

Microbial Water Source for the Desert Plants

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Abstract: Finding water resources for the desert plants is one of the important research areas since it enables saving water resources. *Bromus inermis* plant was noticed to keep 5% of moisture in its rhizosphere zone; therefore, this study aimed to identify this source of water. Thirteen endophytic bacteria were isolated from the root of *B. inermis* and identified. Their specific respiration rate was determined. *Alcaligenes faecalis* showed the highest specific respiration rate. It is a facultative chemoautotrophic hydrogen-fixing bacterium that utilizes the hydrogen gas as energy source and the water is produced as an end product. The source of hydrogen gas for this bacterium is not only from air and soil gases but also from the hydrogen-producing bacteria such as *Enterobacter* spp., which was among the isolated bacteria. The hydrogenases synthesizing genes (*HoxC*, *HypA* and *HypB*) were detected in most of the isolated bacteria and roots of four wild plants, out from 18 wild plant samples, especially the grains of the wild wheat plant. This result suggests that the hydrogen-fixing and hydrogen-producing bacteria transfer from the root through the plant to inhabit the grain/seeds. This can help the grain/seeds to germinate in drought environment.

Key words: Desert plants, *Alcaligenes* spp., *Enterobacter* spp., hydrogenase, *HoxC*, *HypA*, *HypB*.

1. Introduction

Drought is one of the most coming world stresses. With the increase in world population, the water resources become insufficient. The search for renewable sources of water, especially for agriculture, is an important aim of many recent researches. Therefore, several recent researches [1-14] have been conducted to identify the water sources for the desert plants, to identify the drought tolerance mechanism or to genetically modify the plant to be drought tolerant.

The known water sources for the desert plants include fog [1-4], clouds [5-10], small rains and dew [11-14]. There is no previous research article discussing the microbial source of water for the desert plants. Transgenic approach is one of the recent techniques that have been studied in many plants to increase the drought tolerance [15-17].

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In the present study, *Bromus inermis* plant grown in El-Esmayelia desert, Egypt, was noticed to keep 5% of water in the rhizosphere area at 20 cm soil depth, where there is no ground water at such depth, in addition to the absence of rains in this area and the soil was of a sandy soil, where the water can be easily leached. This observation was noticed in different growth locations of different *B. inermis* plants along 7 km in the desert of EL-Esmayelia desert road, Egypt. This indicates that this source of water must be produced by the microflora as a result of symbiosis between the plant and some microorganisms.

The major source of water produced by microorganisms is through their aerobic respiration and by the chemoautotrophic hydrogen-fixing bacteria. These bacteria fix the hydrogen gas by hydrogenases. Hydrogenases are found in many microorganisms including bacteria, archaea and some eukarya [18, 19]. It can be classified according to its metal co-factor into three major classes: [NiFe], [FeFe] and [Fe]

hydrogenases [20, 21]. It can be also classified based on the redox coenzyme into NAD(P)⁺, cytochrome (b) or cytochrome (c) hydrogenases. Another classification is based on the location in the cell, where it can be found soluble in cytoplasm (soluble hydrogenase (SH)) or bind with the cell membrane (membrane bound hydrogenase (MBH)) [18, 19].

The Ni-Fe-hydrogenase and Ni-Fe-Se hydrogenases are mostly found in the hydrogen-fixing bacteria, while the Fe-Fe hydrogenase is found in the hydrogen-producing microorganisms. This last class combines electrons with protons to form hydrogen (H₂ evolution) [20]. Other proteins called “regulatory hydrogenases” (RH) that sense presence of hydrogen and regulate the expression of genes required for the biosynthesis of hydrogenases are found in the hydrogen fixers. These proteins composed of two protein subunits: the large subunit (*HoxC*) and small subunit (*HoxB*) [20, 21].

So, the present study strongly suggested the presence of a symbiosis relationship between some chemoautotrophic bacteria and the desert plant *B. inermis*. Through this symbiosis relationship, the bacteria fix hydrogen and produce water molecules as the end product of hydrogen oxidation; these water molecules ensure the life of the desert plants, which produces useful organic compounds that may be required for the microbial growth.

The desert plants tend to produce large number of light, small and hairy seeds that can be spread out in the desert by air. However, a lot of these seeds are falling down on their parent plants, where they find the water and start germination and continue their life cycle. In addition, the grown bacteria produce other organic compounds, such as growth promoters. The microorganisms also may produce organic acids that increase the availability of some elements from the surround environment [22, 23]. So, the desert plants are growing in groups, and it can grow in the rocky or poor sandy environment, where there is no organic matter or water.

