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Role of Bacterial Endophytes Associated with Seaweed Species in Nourishing Mexican Lime Seedlings in South of Iran

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ABSTRACT

Bacterial endophytes associated with algae represent a rich source of bioactive metabolites and biostimulants that can be used practically in agriculture as biofertilizer. We carried out a series of experiments to study the diversity of bacterial endophytes associated with seaweed species of the Persian Gulf (PG) and Oman Sea (OS) and their capability in nourishing Mexican lime seedlings. We collected samples of brown, red, and green seaweed species (62 samples of brown, 79 of red and 49 of green) from intertidal zones of PG and OS in southern coastlines of Iran. The isolated bacteria were identified molecularly, morphologically and physiologically. Among 12 bacterial genera identified, the genus *Bacillus* had the highest frequency (51.51%). In addition to identification, results showed that all bacterial endophytes isolates were negative oxidase, most isolates (81.25 %) were positive catalase and could produce HCN, and all isolates produced IAA, from 0.897 µg mL^{-1} in *Empedobacter falsenii* to 0.085 µg mL^{-1} in *Bacillus zhangzhouensis*. Most isolates (96.77%) were able to grow on medium incorporated with different NaCl concentrations. Results of inoculation showed that lime seedlings colonized by *B. aquimaris* (MT278260), *B. megaterium* (MN626631) and *B. zhangzhouensis* (MN611359) had more growth and intended morphological characteristics than those lacking endophytes.

Keywords: Endophytic bacteria, HCN test, IAA test, Microbial biofertilizer.

INTRODUCTION

Endophytes are a group of microorganisms capable of making a symbiotic relationship with hosts without causing negative effect on them (Strobel *et al.,* 2004). Furthermore, they are a rich source of bioactive metabolites and various bioactive agents practically used in developing natural drugs and other industrial products (Gunathilake, 2017; Gao *et al.,* 2018). Bacterial endophytes ubiquitously colonize plant internal tissues, causing plants to be less damaged by biotic and abiotic stresses (Khan *et al.,* 2020). The growth can be induced by different manners including the solubilization of phosphate, synthesis of phytohormones, nitrogen fixation, siderophore production, and suppression of phytopathogenic microorganisms (Gamalero *et al.,* 2020). In addition, bacterial endophytes cause plants to become more resistant to stress through the reduction of ethylene synthesized when a

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plant experiences biotic stresses, which occurs through the action of the enzyme 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase (Glick, 2014; Gamalero and Glick, 2015). Bacterial endophytes are less exposed to variations in soil properties such as pH, water availability, and competition with the external microbiota (Cerqueira *et al.,* 2019). Salt tolerant microorganisms have shown to enhance crop growth under salinity conditions (Gupta *et al.,* 2020).

Seaweeds are ecologically important primary producers, competitors, and ecosystem engineers that play a central role in coastal habitats ranging from kelp forests to coral reefs (Harley, 2012; Satheesh *et al.,* 2017). Consequently, many seaweeds bacterial associations are essential for both symbiotic partners (Hollants, 2012). Endophytes from marine environment are gaining special interest because of their existence in the harsh conditions of marines and ocean ecosystem such as temperature, light availability, high salinity and osmotic stress (Debbab *et al.,* 2011; Oliveira *et al*., 2012; Peng *et al.,* 2016).

In recent years, special attention has been paid to bacterial-based fertilizers as a main group of biofertilizers that can induce plant growth through increased nutrition uptake. Utilization of endophytes-mediated biofertilizers have several advantages over synthetic fertilizers. For instance, they are cost effective, eco-friendly and renewable source of plant nutrients. Biofertilizers are one of the important components of integrated nutrient management and allocated a significant portion of the fertilizer needed by plants in the near future (Elavarasi *et al.,* 2020).

Due to lack of detailed information regarding spatiotemporal variation in seaweeds-associated microorganisms of the Persian Gulf and Oman Sea, the present study was carried out to identify the endophytic bacterial microbiota associated with seaweed species and whether isolated bacterial endophytes could possibly be used as biofertilizer.

MATERIALS AND METHODS

Sampling and Isolation of Bacteria

During April 2018 to March 2019, 190 samples of brown, red, and green seaweed species were collected from 18 intertidal locations of three provinces of Iran (Table 1). The seaweeds were identified morphologically using available identification key (Sohrabipour and Rabiei, 1996, 1999, 2007, 2008; Sohrabipour *et al.,* 2004; Pirian *et al.,* 2016). Physicochemical properties of sea water including EC, pH and salinity were also measured in the different sampling sites using the multi meter (HACH, HQ 40d USA) and refractometer (ATAGO, S/Mill-E Japan) devices (Table 1).

