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ANTIOXIDANT AND ANTI-CANCER POTENTIAL OF MARINE LACTIC ACID BACTERIA

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ABSTRACT
Lactic acid bacteria (LAB) such as *Lactobacillus* are important micro-organisms in a healthy human micro biotic Environment. LABS are beneficial micro-organisms, which have been associated with several probiotic effects in both humans and animals. The Present study was carried out by collecting marine sediment samples from Vellar estuary, Parangipettai. The cell free extracts of morphologically different Lactic acid bacteria such as L1-L10 were evaluated for its Radical scavenging activity using DPPH. The selected potential strain L6 was produced 94% of scavenging rate and it was identified as *Lactobacillus plantarum* based on its, morphological, physiological and

INTRODUCTION
Lactic acid bacteria (LAB) such as *Lactobacillus* are important micro-organisms in a healthy human micro biotic Environment (Macfarlane and Cummings 2002). LABS are beneficial micro-organisms, which have been associated with several probiotic effects in both humans and animals (Fuller, 1989). Recently, many research studies have focused on anticancer and antioxidative properties as protective adjuncts against diseases (Yang et al., 2001 and Iwai et al., 2004).

Cancer is a dreadful human disease, increasing with changing life style, nutrition and global warming. According to the WHO, 80% of the world's population primarily those of developing countries rely on plant-derived medicines for the health care Gurib-Fakim, 2006. Natural products and their derivatives represent more than 50% of all the drugs in clinical use of the world. Higher plants contribute not less than 25% of the total. Almost 60% of drugs approved for cancer treatment are of natural origin. There is a positive correlation between the increased dietary intake of natural antioxidants and the reduced coronary heart diseases, cancer mortality, as well as longer life expectancy (Halliwell, 2007 and Rios, 2009).

Marine floras include microflora (bacteria, actinobacteria, cyanobacteria and fungi), microalgae, macroalgae (seaweeds), and flowering plants (mangroves and other halophytes). The marine organisms produce novel chemicals to with stand extreme variations in pressure, salinity, temperature, and so forth, prevailing in their environment, and the chemicals produced are unique in diversity, structural, and functional features (Kathiresan, 2008).

Marine microorganisms are a source of new genes, and exploitation of which is likely to lead to the discovery of new drugs and targets. Secondary metabolites produced by marine bacteria have yielded pharmaceutical products such as novel anti-inflammatory agents, anticancer agents and antibiotics. The contribution of probiotic bacteria, such as lactobacilli and bifidobacteria, is mainly in the control of pathogenic microbes, through production of antibacterial protein namely, bacteriocin (DeVugst, 1994 and Kathiresan, 2008) and anticancer substances (Wollowski, 2001). The dietary supplements of lactobacilli are reportedly decreasing the induction of experimental colon cancer B. R. Goldin 1992.

Probiotic lactobacilli have been known for centuries to promote human health and prevent human disease. Lactobacillus is a well characterized genus in the lactic acid bacteria (LAB) group, which is composed of 100 recognized species (Claesson et al., 2008).

The explanation for tumour suppression by lactic acid bacteria may be mediated through an immune response of the host. *Lactobacillus casei Shirota* (LcS) has been shown to have potent antitumour and antimetastatic effects on transplantable tumour cells and to suppress chemically induced carcinogenesis in rodents.

Cancer appears to be a major cause of morbidity and mortality and runs in the top three cause of death worldwide especially in the developed countries (WHO, 2009). Chemotherapy is one of the potent treatments for

prolonging the patient's life. However, many chemotherapeutic drugs are presently placed in a predicament of reduced therapeutic effect due to the problem of drug-resistance (Peters et al., 2002). Moreover, chemotherapeutic drugs also exert toxicity to normal cells which in turn causes the unpleasant side effects to the patients. For these reasons, research and development for new classes of anticancer agents which exhibit efficient and selective toxicity on tumour cells is attracting increased attention. Among various sources of anticancer drugs, microorganisms have more advantages regarding to the potentials in producing diverse compounds and in manipulation of the production.

MATERIALS AND METHODS

Marine sediment samples were collected from Vellar estuary, Parangipettai and they were serially diluted. 0.1ml from 10^{-4}, 10^{-5}, 10^{-6} dilutions were spread plated over the surface of the MRS agar plates. The plates were incubated at 37˚C for two days. The colonies were observed. Morphologically different 10 *Lactobacillus sp.* strains were selected for the screening of potential strain. They were named as L1, L2, L3.....L10.

