Acta Oceanol. Sin., 2022, Vol. 41, No. 3, P. 86–93
https://doi.org/10.1007/s13131-021-1926-8
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Characterization of some bacterial strains isolated from the Egyptian eastern and northern coastlines with antimicrobial activity of *Bacillus zhangzhouensis* OMER4

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Received 7 February 2021; accepted 26 March 2021

Abstract

Marine microorganisms were considered to be important sources of marine bioactive compounds. The major objective of the study was to isolate and characterize bacteria with antimicrobial activities from the various marine environment of Egypt. In this respect, thirty-five bacterial isolates were recovered from sediment samples collected from different spots along the Egyptian Red Sea coastline and Alexandria coastline during the summer season of 2017 and 2018. According to the morphological, physiological, and biochemical characteristics, the bacterial isolates were clustered into 13 groups designated as A, B, ···, M. And, 14 Gram-negative and 21 Gram-positive bacteria were determined. The isolated bacterial strains were screened for their potentiality for antimicrobial agent(s) production against ten indicator strains. Strain Mo13 was showed high antimicrobial activity against all empirical strains. Subsequently, the most promising marine bacterial isolate with code MO13 was identified as *Bacillus zhangzhouensis* OMER4 according to the phenotypic characterization through morphological, physiological, and biochemical tests as well as genotypic characterization through the 16S rDNA technique. The bioactive components were extracted with ethyl acetate, then analyzed using GC-MS and the substantial component was recognized as phenol, 2, 4-bis(1, 1-dimethyl ethyl).

Key words: marine bacteria, natural product, Egyptian coastline, antimicrobial, *Bacillus zhangzhouensis*, GC-MS characterization

Citation: Soliman O. Mohamed, Suleiman B. Waleed, Roushdy M. Mohamed , Elbatrawy N. Eman, Gad M. Ahmed. 2022. Characterization of some bacterial strains isolated from the Egyptian eastern and northern coastlines with antimicrobial activity of *Bacillus zhangzhouensis* OMER4. Acta Oceanologica Sinica, 41(3): 1–8, doi: 10.1007/s13131-021-1926-8

1 Introduction

The bacterial world is full of spectacular impact on the environment as a component of the ecosystem which deal with many functions whether harmful or beneficial some of the beneficial impacts is bioremediation, biosynthesis of natural products, and so on (Abdel-Razek et al., 2020). For more than seven decades, bacteria have been considered a bio-active source of natural products, some of which were developed into medicines to treat infections and other diseases (Sekurova et al., 2019). Currently, about 60% of confirmed small-molecule drugs are associated with natural products, also about 69% of all antibacterial compounds are extracted from natural products (Patridge et al., 2016; Suleiman, 2020). Microorganisms are considered as sources of valuable compounds such as PUFAs and lipids from oleaginous fungi (Hashem et al., 2020a, b, 2021) which could contribute in the field of sustainable development to help in biodiesel production. Natural products, especially antibiotics, have played an important role in drug exploration in the last several decades and have extended the process of developing medicines with a high level of therapeutic effect (Dias et al., 2012). However, widespread resistance was resulted because of the indiscriminate, prolonged, and wide use of antibiotics without physician description (Suleiman, 2017).

Terrestrial sources have previously been widely researched for the detection of new bioactive compounds (Ullah et al., 2019). However, nowadays, the detection of new sources of antimicrobial and other biologically active compounds from the marine habitat is growing increasingly although it is still under research (López et al., 2018). Marine microorganisms are rich sources of natural products with promising biological activities, especially bacteria and fungi (Barzkar et al., 2019). These microorganisms, particularly those of deep-sea origin, are thought to possess higher potential than terrestrial microorganisms for the production of various secondary metabolites with different bioactivities (Skropeta and Wei, 2014). Adaptations to severe environmental conditions such as low or high temperature, high salinity, high pressure, etc. might contribute to the development of structurally novel and biologically essential compounds by marine microorganisms (Manivasagan et al., 2013).

