International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 2, Feb 2016 IF- 2.818 ISSN: (2349-4077)
© Associated Asia Research Foundation (AARF)
Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

# ISOLATION, SCREENING AND CHARACTERIZATION OF CELLULASE PRODUCING MICROORGANISMS AND ITS APPLICATION

**G.Ruby Janet, T. S.Anitha, K. Rajendran and V. Karthikeyan**

Centre for P.G and Research Department Of Botany, Thiagarajar College (Autonomous)
Madurai – 625009, Tamil Nadu, India.

## ABSTRACT

*Isolation, screening and characterization of Cellulase Producing Microorganisms and its application were studied results were tabulated according to the assay of culluase producing and activity against microorganism. Results indicated that Among the organisms studied* ***Klebsiella planticola*** *and* ***Aspergillus niger*** *produced considerably high amount of cellulase when compared to the other two organisms. Cellulase production with* ***Aspergillus niger*** *was highest at temperature 45°C, pH 5.0, incubation time (7 days) and in the presence of substrates (rice straw).*

**Key words:** *Aspergillus niger, Klebsiella planticola*

## INTRODUCTION:

Indian traditional herbal medicines integration with modern technologies was elaborated in detail by Patharajan et al., in 2010. In this study the Cellulose is an organic and most abundant compound with the formula ($C_6H_{10}O_5$). Cellulose is totally insoluble in water (Lederberg, 1992). Cellulases are group of hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer i.e., cellulose to smaller sugar components including glucose subunits. Cellulolytic enzymes are synthesized by a number of microorganisms. Several antimicrobial

work has been done as antifungal activity of Plumbago species against anthracnose fungus *Colletotrichum gloeosporidodes* (Penz) of chilli. was reported by Saravanakumar et al., in 2011.

Among which fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992). Even the task disease like cancer was also to be proved enough in the work as Effect of 1,3d Glucan of *Ganoderma lucidum* on fibro sarcoma induced mice was reported by Saravanakumar et al., in 2010.

Additional potential applications include the production of wine, beer and fruit juice. Nevertheless, all these uses are of rather small magnitude compared with cellulase requirements for bioconversion of lignocellulosic biomass to fuel ethanol (philipidis, 1994). Considering the importance and application of cellulases, this study was aimed to screen the potential microorganisms for the cellulolytic ability, to analyze their activity against plant pathogens and their effect on plant growth. Cellulose is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the biosphere Jarvis.M (2003).

Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and Bacteria are the main natural agents of cellulose degradation**.** The cellulase complex of ***Aspergillus niger*** has been most thoroughly studied. It can convert native cellulose as well as derived celluloses to glucose. Lederberg (1992). Koomnok *et al.,* (2005) stated that, although a large number of microorganisms can degrade cellulose, only a few of them produce significant quantities of free enzymes capable of completely hydrolyzing crystalline cellulose.

Cellulase yields appear to depend upon a complex relationship involving a variety of factors like inoculums size, pH value, temperature, presence of inducers, medium additives, aeration, growth time, and so forth Immanuel *et al.,* (2006). ***Aspergillus niger*** can convert native cellulose as well as derived cellulose to glucose. King *et al*., (1996)**.** Potential of yeast antagonists on *in vitro* biodegradation of ochratoxin was reported by Patharajan et al., in 2011.

Enormous amount of agricultural, industrial and municipal cellulosic wastes have been accumulating or used inefficiently due to the high cost of their utilization processes Lee *et al.,* (2008). Glucose produced from cellulosic substrate could be further used as substrate for subsequent fermentation or other processes which could yield valuable end products such as ethanol, butanol, methane, amino acid, single cell protein etc.., Walsh (2002) The lignocellulosic

complex is the most abundant carbohydrate on the earth and is the main component of the agriculture waste. Lignocelluloses wastes (LCW) may be grouped into different categories such as wood residues including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse, domestic wastes (lignocellulose garbage and sewage), food industry residues, and municipal solid wastes (Roig *et al*., 2006).