Screening for Antioxidant activity (DPPH free radical scavenging activity) of *Lactobacillus sp.*

The scavenging activity for DPPH free radicals was measured according to Zhao et al., 2006. To 2mL of distilled water, 1mL of 0.1mM DPPH solution in ethanol and 0.5mL of cell free supernatant of *Lactobacillus sp.* (L1-L10) was added. The mixture was shaken vigorously and allowed to reach a steady state for 30min at room temperature. Decolourization of DPPH was determined by measuring the decrease in absorbance at 517 nm, and the DPPH radical scavenging effect was calculated according to the following equation:

$$\% \text{ scavenging rate} = [1 - (A1 - A2)/A0] \times 100,$$

Where *A*0 represents the absorbance of the control (DPPH without extract), *A*1 represents the absorbance of the reaction mixture, and *A*2 represents the absorbance without DPPH (DPPH was replaced by the same volume of distilled water).

Identification

The potential strain was selected based on the antioxidant activity. The highest antioxidant activity producing strain L6 was identified according to the method described by Bergey's manual Buchanan *et al.*, 1974.

Optimization

The factors like pH, temperature, salinity and substrate concentration which were optimized for potential strain.

Mass scale production

The optimized conditions, temperature - 35ºC, salinity – 1.0%, dextrose- 1.0%, peptone – 0.5% and pH 8 were maintained in the medium (250 ml). Four such flasks were kept for incubation at 35°C in a shaker for 42 hrs.

Ammonium sulphate precipitation and dialysis

The shake flasks kept for mass scale production were taken after 42hrs and centrifuged at 15,000 rpm for 10min. To the supernatant, the amount of ammonium sulphate required to give 70% saturation was added slowly with stirring. Dialysis was followed in a tubular cellulose membrane against 500ml distilled water for 24hrs at 4°C.

Separation of biomass from culture broth

After mass cultivation, the culture broth was centrifuged 10,000rpm for 5min. Then the pellet was separated and washed with phosphate buffer. Then the washed cells were broken using sonication and the debris was removed by centrifugation at 10,000rpm for 5min. Further it was used for the anticancer activity study.

Lyophilisation

The partially purified cell free extract and biomass was lyophilized in a Vertis lyophilizer and kept for further analysis.

ANTICANCER ACTIVITY

MINIMAL ESSENTIAL MEDIA PREPARATION

Media is defined as a complex source of nutritional supplementation vital for the growth proliferation and maintenance of cells in vitro. The MEM vial is dissolved in the pre sterilized Millipore distilled water and mixed well, closed and sterilized at 15lbs 121ºc for 15mins. Allow ingredients in the quantity, depending on the concentration of foetal calf serum (2% or 10%) mix well by shaking. Take care avoid spills pass CO_2 using sterile pipette, Shake the bottle, check pH and adjust to 7.2 to 7.4. The MEM bottles are kept for 2 days at 37ºc and checked for sterility, pH drop and floating particles they are then transferred to the refrigerator.

1. **Penicillin and Streptomycin (Concentration 100IU of Penicillin and 100 µg 0f Streptomycin)**

Dissolve both antibiotics in sterile Millipore distilled water, so as to give a final concentration 100 IU of penicillin and 100µg of streptomycin/ml. Mix well and distribute in 1ml aliquots. Store at -20ºC and check sterility.

2. **Fungizone (amphotericin B)**

Dissolve in sterile Millipore distilled water so as to give a final concentration of 20µg/ml and distribute in 1ml aliquots in vials. Store at -20ºc. Check sterility.

3. **L.glutamine: 3%**

Weigh 3g of l-glutamine accurately and dissolve in 100ml sterile Millipore distilled water and mix well. Filter through Millipore membrane filter 0.22µ and distribute in 5ml aliquots in vials. Store at -20ºc. Check sterility.

4. **7.5% sodium-bi-carbonate**

Weigh requisite quantity of sodium-bi-carbonate (to give 7.5% solution) accurately and dissolve in 100ml of

sterile Millipore distilled water. Filter through what man filter paper No.4, distribute into bottles and at 121ºc, 15lbs, 15mins. Cool and store at +4ºc.

