRS2HaP9 (Figure 7).

### *Production of secondary metabolites*

In the genome of *Halorubrum lacusprofundi* HL1RP11, antimicrobial gene clusters including thiopeptides (thiazolyl peptides), bacteriocins and terpenes were identified (Figure S3). Secondary metabolites encoding gene clusters such as siderophore, thiopeptides and terpenes were identified in the genome of *Halobacterium noricense* NRS2-HaP9 (Figure. S3). These genes might be involved in plant growth improvement and biocontrol mechanisms.

## Discussion

The rich microbial diversity of halophyte rhizospheres helps these plants cope with high salinity and drought (Mukhtar et al. 2018). In the current study, culturable halophilic archaeal diversity was analyzed from the rhizosphere and non-rhizospheric soils of halophytes including *S. stocksii* and *A. amnicola*. Plant growth promoting traits of the isolated archaeal strains were screened using different selective media and genes related to plant growth promotion, secondary metabolism and osmoregulation were identified through whole genome sequence analysis of *Halorubrum* HL1RP11 and *Halobacterium* NRS2-HaP9 strains. Previously, only a few studies have investigated the role of archaea in plant health and productivity. To the best of knowledge, this study is the first report of comparative analysis of PGP related traits under invitro conditions and identifications of PGP genes in haloarchaeal genomes.

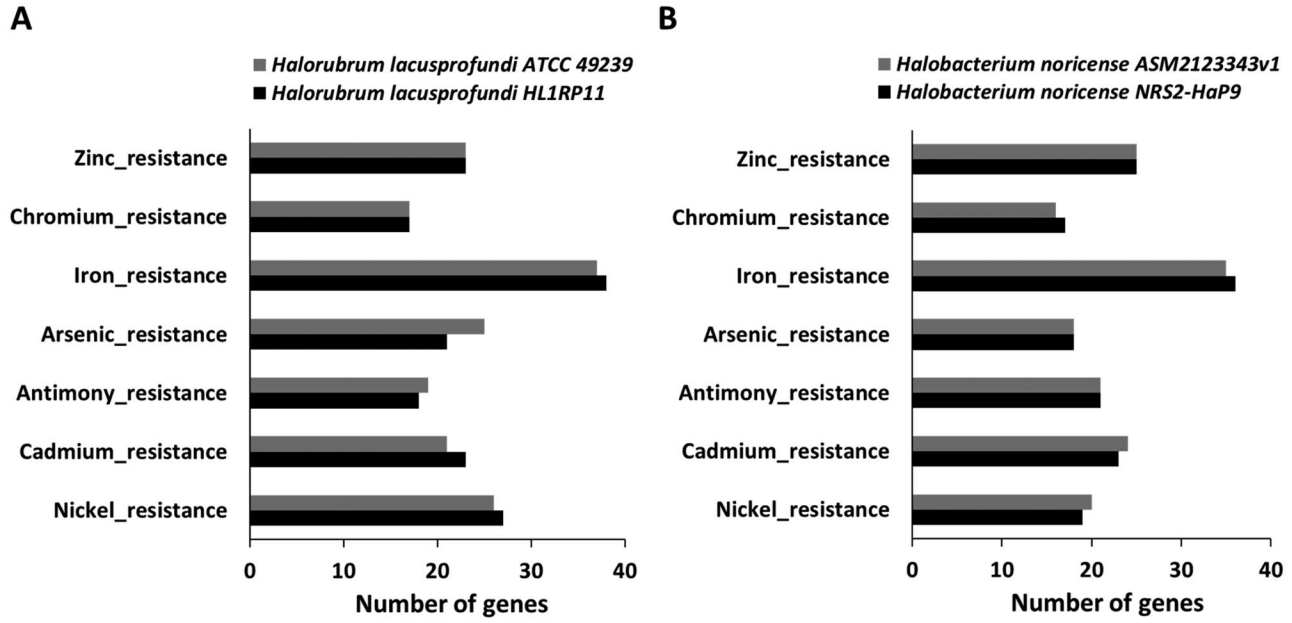


**Figure 7.** Gene ontology of heavy metal resistance related proteins in the genomes of *Halorubrum lacusprofundi* HL1RP1 (A) and *Halobacterium noricense* NRS2-HaP9 (B) with the comparison of their reference strains.