The sterilization protocol was followed by washing the samples in 70% ethanol for 5 seconds and immersion in sterile distilled water for 10 seconds (Zhang *et al.,* 2009). Sterile tissues were cut to 0.5 cm^2 segments, then, 3–4 segments of each seaweed were placed onto Petri plates containing Nutrient Agar (NA). The Petri plates were incubated in a light chamber at 26±2°C for 4 weeks under 12:12 hours (L: D) photoperiod (Suryanarayanan, 1992). The Colonization Frequency (CF) was calculated as Yuan *et al.* (2010) method.

Physiological Characteristics of Bacterial Endophytes

Quantification of Indole-3-Acetic Acid (IAA) in the Bacterial Isolates

The quantification of IAA produced by the endophytes in broth culture media was carried out using the colorimetric Salkowski assay (Patten and Glick, 2002; Rahman *et al.,* 2010).

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Table 1. List of provinces, locations and their GPS coordinates as well as chemical properties of seawater in different sampling sites.

No	Province	Location	Longitude (E)	Latitude (N)	EC (mS cm^{-1})	pH	Salinity (PPT)
1	Hormozgan	Bandar Abbas	56 15 29	27 10 11	56.9	7.49	39.3
2	Hormozgan	Bandar-e Lengeh	54 53 41	26 33 34	56.6	7.97	38.4
3	Hormozgan	Bandar-e Kong	54 57 06	26 35 51	57.9	7.99	39.6
4	Hormozgan	Qeshm Island	56 13 57	26 58 54	56.6	7.94	38.9
5	Hormozgan	Hormoz Island	56 26 45	27 02 17	56.5	7.52	39.0
6	Hormozgan	Sirik	57 04 52	26 31 47	54.5	7.44	38.2
7	Sistan and Baluchistan	Chabahar	60 38 48	25 16 47	52.8	8.1	37.6
8	Sistan and Baluchistan	Gavater	62 15 27	25 12 22	54.5	7.12	36.9
9	Sistan and Baluchistan	Ramin	60 44 51	25 16 07	56.3	7.87	38.6
10	Sistan and Baluchistan	Darya-ye bozorg	60 39 57	25 16 37	55.4	8.13	38.2
11	Sistan and Baluchistan	Kachu	60 53 56	25 13 56	56.4	8.2	38.1
12	Sistan and Baluchistan	Beris	61 10 53	25 08 04	55.5	7.98	38.3
13	Sistan and Baluchistan	Konarak	60 24 22	25 22 29	56.4	7.88	38.9
14	Bushehr	Bushehr	50 48 33	28 55 05	62.7	8.27	**42.0***
15	Bushehr	Shif Island	50 51 59	29 01 59	49.8	7.74	33.9
16	Bushehr	Nuclear Power plant	50 52 50	28 49 42	60.5	8.06	**41.0**
17	Bushehr	Sabz Abad park	50 52 24	28 50 13	60.2	8.06	**41.0**
18	Bushehr	Airport seaside	50 48 34	28 57 36	49.9	7.80	33.9

* Seawater samples with ≥ 40 PPT salinity were detected by refractometer device.

Hydrogen Cyanide (HCN) Test in the Isolated Bacteria

For this test, first King's B medium (44 g glycine in 1 litter NA) and HCN regent [2 % Na_2CO_3 and 0.5% C6H3N3O7 (picric acid)] were prepared. A colony of each isolate picked and streaked on the plates of King's B medium and the saturated filter papers with HCN regent were placed on the lids. The plates were put in an incubator at 28°C for 7 days. The color changes were graded from 1 to 3 points (from white to dark orange colors) (Marakana *et al.,* 2018).

Catalase Test

First, a drop of the isolate test culture was put on the slide, then, 4 to 5 drops of 3% hydrogen peroxide were added. Formation of gas bubbles confirmed that the test isolate was catalase positive (Marakana *et al.,* 2018).

Oxidase Test

A small piece of filter paper was soaked in 1% Tetramethyl-p-Phenylenediamine Dihydrochloride (TMPD) oxidase reagent and let dry. Then, a loop of the fresh bacteria colony was picked up and rubbed onto treated filter paper. When the filter paper color changed to dark purple within 5 to 10 seconds, it meant that the isolate was oxidase positive (MacFaddin, 2000).