5. Foetal calf serum

Bring FCS at room temperature and inactivated at 56ºC in water bath for½ hour and cool at room temperature. If floating particles are seen filter through Seitz filter. Distribute in 100ml, 50ml, 20ml quantities in sterile bottles. Store at -20ºc.

6. Trypsin, PBS, versene, glucose solution (TPVG)

2% Trypsin: 100ml

Weigh 2g of trypsin accurately; dissolve in 100 ml sterile Millipore distilled water with magnetic stirrer for ½ hour. Filter through membrane filter. Store at -20ºc.

0.2%EDTA (versene)

Weigh 200mg of EDTA accurately. Dissolve in 100 ml of sterile Millipore distilled water. Autoclave at 15lbs/15mins.

10% Glucose -100ml

Weigh 1g of glucose accurately. Dissolve in 100 ml of sterile Millipore distilled water and filter through whatman filter paper and autoclave at 15lbs/15mins. All ingredients were mixed and the pH was adjusted to 7.4 with 0.1 N HCl or 0.1 N NaOH. Distributed as 100 ml aliquots and were stored at -20^{0}C.

SUBCULTURING AND MAINTENANCE OF CELL LINE

The medium and TPVG were brought to room temperature for thawing. The tissue culture bottles were observed for growth, cell degeneration, pH and turbidity by seeing in inverted microscope. If the cells were become 80% confluent it goes for sub culturing process. The mouth of the bottle was wiped with cotton soaked in spirit to remove the adhering particles. The growth medium was discarded in a discarding jar. Then 4 – 5 ml of MEM without FCS was added and gently rinsed with tilting. The dead cells and excess FCS were washed out and then the medium was discarded. TPVG was added over the cells and incubated at 37ºC for 5 minutes for disaggregation. The cells were become individual and it was present as suspension. 5ml of 10% MEM with FCS was added by using serological pipette. Gently passaging was given by using serological pipette. If any clumps was present then the process was repeated. After passaging the cells were split into 1:2, 1:3 ratio for cytotoxicity studies for plating method.

"Seeding of cells"

After homogenization one ml of suspension was taken and poured in to 24 well plates. In each well 1ml of the suspension was added and kept in desiccators in 5% CO_2 atmosphere. After 2 days incubation observe the cells in inverted microscope. If the cells became 80% confluent.

Cytotoxicity assay

In order to study the antitumor activity of a new drug, it is important to determine the cytotoxicity concentration of the drug. Cytotoxicity tests define the upper limit of the extract concentration, which is non-toxic to the cell line. The concentration nontoxic to the cells is chosen for antiviral assay. After the addition of samples (Cell free extract and cells), cell death and cell viability was estimated. The result was confirmed by additional metabolic intervention experiment such as MTT assay.

PERFORMANCE OF DRUG CYTOTOXICITY ASSAY

Cytotoxicity is the toxicity or damage caused to the cells on addition of drug (Samples). After the addition of the drug, cell viability is estimated by staining techniques, whereby cells are treated with Trypan blue. Trypan blue is excluded by live cells, but stains dead cells blue. The results are confirmed by additional metabolic intervention experiments such as MTT assays.

Stock drug concentration

100 mg of samples (Cell free extract and cells) were dissolved in 10 mL of serum free MEM giving a concentration of 10mg / 1 mL. The stock is prepared fresh and filtered through 0.45µl filter before each assay. Working concentrations of drug ranging from 10mg/ml to 0.3125mg/ml are prepared as follow

Preparation of working stock of 1 mg /mL

To 4.5 ml MEM add 0.5 mL of stock to give a working concentration of 1mg/mL Samples (Cell free extract and cells) concentration can be prepared from the working stock in MEM without FCS. Prepare required volume of test sample for each concentration. 48hr monolayer culture of Hep2 cells at a concentration of one lakh /ml /well (10 cells / ml / well) seeded in 24 well titer plate. The plates were microscopically examined for confluent monolayer, turbidity and toxicity if the cells become confluent. The growth medium (MEM) was removed using micropipette. Care was taken so that the tip of the pipette did not touch the cell sheet. The monolayer of cells was washed twice with MEM without FCS to remove the dead cells and excess FCS. To the washed cell sheet, 1ml of medium (without FCS) containing defined concentration of the drug in respective wells was added. Each dilution of the drug ranges from 1:1 to 1:64 and they were added to the respective wells of the 24 well titre plates. To the cell control wells 1ml of MEM (w/o) FCS was added. The plates were incubated at 37ºc in 5% CO_2 environment and observed for cytotoxicity using inverted microscope.

