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Research Article

Isolation and Identification of Antibiotic-Producing Halophilic Bacteria from Dagh Biarjmand and Haj Aligholi Salt Deserts, Iran

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ABSTRACT

Background: Halophilic bacteria are potent organisms in production of novel bioactive antimicrobial compounds which might be considered in drug innovation and control of plant pathogens. Salt deserts in Semnan province are of the most permanent hypersaline areas in the North of Iran. Despite the importance of these areas, there is no scientific report regarding the biodiversity and potency of their halophilic bacteria. Thus, aforementioned areas were selected to detect the halophilic bacteria.

Methods: Here, seven strains were isolated and cultured on their molecular and biochemical properties were characterized. To determine the antibiotic potency of the isolates, agar well diffusion method was conducted. Phylogenetic analysis was done to reveal the isolates relationship with previously known strains.

Results: As a result, growth of the strains in the medium containing 5 to 20% (w/v) NaCl determined that the majority of the isolates were moderately halophile. Catalase activity of all strains was positive. The results represented that D6A, Dar and D8B have antimicrobial effects against different plant and human pathogens. Phylogenic tree analysis also showed that two strains of D6A and Dar are belonged to *Bacillus subtilis* and D8B is belonged to *Virgibacillus olivae*. The bacteria extracts were evaluated for their antifungal and antibacterial activities on human and Plant pathogenic strains. The MIC of the extract *B. subtilis* against was found active against human pathogenic fungi and Plant pathogenic bacteria and fungi, ranging from 12.5 to 25 µg/mL.

Conclusion: This study highlights the therapeutic and prophylactic potential of *B. subtilis* extracts as antibacterial and antifungal agents.

Introduction

Upon the adventure of hypersaline environments, scientists were of the conviction that such environments are devoid of any existing microorganisms. By studying such environments, it was recognized that many microorganisms called extremophile can live in and adapt to such conditions.

These bacteria employ certain mechanisms to survive in hypersaline conditions which make them the best settlers in salty environments.[1] Halophilic bacteria distributed in hypersaline environment tolerate several ranges of saline. Based on NaCl tolerance, these bacteria fall into three categories: slightly, moderately and extremely halophile with optimal growth rates at 2-5%, 5-20% and 20-30% NaCl, respectively.[2] Among halophils, moderatly halophilic bacteria are the most heterogeneous group containing many genera, such as *Salinivibrio* and *Halomonas* that the majority of their characteristics- such as ecological, physiological, genetics and biochemical- have been studied.[3,4]

An emerging field of research in recent years showed that the moderately halophilic bacteria have good and strong potential in valuable applications such as compatible solutes, enzymes[5] and polymer sources.[6] They can also be used in degradation of toxic compounds.[7,8]

Nowadays, multi antibiotic-resistant pathogens are an important health problem in medicine. Therefore finding novel sources of antimicrobial compounds is essential to introduce effective agents against resistant pathogen microorganisms.[9-11]

In this era, halophilic bacteria can be a novel source of antimicrobial compounds,[12] and up to now, various antimicrobial and antitumoral materials have been identified from slight and extremely halophilic Prokaryotes such as Halocins, a proteinaceous antimicrobial compound.[13] In 2012, a new source of bacterial biodiversity was isolated from soil of Daecheon Beach and Saemangeum Sea of Korea where the most of isolates were actinobacteria that were capable of producing antibacterial and antifungal metabolites.[2]

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Bacteria which were isolated from Ratnagiri coastal region have both antibacterial and antifungal effects. Overall, antimicrobial producing halophilic bacteria are plentiful in environments such as salt in lakes, saline soils. Besides that, the antimicrobial assays done within halophiles (protein crude extract) have represented that, the marine environments have special ability of producing new antimicrobial and antifungal agents.[14] In 2002, three new cyclopeptides discovered from a marine sediment-derived *Halobacillus litoralis* YS3106 and displayed moderate antifungal activities.[15]

