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Research Article
Molecular Identification, Characterization and Antioxidant Activities of Some Bacteria Associated with Algae in the Red Sea of Jeddah

[1]Doaa Baker, [1]Sawsan Basondwah, [1]Ebtihaj Jambi, [1]Sawsan A. Rahimuddin, [2,4]Mohammad Abuzaid and [2,3]Magda Aly

[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University, 80200-21589 Jeddah, Saudi Arabia
[2]Department of Biology, Faculty of Science, King Abdulaziz University, 80200-21589 Jeddah, Saudi Arabia
[3]Department of Botany, Faculty of Science, Kafrelsheikh University, 33516 Kafr El-sheikh, Egypt
[4]Genetic Engineering and Biotechnology Division, Department of Botany, National Research Center, 12622 Giza, Dokki, Egypt

Abstract

Background and Objective: Algae-associated bacteria produce secondary metabolites that have a great biological impact. The aim of this study was isolation, identification and evaluation the antioxidant activities of the associated bacteria of seven algae, *Padina pavonica*, *Dictyota dichotoma*, *Cystoseira myrica*, *Halimeda opuntia*, *Ulva lactuca*, *Digenea simplex* and *Jania* sp. The bacteria were isolated, characterized and identified. Identification was carried out using 16S rRNA gene sequencing. **Materials and Methods:** The identified bacteria were belonging to 6 families, Alteromonadaceae, Bacillaceae, Lactobacillaceae, Pseudomonadaceae, Rhodobacteraceae and Vibrionaceae and 9 genera. The identified bacteria were belonging to genera, *Alteromonas*, *Bacillus*, *Lysinibacillus Vibrio*, *Lactobacillus*, *Paracoccus*, *Leisingera*, *Pseudomonas* and *Pseudovibrio*. The antioxidant activities of the bacterial ethyl acetate extracts was examined by scavenging DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) methods. **Results:** Out of the 17 isolated bacteria, *Lactobacillus plantarum* showed 95.7% free radical scavenging with EC_{50} = 17.7 μg mL^{-1}, which is nearly similar to the positive control (Butylated Hydroxytoluene, BHT). The FRAP value of *Lactobacillus* extract was 2.00 mM ferric equivalent/mg of the extract. Phytochemical analysis of the bacterial extract revealed the presence of some secondary metabolites such as steroids, saponins, tannins, flavonoids, anthocyanin and betacyanin in all tested extracts. **Conclusion:** The Red Sea algal associated bacteria have a great antioxidant potential that can be used in pharmaceutical industries.

Key words: Marine bacteria, antioxidant, DPPH, FRAP, phytochemicals, ethyl acetate extract, red sea, algae, *Lactobacillus plantarum*

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Corresponding Author: Magda Aly, Department of Biology, Faculty of Science, King Abdulaziz University, 80200-21589 Jeddah, Saudi Arabia
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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The red sea environment contains an enormous diversity of life forms that have great biological potentialities. Marine ecosystem was exposed to a harsh condition as ultraviolet radiation and heavy metal variations which trigger gradual production and accumulation of reactive oxygen species (ROS)[1]. As a defense response, marine organisms produce many phytochemicals such as flavonoids, steroids, tannins, saponins, anthocyanin and betacyanin[2,3]. While marine algae from the Red Sea and their biological activities were studied before[4,5], their associated bacteria were poorly studied. These marine bacteria may represent an endless source of pure materials with biological impacts in pharmaceutical, medicine, food industries and cosmetics[6-8]. The Marine bacteria, *Alteromonas* sp., *Bacillus* sp., *Vibrio* isolated from Portugal, Egypt or China showed antioxidant properties *in vitro* [2,8-10].

Excess stimulation of free radicals causes oxidative stress that might play a role in the pathophysiology of many human diseases such as diabetes, atherosclerosis, Alzheimer and cancer diseases[11]. Phytochemicals and other secondary products which produced from marine bacteria could be used as food preservative or as natural antioxidants to decrease toxicity, health problems and carcinogenic effect[12]. Therefore, there is a need to search for new natural inhibitors against the oxidation process with the least side effects.

