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Anticancer property of sediment actinomycetes against MCF-7 and MDA-MB-231 cell lines

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ABSTRACT

Objective: To investigate the anticancer property of marine sediment actinomycetes against two different breast cancer cell lines. **Methods:** *In vitro* anticancer activity was carried out against breast (MCF-7 and MDA-MB-231) cancer cell lines. Partial sequences of the 16s rRNA gene, phylogenetic tree construction, multiple sequence analysis and secondary structure analysis were also carried out with the actinomycetes isolates. **Results:** Of the selected five actinomycete isolates, ACT01 and ACT02 showed the IC_{50} value with (10.13±0.92) and (22.34±5.82) μg/mL concentrations, respectively for MCF-7 cell line at 48 h, but ACT01 showed the minimum (18.54±2.49 μg/mL) level of IC_{50} value with MDA-MB-231 cell line. Further, the 16s rRNA partial sequences of ACT01, ACT02, ACT03, ACT04 and ACT05 isolates were also deposited in NCBI data bank with the accession numbers of GQ478246, GQ478247, GQ478248, GQ478249 and GQ478250, respectively. The phylogenetic tree analysis showed that, the isolates of ACT02 and ACT03 were represented in group I and III, respectively, but ACT01 and ACT02 were represented in group II. The multiple sequence alignment of the actinomycete isolates showed that, the maximum identical conserved regions were identified with the nucleotide regions of 125 to 221st base pairs, 65 to 119th base pairs and 55, 48 and 31st base pairs. Secondary structure prediction of the 16s rRNA showed that, the maximum free energy was consumed with ACT03 isolate (-45.4 kkal/mol) and the minimum free energy was consumed with ACT04 isolate (-57.6 kkal/mol). **Conclusions:** The actinomycete isolates of ACT01 and ACT02 (GQ478246 and GQ478247) which are isolated from sediment sample can be further used as anticancer agents against breast cancer cell lines.

1. Introduction

Cancer still remains one of the most serious human health problems and breast cancer is the second most universal cause of cancer deaths in women[1]. Therapeutic methods for cancer treatment are surgery, radiotherapy, immunotherapy and chemotherapy[2] and these techniques are individually useful in particular situations and when combined, they offer a more efficient treatment for tumour. Many of the antitumor compounds from marine drugs are derived from algal metabolites[3] and these metabolites play an important role in identification of new pharmaceutical compounds[4,5]. Actinomycetes are one of the most important sources for new bioactive compounds such as antibiotics and enzymes[6-8] which have diverse clinical effects against many pathogenic organisms *viz.*, bacteria, fungi and parasite, *etc*. In fact, more than 60% of approved drugs are derived from natural compounds[9] in that, 50% of the natural antibiotics are produced from actinomycetes, among them marine actinomycetes have been reported to have antifungal, antibacterial and anti-inflammatory activities[10]. But, studies related with the biopotential activities of anticancer metabolites from marine actinomycetes based drug discovery are too limited. In this connection, the present study was made an attempt to find out the anticancer property from marine sediment actinomycetes against two different breast cancer cell lines.

2. Materials and methods

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2.1. Isolation and identification of actinomycetes from marine sediments

Mangrove sediment sample was collected from Manakkudi mangrove ecosystem (Lat.8° 05′E; Long.77° 46′E), Kanyakumari district, Tamilnadu, India. Samples were immediately transferred to the laboratory and 1 g of soil sample was aseptically transferred into 99 mL of presterilized 50% sea water and kept for continuous shaking (100 rpm) for 1 h. About 100 μL of diluted samples were transferred to molten starch casein agar medium (10 g/L soluble starch, 1 g/L of casein and 18 g/L of agar made up with 50% of aged sea water) and incubated at (27±2) ℃ for 7 days. After incubation, colonies appeared on the agar medium were re-streaked in the same agar medium and further subjected for the identification by using conventional[11] and molecular[12] techniques.

2.2. Extraction of bioactive compounds

Mass cultivation of actinomycetes isolates was performed with fermentor. Briefly, 20 mL of overnight culture of actinomycetes (ACT01, ACT02, ACT03, ACT04 and ACT05) isolates were transferred into fermentor containing 1 000 mL of Bennet medium (previously sterilized with steam explosion) individually and incubated for 7 days under continuous shaking. The parameters for our fermentation process were properly set using external controlling device [temperature 24 ℃, dissolved oxygen 3, the agitator at 200 rpm and pH (7.0±0.2)]. Further, the mass cultured broth was adjusted to pH 5.0 (using 1 N HCL) and filtered through cheese cloth to remove mycelia biomass. Further, equal volume (1:1) of ethyl acetate was added and mixed by vigorous shaking and kept without disturbance. The organic phase was collected and evaporated in an incubator at (60-70) ℃ and the residue was stored at -20 ℃ for further use. The biochemical constituents such as sugars, phenol, proteins, amino acids, quinines and alkaloids were analyzed[10].