Studies show that, some moderately halophilic bacteria also produce antimicrobial compounds[9], Sawale *et al.*,[16] introduced two *Bacillus pumilus* strains (NKCM 8905and AB211228) with antibacterial activity from **s**oil samples of coastal area of Arabic ocean. Studies also indicated that some *Bacillus* strains isolated from different hypersaline soils were able to inhibit some plant pathogenic fungi.[17,18] Up to now, controlling plant pathogens by hazardous chemical pesticides remains as a common concern. To date, researchers have introduced different biological strategies to overcome this concern and control destructive effects of plant pathogens. Extraction of antimicrobial compounds from halophilic bacteria is one of the effective strategies which have been mostly noticed recently.[19]

Till now, different environments in all over the world have been studied to identify effective halophilic bacteria. Iran, situated in Middle East of Asia, contains wide areas of salty lands which are suitable for isolation of halophiles. Previous studies have also proved the potency of isolated halophils in some regions.[4,17,18] Whereas there are still more unknown regions with the possibility of having new strains with novel properties.

In the present study, the antimicrobial potency of the moderately halophilic bacteria of Haj Aligholi salt desert and Dagh Biarjmand of Shahrood in Iran were detected and the biodiversity of the halophilic isolates were determined by 16S rRNA sequencing.

Materials and Methods

Sample collection and growth conditions

Soil samples were collected from Haj Aligholi salt desert and Dagh Biarjomand Shahrood. Upper 10 cm of soli was used and then samples were transported to the laboratory by polyethylene sterile bags. Enrichment culture and isolation were done after 18 to 24 h. Samples were cultured in the saline nutrient broth, containing(g/L), NaCl 81, $MgSO_4.7H_2O$ 9.7, $MgCl_2.H_2O$ 7.0, $CaCl_2$ 3.6, KCl 2.0, $NaHCO_3$ 0.06 and NaBr 0.026, where pH was adjusted to 7.3 before autoclaving. Cultures were incubated at 25°C in an orbital shaker, at 150 rpm for 72 hours. In order to culture on solid media 12-15 gL^{-1} agar was added in to new saline nutrient broth, then it was incubated at 25°C for 48 hours. In order to purify single colonies of halophilic bacteria, streak plate method was done.[4] Cell morphology and biochemical tests were carried out to identify isolates.[20] Enrichment cultures were subcultured several times under the same conditions with different NaCl concentration (0%, 5%, 10%, 15%, 20%, 25%).

Antimicrobial assay

Antimicrobial properties of isolates were assayed by agar well diffusion method. To achieve this, the pathogens from human and plants were selected. In this study, *Bacillus cereus* (ATCC 11778), *Escherichia coli* O157 (PTCC 1276), *Klebsiella pneumoniae* (PTCC 10031), *Shigella flexneri* (PTCC 1234), *Pseudomonas aeroginosa* (ATCC 10231), *Streptococcus mutans* (ATCC 35668) and *Candida albicans* (ATCC 10231) were studied as human pathogenic organisms and *Fusarum oxysporum* (IBRC-M 30067), *Aspergillus flavus* (IBRC-M 30029), *Neurospora crassa* (IBRC-M 30138), *Botrytis cinerea* (IBRC-M 30162), *Erwinia amylovora* (IBRC-M 10748), *Psudomonas syringea pv. Syringae* (IBRC-M 10702), *Xanthomonas campestris* (IBRC-M 10644) and, *Rhizobium radiobacter* (IBRC-M 10701) were used as plant pathogenics.

According to agar well diffusion method, described by Ennahar *et al.*, the bacterial culture supernatant were filtered through a 0.22 µm membrane filter after centrifugation at 5000 rpm for 10 min. The pathogenic microorganisms (10^7 CFU/mL) were inoculated into a sterile plate with 20 mL of their selective media. Subsequently, the plate was gently shaked for even spread and suitable mixing of the microorganisms and media. It was allowed to solidify, afterword 5 wells of about 6 mm in diameter were prepared on the surface of the agar plates using a sterile cylinder and the plates were then turned upside down and the wells were labeled with a marker. Each well was occupied with 0.1 mL of the bacterial extracts. Then, the plates were incubated at 37°C for 24 hrs and the inhibition zone was measured. Finally, the results were then organized.[21]