Based on 16S rRNA gene analysis, a total of 24 halophilic archaeal isolates were identified from the rhizospheric and non-rhizospheric soil samples of *S. stocksii* and *A. amnicola*. In this study, 9 halophilic genera including *Halobacterium, Halomicrobium, Halorubrum, Halococcus, Haloferax* and *Halalkalicoccus* were identified. *Halobacterium, Halomicrobium, Halococcus* and *Natrinema* were more abundant as compared to other genera from the rhizosphere of halophytes. Several previous studies have reported the halophilic archaeal genera such as *Halobacterium, Halococcus, Halomicrobium, Haloferax* and *Halalkalicoccus* from various aquatic hypersaline environments (Vreeland 1993; Ventosa et al. 2008; Oren 2010, Menasria et al. 2018) but only two studies including Yadav et al. (2015) and Dubey et al. (2016) reported the PGP halophilic archaea from the rhizosphere of halophytes. Haloarchaeal strains isolated in this study were extremely halophiles and a few strains were able to grow under 2 M of total salt but grew optimally at 2.5-4.0M of salt (NaCl).

In this study, most of the strains showed more than two PGP abilities. About 87.5% of haloarchaeal strains showed P-solubilization activity with a range from 8.15 to 87.77 µg/mL, 62.5% of strains showed production of IAA with a range from 2.11 to 63.42 µg/mL, and 33% strains showed positive results for siderophore production assay. Halophilic archaea such as *Natrialba, Natrinema, Haloarcula,* and *Halococcus* with the ability to solubilize phosphate, produce IAA, and siderophores characterized in this study showed better PGP potential as compared to previously reported haloarchaeal strains. Some previous studies also showed that archaeal strains have ability of mineral phosphate solubilization by the production of organic acids such as oxalic, acetic, citric, and succinic acid (Yadav et al. 2015; Selim et al. 2022).

A recent study also showed that haloarchaea have the ability to produce indole acetic acid and other phytohormones (Taffner et al. 2019). Haloarchaea use iron acquisition and produce siderophores such as the genome of *Haloferax volcanii* contains four genes that are annotated as iuc (iron uptake chelate) genes based on the similarity of the encoded proteins to bacterial siderophore biosynthesis proteins (Hubmacher et al. 2007; Shafiee et al. 2019; Niessen and Soppa (2020). Some archaeal groups play an important role in nitrogen cycle. A number of previous studies have reported the ammonia oxidizing methanogens such as *Nitrososphaera, Nitrosopumilus*, and *Nitrosotalea* (Herrmann et al. 2008; Trivedi et al. 2019). Archaeal plant interactions based on nutrient supply, phytohormones production, nitrogen fixation and biocontrol activity were identified through shotgun metagenomic analysis of rhizosphere soils (Taffner et al. 2019).

The whole genome analysis of *Halorubrum* HL1RP11 and *Halobacterium* NRS2-HaP9 revealed that there were 3431 and 3155 protein coding sequences respectively. A large number of proteins were annotated as hypothetical proteins. The functional analysis of *Halorubrum* HL1RP11 and *Halobacterium* NRS2-HaP9 genomes using KEGG pathway database showed that it has an important role in metabolism of carbohydrates, amino acids, lipids, energy, cofactors and vitamins, inorganic ions, glycan biosynthesis and metabolism, secondary metabolites, signal transduction and cellular processes, DNA replication and repair, cell motility, transcription, translation, ribosomal biogenesis, abiotic stresses and bioremediation of different toxic compounds. Some recent studies on genome sequence analysis of haloarchaea revealed that these microorganisms have a large diversity of proteins and enzymes involved in different metabolic pathways, abiotic stress management and plant growth promotion (Ludt and Soppa 2019; Moissl-Eichinger et al. 2018; Vinogradov and Suga 2020).

The functional analysis of *Halorubrum* HL1RP11 and *Halobacterium* NRS2HaP9 genomes revealed the identification of various genes involved in plant growth promotion. Forty seven genes in HL1RP11 and 53 genes in NRS2HaP9 genomes involved in P-solubilization. The presence of genes

including *PstA, PstB, gcd, pqq_1, PiT* and *phoU* related to P-solubilization (PQQ-dependent alcohol dehydrogenase) were predicted in the genomes of haloarchaea only in this study. Genes including involved in tryptophan biosynthesis (indole acetic acid production) related, and tyrosine were also identified by genome analysis of both HL1RP11 (43 genes) and NRS2HaP9 (37 genes). Eleven genes involved in iron metabolism and siderophore production were identified in the genomes of both haloarchaeal strains.