As it is well known, algal surface host a variation of bacterial strains which may produce potential antioxidant activities but the researches on this particular field are very limited, especially in Saudi Arabia. Finding new antioxidants from sustainable local sources such as macroalgae associated bacteria may help developing new drugs and supplementary foods. The red sea is a suitable environment for new bacteria, waiting to be discovered. This study aimed to isolate, identify and evaluate the antioxidant activities of the associated bacteria with different common macro-algal genera of the Red Sea from Jeddah, Saudi Arabia.

MATERIALS AND METHODS

Algal associated bacteria sampling and isolation: Algae were collected during Summer 2017 by a local diver from a depth of 2-5 m of Obhor shores of Jeddah, Saudi Arabia. Algae were transferred in an ice box then washed with sea water then with distilled water to remove all suspended materials[13]. Algae were identified at Biology department, Faculty of Science, King Abdulaziz University, Saudi Arabia. Bacterial isolates were obtained from the 7 algae by swabbing with a sterile cotton-tipped swab. The swabs were inoculated directly in marine agar medium (Zobell Marine agar 2216, Himedia, India). The plates incubated at 27°C for 24-48 h. Colonies were selected for further purification by streaking technique based on colony characters and morphology (shape, color, margin and surface properties)[9]. All bacterial isolates were preserved in glycerol stock at -20°C for further studies.

Bacterial characterization: Morphological characteristics and some biochemical tests of the bacterial samples were carried out by conventional methods which are used in the microbiology laboratories. Identification was confirmed using molecular techniques.

Morphological characterization and biochemical tests: The morphological characteristics were observed after 24 h of growth on plates which included colony color, growth and shape. The shape of bacteria was examined under the microscope (CX21FS1, Olympus, China). Gram reaction, spore type and motility were also recorded[14]. Oxidase, catalase for all the isolates were carried out[15].

Molecular identification: The DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Germany) according to the instructions of the manufacturer[16]. The NanoDrop spectrophotometer 2000 (Thermo Fisher Scientific, USA) was used to evaluate the DNA purity and quantity, then DNA extracts were saved at -20°C for further analysis. The 16S rRNA genes sequences of bacterial DNA extracts were amplified by Labnet Multigene Mini Thermal Cycler (Sigma Aldrich, USA) and Internal Universal Primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 511 R (5'-GCG GCT GCTGGC ACR KAG T-3'). The 16S rRNA gene sequencing was conducted at Macrogen Laboratory, Korea. The sequences were inserted to the Advanced BLAST search program to identify the sequences of any closely related organisms and phylogenetic analysis was performed by nucleotides blast of NCBI[17].

Bacterial extraction for antioxidant assays: The bacterial isolates were cultured in 500 mL flasks containing 100 mL of marine broth medium BD Difco™ Dehydrated Culture Media: Marine Broth 2216 (Fisher Scientific Company, USA) and incubated under shacking at 120 rpm for 7 days, at 25°C. After fermentation period, the cells were separated from the culture medium by centrifuging at 5000 rpm for 10 min. The supernatants were mixed with equal amount of ethyl acetate

and agitated at for 12-18 h at room temperature. The ethyl acetate was evaporated at 50°C by Rotatory vacuum evaporator HS-2005S-N, (Hahnvapor, Hahnshin scientific, Korea) under reduced pressure. The collected dried extracts were saved in dark glass bottles and stored at -20°C for antioxidant analysis[8]. The dry extracts (0.1 g) were used for phytochemical analysis after dissolving in 5% dimethyl sulfoxide (DMSO).