Gram Test

One drop of KOH (3%) was added on top of the colonies, left it for 15 seconds. Then, using a cool loop, the mixture was drawn up from the bottom of the mixture. If the colony produced viscous strings, it was Gram negative and formation of cloudy liquid, confirmed a positive Gram test (Buck, 1982).

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Salinity Resistance Test

The resistance of bacterial endophytes to saline conditions was investigated using a method described by Vatsyayan and Ghosh (2013). In brief, isolated bacteria that grew in Nutrient Agar (NA), were supplemented with different concentrations of sodium chloride (1, 2 and 3 mol NaCl) and the ability of bacteria to form colonies was checked after 120 hours.

Molecular Identification and Phylogenetic Analysis

The DNA was extracted using boiling method suggested by Dashti *et al.* (1998). The isolated bacteria were identified by amplifying and sequencing a near full-length 16S rRNA gene. The 16S rRNA gene was amplified using the primer pair, bAS-F (5′CCGAATTCGTCGACAACAGAGTTT GATCCTGGCTCAG-3′) and bAS-R (5′-CCCGGGATCCAAGCTTACGGCTACCT TGTTACGACTT-3′), as described by Partida-Martinez and Hertweck (2005). The polymerase chain reaction was performed in a VeritiThermal Cycler (BIO RAD) programmed at 95°C for 4 minutes followed by 33 cycles of 96°C for 60 seconds, 52°C for 30 seconds, and 72°C for 120 seconds and a final extension at 72°C for 10 minutes (Sambrook and Russell, 2001). The base substitution model was implemented using MrModeltest2 (Nylander *et al*., 2004). Phylogenetic relationships and the related tree were constructed using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). The Markov Chain Monte Carlo (MCMC) method within a Bayesian framework was run for 10 million generations (Larget and Simon, 1999) using the 50% majority rule.

Bacterial Inoculation as Biofertilizer

Three *Bacillus* species, including *B. aquimaris* (MT278260), *B. megaterium* (MN626631) and *B. zhangzhouensis* (MN611359), hereinafter known as B5, B9 and B12, respectively, were inoculated to eight-month-old Mexican lime seedlings. The plants were inoculated with soil drenching and foliar spraying with cell density of 1×10^8 CFU mL^{-1} in NB medium. After 120 days, morphological and physiological characteristics of seedlings (trunk width, number of leaf and branches, stem length, leaf fresh/dry weight, root fresh/dry weight, stem fresh/dry weight, root length, root width, Fv/Fm, SPAD) were analyzed. The experiments were carried out in a completely randomized design with four treatments, each replicated three times. One-way ANOVA was used to analyze the data using PROC GLM (SAS, 1988) and, when significant differences were observed, mean grouping was made by the Least Significant Difference (LSD) test ($P< 0.05$).

RESULTS

Colonization Rates

From 190 seaweed samples (including 79 red, 62 brown and 49 green species), in total 1722 isolates were recovered from 1692 seaweed tissue segments. The red, brown, and green species allocated 749, 511, and 462 isolates, respectively. Hormozgan and Chabahar with 624 and 583 isolates, respectively, had the highest number of isolates (Table 2).

The 16S rRNA sequences of the bacterial endophytes and an outgroup sequence from GenBank (*Arthrobacter_crystallopoietes*_PCJS03) were used in the phylogenetic analyses. In addition, 44 sequences from GenBank were included in the phylogenetic analyses. The phylogenetic tree reconstructed from the alignment of 77 16S rRNA sequences using Bayesian inference grouped all isolates in 12 genera. Detailed information regarding 33 sequenced isolates and other supplementary information are shown in Table 3.

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Table 2. Colonization rate in the different seaweed species collected seasonally from different sampling sites.