The current study was based on three main goals. Firstly, the examination of antimicrobial activity by marine bacteria isolated from sediment samples. Secondly, producing an antimicrobial

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agent(s) by marine samples isolated from the Egyptian eastern and northern coastlines. Thirdly, the bioactive compounds produced by marine origin extracts were performed using GC-MS for mass spectroscopic characterization.

2 Materials and methods

2.1 *Isolation of marine bacteria*

Isolation of marine bacteria from the sediment samples of the Red Sea and Mediterranean Sea (Alexandria, Egypt) (Table 1) was carried out by the spread plate method and serial dilution. One gram of collected samples was serially diluted in sterilized seawater to get a population range from 10^{-1} to 10^{-6}. Each dilution (1 mL) was platted out on marine nutrient agar plates that were incubated at 30°C for 48 h, and then counting aerobic bacteria. Colonies were collected depending on the morphological variations and Gram reaction among them, numbered and cultured on slants for further work. The bacterial isolates were further sub-cultured on nutrient agar (NA) medium in order to obtain pure cultures. For more studies, all pure isolates were maintained on slants at 4°C in the refrigerator for further studies.

2.2 *Numerical analysis and statistical analysis*

The similarity matrix was constructed based on the positive or negative of phenotypic characteristics (such as Gram staining, motility, cultural characteristics), physiological characteristics (such as temperature, pH tolerance range, and salinity tolerance range) and biochemical characteristics (such as catalase, oxidase, and IMViC test) for each isolate which was scored as 1 and 0, respectively. The data were analyzed and a dendrogram of cluster analysis was obtained using PRIMER v5.5 software (Rohlf, 1987).

2.3 *Antimicrobial screening of cell-free supernatants*

All marine bacterial isolates were tested for their ability to exhibit antimicrobial activities against three Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 25923) and four Gram-negative bacteria (*Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *and Vibrio cholerae* ATCC 14033). The antifungal screening was carried out against (*Candida albicans* ATCC 10231, *Aspergillus terreus* ATCC 10690, and *Fusarium solani* ATCC 36031). These pathogens were kindly provided by microbiology lab and marine microbiology lab collections at the National Institute of Oceanography and Fisheries (NIOF), Egypt.

The antagonistic activity of the marine bacterial cell-free supernatants was tested by a well-cut diffusion technique (Gad et al., 2016). Nutrient agar plates were swabbed with 100 μL of each strain of pathogenic bacteria using a sterile cotton swab. Approximately 5 mm diameter of the well was made with the help of a sterile gel puncture. Cell-free supernatants (50 μL) were loaded on well then, the plates were incubated at 30°C for 24 h. Antibacterial activity was expressed as the diameter (mm) of the inhibition zone (Amer and Ibrahim, 2019). The diameters of the inhibition zones produced by the supernatant were then compared with the standard antibiotic ciprofloxacin (5 μg/disc) used as positive control and dimethyl sulfoxide (DMSO) used as a negative control by displaying no inhibition zone.

By the same technique, the antifungal activity was performed against the three pathogenic fungi. Subsequently, the plates were incubated at 30°C for 48 h for *Candida albicans* whilst, 72 h or more in case of filamentous fungi. The activity was determined through the use of amphotericin B (100 units/disc) as a positive guide and DMSO used as a negative control by displaying no inhibition zone.