Molecular Identification

Genomic DNA extraction was done by DNA extraction Kit (Cinagene DNa plus, South Korea), according to manufacturer's protocol. Universal primers used forward 16F 5'- AGAGTTTGATCCTGGCTCAG- 3' and reverse 16R for Gram-negative bacteria; 5' – ACGGCTACCTTGTTACGACTT- 3' and was sequenced.[22] Each amplification reactions contained, 1µL of each primer, dNTPs (10 mM) 0.5µm, PCR buffer 2.5 µL, $MgCl_2$ (50 mM) 0.75 µL, template DNA 1 µL, smartaq DNA polymerase 0.2 µL, and dH_2O 19.05 µL, in a final volume of 25µL. The 16S rRNA gene amplification protocol was as: 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, 55°C for 1 min and 72°C for 1.5 min, with final 10 min extension at 72°C [23]. Gel PCR purification Kit (Vivantis, Malaysia) was used for PCR product purification. The purified PCR product was sequenced (Macrogen, South Korea). The phylogenic relationship of the isolates was determined by comparing the sequencing data with the related 16S rRNA gene sequences in the GenBank database of the National Center for Biotechnology Information (NCBI), via

BLAST search. Phylogenetic analysis was performed using of the CLC software.[4]

Antimicrobial assays

Firstly, each colony of bacteria was cultured in 50 mL of liquid nutrient medium and incubated at 30ºC with shaking for 72 hours. Then, to separate the extract from the medium, the culture, was centrifuged in 4000 rpm for 20 minutes. Subsequently, the supernatant was collected and filtered through a 0.22 µm membrane filter.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The MIC of the extract against bacteria was determined using micro broth dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The MIC value is considered as the lowest concentration that completely inhibited bacterial growth after 48 hrs of incubation at 30ºC. The various concentrations (10^{-1} g / ML to 10^{-8} g / ml) of the extract were used to detect the MIC value against tested pathogens. 16 µl suitable medium was added into the each well. Then, 20 µl of the 0.5 McFarland suspension of pathogen bacterial (1:10 diluted) were added into the wells. Finally, different concentration of the extract (20 µl) was added into the same wells and sterile broth was added into the wells for makeup to 300 µL. The positive control wells contain 0.5 McFarlend (OD: 600) of the pathogen and culture medium and the negative control wells contained an antimicrobial extract and culture media. After cultivation, the microplates were mixed well to make the mixture quite uniform and placed in an incubator for 24 hrs at 30°C. The MIC value was determined by the first well in which the visible growth of microorganisms was inhibited.

For the determination of MBC, a portion of liquid (5 µl) from each well that exhibited no growth were taken and then incubated at 30ºC for 24 hrs. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

To determine the MIC value for bacterial extract against fungal pathogens, macro broth dilution method was used. Briefly, serial dilutions of bacterial extract ranging from 10^{-1} g/mL to 10^{-8} g/mL were prepared in tube 1 to tube 8. Then, 50 mL suitable medium was added to 10 sterile tubes. Then, 1 mL extracts suspension (1000 mg/mL in medium) was added to tube 1. The contents were mixed and 5 mL was transferred to tube 2. This serial dilution was repeated through to tube 10. Nine hundred microliter of inoculum was transferred to tubes 1–10.

The positive control (tube 9) contains 0.5McFarlend of the pathogen and culture medium and the negative control (tube10) contained an antimicrobial extract and culture media. Then, all tubes were incubated at suitable temperature. After 48 hours, tubular opacity and growth of fungi were evaluated in comparison with control tube.

To obtain the MFC, 10 mL of each dilution was taken from each well and spread on Suitable medium. Plates were incubated at 28 ºC for 72 h. The MFC was defined as the lowest concentration that yielded three or fewer colonies.

Statistical analysis

Statistical analysis was done by using SPSS software version 16 for calculating the mean of zones of inhibition on tested microorganisms.

Results

Biochemical characterization of halophilic bacteria

In the present study, seven halophilic bacteria were isolated from Dagh Biargemand of Shahrod and Haj Aligholi salt deserts. Halophilic bacteria were identified based on the colony, pigment and microscopy shape. Seven strains were selected for further assays and their biochemical characteristics are summarized in Table 1 and 2. Halophilic bacteria were categorized on the basis to tolerance of different NaCl concentration into slightly, moderately and extremely halophilic bacteria. According to our study, Table 1 shows growth of isolated colonies at different salt concentration. In this study, the majority of the isolates were moderately halophile.

