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Isolation, Identification and Characterization of marine bacteria from the deep sea sediment of Bay of Bengal, India, and their antimicrobial and cytotoxicity potential.

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Abstract

A total of 108 marine bacteria were isolated from the deep sea sediment of Bay of Bengal, India. Of which, 15 bacteria showed antimicrobial activity against human pathogenic bacteria. These antagonistic marine bacteria were characterized phenotypically and their taxonomic affiliations were made on the basis of 16S rRNA gene homology and molecular phylogeny tree analysis, the antagonistic marine bacteria were identified as the species of *Bacillus*, *Halobacillus*, *Staphylococcus*, *Marinobacter*. The extracts of potent marine bacteria exhibited differential cytotoxicity towards lung (A549), cervical (HeLa), breast (MCF-7) and colon (HT-29) cancer cells. The acridine-orange and ethidium bromide (AO/EB) staining of the extract of strain MB30-treated cancer cells showed typical characteristics of apoptosis such as nuclear condensation, cell shrinkage and formation of apoptotic bodies. Present investigation, reports potent marine bacteria from the deep sea sediment of Bay of Bengal that exhibit broad-spectrum antimicrobial and cytotoxicity potential. Due to their innate bioactive potential, these bacteria can be used as the source of potent molecules.

Running Title: Antimicrobial and Cytotoxicity activity

Keywords: Marine bacteria, Molecular phylogeny, Phenotypic characterization, Antimicrobial activity, Cytotoxicity potential, AO/EB staining.

Introduction

Natural resources such as microbes, plants, vertebrates and invertebrates, are valuable sources of bioactive compounds. A large number of drugs have been developed in medicinal practice from natural products.[1] Potential bioactive compounds have been obtained from marine and terrestrial sources.[2] The marine environment covers more than 70% of the world's surface and is a rich source of both biological and chemical diversity.[3] Marine organisms are potential in terms of their ability to produce secondary metabolites which can be utilized as lead compounds in drug discovery. Anticancer compounds such as bryostatins, discodermolide, eleutherobin and sarcodictyin and anti-inflammatory compounds such as pseudopterosins, topsentins, and manoalide, have been reported.[4–9] Zheng et al. reported an antimicrobial alkaloid producing *Pseudoalteromonas piscicida* strain.[10] Lin et al. observed potential apoptosis effects of marine bacterial metabolites on human HeLa cells line.[11] Marine *Streptomyces*,[12] *Cyanobacteria*,[2] *Bacillus*,[13] *Pseudomonas*,[14] *Halomonas* and *Sulfitobacter*,[15] were also shown to exhibit a wide range of antimicrobial and anticancer activities. Recently, the deep sea hydrothermal microbes have been reported.[16] Although a rich potential heritage of therapeutic resource has been exploited for effective and beneficial use against human cancer either in prevention strategy or therapeutic armamentaria to kill tumor cells, the bioactive potential of marine bacteria is yet to be explored considering their rich biodiversity. Therefore, in the present study, we report the isolation and characterization of deep sea sediment of Bay of Bengal bacteria that show antimicrobial and cytotoxic potential.

Materials and Methods

Cell cultures and maintenance

Cell lines, A549, HeLa, MCF7, and HT-29, were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with streptomycin (0.75 µg mL^{-1}), and penicillin (120 U mL^{-1}), amphotericin B (3 µg mL^{-1}) and gentamicin (160 µg mL^{-1}) and 10% Fetal Bovine Serum (FBS) was maintained at 37 °C with a humidified atmosphere of 5% CO_2.

Isolation of marine bacteria

Deep sea sediments (position 13'02.95'N 80'52.29'E, depth; 20 m) were collected from the Bay of Bengal. In order to isolate marine bacteria 1 g sediment was added to 100 mL of saline water. Ten fold dilution (10 µL) was spread plated on Zobell marine agar (ZMA) medium with 1.5% (w/v) Sodium chloride (NaCl). After incubation at 37ºC for 48 h, single colonies were isolated and subcultured to obtain pure cultures. Stock cultures were made in Zobell marine broth containing 50% (v/v) glycerol and stored at – 80°C. [17]

Antagonistic potential of marine bacteria

Screening of marine bacteria for antagonistic activity towards human pathogenic bacteria such as *Aceintobacter baumannii, Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Vibrio cholera*, *Streptococcus pyogenes* and *Candida albicans* was done by employing agar-spot method. Briefly, nutrient agar (NA) plates were over laid with soft agar (1%) seeded with 50 µL aliquot of overnight cultures of pathogenic bacteria. The agar was allowed to solidify and then the marine bacteria were spot-

inoculated on the lawn of pathogenic bacteria and the plates were incubated at 37ºC for 24 h and observed for the growth inhibition zones around the bacterial colony.[18]

Phenotypic characterization of marine bacteria

According to Bergey's manual of determinative bacteriology phenotypic characterization was done.[19–21] Gram reaction was determined by the nonstaining (KOH) method as described in the standard method.[22] Biochemical tests such as production of oxidase, catalase, urease, gelatin hydrolysis, nitrate reduction and carbon source utilization profiles were tested as described.[18,20–21] The results were recorded assigning 1 to positive values and 0 to negative values and the pairwise coefficients of similarity (Dice) were clustered with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm of Numerical Taxonomy System (NTSYS-pc2) software.

Molecular characterization of marine bacteria by 16S rRNA gene analysis

Genomic deoxyribonucleic acid (DNA) was extracted by using standard Sodium Dodecyl Sulfate (SDS) lysis methods.[23] The 16S rRNA (ribosomal ribonucleic acid) genes were amplified by PCR (Polymerase chain reaction) using universal primers, fD1 (5'-AGTTTGATCCTGGCTCA-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3'). The PCR cocktail (50 µL) contained 20 pM of primer, 50 ng of DNA, 1x *Taq* DNA polymerase buffer, 3 U of *Taq* DNA polymerase (Sigma, USA), 0.2 mM of each Deoxynucleotide (dNTPs) , and 1.5 mM Magnesium chloride ($MgCl_2$). PCR conditions consisted of an initial denaturation at 94ºC for 5 min, 30 cycles of denaturation at 94ºC for

1 min, annealing at 59ºC for 1 min and extension at 72ºC for 2 min with a final extension at 72ºC for 5 min. The amplification was examined by 0.8% agarose gel electrophoresis and purified using Quick PCR purification kit (Sigma-Aldrich, USA). The complete 16S rRNA gene was sequenced with automated DNA sequencer with specific primers using the facility at Macrogen Inc (Seoul, Korea).