Table 1. Coordination of sampling sites along the Red Sea and Alexandria coastline

Station	Latitude	Longitude
Eastern Harbor	31.210 9°N	29.883 8°E
El-Shatby	31.211 1°N	29.911 5°E
Sidi Bishr	31.263 1°N	29.981 5°E
Abuqir	31.318 7°N	30.046 9°E
El-Gouna	27.401 6°N	33.682 6°E
NIOF Hurghada	27.238 5°N	33.866 6°E
Safaga Station	26.866 7°N	34.009 4°E
Marsa Alam	25.091 6°N	34.926 8°E
Shalatin Station	23.262 8°N	35.573 6°E

2.4 *Characterization of the selected marine bacteria*

2.4.1 *Phenotypic characterization*

Phenotypic characteristics such as Gram staining, motility, cultural characteristics, catalase, oxidase, and IMViC test were determined for all the marine bacterial isolates according to the standard methods. The effect of sodium chloride, pH level, and temperature on growth was also tested (Al-Allaf, 2011). Cell morphology was microscopically examined using electron microscope model JEOL-JSM 5300 at electron microscope unit of central laboratory of NIOF (El-Naggar et al., 2006).

2.4.2 *Genotypic characterization*

DNA of the promising bacterial isolates was extracted and purified using the genomic DNA extraction protocol of Gene JET™ genomic DNA purification Kit (Fermentas). The region of 16S rDNA was enlarged using Maxima Hot Start polymerase chain reaction (PCR) Master Mix (Fermentas) and cleaned using Gene JET™ PCR Purification Kit (Fermentas) using the protocol of Sigma Scientific Services Co., Egypt. The sequencing of the PCR product was performed by GATC Company using ABI 3730xl DNA sequencer by using the 16S DNA universal primer (27F: AGAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACTT).

2.5 *Extraction of the bioactive compounds*

Various solvents with different polarities (ethyl acetate, chloroform, n-hexane (95%), and n-butanol) were roughly selected for extracting the antimicrobial agents. One hundred milliliter of different organic solvents were added to 100 mL filtrated fermented broth in a 500 mL separating funnel. The mixture was shaken for 20 min and kept to separate the solvent from the aqueous phase. The organic phase was collected and evaporated, and then dissolved in an appropriate solvent. Antimicrobial activity was determined each time using the well-cut diffusion technique.

2.6 *GC-MS analysis*

The active extract was analyzed by using GC-MS (Suleiman et al., 2019; Elkhateeb et al., 2020). Preliminary identification of components was described using Wiley mass spectral database library according to the results of Hassan et al. (2016).

3 Results and discussion

The Mediterranean Sea is one of the world's biodiversity hotspots due to its high qualitative resources, in addition to a signi-

ficant ratio of endemics (Lejeusne et al., 2010), as well as the Red Sea serves as a unique resource of biological diversity by exporting organisms and genetic lines for global aquatic biodiversity systems (Bowen et al., 2013; DiBattista et al., 2013). During the last five years, the majority of the recorded marine species were obtained from the coasts of Egypt, accounting for 58% compared with many other sites on the Red Sea coasts. This may be due to the high biodiversity of species in the Egyptian environment (El-Hossary et al., 2020).

3.1 *Colony-forming unit investigation*

In our study, colony counts of the samples varied between 425 CFU/gm (ie., colony-forming units/granulocyte macrophage) and 2.7×10^6 CFU/gm of sediment. Eastern Harbor sediment harbored the highest average count (2.7×10^6 CFU/gm) followed by Abuqir (8.8×10^5 CFU/gm) and the lowest in Marsa Alam Station (425 CFU/gm). Sediments harbored more bacteria because of the richness in nutrients (Rédou et al., 2015). Eastern Harbor Station harbored more counts than the other stations, suggesting that it may be more polluted. These results are closed to what was observed by El-Bestawy et al. (2011) that the bacterial counts were extremely located in the central part of the coast of Alexandria, particularly during spring and winter. Bacteria with antagonistic activity highly existed in sediments of Eastern Harbor, El-Shatby, Sidi Bishr, and Abuqir as highly polluted sites, while in the other coasts (the Red Sea coasts) it existed less. These data may also suggest that the Mediterranean Sea coastline is more polluted than the Red Sea coastline. In agreement with our results, previous researches have described significant changes and lower diversity in aquatic bacterial communities, compared to comparatively more polluted coastal areas (Schauer et al., 2000; Ghiglione et al., 2005; Ullah et al., 2019).