Table 1. Physicochemical characteristic of isolated halophilic bacteria.

Property	1NB	2NB	3NA	6(2)A	D6A	D8B	Dar
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Colony color	Cream	Cream	Yellow	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+	+	+
Range of NaCl (%) for growth (Optimum)	1-25%	1-25%	1-25%	1-25%	0-15%	1-25%	0-15%
Enzyme							
Oxidase	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Protease	-	-	+	-	-	-	+
Amylase	-	-	+	-	+	-	+
DNase	-	+	+	-	+	-	+
Gelatinase	-	-	+	-	-	-	-
Acid production from:							
Glucose	-	-	-	-	-	-	+
Mannitol	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-
Xylanase	-	+	-	-	-	-	-

Table 2. Sensitivity of the isolated halophiles against different antibiotics (mm).

Antibiotic	1NB	2NB	3NA	6(2)A	D6A	D8B	Dar
Azithromycin	35	30	40	26	40	45	35
Rifampin	40	35	30	35	25	0	40
Vancomycin	20	10	20	20	40	25	20
Amikacin	15	10	17	10	30	10	10
Ciprofloxacin	30	30	30	30	30	40	30
Tetracycline	25	28	30	25	10	30	30
Cefixime	40	35	22	30	0	11	11
Gentamicin	14	15	15	15	15	13	15
Cefalexin	29	30	25	34	26	20	25
Ampicillin	22	32	25	32	30	12	31

The strains including 1NB, 2NB, 3NB, 6(2)A and D8B were moderately halophilic bacteria and two of them were halotolerant. Catalase activity of all of the strains was positive. Catalase is an important enzyme which protects the cell from oxidative damage by reactive oxygen species (ROS). Therefore, catalase activity of these strains keeps them safe against environmental stresses. In this research, the antibiotic resistance of isolated bacteria towards different antibiotics was also determined for phenotypical characterization of isolates. As shown in Table 1, D6A strain was highly resistant to Cefixime, while Cefixime is a broad-spectrum antibiotic and is active against a wide variety of bacteria. All the strains tested demonstrated a low sensitivity against Gentamicin. Among isolates, the highest sensitivity was observed for D8B strain against Azithromycin. Figure 1 depicts the resistance of Dar and D8B strains against different antibiotics.

Identification of halophilic bacteria

The moderately halophiles isolates from Dagh Biarjmand and Haj Aligholi salt desert were identified to belong to *Bacillus*, *Virgibacillus and Halobacillus* genus on the basis of phylogenetic analysis . Six of the seven isolated strains belong to *Bacillus* and *Virgibacillus* genus. According to the phylogenic tree construction analysis, D8B is closely related to *Virgibacillus olivae*. Besides 1NB and 2NB showed close relationship with *Virgibacillus halodenitrificans*. 6(2) A and 3NB strains respectively belonged to the genus *Halobacillus* and *Bacillus Hemicentroni*. Furthermore, D6A and Dar belong to *Bacillus subtilis* subsp. Phylogenetic tree is shown in Figure 2, Table 3.

Table 3. Identification of Seven bacterial strains associated based on 16SrRNA gene sequence identity obtained by BLAST analysis.

Isolate	Bacteria	Similarity	NCBI ID
D6A	*Bacillus subtillis*	99%	MH885315
DAR	*Bacillus subtillis*	99%	MH885314
6(2)A	*Halobacillus trueperi*	99%	MH879814
1NB	*Virgibacillus halodentrificans*	99%	MH879813
2NB	*Virgibacillus halodentrificans*	99%	MH879811
3NB	*Bacillus hwajinpoensis*	99%	MH879816
D8B	*Virgibacillus olivae*	99%	MH879812

Antimicrobial activity of Halophilic bacteria

To probe the possible antimicrobial activity of halophilic bacteria, agar well diffusion method was conducted as mentioned previously. *B. cereus, E. coli* O157, *K. pneumoniae, S. flexneri, S. mutans and C. albicans* were selected as human pathogens to cover the different spectrum of antimicrobial use.