Construction of phylogenetic tree

The retrieved gene sequence was compared with other bacterial sequences by using (National Center for Biotechnology Information) NCBI BLAST (Basic Local Alignment Search Tool) search for their pair wise identities. Multiple sequence alignment and the phylogenetic tree were constructed with Molecular Evolutionary Genetics Analysis (MEGA 4.0) software by using the neighbor-joining (NJ) method with 1000 replicates using bootstrap.[24] The 16S rRNA sequence was submitted to the GeneBank (http://www.ncbi.nlm.nih.gov).[18,21]

Extracellular extracts of marine bacteria

Potent marine bacteria were grown in Zobell marine broth (500 mL) in 1000 mL Erlenmeyer flasks on a rotary shaker at 180 rpm for 72 h at 37 ºC. The cell-free culture supernatant was prepared by centrifuging the culture at 8000 rpm for 20 min at 4 ºC. To the cell free culture supernatant, equal volume of ethyl acetate was added and vigorously shaken for 5 min. Then, the organic (upper) layer was separated and evaporated to dryness in a rotary evaporator and the extract was dissolved in Dimethyl sulfoxide (DMSO). [25]

Cytotoxicity assay

Cell survival rate was determined by employing the 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Exponentially grown A549, HeLa, MCF-7 and HT-29 cells were seeded at a density of 0.2 $\times 10^5$ cells in 96-well plate with a volume of 200 µL per well. Cells were incubated with different concentrations (1, 10, 50 and 100 µg mL^{-1}) of extracts of bacteria and incubated at 37ºC for 24 h. At the end of the incubation periods, 10 µL of MTT stock solution (5 mg mL^{-1}) was added to each well and the plates were further incubated for 24 h at 37 ºC. The formazan crystals that formed due to the cleavage of tetrazolium salt were dissolved by the addition of 100 µL of dimethyl sulfoxide (DMSO) per well. The soluble formazan produced was quantified spectrophotometrically using an Enzyme Linked Immunosorbent Assay (ELISA) reader at 570 nm using the following formula.[25] Cell proliferation inhibition (%) = [1– (A value of the experimental samples/ A value of the 10 control)] ×100%.

Morphological observation

Live cell imaging-Cancer cells were seeded at 0.2 $\times 10^6$ cells per well in 24-well tissue culture plate treated with the half maximal inhibitory concentration (IC_{50}) of extracts for 24 h. After incubation, cells were observed under inverted phase contrast microscopy for morphological changes. [26]

Acridine orange (AO) and ethidium bromide (EB) staining-Cancer cells were seeded at 0.2 $\times 10^6$ cells per well in 24-well tissue culture plate treated with the IC_{50} concentration of extracts and incubated for 24 h. After incubation, the cells were washed thrice with

phosphate buffered saline (PBS), stained with 10 µl of dye mixture (10 mg mL^{-1} AO and 10 mg mL^{-1} EB) and cells were examined under fluorescence microscope. Digital images were obtained using the image acquiring software program (Eclipse TS, Nikon, USA).[26]

Antimicrobial activity

Extracellular extracts of marine bacteria were screened for their antimicrobial activity against human pathogenic bacteria *viz., Aceintobacter baumannii, Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Vibrio cholera*, *Streptococcus pyogenes,* and *Candida albicans* by employing agar well diffusion method.[10] Overnight grown pathogenic bacterial culture (0.1 ml) was mixed with 20 ml of sterile Müller-Hinton agar (MHA) at 45°C and poured into Petri dishes. Agar wells of 6 mm diameters were made in the solidified MHA agar, the ethyl acetate extracts (20 µg) were added in the wells and the plates were incubated at 37°C for 24 h. Antibiotics, erythromycin and rifampicin were used as positive controls for Gram positive and Gram negative bacteria and the solvent, ethyl acetate was used as a negative control.

Statistical analysis

The statistical difference between the groups was determined by Analysis of variance (ANOVA) and Duncan multiple range test. The level of $p < 0.05$ was considered to be statistically significant.

Results

Isolation of marine bacteria and screening for their antagonistic potential

A total of 108 bacteria isolated from the deep sea sediment of Bay of Bengal, India. Among them, 15 bacteria exhibited antagonistic activity against human pathogenic bacteria. Of which, 10 bacteria (strains, MB30, MB2, MB6, MB8, MB18, MB30, MB31, MB45, MB86 and MB106) were tested positive against *Aceintobacter baumannii*, 10 bacteria (strains, MB1, MB2, MB3, MB6, MB8, MB9, MB18, MB30, MB31 and MB45) showed activity against *Bacillus subtilis*, 3 bacteria (strains, MB2, MB30 and MB45) inhibited *Candida albicans*, 5 bacteria (strains MB3, MB6, MB18, MB30 and MB69) showed activity against *Escherichia coli*, 2 bacteria (strains, MB2 and MB39) showed activity against *Salmonella typhi*, 8 bacteria (strains, MB1, MB2,MB3, MB6, MB30, MB31, MB32, MB45) showed activity against *Staphylococcus aureus*, 4 bacteria (strains, MB8, MB9, MB12 and MB18) were activity against *Vibrio cholera* and 1 bacteria (strain MB30) showed inhibition against *Streptococcus pyogenes*.