On the other hand, these bacteria can be transferred from the parent plant into its seeds to enable germination of these seeds, regardless of the environmental conditions where it will be falling down. The present study aimed to identify the microbial source of water for the wild desert plant *B. inermis*.

2. Materials and Methods

2.1 Plant Materials

Eighteen wild plant samples were collected from three different desert locations at Egypt, for four years, and identified at Weeds Research Institute, Agricultural Research Center, Giza, Egypt. Three seed types from three different plants (seeds of *B. inermis* and *Convolvulus arvensis* and grains of wild wheat) were included in this study (Table 1).

2.2 Soil Analysis

The chemical and physical analysis of soil collected from the rhizosphere of *B. inermis* from El-Esmaylya desert road was determined at the Central Lab of Soil, Water and Environment Research Institute, Agricultural Research Center, Egypt.

2.3 Isolation of Endophytic Bacteria

The endophytic bacteria were isolated from the roots of *B. inermis* by the method described by Gagne *et al.* [24].

2.4 Bacteria Identification

Universal 16S rRNA bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and U1492R (5'-GGT TAC CTT GTT ACG ACT T-3') were used [25].

2.5 Determination of Bacterial Specific Respiration Rate

Triphenyl tetrazolium chloride (TTC) reduction method was used. Standard curve was prepared by different concentrations of TTC formazan (Sigma).

The extinction coefficient (ϵ_{485}) was 4,890.7 $\mu\text{M}/\text{cm}$. The bacterial total count was determined in the taken volume from each bacterial suspension by using the pouring plate technique. The specific respiration rate was determined as $\mu\text{m TTC formazan}/\text{CFU}/\text{h}$. All samples were analyzed in triplets.

2.6 Calculation of the Microbial Water Production

The amount of water produced by each microbial strain was calculated from the number of moles of the reduced TTC. One mole of TTC is reduced by one mole of hydrogen gas. The number of moles of hydrogen gas equals to the number of moles of water in the respiration process. So, the mole number of reduced TTC equals to the mole number of water. The amount of water was calculated as $\text{mg}/10^6 \text{ CFU}/\text{h}$ from the specific respiration rate determination data.

2.7 Hydrogenase Detection in the Bacterial Isolates, Plants and Seeds

A primer pair for the subunit *HoxC* and two primer pairs for *HypA* and *HypB* genes were designed for detection of these genes in the isolated bacteria, the rhizosphere of the plants and seeds collected from different desert locations in Egypt (Table 1). The sequences of these primer pairs are as follows:

HoxC: F (GTG AAT GCG CCA ATG TTT CG) and R (GGT TCT CTG TTG CGA GCA TC);

HypA: F (PRIMGCA CTG GAA CTC ATC GAA CAG) and R (GTG ACG TAC TGC TGA CAC TG);

HypB: F (CCT GTA TAT CGA AGG GGA TGA AC) and R (TGA TTT CCA CGT TCA GCA TCT G).

2.8 DNA Extraction from the Bacterial Cells, Plant Roots and Seeds

CTAB method was used to extract DNA from the bacterial isolates and plant samples [25].

2.9 Polymerase Chain Reaction (PCR) Conditions

PCR reaction was conducted to confirm the presence of the genes of interest in the genomic DNA.

The reaction conditions were optimized as following: dNTPs (2.5 mM) 4.0 μL , MgCl_2 (25 mM) 3.0 μL , $10\times$ buffer 5.0 μL , forward primer (10 pmol/ μL) 5.0 μL , reverse primer (10 pmol/ μL) 5.0 μL , template DNA (50 ng/ μL) 1.0 μL , Taq (5 U/ μL) 0.3 μL , add H_2O up to 50 μL . Amplification was carried out in a Hybaid PCR Express programmed for 37 cycles as follows: 94 $^\circ\text{C}/5 \text{ min}$ (1 cycle); 94 $^\circ\text{C}/20 \text{ s}$, 55 $^\circ\text{C}/20 \text{ s}$, 72 $^\circ\text{C}/40 \text{ s}$ (10 cycles); 94 $^\circ\text{C}/20 \text{ s}$, 57 $^\circ\text{C}/20 \text{ s}$, 72 $^\circ\text{C}/40 \text{ s}$ (10 cycles); 94 $^\circ\text{C}/20 \text{ s}$, 56 $^\circ\text{C}/20 \text{ s}$, 72 $^\circ\text{C}/40 \text{ s}$ (15 cycles); 72 $^\circ\text{C}/5 \text{ min}$ (1 cycle); 4 $^\circ\text{C}$ (infinite).

