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	Contents	Page No.
1.	Report on fungi from some of the rotten vegetables. *Dr Regi Raphael K*	1-8
2.	Qualitative analysis of ground water of Guruvayoor. *Dalie Dominic A and Swapna Johny*	9-12
3.	Biochemical changes in the four morphogenic pathways of *in vitro* cultures in *Datura metel* Linn. *Manju Madhavan and Joy P Joseph*	13-16
4.	Effect of fungicide Carbendazim on the growth and yield of *Phaseolus aureus* Roxb. *Rekha K and Merlee Teresa M V*	17-19
5.	Optimization of growth conditions for the production of xylanase enzyme by Streptomyces species and its application. *Anjana J C and Sumathi R K*	20-26
6.	A study on partial characterization of Lindane degrading Lin- A gene product from Bacillus species. *Ruveena T N and Thamarai Selvi*	27-32
7.	Extracellular polysaccharides isolated from *Saccharomyces cerevisiae* inhibit acute and chronic inflammations in mice. *Meera C R and Janardhanan K K*	33-38
8.	Lipase producing thermophilic microorganisms from different organic sources. *Wilsy Wilson, Balasundaran M and Nishad V M*	39-41
9.	Pharmacogenetics- The concept of individualized medicine. *Anjaly Anto M*	42-45
10.	Free radicals and antioxidants *Priya U and Theji Mohan M*	45-47
11.	Neocarzinostatin induces and effect p53 – dependent response in HPV positive cervical cancer cells. *Rajitha K P*	48-49
12.	Statistics and Ethics in Medical Research. *Anitha Chelat*	50-53

Report on fungi from some of the rotten vegetables

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Abstract

Fungal organisms cause diseases in plants, animals and human beings. They are responsible for the deterioration of organic objects. Isolation of fungi associated with the rotten vegetables has been done. Isolations were made from 7 rotten vegetables collected from Sakthan market. These vegetables yielded various fungi pathogens including *Fusarium sp, Rhizopus sp, Trichoderma sp, Aspergillus niger, Aspergillus flavus* and Aspergillus *fumigatus*. The fungi were isolated on potato dextrose agar, described and illustrated.

Key words: Fungal diseases, pathogens, rotten vegetables.

1. Introduction

Plant diseases are known from times preceding the earliest writings. Fossil evidence indicates that plants were affected by disease 250 million years ago. The Bible and other earlier writings mention diseases such as rusts, mildews, blights and blast that have caused famine and other drastic changes in the economy of nations since the dawn of recorded history.

The advances made in the food production in developing countries due to green revolution can be lost if proper attention is not given to plant diseases and other pests. The higher and increased yields of green revolution are based on practices which are generally favorable to attacks by plant pathogens. Today there is an increasing awareness on the part of scientific agricultural community that only through deeper and more fundamental understanding of all the interacting components of agriculture can be expect to improve our capabilities of feeding the expanding world population. Therefore the role of plant pathologists is assuming greater importance in present-day world.

Fungi are a diverse group of either single celled or multicellular organisms that obtain food by direct absorption of nutrients. The fungi is dissolved by enzymes that the fungi excrete which is then absorbed through thin cell wall, is distributed by simple circulation or streaming of the protoplasm. Some fungi are parasitic on living matter and cause serious plant and animal diseases (Mishra et al., 2005).

Some of the fungi, parasitic or saprophytic, associated are known to cause ample damage to the seeds during storage and also adversely affect the crop health (Neergaard.,1970; Kanaujia.,1974). The major types of losses caused by fungi in stored seeds are of loss of viability, discolouration, heating, production of toxins and loss of weight (Narayana Reddy.,1983). Associated fungi appreciably affected the germination of the seeds and there are many reports on 80-100% reduction of germinability of stored seeds (Mishraand Kanaujiia., 1973). The

factors that influence fungal activity in seeds include moisture content, humidity, temperature, aeration and duration of storage and according to one estimate in tropical climates the annual loss during storage is around 45% of annual production.

Fungal spoilage of food commodities and their products is responsible for the significant loss in the world food supply (Mislivec et al., 1972). Fungal infection during pre harvest condition and subsequent colonization during storage bring about grain deterioration. Fungal growth on food has two consequences such as deterioration in quality and production of secondary metabolites known as mycotoxins, which are carcinogenic, mutagenic and teratogenic. Food and feed borne fungi not only cause health hazards in animals but they also affect trade between two trading nations. Therefore enumeration and correct identification of spoilage fungi is very important in alleviating the loss due to biodeterioration and also to reduce the exposure of human and animal life to toxic substances.

Moreover, many microorganisms, including a number of moulds and a part of bacteria and yeast are able to produce toxins in certain conditions. *Fusarium*, *Aspergillus*, *Pencillium* and *Alternaria* are most common toxicants in stored grain. The primary contamination of stored fungi appeared to be at the source at or after harvest and in storage prior to export. Some of the contaminants were toxigenic and were therefore, dangerous.

The purpose of present work was to isolate and identify different fungi associated with the rotten vegetables.

2. Materials and Methods

Rotten vegetables were collected from Sakthan Market, Thrissur. They were *Lycopersicum esculentus* (Tomato), *Solanum tuberosum* (Potato), *Daucus carota* (Carrot), *Momordica charantia* (Bitter Gourd), *Cucumis sativus* (Cucumber), *Benincasa cerefera* (Ash Gourd) and *Brassica oleracea var. capitata* (Cabbage).

2.1. Sterilization

The needle, scalpel and scissors are sterilized by heating over a flame for a few seconds. Petridishes and flasks of media are autoclaved at 15 lb pressure for 15 minutes.

2.2. Isolation and identification of fungi

Vegetable samples inspected for any visible sign of rotting were used for this study. They were cut into small pieces and surface sterilized with 70 % ethanol for one minute. Immediately after this treatment they are transferred to dishes containing sterile distilled water and washed thoroughly to free them from the chemicals. The bits of tissues thus surface sterilized are transferred on to PDA medium in petridishes and examined daily for the growth of the organism. Pure cultures of samples were made by colony sampling method and were subsequently maintained on PDA (Anonymous., 1966).

Place a drop of lacto phenol cotton blue on a clean slide. Transfer a small tuft of the fungus, preferably with spores and spore bearing structures, into the drop, using a flamed, cooled needle. Gently tease the material using two mounted needles. Mix gently the stain with the mould structures. Place a cover slip over the preparation taking care to avoid trapping air bubbles in the stain. The excess fluid is blotted out and the cover slip sealed with finger nail polish. Allow the preparation to dry overnight. Apply a second coat of nail polish over the first coat. Examine the preparation under low-power and high-power objectives. Major morphological characters of the fungi were observed by using Olympus research microscope.

3. Results and Discussion

Mycelial growth on nutrient medium from the infected tissues indicates that the disease may be due to a fungal pathogen that can grow on a nutrient medium. The fungal cytoplasm is seen as a lightly stained blue region forming a layer inside the unstained cell wall of hyphae, conidia that surrounded by a light blue background on the slide. In the present study 6 fungal species were identified (Table 1).

3.1. Fusarium sp.

The aerial mycelium first appears white, and then may change to pink (Fig 1a). From the reverse, it is colorless. *Fusarium* produces two types of asexual spores: microconidia and macroconidia (Fig 2a). Microconidia are one or two celled, and are the type of spore most abundantly and frequently produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macroconidia are three to five celled, gradually pointed and curved toward the ends.

3.2. Rhizopus sp.

Colonies of *Rhizopus* grow very rapidly, fill the Petri dish, and mature in 4 days. The texture is typically cotton-candy like. From the front, the color of the colony is white initially and turns grey to yellowish brown in time (Fig 1b). The reverse is white to pale. Pathogenic species of *Rhizopus* can grow well at 37°C.

Nonseptate or sparsely septate broad hyphae, sporangiophores, rhizoids (root-like hyphae), sporangia, and sporangiospores are visualized. Sporangiophores are brown in color and usually unbranched. They can be solitary or form clusters. Rhizoids are located at the point where the stolons and sporangiophores meet. Sporangia are located at the tip of the sporangiophores. They are round with flattened bases. Apophysis is absent and columellae are hemispherical. Sporangiospores are unicellular, round to ovoid in shape, hyaline to brown in color, and smooth or striated in texture (Fig 2b).

3.3. Trichoderma sp.

The colonies are in shades of dull to dark green (Fig 1c). Conidiophores are highly branched and compactly tufted, often formed in distinct concentric rings. Main branches of the conidiophores produce lateral side branches that may be paired or not. The branches may rebranch, with the secondary branches often paired and longest secondary branches being closest to the main axis. All primary and secondary branches arise at or near 90° with respect to the main axis. The typical *Trichoderma* conidiophore, with paired branches assumes a pyramidal aspect. Conidia typically appear clear green and globose (Fig 2. c).

3.4. Aspergillus niger

Mycelium is submerged with more or less yellow colour in hyphae. Conidiophores arise from substratum, smooth and nonseptate. Conidial heads are blackish brown to carbonous black (Fig 1.d) and heads are globose, phialides typically in two series thickly covering the vesicle (Fig 2.d). Conidia globose at first smooth, later spinulose with colouring substance.

3.5. Aspergillus flavus

When young, the conidia of *A. flavus* appear yellow green in color. As the fungus ages the spores turn a darker green. (Fig 1.e). Conidiophores arise separately, broadening upward; walls pitted appeal rough or spiny and form a spherical vesicle. Conidial heads are spherical with dome shaped vesicles and single or double series of phialides (Fig 2.e). Conidia pyriform to globose, colourless to yellow green.

3.6. Aspergillus fumigatus

Colonies are bluish green (Fig 1.f) with strictly columnar conidial heads. Pigmented conidiophores with clavate vesicles arising from clearly differentiated thick walled foot cells (Fig 2.f). Conidia globose to subglobose and uninucleate.

Fusarium is a large genus of filamentus fungi widely distributed in soil and in association with plants. Most species are harmless saprobes and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. Mass casualties occurred in the Soviet Union in the 1930s and 1940s when *Fusarium*-contaminated wheat flour was baked into bread, causing 60% mortality rate.

Solanaceous crop plants may be infected at any age by the fungi that cause Fusarium wilt. Potato tubers may show browning of the vascular ring as well as browning at the stem end and decay where stolons are attached. If soils are severely infested, production of solanaceous crops may not be possible unless soil fumigation is an option.

Rhizopus is a cosmopolitan filamentous fungus found in soil, decaying fruit vegetables, animal faeces and old bread. While *Rhizopus sp* is common contaminants, they are also occasional causes of serious infections in humans. Some species are plant pathogens.

Trichoderma are in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants.

Aspergillus is a fungus whose spores are present in the air we breathe, but do not normally cause illness. However an individual with a weakened immune status may be susceptible to infection. Aspergillosis is a group of diseases whcich can result from *Aspergillus* infection.

A. niger causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. The fungus also causes disease in peanuts and in grapes.

A. flavus is a common mold in the environment, and can cause storage problems in stored grains. It can also be a human pathogen, associated with aspergillosis of the lungs. Many strains produce significant quantities of aflatoxin, a carcinogenic and acutely toxic compound. *A. flavus* spores are allergic.

A. fumigatus is a fungus of the genus *Aspergillus,* which is widespread in nature, typically found in soil and decaying organic matter such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce thousands of minute grey-green conidia from conidiophores that readily become airborne. Its spores are ubiquitous in the atmosphere and it is estimated that everybody inhales several hundred spores each day; typically, however, these are quickly eliminated by the immune system in healthy individuals. In immunocopromised individuals such as transplant patients and people with AIDS or leukaemia the fungus is capable of becoming pathogenic.

Govindaswamy et al reported that 1957 reported that Species of *Aspergillus* were mostly responsible for the reduction in seed germination and viability loss in rice. Similar situation of high percent association of *Aspergillus* species has been observed in green gram (Varsha et al., 1997). *A.*

flavus species were known to elaborate toxins which inhibit the germination of seeds of many crop plants (Vidyasekaran and Subramanian, 1970).

For each species of fungi, there is a minimum level moisture content below which cannot grow in products. Carbohydrates, protein, fat, reducing sugars, and total nitrogen content were reduced due to mold infection and it was understood that the carbohydrate metabolism is altered by pathogenic invasion. It was also reported that changes in proteins and amino acids in peanuts infected with *Aspergillus parasiticus* and also observed that the proteins were hydrolyzed first to small polypeptides or insoluble components and then to free amino acids due to the infection.

Reports of fungi from seeds, fruits and vegetables in storage are common. Reports of various species of *Aspergillus* are on record from the seeds of vegetables.

Air is made up of many types of spores and other gases, the fungal species which colonized the vegetables were contaminants from the atmosphere. There is a considerable economic loss to the nation due to decaying and rotting of vegetables because of fungi and other microorganism. The great ability of many fungi to adapt to the most diverse habits is well known. Fungi are involved in the necrotisation and these fungi could be easily identified by their fruit bodies. Due to their inability to synthesize their food, they are forced to live on saprophytes on dead organic matter or parasites on other living organisms.

The fungi not only cause seed deterioration but also serve as sources of primary inoculums of many diseases like seedling blight, damping off and wilts in nursery beds and fields. Scientists have found that it is not the occurrence but the number of spores per unit area which has a direct effect on development of diseases in fields as well as in markets.

It has been reported that the fungi found on the surface of the vegetables seeds are pathogenic and effect the germination of seeds.

4. Conclusion

Present study shows that the members of *Aspergillus* are dominant colonizers of rotten vegetables. *Fusarium sp* is present in all the rotten vegetables and *Trichoderma sp* in *Momordica charantia* and *Cucumis sativus*. *Rhizopus sp* is identified in *Solanum tuberosum, Daucus carota, Benincasa cerefera and Brassica oleracea var capitata*. The abundance of *Aspergillus* on vegetables can be attributed to their ubiquitous nature and ability to grow over wide range of temperatures, PH, moisture content and range of substrates. The moisture content is an important factor that influences growth of microorganisms. The high pH values of the tissues of many vegetables make them more susceptible to fungal invasion than fruits.