Phenotypic characterization of marine bacteria

Marine bacteria reported in this study displayed a varying degree of phenotypic traits such as gram staining, production of oxidase, catalase, urease, gelatin hydrolysis, and nitrate reduction. In addition, bacteria exhibited a varying degree of utilization towards different carbon sources such as lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-Arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, α-

Methyl-D-glucoside, rhamnose, cellobiose, melezitose, α-Methyl-D-mannoside, xylitol, ortho-Nitrophenyl-β-galactoside (ONPG), esculin hydrolysis, D-Arabinose, citrate, malonate and sorbose. The Dendrogram of marine bacteria based on their phenotypic traits resulted into 7 major phenons (Table 1; Figure 1). Phenons I, II, III and V consisted of strains MB1, MB2, MB3, and MB30 respectively. The phenon IV consisted of 5 strains (MB6, MB8, MB9, MB12, and MB18). Phenon VI and VII both consisted of 2 strains (MB31 and MB32) and 4 strains (MB45, MB69, MB86 and MB10) respectively.

Molecular characterization of marine bacteria by 16S rRNA gene homology and phylogenetic tree analysis

On the basis of 16S rRNA sequence homology, the marine bacteria were identified as various species of the four different genera, which include *Bacillus boroniphilus* (strain MB2), *B. hwajinpoensis* (MB6), *B. safensis* (MB8), *B. hwajinpoensis* (strain MB9), *B. tequilensis* (strain MB12), *B. vietnamensis* (strain MB18), *B. anthracis* (strain MB31), *B. stratosphericus* (strain MB69), *B. safensis* (strain MB106), *Halobacillus trueperi* (strain MB1), *H. trueperi* (strain MB86), *Marinobacter litoralis* (strain MB3), *Staphylococcus* sp. (strain MB30), *S. haemolyticus* (strain MB32) and *S. equorum* subsp. *linens* (strain MB45). The taxonomic affiliation of marine bacteria was confirmed by 16S rRNA sequence homology and subsequent phylogenetic tree analysis. The16S rRNA sequence of marine bacteria were deposited in the GenBank database under accession number KJ531634 to KJ531648 (Table 2; Figure 2).

Extracellular extracts of marine bacteria

Upon the extraction of supernatant from marine bacteria with ethyl acetate and subsequent dryness in rota evaporator, the extract ranged from 8 to 10 mg was obtained.

Cytotoxicity of extracts

Cytotoxicity of ethyl acetate **extracellular** extracts of marine bacteria and doxorubicin (control) was evaluated using MTT reduction assay on A549, HeLa, MCF-7, and HT-29 cell lines. Results confirmed the dose-dependent inhibitory activity of **extracellular** extracts of marine bacteria. The IC_{50} concentration of each extracts varied with respect to individual bacteria (Table 3). Although the extract of strain MB2 was not effective against A549 and HeLa, it exhibits potent cytotoxicity against HT-29 and MCF-7 with IC_{50} concentration of 9.16±0.2 and 45.3±0.5 respectively. Similarly, the extract of strain MB8 also showed potent cytotoxicity against HT-29 and MCF-7 with the IC_{50} value of 40.16±0.2 and 66.6±2.8 respectively. The extract of strain MB69 revealed potential cytotoxicity against A549 and HeLa with IC_{50} concentration of 0.86±0.5 and 9.33±0.28 respectively. The extract of strain MB30 revealed cytotoxicity against four different cell lines such as A549, HeLa, MCF-7 and HT-29 with the IC_{50} concentration of 90.42±0.21, 42.6±0.57, 49.16±0.28 and 40.16±0.28 µg mL^{-1} respectively (Figure 3).

Morphological observations

The live cell imaging of extract of strain MB30-treated cancer cells showed the dead and floating cells compared to the control cells (Figure 4). The fluorescent image of

acridine-orange (AO) and ethidium bromide (EB) stained cancer cells showed typical characteristics of apoptosis. Uniformly green live cells with normal morphology were observed in the control group, where as different stages of apoptosis such as yellowish green (early) and orange (late) colored cells were observed compared to the control cells (Figure 5).

Antimicrobial activity of extracts

Among the 15 bacteria, the extract of strain MB30 displayed its inhibition towards a total of 6 human pathogenic bacteria. Whereas the extracts of strains MB2, MB6, MB18 and MB45 revealed their inhibitory activity towards 4 bacterial pathogens. The extracts of strain MB1, MB3, MB8, MB9 and MB31 showed activity against 3 bacterial pathogens. The extracts of strain MB12, MB32, MB69, MB86 and MB106 exhibited inhibitory activity towards the pathogens such as *Escherichia coli, Salmonella typhi, Vibrio cholera* and *Aceintobacter baumannii* respectively. The zone of inhibition was ranged from 7.3 to 16 mm and the highest activity was observed from extract the strain MB30 against *Staphylococcus aureus* and *Bacillus subtilis* (Table 4).

Discussion

Marine resources are well known potent resource of antimicrobial, anticancer and anti-inflammatory metabolites. Screening of marine sediment from Bay of Bengal revealed presence of 108 polymorphic marine bacterial colonies and 15 bacterial isolates were found to be antagonistic against bacterial pathogens. The 16S rRNA sequence homology

and subsequent, molecular phylogeny analyses confirmed the affiliation of marine bacteria of different genera such as *Bacillus*, *Staphylococcus*, *Halobacillus* and *Marinobacter.* The present investigation revealed that extracts obtained from different marine bacterial strains possessed differential antimicrobial activity towards pathogenic bacteria. Antibacterial properties were detected in 15 bacteria. Previous reports have reported the antimicrobial activities of deep-sea hydrothermal bacteria, rhizospheric soil bacteria and gastrointestinal tract inhabiting microbes.[16,27–28] Several antagonistic marine bacteria.[25] Ethyl acetate extract of marine actinomycetes, *Gordomia tearrae* revealed potential antimicrobial activity against human pathogenic bacteria.[29] Subashini et al. reported a *Streptomyces* sp. that exhibit both antimicrobial and antifungal activity.[30] For the first time, present study reported the antagonistic marine bacteria from the deep sea sediment of Bay of Bengal.