Antioxidant activities

DPPH free radical scavenging activity: Free radical scavenging activities of the bacterial extracts were determined[13]. In 96-well plate,100 µL, 0.1 mM DPPH (Sigma-Aldrich, USA) in methanol solution was added to 100 µL of different concentration (1000, 500, 250, 62.5, 31.25 and 15.5 µg mL^{-1}) of bacterial extracts. The plate was incubated in a dark at room temperature for 30 min. Ascorbic acid and butylated hydroxytoluene (BHT) (Sigma-Aldrich, USA) were used as positive controls while DPPH solution was used as a negative control. The decrease in absorbance of DPPH due to the antioxidation of the bacterial extract was measured at 515 nm using Synergy HTX Multi-Mode Microplate Reader (Bio-Tek, USA). Experiment was performed in triplicate and the ability to scavenge the DPPH radical was calculated as a percent of DPPH scavenging using the following Equation:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the DPPH control and A_1 is the absorbance of the sample. Extract effective concentration that produce a 50% reduction of DPPH (EC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Ferric reducing antioxidant power (FRAP): The FRAP assay was performed according to the manufactured kit OxiSelect™ ferric reducing antioxidant power (FRAP) (Cell Biolabs, USA). Serial dilutions of 1 mM of Fe^{2+} standards solution were prepared. Assay Buffer (1X) was prepared and used for further preparing of kit reagents. The reaction reagent was prepared just before use and prepared only enough for immediate applications. Equal amounts of colorimetric probe and ferric chloride solution (500 µL) were added to 4 mL of 1X Assay Buffer and was completed to 5 mL total. In 96-well plate, 100 µL of reaction reagent was added to 100 µL of bacterial extracts that prepared freshly in methanol (1 mg mL^{-1}) and to 100 µL of standard serial dilution[18]. Experiment was performed in triplicate and the color changing has read at 540 nm by Synergy HTX Multi-Mode Microplate Reader (Bio-Tek, USA). Results are expressed as mM ferrous equivalent mg^{-1} of extract.

Preliminary phytochemical analysis: Bacterial extracts were analyzed for the presence of any active component. Primary studies were carried out on the chemical analysis of those extracts using methods described by Fadeyi *et al.*[19] and Varadharajan *et al.*[20]. The prepared bacterial extracts were analyzed for the presence of saponins, flavonoids, anthocyanins, betacyanins, steroids and tannins.

Statistical analysis: Values represented as the mean of triplicates±SD. All statistical analyses were carried out using SPSS version 22.0. To determine whether there were any differences among activities of samples, one way-ANOVA and Turkey *post hoc* test ($p<0.05$) were applied to the results.

RESULTS

In this study, 7 algal samples were collected from the red sea water and they were identified as two species of Chlorophyte (*Halimeda opuntia* and *Ulva lactuca*), 3 species of Phaeophyte (*Cystoseira myrica*, *Dictyota dichotoma* and *Padina pavonica*) and finally, two species of Rhodophyta (*Digenea simplex* and *Jania* sp.) as shown in Table 1. The associated bacteria were isolated from the seven identified algal samples.

All bacterial strains were examined and some morphological characterization and biochemical tests were described in Table 2. Most of the strains were Gram negative, motile and non-spore forming bacteria.

Molecular characterization: The DNA was extracted and amplified for all bacterial strains which were associated with the seven algal samples. The genes of 16S rRNA were sequenced and compared to the GenBank database. Table 3 showed the molecular identification to the species level and the accession numbers of the different bacterial isolates.

Table 1: Types of the obtained algal species from the Red Sea

Type of algae	Used algal species	No. of isolated bacteria
Green	*Halimeda opuntia*	
	Ulva lactuca	14 (DBG1-14)
Brown	*Cystoseira myrica*	
	Dictyota dichotoma	
	Padina pavonica	11 (DBB1-11)
Red	*Digenea simplex*	
	Jania. sp.	2 (DBR1-2)

Table 2: Morphological characterization and biochemical tests of the isolated bacteria from different algal species

Algae type	Species	No. of isolates	Shapes	Gram stain	Motility	Spore formation	Catalase	Oxidase
Green algae	*Halimeda opuntia*	DBG1	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBG2	Rod shape	+ve	Non-Motile	+ve	ve	+ve
		DBG3	Filamentous	+ve	Motile	+ve	+ve	-ve
		DBG4	Rod shape	+ve	Motile	+ve	+ve	+ve
	Ulva lactuca	DBG5	Curved-rod shape	-ve	Motile	-ve	-ve	+ve
		DBG6	Rod shape	-ve	Motile	-ve	-ve	+ve
		DBG7	Rod shape	+ve	Motile	+ve	+ve	-ve
		DBG8	Rod shape	-ve	Motile	-ve	+ve	+ve
		DBG9	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBG10	Cocci or short rod	-ve	Non-Motile	-ve	+ve	+ve
		DBG11	Rod shape	+ve	Non-Motile	+ve	-ve	+ve
		DBG12	Rod shape	-ve	Polar Flagella	-ve	+ve	+ve
		DBG13	Rod shape	+ve	Non-Motile	+ve	-ve	+ve
		DBG14	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
Brown algae	*Cystoseira myrica*	DBB1	Rod shape	+ve	Motile	+ve	+ve	+ve
	Dictyota dichotoma	DBB2	Rod shape	-ve	Motile	-ve	+ve	+ve
		DBB3	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBB4	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBB5	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBB6	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBB7	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
		DBB8	Rod shape	-ve	Motile	-ve	+ve	+ve
		DBB9	Rod shape	+ve	Motile	+ve	-ve	+ve
	Padina pavonica	DBB10	Rod shape	-ve	Motile	-ve	+ve	+ve
		DBB11	Curved-rod shape	-ve	Motile	-ve	+ve	+ve
Red algae	*Digenea simplex*	DBR1	Rod shape	+ve	Non-Motile	-ve	-ve	-ve
	Jania sp.	DBR2	Curved-rod shape	-ve	Motile	-ve	+ve	+ve