3.2 *Isolation and screening of marine bacteria from the coastlines*

Based on locations, bacterial isolates (MO1–MO35) were isolated from sediment samples collected from the previously mentioned sites (Table 2). Out of the total isolates, 14 Gram-negative (40%) and 21 Gram-positive bacteria (60%) were detected. This is an adverse percentage with the results obtained by Hassan et al. (2016) as the isolates were observed to be Gram-negative (59.1%), while 40.9% displayed the Gram-positive organisms. However, some studies revealed that all isolates were Gram-positive (Attimarad et al., 2012; Syakti et al., 2019), while other studies revealed that all isolates were Gram-negative (Gupta et al., 2015a, b). After isolation, the total isolates were screened for antimicrobial agent(s) production.

3.3 *Numerical classification and characterization of the marine bacterial isolates*

Numerical taxonomy includes the collection of identifiable characteristics of the microorganisms, involving genetic relationships and has been applied for microbial taxonomy leading to reliable improvement in isolates identification (Kim, 2010).

The representative of all bacterial morphotypes observed from each sample was picked from primary recovered cultures and then stepwise purified on NA medium. Out of 122 bacterial colonies, 35 colonies were selected for numerical classification and antimicrobial activity studies (isolates codes MO1, MO2, MO5, MO7, and MO12 from the Eastern Harbor; isolates codes MO3, MO4, MO11, and MO13 from El-Shatby; isolate codes MO6, MO8, MO19, MO22, and MO26 from Sidi Bishr; isolates codes MO9, MO15, MO23, and MO35 from Abuqir; isolates code MO10 from El-Gouna; isolates codes MO14, MO16, and MO30 from NIOF Hurghada; isolates codes MO17, MO18, and MO33 from Safaga Station; isolates codes MO20, MO21, MO27, and MO29 from Marsa Alam; and isolates codes MO24, MO25, MO28, MO31, MO32, and MO34 from the Shalatin Station). All isolates were identified depending upon the physiological, morphological, and microscopical studies. Das et al. (2019) indicated that the morpho-physio-biochemical characters including the colony characters were not identical which proved that the structure and functions of the bacteria were different.

In our study, physiological and biochemical characteristics were detected for all 35 marine isolates. No unique physiological and biochemical features were detected among these isolates. A similarity matrix was constructed based on the positive or negative physiological and biochemical characteristics for each isolate which was scored as 1 and 0, respectively. At 85% similarity level, 13 clusters were obtained (A, B, C, D, E, F, G, H, I, J, K, L, and M). The colony colors of all isolates were varied between yellow, golden-yellow, or slightly yellow color. The cluster analysis of the 35 isolates was carried out using PRIMER MeV 4.9.0 software according to their various characters. The dendrogram for 35 isolates was carried out using MEGA_X_10.1.6 software.

3.4 *Screening for empirical antimicrobial agent(s) producer strains*

Bacteria produce protective compounds to protect themselves from any foreign organisms some of these have antimicrobial activity (Bhatnagar and Kim, 2010). The isolated bacterial strains were screened for their potentiality for antimicrobial agent(s) production against ten indicator strains. Displaying the varied spectra of antimicrobial actions was the aim behind the choice of these pathogens (Al-Amoudi et al., 2016). This wide spectrum activity is very common in marine bacteria (Abdel-Shakour et al., 2015). In our study, of the 35 isolates screened, 28 isolates showed potential antimicrobial activity evidenced through growth inhibition against at least one of the indicator strains as shown in Table 2. MO13 isolate exhibited both antibacterial and antifungal activity against the tested pathogenic indicators. Accordingly, the MO13 isolate was selected as the most potent isolate with a broad spectrum of antimicrobial activity in this study. According to Helal et al. (2018) who reported that 7 out of 80 bacterial isolates from the Red Sea showed antimicrobial activity against 5 reference strains, and the most potent isolate had been identified as *Bacillus amyloliquefaciens* which exhibited significant results against Gram-negative bacteria, Gram-positive bacteria, and yeasts. As well, fifty bacterial isolates were recovered from the invertebrates from the Red Sea, out of them, 5 isolates showed antifungal activities against *Candida albicans* (El Samak et al., 2018).

