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Isolation and characterization of marine-derived actinomycetes with cytotoxic activity from the Red Sea coast

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ABSTRACT

Objective: To isolate and evaluate the cytotoxic activity of different actinomycetes species isolated from the Red Sea coast in Sharm el-Sheikh, Egypt.

Methods: Forty actinomycetes strains were isolated from different sediments and seawater samples collected from the Red Sea coast in Egypt. Actinomycetes were recognized by morphological and microscopic examinations. Cell viability and cytotoxicity induced by the crude extracts on breast cancer cell lines MDA-MB-231 were assessed using methylene blue assay. The strains with promising cytotoxic activity were identified by sequencing and amplifying the 16S rRNA genes. The antibacterial activities of the crude extracts were performed using Kirby–Bauer disc diffusion method.

Results: The results indicated that five ethyl acetate extracts exhibited cytotoxicity towards breast cancer cell lines MDA-MB-231. The highest cytotoxic activity was found for the ethyl acetate extracts of EGY2 and EGY39. The isolate EGY3 was identified as a new *Streptomyces* species, while the actinomycete EGY22 was found to be a member of the genus *Nocardiopsis* sp. The crude extract of the isolate EGY8 showed slightly high antimicrobial activity against different test microorganisms.

Conclusions: The results of the present study reveal that marine sediments of the Red Sea are a potent source of novel species of actinomycetes. The isolates may be useful in discovery of novel bioactive compounds and an important step in the development of microbial natural product research.

1. Introduction

Breast cancer is one of the leading causes of cancer death among women in worldwide [1]. Incidence rates are high in more developed countries and low in less developed countries. In Egypt, breast cancer is the most common cancer among women and representing 18.9% of total cancer cases [2]. As per literature, the natural products derived from microorganisms provide an excellent source of cancer medication [3]. Among such microorganisms, Gram-positive actinomycete bacteria are of special interest. They are known to produce chemically diverse compounds with a wide range of biological activities [4–8]. From the ecological point of view, several species of actinomycetes (*i.e.*, *Streptomyces* and *Nocardiopsis*) are frequently distributed in marine environments such as oceans, rivers and seas. They are dwelled in sponges, marine sediments, sea sands and water [9].

The Red Sea is one of the most spectacular coastal and marine environments in Egypt and has a rich biodiversity. The sea has a number of unique marine habitats including sea-grass beds, salt-pans, mangroves, coral reefs, salt marshes, numerous

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fish species and different microbial communities [10,11]. According to best of our knowledge, little studies have been conducted to isolate actinomycetes from the Red Sea's seawater and sediments [12,13]. This study aimed to isolate different actinomycetes from seawater and marine sediments of the Red Sea and evaluate the cytotoxic activity of their crude extracts against breast cancer cell line (MDA-MB-231). Additionally, the extracts were assayed against different microbial pathogens. The strains that have cytotoxic activity were phylogenetically characterized based on the 16S rRNA gene sequencing.

2. Materials and methods

2.1. Samples collection

Twenty two sediments and sea water samples were collected from different parts of the Red Sea in Sharm el-Sheikh, South Sinai, Egypt. The collection sites were Sharks Bay, Umm Marikha Bay, Ras Mohammed and Ras Um Sid. Samples were collected at ~10 m depths below the water surface and kept at 4 °C for further working up.

2.2. Isolation of actinomycetes

From each sample, 1 g was dispersed in 9 mL of sterilized water and vortexed for 2 min. The samples were subjected to heat treatment in a water bath at 60 °C for 10 min to eliminate non-sporulating bacteria. Following serial dilution (10^{-1}, 10^{-2} and 10^{-3}) of the suspension with sterile water, a 100-μL aliquot was spread on humic acid-vitamin agar [14] and starch-casein agar [15]. All media were prepared in 50% sea water and supplemented with nalidixic acid (75 μg/mL) and cycloheximide (50 μg/mL). The plates were incubated at 30 °C for 7–30 days until the colonies appeared. Forty actinomycete strains were picked up and took a voucher numbers (EGY1–EGY40).

