PLANT ECOPHYSIOLGY

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Plant Ecophysiology 2 (2023) 1-16

Isolation and identification of salinity and drought tolerant bacteria from Jiroft agricultural fields

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https://zenodo.org/doi/10.5281/zenodo.10461080

Abstract
One of the new ways to cope with salinity and reduce its harmful effects is to introduce salt-tolerant bacteria. In this research, indigenous bacteria resistant to salinity and drought were isolated and identified. The growth rate of isolates at different concentrations of salinity (5%, 10%, 15%, 20%, 25%, 35% and 40%), drought(0.50, -0.15, -0.03, -0.49 and -0.73 MPa), pH and 50°C. Then, the production of Auxin, Siderophore, hydrogen cyanide and phosphate solubility were investigated. A total of 42 soil samples were collected, 50 bacteria were obtained. The K4, K10, K12, K14, K15 codes for the Konar-sandal, C18, C10, C11 codes for Karim-abad, A2, A3, A4 codes for Anbar-abad were resistant to salinity. Also, the K4, K14, K15, C8 codes had the ability to grow in water potential at -0.73. The C8 and K4 isolates had the ability to grow at high pH and 50°C. Also, isolate C8 produced the highest amount of Auxin (1.8 µg/ml) and it was the only isolate that dissolved phosphate. The isolates C8 and K14 had the ability to produce Siderophore. Therefore, C8 isolate was *Bacillus subtilis* as the superior isolate.

Keywords: Drought, Jiroft, Identification, Resistant bacteria and Salinity

Introduction

In general, environmental stresses reduce about 71% of crop yield, among which yield loss due to high temperature is 15%, low temperature is 40%, drought stress is 17% and salinity stress is estimated to be 20% (Ashraf and Harris, 2005). Soil salinity is considered as one of the limiting factors of crop yield all over the world, which is one of the most basic problems of the agricultural sector, especially in arid and semi-arid regions. It is even said that the destruction of the Sumerian civilization in Mesopotamia was due to the salinity phenomenon (Jacobsen and Adams, 1958). Currently, saline soils occupy 7% of the earth's surface. In Iran, about 20% of the country's land (34 million hectares) is affected by salinity (Jafari, 2013).

The most important effects of salinity stress on plant growth are: the effect of osmotic stress, the disruption of ion balance in the plant, and the effect of ion toxicity (Abrol *et al*., 1988). Therefore, in order to make optimal use of these lands and growth resources, various methods are carried out, including leaching of saline soils, proper management of irrigation and cultivation, use of plant varieties resistant to salinity, genetic manipulation, and identification and

use of biological solutions (Pirasteh-Anosheh *et al*., 2015). The high cost of remediation, insufficient drainage, the high cost of soil amendment materials and the unfavorable quality of irrigation water are among the factors involved in this direction. Therefore, it is one of the new strategies to deal with salinity in plants and reduce its harmful effects. It is the introduction of salt-tolerant microorganisms that also improve plant growth (Madigan and Oren, 1999). In harsh natural conditions, such microorganisms deal with many stresses, including high salt concentrations, extreme dryness, and ultraviolet radiation from the sun, and ensure their survival by adjusting the osmotic pressure. In recent years, the potential of using these organisms in various fields of pharmaceutical biotechnology, bioremediation, biofertilizers, etc. has been highly considered (Yadav *et al*., 2015). One of the mechanisms through which bacteria help plant growth in stressful conditions is to reduce the plant's stress ethylene level through the production of the enzyme ACC (aminocyclopropane 1-carboxylate)-deaminase. In stressful conditions, the amount of ACC, which is a precursor to ethylene production, increases inside the plant. Some *Azospirillium* species use ACC deaminase for mechanisms other than producing increased plant resistance against stress. These species may produce indole acetic acid IAA and promote growth in saline conditions (Yadav *et al*., 2015). Salt-resistant bacteria comprise a wide range of bacteria that are resistant to salt, which means that they can grow in the presence or absence of salt, and include some types of Bacillus, Micrococcus, Corynebacterium, and Streptococcus (Safdarian *et al*., 2019).

Halophilic (salt-loving) and halotolerant (salt-tolerant) bacteria play an important role in plant growth. Exopolysaccharide production is one of these roles, which limit Na absorption and stimulate plant growth. Another role of these microorganisms is the reduction of antioxidant enzymes of plants under salt stress. Halotolerants and halophiles also maintain nutrient cycling in saline soils with full functioning enzymes and metabolites in the soil (Munns and Tester, 2008). Today, humans need methods that, while enjoying economic dynamism, improve the environment and optimal use of available resources to meet human food needs, and play a significant role in improving the quality of life of human societies. The activities of the agricultural sector play a very important role in the economy of Jiroft city. So that the dependence of more than 80,000 people of the city's population on the products of this part of the region's economy is an emphasis on this claim. Jiroft city is the hub of agricultural production in the country. Due to the great importance of agricultural products in providing people's food security, it is necessary to pay attention to the stabilization of agricultural operations in order to continue the production ability of the city's farms and preserve the environment (Adali Sardoi *et al*., 2019). The purpose of this research is to isolate salinity and drought resistant bacteria from the soil of Jiroft fields and to measure the amount of auxin and Siderophore production by each bacterial strain, as well as to determine the optimal temperature and pH for the growth of superior salinity and drought resistant bacteria, which has not been done in the region so far.