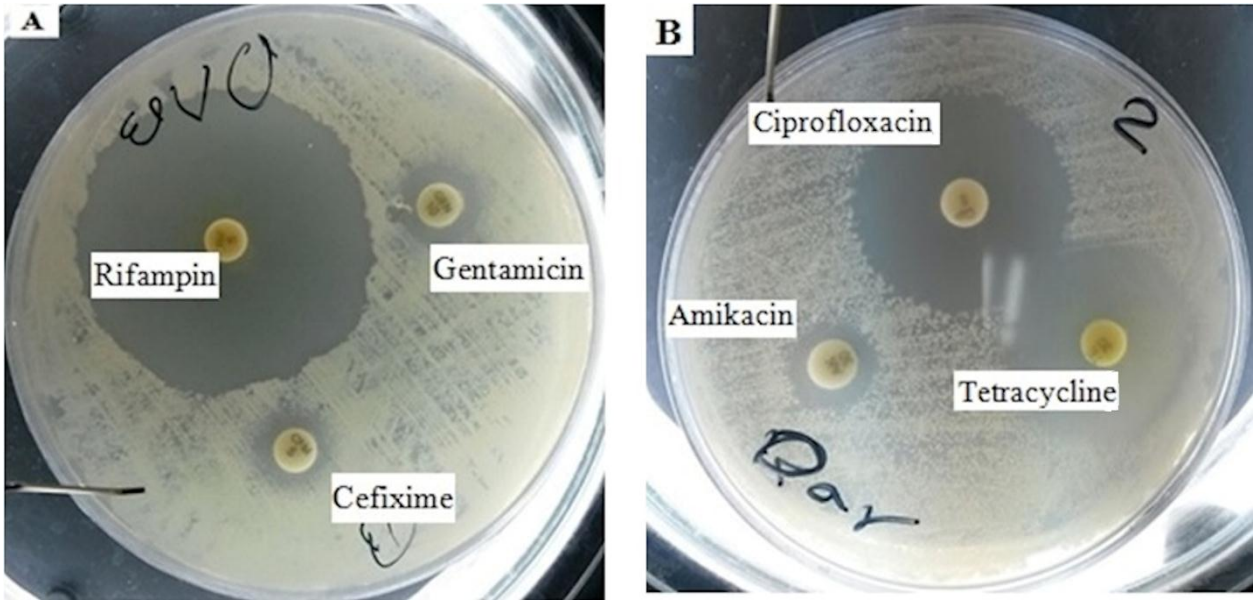

Figure 1. Sensitivity of the isolated halophiles against different antibiotics. A) D8B strain sensitive against Rifampicin whereas resistant against Gentamicin and Cefixime, B) Dar strain sensitive against Tetracycline and Ciprofloxacin whereas resistant against Amikacin.