+ve: Positive result, -ve: Negative result

Table 3: Marine bacterial isolates associated with 7 algae species from the Red Sea

Algae type	No. of isolates	Bacterial identification	Accession No.
Green algae	DBG1	*Vibrio parahaemolyticus*	KT986101.1
	DBG2	*Lysinibacillus* sp. strain 1	KU159206.1
	DBG3	*Bacillus algicola*	KX816439.1
	DBG4	*Bacillus niacin*	KT350462.1
	DBG5	*Vibrio neocaledonicus*	KP236342.1
	DBG6	*Vibrio harveyi*	NR_113784.1
	DBG7	*Bacillus horneckiae*	KT719562.1
	DBG8	*Pseudomonas gessardii* strain 1	LC027455.1
	DBG9	*Alteromonas australica*	CP008849.1
	DBG10	*Paracoccus marcusii*	NR_044922.1
	DBG11	*Lysinibacillus* sp. strain 2	KU159215.1
	DBG12	*Pseudomonas gessardii* strain 2	KT184489.1
	DBG13	*Lysinibacillus* sp. strain 1	KU159206.1
	DBG14	*Alteromonas tagae*	NR_043977.2
Brown algae	DBB1	*Bacillus subtilis*	KY820935.1
	DBB2	*Phaeobacter caeruleus*	NR_042701.1
	DBB3	*Vibrio alginolyticus* strain 1	CP006718.1
	DBB4	*Vibrio alginolyticus* strain 1	CP006718.1
	DBB5	*Vibrio alginolyticus* strain 2	KY941137.1
	DBB6	*Vibrio alginolyticus* strain 1	CP006718.1
	DBB7	*Pseudovibrio ascidiaceicola*	NR_041040.1
	DBB8	*Phaeobacter caeruleus*	NR_042701.1
	DBB9	*Lysinibacillus fusiformis*	KP762327.1
	DBB10	*Phaeobacter caeruleus*	NR_042701.1
	DBB11	*Vibrio alginolyticus* strain 1	CP006718.1
Red algae	DBR1	*Lactobacillus plantarum*	KX710325.1
	DBR2	*Vibrio alginolyticus* strain 1	CP006718.1

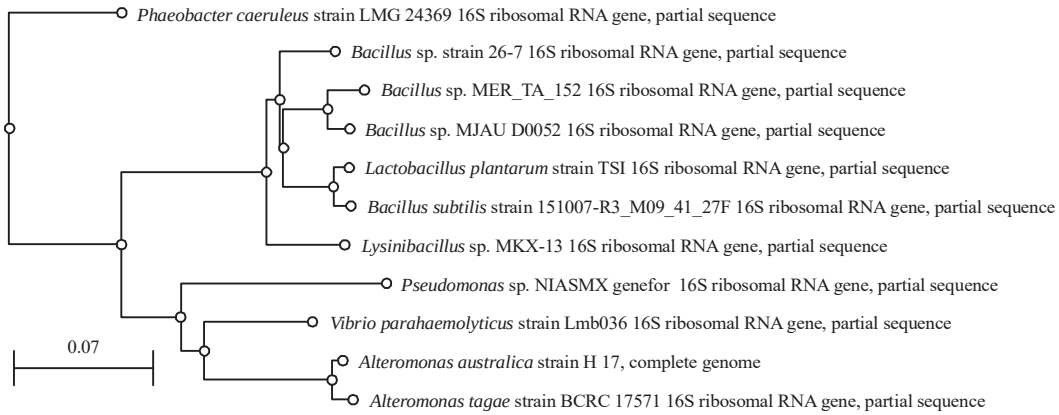

Fig. 1: Phylogenetic tree of the isolated bacteria from different algal species collected from the Red Sea

