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STUDY OF ANTICANCER ACTIVITY OF SECONDARY METABOLITES OBTAINED FROM STREPTOMYCES SPECIES ISOLATED FROM MARINE ENVIRONMENTS

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ABSTRACT

Omnipresent Streptomyces, yields medically important antibiotics, antitumor agents, immunorsuppressive agents and enzymes as secondary metabolites. Antitumor agents are isolated and extracted from various sources. The present study was designed to detect the presence of antitumor agents Actinogan, Fredericamycin and Lactonamycin in Streptomyces isolated from the sea shore. The Streptomyces was grown in pH pH 5.1, 6.0 and 7.3 to trigger expression of Actinogan, Fredericamycin and Lactonamycin respectively. The efficacy of the agents tested on Cancerous 'Hep2' cell lines and normal Vero cell lines. The results revealed that compounds were produced in the Streptomyces and were able to efficiently arrest the growth cell lines in in-vitro studies.

INTRODUCTION

The present century has dedicated in search for anti cancerous agents from various sources (Anand, 2008b.). Our traditional methods of treatment have proved to solve some aliments and give a good quality of life (Anand, 2008a; Anand and Amudha. 2009). The bioactive compounds isolated from the traditional/ aurvedic compounds have proved to be toxic in prolonged usage (Nadia 2012). The microorganisms are known to produce secondary metabolites which are not useful for their own survivability but has given man kind victory over many diseases. One class of such organism is filamentous, non-motile Gram-positive bacteria Actinomycetes. The largest genus of Actinobacteria is represented by Streptomyces.

Streptomyces, appear similar to fungi in their branching filamentous structure. These have high GC-content and produce spores from aerial filaments (sporophores) (Cummins 1958). These are characterized by tough, leathery, frequently pigmented colonies and oxidative type of metabolism (Jensen and Fenical, 1991). These are chemoheteroorganotrophs, growing best at 25°C and pH 8-9. Unlike other bacteria, Streptomyces has linear chromosomes in the cell (Lin, 1993). Normally Streptomyces are non pathogenic, with few exceptions like mycetoma caused by *S. somaliensis*, scabies by *S. caviscabies, S. scabies* in humans (Kämpfer P, 2006).

Streptomyces species are omnipresent due their ability to use any complex organic materials as carbon and energy sources (Cross, 1981). This unique property to breakdown any breakdown compound has guarded the fertility of soil on earth for ages. These churn out two thirds of the clinically useful antibiotics of natural origin (Matsumoto et al, 1986; Berdy, 2005). They are responsible for more than half of the discovered bioactive secondary metabolites acting as antibiotics, antitumor agents, immunorsuppressive agents and enzymes (Goodfellow et al., 1984; Hochlowski et al., 1991; Maskey et al, 2004). Streptomyces yield many commercially used antibiotics (Erythromycin, Neomycin, Streptomycin, Tetracycline, Vancomycin, Rifamycin etc.), antifungal compounds (Nystatin, Amphotericin B, Natamycin, etc.) and anti-cancer compounds (Migrastatin, Aureoverticillactam, Caprolactones, Chinikomycins, etc.) (Geran et al., 1972; Matsumoto 1996; Donia and Hamann, 2003, Maskey et al., 2004)

Cancer is a class of diseases in which a group of cells display the traits of uncontrolled growth (Anand, 2008b). Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents (National Cancer Institute, 2004). Other cancer-prompting genetic abnormalities may be randomly

acquired through errors in DNA replication, or are inherited (Lodish H, 2000). The common mechanism underlying the cytotoxicity of most antitumor agents is cell cycle arrest. Antitumor agents primarily target new plastic cells at the surface of the cancer tumour and smaller tumours with short mass-doubling time (Waun, 2010). Several antitumor agents work by inhibiting DNA replication and terminating cell division at S phase (Saiki et al., 1988).