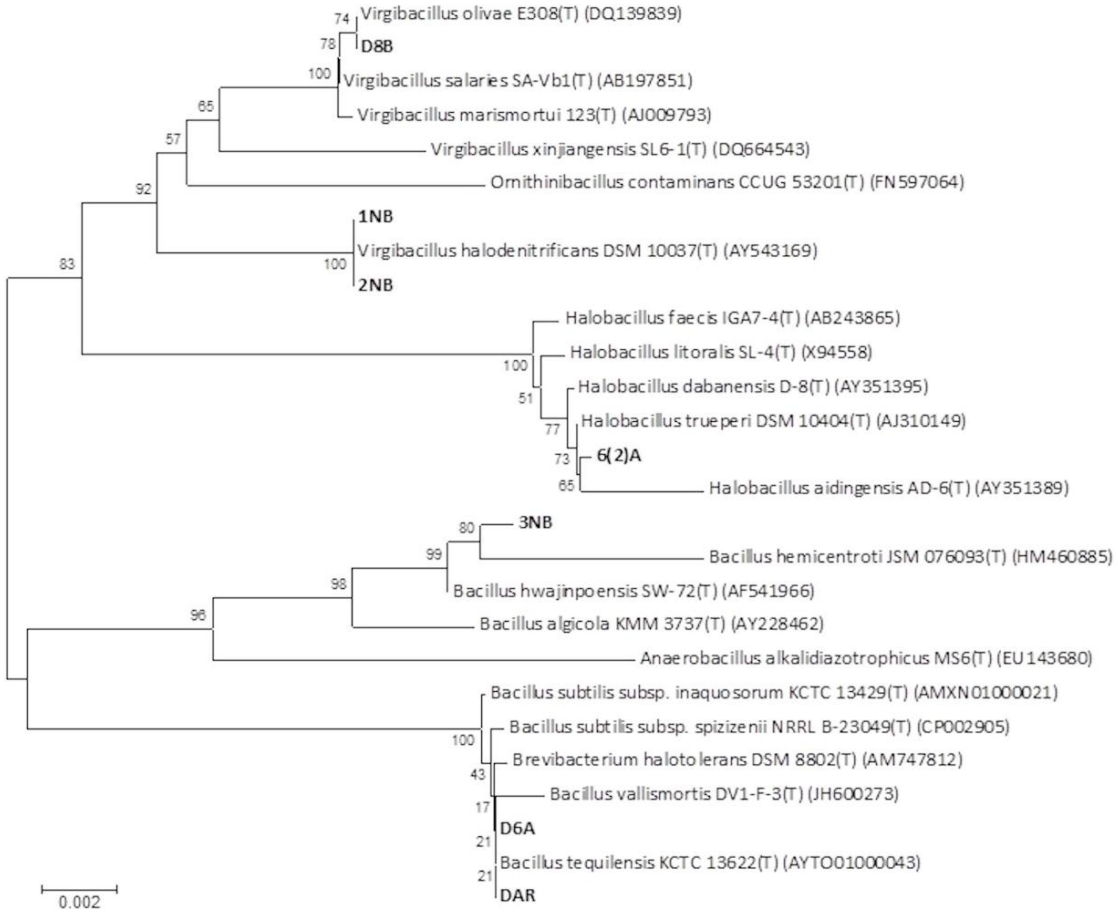

Figure 2. Neighbor-joining phylogenetic tree derived from 16S rRNA gene sequence data showing the positions of seven isolated bacteria including 1NB, 2NB, 3NB, 6(2)A, D6A, DAR and D8B among related bacteria. Numbers at branch points are bootstrap percentages based on 1000 replicates. Bar 0.002 changes per nucleotide position.

Additionally, antibacterial and antifungal activities of isolated strains were screened against plant pathogens including *F. oxysporum*, *A. flavus*, *N. crassa*, *B. cinerea*, *P. syringe pv syringe*, *X. campestris*, *E. amylovora* and *R. radiobacter*.

In this study, among seven isolated halophilic bacteria, only three isolates (D6A, D8B and Dar) were able to produce inhibition zones against the pathogenic microorganisms. *Bacillus* D6A strain depicted antimicrobial effect against pathogenic *F. oxysporum*, *A. flavus*, *N. crassa*, *B. cinerea and C. albicans*. This isolate showed the highest inhibition zone (39±0.1 mm) against *C. albicans. Bacillus* Dar strain was able to produce inhibition zones against *F. oxysporum*, *A. flavus*, *B. cinerea*, *N. crassa* and *C. albicans.* Moreover, D8B had good antibacterial effect against *P. syringea pv. Syringae* (Table 4). Figure 3 reveals the antimicrobial effect of D6A against *C. albicans and B. cinerea.*

The MIC and MBC values (Table 5) indicate that D8B, Dar and D6A display antimicrobial activity against four bacterial, four fungi and one yeast (ATCC) strains.

Table 4. Inhibition zones (mm) against different pathogenic microorganisms.