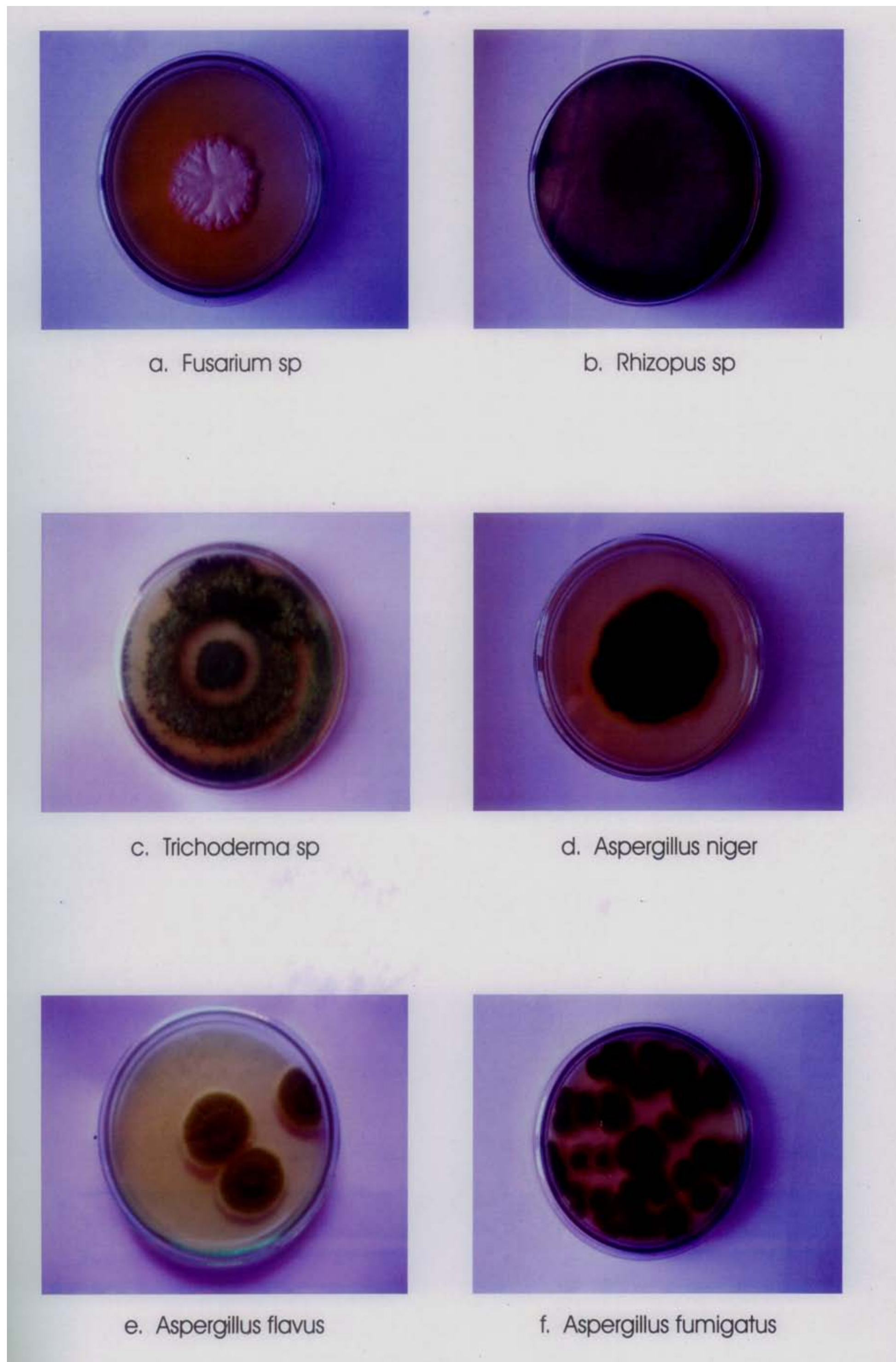
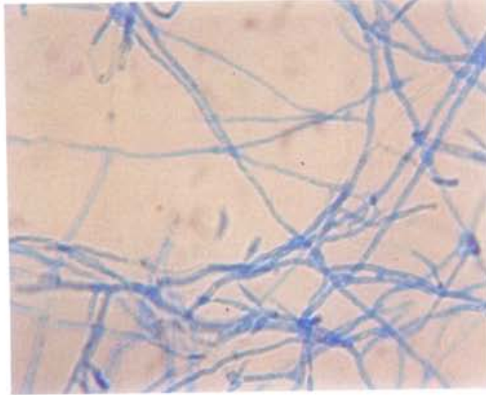
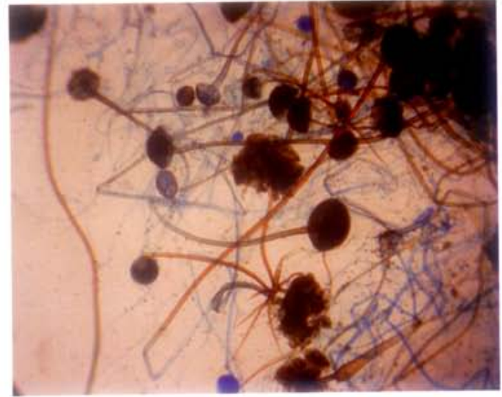


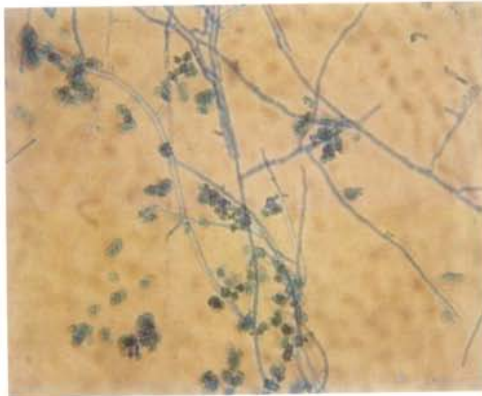
Figure 1. Various isolated fungi growing on medium.



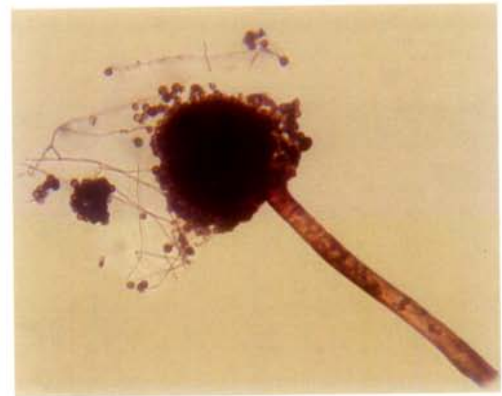
a. Fusarium sp



b. Rhizopus sp

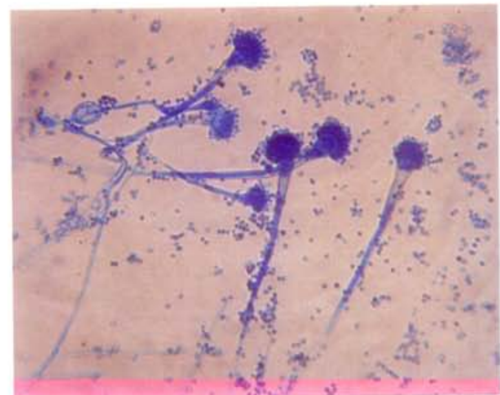


c. Trichoderma sp



d. Aspergillus niger

e. Aspergillus flavus



f. Aspergillus fumigatus

Figure 2. Spores and spore bearing structures of isolated fungi.

Table 1. Fungi isolated from rotten vegetables

SI. No	Host and Family	Pathogen
1	*Lycopersicum esculentus* (Solanaceae)	*Fusarium sp*
2	*Solanum tuberosum* (Solanaceae)	*Fusarium sp, Rhizopus sp*
3	*Daucus carota* (Apiaceae)	*Fusarium sp, Rhizopus sp*
4	*Momordica charantia* (Cucurbitaceae)	*Fusarium sp, Aspergillus niger, Aspergillus fumigatus, Trichoderma sp*
5	*Cucumis sativus* (Cucurbitaceae)	*Fusarium sp, Aspergillus niger, Trichoderma sp*
6	*Benincasa cerefera* (Cucurbitaceae)	*Fusarium sp, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Rhizopus sp.*
7	*Brassica oleracea var. capitata (*Brassicaceae)	*Rhizopus sp, Fusarium sp*

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Qualitative analysis of ground water of Guruvayoor

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Abstract

The world is today facing many pollution problems. With this view the study was conducted to analyze the status of groundwater quality of Guruvayoor. Various parameters like color, odour, pH, oxygen, carbon dioxide, hardness, chlorides and TDS were studied. The values of most of the parameters were above the permissible limits indicating pollution.

Key words: Ground water quality, Guruvayoor, hardness, oxygen, carbon dioxide, TDS

1. Introduction

The world's water resources form a single entity. About 90% of the earth's water is found in oceans. At present this water is not directly available for use. The rain water is directly used in first instance, as it falls from skies much of this percolates through the surface and subsoil replenish the ground water supply from which some of it is taken for use before it is eventually discharged via rivers into oceans.

The abundance of water of India surprised the Persian when they first set foot in India. They called the area "Hindusthan" which in ancient Persian means "Land of water". According to the report published by the National Commission of Integrated Water Resources Development plan, the total water resources for India that can be considered usable came only to 1086 billion cubic meters (bcm). This includes river, freshwaters and replenishable ground water.

India faces great pollution problems, hardly 10-20% of the people get clean water, 80% of the people quench their thirst from polluted water, and the rest are left with no source of water.

Kerala even though called God's own country still faces problem of water shortage, quality degradation and son on. The quality of water is of vital concern for mankind since it is directly linked with human welfare. The ground water and surface water contains dissolved minerals from the soil layers through which it passes. In the process of seepage through the ground, the water gets depleted most of the microorganisms originally present in surface water.

Even though, three fourth of the earth his covered with water, yet paradoxically water problems exist around the world. Faecal pollution of drinking water has in the past caused many water borne diseases which wiped out entire population of cities. At present, the menace of water borne, diseases and epidemics still looms large on the horizons of developing countries. Polluted water is the culprit in all such cases. The major sources of water pollution are domestic waste from urban and rural areas and industrial

wastes which are discharged into natural water bodies.

2. Materials and Methods

2.1. Sample Collection

The area selected for the study extended to about 3 KM around Guruvayoor. About ten sample collecting areas were selected. The selection was done keeping in mind the uniform distribution of selection sites, the water from the wells were collected and analysis of their physico-chemical characters were carried out to study their suitability for drinking purposes. For collection plastic bottles of 1 liter were used.

3. Results and Discussion

The results are represented in Table 1. The permissible limits for the various parameters are represented in Table 2. The table indicates color of the water varied from colorless to yellowish; this is due to residues of decomposing organic matter. The colorless condition required for drinking water has been surpassed here. Sample 3 and 6 were yellowish. Inspite 1 sediments were visible as brown suspensions. Even though the water samples were without any objectionable odour, the result indicated drastic polluted condition.

The pH of the water sample ranged from 6 to 8. This also is indicating a polluted condition as this is above the permissible limit.

The dissolved oxygen is a measure of the ability of water body to support a well balanced aquatic life. Insufficient dissolved oxygen leads to onset of anaerobic conditions and release of obnoxious gases. Here the reports show that the oxygen level in most of the samples were very low with a least in sample 6 and sample 8. Dr. Rajamani of Cherthala N.S.S. College has reported low oxygen levels in waters of Thaneermukkam panchayath (Vanitha, Jan. 2004). She has reported an increase in the incidence of cancer there due to drinking of water containing chemical elements above the permissible limits. Therefore it is essential that the dissolved oxygen and other water qualities should be maintained within the permissible limit.

The dissolved carbon dioxide to a certain limits is not harmful but above 60 ppm it is harmful. Here the reports show that the carbon dioxide amount went high in sample 3 to 100 ppm and it was 60 ppm in sample 2 indicating pollution.

The bicarbonates, chlorides and sulphates dissolved in the water primarily account for the water hardness. The hardness caused by bicarbonates is called carbonate hardness or temporary hardness. Hardness caused by sulphates and chlorides of calcium and magnesium is called permanent hardness; this cannot be removed by heating. The hardness of the most of the samples (S3, S4, S5, S8, and S10) is above the permissible limit indicating pollution. Studies conducted by Jayan et al have brought to light the greater incidents of kidney stones in patients who used well water containing higher amounts of total hardness and calcium (Hindu).

In many areas the level of chlorides in natural water is an important consideration. In places where brackish waters must be used for domestic purposes, the amount of chlorides present is an important factor in determining the quality. It is is used to control pumping ground water from locations where intrusion of sea water is a problem. The chloride there ranged from 6 mg/ liter to 29 mg/liter.

The total dissolved solids in the various samples were also above the permissible limit. When the limits were 500 mg/liter, many samples showed TDS content above this and it increased to 1250 mg in some samples. The well water users with high TDS are also at the risk of kidney disorders.

Thus the water of the area is heavily polluted in terms of all parameters. The waters require immediate treatment and various ways of treatment have to be scheduled, proper drainage facilities have to be immediately charted out. Strategies have to be implemented in different regions depending on water problems faced by the region due to inefficient use or pollution. Both local people and the government need to work together to provide pure water to the locality.

Table 1. Various physico – chemical parameters of the drinking water analyzed at various sites in Guruvayoor.

SI No.	Colour and odour	Dissolved Oxygen (ppm)	Dissolved Carbon dioxide (ppm)	Total Hardness (mg/l)	Calcium (mg/l)	Chloride (mg/l)	TDS (mg/l)
1	Brownish Odourless	2	17	132	52.90	20	500
2	Colourless Odourless	8	60	52	20.84	17	750
3	Yellowish Odourless	2	100	260	104.20	29	1250
4	Colourless Odourless	9	36	152	60.92	6	250
5	Colourless Odourless	3	20	152	60.92	9	750
6	Yellowish Odourless	1	9	44	17.63	12	1250
7	Colourless Odourless	2	13	28	11.22	18	500
8	Colourless Odourless	1	22	180	72.14	24	500
9	Colourless Odourless	2	8	84	33.66	6	500
10	Colourless Odourless	7	3	192	76.94	11	1000

Table 2. Standard values for Physico- chemical parameters

SI No.	Physico-chemical parameters	Permissible Limits		
		Drinking	Bathing	Irrigation
1	Colour, Odour and Taste	Colourless Odourless Tasteless	-	-
2	pH	6.5 - 8.5	6.5 - 8.5	6.0 - 8.0
3	Dissolved Oxygen	6	5	-
4	Carbon Dioxide	-	-	-
5	Total hardness	100	-	-
6	Calcium	100	-	-
7	Chlorides	250	-	-
8	Total dissolved solids	500	500	-

4. Conclusion

Thus of the all sample analyzed only a few were unpolluted and the other indicated the analyzed parameters far from the permissible limits. If this water is used without treatment the population of the area will soon be prone to many health hazards as indicated by many case studies. This project has brought forth the typical pathetic condition of the area as far as water quality is concerned. The treatment of the water is the immediate necessity of the hour.

The resolutions of the Governments to provide water to all citizens should not restrict to just provide water. It should extend to the level of pure and safe water. The quantity of the water being utilized should be maintained within the permissible limit by prevention of pollution and contamination.

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Biochemical changes in the four morphogenic pathways of invitro cultures in *Datura metel* Linn

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Abstract

The quantitative changes in the primary metabolites like soluble sugars and starch in the organogenic and non organogenic pathways of *in vitro* culture in *Datura metel* Linn was studied. Primary metabolites showed a different trend of accumulation in organogenic pathways and non-organogenic cultures. In organogenic pathways the first phase was steady followed by a rapidly increasing phase and then a rapidly declining phase. In non organogeinc cultures the rapidly declining phase 3rd phase was absent.

Key words: In vitro, Datura metel, organogenic callus, non organogenic callus.

Abbreviations: BAP – 6- Benzylaminopurine, IAA –Indole 3 acetic acid, Kn – Kinetin, TDZ-Thidiazuron, 2, 4-D - 2, 4-Dichlorophenoxyacetic acid.

