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# Isolation, distribution and evaluation of cytotoxic and antioxidant activity of cultivable actinobacteria from the Oman Sea sediments

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**Abstract**

Screening bioactive natural products from bacteria is a determinative step in the drug discovery programs. The present study aim to isolate actinobacteria from the Oman Sea sediments for determining the effects of different culture media and treatments on the yield of the isolation process, and measure the DPPH radical scavenging and Artemia cytotoxic activity of culture extracts of the actinobacterial isolates. A total of 290 actinobacterial isolates were collected from 14 sediment samples. Heat treatment (40.68%) and $M_4$ medium (29.31%) exhibited the maximum isolation rates of actinobacteria. *Streptomyces* isolates were dominantly distributed in all of the investigated stations according to 16S rRNA gene sequencing. The distribution pattern of *Streptomyces* followed a depth-dependent frequency trend, whereas the members of rare genera including *Micromonospora*, *Nocardia Actinoplanes*, *Nocardiopsis*, *Saccharopolyspora* and *Crossiella* were distributed in deeper stations. Approximately, 25% of the examined isolates could scavenge 90% of $10^{-4}$ mol/L DPPH solutions at 1 250 μg/mL final concentration of their ethyl acetate culture extracts. Furthermore, the most potent extracts could scavenge DPPH radicals with $IC_{50}$ ranges from 356.8 to 566.4 μg/mL. Brine shrimp cytotoxicity tests showed that 38.88% of the examined culture extracts exhibited $LC_{50}$ lower than 1 000 μg/mL against the Artemia cells. Moreover, the most potent culture extracts exhibited $LC_{50}$ range from 335.4 to 534.4 μg/mL. Phylogenetic analysis by 16S rRNA gene sequence revealed that the OS 005, OS 263 and OS 157 closely related to *Streptomyces djakartensis*, *Streptomyces olivaceus* and *Nocardiopsis dassonvillei* respectively. These results suggested the widespread distribution of the antioxidant and cytotoxic producing actinobacteria in the Oman Sea sediments, which could be considered as promising candidates for the discovery of microbial bioactive compounds.

**Key words:** antioxidant activity, cytotoxic activity, marine actinobacteria, Oman Sea, phylogenetic analysis

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## 1 Introduction

Actinobacteria are gram positive and high G+C content bacteria that form the largest phylum in the bacterial domain. Moreover, they are considered as the most important bacteria in microbial biotechnology (Manivasagan et al., 2014). They also have produced the maximum percentage of bioactive/non-bioactive constituents (47.01%) that is more than the average proportion among the whole marine organisms (28.39%) (Olano et al., 2009). These fascinating characteristics attracted the attention of researchers to isolate actinobacteria from marine habitats especially from sponges, corals, mangroves and sediments for their therapeutic compounds (Gozari et al., 2019a; Mahmoud and Kalendar, 2016; Ser et al., 2017). Some of the cytotoxic agents that produced by sediments inhabiting actinobacteria are salinosporamide A, carpatamides, daryamides and pyridinium (Asolkar et al., 2006; Dasari et al., 2012; Fenical et al., 2009; Fu et al., 2014). In addition, the secondary metabolites of marine actinobacteria were also reported as potent antioxidant agents. For instance, *Streptomyces malaysiense* sp. strain MUSC 136 isolated from mangrove forest soil showed DPPH radical scavenging activity with inhibition percentage of 27.24% at 2 mg culture extract per milliliter of 0.016% DPPH solution (Ser et al., 2016). Since the adaptation to harsh environmental conditions in marine microenvironments could promote genetic changes in the actinobacteria, new biosynthesis pathways could develop for unique bioactive compounds to cope with these conditions

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(Gontang et al., 2010; Zotchev et al., 2017). Hence, isolation and preservation of the actinobacteria from a less-discovered environment such as the Oman Sea are essential to conserve their valuable biotechnological potentials. The Oman Sea is a part of the Indian Ocean that has various environmental conditions with particular hydrological pattern and its sediments derived from different sources (Karamouz et al., 2012), which rarely has been explored for the sediment inhabiting actinobacteria. Consequently, the main goal of the present investigation was the selective isolation of the actinobacteria from the Oman Sea sediments. Various sample treatments and culture media were used to increase the recovery rate of the cultivable actinobacteria. The antioxidant and cytotoxic activity of the isolated actinobacterial populations in the sediments were measured, and the most potent isolates were identified genetically.