Pathogenic microorganism	1NB	2NB	3NA	6(2)A	D6A	D8B	Dar
Bacillus cereus	0	0	0	0	0	0	0
Escherichia coli O157	0	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	0	0	0	0
Shigella flexneri	0	0	0	0	0	0	0
Streptococcus mutans	0	0	0	0	0	0	0
Fusarium oxysporum	0	0	0	0	8±0.4	0	13±0.2
Aspergillus flavus	0	0	0	0	11±0.1	0	13±0.1
Neurospora crassa	0	0	0	0	13±0.2	0	16±0.1
Botrytis cinerea	0	0	0	0	14±0.2	0	13±0.2
Candida albicans	0	0	0	0	39±0.1	0	0
Pseudomonas syringae pv. Syringae	0	0	0	0	0	29±0.1	0
Xanthomonas campestris	0	0	0	0	0	0	0
Erwinia amylovora	0	0	0	0	0	0	0
Rhizobium radiobacter	0	0	0	0	0	0	0

The values shown represent the average of three determinations ± standard deviations.

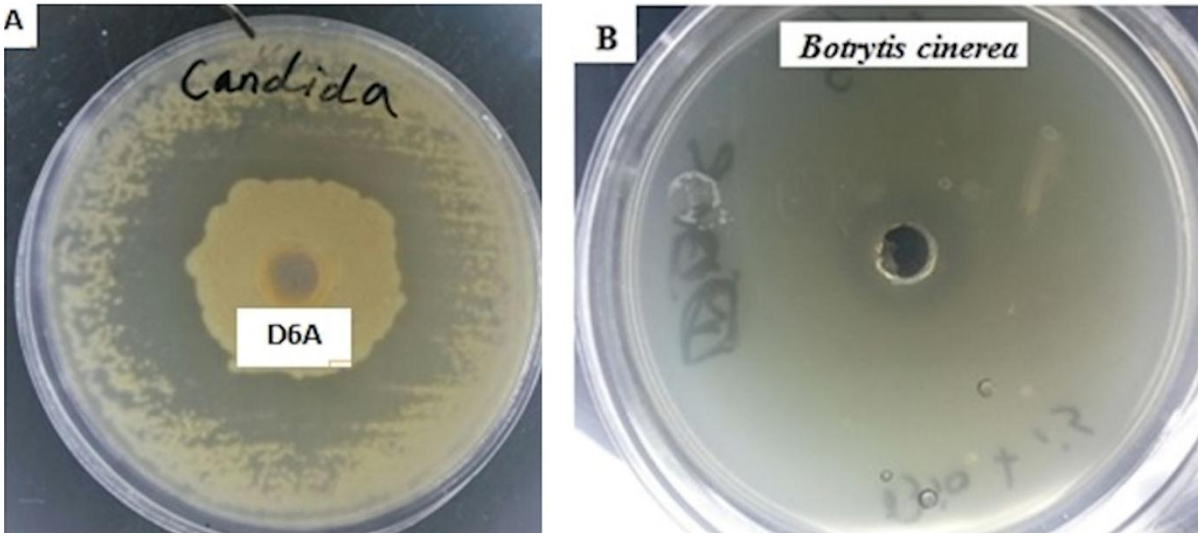

Figure 3.Antimicrobial effect of D6A bacterial strain against **A**) *Candida albicans*. ***B****) Botrytis cinerea.*

Table 5. MIC, MBC and MFC bacterial extract against Human and Plants microbial of the most effective.

Pathogen	MIC[a]	MBC[b]	MFC[c]
Fusarium oxysporum	12.5 µg/ml	-	12.5 µg/ml
Aspergillus flavus	25 µg/ml	-	25 µg/ml
Neurospora crassa	25 µg/ml	-	25 µg/ml
Botrytis cinerea	25 µg/ml	-	25 µg/ml
Candida albicans	25 µg/ml	-	25 µg/ml
Pseudomonas syringae	25 µg/ml	25 µg/ml	-

[a] Minimum inhibitory concentration
[b] Minimum bactericidal concentration
[c] Minimum fungicidal concentration

The activity of *B. subtilis* against Plant and human tested fungi was considerable, with the highest values for *F. oxysporum* (MIC=12.5 µg/ml), *A. flavus* (MIC=25 µg/ml)., *N. crassa* (MIC=25 µg/ml)., *B. cinerea* (MIC=25 µg/ml), and *C. albicans* (MIC=25 µg/ml). *V. olivae* was also active against plant bacteria *P.syringe* (MIC=25 µg/ml). The extract was inactive against human bacteria.