Bacteria and archaea are the dominant microorganisms in salt marshes (Margesin and Schinner, 2001). Their compatibility can be due to the high osmotic pressure, accumulation of organic solutes in their cytoplasm and creating osmotic balance. Very salty and dry soils are the natural habitat of salt-loving bacteria and archaea (Holt *et al*., 1994). In a research related to the growth of *Pseudomonas* inoculated with flax seedlings (*Gossypium hirsutum* L.) against *bacterium putida* strain Rs-198 of

salt stress, it was found that this bacterium can increase the absorption of magnesium, potassium and calcium elements and decrease the absorption of sodium from the soil. the result of increasing the growth of seedlings (Yao *et al*., 2010. In another study, *Azospirillum brasilense* and *Pantoea dispersa* bacteria increased the dry weight of the plant under saline conditions and also improved the rate of photosynthesis and stomatal conductance of sweet pepper (*Capsicum annuum* L.) seedlings. (Del Amor and Cuadra-Crespo, 2011). In a study related to the inoculation of Staphylococcus EY37, Bacillus EY30 and Kocuria EY43 bacteria, the harmful effect of salinity on the severe reduction in growth, yield and nutrition of strawberry (*ananassa Fragaria*) seedlings has been reported (Karlidag *et al*., 2011).

In the model presented by Mayak *et al*., (2004), it was suggested that rhizospheric bacteria with the ability to produce ACC and aminase enzymes can reduce plant ethylene and moderate the effects of salinity. In the region of Jiroft, there has been no report of identification of bacteria resistant to salinity and drought. For this reason, the current research, which aims to isolate and identify bacteria resistant to salinity and drought, is new in this region.

Materials and methods

Collecting soil samples

Soil samples were selected from a depth of 30cm in the dry and salty parts of Jiroft farms (Kanarsandal, Jazsaleh, Anbarabad and Karimabad areas) in the crop year of 2019 and were transferred to the laboratory in sterile nylon bags and kept in the refrigerator at a temperature of 4°C until further tests. (Roesch *et al*., 2007).

Isolation of soil bacteria

First, ten grams of each soil sample was poured into 90ml of sterile physiological serum and placed on a shaker at 100 rpm for one hour, and then serial dilutions up to five dilutions were prepared from the soil suspensions. Finally, 60 microliters of each dilution was added to the minimal base culture medium prepared in advance and spread with a glass culture rod. To prepare a stock of bacterial isolates, they were cultured on TSA medium in a gradient in screw-cap test tubes, and after 24 hours of growth of the isolates at 30°C, the isolates were kept in a refrigerator at 4°C (Roesch *et al*., 2007).

Screening of salt resistant bacteria

To check the salinity resistance of the isolates, they were cultured in nutrient broth with concentrations of 5%, 10%, 15%, 20%, 25%, 35% and 40% sodium chloride. In order to make the respective environments, five, 10, 15, 20, 25, 35 and 40 grams of sodium chloride salt were poured into the balloon, and then the volume of each sample was brought to 100ml with nutrient broth culture medium. No salt was used in the control sample. The growth rate of the isolates (by measuring the optical density at 660nm) was measured using a spectrophotometer (Safdarian *et al*., 2019).

Screening of drought resistant bacteria

Different water potentials (0.50, -0.15, -0.03, -0.49 and -0.73 MPa) were prepared by adding calculated amounts of polyethylene glycol 6000 per liter of nutrient broth culture medium, respectively, according to the following formula became.

Water potential(wp)=-(1018e-2)c-(1.18e-4)$c^8$2+(2.67e_4)^{ct}+(8.39e-7)$c^8$2T

T: Kelvin temperature **c:** polyethylene glycol concentration **t:** ambient temperature **e:** constant coefficient **wp:** water potential

Then 0.1ml of bacterial liquid culture was added to different water potentials and three replicates were prepared from each

concentration. The cultured water potentials were incubated at 30 ± 2°C at 120rpm for 24hours. Bacterial growth was measured by measuring the optical density at 600nm with a spectrophotometer (Rajput *et al*., 2013). Polyethylene glycol was not used in control samples.

Investigating the resistance of the salt and drought resistant isolates to high pH and 50°C

Normal sodium hydroxide and normal hydrochloric acid were used to make liquid broth nutrient culture medium with different pHs of 5, 5.5, 6, 6.5, 7, 7.5, and 9. Then, the isolates resistant to salinity and drought of the previous stage were inoculated in culture media and kept at 30 ± 2°C for 24 hours, and their optical absorption was read at 600 nm (Rehman and Nautiyal, 2002) and in order to Examining the effect of heat stress, a loop of fresh bacterial culture was inoculated in 30ml tubes containing 10ml of nutrient broth and placed in an incubator shaker for eight hours until the bacterial population reached $1x10^8$ cfu/ml (McFarland's half). Then the cultured tubes were placed at 50°C for 24 hours and their optical absorbance was read at 600nm (Hassanshahian and Mohamadian, 2011).

Quantitative estimation of indole-3-acetic acid by salt and drought resistant isolates

Estimation of Auxin content was done by break method and using spectrophotometry. First, bacterial isolates were cultured for 72hours in nutrient broth culture medium with L-tryptophan (500 µg/ml) and 2% sodium chloride. Then they were kept in an incubator at 30°C for 72 hours. After the incubation period, the samples were centrifuged at 1000 g for 15 minutes. Then, take one cc from the supernatant solution and mix it with 4cc of Salkowski's reagent (150 ml of concentrated sulfuric acid, 250 ml of distilled water and 7.5ml of 0.5M iron chloride) and after 20 minutes, absorb the isolates at a wavelength of 530nm was read (Hujjat Nughi *et al*., 2012).