3. Results and Discussion

The soil sample collected from the rhizosphere zone of *B. inermis* wild plant was found to contain 5% of moisture. The physical and chemical analyses of this soil sample were illustrated in Table 2. The isolated and identified endophytic bacteria from the roots of *B. inermis* were listed in Table 3.

3.1 The Water Source in the Rhizosphere of *B. inermis* Wild Plant

The present investigation suggested that the water source in the rhizosphere zone of the desert plants is a microbial product. In absence of organic matter in the desert soil and rocks, only the chemoautotrophic bacteria are able to grow, especially the CO_2 and hydrogen fixers. This last process is performed via hydrogenase enzymes. The hydrogen-fixing bacteria oxidize hydrogen through two types of hydrogenases: MBH and soluble NAD-dependent hydrogenase (SH) [28]. The SH reduces the NAD^+ into $\text{NADH}+\text{H}^+$, while the MBH transfers the electrons from H_2 to co-enzyme Q [26-31]. Both $\text{NADH}+\text{H}^+$ and the reduced co-enzyme Q continue the electron transport chain (ETC) to the last electron acceptor (oxygen atom) to produce water as an end product. Through the ETC, the ATP is generated, since the hydrogen is fixed by these bacteria as an energy source [32].

Table 1 Location, collection date and names of the collected plant samples.

Ser.	Plant name	Collection date	Location*
1	<i>Bromus inermis</i>	3/2014	A
2	<i>Salicornia fruticosa</i>	8/2017	A
3	<i>Farsetia burtonae</i>	8/2017	A
4	<i>Zhygophyllum decumbens</i>	8/2017	A
5	<i>Z. coccineum</i>	8/2017	A
6	<i>F. burtonae</i>	8/2017	A
7	<i>B. inermis</i>	8/2017	A
8	<i>Z. simplex</i>	8/2017	A
9	<i>Calotropis procera</i>	8/2017	A
10	<i>Z. decumbens</i>	8/2017	B
11	<i>S. fruticosa</i>	8/2017	B
12	<i>B. inermis</i>	8/2017	C
13	Unidentified	8/2017	C
14	Wheat plant	3/2018	A
15	Wheat grains	3/2018	A
16	<i>Convolvulus arvensis</i>	3/2018	A
17	<i>B. inermis</i> seeds	3/2014	A
18	<i>C. arvensis</i> seeds	8/2017	A

*Location (A) is the Esmayelia desert road, (B) Abuo-Zahabl desert area and (C) the desert of October city.

Table 2 Chemical and physical analyses of the soil sample from the rhizosphere zone of *B. inermis*.

Chemical analysis		Physical analysis	
EC*	2.99 dS/m	Coarse sand	9.56%
Ca	20.4 me/L	Fine sand	86.6%
Mg	4.4 me/L	Silt	2.7%
Na	3.9 me/L	Clay	1.09%
K	0.69 me/L		
HCO ₃	4.6 me/L		
Cl	10.4 me/L		
SO ₄	13.89 me/L		
pH	7.98 me/L		
SP	36.7%		

*EC: electric conductivity; SP: saturation percentage.

Table 3 The endophytic bacteria isolated from the roots of *B. inermis* wild plant.

Isolate No.	Bacteria name	Identification quality %	Gene length (base pairs)
1	<i>Enterobacter tabaci</i>	97	1,212/1,244
2	<i>Bacillus cereus</i> ATCC 14579	99	942/956
3	<i>B. amyloliquefaciens</i> strain BCRC 11601	96	1,316/1,373
4	<i>Myroides gitamensis</i> strain BSH-3	92	623/674
5	<i>B. safensis</i> strain NBRC 100820	98	592/606
6	<i>E. mori</i> strain R18-2	91	221/242
7	<i>Pantoea agglomerans</i> strain JCM1236	96	1,122/1,174
8	<i>E. tabaci</i> strain YIM Hb-3	97	1,175/1,207
9	<i>Acinetobacter johnsonii</i> strain ATCC 17909	97	1,043/1,071
10	<i>E. cloacae</i> strain DSM 30054	98	1,178/1,208
11	<i>Alcaligenes faecalis</i> strain RS-19	98	1,082/1,107
12	<i>B. pumilus</i> strain HN-10	99	942/952
13	<i>Cronobacter sakazakii</i> strain CCFM8307	88	202/256