Table 4: Antioxidant activities of 17 algae associated bacterial strains, ascorbic acid and BHT

Bacterial extract (1 mg mL^{-1})	DPPH reducing (%)	FRAP value (mM FE mg^{-1} of extract)
Alteromonas australica	48.98±0.01*[a]	0.021±0.011*[a]
Alteromonas tagae	56.48±0.02*[a]	0.009±0.008*[a]
Bacillus algicola	56.56±0.02*[a]	0.010±0.005*[a]
Bacillus horneckiae	52.14±0.02*[a]	0.009±.005*[a]
Bacillus niacini	61.52±0.02*[a]	0.024±0.012*[a]
Bacillus subtilis	52.87±0.002*[a]	0.009±0.003*[a]
Lactobacillus plantarum	95.69±0.13	2.000±0.003
Lysinibacillus fusiformis	62.66±0.06*[a]	0.014±0.007*[a]
Lysinibacillus sp.	57.99±0.01*[a]	0.010±0.012*[a]
Paracoccus marcusii	59.83±0.02*[a]	0.019±0.028*[a]
Phaeobacter caeruleus	52.05±0.02*[a]	0.013±0.014*[a]
Pseudomonas gessardii	47.86±0.01*[a]	0.009±0.018*[a]
Pseudovibrio ascidiaceicola	52.19±0.01*[a]	0.008±0.006*[a]
Vibrio alginolyticus	44.81±0.03*[a]	0.010±0.006*[a]
Vibrio harveyi	53.98±0.01*[a]	0.030±0.018*[a]
Vibrio neocaledonicus	40.57±0.09*[a]	0.014±0.019*[a]
Vibrio parahaemolyticus	60.31±0.06*[a]	0.010±0.002*[a]
Ascorbic acid (control)	97.23±0.13	4.800±0.07
BHT (control)	95.18±0.13	2.500±0.05

Values are the Mean±SD of three independent experiments of mg mL^{-1},*Highly significant differences at $p<0.01$ compared to *Lactobacillus plantarum*, [a]Highly significant differences at $p<0.05$ compared to ascorbic acid and BHT

The identified isolates were belonging to 6 families, Alteromonadaceae, Bacillaceae, Lactobacillaceae Pseudomonadaceae, Rhodobacteraceae and Vibrionaceae and 9 genera. Out of the 37 identified bacteria, 17 bacterial isolates were different, 2 isolates belong to genus *Alteromonas*, 4 isolates to the genus *Bacillus*, 2 isolates to *Lysinibacillus*, 4 isolates to genus *Vibrio* and one isolate to the genera *Lactobacillus*, *Pseudomonas*, *Paracoccus*, *Phaeobacter* and *Pseudovibrio*. The phylogenetic tree of the obtained bacteria was shown in Fig. 1. It was clear that the most abundant strains associated with algae were belonging families Vibrionaceae and Bacillaceae while bacteria of families Alteromonadaceae, Pseudomonadaceae, Rhodobacteraceae were mainly isolated from green and brown algae. The isolate DBR1 was belonging to genus *Lactobacillus* and identified as *L. plantarum* with 98% similarity level.

Antioxidant analysis

DPPH Free radical scavenging activity: The DPPH scavenging activities was significantly various among bacterial species ($p<0.01$). The percentage of reduced DPPH radical by bacterial ethyl acetate extracts at concentration of 1 mg mL^{-1} is shown in Table 4. Evaluating the effectiveness of the antioxidant quality of the bacterial samples was given by the parameter EC_{50}. A significant difference was found between *Lactobacillus plantarum* and the other bacterial extracts at

Table 5: Preliminary phytochemical analysis of 17 algae associated bacteria ethyl acetate extracts of three independent experiments