Evaluation of phosphate solubility by salt and drought resistant isolates

Bacterial isolates were cultured on Picosky solid culture medium. Then the culture plates were kept at 30±2°C for five days. To identify the power of phosphate solubilization, the characteristic of clarifying the environment around the colony is used. Then, the isolate that has dissolved phosphate in the medium was inoculated in the Picosky liquid culture medium and incubated for 100hours (at a speed of 100rpm) at 30±2°C. At the end, two milliliters of the culture medium were removed and transferred to two milliliter vials. Then, to remove bacteria and solids in the culture medium, the vials were centrifuged for 10 minutes at 12,000rpm and at 25°C. The clear supernatant solution of each vial was transferred to new vials. Finally, usable phosphorus was measured by the method proposed by Watanabe and Olsen (Watanabe and Olsen, 1965).

Siderophore production by salt and drought resistant isolates

According to the Casschuttle method, the desired isolate was first cultured in a minimum iron medium at 30°C. Then the sample was centrifuged for 15 minutes at 27,000rpm and the Cass solution was added to the culture supernatant in equal proportions and incubated for 20 minutes. In the presence of Siderophore, iron was removed from the color composition and caused a decrease in the intensity of the blue color. The color intensity was read by a spectrophotometer at 630nm (Adav *et al*., 2014).

Measurement of hydrocyanic acid by isolates resistant to salinity and drought

Hydrocyanic acid production was investigated by the method of organic or inorganic salts of picric acid. Bacteria were cultured in nutrient broth containing 4.4% glycine. Then filter paper (Whatman No. 1) soaked in a solution of 2% sodium carbonate and 0.5% picric acid was placed on the culture medium. It was placed in an incubator at 28 ± 2°C for 96hours. The production of HCN was indicated by the color change of the filter paper from yellow to orange-brown (Castric, 1974)

Biochemical identification of the superior isolate resistant to salinity and drought

Identification of superior isolates was done by gram staining, catalase test, oxidase test, motility test, cultivation in TSI medium and cultivation in Simon citrate medium.

Molecular identification of the superior isolate resistant to salinity and drought

First, DNA extraction was done by CTAB method, and finally, the DNA sample was stored in a -20°C freezer until use. Diluted was removed and sterile double distilled water was added to it. In total, there were ten lands. The sequence of the forward primer and the sequence of the reverse primer are given in Tables 1 and 2.

Table1. Primer sequence of 27f

SEQ	5-AGAGTTTGATCMCTCAG-3
GC%	45
Tm	56.4

Table2. Reverse primer sequence 1525r

SEQ	5-AAGGAGGTGWTCCARCC-3
GC%	52.9
Tm	52.4

PCR reaction

In order to prepare the PCR materials, one LANDA of the diluted primer and one LANDA of the back diluted primer were mixed with one LANDA DNA and 5µM of Mastermix (Genel Co., South Korea) along with 17µM of water and a total volume of 25 became micromolar.

Electrophoresis step

After that, with the help of Gel documentation device under UV light, the presence of the target gene was checked based on its band size and then a photo was taken.

Preparation of samples to determine the nucleotide sequence

The samples of PCR products, which were confirmed by electrophoresis, were used to determine the sequence. Sequence determination was done by South Korea's Macrogen Company using reverse primers.

Bioinformatics studies

The 16srDNA gene sequence of bacteria isolated in NCBI gene bank was compared through BLASTN program and aligned with MEGA6 software. Phylogeny analysis and phylogenetic tree drawing was done based on Neighbor-joining phylogeny algorithm.

Analysis of information

Graphs were drawn with Excel 2013 software and data analysis was done with SPSS 16 statistical program. The obtained results were analyzed using one-item statistical tests.

Results:

Collecting soil samples

A total of 42 soil samples were collected from Kanar Sandal (four samples), Jazsaleh (five samples), Anbarabad (five samples), and Karimabad (six samples) farms in 2019.

Isolation of soil bacteria

Based on the morphological characteristics of colonies grown on TSA medium, different bacterial isolates were selected and coded in terms of colony color, colony height, colony shape, colony margin, and colony size. A total of 50 bacteria were obtained from Jazsaleh (8 isolates), Kanar Sandal (16 isolates), Karim abad (16 isolates) and Anbar abad (10 isolates). The code of bacteria obtained from each region is given in Table 3.

Table3. Code of bacteria obtained from different salty and dry areas of Jiroft

Collection area	Bacteria code	Collection area	Bacteria code	Collection area	Bacteria code	Collection area	Bacteria code
Karimabad	C16	Karimabad	C3	Kanar Sandal	K6	Jazsaleh	J1
Anbarabad	A1	Karimabad	C4	Kanar Sandal	K7	Jazsaleh	J2
Anbarabad	A2	Karimabad	C5	Kanar Sandal	K8	Jazsaleh	J3
Anbarabad	A3	Karimabad	C6	Kanar Sandal	K9	Jazsaleh	J4
Anbarabad	A4	Karimabad	C7	Kanar Sandal	K10	Jazsaleh	J5
Anbarabad	A5	Karimabad	C8	Kanar Sandal	K11	Jazsaleh	J6
Anbarabad	A6	Karimabad	C9	Kanar Sandal	K12	Jazsaleh	J7
Anbarabad	A7	Karimabad	C10	Kanar Sandal	K13	Jazsaleh	J8
Anbarabad	A8	Karimabad	C11	Kanar Sandal	K14	Kanar Sandal	K1
Anbarabad	A9	Karimabad	C12	Kanar Sandal	K15	Kanar Sandal	K2
Anbarabad	A10	Karimabad	C13	Kanar Sandal	K16	Kanar Sandal	K3
		Karimabad	C14	Karimabad	C1	Kanar Sandal	K4
		Karimabad	C15	Karimabad	C2	Kanar Sandal	K5