1. Introduction

Datura metel Linn is a tropically distributed and popular medicinal plant widely used in India and is of considerable toxicological importance. The entire plant is used as a narcotic, aphrodisiac, febrifuge, anthelmintic and topically applied to remove tumors. The plant contains traces of alkaloids hyocyamine and atropine. Due to over exploitation, low seedling survival and reduction in the wastelands where it grows, there has been a considerable decline in the availability of *D. metel* during recent times. As *D. metel* is a plant of great demand in ayurvedic medicine and also in the pharmaceutical industry, *in vitro* studies are of immense potential.

This paper reports the quantitative changes in the primary metabolites like soluble sugars and starch in the organogenic and non organogenic pathways of *Datura metel* Linn.

2. Materials and Methods

D. metel growing in the medicinal garden of Ayurveda college Tripunithura, Kerala was collected for the present study. For establishment of *in vitro* culture young leaf explants were surface sterilized with 0.1% (w/v) HgI_2 for 6 minutes and rinsed thoroughly with sterile distilled water. Surface sterilized explants were cut into small pieces and placed on media containing MS (Murashige and Skoog., 1962) mineral salts, 3% (w/v) sucrose, 0.8% (w/v) agar. The medium pH was adjusted to 5.8 and autoclaved at 121° C

for 20 minutes at 15 lbs. All the cultures were maintained at 25± 2 °C with a photoperiod of 16 /8 h light /dark cycle. The culture room was equipped

Table 1. Morphogenic pathways with best responding media.

Sl No	Pathways	Best Medium	Time for shoot (days)
1	Non organogenic callus	2mg/l2,4-D +0.5mg/lTDZ	-
2	Organogenic callus	1.5mg/lIAA+3mg/lKn	19
3	Direct plant regeneration	2mg/lIAA+0.5mg/lBAP+0.5mg/l Kn	16
4	Development of Protocorm like structure	4mg/lIAA+2mg/l BAP	21

with white fluorescent lamps with a light intensity of 2000 lux.

In vitro culture of four morphogenic pathways were established from leaf explants in different hormonal combination (Data not shown). The morphogenic pathways and the best responding media and time to respond are given in Table 1. One 100 mg samples were collected at different time intervals for estimation of starch and soluble sugar content from all the four culture pathways.

For quantitative estimation of starch and soluble sugars 100 mg dry weight samples were collected and extracted with 5 ml of methanol. This was extracted once again and combined extract was reduced to dryness. An aliquot of 0.5ml of this solution was employed for the determination of soluble sugars by anthrone calorimetric method. Starch was extracted from the residue in boiling distilled water and determined using I_2 KI method (McCready *et al*., 1950).The quantity of metabolites in the respective stages represented as mg/gm of samples.

3. Results and Discussion

Biochemical estimation of soluble sugars and starch was carried out in the four different pathways of morphogenesis. In organogenic calli estimations were done at an interval of 10 days from date of inoculation. During indirect organogenesis estimations were carried out at an interval of 5 days from the date of inoculation till the shoot develop 2-3 leaves. In case of direct regenerations estimations were done at an interval of 3 days starting from the date of inoculation. In the case of regeneration through intermediary structures estimations were carried out at an interval of 5 days till the shoot develop 2-3 leaves.

Metabolite mobilization in organogenic callus, direct regeneration, regeneration via protocorm stage showed a similar trend. In organogenic pathways there was an initial steady followed by an increasing and then a decline phase in

the accumulation of metabolites both starch (Fig 1) and soluble sugars (Fig 2). In both organogenic and non organogenic routes the initial steady phase is similar and is because the cells in culture require a conditioning phase that is the tissue has to get established with the medium. After establishment the cells depending on the media and hormonal supplements become metabolically active and start synthesizing and storing compounds. This explains the steep increase in the metabolites in the second phase. In the third phase organogenic cultures start devloping meristematic points and tissue differentiation were high energy is required. Krishnan *et al.,* (1993) explained the starch accumulation just prior to the formation of meristematic centres and depletion during the later stages of shoot bud development in *Spathoglottis plicata.* Starch metabolism is associated with organogenic and embryogenic processes has been investigated in tissue cultures of Tobacco (Thorpe 1974) *Solanum surattense* (Swanker *et al.*,1985) *Papaver oriental* (Hara *et al* ., 1985). In all the studies a positive correlation has been observed between starch accumulation and organ development as observed in the present investigation. Starch functions as a readily or as an osmotic agent in the form of free soluble sugars essentially required for the development (Thorpe, 1974; Seeni and Gnanam., 1981; Kelker *et al.,* 1996). Thus starch serves as an energy source for the high energy source for the high energy requiring process of shoot bud differentiation. Accumulation of soluble sugars in the second phase may be formed from sucrose hydrolysis and is a pre requisite in an otherwise non photosynthetic and energy deficient tissue for the synthesis of starch and other cellular macromolecules required for initiating cell division and tissue growth. Evidence for direct hydrolysis of sugars from sucrose in the medium and their subsequent accumulation and utilization is well established (Swarnkar *et al.*, 1986; Hara *et al.*, 1985; Gopalan *et al.*, 1992).

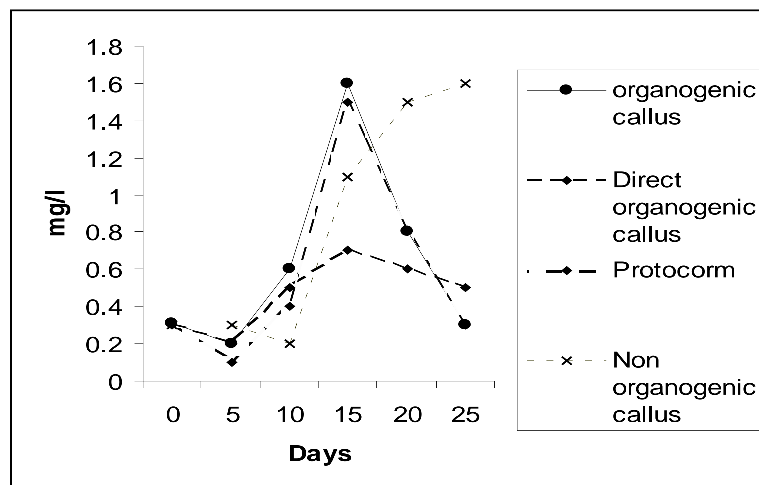

Figure 1. Starch mobilization in different morphogenic pathways in *Datura metel in vitro* cultures.

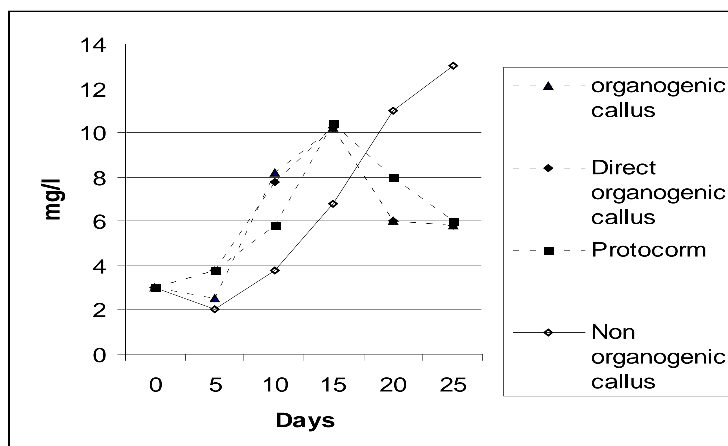

Figure 2. Soluble sugar mobilization in different morphogenic pathways in *Datura metel in vitro* cultures.

4. Conclusion

Metabolite mobilization in organogenic cultures showed a similar trend. There is an initial steady phase followed by an increasing and then a decline phase. The non organogenic cultures vary only in the third phase. In conclusion, the organogenic and non organogenic cultures vary only in the third phase that is depending on the whether the cells are proceeding for differentiation or not. If the tissue is not for differentiation the accumulation continues and if there is differentiation metabolite utilization takes place.

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Effect of fungicide Carbendazim on the growth and yield of *Phaseolus aureus* Roxb

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Abstract

Phaseolus aureus Roxb. was treated with different concentrations of the fungicide Carbendazim viz. 0.5mg/kg, 1.0mg/kg, 2.5 mg/kg of potting mixture. The plant grown in the pot filled with potting mixture (soil, sand and cow dung in 1:2:1 proportion) alone was treated as control. The parameters studied were shoot length, number of leaves, yield and the number of root nodules. All the parameters showed maximum values in the plant treated with 0.5mg/kg of carbendazim indicating the stimulatory effect of the fungicide in lower concentrations. The plants exposed to 1.0mg/kg, 2.5mg/kg of fungicide showed decreased values for all the parameters revealing the toxic effect of carbendazim in higher concentrations.

Key words: Carbendazim, fungicide, *Phaseolus aureus* Roxb., shoot length, yield, root nodules

1. Introduction

Nature has provided a clean environment for the growth of all living organisms including plants, animals and man. Any kind of disturbance in the environment has harmful effect on the ecosystem. The human race is madly rushing towards industrialization and modernization without giving any attention to the deteriorating quality of the environment that will affect the life of future generation. In order to increase the agricultural productivity a wide range of synthetic chemicals including fertilizers, insecticides, pesticides, fungicides etc are used without any discrimination. According to Horrigan et al. (2002), pesticide related death worldwide is estimated as 10,000 per year with about three-fourth of these occurring in developing countries. Studies have also shown that the excessive use of pesticides adversely affect plant growth and productivity. In this context, the present study has undertaken with the intention of finding out the degree of toxicity of a commonly used fungicide, carbendazim on the growth and yield of green gram, *Phaseolus aureus*, Roxb.

2. Materials and Methods

Good quality seeds of *Phaseolus aureus* Roxb were kept for germination in a pot filled with potting mixture. With the onset of two leaves these plantlets were transplanted to four pots with three plants /pot.

The first pot filled with soil, sand and cowdung in the ratio 1:2:1 was kept as control. In the remaining three pots, different concentrations of fungicide carbendazim viz. 0.5mg/kg, 1.0mg/kg, and 2.5mg/kg of potting mixture respectively was added. The parameters studied were:

a. Length of shoot
b. Number of leaves
c. Yield (Number of fruits per plant)
d. Root nodules

After the completion of growth and fruit setting, the plants from each pot were uprooted to count the number of root nodules on the root system.

3. Results and Discussion

The results of investigation have shown that lower concentrations of carbendazim had a stimulatory effect on *Phaseolus aureus*, Roxb. as the growth and yield of the plant showed higher values than the control when treated with 0.5mg/kg fungicide. With the increasing concentrations of carbendazim, there was a decreased growth and yield indicating the toxic nature of the fungicide at higher concentrations (Table 1).

The studies conducted on the influence of endosulfan on the growth and productivity of *Vigna radiata*, by Trivedi *et al.* (1990) showed the harmless nature of endosulfan at lower concentration, while higher concentrations appeared to be toxic to the plant growth. Reduction in growth may be due to the presence of excessive amount of trace metals and other pollutants at higher concentrations of fungicide. According to Agarwal and Beg (1982), reduced plant growth when exposed to higher levels of fungicide is due to the interference in auxin regulated elongation of cells.

Reduced leaf formation along with retarded primary productivity due to decreased pigment production may have caused lesser yield in the experimental plant exposed to higher levels of carbendazim. Inhibition to chlorophyll synthesis in the plant treated with higher concentrations of endosulfan was noticed by Trivedi et al (1990).

In the present study, the lower dose of carbendazim showed no phytotoxic effect on *Phaseolus aureus* Roxb. which is in agreement with the statement of Nene and Thapliyal (1979) and Chandra et al (1983) that the carbendazim releasing fungicides when used at the optimum concentrations do not cause phytotoxicity. Ghosh and Srivastava (1994), demonstrated that bavistin, a carbendazim releasing fungicide exhibited least phytotoxic effect and acted as a growth stimulatory compound at certain concentrations. This is in accordance with the results of current study.

Table 1. Effect of fungicide Carbendazim on the growth and yield of *Phaseolus aureus*, Roxb.

Observation (after 60 days of growth)	**Concentration of Carbendazim**			
	Control	**0.5mg/kg**	**1.0mg/kg**	**2.5mg/kg**
Shoot length(cm)	19.5	19.8	18.2	17.4
Average no. of leaves/plant	6	8	7	5
Average no. of fruits/plant	3	5	4	2
Average no. of root nodules/plant	8	12	6	3

4. Conclusion

Carbendazim is advantageous to *Phaseolus aureus*, Roxb. In two ways: a) by protecting the plant from fungal infections b) by acting as a growth stimulatory agent at lower concentrations. However, beyond certain limit, it is proved to be highly toxic to plant growth and productivity.

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Optimization of growth conditions for the production of xylanase enzyme by *Streptomyces species* and its application

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Abstract

The present study aims at the isolation, identification and optimization of growth conditions for the production of xylanase enzyme. The *Streptomyces sp* was isolated from from the soil sample. The enzyme activity of extracted crude enzyme was estimated and was then subjected to partial purification by ammonium sulphate purification, dialysis SDS-PAGE. The application of xylanase in clarification of sugarcane juice was performed. Thus the fundamental studies should enable more effective biotechnological exploitation of xylanases. The xylanase has a number of applications in different field. Thus the fundamental studies should enable more effective in biotechnological exploitation of xylanases.

Keywords – *Xylanase, Streptomyces sp, optimization, submerged fermentation*

1. Introduction

Xylan, the major constituent of the hemicellulose of plant cell walls and grasses are heteropolymers and is the second most abundant renewable source (Goheen, 1982). Lignocellulosic materials are wide spread in nature and xylan is a polysaccharide found on the hemicellulosic fraction of lignocellulose (Chivero *et al.*, 2001). Xylanase enzymes deconstruct plant structural material by breaking down the hemicellulose, a major component of cell wall (Belfaquih *et al.*, 2002). The hydrolysis of the characterstic backbone consisting of β -1, 4 linked Dxylosyl residues involves β-1, 4 - Xylanases (1,4, β - D- xylan xylanohydrolase ; EC 3.2.1.8) and β - Xylosidases (1,4, β – D- Xylan Xylohydrolase ; EC 3.2.1.37).

Xylanase production has been reported by many microorganisms such as *Aspergillus niger, Bacillus subtilis, Bacillus pumilus, Cryptococcus albidus, Trichoderma, ressei, Streptomyces* sp, *Chaetomium trilaterale* and many more. Most fungal species produce xylanase during growth with agricultural waste material as the main carbon source The fungus produce enzyme extracellularly using various substrates both in submerged and solid state fermentation though solid state fermentation was found to be more economical mainly due to cheap and abundant availability of agricultural wastes which can be used as substrates (Nascimento *et al.*, 2003).

High endoxylanase titers were observed when *Streptomyces malaysiensis* was grown in larchwood liquid medium. Optimization studies for better xylanase yields have also been carried by varying different parameters such as pH, temperature, aeration and medium composition. The expressed xylanase may be recovered there from by centrifugation or filtration, precipitating proteinaceous components by means of salts such as ammoninum sulphate

followed by chromatographic procedures (Morosoli *et al.,* 1986).