Province	Season	Seaweeds	Color	segments	Isolates	Colonization (%)
Hormozgan	Spring	18	5 red	45	30	66.66
			7 brown	63	52	82.53
			6 green	54	40	74.07
	Summer	21	8 red	72	78	108.33
			7 brown	63	75	119.04
			6 green	54	66	122.22
	Winter	37	15 red	135	101	74.81
			15 brown	135	121	89.62
			7 green	63	61	96.82
	Total	76	**76**	**684**	**624**	**91.22**
Chabahar	Spring	2	1 red	9	5	55.55
			1 green	9	13	144.44
	Summer	45	24 red	216	262	121.29
			6 brown	54	66	122.22
			15 green	135	139	102.96
	Winter	14	3 red	27	16	59.25
			7 brown	63	46	73.01
			4 green	36	36	100
	Total	61	**61**	**549**	**583**	**106.19**
Bushehr	Spring	11	5 red	45	88	195.55
			5 brown	45	63	140
			1 green	9	9	100
	Summer	17	9 red	81	70	86.41
			7 brown	63	48	76.19
			1 green	9	5	55.55
	Winter	25	9 red	81	99	122.22
			8 brown	54	40	74.07
			8 green	54	93	172.22
	Total	53	**53**	**441**	**515**	**116.78**
	Total+	190	**190**	**1674**	**1722**	**102.86**

Physiological Characteristics

HCN was produced by 81.81% of bacteria. *Pseudomonas* sp. strain B-1-47**,** *Bacillus pumilus*, *B. subtilis* and *Shewanella algae* produced higher HCN compared with the other bacteria (*B. velezensis* strain QT2, *B. axarquinsis* strain BPRIST011, *Bacillus* sp., *Enterobacter hormaechei* strain E2, *B. subtilis* (B34) and *B. licheniformis* strain MP-6 did not produce HCN) (Table 4). All isolates were oxidase negative and were able to produce IAA, ranging from 0.085 μg mL^{-1} in *B. zhangzhouensis* strainTv91C to 0.897 μg mL^{-1} in *E. falsenii*). In addition, most of isolates were catalase negative and about 81.25% of them were Gram positive (Table 4).

Salinity Test

All isolates grew successfully on medium incorporated with different NaCl concentrations. While in 1 mol NaCl, most of isolates (96.77%) grew very well and were ranked in group 8, in 2 and 3 mol NaCl only 38.7 and 6.45% of isolates were able to grow, respectively (Table 4).

Role of Isolated Bacteria as Biofertilizer

All B5, B9, and B12 isolates could successfully improve the morphological characteristics of the Mexican lime seedlings tested (the analyses were made 120 days

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Table 3. Diversity and spatiotemporal distribution of 33 bacterial endophytes associated with seaweed species.

No	Code	Isolates	Accession No	Host	Sampling site ID[a]	Sampling time
1	B2	*Kocuria indica* strain **BT121**	MN611310	*Dictyota* sp.	Q.I	Spring
2	B3	*Exiguobacterium profundum*	MN628624	*Hypnea* sp.	Q.I	Spring
3	B5	*Bacillus aquimaris* strain **OD14**	MT278260	*Rosenvingea orientalis*	Q.I	Spring
4	B7	*Bacillus subteraneus* strain **HWG-A11**	MN611354	*Hypnea* sp.	Q.I	Spring
5	B8	*Bacillus thuringiensis* strain **UQPM24**	MN611357	*Digenenia simplex*	BU	Spring
6	B9	*Bacillus megaterium* strain **JX285**	MN626631	*Gracilaria foliifera*	BU	Spring
7	B11	*Bacillus velezensis* strain **QT2**	MN611358	*Laurencia* sp.	B.L	Summer
8	B12	*Bacillus zhangzhouensis* strain **Tv91C**	MN611359	*Cladophoropsis* sp.	Q.I	Spring
9	B13	*Oceanimonas smirnovii* strain **ST3**	MN626636	*Champia glubliifera*	Q.I	Spring
10	B14	*Empedobacter falsenii*	MN625702	*Jania robens*	Q.I	Spring
11	B15	*Pseudomonas* sp. strain **B-1-47**	MN611360	*Hypnea* sp.	Q.I	Spring
12	B16	*Brevundimonas diminuta* **JCM2789**	MN611361	*Caulerpa taxifolia*	Q.I	Spring
13	B17	*Bacillus cereus* strain **QGC-12**	MN626635	*Champia parvulla*	BU	Summer
14	B19	*Bacillus cereus* strain **Xpq-15**	MN611362	*Ulva* sp.	Q.I	Spring
15	B20	*Bacterium* sp. strain**YC-ZSS-LKJ-*184***	MN611363	*Ulva* sp.	Q.I	Spring
16	B21	*Bacillus halotolerans*	MN611364	*Chaetomorpha* sp.	SI	Summer
17	B22	*Bacillus subtilis*	MN611365	*Cladophoropsis* sp.	Q.I	Spring
18	B23	*Bacillus* sp.	MN625740	*Sargassum boveanum*	BU	Summer
19	B25	*Pseudomonas stutzeri* strain **GR45**	MN626637	*Champia glubliifera*	Q.I	Spring
20	B26	*Bacillus subtilis*	MN611366	*Rosenvingea orientalis*	Q.I	Spring
21	B27	*Empedobacter hormaechei*s train **E2**	MN626637	*Jania robens*	Q.I	Spring
22	B28	*Alcaligene aquatillis* strain **BUN 33**	MN611367	*Iyengaria stellate*	Q.I	Spring
23	B29	*Empedobacter hormaechei*	MN626630	*Jania robens*	Q.I	Spring
24	B30	*Wautersiella falsenii* strain **NF1159**	MT111593	*Enteromorpha* sp.	Q.I	Spring
25	B31	*Bacillus pumilus*	MN626633	*Gracilaria foliifera*	BU	Summer
26	B32	*Streptomyces mutabilis* strain **STR66**	MN611368	*Digenenia simplex*	BU	Summer
27	B33	*Bacillus* sp.	MN611369	*Laurencia* sp.	GG	Winter
28	B34	*Bacillus subtilis* strain **B-47**	MN611370	*Gracilaria corticata*	CH	Summer
29	B35	*Shewanella algae*	MN626629	*Jania robens*	GG	Summer
30	B36	*Shewanella algae*	MN611371	*Cladophoropsis* sp.	GG	Summer
31	B40	*Shewanella algae* strain **A60**	MN611373	*Cladophoropsis* sp.	GG	Summer
32	B37	*Bacillus* sp.	MN626632	*Gracilaria acerosa*	CH	Summer
33	B38	*Bacillus licheniformis* strain **MP-6**	MN611372	*Chalerpa peltata*	CH	Summer