## 2 Materials and methods

### 2.1 *Collection of samples*

Marine sediment samples were collected from 14 locations along the northern part of the Oman Sea in Hormozgan Province, Iran in December 2015. The exploration area was located between longitudes 57° to 59°30′E and latitudes 25° to 27°N. Depth and position of each station were recorded (Fig. 1). A Van Veen Grab sampler (Hydro-bios, Germany) was used for collecting sediments samples. The collected samples were transferred to 50 mL sterile collection vials, and kept refrigerated until processing (usually within 24 h) (Maldonado et al., 2009). The depth of sampling stations and some physicochemical properties of water were recorded by CTD system.

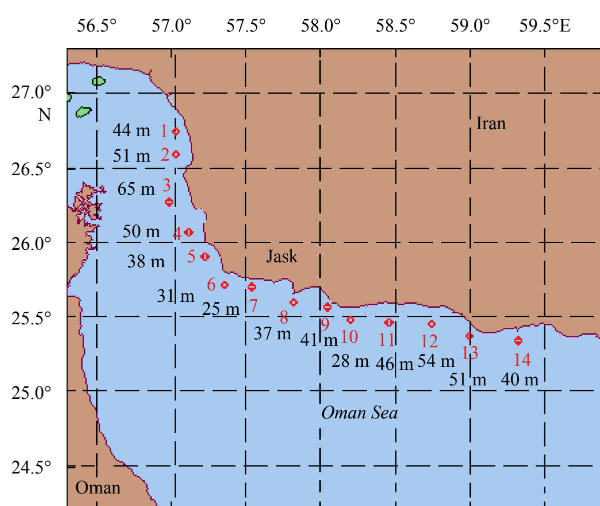


**Fig. 1.** Geographical locations and depth of sampling stations in the Oman Sea.

### 2.2 *Isolation of actinobacteria*

2.2.1 *Physical and chemical treatments*

Five physical and one chemical treatments were applied to the samples. Heat treatment was applied by incubating the diluted samples at 50°C in water bath for 1 h (Hameş-Kocabaş and Uzel, 2012). Desiccation treatment was done by drying samples under laminar flow for 2 weeks (Jensen et al., 2005). Sediment samples were irradiated by UV 254 nm at 20 cm distance on the samples for 30 s in a UV chamber (Bredholt et al., 2007). Ultrasonic treatment of the samples was taken in an ultrasonic bath with 40 kHz power for 2 min and at 30°C (Qiu et al., 2008). Sediments samples were treated at –21°C for 24 h in a freezer for the freezing treatment (Jensen et al., 2005). Chemical treatment was done by adding 1.5% phenol in the final sample solution and incubated at 30°C for 30 min (Bredholt et al., 2008).

2.2.2 *Inoculation of the sediments samples in the isolation media*

A total of 200 µL of serially diluted samples ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$) in sterilized seawater were inoculated in the following culture media. $M_1$ (1% starch, 0.03% casein, 0.2% $KNO_3$, 0.2% $K_2HPO_4$, 0.005% $MgSO_4{\cdot}7H_2O$, 0.002% $CaCO_3$, 0.001% $FeSO_4{\cdot}7H_2O$, 1.8% agar in filtered seawater), $M_2$ (0.5% peptone, 0.1% yeast extract, 0.08% $MgCl_2$, 0.6% $CaCl_2$, 1.5% agar in filtered seawater), $M_3$ (10% aqueous extract of marine sediments, 1.5% agar in filtered seawater), $M_4$ (0.5% glucose, 0.1% asparagine, 0.1% $K_2HPO_4$, 1.8% agar in filtered seawater), $M_5$ (1.8% agar in filtered seawater) and $M_6$ (0.5% glycerol, 0.1% arginine, 0.1% $K_2HPO_4$, 1.8% agar in filtered seawater). All the isolation media were supplemented with 100 mg/L cycloheximide and 25 mg/L nalidixic acid. Inoculated media were incubated at 28°C and examined for growth up to 4 weeks.