Alcalegenes sp. was among the isolated and identified bacteria from rhizosphere of *B. inermis*. This bacterium is a facultative chemoautotrophic bacterium that produces NAD-dependent hydrogenase [32] in addition to the soluble hydrogenase enzyme [33]. Most [Fe-Fe] and [Ni-Fe] hydrogenases react with O₂ in addition to H₂. The reaction of [Fe-Fe] hydrogenases with O₂ is irreversible, resulting in enzyme damage; while [Ni-Fe] hydrogenases react with O₂ to yield a product, which is a complex of the oxygen atom and Fe-Ni co-factor. This product can be reactivated upon reduction of the oxygen atom to the end product, which is water [34, 35]. Therefore, the bacteria adapt some mechanisms to save the hydrogenase from oxygen. This includes either increase in the respiration rate or the rate of direct reaction between the protons generated from the hydrogen oxidation by hydrogenase and the oxygen molecules to form water as an end product [35]. For this reason, the hydrogen-fixing bacteria are characterized by high specific respiration rate. Therefore, the specific respiration rate was determined for all of the isolated bacteria in the present study.

3.2 Specific Respiration Rate Determination

Since the major source of microbial water is

through the respiration process, the specific respiration rate of all isolated 12 bacteria was determined (Table 4). The bacterial strain *Alcaligenes faecalis* showed the highest respiration rate after 1 h and 3 h (Fig. 1). The strain is a facultative chemoautotrophic bacterium. This bacterium requires to increase its respiration rate to avoid inactivation of its Ni-Fe hydrogenase content, which is the basic enzyme for energy production through hydrogen fixation.

3.3 Microbial Water Production by Each Microbial Strain

The calculated amount of water showed that the highest amount of water (5.5 mg water/10⁶ CFU/h) was produced by *Alcalegenes* sp. This was followed by *Enterobacter cloacae* (1.9 mg water/10⁶ CFU/h) (Fig. 2).

3.4 The Source of Hydrogen Gas for the Hydrogen Fixers in the Soil

Since the hydrogen concentration in air is only about 0.55 ppm [36], this concentration is not enough to produce sufficient amount of water for plant growth. To produce 1 mole of water (18.01 g), it needs 1 mole of hydrogen gas, which is 22.4 L under that standard condition. Atmosphere air contains about 0.55 ppm of

Table 4 The specific respiration rate (μM formazan formed/CFU) of the 12 endophytic bacteria isolated from the roots of *B. inermis* after 1 h and 3 h.

Bacterial code	Bacteria	$\mu\text{M}/\text{CFU}/\text{h}$	$\mu\text{M}/\text{CFU}/3\text{ h}$
B1	<i>E. tabaci</i>	7.76239E-05	7.87273E-05
B2	<i>B. cereus</i> ATCC 14579	3.37548E-09	2.90547E-09
B3	<i>B. amyloliquefaciens</i> strain BCRC 11601	3.53442E-11	1.20964E-10
B4	<i>M. gitamensis</i> strain BSH-3	5.15367E-07	3.54629E-07
B5	<i>B. safensis</i> strain NBRC 100820	1.93581E-09	1.11796E-10
B6	<i>E. mori</i> strain R18-2	5.71027E-05	3.41178E-05
B7	<i>P. agglomerans</i> strain JCM1236 <i>E. tabaci</i> strain YIM Hb-3	3.41537E-06	2.05866E-06
B8	<i>E. cloacae</i> strain DSM 30054	2.99063E-05	1.73066E-05
B9	<i>E. tabaci</i> strain YIM Hb-3	1.81356E-05	1.73652E-05
B10	<i>A. johnsonii</i> strain ATCC 17909	0.000109192	5.71862E-05
B11	<i>A. faecalis</i> strain RS-19	0.00030483	0.000154787
B12	<i>B. pumilus</i> strain HN-10	0.000109192	5.71862E-05
B13	<i>C. sakazakii</i> strain CCFM8307	1.72127E-06	1.43551E-06