Some previous studies also reported the role of archaea in plant growth promotion with their ability to solubilize inorganic phosphate and produce phytohormones and siderophores (Al-Mailem et al. 2010; Yadav et al. 2015; Song et al. 2019). The ammonia monooxygenase genes were identified in ammonia-oxidizing archaea that were isolated from the rhizosphere of *Littorella uniflora* (Trivedi et al. 2019).

Halophilic archaeal genera including *Haloarcula, Halococcus, Haloferax, Halobacterium* and *Natronococcus* have the ability to produce exopolysaccharides. Various sugars such as glucose, rhamnose, galactose, mannose, galactosamine, and glucopyranosiduronic acid are involved in the biosynthesis of exopolysaccharides in archaea (Schmid et al. 2016; Hamidi et al. 2019). Identification of genes related to PGP traits has been previously reported in some archaea, however, this study is the first report that described the characterization of PGP haloarchaeal strains from the rhizosphere of halophytes and identification of related genes through whole genome analysis of two haloarchaeal strains *Halorubrum* HL1RP11 and *Halobacterium* NRS2HaP9.

About 40-63% of archaeal strains showed heavy metal tolerance for Cd, Ni, Cr and Zn at a concentration of 5.0 mM. In this study, haloarchaea showed more tolerance for chromium as compared to other metals. A number of previous studies also showed that halophilic archaea including *Haloferax*, *Halobacterium*, *Halococcus*, *Haloarcula*, and *Halorubrum* have heavy metal resistance genes on their plasmids and chromosomal DNA. These microorganisms help plants to grow under saline polluted soils. The phenomenon of archaeal heavy metal resistance has fundamental importance and is particularly relevant in microbial ecology, especially in connection with the roles of microbes in biogeochemical cycling of heavy metals and in the bioremediation of metal-contaminated environments (Das et al. 2014; Voica et al. 2016; Moopantakath et al. 2023).

Genome annotation analysis showed that siderophore, thiopeptides and terpenes were commonly identified from both *Halorubrum* HL1RP11 and *Halobacterium* NRS2-HaP9. Thiopeptides (thiazolyl peptides) are a class of pretentious antibiotics produced by bacteria and archaea. These antibiotics usually show positive activity against Gram-positive bacteria such as *Bacillus* and *Staphylococcus* genera (Just-Baringo et al. 2014; Vinogradov and Suga 2020). Terpenes and terpenoids are important antimicrobial compounds identified and characterized in bacteria, archaea, and plants. Terpenes and terpenoids play a role in biosynthesis of the cell membrane and cell wall, electron transport and conversion of light into chemical energy including chlorophylls, bacteriochlorophylls, rhodopsins, and carotenoids (Boronat and Rodríguez-Concepción 2015; Verma et al. 2020). Some recent studies reported the identification of gene clusters related to only carotenoids in haloarchaea (Wang et al. 2019; Serrano et al. 2022), however, this study is the first report of identification of gene clusters related to siderophores, terpenes and terpenoids in halophilic archaea.

## Conclusion

The main objective of the current study was to evaluate the haloarchaeal diversity in the rhizosphere and non-rhizospheric soils of halophytes (*S. stocksii* and *A. amnicola*) through culturable techniques. Genes related to PGP traits were identified from two haloarchaeal strains *Halorubrum* HL1RP11 and *Halobacterium* NRS2HaP9. Our results suggest that *Halobacterium, Halococcus, Halorubrum, Halobacterium* and *Natrinema* were dominant in all soils. Most of the strains identified in this study showed more than two PGP traits. More than 60% strains demonstrated positive results for P-solubilization, IAA production and heavy metal resistance. The genomic annotation of *Halorubrum* HL1RP11 and *Halobacterium* NRS2HaP9 revealed the identification of genes involved in PGP, e.g., phosphate solubilization, IAA production, and exopolysaccharides production; heavy metal resistance and secondary metabolism, e.g., phenazine, siderophore production and terpene related gene cluster. We suggest that PGP haloarchaeal strains may be useful for increasing the growth and production of important crops in agriculture lands.

## Acknowledgments

We are highly thankful to Pakistan Academy of Sciences for a project entitled, "Whole genome analysis of halophilic archaea and identification of genes encoding for plant growth promoting traits and osmoregulation".