### 2.3 *Preliminary characterization of actinobacteria*

All of the colonies were preliminary characterized based on macromorphological properties including color, shape, size and the form of colonies and micromorphological description such as shape and arrangements of the isolates in Gram staining (Whitman et al., 2012). Finally, the distinct isolates were selected for identification by 16S rRNA sequencing.

### 2.4 *Production and extraction of bioactive metabolites from culture medium*

The purified distinct isolates were inoculated in 100 mL $M_2$ medium and incubated in a shaking incubator at 28°C and 220 r/min. After the five days of the incubation, the filtered fermentation broths were extracted with equal volume of ethyl acetate twice (1:1, v/v) and evaporatedin rotary evaporator. The culture extracts of the distinct isolates were examined for antioxidant and cytotoxic activity after concentration and preparation (Seidel, 2006).

### 2.5 *Antioxidant bioassay for the culture extracts of the actinobacteria*

The DPPH radical scavenging activity of the culture extracts of the distinct isolates was determined by the microdilution method at the final concentration of 1 250 µg/mL (Gozari et al., 2018; Leong and Shui, 2002). $IC_{50}$ of the most potent isolates with >90% scavenging activity was determined at seven final concentration (1 250, 625, 312, 156, 78, 39, 19.5 µg/mL). Five microliters of each primary concentration were added to 195 µL DPPH solution at 100 µmol/L concentration in methanol. The 96 well microplates were incubated at room temperature in the dark for 30 min. The absorbance of samples was measured by Microplate Reader (BioTech instrument) at 517 nm. Ascorbic acid was taken as the positive control. The scavenging activity (%) of the samples was calculated by the following equation:

$$\text{scavenging activity} = (I_0 - I_s/I_0) \times 100\%,$$

where $I_0$ is the absorbance of the untreated DPPH solution, and $I_s$ is the absorbance of the samples or the standard control in the DPPH solution.

### 2.6 *Cytotoxicity of the culture extracts of the actinobacteria*

Cytotoxic activity of the culture extracts of the distinct isolates was assayed by brine-shrimp microwell cytotoxicity method (Atta-ur-Rahman et al., 2001). One gram of *Artemia franciscana* cysts (INVE ™) was inoculated in 2 L filtered seawater with salinity 30 and incubated at 22–29°C under white light for 48 h. After the collection of nauplii cells, 100 μL nauplii suspension (10–15 nauplii/100 μL) was added to 100 μL extracts at 1 000 μg/mL in each 96-well microplate and incubated at 25°C for 24 h. The number of live and dead nauplii was recorded and potent extracts were determined. $LC_{50}$ of the most potent isolates with >90% cytotoxic activity was determined at lower concentration (500, 250, 125 and 62.5 μg/mL) by the following equation:

$$\text{cytotoxic activity} = (N_{\text{control}} - N_{\text{test}}/N_{\text{control}}) \times 100\%,$$

where $N_{\text{test}}$ is the number of live nauplii at treated well, and $N_{\text{control}}$ is the number of live nauplii at untreated well.

### 2.7 *Molecular identification and phylogenetic analysis of potent isolates*

Extraction of genomic DNA of the distinct isolates was performed based on CTAB method defined by Kieser et al. (2000). The 16S rRNA gene was amplified through PCR reaction using 16S universal primers 27F (5′ to 3′AGAGTTTGATCCTGGCTCAG) and 1492R (5′ to 3′ACGGCTACCTTGTTACGA). The thermal cycles were optimized as follow: (95°C for 4 min, 95°C for 1 min, 60°C for 1 min and 72°C for 2 min) × 35 cycles followed by final extension at 72°C for 10 min. The amplified PCR products were purified by Qiaquick PCR purification kit (QIAGEN, USA). Consequently, the 16S rRNA gene was sequenced by Macrogen Company (Seoul, Korea and were analyzed using the BLAST program at NCBI (National Centre for Biotechnology Information) server (Madden, 2013). Phylogenetic analyses of the 16S rRNA gene of the distinct isolates were constructed using neighbor joining algorithm (Saitou and Nei, 1987) with MEGA 7 program (Kumar et al., 2016). The 16S rRNA gene sequences were registered to NCBI GenBank database.