[a] Q.I, BU, CH, GG, SI and B.L, denote Qeshm Island, Bushehr, Chabahar, Gulf of Gavater, Sirik and Bandar-e Lengeh, respectively.

post inoculation). B12 isolate, had the best results in the most of analyzed characterizes; SPAD, stem fresh/dry weight, root fresh/dry weight, leaf fresh/dry weight, trunk width, leaf number and stem length (29.11 %, 157.55 %, 109.44 %, 206.56 %, 190.44 %, 143.28 %, 660 %, 113.96 %, 239.66 % and 153.3 % respectively than control). B9, can improve root width than control (82.07 %) (Table 5).

DISCUSSION

Endophytic microorganisms may help hosts to cope with physiological disturbances, or to increase their tolerance to environmental changes (Soltani *et al.,* 2016). Based on 16S rRNA gene sequences, 12 bacterial genera including *Bacillus*, *Kocuria*, *Pseudomonas*, *Oceanimunas*, *Wautersiella*, *Brevundimonas*, *Bacterium*,

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Table 4. Physiochemical characteristics of bacterial endophytes isolated from different seaweed species.[a]

No	Code	OT	CT	L-Trip (0, mg L^{-1})	L-Trip (100, mg L^{-1})	HCN	Gram	Nacl (1 mol)	Nacl (2 mol)	Nacl (3 mol)	Colony color
1	B2	-	+	0.068	0.094	1	+	8	2	2	Yellowish
2	B3	-	+	0.059	0.174	1	+	8	1	1	Orange
3	B5	-	+	0.123	0.158	1	-	8	8	5	Pink
4	B7	-	+	0.077	0.097	1	-	8	1	1	Yellowish
5	B8	-	+	0.043	0.372	1	+	6	1	1	Creamy
6	B9	-	+	0.010	0.126	1	+	8	5	2	Beige
7	B11	-	+	0.002	0.135	0	+	8	5	3	Beige
8	B12	-	+	0.000	0.085	1	+	8	7	2	Creamy
9	B13	-	+	0.017	0.224	1	+	8	1	1	Orange
10	B14	-	+	0.035	**0.897**	1	-	8	2	2	Yellowish
11	B15	-	+	0.032	0.295	**2**	-	6	1	1	Creamy
12	B16	-	+	0.102	**0.733**	1	-	8	2	2	Creamy
13	B17	-	-	0.086	0.199	1	+	8	3	3	Beige
14	B19	-	+	0.125	0.152	1	-	8	4	2	Creamy
15	B20	-	+	0.004	0.198	1	+	7	1	1	Creamy
16	B21	-	+	0.139	0.245	0	+	8	5	1	Beige
17	B22	-	-	0.131	0.228	0	+	8	2	2	Creamy
18	B23	-	+	0.034	0.143	1	+	8	8	1	Pink
19	B25	-	+	0.016	0.168	1	+	8	6	2	Pink
20	B26	-	-	0.010	0.123	1	+	8	4	3	Brown
21	B27	-	-	0.022	0.185	0	+	8	3	2	Creamy
22	B28	-	+	0.020	0.157	1	-	7	7	1	Yellowish
23	B29	-	+	0.022	**0.622**	1	-	8	1	1	White
24	B30	-	+	0.002	**0.600**	1	-	8	7	1	Beige
25	B31	-	+	0.019	0.102	**2**	+	8	2	2	White
26	B32	-	-	0.001	0.104	1	+	1	1	1	White
27	B33	-	+	0.006	0.149	**2**	-	8	4	3	White
28	B34	-	-	0.011	0.121	0	+	8	3	2	White
29	B35	-	+	0.015	0.279	**2**	-	8	2	2	Beige
30	B36	-	+	0.019	0.155	1	+	8	5	5	Beige
31	B37	-	+	0.056	**0.724**	1	-	8	2	2	White
32	B38	-	+	0.017	0.225	0	+	8	2	2	Beige
33	B40	-	-	0.029	0.179	1	-	7	1	1	Beige