## MATERIALS AND METHODS

**1) ISOLATION OF MICROORGANISMS FROM SOIL:** Soil sample was collected from place where baggases are thrown. One gram of soil sample was aseptically transferred to 99ml of sterile water taken in a conical flask and mixed thoroughly. From this soil mixture, 1ml was transferred aseptically with the help of sterile pipette to a test tube containing 9ml of sterile water to make the dilution 10-2. Similarly, the serial dilutions were made up to 10-5. From the respective dilution, 1ml of inoculum was aseptically transferred to sterile petriplates. In the case of bacterial isolation, sterilized and cooled nutrient agar medium was poured aseptically into the plates. Similarly the potato dextrose agar medium was poured on plates containing inoculums of respective dilutions for fungal isolation. The plates were incubated at room temperature. Nutrient agar plates were examined after one day for bacterial colonies and potato dextrose agar plates were examined after 4 days for the development of fungal colonies.

**2) SCREENING - CLEARING ZONE ASSAY:** To find out the cellulase producing bacteria and fungi, the isolated colonies were inoculated in CMC agar media. To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 0.1% Congo red for 15 minutes and washed with 1M NaCl. (Apun *et al*., 2000).

**3) ENZYME PRODUCTION:** The enzyme production medium was prepared and 50 ml of medium were taken in 100ml conical flasks and the flasks were sterilized. After cooling, the flasks were inoculated with the isolated fungal and bacterial cultures respectively. The inoculated medium was incubated at 37°C in shaker incubator for 24h in case of bacteria and for seven days in case of fungal cultures. At the end of the fermentation period, the culture medium was centrifuged at 5000rpm for 15minutes to obtain the crude extract, which served as enzyme source.

**4) ENZYME ASSAY:**

**a) SPECTROPHOTOMETRIC METHOD: (Miller, 1959)** A reaction mixture composed of 0.2ml of crude enzyme solution plus 1.8Ml of 0.5% carboxymethyl cellulose (CMC) in 50mM sodium phosphate buffer (pH 7) was incubated at 37°C in a shaking water bath for 30minutes. The reaction was terminated by adding 3mL of DNS reagent. The colour was then developed by boiling the mixture for 5minutes. OD of the samples was measured at 575 nm against blank containing all the reagents excluding the crude enzyme.

b) **COTTON ASSAY:** Cotton degrading activity was determined by the method of Mandels et al., (1976). In a test tube 100mg of cotton was transferred and 0.1ml of 1% sodium citrate buffer was added. 2ml of enzyme was added and mixed by squeezing out air bubbles with spatula. Tubes were incubated at 37°C in water bath for 24 hours followed by addition of 3ml DNS reagent. Tubes were then placed in boiling water bath for 5 minutes, cooled in ice bath and 14.4ml of water was added to the tubes and absorbance at 550nm was noted.

**5) ACTIVITY AGAINST PATHOGENS (DUAL CULTURE METHOD):** Blocks of well grown cultures of ***Fusarium oxysporum*** and ***Rhizoctonia solani*** were placed separately at one end of the petriplates containing sabouraud dextrose agar medium. Discs of isolated cellulase producing fungal cultures were placed aseptically using a cork borer on the other end of the plates. In case of bacterial cultures, four way streaking was done on the SDA medium and in the center of the plate the pathogens were placed. The plates were incubated for 3-5 days. Replicates were maintained for each treatment. Medium inoculated with pathogens alone served as control.

**6) GROWTH PROMOTING ACTIVITY:** soil moisture. Ten days after germination, the plants were uprooted carefully, shoots and root lengths were measured.

**d) Estimation of photosynthetic pigments:** To find out the amount of photosynthetic pigments in treated plants, the following experiments were carried out as described by **Arnon (1949).** One gram of fresh leaf materials from the treated plants were ground with 80% acetone in a morter and pestle. The extract was filtered through a filter paper and centrifuged at 5000rpm for 5 minutes to get a clean supernatant. The pellet was repeatedly extracted with 80% acetone, until it becomes colourless. The supernatant were cooled and made upto a final volume. The absorbance of the extract was measured at 620 and 660nm against acetone as blank. The chlorophyll content of the sample was calculated using the formula,

Chl a (mg/g) = ((12.7(A620nm)-2.69(A660nm)) / (1000× W) ×V

Chl b (mg/g) = ((22.9(A620nm)-4.68(A660nm)) / (1000 ×W) ×V

Total Chl (mg/g) = ((20.2(A620nm) + 8.02(A660nm)) / (1000 ×W) ×V

Where

V = total volume of the chl content.