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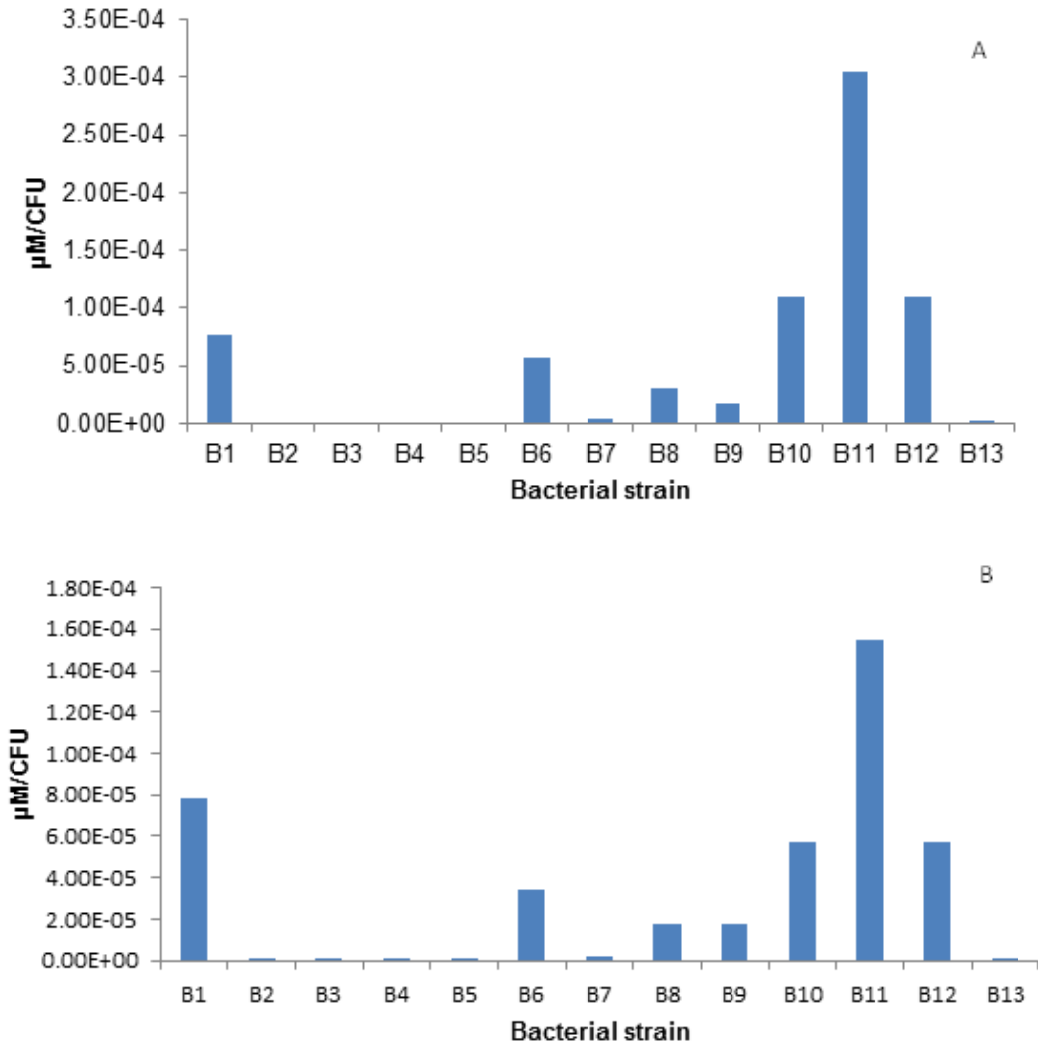


Fig. 1 The specific respiration rate (µM of formazan/CFU) for the 13 endophytic bacteria isolated from the roots of *Bromus inermis* after 1 h (A) and 3 h (B).

The strains names were illustrated in Table 3.