3.5 *Identification of the most promising marine bacterial isolate*

The most promising marine bacterial isolate (MO13) regarding the antimicrobial activity was submitted to the phenotypic characterization through morphological, physiological, and biochemical tests and submitted to genotypic characterization through the 16S rDNA technique.

3.5.1 *Phenotypic characterization*

The isolate (MO13) was grown on a nutrient agar plate with seawater. Some phenotypic characteristics of the selected isolate including colony and cell morphology, Gram reaction, catalase, and oxidase test in addition to some physiological and biochemical experiments have been depicted in Table 3. The results showed that isolate MO13 is a Gram-positive, *Bacillus* bacterium.

Table 2. Antimicrobial activity spectra of the marine isolates against different test microorganisms

Isolate code	Diameters of inhibition zones/mm									
	BS	EF	ST	V	EC	SA	PA	CA	FS	AN
MO1	21	15	–	20	17	19	–	26	17	20
MO2	20	19	–	20	17	18	17	24	16	23
MO3	25	20	19	25	–	–	19	20	–	21
MO4	16	17	18	36	16	17	17	18	–	21
MO5	21	26	19	30	42	28	17	–	19	19
MO6	18	20	14	37	32	40	24	–	13	–
MO7	23	–	22	38	20	36	25	–	–	–
MO8	17	–	28	28	29	34	20	18	10	18
MO9	14	19	29	28	36	31	13	13	19	20
MO10	19	18	20	21	31	35	19	21	29	19
MO11	21	26	19	27	29	29	21	19	18	12
MO12	31	28	20	33	37	31	14	19	19	10
MO13	45	48	30	41	41	35	25	30	19	29
MO14	21	09	18	29	09	29	29	–	9	9
MO15	21	12	–	27	07	17	26	21	–	–
MO16	–	09	09	31	14	18	19	09	–	–
MO17	–	21	19	28	13	20	18	08	9	–
MO18	17	–	–	18	–	–	–	–	–	–
MO19	19	29	29	19	09	20	28	28	26	13
MO20	17	17	18	14	17	–	18	17	10	19
MO21	22	14	–	20	29	21	22	21	19	–
MO22	19	09	–	–	–	–	–	–	–	–
MO23	–	–	–	–	–	–	–	–	–	–
MO24	–	–	–	–	–	–	–	–	–	–
MO25	18	17	09	–	–	09	–	–	–	–
MO26	27	20	19	–	–	08	09	–	08	9
MO27	22	21	23	28	–	29	27	18	09	1.9
MO28	27	20	19	–	–	08	09	–	08	0.9
MO29	22	21	23	28	–	29	27	18	09	1.9
MO30	–	–	–	–	–	–	–	–	–	–
MO31	–	–	–	–	–	17	–	–	–	–
MO32	–	–	–	–	–	–	–	–	–	–
MO33	–	–	–	–	–	–	–	–	–	–
MO34	–	–	–	–	–	–	–	–	–	–
MO35	–	–	–	–	–	–	–	–	–	–

Note: Data expressed unit in mm. Test organisms: BS was assigned for *Bacillus subtilis*; EF, *Enterococcus faecalis*; ST, *Salmonella typhi*; V, *Vibrio cholerae*; EC, *Escherichia coli*; SA, *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; CA, *Candida albicans*; FS, *Fusarium solani*; AN, *Aspergillus niger*. And – represents not detected.