MTT ASSAY

MTT assay is called as (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide.MTT assay was first proposed by Mossman in 1982. After incubation, remove the medium from the wells carefully for MTT assay. In each well wash with MEM (w/o) FCS for 2 – 3 times. And add 200µl of MTT conc of (5mg/ml). And incubate

for 6-7hrs in 5% CO2 incubator for Cytotoxicity. After incubation add 1ml of DMSO in each well and mix by pipette and leave for 45sec. If any viable cells present formazan crystals after adding solublizing reagent (DMSO) it shows the purple color formation. The suspension is transferred in to the cuvette of spectrophotometer and an O.D values is read at 595nm by taking DMSO as a blank.

- Cell viability (%) = Mean OD/Control OD x 100

RESULTS

Lactic acid bacteria were isolated from sediment samples, Vellar estuary using MRS agar. The colonies were creamy white, transparent and smooth round in shape. In the present study cell free extracts of morphologically different Lactic acid bacteria such as L1-L10 were evaluated for its Radical scavenging activity using DPPH. The effects of the *Lactobacillus sp.* cell free extract on the antioxidant activity *in vitro*, the DPPH scavenging rate of the cell free extract was examined. When the cell free extract concentration was 10mg/mL, the DPPH scavenging rate 40%, 55%, 45%, 60%, 80%, 94%, 72%, 70%, 65%and 79% were respectively (Fig. 1).

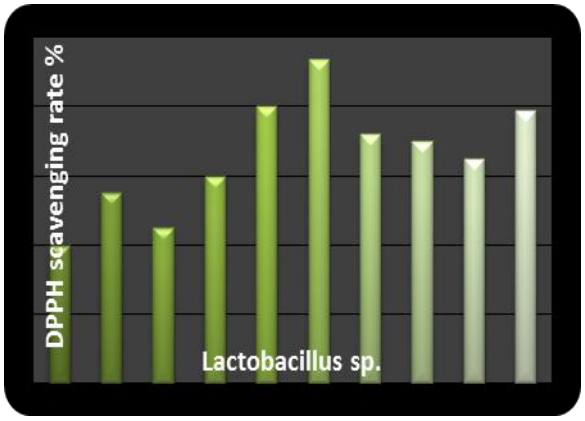


Fig1: Scavenging effect on DPPH radicals by cell free extracts of *Lactobacillus sp.*

The selected potential strain L6 was produced 94% of scavenging rate and it was identified as *Lactobacillus plantarum* based on its, morphological, physiological and biochemical characteristics. The strain was gram positive, non motile, non spore forming and rod shaped. It showed positive reaction in oxidase, fermentation in fructose, glucose and mannitol. It was catalase negative and negative in MR-VP test and nitrate reduction.

Culture conditions like temperature (25ºC, 30ºC, 35ºC and 40ºC), pH (6, 7, 8, 9, 10 and 11), and salinity (0.5%, 1.0%, 1.5%, 2.0% and 2.5%, nutrients like carbon sources (1.0%) (Starch, cellulose, sucrose, maltose and dextrose) and nitrogen sources (0.5%) (Yeast extract, casein, peptone, ammonium sulphate and beef extract) were found influence on growth. The identified optimized parameters were as follows, pH-8, temperature-35°C, salinity-1.0%, dextrose-1% and peptone-0.5% and incubation time 42hrs were found to be ideal conditions for the strain used in the present study for mass scale production.

The shake flasks kept for mass scale production were taken after 42hrs.After mass cultivation; the culture broth was centrifuged at 10,000rpm for 5min. Then the pellet and supernatant was separated. To the supernatant, the amount of ammonium sulphate required to give 70% saturation was added slowly with stirring. Dialysis was performed in a tubular cellulose membrane against 500ml distilled water for 24hrs at 4°C.

The pellet was separated and washed Phosphate buffer. Then the washed cells were broken using sonication and the debris was removed by centrifugation at 10,000rpm for 5min. Further it was used for the anticancer activity study. The partially purified proteins and cells was lyophilized and used for further analysis.