	Phytochemicals					
Bacteria strain	Saponins	Flavonoids	Anthocyanin	Betacyanin	Tannins	Steroids
Alteromonas australica	+	+	-	-	+	+
Alteromonas tagae	-	+	-	+	+	+
Bacillus algicola	-	-	+	-	-	+
Bacillus horneckiae	-	-	-	-	-	+
Bacillus niacini	-	+	-	+	+	+
Bacillus subtilis	+	+	+	-	-	+
Lactobacillus plantarum	-	+	+	-	-	+
Lysinibacillus fusiformis	-	-	+	-	-	-
Lysinibacillus sp.	-	+	+	-	-	+
Paracoccus marcusii	-	-	-	-	-	+
Phaeobacter caeruleus	+	+	+	+	+	+
Pseudomonas gessardii	-	+	+	+	+	+
Pseudovibrio ascidiaceicola	-	+	+	+	+	+
Vibrio alginolyticus	-	+	+	+	+	+
Vibrio harveyi	-	-	-	+	+	-
Vibrio neocaledonicus	-	+	-	+	+	+
Vibrio parahaemolyticus	+	+	-	-	+	+

+: Presence of the compound, -: Absent of the compound

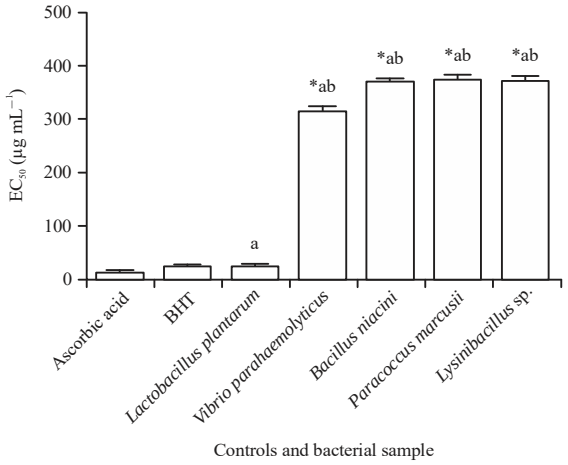

Fig. 2: EC_{50} value expressed as µg mL^{-1} for the most active bacterial isolates

EC_{50} value is the effective concentration of antioxidant necessary to reduce DPPH radicals by 50%, *Significance differences at p<0.01 compared to *Lactobacillus plantarum*, [a]Significance differences at p<0.01 compared to ascorbic acid, [b] Significance differences at p<0.01 compared to BHT

p>0.05. The highest scavenging percentage (96%±0.13) was of *Lactobacillus plantarum* which was obtained from the red alga *D. simplex* and the lowest was of *Vibrio neocaledonicus* (41%±0.09). The EC_{50} of the active bacteria that gave more than 60% inhibition of DPPH were calculated and compared to controls (Fig. 2). *Lactobacillus plantarum* has EC_{50} = 17.71 µg mL^{-1} that was relatively close to the synthetic antioxidant BHT (EC_{50} = 18.71 µg mL^{-1}).

Ferric reducing antioxidant power (FRAP): The FRAP assay was significantly different between bacterial species (p<0.01). Bacterial antioxidant abilities were determined after reducing Fe^{+3} to Fe^{+2} and the results were shown in Table 4. There is a significant difference in FRAP value of *Lactobacillus plantarum* and other bacterial extracts (p<0.01) . The highest Fe^{2+} concentration was recorded for *Lactobacillus plantarum* extract (2.0±0.003 mM FE mg^{-1} of extract), followed by *Vibrio harveyi* (0.030±0.05) then, *Bacillus niacini* (0.024±0.073), *Alteromonas australica* (0.021±0.01) and finally *Pseudovibrio ascidiaceicola* (0.008±0.01).

Preliminary phytochemical analysis: The primary analysis of the bacterial extracts revealed the presence of many secondary products (Table 5) like saponins, flavonoids, anthocyanins, betacyanins, steroids and tannins.

DISCUSSION

In the present study, 17 different bacterial strains that associated with 7 algae species have been identified. These algal samples were collected from the Red Sea. Bacteria were isolated, identified and screened for their antioxidant activities. The antioxidant activities of bacterial extracts were evaluated by two methods, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), Preliminary phytochemical analysis was performed to understanding such activities.