It grew at 10–45°C, pH range 5–9, catalase, oxidase, Voges Proskauer, and glucose utilization are positive as shown in Table 3.

3.5.2 *Genotypic characterization*

Genomic DNA of the bacterial isolates MO13 was prepared, and the gene coding for the 16S rRNA was partially amplified using the universal primers (16S 27F and 16S 1492R). The amplified PCR fragment was purified and then sequenced. A valid sequencing fragment data (1 492 bp) was aligned against the 16S rRNA sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). According to the analyses of the 16S rRNA sequence of the bacterial isolate MO13; it showed a high similarity to level with *Bacillus* species; in particular, *B. zhangzhouensis*. Subsequently, its rDNA sequence had been submitted on gene bank and it takes an accession number of MT269497.1 and finally designated as *B. zhangzhouensis* strain OMER4 (Fig. 1). The phylogenetic tree of *B. zhangzhouensis* strain OMER4 was carried out using MEGA_X_10.1.6 software.

Bacillus zhangzhouensis first isolated from shrimp farm aquaculture water in Zhangzhou, China. The DNA G+C content of the type strain is 41.4% (mole percentage) (Liu et al., 2016). Recently, Karagoz et al. (2018) identified *B. zhangzhouensis* by 16S rRNA gene sequencing and the isolate has been proven to be an important agent in biocontrol of popular scab disease caused by *Streptomyces scabies* and have the ability of solubilizing phosphate and fixing nitrogen. *Bacillus* has gained a high degree of interest among bacterial genera because it produces a wide range of beneficial biomaterials that are very useful for the agricultural, food, chemical, and pharmaceutical sectors (Roongsawang et al., 2011).

Members of the genus *Bacillus* have recently been shown to generate a wide range of antimicrobial compounds against several microbial pathogens (Wu et al., 2018; Kelany et al., 2019). In agreement with our study, numerous previous researches have indicated that many *Bacillus* species isolated from sea sediments have shown more antagonism to Gram-positive bacteria, but less to Gram-negative bacteria (Kannahi and Eshwari, 2016; Sawale and Thivakaran, 2013). It may be attributed to variations in the

Table 3. Phenotypic characterization of the most promising marine bacterial isolate

Test isolate	MO13
Morphological characterization	
Shape	rod shape
Colony color	slightly yellow
Gram reaction	Gram-positive
Physiological characterization	
Temperature	10–45°C
Optimal growth temperature	30°C
pH tolerance range	5–9
Salinity tolerance range (NaCl)	0–10%
Biochemical characterization	
Catalase	+
Oxidase	+
Indole test from tryptophan	–
Methyl red test	–
Voges Proskauer test	+
Citrate test	–
H_2S production test	–

Note: + and – represent positive and negative reactions, respectively.

composition of Gram-positive and Gram-negative bacteria in the cell wall. Apart from Gram-positive bacteria, which has only one thick cell wall, Gram-negative bacteria have much more complicated structures in which antimicrobial peptides have to be crossed to associate with their molecules required during cell wall biosynthesis (Malanovic and Lohner, 2016).

4 Spectroscopic analysis of the crude extract using gas chromatography-mass spectroscopy (GC-MS)

Different solvents including ethyl acetate, chloroform, n-hexane (95%), and n-butanol were tested for their efficiency to extract the desired bioactive compounds. The crude extract resulting in using solvent was tested for its antimicrobial activity against the previously mentioned microbial indicators. The results revealed that ethyl acetate was the most efficient solvent for extracting the desired compounds and realized the highest antimicrobial activity. In agreement with our findings, ethyl acetate has been used as the best solvent for extraction of antimicrobial compounds from several *Bacillus* sp. (Berić et al., 2012; Malash et al., 2016; Farag et al., 2019).