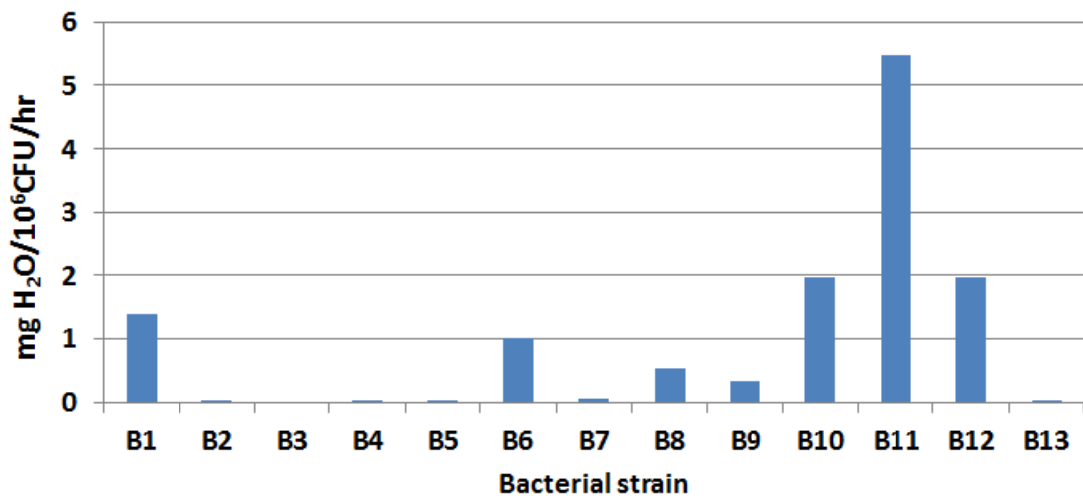


Fig. 2 The amount of water produced by 10⁶ CFU of each microbial strain per hour.

hydrogen gas (0.000055%). This means that to produce 18.01 g water, bacteria need to use all the hydrogen gas in 40,727,273 L of air. *E. tabaci*, *E. cloacae* and *E. mori*, which were among the isolated bacteria, are hydrogen-producing bacteria [37]. The hydrogen-producing bacteria tend to reduce the protons facilitated by hydrogenase and electron carriers into hydrogen to dispose the excess of reducing equivalents generated during the fermentation process [38]. Hydrogen production needs anaerobic conditions, so that the oxygen is removed by *Alcaligenes* sp. as well as the plant roots respiration optimizes the conditions for hydrogen production by *Enterobacter* spp. The soil gases can be also considered as a major source of hydrogen. Randolph *et al.* [39] reported that the hydrogen gas concentration found in several fault soil samples ranged from 80 ppm to 700,000 ppm. Hydrogen gas can be released by chemical and mechanical changes in crustal rocks [39]. This result supports utilization of the soil hydrogen gas as a source of energy and water by *Alcaligenes* sp.

3.5 Detection of Hydrogenases in the Isolated Bacteria, Plant Roots and Seed Samples

The hydrogen sensing in *A. eutropha* is achieved by a hydrogenase-like protein called RH, which controls the hydrogenase gene expression. This protein is composed of two subunits *HoxC* (large subunit) and *HoxB* (small subunit) [40]. On the other hand, the maturation of Ni-Fe hydrogenase (assembly of the Ni-Fe active site) is a complex process that requires several accessory proteins encoded by the genes *HypA*,

HypB, *HypC*, *HypD*, *HypE*, *HypF* and others [41, 42]. The *HypA* and *HypB* genes were previously identified in the hydrogen fixer *A. eutropha* [43, 44] and hydrogen-producing bacteria such as *Enterobacter* spp. and *Escherichia coli*, since all of these bacteria contain Ni-Fe hydrogenase type [45, 46]. Therefore, a primer for each of the following subunits (*HoxC*, *HypA* and *HypB* genes) was designed in the present study for detection of these genes in the isolated bacteria, the plant roots and seed samples.

The *HoxC* gene was successfully detected in *A. faecalis* (lane 6, Fig. 3a) at about 200 bp. The *HypA* gene was detected in *A. faecalis*, *Pantoea agglomerans*, *E. cloacae* and *Bacillus pumilus* (lanes 6, 9, 14, 16, Fig. 3b). This result is in agreement with previously published data, which showed that the *HypA* gene is required for the maturation of Ni-Fe hydrogenase produced by *Alcaligenes* sp., *Enterobacter* spp. and *P. agglomerans*. *P. agglomerans* is one of the Enterobacteriaceae and hydrogen-producing bacterium. *B. pumilus* is found to produce a hydrogenase E.C. 1.12.7.2 (Brenda Database: <https://www.brenda-enzymes.org/enzyme.php?ecno=1.12.7.2>). The *HypB* was detected in the *E. cloacae* strains (lanes 13 and 14, Fig. 3c).