The screening of xylanase enzyme with useful or novel characteristics is done and since high temperature is used in many industrial purposes, thermostability is one of the desirable enzyme characteristics.

The xylanase has a number of applications in different field. This enzyme can be used as an agent for degradation or modification of plant cell walls or any xylan containing material. The xylanase enzyme used in biobleaching paper pulp. Xylanases may be used in the baking industry to improve the elasticity and stability of the dough. This enzyme also aid in increasing juice yields from fruits or vegetables. The judicious use of xylanolytic enzymes could result in cleaner reactions, higher yields, lower consumption of enzyme and energy, parameters vital to the economic feasibility of industrial processes.

2. Materials and Methods

2.1. Collection of soil sample

The soil samples were collected from different places in and around Coimbatore. In an aseptic condition transferred to the laboratory.

2.2. Isolation of microorganism and screening for xylanase activity

Starch casein nitrate agar plates were inoculated by spread plate technique.The oats spelt agar medium plates were prepared and sterilized. The 15 actinomycete isolates were inoculated on xylan agar plates and incubated for 4 days.The plates for isolates were flooded with absolute ethanol and left for 16 hours at room temperature to precipitate xylan. Colonies producing xylanase enzyme were surrounded clear zones against an opaque background of non-hydrolyzed media (Nanmori *et al*., 1990; Rifaat Rawashdeh *et al.*, 2005).

2.3. Identification of the selected isolates

The colony morphology was studied by observing the colonies in starch casein nitrate plates.The microscopic examination of the isolate was also studied by slide culture technique.

2.4. Selection of fermentation medium

The solid and liquid mediums were prepared for the isolate.

2.5. Solid state fermentation

Rice bran, wheat bran, corncob and combinations of all 3 were used as substrate for the fermentation. The solid substrate was moistened with 20 ml of mineral salt medium. The medium was inoculated with *Streptomyces* sp.

2.6. Submerged Fermentation

The *Streptomyces sp* was grown on mineral medium with xylan for xylanase production and activity was determined.

2.7. Enzyme recovery assay

The enzyme was extracted after the completion of fermentation. For *Strepromyces sp,* the fermentation medium was kept in rotary shaker for 4 days. After cultivation, the growth was removed by centrifuging the medium. The supernatant was used as source of crude enzyme preparation. Xylanase activity was determined by measuring the release of reducing sugars from oat spelt xylan as substrate. The reaction mixture composed of 1.8 ml substrate of 0.5% xylan in 50 mM sodium phosphate buffer (pH 7.0) and 0.2 ml of crude enzyme from *Streptomyces sp* was incubated in water bath at 60^0 c for

15 minutes.The releasing reducing sugar was measured by 3,5 dinitrosalicylic acid method (DNSA method) (Miller, 1959).

2.8. Optimization of growth conditions for the production of xylanase enzyme

The study of enzyme activity at different growth period – The study of optimization of growth conditions optimize liquid medium for maximum enzyme production by *Streptomyces sp* the sample was analysed at different growth period. The analysis of samples was drawn at regular intervals showed that the enzyme production started after 48 hours and continued exponentially upto 216 hours. The enzyme activity was measured by DNSA method.

2.9. Effect of pH on xylanase activity

The optimum pH was determined by incubating the *Streptomyces sp* in the liquid medium at various pH ranging from 4-9. The enzyme activity was measured by DNSA method.

2.10. Effect of temperature on xylanase activity

The optimum temperature was determined by inoculating the *Streptomyces sp* in the liquid medium at different temperature ranging from 28°C, 37°C, 45°C, 50°C, 60°C. The enzyme activity was determined by DNSA method.

2.11. Effect of carbon source on xylanase production

Five different carbon sources namely glucose, sucrose, maltose, lactose and xylose were substituted and incorporated into production medium inoculated with *Streptomyces sp*. The DNSA method was used to assay the xylanase activity.

2.12. Effect of nitrogen source on xylanase production

Four nitrogen sources like sodium nitrite, ammonium sulphate, ammonium chloride and urea were substituted individually. The sources were incorporated into the production medium inoculated with *Streptomyces sp.* and incubated for 4 days. The xylanase activity was determined by DNSA method.

2.13. Effect of metal ions on xylanse production

The metal ions like ammonium ions, copper ions, sodium ions, magnesium ions, ferric ions and calcium ions were substituted individually. The sources were incorporated into the production medium inoculated with *Streptomyces sp* and was incubated for 4 days. The xylanase activity was determined by DNSA method.

2.14. Partial purification

The crude enzyme from isolate was extracted. In case of *Streptomyces* sp, the ammonium sulphate was added with 70% saturation by continuous stirring and kept overnight at 4°C for 24 hrs. The resultant precipitate was collected by centrifugation and the precipitate was dissolved in 0.05 M Tris HCl buffer pH 7.6 for *Streptomyces sp*. The partially purified sample obtained after ammonium sulphate fractionation from *Streptomyces* sp was then dialyzed overnight against 0.05 M Tris HCl buffer pH 7.6 at 4°C.

The purified enzyme thus obtained was subjected to SDS-PAGE for further purification.

2.15. Application of partially purified xylanase in the clarification of sugarcane juice

The extracted sugarcane juice was obtained in a beaker. About 2 ml of

xylanase enzyme was added to the juice and kept for overnight incubation along with a control. The changes were observed visually.

3. Results and Discussion

About fifteen actinomycetes from the soil samples were isolated and were plated to the culture medium. The fifteen actinomycetes were screened for xylanase activity. The isolated actinomycetes were inoculated on to minimal xylan agar plates. A clear zone against an opaque background of non hydrolyzed media was observed. From this one actinomycete of bigger zone were selected for further studies. In case of actinomycete the gray colour organism with branced mycelium and spores were observed .Under microscopic examination the organism was found to resemble *Streptomyces sp*. The isolate was subjected to solid state fermentation and submerged fermentation. But for *Streptomyces* sp the submerged fermentation was found to be best for xylanase production.

The crude enzyme of *Streptomyces sp*. was extracted. They enzymes were assayed for xylanase activity by DNSA method. The optimization of the growth conditions for the production of the xylanase enzyme was then studied. The enzyme activity at different growth period was first studied. Results showed the xylanase production increased and reached maximal value after 4 days (Fig 1). Then the activity decreased on increasing the fermentation period for the organism. In case of effect of pH the *Streptomyces* sp., showed stable enzyme production from pH 6 to 9 and maximum at pH 7 (Fig 2).The *Streptomyces* sp., were grown at different temperature ranging from 28°C, 37°C, 40°C, 50°C. In this case the enzyme production was high at 28°C (Fig 3). After 40°C the growth and production of enzyme were less. The effect on addition of carbon source glucose, sucrose, maltose, lactose and xylose to the liquid medium for *Streptomyces* sp., was studied. The enzyme activity was measured and studied. When xylose was used as carbon source, high xylanase activity was measured (Fig 4). The effect on addition of nitrogen sources such as ammonium sulphate, ammonium chloride, sodium nitrate and urea were studied. The ammonium sulphate was the nitrogenous compound which yielded maximum activities for *Streptomyces* sp (Fig 5).The effect of ions on xylanase production were studied and activity was measured. The ammonium ions increase the xylanase production (Fig 6).

The partial purification with ammonium sulphate fractionation of enzymes was done.The dialysis of sample was done and in case of the *Streptomyces* sp., a 1.5 fold increase in the activity was obtained. The molecular weight determination of *Streptomyces* sp was done. For *Streptomyces* sp 42 kilo Dalton band was observed. The application of the enzyme in the clarification of the sugarcane juice was also studied. The juice was clarified and became less turbid with the addition of the enzyme after 24 hours of incubation.

The xylanase of interest may be over expressed by introducing and expression. Thus given their mode of action and great variation in substrate or product specificities, xylanolytic enzymes are major focus of applied research and great interest for biotechnology applications (Sunna *et al.,* 1997). The present study deals with the production of xylanase by *Streptomyces* sp in submerged fermentation. In case of *Streptomyces sp*, the submerged medium produced more xylanase enzyme compared to solid substrates with agricultural

residues. The aeration was also provided by fermenting it in a rotary shaker.

The production of xylanase enzyme was found to be affected by many factors such as growth period, pH, temperature, carbon sources, nitrogen sources and metal ions. The xylanase production started after 72 hours of incubation and continued exponentially up to 168 hours and then decreased. The pH optimization was done and was stable at broad range of pH. The xylanase production was maximum at alkaline pH of 7.5, for *Streptomyces* sp. The result agrees with Belfaquih *et al.,* (2002) who stated that xylanase produced by *S. achromogenes* strain 5028 exhibited high activity from 4.5 to 8.5 certain xylanase from *Aspergillus kawachii* and *Pencillium herque* exhibit an optimum pH more on the acidic side 92.0-6.0 (Funaguma *et al.,* 1991).

The xylanase enzyme production was found to be maximum at room temperature and continued production till 40^0C but then it decreased for *Streptomyces* sp. The different carbon sources like glucose, sources, maltose, lactose and xylose were individually supplemented on to the medium. The xylanase production was maximum when xylose was added for *Streptomyces* sp. Xylose has been described as an effective inducer and carbon source for xylanase production in several micro organisms including *Fusarium oxysporum* (Singh *et al.,* 1992) and *T.lanuginosus*. When xylose and xylan together were used as carbon source, the resultant high xylanase activity suggested that xylanase production was not subject to xylose repression (Srivastava and Srivastava, 1993). For the *Streptomyces* sp the ammonium chloride was found to produce higher yield. The ammonium ions were found to increase the xylanase production for *Streptomyces* sp.

After optimizing the cultural condition for the xylanase production of the organism, the extracted enzyme was subjected to partial purification. The enzymes were subjected to fractionation with ammonium sulphate salt and then dialyzed. The enzyme activity of dialyzed enzyme showed a 1.8 fold increase in activity in case of *Streptomyces* sp. Thus increase indicates that inhibitory material present in crude extract has been removed. The dialyzed enzymes were further subjected to SDS –PAGE. The discrete bands were obtained for the enzymes. For *Streptomyces* sp about 42 Kda band was obtained. There are low MW/ basic and high MW/ acidic xylanase in Clostridium sp and *Streptomyces* sp Microbial xylanases are single submit proteins with molecular masses within a range of 8-145 Kda.

The application of partially purified enzyme in sugarcane juice was done. In the sugarcane juice, the enzyme reduced the turbidity, colour and increased amount of reducing sugars in it. Thus the extracted enzyme was found to be industrially important and mainly applied in food industry.

4. Conclusion

The present enzymes can be used industrially but for commercialization, the enzyme should undergo further purification procedures and laboratory tests. Gene cloning technology and DNA recombination techniques have opened new research areas in biotechnology, permitting the production of efficient microbial strains with specific desired enzymes systems in the near future. The future studies will be directed towards characterization of the enzyme complex

and identification and manipulation of the encoding genes. Thus the fundamental studies should enable more effective biotechnological exploitation of xylanases.

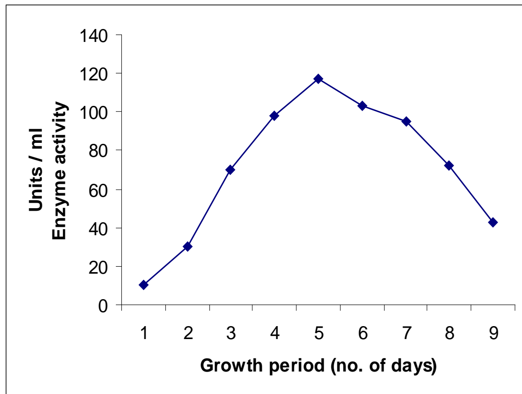

Figure 1. Effect of growth period

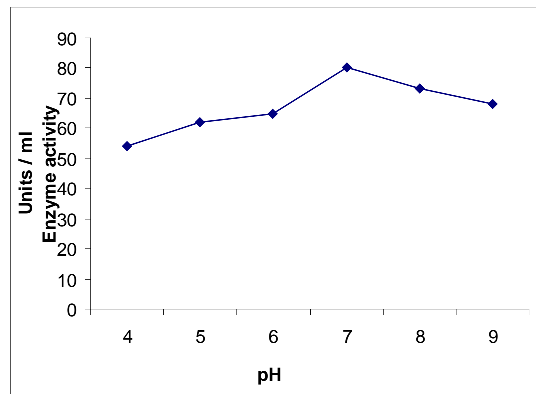

Figure 2. Effect of pH

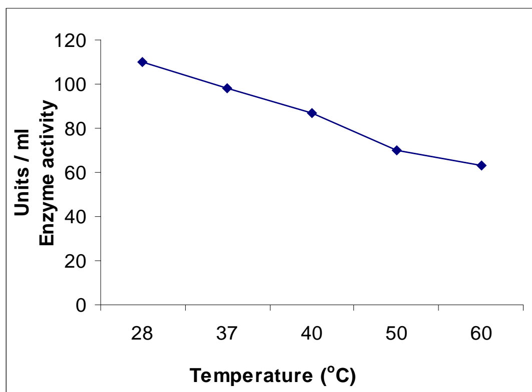

Figure 3. Effect of temperature

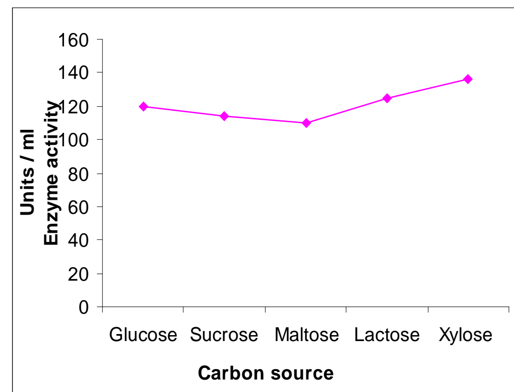

Figure 4. Effect of carbon source

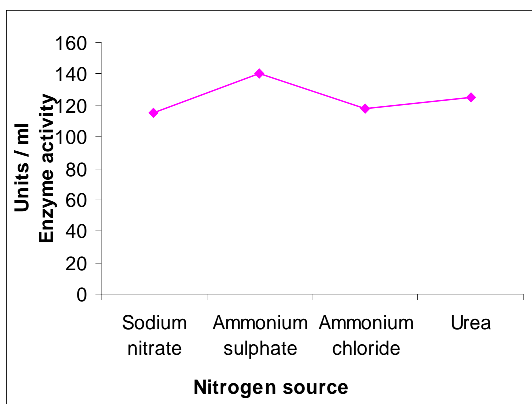

Figure 5. Effect of nitrogen source

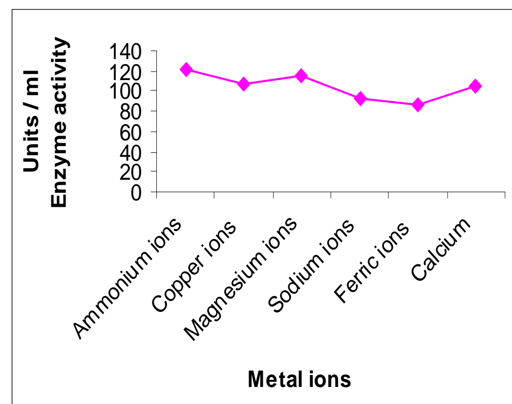

Figure 6. Effect of metal ions

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A study on partial characterization of Lindane degrading Lin- A gene product from *Bacillus species*

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Abstract

Characterization of Lindane degrading γ-HCH dehydrochlorinase from *Bacillus* sp was carried out. γ-HCH degrading *Bacillus* sp was isolated from the soil sample which was taken from the Lindane exposed fields. Screening of Enzyme activity was done in MSLP medium.After extraction and partial purification of the enzyme, characterization was done by SDS-PAGE.15 K Da band was observed and presence of Lin A gene product was conformed. The metabolite γ -PCCH was detected in MSLP medium and optimization of growth conditions for maximum activity of enzyme was checked with different temperature, pH and metal ions. Immobilization of cells increased the enzyme activity. Results suggest the use of immobilized cells in the prevention of bioaccumulation of Lindane in soil.