The components of ethyl acetate crude extract were exposed to GC-MS analysis to preliminarily identify its components. As shown in Fig. 2a, the major constituent in the ethyl acetate crude extract at retention time 13.873 min was expected as Phenol, 2, 4-bis(1, 1-dimethyl ethyl). Figure 2b corresponds to molecular weight 206.16 g/mol and formula $C_{14}H_{22}O$. The mass spectrum of Phenol, 2, 4-bis(1, 1-dimethyl ethyl) is presented in Fig. 3.

The marine environment has a variety of macro-and microorganisms that have evolved specific metabolic capabilities to ensure their survival in varied and hostile environments, resulting in the biosynthesis of an array of secondary metabolites with specific activities. Several of these metabolites are high-value commercial products for the pharmaceutical and cosmeceutical industries (Martins et al., 2014). Phenol, 2, 4-bis(1, 1-dimethyl ethyl) (PD) (Fig. 3) also called 2, 4-Di-tert-butylphenol, 2, 4-di-t-Butylphenol, 1-Hydroxy-2, 4-di-tert-butylbenzene, Antioxidant No. 33, Prodox 146A-85X and 2, 4-bis(tert-butyl) phenol. PD has been re-

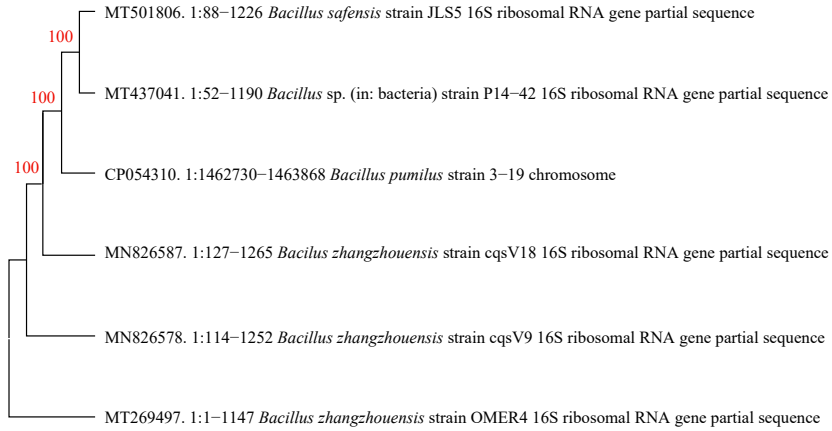

Fig. 1. Phylogenetic tree of *Bacillus zhangzhouensis* strain OMER4, 16S rDNA-based related bacterial species.

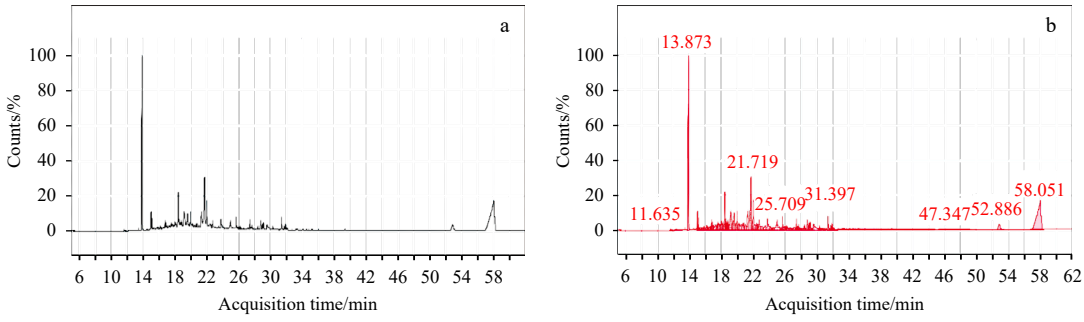

Fig. 2. GC-MS analysis of the ethyl acetate extract produced by *Bacillus zhangzhouensis* strain OMER4 (a) and mass spectrum of the major component (b).