Cytotoxicity in partially purified protein sample seeded with the cell line Concentration (µg/ml) and cell viability (%) was 1000 (35.63), 500 (41.22), 250 (48.55), 125 (52.33), 62.5 (61.45) 31.25 (65.23), 15.625 (77.88), 7.8125 (81.25) and3.906 (96.32)for HeP2 as given in Fig 2. Fig 3 shows the cytotoxicity of cells to HeP2 cell lines. The cytotoxicity for Hep2 cell lines it was found to be 12.33, 15.69, 20.89, 25.69, 32.58, 51.02, 59.63, 65.24 and 78.96%. These cytotoxicity ranges were observed for the concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.906(µg/ml).

The partially purified proteins and cellswere present in *Lactobacillus plantarum* isolated from the Vellar estuary. Samples were seeded with the Cancerous Hep2 (Hepato cellular liver carcinoma) cell lines. Observation reveals that the sample treated with Hep2 cell lines give out different ranges of cytotoxicity as shown in Fig 4 and 5.

Hence the present study has solved the purpose to find the cheap natural source of antitumor agents from *Lactobacillus plantarum* isolated from marine environment.

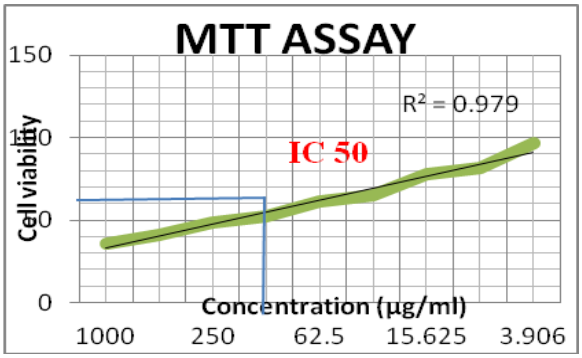


Fig 2 MTT assay of Cell free extract *Lactobacillus plantarm*

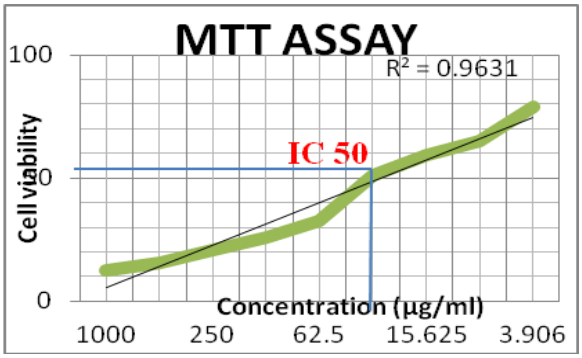
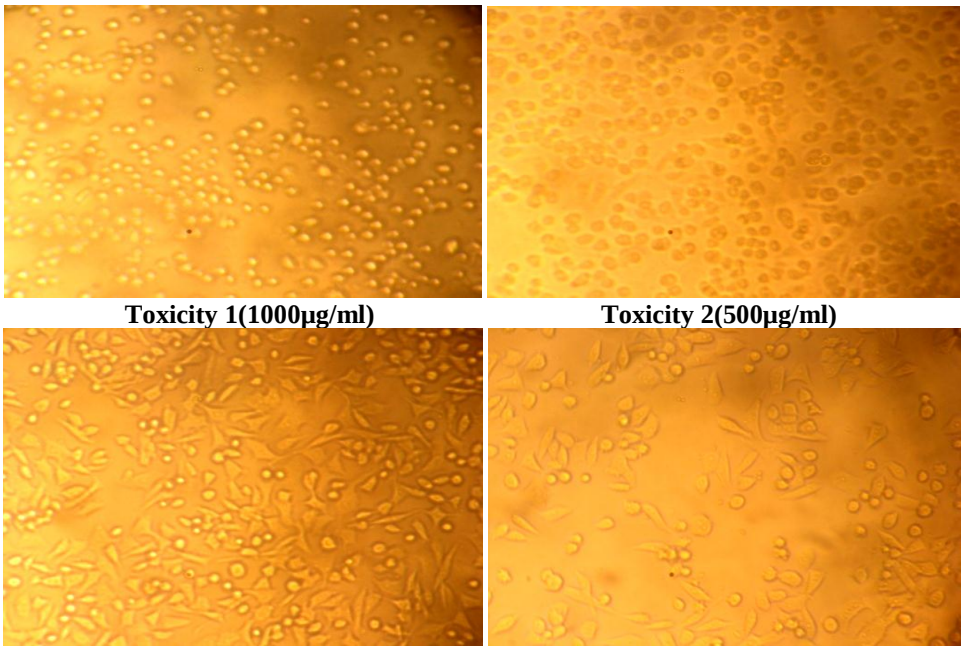