Monolayer cultures of cells, A549, HeLa, MCF-7, HT-29, were screened using various concentrations of the extracts of different marine bacteria to study their differential cytotoxicity potential. The ethyl acetate extract of strain MB30 was found to be cytotoxic against A549, HeLa, MCF-7 and HT-29 cells with respective IC_{50} values of 90.42±0.21, 42.6±0.57, 49.16±0.28 and 40.16±0.28 µg mL^{-1}. Antimicrobial and cytotoxic potential of the bacteria may be due to their potential to produce bioactive compounds.[14] The extract of *Bacillus* sp. was identified to be cytotoxic against human cancer cell lines viz., HepG2, HCT and MCF-7 with the IC50 values of 46.9, 28.6 and 21.3µg mL^{-1} respectively. Earlier studies have also demonstrated the ability of marine bacteria, *Pseudomonas* sp. NO.7; *Pseudomonas* sp. MNO5) for antimicrobial activity against human pathogens and cytotoxicity activity against lung cancer cell lines.[14,31] Recently, Sun et al. showed that the

bacteria from sediment hydrothermal vent were able to produce bioactive metabolites and enzymes.[16] *Pseudoalteromonas* from the sediments of South China Sea and *Bacillus* spp. from the coastal sediments of King George island produced extracellular metabolites and enzymes.[32–34] In conclusion, the present investigation reported Bay of Bengal bacteria that exhibit broad-spectrum antimicrobial and anticancer activities for the first time. Further studies on the delineation of genes and their bioactive constituents could pave way for identification of novel drug metabolites.

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Table Legend

Table 1. Biochemical characterization of marine bacteria.

Table 2: The 16S rRNA sequence homology based identification of marine bacteria.

Table 3: *In vitro* cytotoxicity activity of the **extracellular** extracts of marine bacteria.

Table 4: Antimicrobial activity of the **extracellular** extracts of marine bacteria.

Table 1. Biochemical characterization of marine bacteria.

Traits	Strains							
	MB1	MB2	MB3	MB6	MB8	MB9	MB12	MB18
Primary characteristics								
Gram staining	−	+	−	+	−	+	+	+
Oxidase	+	+	−	−	−	−	−	−
Catalase	+	+	+	+	+	+	+	+
Urease	−	−	−	−	−	−	−	−
Gelatin hydrolysis	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+
Assimilation of								
Lactose	−	−	−	−	−	−	−	−
Xylose	−	−	−	−	−	−	−	−
Maltose	+	+	+	+	+	+	+	+
Fructose	−	−	+	+	+	+	+	+
Dextrose	−	+	+	+	+	+	−	+
Galactose	+	+	−	+	+	+	−	−
Raffinose	−	−	−	+	+	+	+	+
Trehalose	+	+	+	+	−	+	+	−
Melibiose	−	−	−	−	−	−	−	−
Sucrose	−	+	+	+	+	+	+	+
L− Arabinose	+	+	+	−	+	−	+	+
Mannose	+	+	+	+	+	+	+	+
Inulin	−	−	+	−	−	−	−	−
Sodium gluconate	−	−	−	−	−	−	−	−
Glycerol	−	+	+	+	+	+	+	+
Salicin	+	−	+	−	−	−	−	−
Dulcitol	+	−	−	−	−	−	−	−
Inositol	+	−	+	+	+	+	+	+
Sorbitol	−	−	+	−	−	−	−	−
Mannitol	+	−	+	+	+	+	+	+
Adonitol	+	−	−	−	−	−	−	−
Arabitol	+	−	−	−	−	−	−	−
Erythritol	+	−	−	−	−	−	−	−
α −Methyl−D−glucoside	+	−	−	−	−	−	−	−
Rhamnose	−	−	+	+	+	+	+	+
Cellobiose	+	−	−	+	+	+	+	+
Melezitose	−	+	−	−	−	−	−	−
α −Methyl−D−mannoside	−	−	+	−	−	−	−	−
Xylitol	−	+	−	−	−	−	−	−
ONPG	−	−	−	−	−	−	−	−
Esculin hydrolysis	−	+	+	−	−	−	−	−
D− Arabinose	−	−	+	+	+	+	+	+
Citrate utilization	−	−	−	−	−	−	−	−
Malonate utilization	−	−	−	−	−	−	−	−
Sorbose	−	−	−	−	−	−	−	−

+ Positive; − Negative

Table 1: Biochemical characterization of marine bacteria (Contd….).

Traits	Strains						
	MB30	MB31	MB32	MB45	MB69	MB86	MB106
Primary characteristics							
Gram staining	+	+	+	+	+	−	+
Oxidase	+	−	−	−	−	−	−
Catalase	+	+	+	+	+	+	+
Urease	−	−	−	−	−	−	−
Gelatin hydrolysis	−	+	+	+	+	+	+
Nitrate reduction	−	+	+	+	+	+	+
Assimilation of							
Lactose	−	−	−	−	−	−	−
Xylose	−	−	−	+	+	+	+
Maltose	+	−	−	−	−	−	−
Fructose	+	−	−	−	−	−	−
Dextrose	+	−	−	+	+	+	+
Galactose	+	−	−	+	+	+	+
Raffinose	−	−	−	+	+	+	+
Trehalose	−	−	−	−	−	−	−
Melibiose	−	−	−	−	−	−	−
Sucrose	+	+	+	−	−	−	−
L− Arabinose	−	−	−	−	−	−	−
Mannose	−	−	−	−	−	−	−
Inulin	−	−	−	−	−	−	−
Sodium gluconate	−	−	−	−	−	−	−
Glycerol	+	+	+	−	−	−	−
Salicin	−	−	−	+	+	+	+
Dulcitol	−	+	+	+	+	+	+
Inositol	−	+	+	+	+	+	+
Sorbitol	−	−	−	−	−	−	−
Mannitol	+	−	−	−	−	−	−
Adonitol	−	+	+	−	−	−	−
Arabitol	−	−	−	−	−	−	−
Erythritol	−	−	−	−	−	−	−
α −Methyl−D−glucoside	−	−	−	−	−	−	−
Rhamnose	−	−	−	−	−	−	−
Cellobiose	−	+	+	−	−	−	−
Melezitose	−	−	−	−	−	−	−
α −Methyl−D−mannoside	−	−	−	−	−	−	−
Xylitol	−	−	−	+	+	+	+
ONPG	−	−	−	−	−	−	−
Esculin hydrolysis	−	−	−	+	+	+	+
D− Arabinose	−	−	−	+	+	+	+
Citrate utilization	−	+	+	−	−	−	−
Malonate utilization	−	−	−	−	−	−	−
Sorbose	−	−	−	−	−	−	−

+ Positive; − Negative

Table 2: The 16S rRNA sequence homology based identification of marine bacteria.