[a] Samples coded by 0, 1 and 2 denote no HCN production; medium production (yellow in color); and relatively high production (orange in color), respectively. For IAA, 0 and 100 mg L^{-1} concentrations of L-Trip were used. For salinity test, 1, 2, 3, 4, 5, 6, 7 and 8 denote very weak, weak, relatively weak, relatively intermediated, intermediated, relatively severe, severe, and very severe, respectively. OT (Oxidase Test), CT (Catalase Test).

Exiguobacterium, *Enterobacter*, *Alcanigene*, *Streptomyces* and *Shewanella* were isolated from the seaweed species. The majority of the identified bacteria belonged to the genus *Bacillus* (51.51%), which was consistent with findings of Fuhrman and Hagstrom (2008) and Ettoumia *et al*. (2010), who demonstrated the predominance of *Bacillus* in most seaweed species. We identified a complex of *Bacillus* species (*Bacillus aquimaris, B. thuringiensis, B. subtilis, B. zhangzhouensis, B. subterraneus, B. cereus, B. megaterium, B. velezensis, B. halotolerans, B. pumilus*, *B. licheniformis* and *Bacillus* sp.) that were in accordance with the results of Siefert *et al*. (2000), Yoon and Oh (2005), Sutha *et al*. (2011), and Lee *et al*. (2006).

Actinobacteria is another important group of endophytic bacteria in marine ecosystem due to their ability to produce bioactive compounds (Rohwer *et al.,* 2001). *Kocuria*

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Table 5. Mean (±SE) of the studied attributes in the bacterial colonized Mexican lime seedlings compared to non-inoculated seedlings. [a]

Characterizes	N	B5	IRCC	B9	IRCC	B12	IRCC
SPAD	53.93±0.01^{***c}	69.00±0.01^{***a}	26.22	66.56±0.01^{***b}	23.41	69.63±0.01^{***a}	29.11
Trunk width	3.58±0.01^{***d}	6.54±0.00^{***b}	82.68	4.9±0.04^{***c}	36.87	7.66±0.00^{***a}	113.96
No. Leaf	21.00±0.00^{***d}	64.66±0.02^{***b}	207.9	36.00±0.00^{***c}	71.42	71.33±0.02^{***a}	239.66
No. Branch	3.66±0.00^{***d}	10.00±0.01^{***a}	173.22	7.33±0.00^{***c}	100.27	8.66±0.00^{***b}	136.61
Stem length	20.00±0.01^{***c}	35.00±0.01^{***b}	75	32.00±0.00^{***b}	60	50.66±0.01^{***a}	153.3
Fv/Fm	0.78±0.00^{**b}	0.82±0.00^{**a}	5.12	0.81±0.00^{**a}	3.84	0.8±0.00^{**a}	2.56
Leaf FW	4.99±0.01^{***d}	9.08±0.00^{***b}	81.96	7.53±0.02^{***c}	47.29	12.14±0.00^{***a}	143.28
Leaf DW	0.45±0.00^{***d}	1.74±0.01^{***c}	286.66	2.57±0.00^{***b}	471.11	3.42±0.01^{***a}	660
Root FW	5.03±0.00^{***c}	7.43±0.01^{***b}	47.713	8.77±0.01^{***b}	74.35	15.42±0.01^{***a}	206.56
Root DW	1.36±0.02^{***c}	2.03±0.00^{***b}	49.26	2.18±0.01^{***b}	60.29	3.95±0.01^{***a}	190.44
Stem FW	3.11±0.02^{***d}	5.61±0.00^{***b}	80.38	5.03±0.00^{***c}	61.73	8.01±0.00^{***a}	157.55
Stem DW	1.53±0.00^{***b}	1.76±0.02^{***b}	15.03	1.9±0.01^{***b}	24.18	3.21±0.01^{***a}	109.80
Root length	29.5±0.00^{***d}	53.33±0.02^{***a}	80.77	45.93±0.2^{***b}	73.32	39.86±0.00^{***c}	35.11
Root width	27.00±0.00^{***c}	37.33±0.01^{***b}	38.25	49.16±0.02^{***a}	82.07	46.5±0.02^{***a}	72.22