Key words: γ Hexa Chlorocyclo Hexamine, Lindane, Minimal Salt Lindane Peptone Medium, SDS-PAGE, Bacillus sp.

1. Introduction

The insecticidal properties of hexachlorocyclohexane (HCH) were discovered independently by Dupire and Thomas in the early 1940.In the same year lindane was registered as an agricultural insecticide with the U.S. Department of Agriculture (USDA), and in 1951 it was approved by the U.S. Food and Drug Administration (FDA) for medical use in the treatment of scabies and lice. Since this time, the vast majority of lindane use more than 99% has been in agriculture and much of the safety and environmental concerns have related to this application. Most exposure of the general population to lindane results from agricultural uses and the intake of contaminated foods, such as produce, meats and milk (Vogel *et al* .,1987).The microorganisms shown by screening experiments to be capable of metabolizing and degrading lindane are as follows :Bacteria such as *Bacillus* sp, *Pseudomonas* sp, *Citrobacter* sp, and *Arthrobacter* sp .Fungi such as *Penicillium* sp and the *Rhizopus* sp also can degrade lindane.This paper describes the partial characterization of lin Agene product form *Bacillus* sp (Tu, 1976).

2. Materials and Methods

2.1. Isolation and enrichment of Lindane degrading bacterium

Samples were collected from Lindane exposed sugarcane field in and around Coimbatore. Lindane degrading bacterium was isolated from the soil sample in Minimal Salt Lindane Peptone agar plates. Screening of γ HCH degrading enzyme was done on Luria Bertani agar plate with Lindane. Morphologial, microscopic and cultural

characteristics were checked to identify the bacterium.

2.2. Effect of Lindane concentration on the growth of Bacillus sp.

Production of the γ HCH dehydrochlorinase was carried out in 250 ml Erlenmeyer flasks containing 100 ml MSLP broth with different Lindane concentration. Lindane was added in various concentrations such as 500 ppm, 1000 ppm, 1500 ppm & 2000 ppm. A set of production medium was prepared for the growth of *Bacillus* sp . The flasks were incubated at 37°C and 120 rpm in a shaker. The growth rate of the isolate was measured after 2nd, 4th, 6th, 8th & 10th day of incubation. Growth was measured by OD values.

2.3. Enzyme recovery assay

The enzyme is extracted from the production medium which showed maximum growth of the isolate. This enzyme extract was taken for further studies. At the end of the fermentation period, the enzyme was obtained by centrifugation and it was stored at 4°C till use. (Jagnow *et al* .,1977)

2.4. Characterization of Lin A gene product

The crude enzyme extracts from *Bacillus* sp was extracted. Ammonium sulphate precipitation and dialysis were done for the partial purification of γ HCH dehydrochlorinase. The product of Lin A was measured by sodium dodecyl sulphate –polyacrylamide gel electrophoresis. Protein sample was heated in boilimg water for 2 minutes before electrophoresis on 0.75 mm thick, 12% (wt/vol) polyacrylamide overlaid with an SDS 4.5% (wt/vol) polyacrylamide stacking gel. The weight of degradation enzyme was determined by using various standards.

2.5. Assay of the enzyme

Assay of enzyme was performed by indirect method such as identification of metabolites. Identification of γ-PCCH metabolite was done by adding 30μl of enzyme extract in the wells on Luria Bertani agar plates and γ HCH dissolved in ether (10% wt/vol) was sprayed onto the plates in a thin layer. Degradation of γ HCH was detected by the appearance of cleared zones. (Imai *et al*., 1991). This method is also used for checking the enzyme activity.

2.6. Optimisation of growth condition for the maximum activity of γ HCH dehydrochlorinase

2.6.1. Effect of different temperature on γ HCH dehydrochlorinase activity

The optimum temperature for *Bacillus* sp was determined by incubating in MSLP medium at different temperature ranging from 25°C, 35°C, 45°C, 55°C and 65°C. After the incubation the enzyme activity was determined on 8th day of incubation by extracting the enzyme. 30 μl of partially purified enzymes from isolate 1 and 2 were added to well in the LB pates. The zone of degradation was checked.

2.6.2. *Effect of different pH on γ HCH dehydrochlorinase activity*

The optimum pH of the organisms were determined by inoculating the organism in the production media which was maintained at different pH ranges from 5 to 10 and they were incubated at 37°C for 8 days. The enzyme activity was determined after the 8th day of incubation by extracting the enzyme. Partially purified enzymes were added to well in the LB pates. The degradation zone was checked.

2.6.3. Effect of different metal ion on γ HCH dehydrochlorinase activity

The different metal ions namely calcium ions, zinc, ions, mercuric ions,

sodium ions and ferric ions were substituted individually. The sources were incorporated in the production medium and were inoculated with *Bacillus* sp and were incubated at 37°C for 8 days. The enzyme activity was determined on 8th day of incubation. Zone of degradation was checked.

2.6.4. Effect of immobilization on enzyme activity

Immobilisation was done with sodium alginate. The beads were kept for curing for 1 hour at 4°C. The cured beads were washed 3-4 times with distilled water and they were stored in the solution of 0.9% sodium chloride till use in refrigeration. Effect of immobilization on activity was checked by the enzyme assay technique.

3. Results and Discussion

Isolate was observed as abundant opaque white waxy growth. A zone of clearance was observed with the isolate along the streak line in the MSLP medium. This indicated the presence of γ-HCH dehydrochlorinase. The gram stained smear of the isolate appeared as Gram positive rods. Biochemical test results of the isolate were resembled the standard results of Bacillus sp. Maximum growth rate of isolate was observed in production medium with 1500 ppm lindane in 8th day (table 1). Highest OD value was observed in this lindane concentration.

The molecular weight determination of the enzyme extract from *Bacillus* sp species was done. The enzyme from isolate showed approximately 15 kilo Dalton (KDa) band. Zones were observed around the well on LB plate which was spread with γ-HCH & ether. The well contains 30µl of enzyme extract. This shows the degradation activity of the dehydrochlorinase enzyme.The presence of γ-PCCH was conformed.

The isolate was grown at the different temperatures ranging from 25^0 C, 35^0C, 45^0 C, 550C and 65^0C. The enzyme production was found to be the maximum at 37^0C (Table 2). The isolate was grown in the medium with different pH ranging from 5 to 10. The maximum dehydrochlorinase activity was exhibited with the pH 8 and it was found to gradually decrease after pH 8. Incorporation of the different metal ions to the fermentation medium and their activity was studied. Sodium ions exhibited an increased amount of the dehydrochlorinase activity.

The involvement of the immobilization technique for the whole cell increased the dehydrochlorinase production when the immobilized beads were inoculated to the production medium rather than with the inoculation of the free cells in the production medium (Table 3).

The use of micro-organism for bioremediation requires an understanding of all the physiological and biochemical in present situation aspects involved in pollutant transformation. In present situation identification and isolation of an enzyme system, study of its regulation and optimization of the condition and modern molecular approaches for application in the soil and natural water systems has its own importance (Dorgra *et al.*, 2004).

The soil samples were collected from lindane exposed field for the isolation of lindane degrading microorganisms. In MSLP medium Lindane is act as the sole source of carbon. The organism which can utilize lindane only will grow in MSLP. Morphologically distinct bacterial colonies thus obtained were screened for their lindane degradation ability. In Luria Bertani

agar 50mg liter^{-1} γ HcH was added. At this γ HcH concentration part of the γ HCH was insoluble and thus visible as a white precipitate. Cells that have grown on such plates and which is able to degrade γ HcH have such a halo. The clear zone was observed around the streak line of *Bacillus* sp. Good growth was observed in production medium with 1500 ppm lindane in 8th day. So this concentration was selected for further studies.

Partially purified enzyme samples from *Bacillus* sp was run in SDS PAGE for the characterization of the Lin A like gene products. The presence of γ HCH dehydro chlorinase was conformed 15.4 KDa bands. The mass may be deduced to form the nucleotide sequence of Lin A gene. The presence of γ PCCH metabolite was conformed by zone of clearance. This also shows the activity of enzyme (Heritage *et al*., 1977). The immobilized will contain more amounts of cells in single beads. So the immobilized beads showed higher enzyme activity than the free cells. The extracellular enzymes can easily immobilized beads. Due to the introduction of lindane into the environment the presence of this pollutant has been observed frequently in soil and ground water all over the world (Yule *et al*., 1967).

Lindane constitutes a major environmental problem due to its widespread use in the past, its pronounced persistence against chemical and biological degradation and its tendency to bio accumulate in the food chain. On August 5^{th,} 2003 the centre for science and Environment (CSE) in India found that pepsi Co and coca cola brands in Delhi contained lindane residues.

4. Conclusion

The findings suggest that encapsulation is a promising tool for soil bioremediation, also for sites contaminated with lindane. More studies should be done for the purification of γ HCH dehyrochlorinase and immobilization of the enzyme. These immobilized cells can prevent the bio accumulation of lindane in soil and ground water (Nagata *et al*., 1993).

Table 1. Effect of Lindane concentration on the growth of *Bacillus sp*.

Lindane Concentration	No of Days				
	2nd	4th	6th	8th	10th
Control + Isolate	0.65	0.70	1.10	1.15	1.14
500 ppm + Isolate	0.69	0.74	1.12	1.18	1.17
1000ppm + Isolate	0.7	0.73	1.14	1.2	1.11
1500ppm + Isolate	0.85	0.91	1.18	1.25	1.24
2000ppm + Isolate	0.67	0.73	1.11	1.15	1.14

Table 2. Effect of different temperature on enzyme activity

Temperature	Zone measurement of the isolate (mm)
25^0 C	29
35^0 C	32
45^0 C	30
55^0 C	20
65^0 C	15

Table 3. Effect of immobilization on enzyme activity

Cells	Zone measurement of the isolate
Free cells	28
Immobilized cells worth sodium alginate	32

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Extracellular polysaccharides isolated from *Saccharomyces cerevisiae* inhibit acute and chronic inflammations in mice

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Abstract

Saccharomyces cerevisiae grown in submerged culture produced significant amount of extracellular polysaccharides. Two fractions of exopolysaccharides, neutral exopolysaccharides (nEPS) and acidic exopolysaccharides (aEPS), were isolated from the culture broth by ethyl alcohol precipitation, deproteination and DEAE-cellulose column chromatography. The anti-inflammatory activities of both the fractions were determined by carrageenan induced acute and formalin induced chronic inflammatory models in mice. nEPS and aEPS showed significant anti-inflammatory activity in both the models, comparable to the activity of clinically used standard drug, diclofenac. The aEPS was found to be more effective to inhibit carrageenan as well as formalin induced inflammations than the nEPS. The results thus indicate the profound anti-inflammatory activity of yeast exopolysaccharides and their potential therapeutic use.

Keywords – Acidic exopolysaccharides, Anti-inflammatory activity, Extracellular polysaccharides, Neutral exopolysaccharides, *Saccharomyces cerevisiae*

1. Introduction

Extracellular polysaccharides or exopolysaccharides (EPS) occur widely among the bacteria, micro algae, fungi and yeasts. In the continuing search for novel, natural, water-soluble polysaccharides, special attention has been directed in recent years to the production of extracellular polysaccharides of microorganisms. *Saccharomyces cerevisiae* is genetically well defined yeast that has traditionally provided a good model system for yeast cell wall study. The major components of fungal cell walls are polysaccharides and glycoproteins. Three main groups of polysaccharides forming the yeast cell wall are polymers of mannose covalently linked to peptides (mannoproteins, ~ 40%), polymers of glucose (β-glucans, ~60%) and polymers of N-acetylglucosamine (chitin, ~2%) (Orlean, 1997).

Inflammation, a fundamental protective response; may be harmful in conditions such as life threatening hypersensitive reactions to insect bites, drugs, toxins and in chronic diseases such as rheumatic arthritis, atherosclerosis, lung fibrosis and cancer (Collins, 1999). Recent studies suggest that inflammatory tissue damages are due to liberation of reactive oxygen species (ROS) at the inflammation sites (Conner and Grisham, 1996). It is also generally assumed that most of the antioxidants possess anti-inflammatory effect. Extensive studies have been done during the recent years on the free radical scavenging activity of yeast cell wall polysaccharides, mainly with immunomodulating β-glucans.

Saccharomyces cerevisiae is an extensively used microorganism in food and fermentation industries. We examined the anti-inflammatory activity of neutral and acidic exopolysaccharides isolated from the submerged culture of *S. cerevisiae* and the results are reported in this communication.

2. Materials and methods

2.1. Yeast strain

Saccharomyces cerevisiae (MTCC-174) strain obtained from the culture collection of Institute of Microbial Technology, Chandigarh, India was used in this study. Culture was maintained on Yeast extract Peptone Dextrose Agar {1% yeast extract (w/v), 2% peptone(w/v), 2 % dextrose (w/v), 2 % agar (w/v)} medium at 4°C.

2.2. Production of exopolysaccharides

Seed cultures were prepared by inoculating cells grown on a Yeast extract Peptone Dextrose (YPD) agar slant into a 250 ml flask that contained 50ml of YPD Broth (pH 7.0), with subsequent incubation at 28°C for 24 hours with shaking at 200-210 rpm. Five milliliters of the culture were transferred into the 250ml flasks containing 50ml of the production medium (pH 7.0). The culture was shaken at 28°C and with 200-210 rpm for 60 hours. Cells were harvested by centrifugation at 12000 rpm and 4°C for 8 minutes to remove cells. The supernatants were pooled, vacuum filtered and concentrated in a vaccum evaporator to about one fourth of its original volume, precipitated by a 3-fold volume of chilled ethanol and kept at 4°C for 24 hours (Krizkovn *et al., 2001).* The crude exopolysaccharides (cEPS) precipitated were separated by centrifugation, dissolved in distilled water and then deproteinized by Sevag's method (Staub, 1965).