2.3. Cytotoxicity assay

The breast cancer cell lines such as MCF-7 and MDA-MB-231 were obtained from National Centre for Cell Science, Pune, India. Cells were grown as monolayer culture in RPMI 1640 medium (10% fetal bovine serum, 1 mM sodium pyruvate and 100 U/L of penicillin/streptomycin) and incubated at 37 ℃ in a 5% of CO_2 atmosphere. Cell lines (100 μL) were seeded in 96 well plates at a concentration of 5×10^3 cells/mL for 24 h. After that, culture medium was replaced with 100 μL serum free medium containing various concentrations (25, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 μg/mL) of sponge associated actinomycete extracts at 24 h and 48 h. Later, the medium was refreshed with 100 μL of serum free medium (RPMI 1640) and 20 μL of MTT (5 μg/mL of (3, 4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazo liumbromide). The microtitre plates were incubated for 3 h in dark and the developed color was measured with ELISA reader at 570 nm. Triplicates were maintained for each treatment. Inhibitory concentration (IC_{50}) values were directly determined by linear regression analysis with office XP (SDAS) software.

2.4. 16S rRNA gene amplification and sequencing

Genomic DNA was isolated by using standard method[13] and amplified by PCR with forward primer-F243 (5′-GGATGAGCCCGCGGCCTA-3′) and reverse primer R513GC-(5′-CGGCCGCGGCTGCTGGCACGTA-3′). The reaction mixture contained 25 to 50 ng of DNA, ExTaq PCR buffer, 1.5 mM $MgCl_2$, 10 mM deoxynucleoside triphosphate mixture, 50 pmol of each primer, and 0.5 U of ExTaq polymerase. PCR conditions consisted of an initial denaturation at 94 ℃ for 5 min; 30 cycles at 94 ℃ for 1 min, annealing at 63 ℃ for 1 min and 72 ℃ for 1 min; and final 5 min extension at 72 ℃. The amplification products were examined by agarose gel electrophoresis and purified by using a QIA quick PCR clean up kit with the protocol suggested by Qiagen Inc. The complete 16S rRNA gene was sequenced by using the PCR products directly as sequencing template with above mentioned primers. All sequencing reactions were carried out with an ABI 377 automated DNA sequencer.

2.5. Construction of phylogenetic tree

The retrieved gene sequences were compared with other bacterial sequences by using NCBI BLAST search for their pair wise identities. Multiple sequence alignment and the phylogenetic tree were constructed with MEGA 4.0 software (http://www.megasoftw are.net) by using the neighbor-joining (NJ) method with 100 replicates as bootstrap value and NJ belongs to the distance-matrix method[13]. The 16S rRNA sequence was submitted to the GenBank. The RNA secondary structure of the isolates was predicted by using Genebee online software (http://www.genebee.msu.su/services/rna2_reduced.html).

3. Results

Five different sediment actinomycetes were isolated from the Manakkudi mangrove ecosystem. The mycelial characteristics of the isolated actinomycetes were varied as long branched hyphae, curling rod like, branched hypae with pink coloured spores and white coloured spores. Further, the colour of the aerial mycelium was identified as pale pink, white gray, dull white, pale pink and greenish white. Moreover, the riverside colour of the aerial mycelium was differed from white colour to yellowish colour. Size of the colony was differed from 3 cm to 6 cm (Table 1). The anticancer property of the fermented cell free extracts against breast cancer cell lines revealed that, the cytotoxic index of all the five isolates on breast cancer lines (MCF7 and MDA-MB-231) exhibited dose and time dependant growth inhibition (Figure 1 and 2). Among the five isolates, ACT01 showed the minimum concentration of IC_{50} value (10.13 ±0.92 μg/mL) followed by ACT02 (22.34±5.82 μg/mL) at 48 h for MCF-7 cell lines. Similarly, ACT01 showed the minimum concentration of IC_{50} value (18.54±2.49 μg/mL) for MDA-