[a] Seaweed bacterial endophytes could improve the morphological characterizes in the inoculated Mexican lime seedlings over the control samples (P< 0.0001). IRCC denotes increase rate compared to the control and N denotes seedlings without inoculation. FW and DW means: fresh and dry weight, respectively.

palustris has exclusively been found in some seaweeds (*Sargassum muticum, Rhodomela confervoides*) and some marine sponge species. Here, *K. indica* was isolated from *Dictyota* sp., an important seaweed species found across intertidal zones of the Persian Gulf and Oman Sea. Mutualistic relationship between *K. indica* and *Dictyota* sp. can be successfully used to produce bioactive compounds from this seaweed species. The strain *K. palustris* isolated from *S. muticum*, has been widely used in biodegradation of organic matter (Kovacs *et al.,* 1999). Seaweed endophytic bacterial are able to produce novel bioactive compounds because of their ability to inhabit millions of unique biological niches in many unusual environments (Villarreal-Gomez *et al.,* 2010). Some isolated bacteria such as *Shewanella* sp. belong to the order Alteromonadales are able to detoxify heavy metals that enter the algae body and protect their hosts against metal ions (Konishi *et al.,* 2007; Seshadri *et al.,* 2012; Yokesh Babu *et al.,* 2014). *Streptomyces variabilis* is another seaweed-associated bacterium that produces ammosamide, an anti-cancer agent metabolite (Pan *et al.,* 2012). Here, we identified a different species of *Streptomyces* (*S. mutabilis*) isolated from *Digenenia simplex* that may have anti-cancer properties similar to what has been reported for *S. variabilis*.

Accordingly, it was revealed that all bacteria were able to produce IAA. These findings are in agreement with previous studies (Fernandes Galdiano Júnior *et al.,* 2011). Previously, Lwin *et al*. (2012) found that five *Bacillus* species isolated from rhizospheric soil, produced IAA ranged from 0.53 to 0.71 μg mL^{-1}. Probably, several Indole compounds present in the liquid culture supernatants are more reactive to IAA alone when in Salkowski's solution (Tsavkelova *et al.,* 2007b).

In the present study, most of the bacterial isolates produced HCN (81.81 %) (*Pseudomonas* sp., *B. subtilis, B. pumilus* and *S. algae* produced twice as much HCN as other bacteria). According to Stutz *et al*. (1986), HCN is one of the most important volatile compounds produced by *Pseudomonas* sp. to biologically control plant diseases (Masumi *et al.,* 2014). Herein, *Pseudomonas* sp. isolated from *Hypnea* sp. produced high level of HCN. Capability to

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produce HCN by *Pseudomonas* species may be a good opportunity for these bacteria to be used as a biocontrol agent against plant diseases. In addition, it can be used as a biopriming agent on different crops to prime resistance against biotic stresses (Haas and Défago, 2005).

Some bacteria produce the enzyme catalase, which facilitates cellular detoxification (Reiner, 2010). In addition, catalase neutralizes the bactericidal effects of hydrogen peroxide (Wheelis, 2008) and its concentration in bacteria is correlated with pathogenicity (Mahon *et al.,* 2007). We showed that most of bacterial isolates identified in this project (81.25%) were catalase positive.