Many algal species from the Red Sea have been isolated and studied for their biological impact[21-23]. However, this study could be the first one interested in screening the antioxidant activities of the algal associated bacteria from the Red Sea of Jeddah, Saudi Arabia. These associated bacteria with marine algal species may give them protection or produce active compounds with biological activities, such as antimicrobial agents, antioxidant, anti-inflammation, enzymes and/or plant growth regulators. Bacterial identification was performed by 16s rRNA gene sequencing technique. The genera *Alteromonas*, *Bacillus*, *Lactobacillus*, *Lysinibacillus*, *Paracoccus leisingera*, *Pseudomonas, Pseudovibrio* and *Vibrio* were identified. As shown in results, *Vibrio* sp. and *Bacillus* sp., were the most common bacteria in the seven investigated algae, these genera in particularly abundant on the surface of marine organisms, where they form commensal, symbiotic or pathogenic associations and can tolerate adverse conditions such as high temperature, pressure, salinity and pH[24]. Most of our finding of bacterial strains is compatible with[2,25–31] who isolated the same genera from the marine environment worldwide.

Due to the harsh conditions, marine organisms produce potent bioactive compounds to fight off their competitors or to escape from micropredation[32]. Marine bacteria proved their antioxidant activities in different locations around the world[33,34]. Species of *Bacillus*, *Lactobacillus* and *Alteromonas* showed potential antioxidant activities[2,10,26,34,35].

The majority of current bacterial extracts showed significant activities in different degrees. The most interesting results were that revealed $\geq$60% DPPH scavenging activities. The investigated *Lysinibacillus fusiformis*, *Bacillus niacini* and *Vibrio parahaemolyticus* results are in agreement with the results of *Vibrio* sp., obtained from a brown alga in Portugal[9] and *Bacillus* sp., isolated from marine sediment[36]. *Bacillus* strains are also considering as a potential probiotic which could promote human health[37,38]. It was reported that *Bacillus halodurans, Bacillus licheniformis* and *Vibrio* sp., showed excellent antioxidant activities[10,38,39] which may be due to the presence of exopolysaccharides and phenolic compounds[2,40].

Lactobacillus plantarum are useful bacteria, previously isolated from marine environment and considered a producers of many health benefit products[41-43,]. In this research, its extract has the best activity in antioxidant production compared to the other bacterial extracts. Because lack of information about marine *Lactobacillus* as an antioxidant, our result is compatible to the study that revealed the antioxidant activities of *L. plantarum* against free radical DPPH from Kimchi, a Korean traditional fermented food. They suggested that the exopolysaccharides which was isolated from *L. plantarum* could be the reason of activities and they consider *L. plantarum* extract as natural antioxidants that play an important role against oxidative damage caused by free radical[43,44], thus the extract this isolate could be used as a replacement of synthetic antioxidant in food and drug applications.

Finally, phytochemicals are natural antioxidants present in bacterial extracts[34]. According to our results of the primary phytochemical revealed the presence of steroids, flavonoids, saponins, anthocyanin, betacyanin and tannins. Steroids, flavonoids and tannins in the most of bacterial extracts which may explain the antioxidant activities of them, in addition to other such as polysaccharides, fatty acids and proteins[23,45,46]. Many studies indicated a positive correlation between the antioxidant activities and the total phenolic compounds[47-49]. The type and quantity of the primary phytochemical depend on many factors such as the habitat, nature, season of collection, pollution[7,50,51]. In conclusions, the Red Sea is rich with various algal species that might be associated with many types of bacterial strains with antioxidant activities.

CONCLUSION

Antioxidant activities of the bacteria isolated from macro-algae of the Red Sea coasts clearly indicated their important potentiality. To our knowledge this could be the first study that has been done in Saudi Arabia on antioxidant activity of some associated bacteria with some marine algae. Although *Lactobacillus plantarum*, *Alteromonas australica*, *Bacillus niacini*, *Lysinibacillus fusiformis*, *Vibrio harveyi*, *V. parahaemolyticus*, exhibited great potential as antioxidant, *L. plantarum* was the superior in antioxidant activities as well as the synthetic antioxidant BHT. The phytochemicals revealed the active compounds in bacterial extracts that could be the reason for the activities. These extracts could be used in cosmetically, therapeutically and industrially sectors. More investigations are recommended to learn about the gold mine hidden in the algal associated bacteria of the Red Sea.

SIGNIFICANCE STATEMENT

This study sheds light for the first time on the bacteria associated with algae in the Red Sea and their antioxidant activities.

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