2.3. Isolation of neutral and acidic EPS

Neutral and acidic fractions of polysaccharides were isolated from cEPS by DEAE – cellulose column

chromatography. The cEPS (3g) was loaded on to the DEAE - cellulose column (bed volume = 50 ml). The column was eluted with 25 ml fractions of deionized water followed by 2M NaCl. Each fraction was tested with anthrone reagent (Yemm and Wills, 1954). The fractions obtained by deionized water elution showing positive anthrone test were designated as the neutral EPS (nEPS). Those fractions were combined, concentrated to 10 ml volume and then dialyzed against deionized water for 48 hours to remove small molecules in the solution.

After dialysis the fraction was re precipitated with chilled ethanol and the residual ethanol was removed by centrifugation and dried at 40-50°C which gave a white powder (0.52 g). The fractions eluted with 2M NaCl, showing positive anthrone test were designated as acidic EPS (aEPS). The fractions were combined, concentrated to 10ml volume and then dialyzed against deionized water for 48 hours. The fraction after dialysis was re precipitated with chilled ethanol and dried at 40-50°C which gave a brownish powder (0.78 g). Both nEPS and aEPS were tested by Lowry – Folin's test (Lowry *et al.*, 1951) to detect proteins, if present.

2.4. Test Animals

Female Swiss albino mice were purchased from Small Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Trichur. They were kept for a week under environmentally controlled conditions with free access to standard food (Sai Durga, Bangalore) and water. Mice weighing 20 – 22 g were used for this study. The animals were maintained according to the guidelines recommended by Animal Welfare Board and experiments were carried out with the approval of Institutional Animal Ethic Committee.

2.5. Carrageenan induced paw oedema

Animals were divided into 6 groups of 6 animals each. In animals of all the groups, acute inflammation was produced by the subplanar injection of 0.2 ml of freshly prepared 1 % (w/v) suspension of carrageenan in normal saline in the right hind paw of the mice (Winter *et al.*, 1962). The paw thickness was measured using Vernier calipers before and 3 hr after carrageenan challenge in each group. Group 1 was kept as control. The group 2 and 3 animals were pre-medicated with nEPS (0.125 and 0.25 mg/animal, p.o.), group 4 and 5 with aEPS (0.125 and 0.25 mg/animal, p.o.) and the group 6 with standard reference drug diclofenace (0.5 mg/animal, p.o.) one hour before carrageenan injection.

2.6. Formalin induced paw oedema

The animals were treated in the same way as in the above model, except that formalin (0.2ml of freshly prepared 2 % formalin) was used as the oedematogenic agent. The drug treatment continued for 6 consecutive days (Ajith and Janardhanan, 2001). Diclofenac (0.5 mg/animal) was used as the reference drug. In both the models the degree of oedema formation was determined as increase in paw thickness. The increase in paw thickness and percentage inhibition was calculated as follows:

Increase in paw thickness in control/treated

$$P_c / P_T = P_t - P_0$$

Where, P_t = paw thickness at time t,

P_0 = initial paw thickness

Percentage inhibition = $(P_C - P_T) / P_C \times 100$

Where, P_C = increase in paw thickness of control group,

P_T = increase in paw thickness of treated groups.

2.7. Statistical analysis

The data were statistically analyzed using Student's *t* test and P values less than 0.001 were considered significant. All data were represented as mean ± SD.

3. Results and Discussion

Saccharomyces cerevisiae produced significant amount of extracellular polysaccharides when grown in submerged culture. Purification of crude polysaccharide afforded to yield both neutral and acidic fractions. Both nEPS and aEPS were strongly anthrone positive and weakly positive to Lowry-Folin's test. The nEPS and aEPS significantly reduced the carrageenan induced paw oedema ($P < 0.001$). The anti-inflammatory activity of aEPS at a dose of 0.25mg / animal was found to be higher than that of the activity of the reference drug, diclofenac at 0.5mg / animal (Figure 1). Both the nEPS and aEPS were also effective in ameliorating formalin induced chronic inflammation. Formalin induced paw oedema was inhibited significantly ($P< 0.001$) by nEPS and aEPS. The inhibition of inflammation by nEPS at a dose of 0.25mg / animal and aEPS at a dose of 0.125 and 0.25mg / animal was found to be higher than the inhibitory effect of reference drug diclofenac at 0.5mg / animal (Figure 2). In both the models, the aEPS isolated from *S. cerevisiae* was found to be more effective to inhibit inflammation than the nEPS.

Yeasts and other fungi produce large quantity of exopolysaccharides. The fungal polysaccharides are acidic, alkaline or neutral in nature. It has been reported that mannan is found to be the main component (90%) of yeast exopolysaccharides. Yeast wall mannoproteins are highly glycosylated polypeptides, often 50 to 95 % carbohydrate by weight and thus it is often considered as yeast proteoglycans (Orlean, 1997; Van der Vaart *et al.,* 1995). Both nEPS and aEPS isolated from *S.cerevisiae* culture broth showed typical color reaction with anthrone reagent and slight positive reaction with Lowry-Folin's reagent even after deproteination by Sevag's method. Since the protein moiety is not completely removed by Sevag's reagent, it appears that both nEPS and aEPS are protein bound polysaccharides.

Results of the investigation showed that both neutral and acidic exopolysaccharides of *S.cerevisiae* possess significant inhibitory effect on carrageenan and formalin induced oedema in mice. Inflammation is a complex series of events resulting from a complex network of cell-cell, cell-mediator and tissue interactions. Many substances have been proposed as inflammatory mediators, released locally at the site of inflammation and having biological properties that cause or enhance the signs and symptoms of inflammation. A very important event elicited by an inflammatory stimulus is the perturbation of the neutrophil membrane. Membrane activation produces highly reactive oxygen products, including superoxide, and the release of lysosomal enzymes (Vinegar *et al.*, 1987). The reactive oxygen species (ROS) such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) have been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer (Hemnani and Parihar, 1998). The inflammatory tissue damages are mainly due to the

liberation of reactive oxygen species from phagocytes invading the inflammation sites (Conner and Grisham, 1996; Winrow *et al.,* 1993; Parke and Sapota, 1996). The aEPS of *S.cerevisiae* showed higher inflammation inhibitory activity compared to nEPS in both the carrageenan and formalin induced paw oedema models in mice.

The content of proteinous substances in the polysaccharide molecules potentiates their free radical scavenging activity (Liu *et al., 1997)*. This kind of free radical scavenging activity is demonstrated by a variety of carbohydrates. The antioxidative property of protein bound polysaccharides may be one of the factors responsible for the anti-inflammatory activity of nEPS and aEPS isolated from the submerged culture of *S.cerevisiae*.

4. Conclusion

Non-steroidal anti-inflammatory drugs are extensively used in the recent years for treatment and management of inflammatory diseases. However for the treatment of several chronic diseases, life long dependency of drug is often necessary and most commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) suffer several side effects. Hence natural, nontoxic polysaccharides of microbial origin are of significant value in the treatment of inflammation and related diseases. In this context the exopolysaccharides isolated from Baker's yeast, *S.cerevisiae* has potential use in anti-inflammatory therapy.

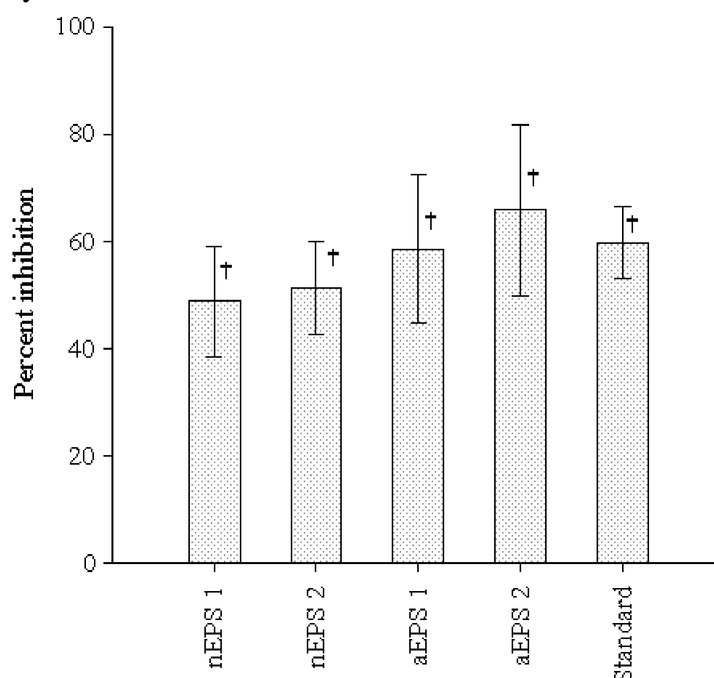

Figure 1. Inhibition of carrageenan induced inflammation by neutral and acidic exopolysaccharides of *S.cerevisiae.* All values are ± standard deviation from 6 mice; † $P < 0.001$ with respect to control. (Along the X-axis, the suffixes 1 and 2 indicates the concentrations of 0.125 mg and 0.25 mg/animal respectively; standard means the concentration of Diclofenac 0.5mg/animal)

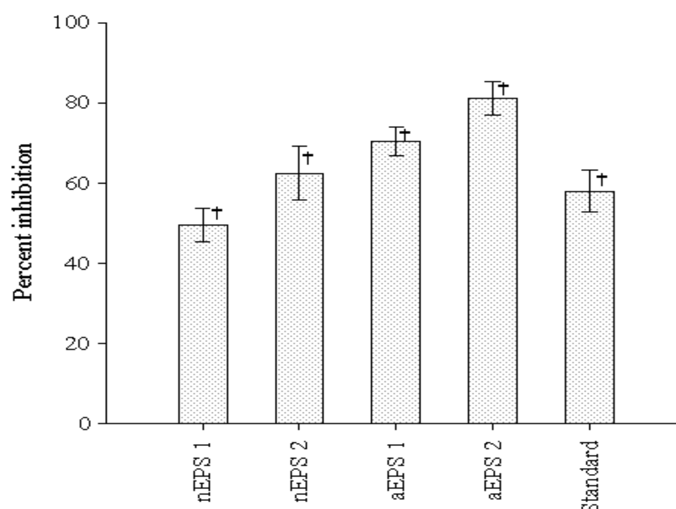

Figure 2. Inhibition of formalin induced inflammation by neutral and acidic exopolysaccharides of *S.cerevisiae.* All values are ± standard deviation from 6 mice; † P < 0.001 with respect to control. (Along the X-axis, the suffixes 1 and 2 indicates the concentrations of 0.125 mg and 0.25 mg/animal respectively; standard means the concentration of Diclofenac 0.5mg/animal)

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Lipase producing thermophilic microorganisms from different organic sources

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Abstract

Lipase producing thermophillic microorganisms were isolated from Laloor trenching ground, Ayurvedic factory, dairy plant, and from garden soil. Lipolytic microorganisms present in the sample were estimated using dilution plate technique. Lipase producing organisms were more in Ayurvedic factory waste compared to other samples. The morphologic and microscopic characteristics of thermophilic lipolytic bacteria were carried out by Rhodamin B Lipase agar plating and Gram staining, Spore staining methods respectively. Most of the organisms were bacteria. Both Gram positive and Gram negative spore forming bacteria were found to be dominant. The present study shows the isolated strains can be useful in industrial purposes.

Keywords – Lipase, Rhodamine B lipase agar.

1. Introduction

The soil environment is unique in several ways. It contains a vast population of bacteria, actinomycetes, fungi, algae and protozoa. They can degrade the different substrate by producing various enzymes. Temperature governs all biological process, and it is thus a prime factor of concern to the bacteria. Thermophiles are organisms which grow readily at temperature of 45 to 65^{0}C, and some, the obligate thermophiles, are incapable of multiplying below 40^{0}C. These organisms flourish in many habitats including composts, self-heating hay stacks, hot water lines, and hotsprings (Prescott *et al.*, 2005). Enzymes are attractive because of their variety; they have the potential to catalyze a vast range of industrially important chemical reactions. Lipases are produced by various types of bacteria, fungus and actinomycetes. Lipases have a wide array of industrial applications in the production and processing of detergents, oils, fats and dairy-products.

In the present study, estimation of the lipolytic microorganisms present in compost samples from municipal solid waste, diary industry effluent, herbal waste from ayurvedic factory and from garden soil was done. The morphological and microscopic characterization of thermophilic lipolytic bacteria was also carried out.

2. Materials and Methods

The waste samples were obtained from Laloor trenching ground, Ayurvedic factory, dairy plant, and from garden soil. The lipase producing thermophilic microorganisms were estimated by dilution plate technique using Rhodamin B Lipase Agar medium. The lipase producing microorganisms were isolated from the four sources by dilution plate technique, total microbial colonies and the colonies showing zone of clearance were counted using colony counter. Culture characters such as form, shape, and appearance were studied. The morphological features of the selected microorganisms were studied using Gram staining and Spore staining methods.

3. Results and Discussion

The microbial population counts are provided in Table 1. The numbers of lipase producing microorganisms were more in ayurvedic waste sample compared to other samples. This could be due to the fats and lipid content of the sample. Different industrial by products were also used as carbon and nitrogen sources in the production of lipase by Fadiloglu and Erkmen, 2001. The highest yield of lipase was obtained with lipids or fatty acids as carbon sources (Dalmau et al., 1998).

The effect of temperature on lipase production was determined by incubating the bacterial isolates at temperature ranging from 45^{0}C - 65^{0}C. All the isolates produced lipase at 45^{0}C temperature. Three organisms were found to produce the enzymes even at 65^{0}C. All the lipase producing isolates in the present study were bacteria. Colony characteristics of lipase producing thermophilic organisms are given in Table 2.

4. Conclusion

Of all known enzymes, lipases have attracted the most scientific attention. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyze esterification, interesterification, and transesterification reactions in nonaqueous media. This versatility makes lipases the enzymes of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries. The most significant industrial

applications of lipases have been mainly found in the food, detergent, and pharmaceutical sectors. Hence, the isolated thermophilic strains in the present study can be used for industrial applications.

Table1. Mean microbial count and the mean number of lipase producing microorganisms per gm sample obtained in Rhodamin B Lipase Agar medium from the four sources.

Type of sample	**No. of colonies after 16 hr (45°C) (cfu/g)**	**No. of lipase producing colonies after 16 hr (45° C) (cfu/g)**	**No. of colonies after 48 hr (45° C) (cfu/g)**	**No. of lipase producing colonies after 48 hr (45° C) (cfu/g)**
Municipal Solid Waste	14×10^4	1×10^4	14×10^4	2×10^4
Ayurvedic factory waste	5×10^4	2×10^4	19×10^4	3×10^4
Dairy plant waste	3×10^4	0	4×10^4	0
Garden soil	15×10^4	1×10^4	18×10^4	1×10^4

Table 2. Colony characteristics of lipase producing thermophilic organisms from different isolates.