We showed that some isolates such as, *B. megaterium, Bacillus* sp. (B23)*, B. cereus, B. zhangzhouensis, B. axarguiensis, W. falsenii, E. hormaechi, S. algae* and *P. stutzeri* grew well in medium incorporated with 3 mol NaCl, an important attribute for these species to reduce the negative effects of salt in plants cultivated under salinity conditions or in stenohaline plants.

By investigating the biofertilizer activity of some isolates (B5, B9 and B12) as growth promoting bacteria, it was revealed that the lime seedling colonized by these isolates had higher key physicochemical properties compared with those lacking endophytes. When endophyte-colonized seedlings were exposed to temperature stress, they were less damaged than the control and could return to pre-stress conditions faster. This may result from the production of secondary metabolites and hormones such as HCN. Among three bacterial isolates, B12 led to the best results in number of leaves, stem length and root fresh weight in the seedlings. The maximal photochemical efficiency of photosystem II (Fv/Fm ratio) is reflecting the capacity of solar energy use in PSII (Rivero *et al.,* 2014). Isolate B9 had the highest effect on improving the Fv/Fm ratio in the colonized seedlings (Table 5). The history of bacterial endophytes and those acting as plant growth-promoting (Mohammadi and Sohrabi, 2012; Vatsyayan and Ghosh, 2013), indicates that these microorganisms have a high capability to widely use in plants as biostimulants.

CONCLUSIONS

Isolation of high potent nourishing endophytic bacteria from seaweeds may open a new window to focus on this source of interesting microorganisms to produce the next source for biofertilizers. In this context, we need to clarify the relationship between seaweeds and endophytes to find out which seaweed species has high distribution and diversity and which of them may colonize by more intended endophyte species. Our findings prepared a platform for future studies that aim to focus on the physicochemical characteristics of endophytes identified in the present study.

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نقش باکتری‌های اندوفیت مرتبط با گونه‌های جلبک جنوب ایران در تغذیه گیاهچه‌های مکزیکن لایم

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چکیده

اندوفیت‌های باکتریایی مرتبط با جلبک‌ها منابع غنی از متابولیت‌های فعال زیستی و محرک‌های زیستی هستند که عملاً می‌توانند در کشاورزی به‌عنوان کود زیستی مورد استفاده قرار گیرند. در اینجا، ما مجموعه‌ای از آزمایش‌ها را برای مطالعه تنوع اندوفیت‌های باکتریایی مرتبط با گونه‌های جلبک خلیج فارس و دریای عمان و توانایی آن‌ها در تغذیه گیاهچه‌های لیمو انجام داده‌ایم. برای این منظور و شناخت بهتر باکتری‌های اندوفیتی مرتبط با جلبک‌های دریایی، نمونه‌هایی از گونه‌های جلبک قهوه‌ای، قرمز و سبز (۶۲ نمونه قهوه‌ای، ۷۹ نمونه قرمز و ۴۹ نمونه سبز) از نواحی جزر و مدی خلیج فارس و دریای عمان در سواحل جنوبی ایران جمع‌آوری شدند. جدایه‌های باکتریایی به‌روش مولکولی، مورفولوژیک و فیزیولوژیک مورد شناسایی قرار گرفتند. از بین ۱۲ جنس باکتری شناسایی شده، جنس *Bacillus* بیشتر فراوانی را دارا بود (۵۱/۵۱ درصد). علاوه‌براین، دیگر نتایج در مورد شناسایی ویژگی‌های اندوفیت‌های باکتریایی حاصل شد، از جمله، همه جدایه‌ها اکسیداز منفی بودند؛ بیشتر جدایه‌ها (۸۱/۲۵ درصد) کاتالاز مثبت و قادر به تولید سیانیدهیدروژن بودند؛ همه جدایه‌ها ایندول استیک اسید تولید می‌کردند، از ۰/۸۹۷ میلی‌گرم در میلی‌لیتر در *Empedobacter falsenii* تا ۰/۰۸۵ میلی‌گرم در لیتر در *Bacillus zhangzhouensis*. بیشتر جدایه‌ها (۹۶/۷۷ درصد) قادر بودند روی محیط حاوی غلظت‌های مختلف نمک کلرید سدیم رشد کنند. نتایج تلقیح نشان دادند که گیاهچه‌های لیمو کلونیزه شده با جدایه‌های B5، B9 و B12 دارای رشد بیشتر و صفات مورفولوژیک بهتر نسبت به نمونه‌های بدون اندوفیت بودند.

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