### 2.8 *Statistical analyses*

All the experiments were performed in triplicates. The results of the bacterial isolation were reported as the percentage of the *Streptomyces*-like/non-*Streptomyces*-like isolates to the whole actinobacterial isolates. The antioxidant potential and artemia cytotoxicity assay presented as mean $IC_{50}$ and $LC_{50}$ ± standard error (SE), respectively. The $LC_{50}$ and $IC_{50}$ values of the extracted metabolites and their 95% confidence intervals were determined using the non-linear regression by GraphPad PRISM version 6 (GraphPad Software, San Diago, CA). The levels of statistical significance ($p$<0.05) of the data were calculated with oneway ANOVA followed by LSD using SPSS program (Version 24). The topology of the resultant phylogenetic tree was evaluated by bootstrap resampling with 1 000 replicates (Felsenstein, 1985) with MEGA7.

## 3 Results

Overall, 290 isolates of the actinobacteria were derived from 14 sediment samples throughout the northern parts of the Oman Sea in Hormozgan Province, Iran. The actinobacterial isolates were widely spread along the exploration area, especially in the samples collected from Stas 5, 6, 7, 8, 10, 11 and 12 (Fig. 2). Moreover, preliminary characterization exhibited that the *Streptomyces*-like isolates were dominant in most of the stations. Among the selected isolation media, $M_4$ medium significantly resulted in the growth of the highest number of actinobacterial isolates (29.31%), while $M_6$, $M_1$, $M_3$, $M_2$ and $M_5$ media yielded 21.72, 18.96, 14.13, 10.34 and 5.51% of the isolates respectively. Most of the isolated colonies in different media belonged to *Streptomyces*-like isolates (Fig. 3). Comparison of sample treatment performances revealed that heat treatment recovered 40.68% of the actinobacterial isolates at all the culture media and significantly exhibited more efficiency than drying (17.93%), UV (13.10%), phenol (10.68%), freezing (3.79%) and sonication (3.44%) treatments (Fig. 4). Furthermore, *Streptomyces*-like isolates were predominant in heat (64.40%), UV (63.15%), drying (61.53%) treatments, whereas freezing (45.45%), sonication (40%) and phenol (3.22%) treatments promoted the non-*Streptomyces*-like isolates (Fig. 4). According to our preliminary characterization results, 36 distinct isolates were selected for bioassay experiments. The results of antioxidant activity showed that 69.44% extracts of distinct isolates could scavenge more than 50% of DPPH radicals at 1 250 μg/mL. While 25% of them showed more than 90% scavenging activity. The $IC_{50}$ values of the crude extracts of the most potent isolates varied from 356.8 to 566.4 μg/mL (Table 1). Cytotoxicity assay revealed that 13.88% of the distinct isolates showed more than 90% cytotoxic activity against *Artemia franciscana* nauplii at 1 000 μg/mL. The crude extracts of the most potent isolates exhibited $LC_{50}$ values ranged from 335.4 to 534.4 μg/mL (Table 2). Analysis of the 16S rRNA gene sequences of 36 distinct isolates confirmed that *Streptomyces* with 25 strains established dominant group in the structure of the actinobacterial community. However, rare actinobacteria including *Micromonospora* (3 strains), *Nocardia* (3 strains), *Actinoplanes* (2 strains), *Nocardiopsis* (1 strain), *Saccharopolyspora* (1 strain) and *Crossiella* (1 strain) were isolated especially from deeper stations. The

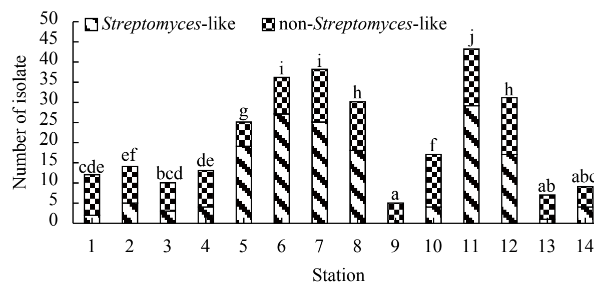


**Fig. 2.** Distribution of cultivable actinobacteria in the sediments of different positions of the Oman Sea. Values carrying same alphabet did not vary significantly from each other (LSD<0.05).