Screening of salt resistant bacteria

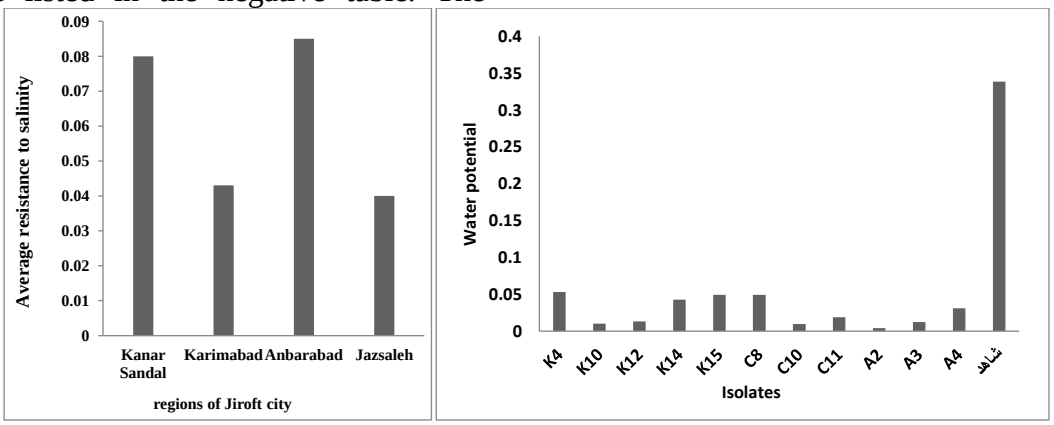
The culture of bacterial isolates in nutrient broth liquid culture media with concentrations of (5%, 10%, 15%, 25%, 35%, 40%) of salt, after 24 hours, the optical absorption of the samples was read at a wavelength of 660 nm. The results are given in Table 4.

Table4. Results of optical absorption of bacterial isolates cultured in different salinity concentrations at 660nm

Salt concentration Bacteria Code	%5	%10	%15	%20	%25	%35	40%	Contral
J1	0/103	0/003	-	-	-	-	-	0/114
J2	0/131	-	-	-	-	-	-	0/133
J3	0/358	0/056	0/040	-	-	-	-	0/372
J4	0/594	0/258	0/088	-	-	-	-	0/595
J5	0/009	0/008	-	-	-	-	-	0/215
J6	0/265	0/131	0/098	-	-	-	-	0/182
J7	0/189	-	-	-	-	-	-	0/216
J8	0/009	-	-	-	-	-	-	0/049
K1	0/320	0/115	0/053	0/019	-	-	-	0/339
K2	0/478	0/207	0/107	0/012	-	-	-	0/889
K3	0/009	0/008	-	-	-	۰/۰۰۸	۰/۰۰۹	0/273
K4	0/295	0/118	0/095	0/089	0/063	0/024	0/004	0/328
K5	0/352	0/110	0/207	-	-	-	-	0/400
K6	0/067	0/060	0/057	-	-	-	-	0/149
K7	0/116	0/097	0/059	-	-	-	-	0/185
K8	0/213	0/090	0/003	-	-	-	-	0/305
K9	0/119	0/49	0/19	-	-	-	-	0/288
K10	0/391	0/310	0/233	0/152	0/082	0/062	0/047	0/389
K11	0/163	0/100	-	-	-	-	-	0/413
K12	0/148	0/112	0/711	0/389	0/235	0/198	0/135	0/193
K13	0/058	0/013	0/012	-	-	-	-	0/168
K14	0/362	0/152	0/120	0/078	0/054	0/034	0/024	0/434
K15	0/123	0/114	0/067	0/051	0/028	0/023	0/018	0/175
K16	0/074	0/051	0/036	-	-	-	-	0/193
C1	0/051	0/009	0/006	-	-	-	-	0/262
C2	0/361	0/125	0/026	-	-	-	-	0/177
C3	0/111	0/083	0/058	0/045	0/018	0/009	-	0/157
C4	0/056	-	-	-	-	-	-	0/158
C5	0/034	-	-	-	-	-	-	0/156
C6	0/018	-	-	-	-	-	-	0/110
C7	0/086	-	-	-	-	-	-	0/156
C8	0/148	0/112	0/711	0/389	0/235	0/198	0/135	0/193
C9	0/058	0/013	0/012	-	-	-	-	0/168

C10	0/372	0/162	0/130	0/088	0/064	0/044	0/034	0/334
C11	0/123	0/114	0/067	0/051	0/028	0/023	0/018	0/175
C12	0/074	0/051	0/036	-	-	-	-	0/193
C13	-	-	-	-	-	-	-	0/174
C14	0/066	0/009	-	-	-	-	-	0/165
C15	0/082	-	-	-	-	-	-	0/197
C16	0/009	0/001	-	-	-	-	-	0/210
A1	-	-	-	-	-	-	-	0/195
A2	0/320	0/274	0/174	0/164	0/076	0/063	0/028	0/339
A3	0/878	0/407	0/400	0/342	0/331	0/049	0/045	0/889
A4	0/009	0/008	0/231	0/196	0/187	0/265	0/069	0/273
A5	0/115	-	-	-	-	-	-	0/199
A6	-	-	-	-	-	-	-	0/234
A7	0/111	0/098	0/005	-	-	-	-	0/184
A8	0/186	0/102	0/006	-	-	-	-	0/193
A9	0/196	0/115	0/091	-	-	-	-	0/213
A10	0/214	0/126	0/060	-	-	-	-	0/245