## Authors' contributions

SMu: conducted the experiment, analyzed the data, and prepared the manuscript. HZS conducted the experiment and helped in data analysis. SMe: guided in the experiment plan and edited the manuscript. KM: supervised the research and edited the manuscript. All authors contributed to the article and approved the submitted version.

## Disclosure statement

The authors declared that they have no conflict of interest in the publication.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, LT634693-LT634709 and JAJNEG000000000 and JAJSOI000000000.

## References

Ahmad K, Hussain M, Ashraf M, Luqman M, Ashraf MY, Khan ZI. 2007. Indigenous vegetation of Soon valley at the risk of extinction. Pak J Bot 39(3):679–690.

Al-Mailem DM, Sorkhoh NA, Marafie M, Al-Awadhi H, Eliyas M, Radwan SS. 2010. Oil phytoremediation potential of hypersaline coasts of the Arabian Gulf using rhizosphere technology. Bioresour Technol 101(15):5786–5792.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19(5):455–477.

Beal J, Farny NG, Haddock-Angelli T, Selvarajah V, Baldwin GS, Buckley-Taylor R, Gershater M, Kiga D, Marken J, Sanchania V, et al. 2020. Robust estimation of bacterial cell count from optical density. Commun Biol 3(1):512.

Berendsen RL, Pieterse CM, Bakker PA. 2012. The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486.

Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29(12):2607–2618.

Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de Los Santos ELC, Kim HU, Nave M, et al. 2017. antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res. 45(W1):W36–W41.

Boronat A, Rodríguez-Concepción M. 2015. Terpenoid biosynthesis in prokaryotes. Adv Biochem Eng Biotechnol 148:3–18.

Dagla HR, Shekhawat NS. 2005. In vitro multiplication of Haloxylon recurvum (Moq.) a plant for saline soil reclamation. J Plant Biol 7:155–160.

Darling AE, Mau B, Perna NT. 2010. Progressive *Mauve*: multiple genome alignment with gene gain, loss, and rearrangement. PLoS One 5(6):e11147.

Das D, Salgaonkar BB, Mani K, Braganca JM. 2014. Cadmium resistance in extremely halophilic archaeon Haloferax strain BBK2. Chemosphere 112:385–392.

DasSarma P, Klebahn G, Klebahn H. 2010. Translation of Henrich Klebahn's damaging agents of the klippfish, a contribution to the knowledge of the salt loving organisms. Saline Syst 6(1):7.

DasSarma S, DasSarma P. 2015. Halophiles and their enzymes: negativity put to good use. Curr Opin Microbiol 25:120–126.

Dave B, Anshuman K, Hajela P. 2006. Siderophores of halophilic archaea and their chemical characterization. Indian J Exp Biol 44(4):340–344.

Dubey G, Kollah B, Gour VK, Shukla AK, Mohanty SR. 2016. Diversity of bacteria and archaea in the rhizosphere of bioenergy crop *Jatropha curcas*. 3 Biotech 6(2):257.

Etesami H, Beattie GA. 2018. Plant-microbe interactions in adaptation of agricultural crops to abiotic stress conditions, in probiotics and plant health. In: Kumar, V, Kumar, M, Sharma, S, Prasad, R, editors. Singapore: Springer, p. 163–200.

Gaba S, Singh RN, Abrol S, Yadav AN, Saxena AK, Kaushik R. 2017. Draft genome sequence of *Halolamina pelagica* CDK2 isolated from natural salterns from Rann of Kutch, Gujarat, India. Genome Announc 5(6):1–2.

Hamidi M, Mirzaei R, Delattre C, Khanaki K, Pierre G, Gardarin C, Petit E, Karimitabar F, Faezi S. 2019. Characterization of a new exopolysaccharide produced by *Halorubrum* sp. TBZ112 and evaluation of its anti-proliferative effect on gastric cancer cells. 3 Biotech 9(1):1.

Hartmann A, Klink S, Rothballer M. 2021. Importance of N-Acyl-Homoserine Lactone based quorum sensing and quorum quenching in pathogen control and plant growth promotion. J Pathology 10(12):1561.

Herrmann M, Saunders AM, Schramm A. 2008. Archaea dominate the ammonia- oxidizing community in the rhizosphere of the freshwater macrophyte Littorella uniflora. Appl Environ Microbiol 74(10):3279–3283.