Detection of these genes in the rhizosphere of the collected plants and seeds/grains showed that *HoxC* gene was detected in the root of *Salicornia fruticosa*, *Zhygophyllum decumbens* and wheat grains collected from EL-Kadesya desert region (lanes 2, 4, 15, Fig. 4a).

The *HypA* gene showed a strong band in the rhizosphere of *C. arvensis* collected from EL-Kadesya desert region (lane 16, Fig. 4b). *HypB* gene was detected

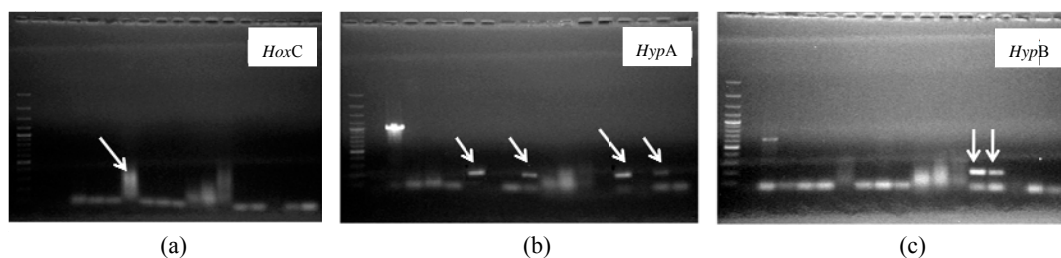


Fig. 3 Detection of *HoxC*, *HypA* and *HypB* genes (a, b, c, respectively) in the isolated bacteria.

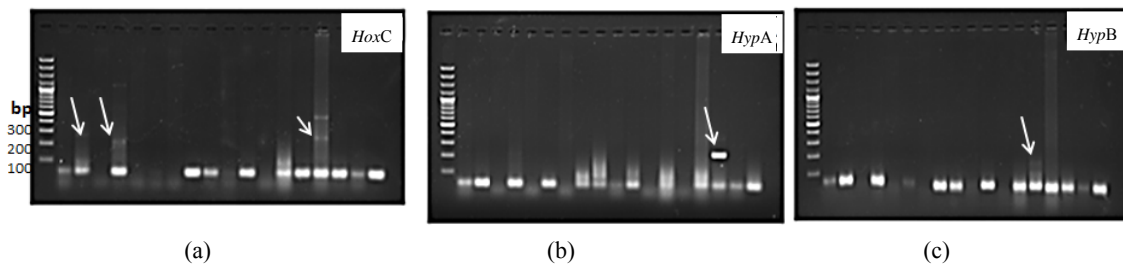


Fig. 4 Detection of *HoxC*, *HypA* and *HypB* genes in the rhizosphere of wild plants and seeds/wheat grins.

The plant samples in lanes from 1 to 18 are listed in Table 1.

in the rhizosphere of wild wheat plants (Fig. 4c). Presence of these genes in the rhizosphere and grains of wild plants strongly suggest that the hydrogen-fixing and hydrogen-producing bacteria transfer from the root through the plant to inhabit the grain/seeds, this can help the grain/seeds to germinate in any environment, in which, it will be fallen down.

Further investigations are required to study the microbiome of the desert plants and the relationship between them and the plant type. Also, a radioactive isotope study by inoculating the soil with both hydrogen-fixing and hydrogen-producing bacteria and cultivation of wheat plant seedlings in presence of tritium environment followed by detection of heavy water in the plant will prove the findings in this present study.

4. Conclusions

The world is facing many challenges, including the limited water supply, especially for planting. Most of researchers are studying new plant varieties, which have drought-tolerance or low water requirements. This present research opens new prospects toward solving the problem of water poverty. *Alcaligenes* sp., a facultative chemoautotrophic hydrogen-fixing bacterium, was isolated from and detected in the rhizosphere of different desert plants and some of their seeds. This bacterium utilizes the hydrogen gas as energy source and produces the water as an end product. This water enhances the seeds germination and plant growth. The hydrogenases genes are detected in the grains of wild wheat and seeds of some desert plants. The hydrogen source for this bacterium

is from air, soil and microbial production from *Enterobacter* spp. The microbial source of water will be material for many applications and a novel trend in scientific research of the agriculture field.

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