Strain	Close relative	Sequence homology (%)	GenBank accession number
MB1	*Halobacillus trueperi*	99	KJ531634
MB2	*Bacillus boroniphilus*	99	KJ531635
MB3	*Marinobacter litoralis*	99	KJ531636
MB6	*B. hwajinpoensis*	99	KJ531637
MB8	*B. safensis*	100	KJ531643
MB9	*B. hwajinpoensis*	99	KJ531638
MB12	*B. tequilensis*	99	KJ531639
MB18	*B. vietnamensis*	99	KJ531640
MB30	*Staphylococcus* sp.	100	KJ531648
MB31	*B. anthracis*	99	KJ531645
MB32	*S. haemolyticus*	100	KJ531644
MB45	*S. equorum* subsp. *linens*	100	KJ531646
MB69	*B. stratosphericus*	100	KJ531647
MB86	*H. trueperi*	99	KJ531641
MB106	*B. safensis*	100	KJ531642

Table 3: *In vitro* cytotoxicity activity of the **extracellular** extracts of marine bacteria.

Bacterial strains	IC_{50} values (µg/mL; Mean ±SD)			
	Cancer cell lines			
	A549	HeLa	MCF-7	HT-29
MB2	–	–	45.3±0.5	9.16±0.2
MB8	–	–	66.6±2.8	40.16±0.2
MB30	90.42±0.21	42.6±0.57	40.16±0.28	49.16±0.2
MB69	0.86±0.5	9.33±0.28	–	–
Doxorubicin	1.01±0.30	0.38±0.15	0.95±0.27	0.88±0.2

A549 = Lung cancer; HeLa = cervical cancer; HT-29 = colon cancer; MCF-7 = breast cancer;

NT – Not tested; SD = standard deviation (± Std error).

Table 4: Antimicrobial activity of the **extracellular** extracts of marine bacteria.

Strain	Zone of inhibition (mm)							
	Tested microorganisms							
	Ab	Bs	Ca	Ec	St	Sa	Vc	Sp
MB1	++	+	–	–	–	–	+	–
MB2	+	+	+	–	–	+	–	–
MB3	–	+	–	+	–	+	–	–
MB6	+	+	+	–	–	+	–	–
MB8	+	+	–	–	–	+	–	–
MB9	–	+	–	+	–	++	–	–
MB12	–	–	–	–	–	–	++	–
MB18	+	+	+	–	–	+	–	–
MB30	+	++	+	+	–	++	–	+
MB31	+	++	–	–	–	+	–	–
MB32	–	–	–	–	++	–	–	–
MB45	+	+	+	–	+	–	–	–
MB69	–	–	–	+	–	–	–	–
MB86	+	–	–	–	–	–	–	–
MB106	+	–	–	–	–	–	–	–

Ab - *Aceintobacter baumannii,* Bs - *Bacillus subtilis,* Ca - *Candida albicans, Ec* **-** *Escherichia coli,* St - *Salmonella typhi, Sa* - *Staphylococcus aureus,* 7. *Vibrio cholera*, Sp - *Streptococcus pyogenes*. NT – Not tested. + Inhibition zone between 10 and 15 mm; ++ Inhibition zone between 15 and 30 mm.

Figure Legend

Fig 1. Dendrogram of marine bacteria isolated from deep sea sediment of Bay of Bengal based on their phenotypic traits. The pair-wise coefficients of similarity (Dice) were clustered with the UPGMA algorithm of NTSYS-pc2. The marine bacteria were grouped into 7 phenons.

Fig 2. Phylogenetic tree analysis of marine bacteria isolated from the deep sea sediment of Bay of Bengal based on the nucleotide sequence of 16S rRNA. The multiple sequence alignment was done in CLUSTAL program. The pair-wise evolutionary distances were calculated using Kimura2-parameter model. The phylogenetic tree was constructed by neighbour-joining (NJ) method with 10,000 replicates using bootstrap.

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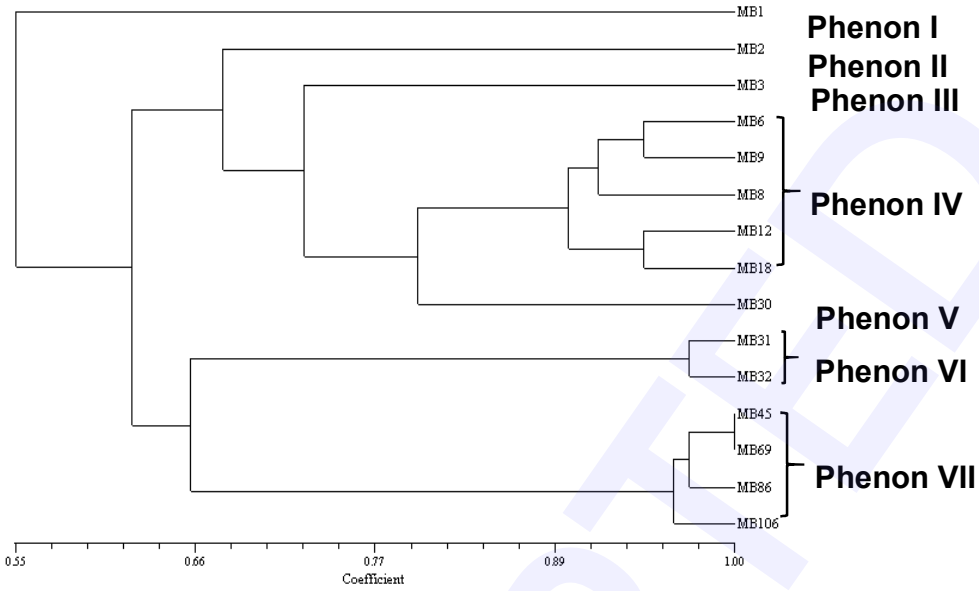
Fig 5. Fluorescent image of acridine-orange and ethidium bromide double staining of cancer cells treated with the extract of strain MB30 showing the induction of apoptosis.