Among the available isolates, codes K4, K10, K12, K14, K15 are related to Kanar Sandal, codes C8, C10, C11 are related to Karimabad, codes A2, A3, A4 are related to Anbarabad due to their growth up to 40% salt concentration and being resistant. They were selected for further studies. Isolates that did not grow after 24 hours of culture at 30°C and no turbidity was observed in their test tubes are listed in the negative table. The average growth of each of the isolates in different percentages of salt (5%, 10%, 15%, 25%, 35%, 40%) was calculated and the total average for each region was estimated. The relevant results are given in chart1. According to the diagram, the average growth of salt resistance isolates in Anbarabad region is higher than the other three regions.



Diagrams1 and 2. Average resistance to salinity and drought of bacterial isolates from four regions of Jiroft

Screening of drought resistant bacteria

The salt resistant isolates of the previous stage in order to screen drought resistance against different water potentials (0.50, -0.15, -0.03, -0.49 and -0.73 MPa) in the presence of polyethylene glycol 6000 at 30°C for 24 hours. Then the results of bacterial growth at 600nm wavelength were read using a spectrophotometer (Table 5).

Table5. The results of reading the optical absorption of bacteria grown in different concentrations of water potentials at 600nm.

water potential(MPa) Bacteria code	0/50	-0/15	-0/03	-0/49	-0/73	contral
K4	0/026	0/029	0/029	0/144	0/038	0/328
K10	0/023	0/017	0/011	-	-	0/389
K12	0/019	0/017	0/012	0/010	0/008	0/193

K14	0/059	0/057	0/055	0/023	0/019	0/434
K15	0/013	0/025	0/041	0/155	0/013	0/175
C8	0/056	0/056	0/046	0/044	0/043	0/193
C10	0/034	0/007	0/004	0/003	-	0/334
C11	0/040	0/039	0/014	-	-	0/175
A2	0/012	0/009	-	-	-	0/339
A3	0/050	0/011	-	-	-	0/889
A4	0/030	-	-	-	-	0/273

Codes K4, K14, K15, C8 had the ability to grow in the water potential of -0.73 and besides being resistant to salinity, they are also resistant to drought. The rest of the codes in the table were removed to perform the next tests. The results related to the cultivation of isolates in different water potentials are given in Figure 2. In the control sample, the average growth of isolates is 0.3383. Compared to the control, the average of the highest growth of isolates in water potentials is related to isolates No. K4, K14, K15, C8. It is concluded that these isolates are drought resistant isolates.

Table 6 shows the comparison of the mean and the mean difference of the optical absorption density results of the growth of salt-resistant isolates in different water potentials (0.50, -0.15, -0.03, -0.49, -0.73).

Table6. Comparison of mean and mean difference of optical absorption density results of growth of salt resistant isolates in different water potentials.

Sources of changes	degree of freedom	mean	mean squared	difference mean	standard deviation	error standard deviation	significance
K4	4	53/20	2830/24	53/20	50/95	22/78	0/080
K10	4	10/20	104/04	10/20	10/23	4/57	0/090
K12	4	13/20	174/24	13/20	4/65	2/08	0/003
K14	4	42/60	1814/76	42/60	19/81	8/86	0/009
K15	4	49/40	2440/36	49/40	60/13	26/89	0/140
C8	4	49	2401	49	6/48	2/89	0
C10	4	9/60	92/16	9/60	13/86	6/20	0/197
C11	4	18/60	345/96	18/60	19/91	8/90	0/105
A2	4	4/20	17/64	4/20	5/84	2/61	0/184
A3	4	12/20	148/84	12/20	21/66	9/68	0/276
A4	4	6/00	36	6/00	13/41	6/00	0/374

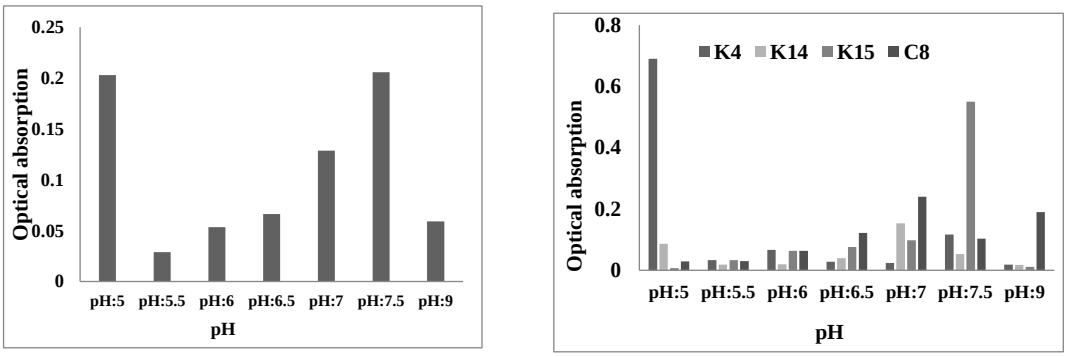
Significant at the 0.5% probability level

Investigating the growth of salt and drought resistant isolates at high pH

The growth of the screened isolates was different in acidic, alkaline and neutral pHs. At pH: 5, the highest growth is related to code K4, at pH: 5.5, code K15 and K4, at pH: 6, the highest growth is related to code K15 and C8, at pH: 6.5, code C8, at pH: 7, code C8, at pH K15 code: 7.5 and C8 code grew the most at pH: 9. The results are given in Table 7. K4 isolate at pH 7.5, K14 isolate at pH 5, K15 isolate at pH 7 and C8 isolate at pH 9 had the highest growth. Therefore, isolate C8 is resistant to high pH. The growth of the isolates is given in Figure 3.