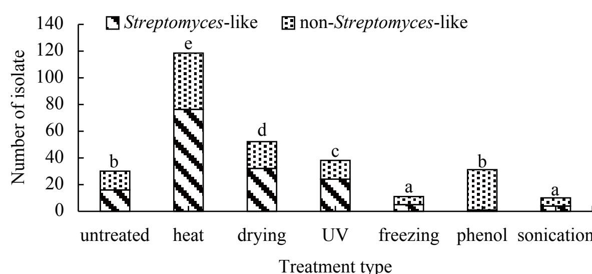


**Fig. 3.** The effect of different pretreatments on isolation of actinobacteria from collected Oman Sea sediments. Values carrying same alphabet did not vary significantly from each other (LSD<0.05).

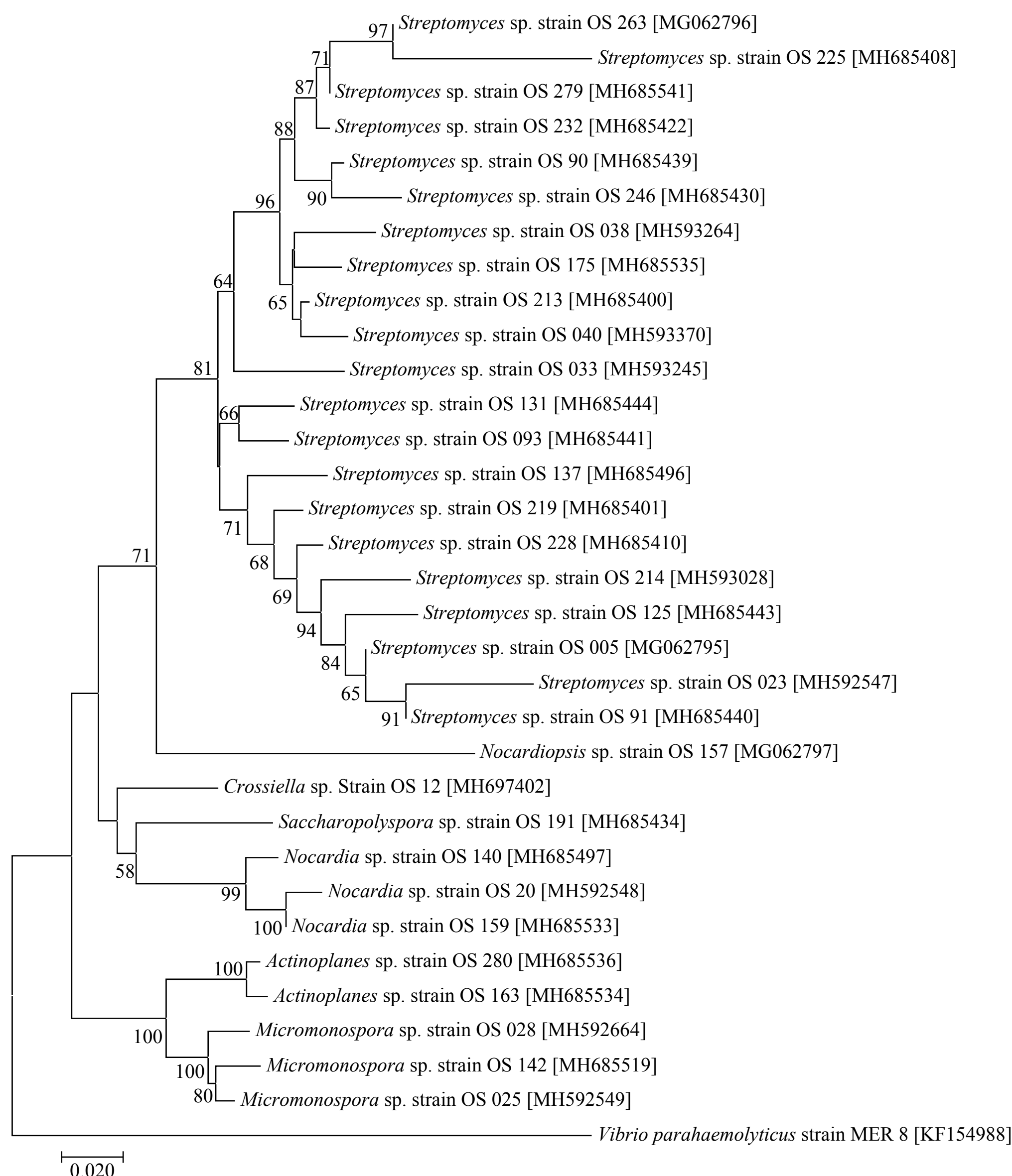


**Fig. 4.** Phylogenetic tree based on 16S rRNA gene sequence analysis, reconstructed from evolutionary distances by using the neighbour-joining method, showing the phylogenetic position of the distinct actinobacterial isolates. *Vibrio parahaemolyticus* mer 8 was used as an out-group. Bootstrap values are indicated at the relevant branching points. Number of branch node are bootstrap value based on 1000 resampling. Bar: 0.05 substitutions per nucleotide position.

phylogenetic analysis showed different similarity values between the most potent strains and their closest type strains. So that, the *Streptomyces* sp. strain OS 005, *Streptomyces* sp. strain OS 263 and *Nocardiopsis* sp. strain OS 157 showed 99%, 98% and 97% similarity with *Streptomyces djakartensis* strain NBRC 15409, *Streptomyces olivaceus* strain NBRC 12805 and *Nocardiopsis dassonvillei* subsp. *albirubida* strain NBRC 13392 respectively. The neighbor-joining tree showed that the distinct isolates clustered separately in different clades. The *Actinoplanes* strains formed shared clade with the strains of *Micromonospora*, which located in different clusters. The *Streptomyces* strains located in a big clade and the strains of *Nocardia*, *Saccharopolyspora* and *Crossiella* located in different clusters of a same clade (Fig. 4).