Galaxy of compounds is reported to posses' antitumor activity. The source of these compounds and extraction are not economically viable. Some compounds like actinogan, Fredercamycin and Lactonamycin elucidated clearly their antitumor activity (Kelly et al., 1986). Actinogan is water soluble where as Fredercamycin is stable at room temperature even under anhydrous conditions. Lactonamycin inhibits dividing cells in the G2/M cell cycle phase (Matsumoto, 1996).Presently these chemicals are produced by chemosynthesis (Bennett 1986). According to the NCI guidelines, antitumor activity of compounds like Lactonamycin should be tested with human cell lines from gastric adencarcinoma (HMO2), breast carcinoma (MCF 7), hepatocellulaer carcinoma (HEP G2) Matsumoto et al., (1996). The antitumor activity is directly reflected by arresting the division of cells in the cell line.

In the present study the efforts were made to know if the Streptomyces isolated from marine soil can serve as economic source antitumor agents, Actinogan, Fredercamycin and Lactonamycin and their efficacy was evaluated.

MATERIAL AND METHODS

Sample collection and isolation

Marine soil sample was collected from Marina beach, Chennai and were transported to the Environmental Biotechnology Laboratory in small plastic containers and were processed within 4 hours after collection (Donia and Hamann, 2003). The soil was allowed to dry in sterile incubator followed by serial dilution. Isolation of Streptomyces is done using spread plate method and characterization of the isolated bacteria is done using Gram Staining and standard identification procedures (Anand 2010).

Separation of proteins and protein precipitation

The Streptomyces were grown in YMG agar broth in three different Fulcan tubes with three different pH 5.1, 6.0 and 7.3 for the production of antitumor compounds Actinogan, Lactanomycin and Fredericamycin respectively. The Protein Precipitation was carried out by using 10% TCA and 1X PBS buffer. SDS-PAGE was done to separate proteins according to their size and to confirm the presence of the three proteins.

Cell Lines

The Hep-2 cell line , derived from laryngeal carcinoma cells were used as a model in to study the antitumor activity The Vero cell line was also grow indefinitely in culture as control. To evaluate the proliferative potential of this line, cytogenetic methodology of Moorhead et al., (1960) were used. The Vero cell line is a continuous cell line, which is aneuploid and will grow indefinitely in culture.

Maintenance of cell line

The cell line was maintained in the medium containing Penicillin and Streptomycin (100µg/ml), Kanamycin acid sulphate (200µg/ml), Fungizone (Amphotericin B) (20µ/ml), L-Glutamine 3%, 7.5% Sodium-bi-carbonate solution, Foetal Bovine Serum (500 ml) in Trypsin PBS Versene Glucose solution (TPVG). The mouth of the bottle was wiped with cotton and soaked in spirit. The medium was removed using a 10 ml pipette. The cells were gently rinsed with PBS. Then 4ml of TVPG (pre warmed to 37°C) was added over the cells. The TPVG was allowed to act for 3-5 minutes. TVPG was pipette out and 5ml of 5% Minimum Essential Media (MEM) was added. The cell clusters were broken by gently pipetting back and forth. The cells were counted with the help of a haemocytometer. The sterile Tissue culture bottles were properly labelled, corked and kept ready. 9ml of growth medium was added to each of the Tissue Culture Bottles. Cell suspension was then added to each of the Tissue Culture Bottles based on the cell count. The bottle was gently shaken so as to allow uniform dispersion of cells. Cell lines, date of seeding, passage number were indicated on the label on the bottle. The newly seeded bottles were tightly closed with the help of a stopper and then it was incubated at 37°C. The cell growth was observed everyday. On fourth (day 4) the medium was made decant and replenished with 10ml and medium maintained with 2% Fetal Calf Serum (FCS).The cells were maintained by dispersion and sub culturing of the cells was done in cell depository.

Cell Count

The cell counting was done with the help of Haemocytometer. The Haemocytometer's each corner square is $0.1mm^2$; Volume $0.1mm^3$ can accommodate 103 or 104 cells per ml.