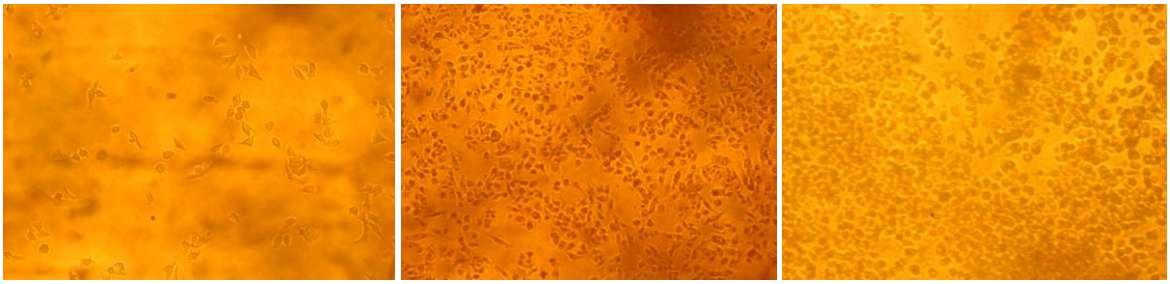
Fig: 3 MTT assay of Cells from Lactobacillus plantarm



Toxicity 1(1000µg/ml) **Toxicity 2(500µg/ml)**

Toxicity 4(31.25µg/ml) **Toxicity 3 (62.5µg/ml)**

Fig:4 Cytotoxicity effect of cell free extract from *Lactobacillus plantarm* on Hep2 cell line



Toxicity 1(1000µg/ml) **Toxicity 2(500µg/ml)** **Toxicity 3(62.5µg/ml)**

Fig: 5 Cytotoxicity effect of cells from *Lactobacillus plantarm* on Hep2 cell line.

DISCUSSION

Probiotics are defined as "living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma, 1998; Tannock, 2002) but interest in this area was initiated by Metschnikov 100 years ago (Metschnikoff,1907). Most probiotic microorganisms belong to Lactic Acid Bacteria (LAB), such as *Lactobacillus* sp, *Bifidobacterium* sp and *Enterococcus* sp (Klein *et al*., 1998). The yeast *Saccharomyces boulardii* has been studied extensively (Elmer *et al*., 1999) and also other bacterial species, like *Bacillus* sp (Senesi*et al*., 2001) and *Clostridium butyricum* (Takahashi *et al*., 2004). In some countries the use of *Enterococcus* sp as a probiotic has been questioned because of safety aspects with regard to transfer of genes conferring antibiotic resistance (Lund and Edlund, 2001). Most scientists agree that probiotic strains shall be able to survive transit through the gastric acid environment as well as exposure to bile and pancreatic juice in the upper

small intestine to be able to exert beneficial effects in the lower small intestine and the colon, although there are convincing data on beneficial immunological effects also from dead cells (Mottet and Michetti, 2005).Best effect is achieved if the microorganisms colonize the intestinal surface mucus layer since they then can affect the intestinal immune system, displace enteric pathogens, provide antioxidants and antimutagens, and possibly other effects by cell signaling. That intake of LAB influences multiple systems was elegantly shown for *Lactobacillus* GG using microarray analysis (Di Caro *et al*., 2005). One month treatment resulted in up-regulation of 334 genes and down-regulation of 92 genes involved in inflammation, apoptosis, cell-cell signaling, cell adhesion and differentiation and signal transcription and transduction.

In recent years, multiple reports have described beneficial effects from various aspects on important diseases, like intestinal infections, inflammatory bowel disease (IBD), and allergy by addition of selected strains to food products, often together with fiber or a prebiotic substance. In many countries, there are now several probiotic products on the market but the documentation is often based upon case reports, animal studies or uncontrolled small clinical trials. Furthermore, there is no general acceptance on how to characterize probiotic microorganisms, and few products declare the actualcontent of live microorganisms.