	Colony characteristics				**Bacterial morphology**	**Gram staining**	**Spore staining**
	Margin	**Appear-ance**	**Surface**	**Nature**			
1	Irregular	Less Mucoid	Flattened	Translucent	Short rods	Positive	Spore forming
2	Circular	Dry	Flattened	Opaque	Long rods	Positive	Spore forming
3	Irregular	Dry	Flattened	Opaque	Long rods	Positive	Spore forming
4	Irregular	Dry	Flattened	Translucent	Short rods	Positive	Spore forming
5	Irregular	Mucoid	Flattened	Opaque	Short rods	Negative	Spore forming
6	Circular	Less Mucoid	Flattened	Translucent	Intermediate rods	Negative	Spore forming
7	Irregular	Dry	Flattened	Opaque	Intermediate rods	Negative	Spore forming
8	Circular	Mucoid	Convex	Opaque	Cocci	Negative	Spore forming

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Pharmacogenetics- The concept of individualized medicine

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1. Introduction

It is well recognized that different patients respond in different ways to the same medication. Variation in drug response can result in therapy failure or adverse drug reactions (ADRs). The clinical consequences range from mild, self limiting side effects to serious illness or death. In US, ADRs occurring in hospital rank among the top six causes of death. Genetic factors are estimated to account for 20 to 95 per cent of inter-patient variability. Although many non-genetic factors influence the effects of medications, including age, organ function, concomitant therapy, drug interactions, and the nature of the disease, there are now numerous examples of cases in which inter-individual differences in drug response are due to sequence variants in genes encoding drug-metabolizing enzymes, drug transporters, or drug targets. Unlike other factors influencing drug response, inherited determinants generally remain stable throughout a person's lifetime.

The influence of genes on outcome of drug treatment is a rapidly evolving field termed "Pharmacogenetics" over 50 years ago by the German geneticist Friedrich Vogel. The ultimate goal of Pharmacogenetics is to use the genetic makeup of an individual to predict drug response and efficacy, as well as potential adverse drug events.

The first observations of genetic variation in drug response date from the 1950s, involving the muscle relaxant suxamethonium chloride, and drugs metabolized by N-acetyltransferase. One in 3500 Caucasians has less efficient variant of the enzyme (butyrylcholinesterase) that metabolizes suxamethonium chloride. As a consequence, the drug's effect is prolonged, with slower recovery from

surgical paralysis. Variation in the N-acetyltransferase gene divides people into "slow acetylators" and "fast acetylators", with very different half-lives and blood concentrations of such important drugs as isoniazid (antituberculosis) and procainamide (antiarrhythmic).

There are no biomarkers at present that can predict which group of patients responds positively, which patients are non-responders and who experiences adverse reactions for the same medication and dose. After the completion of the human genome project, a haplotype map (HapMap) has been recently developed by the International HapMap Consortium with an intention of profiling DNA sequence variations across the human genome. This should provide a powerful tool to understand the genetic variants and The field of Pharmacogenetics began with a focus on drug metabolism, but it has been extended to encompass the full spectrum of drug disposition, including a growing list of transporters that influence drug absorption, distribution, and excretion.

2. Drug metabolizing enzymes

Polymorphism of the genes encoding drug metabolizing enzymes has been the core focus of Pharmacogenetics since its beginnings in the 1950s. There are more than 30 families of drug-metabolizing enzymes in humans, and essentially all have genetic variants, many of which translate into functional changes in the proteins encoded. Among drug metabolizing enzymes, Cytochrome P450 (CYP) proteins are heme-containing enzymes. They are well known for their oxidative degradation of endogenous chemicals present in the diet, environment and medications including immunosuppressive drugs such as cyclosporine and tacrolimus, which have been extensively used to prevent acute rejection following solid organ transplantation. There are as many as 57 CYP genes and among them three families of genes – CYP1, CYP2 and

drug responses (biomarkers). This knowledge may ultimately allow the development of personalized medications based on the genotype of each patient. A considerable body of evidence suggests that single nucleotide polymorphism (SNP) in genes encoding drug transporters, drug-metabolizing enzymes, enzymes involved in DNA biosynthesis and repair might determine drug efficacy and toxicity. More than 1.4 million single-nucleotide polymorphisms were identified in the initial sequencing of the human genome with over 60,000 of them in the coding region of genes. Some of these single-nucleotide polymorphisms have already been associated with substantial changes in the metabolism or effects of medications and some are now being used to predict clinical response.

CYP3—are the major genes contributing to the oxidative metabolism of various compounds. The frequency of variant alleles of CYP families varies among populations according to the race and ethnic background. For instance, there are 78 reported variants of CYP 2D6 that are associated with adverse drug reactions. Many of these polymorphic genes encode inactive enzymes. These inactive enzymes may produce adverse drug reactions among patients because of their poor metabolic activity (e.g. risperidone adverse effect). Similarly, several inactivating genetic polymorphisms have been reported in another member of the CYP family namely CYP2C19 (CYP2C19*2 and CYP2C19*3), which are also associated with adverse drug reactions. This enzyme is responsible for the metabolism of proton pump inhibitors (e.g. omeprazole and lansoprazole). Approximately 2–4% of white and 4% of African Americans have poor metabolism. Additionally, CYP2C9*2 and CYP2C9*3 alleles reduce the clearance of warfarin and increase the risk of bleeding and CYP2C9*13 allele is associated with reduced metabolism of lornoxicam. Similarly, CYP2C8 plays a role in the disposition of therapeutic

drugs. The intestinal epithelium and liver contain the most abundant member of the CYP family namely CYP3A and these enzymes are responsible for the metabolism of more than half of the therapeutic drugs. Its activity also varies among members of a given population. In addition, this enzyme may undergo induction (rifamycins) and inhibition (calcium channel blockers) depending on the drug administration, which may account for its poor or higher metabolic activity. The inter-individual variation in the immunosuppressive drugs cyclosporine and tacrolimus could be due to inter-individual differences in the expression of CYP 3A4 and 3A5 and the drug transporter P-glycoprotein. However, genetic variants identified in the CYP3A4 and CYP3A5 genes have only a limited impact on the CYP3A mediated drug metabolism, and hence the identification of the genotype for the ABCB1 gene may provide further clues for the individualization of immunosuppressive drug therapy.

3. Drug Transporters

Transport proteins have an important role in regulating the absorption, distribution, and excretion of many medications. Members of the adenosine tri phosphate (ATP)–binding cassette family of membrane transporters are among the most extensively studied transporters involved in drug disposition and effects. Genetic variability in drug transporters plays a role in the resistance of malignant cells to anticancer agents. For instance, polymorphism in the ABC-binding cassette (ABC) gene may affect the function and expression of proteins. This may cause certain drug induced side effects and uncertainty in treatment efficacy. A member of the ATP-binding cassette family, P-glycoprotein, is encoded by the human *ABCB1* gene (also called *MDR1*). A principal function of P-glycoprotein is the energy-dependent cellular efflux of substrates, including bilirubin, several anticancer drugs, cardiac glycosides, immunosuppressive agents, glucocorticoids,human immunodeficiency virus (HIV) type 1 protease inhibitors, and many other medications. The expression of P-glycoprotein in many normal tissues suggests that it has a role in the excretion of xenobiotics and metabolites into urine, bile, and the intestinal lumen. At the blood–brain barrier, P-glycoprotein in the choroid plexus limits the accumulation of many drugs in the brain, including digoxin, ivermectin, vinblastine, dexamethasone, cyclosporine, domperidone, and loperamide. A synonymous single-nucleotide polymorphism (i.e., a single-nucleotide polymorphism that does not alter the amino acid encoded) in exon 26 (3435C→T) has been associated with variable expression of P-glycoprotein in the duodenum; in patients homozygous for the T allele, duodenal expression of P-glycoprotein was less than half of that in patients with the CC genotype. CD56+ natural killer cells from subjects homozygous for 3435C demonstrated significantly lower ex vivo retention of the P-glycoprotein substrate rhodamine (i.e., higher P-glycoprotein function). Digoxin, another P-glycoprotein substrate, has significantly higher bioavailability in subjects with the 3435TT genotype. As is typical for many pharmacogenetic traits, there is considerable racial variation in the frequency of the 3435C→T single - nucleotide polymorphism. The 3435C→T single-nucleotide polymorphism is in linkage disequilibrium with a nonsynonymous single-nucleotide polymorphism (i.e., one causing an amino acid change) in exon 21 (2677G→T, leading to Ala893Ser) that alters P-glycoprotein function. Because these two single-nucleotide polymorphisms travel together, it is unclear whether the 3435C→T polymorphism is of functional importance or is simply linked with the causative polymorphism in exon 21. The 2677G →T single nucleotide polymorphism has been associated with enhanced P-glycoprotein function in vitro and lower plasma fexofenadine

concentrations in humans, effects opposite to those reported with digoxin.

4. Drug Targets

Genetic variation in drug targets (e.g., receptors) can have a profound effect on drug efficacy. Sequence variants with a direct effect on response occur in the gene for the β_2-adrenoreceptor, affecting the response to β_2-agonists; arachidonate 5-lipoxygenase (ALOX5), affecting the response to ALOX5 inhibitors and angiotensin-converting enzyme (ACE), affecting the renoprotective actions of ACE inhibitors. Genetic differences may also have indirect effects on drug response that are unrelated to drug metabolism or transport, such as methylation of the methylguanine methyltransferase (MGMT) gene promoter, which alters the response of gliomas to treatment with carmustine. The mechanism of this effect is related to a decrease in the efficiency of repair of alkylated DNA in patients with methylated MGMT. It is critical to distinguish this target mechanism from genetic polymorphisms in drug-metabolizing enzymes that affect response by altering drug concentrations, such as the thiopurine methyltransferase polymorphism associated with the hematopoietic toxicity of mercaptopurine and susceptibility to radiation- induced brain tumors.

The β_2 -adrenoreceptor (coded by the *ADRB2* gene) illustrates another link between genetic polymorphisms in drug targets and clinical responses. Genetic polymorphism of the β_2 adrenoreceptor can alter the process of signal transduction by these receptors. Three single-nucleotide polymorphisms in *ADRB2* have been associated with altered expression, down regulation, or coupling of the receptor in response to β_2 -adrenoreceptor agonists. Single-nucleotide polymorphisms resulting in an Arg-to-Gly amino acid change at codon 16 and a Gln to-Glu change at codon 27 are relatively common, with allele frequencies of 0.4 to 0.6, and are under intensive investigation for their clinical relevance.

5. Conclusion

Inter-individual difference in the efficacy and toxicity of medication is common among patients. This difference in drug response could be due to genetic, environmental factors and dose–response curve of a drug (pharmacokinetic and pharmacodynamic). Knowledge of an individual genetic variability in drug response is, therefore, clinically and economically important. Pharmacogenetics and pharmacogenomics are the two recent developments to investigate inter-individual variation and drug response. This type of genetic profiling of the population provides benefits for future medical care by predicting the drug response, or developing DNA based tests. However, these studies certainly do not suggest that pharmacological basis of drug development is a credible concept and become reality in the future because drug response can be modulated by a number of non-genetic factors such as co-medication and concurrent diseases. These non-genetic factors may increase the complexity in prescribing the medication appropriately. Although in some cases polymorphism in a gene is associated with poor efficacy and adverse drug reactions, their clinical relevance remains to be understood. Moreover, it may not be applicable to all diseases and all treatments. Additionally, this kind of approach may require a genome wide linkage analysis rather than genotyping of single genes and increased enthusiasm and education in the clinical community. In addition, its negative psychosocial consequences, violation of privacy or discrimination by pharmacogenetic testing, knowledge on the variant and disease disposition (e.g. apolipoprotein E4 allele in statin treatment and Alzheimer disease), associated cost and availability and its complexity (extensive geographic variations in genes) and understanding or

explaining the test results may pose a challenge in its public acceptance. It is not clear at present whether data from one ethnic population can be extrapolated to another population. It is also necessary to bring Pharmacogenetics itself to the lay public and explain how they influence drug response. Therefore, incorporation of the pharmacogenetic data into clinical practice (risk assessment and treatment decision) is a challenge for the future.

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http://en.wikipedia.org/wiki/Pharmacogenetics

Free radicals and antioxidants

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1. Introduction

Free radical in an atom or molecule occupies orbital. The outer most orbital contains two electrons, each spinning in opposite directions. The chemical covalent bond consists of a pair of electrons each component of the bond donating one electron each. Free radicals can be defined as chemical species possessing an unpaired electron, which is formed by homolytic cleavage of a covalent bond of molecules by loss of a single electron to a normal molecule. Oxidation is a metabolic process in the body that provides energy for vital cell activities, besides production of unavoidable oxygen derived free radicals.

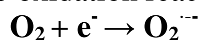
Reactive oxygen species acting as oxidants, the compounds with a tendency to donate oxygen to other substances. When oxygen is partially reduced it become activated and reacts readily with a variety of bio molecules. The sources of reactive oxygen species are xanthine oxidase, which generates super oxides, cyclo oxygenase and lipoxygenase in the cytosol which produce hyroxy and peroxyl radicals. Stimulated Neutrophil produce super oxides, cytochrome oxidase system located in mitochondria produce super oxide radicals during metabolism .The exogenous sources of free radicals include oxidant toxins, ionising radiations, environmental pollutions, cigarette smoke and sun light. Most transition metals are good promoters of free radical reactions.

2. Reactive Oxygen Species (ROS)

There are five possible species super oxide radical (**$O_2^{\cdot -}$**), hydroperoxyl (**$H_2O^{\cdot}$**), hydroxyl radical **($OH^{\cdot}$)** singlet oxygen (**$^1O_2$**), hydrogen peroxide **(H_2O_2).**

2.1. Super oxide anions ($O_2^{\cdot -}$)

Super oxide anion is the first reduction product of O_2. Super oxide is a one electron reduction product of molecule oxygen that is formed during normal respiration in mitochondria and by auto oxidation reactions.

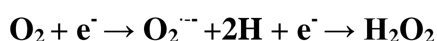


$$O_2 + e^- \rightarrow O_2^{\cdot -}$$

It is capable of initiating per oxidation of unsaturated fatty acids. **$O_2^{\cdot -}$** may be one of the possible factors for increased risk of carcinogenesis.

2.2. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) may be generated by divalent reduction of O_2 or indirectly by univalent reduction of **$O_2^{\cdot -}$**. Hydrogen peroxides (H_2O_2) stimulate the proliferation of smooth muscles .It has also been known to be mutagenic and carcinogenic.



$$O_2 + e^- \rightarrow O_2^{\cdot -} + 2H + e^- \rightarrow H_2O_2$$

2.3. Hydroxyl radical (OH)

These radicals are highly reactive. The reaction of OH^* are site specific. It reacts with an organic substrate at the site or near the sites of its formation. Lipid is very susceptible to OH^* attach and initiate lipid per oxidation. OH^* radical induce conformational changes in DNA including strand break, base modifications, damage to tumour suppressor gene and enhanced expression of proto oncogenes.

2.4. Perhydroxyl radical (OOH^*)

Protonation of **$O_2^{\cdot -}$** gives rise to O OH^*. That can initiate lipid per oxidation.

2.5. Singlet Oxygen (($^1O_2$)

This chemical form of oxygen is not a free radical, but is reported to be an important reactive oxygen species in reaction related to UV exposure. Its toxicity is reinforced when appropriate photo excitable compounds are present with molecular oxygen. Singlet oxygen may form during the degradation of lipid peroxides and may cause the production of other peroxide molecules.