Table7. Results of optical absorption of salt and drought resistant isolates at different pH and 30°C

pH Bacteria code	5	5/5	6	6/5	7	7/5	9
K4	0/69	0/033	0/067	0/028	0/024	0/117	0/018
K14	0/086	0/019	0/020	0/039	0/153	0/053	0/017
K15	0/007	0/033	0/063	0/076	0/098	0/55	0/011
C8	0/029	0/030	0/063	0/122	0/240	0/103	0/190



Diagrams3 and 4. The average growth of salt and drought resistant isolates at different pH

Figure 4 shows the average growth of salt and drought resistant isolates at each pH. Based on this, the highest growth of the isolates was respectively at pH: 7.5 (neutral), pH: 5 (acidic), pH: 7 (neutral), pH: 6.5 (acidic), pH: 9 (alkaline), pH: 6 (acidic). And finally it was at pH: 5.5 (acidic). Comparison of mean and mean difference of optical absorption density results of growth of salt and drought resistant isolates at different pH (5, 5.5, 6, 6.5, 7, 7.5, 9) are given (Table 8).

Table8. Comparison of mean and mean difference of light absorption density growth of salt and drought resistant isolates at different pH

Sources of changes	degree of freedom	mean	mean squared	difference mean	standard deviation	error standard deviation	significance
K4	6	50/85	2585/72	50/58	35/60	13/45	0/009
K14	6	55/28	3055/87	55/28	49/64	18/76	0/026
k15	6	49	2401	49	33/72	12/74	0/009
C8	6	111/0	12321	111/0	80/26	30/33	0/011

Significant at the 0.5% probability level

Resistance of isolates resistant to salinity and drought to 50°C

The optical absorbance of the screened isolates resistant to salt and drought (K4, K14, K15, C8) at 50°C after 24 hours at 600 nm is given in Table 9.

Table9. The results related to reading the optical absorption of salt and drought resistant isolates at 50°C

Bacteria code	Optical absorption (nm)
K4	0/015
K14	0/006
k15	0/002
C8	0/101

According to the absorption of the samples, isolate C8 and isolate K4 have grown the most at 50°C. Figure 5 shows the growth rate of salt and drought resistant isolates at 50°C.

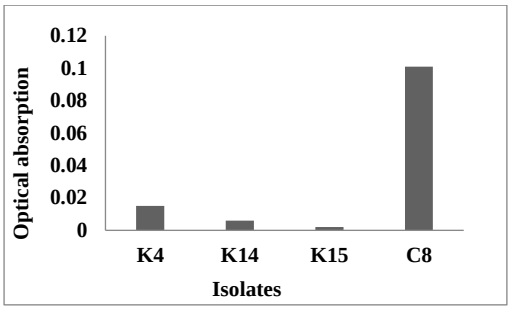


Diagram5. Growth rate of salt and drought resistant isolates at 50°C

Quantitative estimation of indole-3-acetic acid by salt and drought resistant isolates

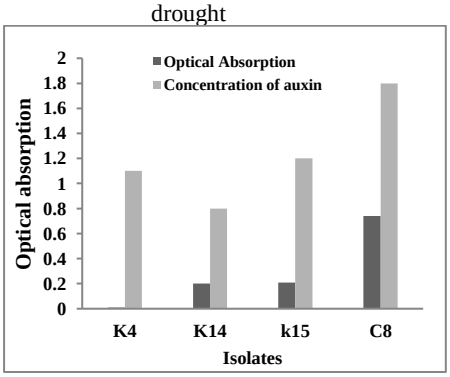
The optical absorbance of salt and drought resistant isolates was read in the presence of L-tryptophan and 2% sodium chloride after 72 hours at 530 nm. The results are given in Table 10. Among the isolates, isolate C8 had the highest optical absorption and isolate K4 had the lowest optical absorption. The concentration of Auxin produced by the isolates was measured with a spectrophotometer. C8 isolate was able to produce 1.8 µg/ml of Auxin after 72 hours of incubation.

Table10. The results of light absorption and Auxin concentration produced by isolates

Bacteria code	Concentration(Mg/ml)	Optical Absorption(Nm)
K4	1/1	0/01
K14	0/8	0/20
k15	1/2	0/21
C8	1/8	0/74

Diagram 6 shows the optical absorption of samples and the concentration of Auxin produced by isolates resistant to salt and drought.

Diagram6. Optical absorption of samples and concentration of Auxin produced by isolates resistant to salinity and drought



Evaluation of phosphate solubility by salt and drought resistant isolates

The investigation of the transparent halo around the isolates cultured in Picosky medium was evaluated after five days to identify the isolates that have phosphatase enzyme and decompose the phosphate in the environment. Among the isolates screened, only isolate C8 was able to dissolve phosphate (Figure 1). The solubility of isolated C8 phosphate was read using a spectrophotometer at 450 nm. The resulting number was 101.