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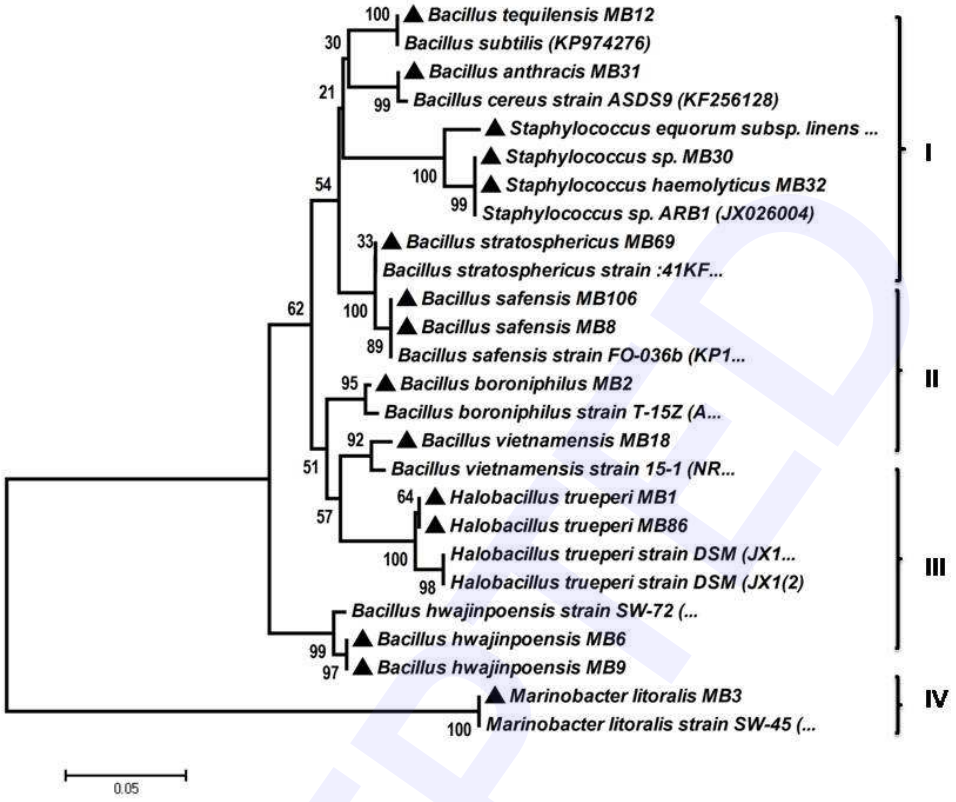

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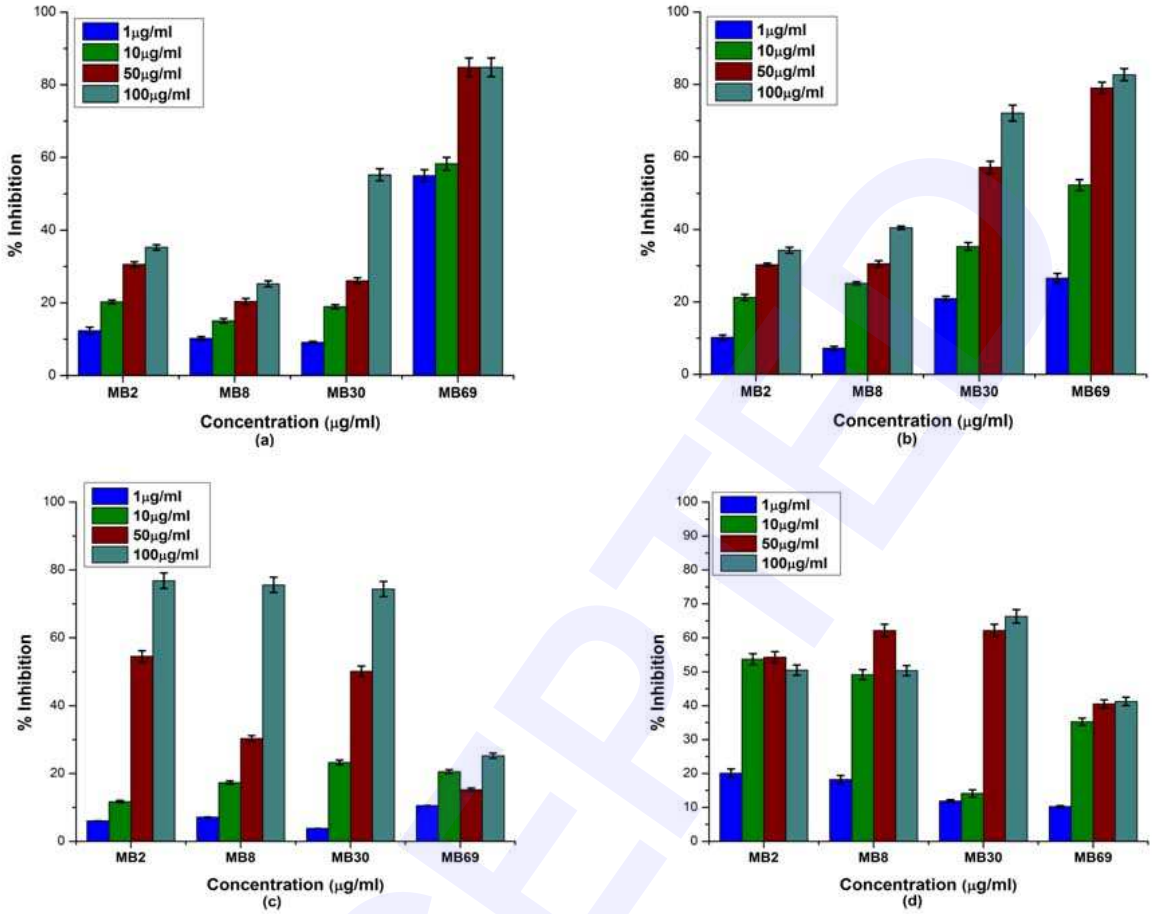