## 4 Discussion

Recovery of the highest rate of biodiversity and frequency of the actinobacterial isolates from less-explored environments can maximize hit rate to find unique bioactive strains (Zotchev et al., 2017). For this purpose, a combination of isolation media and treatments were implemented to describe the distribution pattern of cultivable actinobacteria in the exploration area. This pattern revealed that actinobacterial populations frequently distributed in the shallowest and nearshore stations. Moreover, *Streptomyces*-like isolates followed by a depth dependent frequency pattern (Fig. 2). Hence, the number of *Streptomyces*-like isolates decreased with increasing distance from shore. In spite of the fact that environmental factors could be shaped structure of bacterial community in marine sediments, our results showed that the observed distribution pattern of actinobacteria could not be influenced by environmental factors. Our results revealed that pH, salinity, oxygen and temperature did not show drastic variation between all stations (Table 3). In particular, variation of these en-

**Table 1.** DPPH radical scavenging activity of secondary metabolites extracted from the selected actinobacterial isolates

| Isolate No. | $IC_{50}$ ±SE /μg·mL$^{-1}$ | 95% confidence intervals | Isolate No. | $IC_{50}$ ±SE /μg·mL$^{-1}$ | 95% confidence intervals |
|---|---|---|---|---|---|
| OS* 005 | 358.6±47.20 | 253.4–463.8 | OS 142 | 566.4±11.36 | 542.6–590.2 |
| OS 023 | 398.9±27.53 | 341.3–456.5 | OS 157 | 356.8±15.65 | 324.1–389.6 |
| OS 038 | 501.6±28.49 | 441.9–561.2 | OS 237 | 428.5±12.00 | 403.4–453.6 |
| OS 084 | 448.8±16.95 | 413.3–484.3 | OS 263 | 452.4±24.00 | 402.2–502.7 |
| OS 093 | 516.7±19.28 | 476.4–557.1 | | | |

Note: * The Oman Sea. Values are means of three replicates ±SD and were statistically analyzed by one-way ANOVA and were found to be significantly different as compared to control at 5%.