In the present study *Lactobacillus sp.* was isolated from sediment samples, Vellar estuary using MRS agar. The colonies were creamy white, transparent and smooth round in shape. Anand et al., 2012 detected the presence of antitumor agents Actinogan, Fredericamycin and Lactonamycin in *Streptomyces* sp isolated from the sea shore.

In the present study the effects of the *Lactobacillus sp.* cell freeextract on the antioxidant activity *in vitro*, the DPPH scavenging rate of the cell free extract was examined. When the cell free extract concentration was 10mg/mL, the DPPH scavenging rate 40%, 55%, 45%, 60%, 80%, 94%, 72%, 70%, 65%and 79% were respectively.Monajjemi et al., 2012 testedantioxidative activity of supernatant from fermented soy whey to find out their nutraceutical potential. Fermented soy whey was prepared using *Lactobacillus plantarum*, *Streptococcus thermophilus*and Kefir, fermented at room temperature (25-26°C) for 24 and 48hrs. Antioxidative properties were assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity. All fermented soy whey extracts exhibited a potentially antioxidant activities, yet extract kefir soy whey with 48 hrs incubation gave antioxidant activity 90% compared to others in the present study *Lactobacillus plantarum*produced highest antioxidant activity 94%.

In the potential strain *Lactobacillus plantarum* optimized and identified ideal parameters were as follows, pH-8, temperature-35°C, salinity-1.0%, dextrose-1% and peptone-0.5% and incubation time 42hrs were found to be ideal conditions for the strain used for mass scale production in MRS broth.The shake flasks kept for mass scale production were taken after 42hrs.After mass cultivation, the culture broth was centrifuged 10,000rpm for 5min. Then the pellet and supernatant was separated. To the supernatant, the amount of ammonium sulphate required to give ammonium sulphate 70% saturation was added slowly with stirring. Dialysis was followed in a tubular cellulose membrane against 500ml distilled water for 24hrs at 4°C. Anand et al., 2012reported 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. The Protein Precipitation was carried out by using 10% TCA and 1X PBS buffer.

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).

In the present study cytotoxicity in partially purified protein sample seeded with the cell line Concentration (µg/ml) and cell viability (%) was 1000 (35.63), 500 (41.22), 250 (48.55), 125 (52.33), 62.5 (61.45) 31.25 (65.23), 15.625 (77.88), 7.8125 (81.25) and 3.906 (96.32)for Hep2 as given in table 2. Table 3 shows the cytotoxicity of cells to HeP2 cell lines. The cytotoxicity for HeP2 cell lines it was found to be 12.33, 15.69, 20.89, 25.69, 32.58, 51.02, 59.63, 65.24 and 78.96%. These cytotoxicity ranges were observed for the concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.906(µg/ml).

Anand et al., 2012 detected the presence of antitumor agents Actinogan, Fredericamycin and Lactonamycin in *Streptomyces* isolated from the sea shore. 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. 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).

Luis et al., 2010 evaluated anticancer activities of extracts from the seaweeds *Egregiam enziesii, Codium fragile, Sargassum muticum, Endarachnebin ghamiae, Centroceras clavulatum*and *Laurencia pacifica* collected from Todos Santos Bay, México. Organic extracts were obtained from bacteria-free algae and from surface-associated bacteria. Bacterial strains were isolated from the surface of seaweeds as belonging to the phyla *Firmicutes*, *Proteobacteria* and *Actinobacteria* showed anticancer activity, with IC_{50} valuesof 6.492, 5.531, and 2.843µg/ml respectively.

The marine environment represents a relatively untapped source of functional ingredients that can be applied to various aspects of food processing, storage, and fortification. Moreover, numerous marine-based compounds have been identified as having diverse biological activities, with some reported to interfere with the pathogenesis of diseases. Bioactive peptides isolated from fish protein hydrolysates as well as algal fucans, galactans and alginates have been shown to possess anticoagulant, anticancer and hypocholesterolemic activities. Additionally, fish oils and marine bacteria are excellent sources of omega-3 fatty acids, while crustaceans and seaweeds contain powerful antioxidants such as carotenoids and phenolic compounds. On the basis of their bioactive properties, in the present study focuses on the potential use of marine-derived *Lactobacillus plantarum* isolate as functional food ingredients for health maintenance and the prevention of chronic diseases.

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