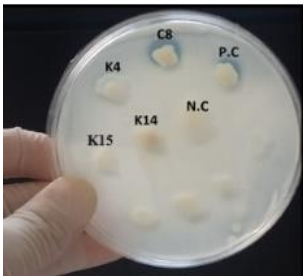


Figure1. Phosphate dissolution by salt and drought resistant isolates P.C (positive control) and N.C (negative control)

Siderophore production by superior isolates

Cultivation of the isolates was carried out in minimal iron medium and then Cass solution was added to it. The amount of color reduction in the samples was measured at 630nm. Among the K4, K14, K15 and C8 isolates, none of them were able to reduce the color intensity.

Measurement of hydrocyanic acid by salt and drought resistant isolates

Among the isolates resistant to salinity and drought, only isolates C8 and K14 had the ability to change the color of filter paper impregnated with picric acid and sodium carbonate to pale brown after four days (96hours).

Biochemical identification of superior isolate resistant to salinity and drought

According to the results obtained by the isolates isolated from the soil samples of farms in Jiroft city, isolate C8 was recognized as the superior isolate, and the biochemical identification of this isolate was done. The biochemical identification results of isolate C8, including gram staining, catalase, oxidase, Simon citrate, TSI and movement are given in Table 11. Gram staining showed short, gram-positive and spore-bearing bacilli. According to the results of biochemical tests of C8 bacteria genus, *Bacillus* was fulfilled.

Table11. The results of biochemical tests related to the superior isolate resistant to salinity and drought

biochemical tests	Gram staining	Catalase	oxidase	Simon Citrate	TSI	movement	H2S	gas production
C8	gram positive-spored	positive	negative	negative	acid/acid	positive	negative	negative
K4	gram positive-none spored	positive	negative	negative	acid/acid	-	negative	negative
K14	gram positive-none spored	positive	negative	negative	acid/acid	-	negative	negative
K15	gram positive-none spored	positive	negative	negative	acid/acid	positive	negative	negative

Molecular identification of the superior isolate resistant to salinity and drought

Optical absorption of extracted DNA

DNA extraction of isolate C8 as the superior isolate was performed using the CTAB method. The optical absorption of the extracted DNA sample was read at 260 and 280 nm using a spectrophotometer (Nanodrop) and then the resulting numbers were divided by each other. In connection with isolate C8, the optical absorption ratio of 260 to 280 was 1.86. Also, the extracted DNA concentration of the C8 isolate was equal to 800 ng/microliter.

After confirming DNA extraction, PCR steps were performed. The gel image of the PCR product is given in Figure 2. According to the ladder, the PCR product formed a band in 1500bp.

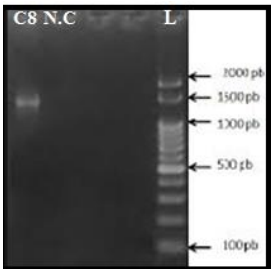


Figure2. Gel image of the PCR product of the superior isolate resistant to salt and drought

The results of Microorganism identification using 16S rDNA translation method

Amplification of 16SrDNA gene fragment was done using 27F and 1492R primers and a 1500 base pair fragment was amplified by PCR method. Then the target fragment was sent to Tekapozist company for sequencing and purification.

Bioinformatics analysis

The results of synonymous determination were analyzed with chromase electrogram format using chromase version 1/41 software, then the final sequence was compared with other bacterial sequences available in NCBIGenbank. After the sequencing of the C8 isolate by the South Korean Bionier company, which was done through the intermediary of the Iranian Tekaposist company, the obtained sequence was blasted on the NCBI site and the phylogeny tree was drawn using the phylogeny software. Based on sequence blast results and phylogeny tree drawing, isolate C8 is *Bacillus subtilis* (Figure 3).

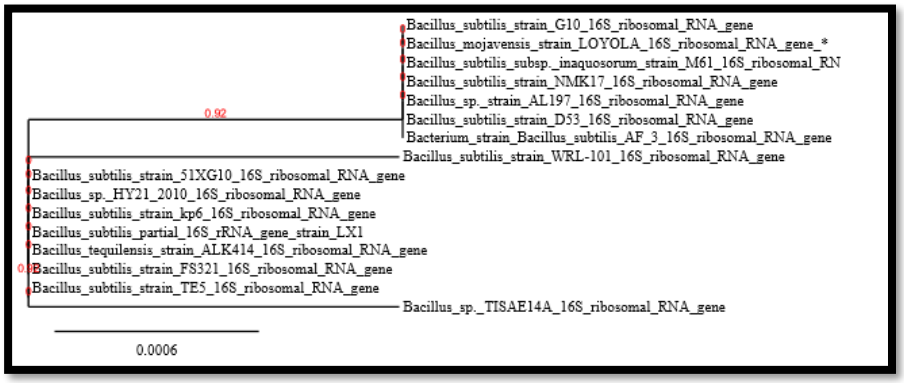


Figure3. Phylogeny tree based on 16SrDNA gene sequence obtained from C8 isolate sequencing

Discussion

The existence of huge water resources and saline soil are considered a major threat to sustainable agricultural production, especially in arid and semi-arid regions of the world (Kafi, 2008). Meanwhile, after India and Pakistan, Iran is at the top with 6 million hectares of saline land. Countries under threat from the point of view of salinity stress are considered (Moameni, 2010). Natural factors and artificial factors go hand in hand and expand the extent of the country's salty lands. The dominant organisms in these soils are mostly salt-loving bacteria and archaea (Margesin and Schinner, 2001). The reason for their compatibility is due to the high osmotic pressure, the accumulation of organic solutes in their cytoplasm and the creation of osmotic balance (Holt *et al*., 1994).