3. Antioxidants

The antioxidants are the substances whose presence in relatively low concentration significantly inhibit or retard the rate of oxidation of oxidisable substances. Human body has its own antioxidants defences to protect against hazardous effect of oxidative stress given by free radicals.

Antioxidants are broadly classified in to two groups. Enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants include enzymes like manganese containing super oxide dismutase, catalase, and selenium containing glutathione peroxidase and glutathione reductase. Nonenzymatic antioxidants like tocopherol, ascorbic acid and reduced glutathione etc.

3.1. Enzymatic antioxidants

3.1.1. Super Oxide Dismutase

Super oxide dismutase is a family of metallo enzymes that convert **$O_2^{\cdot -}$** to H_2O_2 according to the following reactions.

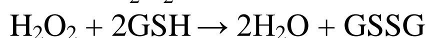
$$O_2^{\cdot -} + O_2 + 2H \rightarrow H_2O_2 + O_2$$

It is an important enzyme because it is formed in all aerobic organisms. Super oxide dismutase is considered to be a stress protein which is synthesised in response to oxidative stress. It protects

the cell from damage caused by super oxide radical.

3.1.2. Glutathione peroxidase

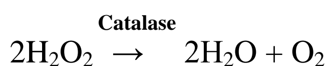
Glutathione peroxidase is well known first line of defence against oxidative stress, which requires glutathione as cofactor. Glutathione peroxides catalyses the oxidation of reduced glutathione to glutathione di sulphide at the expense of H_2O_2.



$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

3.1.3. Catalase

Catalase is an enzyme which is presents in most cells and catalyses the decomposition of hydrogen peroxide to water and oxygen. Glutathione peroxidase and catalase were found to be important in the inactivation of many environmental mutagens.



$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Glutathione reductase is high in liver, kidney and many other mammalian tissues. The function of this enzyme is to regenerate reduced Glutathione which has been converted to Glutathione disulphide by oxidation and by thiol transfer reactions.

3.2. Nonenzymatic antioxidants

Dietary antioxidants may play an important role in retarding several congenitive disorders associated with neuronal diseases including Alzheimer's disease, Parkinson's disease etc.

3.2.1. Vitamin E

Vitamin E occurs in plasma as a variety of tocopherol of which the alpha and gamma isomers are usually the major ones. Alpha tocopherol is biologically the most active isomer in mammals. It is the lipid soluble antioxidant present in antioxidant present in all cellular membranes which protect against lipid peroxidation. Major function is protecting cell membrane lipids from oxidative damage initiated by reactive oxygen metabolites.

3.2.2. Vitamin C

Vitamin C is an effective scavenger of free radicals which include $\mathbf{O_2}^{\cdot -}$, OH^* and other sulphur and nitrogen radicals.

3.2.3.Glutathione

Glutathone is a vital substance in detoxification and cell physiology. Glutathione reduces the formation of toxic peroxides on biological system by acting as substrate for Glutathione peroxidase.

4. Conclusion

In conclusion, antioxidants in physiologic quantities found in natural food are often fighter against harmful free radicals. Antioxidant vitamins naturally present food are a balanced mixture of redox with reduced and oxidised form along with several other compounds which may also beneficial.

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Neocarzinostatin induces and effect p 53 – dependent response in HPV positive cervical cancer cells

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Abstract

Human Papilloma virus (HPV) E6 viral one protein play an important role during cervical carcinogenesis. This once operotian trinds the tumor suppressor protein p 53, leading to its degradation via the ulriquitin – proteasome pathway. Therefore it is generally assumed that in HPV positive cancer cells p 53 function is completely abolished. However, recent findings suggest that p53 activity can be recovered in cells expressing endogenous E6 protein. The capacitu of Hela, INBL Caski, C33A and ViBo cell lines to respond to Neocarzinosation (NCS), a natural product which induces single and double strand breaks in DNA was examined and was fond that NCS treatment inhibits cellular proliferation through G2 cell cycle arrest and apoptosis induction. This effect was proceeded by nuclear annulations of p 53 protein in ViBo cells (HPV negative), nuclear accumulation of transcriptionaly advice p 53 and inhibition of all proliferation are observed after NCS treatment. These results suggests that HPV positive cervical cancer cells are capable of responding efficiently to DNA damage provoked by NCS treatment through a p 53 dependent pathway, inspite of presence of E6 protein.

Key words: Human Papilloma virus, p 53, cervical carcinogenesis

Carcinoma of the uterine cervix is one of the most common neoplasia in women, and more than 90% of all cervical carcinomas are epidemiologically associated with infection by high risk human pappilloma viruses (HPVs) such as types 16,18,31 and 33. These viruses encode two encoprotions, E6 and E7, capable of altering proliferation and apoptosis, as well as inducing immortalization of primary human keratinocytes. E6 speciaffically binds to wild type p 53 protein inducing its cytoplasmic sequestration and subsequently enhancing its degradation in an ATP/ ubiquitin- dependent – manner, resulting in deregulated ill growth and enhanced genomic instability.

P53 is a transcription factor with sequence specific DNA binding activity, and there is evidence that this tumor suppressor plays an important role in the maintenance of genomic stability after DNA damage. Intracellural p 53 protein levels increase following DNA damage, inducing all cycle arrest in G, which allows the cell to undergo efficient DNA repair. In this way, wild type p53 counteracts the emergence of all alones with acquired. DNA mutations. In contrast, mutanl p53 is unalade to induce G, arrest in response to DNA damage, resulting in inefficient DNA repair and the emergence of genertically unstable cells. If damage is not repaired, the cells are climeinated by p53-indeed apoptisis. Therefore the ability to

viduce apoptosis by p53 is of cetral importance to its tumor suppressor activity.

Since p53 protein is potentially functional in cerrvical cancer cell lines, blocking E6 mediated p 53 inadivation is a main therapectice goal. Previous studies have shown that inhibition of E6 mediated degradation of p53 in cervical cancer frequently results in increased levels of p53 expression. However in many cases it is necessary to induce DNA damage so that p53 becomes stable. Thus therapentic strategis that selectively rescue the p53 pathway may help sensilize HPV positive transformed cells to undergo growth arrest or apoptosis.

The endeiynes are a class of DNA strand breaking agents that show promise as anticancer drugs they are extremely selectivity for neoplastic cells relative to normal human bone narrow. NCS a product naturally found as a complex of an enedigne chomophore and a 10- KDa protein was the first member of this family to be described. It has been previously shown to induce both ss and ds breaks in DNA. The olego nucleousomal cleavage of DNA seen after NCS treatment is associated with apoptosis; rather than being the direct result of the stand clearing effects of the drug itself.

NCS is considered a prodrug that requires reduction for activation and its cylotocivity has been demonstrated to vary directly with the self hydryl content of the cell. Interestingly, higher Bd – 2 protein expression, frequently observed after p53 inadivation, enhances NCS effects. Enediynes that equire reductive activation might be clinically useful agents in the theraphy of tumors related to upregulation of the proto-encogene bcl-2 such as neuroblastomes, estrogen – responsive breast cancers and cervical cancer.

In this study we have determined the effect of NCS on stabilization and activation of p53 as well as on cell proliferation and apoptosis induction in HPV positive and negative cervical cancer cell lines. NCS was induction in HPV positive and negative cervical cancer cell lines. NCS was capable of cellular proliferation by inducting G2 cell cycle arrest and apoptosis in both HPV positive and negative cell lines. NCS lethality was higher for tumor cells than for normal human jertinocytes under the same experimental conditions. In addition, we observed a significant increase in nuclear p53 levels and trnacriptional function in HPV positive and also in ViBo (HPV negative) cells. Higher level of p21 transcripts accompanied this effect in all cell types tested. These results suggests that HPV positive cervical cancer cell lines have an effective NCS induced DNA damage response despite E6 expression, suggesting an anticancer potential for this drug against cervical cancer, independently of HPV infection.

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Statistics and Ethics in Medical Research

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1. Introduction: Application of Biostatistics

Biostatistics is simply the application of statistics to the analysis of biological and medical data. The science of biostatistics includes demography, epidemiology, design and analysis of clinical trials in medicine. This has been used in agriculture to improve crops and animal breeding. This is unavoidable in the field of ecology and forecasting. After all, biostatistics is a powerful research mechanism helpful in medical practice and research to address problems in biomedical and human health.

But in medical practice and research we have to ensure certain scientific standards with respect to laws and moral values as it involves risks of public health. Researchers and Medical practioners have a significant role in ensuring the ethical standards and scientific merit of research on human subjects. Research which is having no scientific value is against ethical principles. It is the duty of every researcher to make valid and reliable results from their research work and for which the use of the science of statistics has a significant role.

2. Medical Ethics

Ethical character is a vital module in research, in particular in medical research. Ethics is a subject that deals with values and moral rules. The application of ethics in human research is very essential for detecting the actual risk factors for disease, understanding the symptom of disease and develops new devices and procedures for detecting and monitoring the disease and developing new drugs and effective treatments in order to prevent disease in the advance stage itself. Actually ethics is not a natural science but it is a conception of the human mind. It is significant to clinical research because the risk involved is more there as it relates to public health. Its result may sometimes be beneficial or harmful to a patient. It is to be ensured the protection of the patients as well as the researchers and the medical practioners. How can humanity make possible clinical research and at the same time safeguard people from maltreatment and harm? What standards should it implement and follow? Here comes the importance of ethical values which are to be followed in every clinical research.

There are certain principles and protocols which should be adhered to every researcher for safeguarding the scientific standards and merits in medical research. The research institutes and the world health organizations are actively involving to design the various aspects and methods for improving the scientific standards with respect to laws and moral values. Codes have been developed to establish guidelines for research in human study. The medical investigators are conducting studies using various type medical data for improving the existing system and for making revolutionary changes to the system by applying their

research results. The science of statistics is an important component of medical research, especially in the design of research studies and in the analysis of resultant data. The role of ethics has now been highlighted in many aspects of medical research. But little attention has been only given to the significant role that statistical principles and ethics play in medical research.

3. Statistician and Ethical Codes

In the broader perspective, statistical methods for design and analysis are powerful research tools in the field of medical research and they are widely acknowledged in the modern era. Therefore, it is the utmost duty of statisticians to maintain their professional integrity in all their statistical design and analysis. Their selections and methods designed should be result oriented. They should present their findings openly, completely in a transparent manner regardless of the outcomes. They have to communicate their findings for the advantage of the human being, but during this activity they should try to ensure no harm to any population group. They have to conduct the statistical inquiry with great care, full justification of need, and notification of those involved, in order to ensure the accuracy and precision of the data described in their result. Statistical inquiries concerning biomedical research are rather complicated as it involves human subjects and hence statisticians should be aware of the likely consequences of the errors reflected in their data. There should not be any fear or favour when they challenge a preferred outcome. They have to make all efforts to guard against misinterpretation or misuse of their data. In order to promote and preserve the confidence of the public, statisticians should ensure that they accurately and correctly describe their results. It is the responsibility of statisticians to alert prospective users of the results to the limits of their reliability and applicability.

In the advanced science of biostatistics statistical methods are integrated into medical informatics, public health informatics, and bioinformatics. Hence statisticians have to acquire the knowledge of technological developments, procedures, and standards which are relevant to their field. They have to upgrade their professional knowledge and skills accordingly. The development of information technology has made easy accessibility to huge volume of data. Statisticians should be very careful while dealing with such data. In many cases privileged data is kept confidential. Accessibility to certain scientific methods and procedures are not easy. But statistical methods and procedures utilised to conduct the inquiry or produce published data are to be exempted from such restrictions. But statisticians should not forget that they are bound to the general moral rules of scientific and scholarly conduct while dealing with privileged data and they should not hoodwink or misrepresent the scientific or scholarly research work of others.

Statisticians should always take in to account the prevalent methods and procedures while conducting statistical enquiries. They should conduct impartial assessment and studies and based on which only they have to come to a conclusion. Any attempt to establish a predetermined outcome from a proposed statistical inquiry should be rejected. They should ensure that they have followed all ethical principles in all their undertakings.

4. Ethics Committees and Guidelines

Statisticians are constrained to protect public, individually and collectively,

against potentially harmful effects of predetermined outcome while dealing with human subjects. Therefore, proper evaluation of all medical research using biostatistics is essential from ethical and scientific points of view. There can very few arguments against the need for ethical review of protocols for human research before starting the research and in this regard a biostatistics expert is necessary on research ethics committees. A clinical investigator must obtain permission from ethics committee before starting his research as it involves on human beings.

The application of science attributable to the field of biostatistics is controlled by various guidelines issued by national and international agencies .The guidelines which are related to ethical issues are for the purpose of minimizing harm to the research participants. Unfortunately, the different sources of guidance are sometimes in conflict with one another adding more risk to statisticians.

More positively, several of the larger federal statistical agencies have given more attention to methodological, technological, organizational, and operational safeguards in recent years. For example, the American Statistical Association (ASA) Ethical Guidelines, inclusive of specific strategies for coping with and helping to prevent ethical threats to government statistical work, were written for a very broad audience; including persons working in academia, industry, and government. The International Conference on Harmonization (ICH) in 1996 had also recommended its good clinical practice (GCP) guidelines for adoption. The Indian Council for Medical Research (ICMR) has issued ethical guidelines for biomedical research on human subjects for the institutions in India which carry out any form of biomedical research involving human beings. The Drug Controller General of India has recently decided to make registration of clinical trials mandatory with an intention to bring more transparency and accountability to clinical trials. All these measures are meant for the protection, safety and wellbeing of all research participants and also for discouraging the misuse of resultant outcome of clinical research.

5. Statistics and Medical Journals

Many of our trial results that are published are not up to the international reporting standards. It may be of poor trial design. The poor quality of statistics in published papers is not restrained to medical research and it has been a cause of concern for many years. A review of the work already made before its publication can certainly have fruitful results. The errors can be identified and rectified to a certain extent.

A critical review can have more fruitful results. It is better to valuate the work of one by an expert before publishing it. It is apparent that statistical review before publication ought to be highly effective, because with regard to statistical methods, which the majority of readers of medical papers is not able to judge for themselves and so must take on trust. So, Journal publishers should have to maintain certain ethical standards, especially when deal with statistical data. They should clearly state what their policy is, there should be statistical guidelines for contributors, all research papers should include a separate section on statistical methods, the journals should give priority to well-executed and well-documented studies, authors should be encouraged to include the raw data in their papers and Journal should employ editorial staff with some understanding of statistics.

6. Conclusion

After all, it is difficult to choose the best design for a clinical trial. The result of poor research design will be worthless and waste. Efforts and valuable resources are wasted there for nothing. Wrong analysis of data can also produce false results and conclusions. The standard principles and the methodologies are often being violated in clinical trials for predetermined selections and results. In other ways, the abuse of the standard principles for the desired results is just unethical or clinical misconduct. For clinical research, substandard analysis may lead to great mishaps as it involves human health. These ethical concerns provide more risk to the individuals responsible for the statistical design and analysis. So, the application of ethical values are noteworthy in the practice of statistics and with how statistics could be applied as a tool to human subjects and thus help people face ethical and social issues affecting all of society.

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