2.3. Morphological characterization

Characterization of the isolated strains was performed by morphological methods [16].

2.4. Fermentation and extraction of metabolites

To prepare the cultures, chunks of well-grown agar plate of each strain were used to inoculate 2 × 100 cm^3 Erlenmeyer flasks each containing 100 mL of Waksman medium with 50% sea water. The Waksman liquid medium is consisting of glucose (2.0 g/100 mL), meat extract (0.5 g/100 mL), peptone (0.5 g/100 mL), dried yeast (0.3 g/100 mL), NaCl (0.5 g/100 mL) and $CaCO_3$ (0.3 g/100 mL). The cultures were grown at 28 °C for 3–4 days with orbital shaking at 200 r/min. To construct a library of crude extracts, the culture broths of different strains were extracted consecutively with ethyl acetate. The cell pellets were extracted three times with methanol. The solvents from culture broth and mycelia were evaporated under vacuum and collected together in a small glass vial and stored at −20 °C for further use. Each vial took a serial number identical to the number of its own bacterial strain. Each crude extract was dissolved in dimethyl sulfoxide for further investigation.

2.5. Cell culture

Human breast cancer cell line MDA-MB-231 was obtained from the Institut National de la Santé et de la Recherche Médicale, Dijon, France. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 4.5 g/L of glucose, 4 mmol/L of L-glutamine, and 10% heat-inactivated fatal calf serum.

2.6. Cell viability assay

MDA-MB-231 cells were seeded in 96-well plates (4×10^4 cells per well) with 100 μL of Dulbecco's modified Eagle's medium. Cells were incubated at 37 °C in a 5% CO_2 incubator for 24 h. Crude extracts at different doses were added to each well. After 24 h incubation, the culture media was removed and the cells were washed with 100 μL phosphate-buffered saline. Cells were fixed by adding 100 μL of 70% ethanol and incubated at room temperature for 15 min. After removal of ethanol, 100 μL of methylene blue dye was added. The plates were incubated at room temperature for 15 min. To remove the excess of dye, the plate washed three times with tap water and then incubated for 2 h at 37 °C. Dye was eluted from the attached cells by adding 100 μL of 0.1 mol/L HCl in each well and then incubated for 5 min at room temperature. The developed blue color was measured using a microplate reader at 630 nm. Controls referred to wells containing only cells and medium with and without 10% dimethyl sulfoxide.

2.7. Antimicrobial activity

Two Gram-positive bacteria [*Streptococcus pyogenes* (*S. pyogenes*) and *Staphylococcus haemolyticus* (*S. haemolyticus*)], two Gram-negative bacteria [*Salmonella typhi* (*S. typhi*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)] and fungal yeast [*Candida tropicalis* (*C. tropicalis*)] were used in this study. The microbial strains were obtained and confirmed at the Department of Botany and Microbiology, Faculty of Science, Helwan University. The concentrations of the bacterial crude extracts were adjusted to 50 mg/mL. The antimicrobial activities were performed by using disk diffusion method [17]. Chloramphenicol (10 μg/disc) and ampicillin (5 μg/disc) were used as positive controls. The activity was determined by measuring the diameter of the inhibition zones in millimeters.

2.8. Isolation of genomic DNA of actinomycetes cultures

The actinomycete strains (EGY2, EGY3, EGY22, EGY27, and EGY39) were grown in 100 mL of tryptic soy broth for 24 h at 28 °C. The cells were centrifuged at 10000 r/min and washed two times with Tris–EDTA buffer. The DNA was extracted from actinomycetes strains using gene JET™ genomic DNA purification kit (Thermo Scientific Fermentas, Vilnius, Lithuania).

2.9. Partial 16S rRNA gene amplification, DNA sequencing and phylogenetic analysis

The gene coding 16S rRNA was amplified by PCR from the isolated genomic DNA using the forward (5′-TCACGGA GAGTTT-GATCCTG-3) and the reverse (5′-GCGGCTGCTG GCACGTA GTT-3′) primers. The PCR conditions consisted of