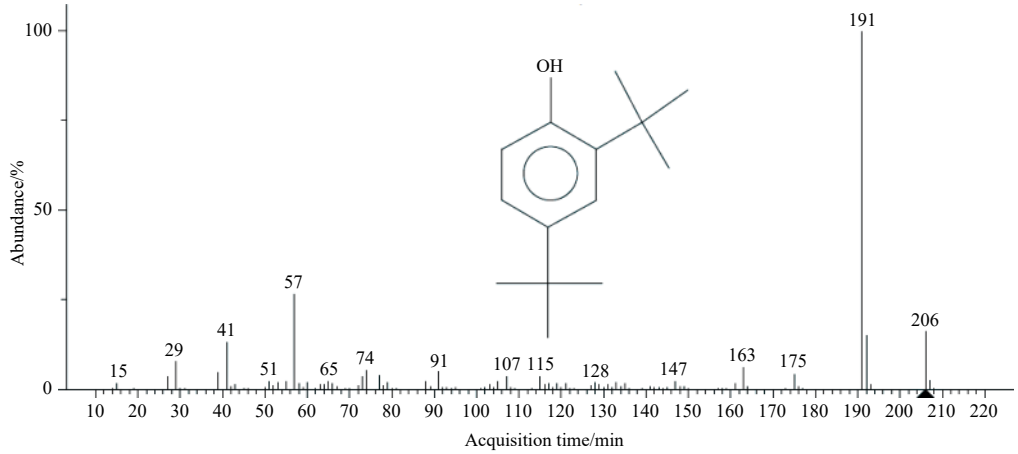

Fig. 3. The mass spectrum of the active metabolite, phenol, 2, 4-bis(1, 1-dimethyl ethyl) by marine bacterial isolate, *Bacillus zhangzhouensis* OMER4.

ported from various bacterial sources with antibacterial, antifungal, antioxidant, anti-biofilm, anticancer, and anti-inflammatory properties (do Amaral et al., 2014; Rao et al., 2015).

Previous studies reported the antifungal ability of PD against *Colletotrichum acutatum*, *Fusarium oxysporum*, and *Phytophthora capsici* (Sang et al., 2011; Sang and Kim, 2012; Dharni et al., 2014). A previous study suggested that PD changes the integrity of cell membranes to produce the antifungal effect against *Phytophthora cinnamomi* (Rangel-Sánchez et al., 2014; Teresa et al., 2014). However, in the other study, the PD of bacterial origin and the commercial compound did not exhibit any bactericidal effects. Apart from inhibiting and destroying biofilm, PD improved the sensitivity of *Serratia marcescens* to gentamicin when given symbiotically, providing another pathway for combination therapy where PD can be used to enhance the efficiency of traditional antibiotics (Padmavathi et al., 2014).

5 Conclusions

Microorganisms still represent a very important tool for multiple purposes, one of those roles is antibiosis by which the bacteria produce potential antimicrobial agents that cause inhibition of a wide range of other microorganisms including other genera of bacteria, yeasts, and other filamentous fungi. In the current study, screening of marine bacteria along two coastal lines in Egypt (the Red Sea and the White Mediterranean Sea) succeeded to isolate *B. zhangzhouensis* strain OMER4 which fortunately has a promising antimicrobial potential against a wide range of the test microorganisms including Gram-positive, Gram-negative bacteria and fungi. Ethyl acetate exhibits better results than the other investigated solvents, GC-MS is a very helpful tool to predict the active metabolites produced by the bacterial strain particularly, by using the most appropriate solvent which could dissolve the active metabolite to a degree that surpasses the other undesirable metabolites. The problem of multidrug resistance to antibiotics increased for several reasons, hence, there is a crucial need to incorporate new effective generations of antibiotics into the pharmaceutical market, and this study attempt to offer a natural effective antimicrobial agent to overcome the antimicrobial resistance. Our findings may contribute as a promising solution to the microbial infections which threaten the lives of humans, animals, as well as plants.

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