**Table 2.** Cytotoxic activity of secondary metabolites extracted from the selected actinobacterial isolates

| Isolate No. | $LC_{50}$ ±SE/μg·mL$^{-1}$ | 95% confidence intervals |
|---|---|---|
| OS 005 | 417.9±20.51 | 372.2–463.6 |
| OS 157 | 335.4±20.92 | 288.7–382.0 |
| OS 237 | 534.4±20.07 | 489.7–579.2 |
| OS 246 | 507.5±41.93 | 414.0–600.9 |
| OS 263 | 475.6±22.01 | 426.6–524.6 |

Note: Values are means of three replicates ±SD, analyzed by one-way ANOVA and were significantly different compared to control at 5%.

vironmental parameters in the deeper stations cannot limit the distribution of *Streptomyces* compared with other actinomycetes. Accordingly, this depth dependent pattern could be influenced by runoff currents such as the Jagin, Minab, Gabrik, Sadij Rivers and seasonal streams that carry terrestrial material including high frequency of *Streptomyces*. The higher prevalence of *Streptomyces* in terrestrial environments rather marine environments was previously approved (Kurtböke, 2017). Therefore, runoff sedimentation can influence the distribution pattern of *Streptomyces* in coastal sediments, although other factors should also be considered. These findings are consistent with the reported distribution pattern of actinobacteria in marine sediments of the Oman Sea, Trondheim Fjord, Persian Gulf and Bahamas (Bredholt et al., 2008; Gozari et al., 2016b; Gozari et al., 2019b; Jensen et al., 1991). The results of selective isolation process showed that $M_4$ medium recovered the highest frequency of actinobacterial isolates, although $M_3$ (56.09%) and $M_5$ (62.50%) media exhibited high isolation rate of the non-*Streptomyces*-like isolates that might be because of their low organic content and their close composition to the marine environment. Hence, many of the undesired fast growing bacteria could not grow on the above mentioned minimal media (Doelle, 2014; Mann and Lazier, 2013). Among the treatments, heating could remove fast growing temperature-sensitive bacteria in the favor of actinobacterial isolates. The efficiency of heat treatment to maximize the isolation rate of actinobacteria was also shown in other studies (Gozari et al., 2016a; Jensen et al., 2005; Mincer et al., 2005). The selective performance of phenol treatment in the isolation of non-*Streptomyces* like isolates is due to the destruction of polyamide polymers in the protein structures of *Streptomyces* by phenol, while many of the non-*Streptomyces* isolates show lower sensitivity to phenol due to their lipid-rich cell wall (Istianto et al., 2012). Qiu et al. (2008) have reported the selective effect of phenol treatment in favor of *Micromonospora* species. Antioxidant activity screening of the culture extracts of the bacteria revealed the high potential of DPPH radical scavenging activity of the isolated actinobacteria. Recent studies showed that the presence of oil pollutants such as polyaromatic hydrocarbons ($PAH_s$), heavy metals and xenobiotics increases the levels of oxidative pressure in marine sediments. Consequently, the marine sediments inhabiting organisms increase their antioxidant activity to neutralize this pressure (Solan and Whiteley, 2016). Determination of antioxidant activity of the most potent isolates indicated that the OS 157 culture extract exhibited the highest activity with $IC_{50}$ value of 356.8 μg/mL (Table 1). In this regard, Lee and his colleagues showed that ethyl acetate culture extract of the *Streptomyces* sp. strain MJM 10778 could scavenge DPPH radicals with $IC_{50}$ value of 92.8 μg/mL (Lee et al., 2014). In another study, the crude extract of the *Streptomyces* sp. LK-3 showed DPPH scavenging activity with $IC_{50}$ value of 41.09 μg/mL (Karthik et al., 2013). The results of cytotoxic activity screening showed that 38.88% extrac-