Anti Tumor Assay

Different pHs were maintained to activate to these proteins (Actinogan, Lactonamycin, and Fredericamycin) in the broth. Presences of these compounds in broth were confirmed using SDS-PAGE for the visualising bands at 13.4 Kd for Actinogan, 10 Kd for Fredericamycin and 12.5 Kd for Lactonamycin. These proteins were extracted and the cell lines were treated with these proteins and tested for antitumor properties by counting cells after the treatment with the three components separately.

RESULTS

The three proteins namely Actinogan, Lactonamycin, Fredericamycin were present as secondary metabolites in Streptomyces species isolated from the sea shore. Samples were seeded with the Cancerous Hep2 cell lines and normal Vero cell lines in a 72 wells titre plate. The Lactonamycin was seeded with the normal Vero cell lines and the cancerous HeP2 cell lines. Observation reveals that the sample treated with Vero cell lines and HeP2 cell lines give out different ranges of cytotoxicity as shown in table 1. In Vero cell lines for the concentrations N, 1:1, 1:2, 1:3, 1:4 the cytotoxicity observed are 25%, 50%, 25% and 40% cytotoxicity were observed for the same concentrations.

Cytotoxicity in both the cell lines seeded with the Fredericamysin was 100%, 0%, 0%, 0% for Vero cell lines through the concentrations N, 1:1, 1:2, 1:3, 1:4 and 25%, 25%, 25%, 50% for HeP2 as given in table 2. Table 3 shows the cytotoxicity of Actionogan to Vero and HeP2 cell lines. The cytotoxicity for Vero cell line was 50%, 0%, 0%, and 0% and for HeP2 cell lines it was found to be 0%, 0%, 50%, and 0%. These cytotoxicity ranges were observed for the concentrations N, 1:1, 1:2, 1:3, and 1:4.

Table 1. Lactonamycin treated against Vero cell lines and Hep2 cell lines

Concentration / Cell Line	N	1:1	1:2	1:4
Vero cell lines	25%	0%	0%	25%
HeP2 cell lines	25%	50%	25%	50%

N, 1:1, 1:2, 1:3, 1:4 are the concentrations of the sample compounds and the cell lines respectively, where N is the neat.

Table 2. Fredericamycin treated against Vero cell lines and Hep2 cell lines

Concentration / Cell Line	N	1:1	1:2	1:4
Vero cell lines	100%	0%	0%	0%
HeP2 cell lines	25%	25%	25%	50%

N, 1:1, 1:2, 1:3, 1:4 are the concentrations of the sample compounds and the cell lines respectively, where N is the neat.

Table 3. Actinogan treated against Vero cell lines and Hep2 cell lines

Concentration / Cell Line	N	1:1	1:2	1:4
Vero cell lines	50%	0%	0%	0%
HeP2 cell lines	0%	0%	50%	0%

N, 1:1, 1:2, 1:3, 1:4 are the concentrations of the sample compounds and the cell lines respectively, where N is the neat.

DISCUSSIONS

Micororganisms have evolved to develop mechanisms to live in any inhabitable environment (Anand et al., 2012). Secondary metabolites Actinogan, Lactonamycin, and Fredericamycin were present in isolated Streptomyces species. The actinogan triggers its action by inhibiting the protein production (Bradner *et al*., 1962). The mode of action of Fredericamysin happens through inhibition of RNA and protein bio synthesis (Misra *et al*., 1982) and lactonamycin inhibits the cell cycle in the G2/m phase (Matsumoto *et al*., 1996). There is a complex interaction between carcinogens and the host genome may explain why only some develop cancer after exposure to a known carcinogen. The genetics of cancer pathogenesis, shows that DNA methylation, and micro RNA's play an important role in disease regulation. A normal cell gets transform into a cancerous cell due to alteration in genes which regulate cell growth and differentiation (Geran, 1972).

These three compounds are already used for malignant tumours, specifically germ cell tumours, Lymphomas, Head and Kaposi's sarcomas. The present study has solved the purpose to find the cheap natural source of antitumor agents from Streptomyces.

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