Some areas of the lands of Jiroft city have been affected by the factors of salinity and drought, and for this reason, they have been the focus of this research. Since one of the

new ways to deal with salinity in plants and reduce its harmful effects is the introduction of salinity-tolerant bacteria (Madigan and Oren, 1999), in this research, these bacteria that are resistant to salinity and drought are isolated and identified. Salt-loving bacteria have the ability to reduce the effects of salinity stress around plant roots and improve soil fertility, in addition to increasing plant resistance to salinity stress and photosynthesis (Ventosa *et al*., 1998).

In the present research, after isolating bacteria from salty and dry areas of Jiroft city, their growth was investigated against different concentrations of salt. According to the numbers obtained from reading the optical absorption of the samples at 600nm, it was found that the growth of bacteria in concentrations without salt was much higher than their growth in concentrations containing salt, and as the concentration of salt increases, the growth of bacterial isolates also decreases. And even some of them did not have the ability to grow in high concentrations of salt. Among the 50 isolated isolates, 11 isolates were able to grow in 40% salt concentration. The average of salt resistant isolates was higher in Anbarabad and Kanar Sandal regions.

In 2019, Safdarian and partners isolated salt-loving bacteria from the saline soils of Golestan province. The salt concentrations used in this research without salt were 5%, 10%, 15%, 25%, 35% and 40%, and among the 45 bacterial strains that were isolated, three isolates grew in 40% salt concentration. Safdarian also studied the tolerance of isolated strains to drought in water potentials of 0.50, -0.15, -0.03, -0.49, and -0.73. It was found that three salinity-resistant isolates had the ability to grow in drought potential of -0.49. In this research, after isolation of salt-resistant bacteria, their growth rates were investigated in different water potentials. Among 11 salinity-resistant isolates, four isolates were evaluated to be able to grow at a potential of -0.73 and fully tolerant.

In 2018, Alikhani and partners isolated isolates tolerant to salinity and drought in the rainy conditions of Maghan Plain and Kohkin Plain. In this article, isolated soil bacteria were investigated in the range of pH 4 to 8.5, and among the isolates, seven isolates resistant to high pH were isolated. Optical absorption density at 600nm was used to check the growth rate of bacteria. In the present research, the results showed that the isolates had the highest growth at pH equal to five, and then the growth was more at pH equal to 7.5. At a pH equal to 9, C8 isolates grow the most and are resistant to alkaline pH.

Since Jiroft city is one of the tropical regions of the country, the resistance of isolates to growth at 50 °C was also investigated. All isolates resistant to salinity and drought were grown at 50°C, but isolates C8 and K4 had the highest growth. The amount of Auxin production by salt-resistant isolates was checked and the highest production amount was related to C8, k4, and k15 isolates at the rate of 1.8, 1.1, and 1.2 µg/ml, respectively. In the article of Safdarian and partners in 2019, they investigated the amount of Auxin production by the isolated strains. Among the isolated strains, the highest amount of Auxin production was equal to 2.4. In the current research, the phosphate solubility of the isolates was also measured.

Among the isolates present in this research, C8 had the ability to dissolve phosphate, but none of the isolates were able to produce cyanohydric acid and Siderophore. In Safdarian's article, none of the isolates were able to produce hydrocyanic acid and Siderophore. In this research, the isolate C8 was recognized as the superior isolate because it was resistant to salinity, drought, high pH, Auxin production and phosphate dissolution in the relevant tests, and the sequencing of this isolate identified *Bacillus subtilis*, which by matching the results of the biochemical tests with the sequence Found, its identification result is correct. In 2008, Kirkwood and partners were able to isolate different species of Phormidium from soil and brackish wetlands in Oklahoma, USA, some of which were able to tolerate 15% of sodium chloride salt. Their results showed that the maximum growth of these strains

occurred at low salt concentrations and their growth decreased drastically with increasing salt concentration. Similarly, the strains isolated in this study grew less with increasing salt concentration. In relation to the investigation of salinity and drought resistant bacteria that can withstand high pH, Auxin production, hydrocyanic acid production and phosphate dissolution, no article has been published in Jiroft city, and for this reason, the current research is new.

Conclusions

About 15-20% of the total area of the country is Includes the saline lands that According to the recent droughts and also the lack of water management and irrigation of agricultural lands, this amount has increased over the past few years and its impact on the daily life of animals is more Reveal. Jiroft city is one of the most important agricultural hubs in Iran. Therefore, the expansion of soil salinity as well as drought affects agriculture in the region. Native organisms such as bacteria have the ability to adapt to new environmental conditions due to having smaller genomes compared to other organisms. Bacteria have the ability to grow under salinity, drought and alkaline conditions and have the ability to coexist with plants. Therefore, plants for their growth need bacteria and their metabolites such as Auxin, Siderophore, cyanideic acid, vitamins, etc.

In the present study, isolation of drought and salinity-tolerant bacteria from Jiroft farms was carried out and finally superior strain C8 was isolated. In addition to resistance to high salinity and drought conditions, this strain also grew at alkaline pH. Production of Auxin (plant growth hormone), dissolution of phosphate (to make phosphorus available for plants) and production of cyanideic acid are among the capabilities of this native strain. Biochemical and molecular identification of this superior isolate was determined by *Bacillus subtilis.*

Offers

Further researches are suggested to investigate the effect of isolates obtained from this study on the growth of plants in the region and plants grown under drought and salinity conditions and how to use them in biological control of plant pathogens in the region and identification of salt and drought resistant fungi.

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