**Table 3.** Samples location information

| Sample No. | North latitude | East longitude | Depth/m | pH | Salinity | Temperature/°C | Date (day/month/year) | Time |
|---|---|---|---|---|---|---|---|---|
| 1 | 26°42′18″ | 57°00′11.9″ | 44.11 | 8.01 | 36.75 | 25.11 | 2/12/2015 | 12:20 |
| 2 | 26°37′08.3″ | 57°01′04.2″ | 51.36 | 7.85 | 36.68 | 24.32 | 2/12/2015 | 15:55 |
| 3 | 26°15′26.3″ | 56°27′12.2″ | 65.18 | 7.91 | 36.65 | 23.94 | 2/12/2015 | 19:40 |
| 4 | 26°05′02.9″ | 57°08′06.3″ | 50.14 | 7.96 | 36.71 | 24.46 | 2/12/2015 | 22:15 |
| 5 | 25°50′25.8″ | 57°12′48.6″ | 38.26 | 8.01 | 36.82 | 24.85 | 3/12/2015 | 01:15 |
| 6 | 25°41′06″ | 57°21′49.4″ | 31.33 | 8.08 | 36.85 | 24.48 | 3/12/2015 | 06:45 |
| 7 | 25°41′22.1″ | 57°35′07.6″ | 25.17 | 7.86 | 36.66 | 26.91 | 3/12/2015 | 07:50 |
| 8 | 25°35′13.7″ | 57°49′04.7″ | 37.26 | 8.04 | 36.58 | 25.94 | 3/12/2015 | 11:25 |
| 9 | 25°36′27.3″ | 58°03′35.4″ | 41.13 | 8.07 | 36.79 | 24.58 | 3/12/2015 | 15:15 |
| 10 | 25°28′42.3″ | 58°12′09.8″ | 28.30 | 7.90 | 36.48 | 25.78 | 3/12/2015 | 18:15 |
| 11 | 25°27′18.4″ | 58°25′09.1″ | 46.22 | 8.01 | 36.63 | 24.63 | 3/12/2015 | 23:50 |
| 12 | 25°25′17.5″ | 58°45′08.6″ | 54.15 | 7.89 | 36.59 | 24.15 | 4/12/2015 | 06:10 |
| 13 | 25°21′21.2″ | 58°59′28.9″ | 51.12 | 7.98 | 36.55 | 24.37 | 4/12/2015 | 11:40 |
| 14 | 25°18′15.5″ | 59°25′16.7″ | 40.29 | 8.05 | 36.44 | 24.90 | 4/12/2015 | 17:55 |

Note: The temperature (°C), salinity and pH of water were recorded by CTD system.

ted secondary metabolites from the distinct isolates exhibited $LC_{50} \leqslant 1\ 000$ μg/mL. Therefore, these results approved their bioactivity according to accepted criteria (Meyer et al., 1982). In this context, the study of Kesavan (2015) showed that 13.46% isolated actinobacteria from marine sediments represented $LC_{50}$ value lower than 1 000 μg/mL by brine shrimp cytotoxicity assay. The result of our study showed that the extracted secondary metabolites from OS 157 isolate exhibited maximum cytotoxic activity with $LC_{50}$ value of 335.4 μg/mL (Table 2). In another study, the extracted metabolites from *Streptomyces* sp. strain CAS isolated from marine sediments exhibited $LC_{50}$ value of 23.5 μg/mL against Artemia nauplii (Palaniappan et al., 2013). Phylogenetic analysis demonstrated that there were significant evolutionary distances between selected potent strains and their closest type strains. Therefore, these putative strains might have undergone mutations in the 16S rRNA gene during the adaptation process with the microenvironments in marine sediments (Orr, 2005). Accordingly, their biosynthetic gene clusters also might be encountered mutation that led to the development of the new biosynthetic pathways and, consequently, the novel bioactive compounds. In conclusion, we have introduced a selective isolation process, including media and treatments that could exploit frequent and diverse taxonomic groups of marine actinobacteria. This study revealed the distribution pattern and diversity of actinobacterial populations in the north part of the Oman Sea sediments in Hormozgan Province, Iran and represented promising strains for the discovery of cytotoxic and antioxidant agents.

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