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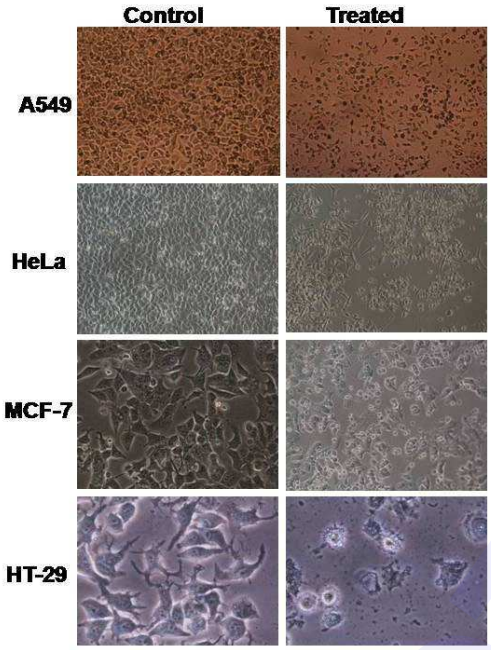

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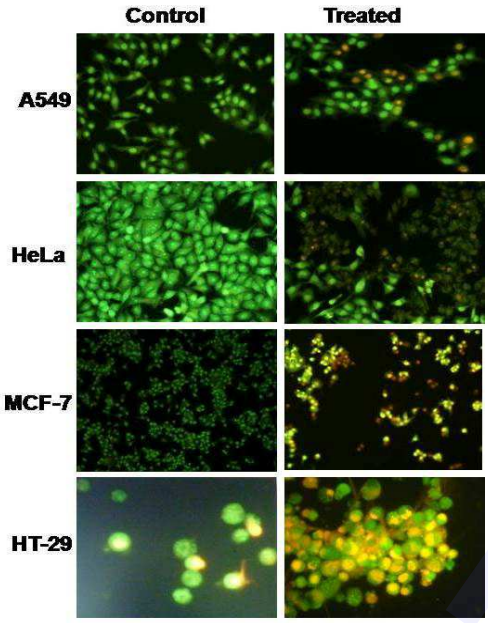

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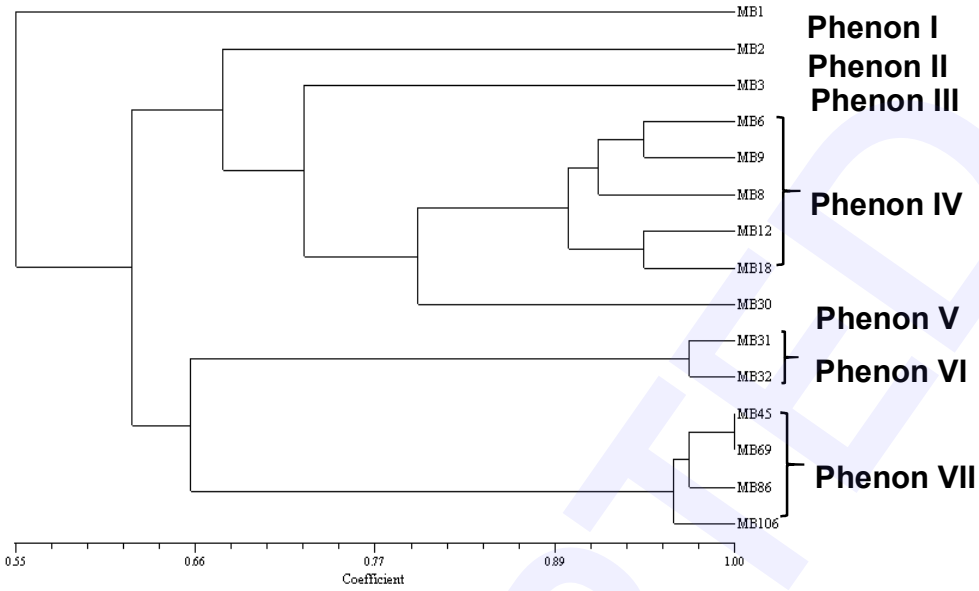

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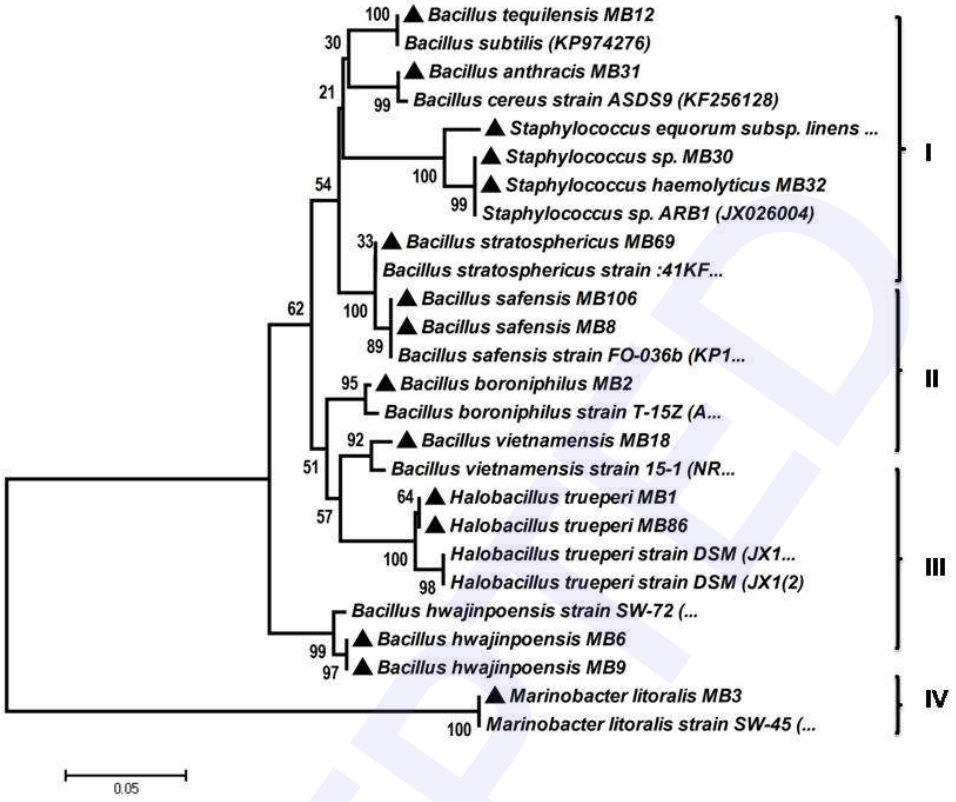