Discussion

The discovery of novel antibiotic is essential subject because probability of multiple resistances on pathogenic microorganisms is very high.[24] Halophilic bacteria have good potential to be a new production source of bioactive compounds such as antimicrobial and antitumoral agents.[17]

Halophilic microorganisms are a wide range of creatures that include several members such as, moderately halophilic bacteria and so forth.[14] One of the most important methods for strain identification and distribution is 16S rRNA sequencing method.[25]

In the present study, the results of 16S rRNA sequencing determined that the isolates belonged to *Virgibacillus* and *Halobacillus.* Previously, Hashemi *et al.*[26] isolated *Bacterium* and *Bacillus* from Maharlu salt Lake in Iran. Also some moderately halophilic bacteria that include *Halomonas, Salinicoccus, Planococcus, Bacillus and Halobacillus* were isolated from Weihai Solar Saltern in China.[17] In another study, Irshad *et al.*[27] were able to isolate *Bacillus, Streptomyces, Microbacterium, Micrococcus, Planococcus and Marinobacter from* foreshore soils in Korea. These researches well confirmed that Bacillus is dominant in all of the studies [16,17,26,27] and in addition, in this research, *Virgibacillus* was isolated as a new which had not been discussed in any other previous studies. There are few articles published on antibiotic production by halophilic *Virgibacillus*[26] and this is the first report on the antibiotic production ability of halophilic *Virgibacillus olivae* species. Antibiotic sensitivity data for D8B strain have some similarities with previously isolated of *Virgibacillus olivae* species.[28] However, in this research D8B strain did not show any sensitivity to Rifampin which is in contrast to *Virgibacillus olivae*. Besides, among the isolates, only the D8B strain showed inhibition zone against *P. syringe* with, maximum zone of inhibition of 29 mm. *P.* syringae is a Gram negative, plant-pathogenic bacterium noticed for its diverse and host-specific interactions with different plant species.[29] As it was previously mentioned, biological control of fruits' and vegetables' diseases via microorganisms suggests an alternative strategy that is significantly potential. Therefore, focusing on different environments to discover novel and potent antimicrobial agents against these pathogenic organisms is essential.

Here, the presented data indicated that D6A and Dar strains belonging to *Bacillus species* had antifungal effect against other plant pathogens including *F. oxysporum, A. flavus, N. crassam* and *B. cinerea.*

Sadfi-Zouaoui *et al.*,[18] previously showed the ability of moderately halophilic bacteria in controlling plant diseases such as Grey Mould tomato fruits caused by *B. cinerea.* This group confirmed that halotolorants mostly affect pathogens by their hydrolytic enzymes such as proteases, chitinases, amylases, laminarinases, lipases and cellulases. In another study, it was indicated that the antibacterial and antifungal properties of halophiles *may* occur as a result of their multiple plasmids.[14]

On the other hand, D6A strain showed a zone of inhibition (39 mm) against *C. albicans*, human pathogenic fungi. In this regard, the antifungal activity of a

moderately halophilic strain of actinomycete HB-11 was detected against different fungal organisms. This strain has been isolated from soil samples of Mumbai sea port, India and characterized as *Streptomyces werraensis*. The maximum zone of inhibition (30 mm) was against *A. parasiticus* NCIM 904 where its inhibition zone for *C. albicans* was 15 mm.[30] These findings confirm that studying new environments in all over the researchers discover potential novel antimicrobial which can specifically affect some pathogens.

Conclusion

All of the studies indicate that different halophilic bacteria in various geographical regions are highly diverse and have good potential to produce novel antimicrobial compounds. In contrary, further studies needed to determine the structure of the antimicrobial compounds by spectroscopic methods for identification of their mode of action. This research was conducted to isolate potent antibiotic producing halophiles from Dagh Biargemand and Haj Aligholi salt deserts in Semnan Province of Iran. The antibacterial and antifungal assays of halophiles showed that, the salty soil of these areas represent a potential source of new antimicrobial and antifungal agents.

Conflict of interests

The authors claim that there is no conflict of interest.

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