Hubmacher D, Matzanke BF, Anemüller S. 2007. Iron-uptake in the Euryarchaeon *Halobacterium salinarum*. Biometals 20(3-4):539–547.

Just-Baringo X, Albericio F, Álvarez M. 2014. Thiopeptide antibiotics: Retrospective and recent advances. Mar Drugs 12(1):317–351.

Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, Kawashima S, Katayama T, Araki M, Hirakawa M. 2006. From genomics to chemical genomics: new developments in KEGG. Nucleic Acids Res 34(Database issue): D354–357.

Kauri T, Wallace R, Kushner DJ. 1990. Nutrition of the halophilic archaebacterium, Haloferax volcanii. Syst Appl Microbiol 13:14–18.

Kibria M, Hoque M. 2019. A review on plant responses to soil salinity and amelioration strategies. OJSS 09(11):219–231.

Kijne JW. 2006. Abiotic stress and water scarcity: identifying and resolving conflicts from plant level to global level. Field Crop Res 97(1):3–18.

Kumar P, Sharma PK. 2020. Soil salinity and food security in India. Front Sustain Food Syst 4:533781.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549.

Kumar V, Tiwari SK. 2017. Halocin HA1: An archaeocin produced by the haloarchaeon *Haloferax larsenii* HA1. Process Biochem 61:202–208.

Lekakis E, Aschonitis V, Pavlatou-Ve A, Papadopoulos A, Antonopoulos V. 2015. Analysis of temporal variation of soil salinity during the growing season in a flooded rice field of thessaloniki plain-Greece. Agron J 5(1):35–54.

Ludt K, Soppa J. 2019. Polyploidy in halophilic archaea: Regulation, evolutionary advantages, and gene conversion. Biochem Soc Trans. 47(3):933–944.

Menasria T, Aguilera M, Hocine H, Benammar L, Ayachi A, Si Bachir A, Dekak A, Monteoliva-Sánchez M. 2018. Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes. Microbiol Res 207:289–298.

Moissl-Eichinger C, Pausan M, Taffner J, Berg G, Bang C, Schmitz RA. 2018. Archaea are interactive components of complex microbiomes. Trends Microbiol 26(1):70–85.

Moopantakath J, Imchen M, Anju VT, Busi S, Dyavaiah M, Martínez-Espinosa RM, Kumavath R. 2023. Bioactive molecules from haloarchaea: Scope and prospects for industrial and therapeutic applications. Front Microbiol 14:1113540.

Mukhtar S, Malik KA, Mehnaz S. 2020a. Osmoadaptation in halophilic bacteria and archaea. Res. J. Biotechnol 15(5):154–161.

Mukhtar S, Mirza BS, Mehnaz S, Mirza MS, Mclean J, Malik KA. 2018. Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome. World J Microbiol Biotechnol 34(9):136.

Mukhtar S, Zareen M, Khaliq Z, Mehnaz S, Malik KA. 2020b. Phylogenetic analysis of halophyte-associated rhizobacteria and effect of halotolerant and halophilic phosphate-solubilizing biofertilizers on maize growth under salinity stress conditions. J Appl Microbiol 128(2):556–573.

Niessen N, Soppa J. 2020. Regulated iron siderophore production of the halophilic archaeon *Haloferax volcanii*. Biomolecules 10(7):1072.

Oren A. 2010. Industrial and environmental applications of halophilic microorganisms. Environ Technol 31(8-9):825–834.

Oren A. 2012. Taxonomy of the family Halobacteriaceae: a paradigm for changing concepts in prokaryote systematic. Int J Syst Evol Microbiol 62(Pt 2):263–271.

Patel RR, Patel DD, Bhatt J, Thakor P, Triplett LR, Thakkar VR. 2021. Induction of pre-chorismate, jasmonate and salicylate pathways by *Burkholderia* sp. RR18 in peanut seedlings. J Appl Microbiol 131(3):1417–1430.

Patel RR, Thakkar VR, Subramanian RB. 2016. Simultaneous detection and quantification of phytohormones by a sensitive method of separation in the culture of *Pseudomonas* sp. Curr Microbiol 72(6):744–751.

Pérez-Miranda S, Cabirol N, George-Téllez R, Zamudio-Rivera LS, Fernández FJ. 2007. O-CAS, a fast and universal method for siderophore detection. J Microbiol Methods 70(1):127–131.

Pikovskaya R. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17:362–370.