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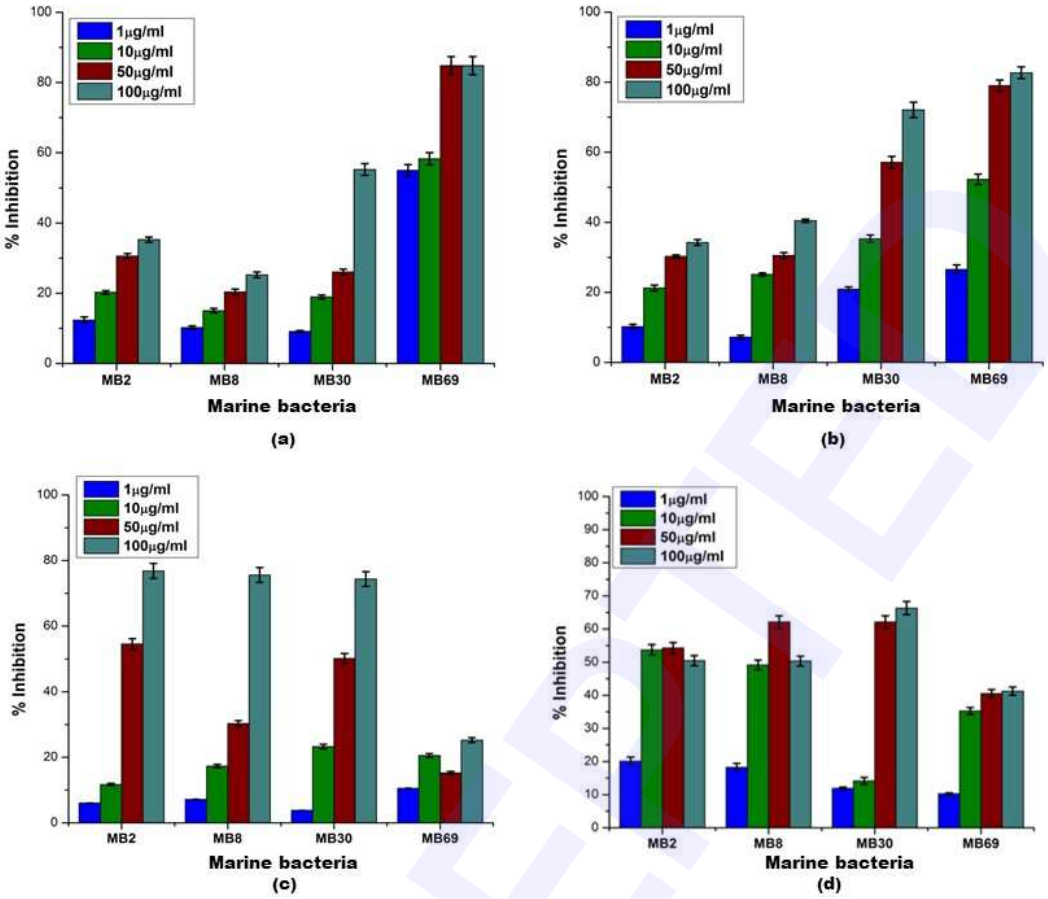

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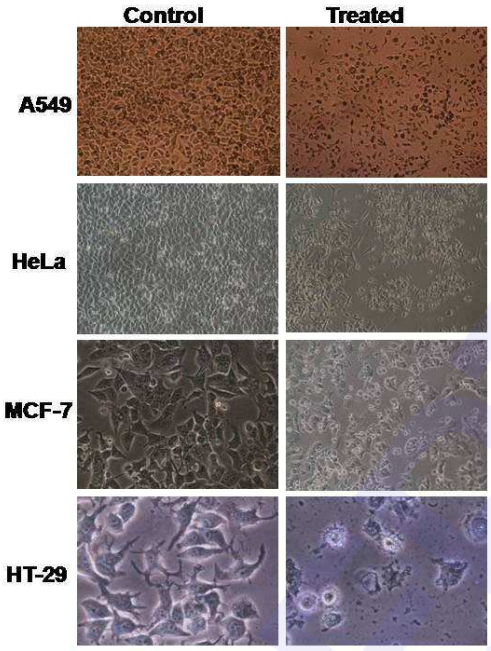

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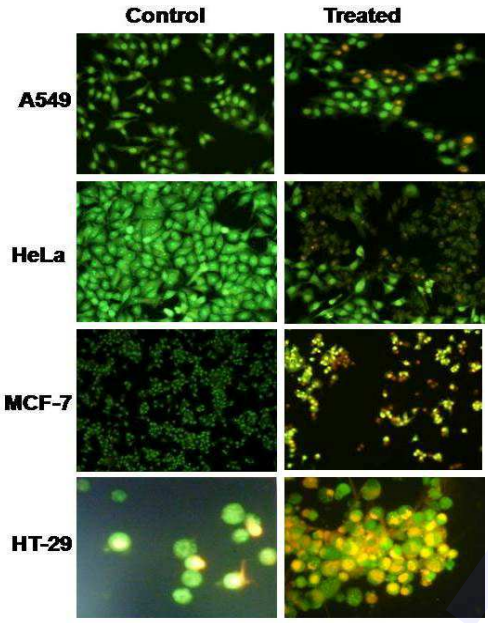

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