Qu Q, Zhang Z, Peijnenburg WJGM, Liu W, Lu T, Hu B, Chen J, Chen J, Lin Z, Qian H. 2020. Rhizosphere microbiome assembly and its impact on plant growth. J Agric Food Chem 68(18):5024–5038.

Reang L, Bhatt S, Tomar RS, Joshi K, Padhiyar S, Vyas UM, Kheni JK. 2022. Plant growth promoting characteristics of halophilic and halo-

tolerant bacteria isolated from coastal regions of Saurashtra Gujarat. Sci Rep 12(1):4699.
Schmid J, Fariña J, Rehm B, Sieber V. 2016. Editorial: Microbial Exopolysaccharides: From Genes to Applications. Front Microbiol 7:308.
Selim S, Akhtar N, Hagagy N, Alanazi A, Warrad M, El Azab E, Elamir MYM, Al-Sanea MM, Jaouni SKA, Abdel-Mawgoud M, et al. 2022. Selection of newly identified growth-promoting archaea *Haloferax* species with a potential action on cobalt resistance in maize plants. Front Plant Sci 13:872654.
Serrano S, Mendo S, Caetano T. 2022. Haloarchaea have a high genomic diversity for the biosynthesis of carotenoids of biotechnological interest. Res Microbiol 173(3):103919.
Shafiee RT, Snow JT, Zhang Q, Rickaby REM. 2019. Iron requirements and uptake strategies of the globally abundant marine ammonia-oxidising archaeon, *Nitrosopumilus maritimus* SCM1. Isme J 13(9):2295–2305.
Shrivastava P, Kumar R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22(2):123–131.
Smith-Moore CM, Grunden AM. 2018. Bacteria and archaea as the sources of traits for enhanced plant phenotypes. Biotechnol Adv 36(7):1900–1916.
Song GC, Im H, Jung J, Lee S, Jung M-Y, Rhee S-K, Ryu C-M. 2019. Plant growth-promoting archaea trigger induced systemic resistance in *Arabidopsis thaliana* against *Pectobacterium carotovorum* and *Pseudomonas syringae*. Environ Microbiol 21(3):940–948.
Taffner J, Cernava T, Erlacher A, Berg G. 2019. Novel insights into plant-associated archaea and their functioning in arugula (Eruca sativa Mill.). J Adv Res 19:39–48.
Trivedi C, Reich PB, Maestre FT, Hu HW, Singh BK, Delgado-Baquerizo M. 2019. Plant-driven niche differentiation of ammonia-oxidizing bacteria and archaea in global drylands. Isme J 13(11):2727–2736.
Ventosa A, Mellado E, Sanchez-Porro C, Marquez MC. 2008. Halophile and halotolerant microorganisms from soils. In: Dion P, Nautiyal CS, editors. Microbiology of extreme soils. Berlin: Springer-Verlag, pp 87–115.
Verma DK, Vasudeva G, Sidhu C, Pinnaka AK, Prasad SE, Thakur KG. 2020. Biochemical and taxonomic characterization of novel haloarchaeal strains and purification of the recombinant halotolerant α-amylase discovered in the isolate. Front Microbiol 11:2082.
Vinogradov AA, Suga H. 2020. Introduction to thiopeptides: Biological activity, biosynthesis, and strategies for functional reprogramming. Cell Chem Biol 27(8):1032–1051.
Voica DM, Bartha L, Banciu HL, Oren A. 2016. Heavy metal resistance in halophilic Bacteria and Archaea. FEMS Microbiol Lett 363(14):fnw146.
Vreeland RH. 1993. The biology of halophilic bacteria. Vreeland, RH, Hochstein LI, editors. Boca Raton: CRC Press, p. 105–134.
Wang S, Zheng Z, Zou H, Li N, Wu M. 2019. Characterization of the secondary metabolite biosynthetic gene clusters in archaea. Comput Biol Chem 78:165–169.
Watanabe F, Olsen S. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Science Soc of Amer J 29(6):677–678.
Winnepenninckx B, Backeljau T, de Wachter R. 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet 9(12):407–412.
Yadav AN, Sharma D, Gulati S, Singh S, Dey R, Pal KK, Kaushik R, Saxena AK. 2015. Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. Sci Rep 5:12293.
Yildiz E, Ozcan B, Caliskan M. 2012. Isolation, characterization, and phylogenetic analysis of halophilic archaea from a salt mine in central Anatolia (Turkey). Pol J Microbiol 61(2):111–117.