W = weight of the leaves.

**e) Estimation of protein:**

About 1 gram of fresh leaf material was taken in a mortar and pestle. To this, 15ml of distilled water was added and ground well. The extract was centrifuged at 3000rpm for 10 minutes. The lower layer was discarded. To the supernatant equal volume of cold 5% TCA was added. It was left for 30 minutes in an ice bath. The pellet was dissolved in 15ml of 0.2N NaOH. From this, 1ml was taken and mixed with 4ml of alkaline copper reagent. It was shaken well and was allowed to stand for 10 minutes at room temperature. Then 0.1ml of diluted Folin's phenol reagent was added and mixed well. After 20 minutes, the OD was measured at 650nm using calorimeter. The amount of protein present in the plant materials were measured using a standard value.

7) **IDENTIFICATION OF BACTERIA:**

**The isolated bacteria were identified by following tests.**

Gram Staining, Indole test, Methyl Red Test, and Proteolytic Activity

**8) IDENTIFICATION OF FUNGI:** The isolated fungi which showed positive for cellulose production were identified by lacto phenol cotton blue staining. Clean glass slides were taken, on each slide, the fungal colonies were placed, then few drops of lacto phenol cotton blue stain was added and then cover slips were placed over it. The slides were observed under microscope.

**RESULTS**

**1) ISOLATION OF MICROORRGANISMS:**

Six different bacterial colonies and five different fungal colonies were isolated from soil. The selected colonies were further analyzed for the production of cellulase enzyme.

2) **SCREENING – CLEARING ZONE ASSAY:**

The isolated bacterial colonies and fungal colonies were incubated in CMC agar plates. Two bacterial colonies B5 and B6 and two fungal colonies F4 and F5 (Plate:1) which produced clear zone after treatment with congo red and NaCl solution were selected for further tests (Dual culture assay, spectrophotometric assay, cotton assay, seed germination and plant growth).

**3) ENZYME PRODUCTION:**

The enzyme production medium was prepared and the isolated cultures were inoculated. After incubation, the medium appeared turbid which indicated the growth of cultures i.e B5, B6, F4 and F5. Culture filtrates were centrifuged and crude enzyme source of the cultures were obtained.

**4) ENZYME ASSAY:**

**SPECTROPHOTOMETRIC METHOD:** The cellulolytic activity of the isolated organisms was analyzed by spectrophotometric method. Cellulose was used as substrate and assay was carried out. The optical density obtained was compared with the standard graph value and amount of cellulose degraded was determined. (Table: 1)

**COTTON ASSAY:** The substrate was changed and the cellulolytic activity of the enzyme of the isolated cultures was determined. Instead of cellulose, cotton was used as substrate and the amount of cellulose degraded was found by observing the optical density and comparing with glucose standard graph. (Table: 2)

**5) ACTIVITY AGAINST PATHOGENS:**

Each of the isolated culture was inoculated in agar plates against the pathogens ***Fusarium oxysporum*** and ***Rhizoctonia solani*** and the level of inhibition was noted. (Plate: 3,4)

**6) GROWTH PROMOTING ACTIVITY:**

a) Enriched enzyme production medium was prepared and taken in separate flasks. The isolated cultures were inoculated in the flasks and kept for incubation. After incubation the culture filtrates were centrifuged. Supernatant of the cultures served as enzyme source for seed germination and pot culture experiment.

**b) Seed germination test:** Uniform shaped pearl millet (***Pennisetum glaucum)*** and fenugreek (***Trigonella foenum-graecum)*** seeds were treated with the enzyme and results were observed after germination. Culture filtrates of B6 and F5 enabled the fast germination when compared to B5 and F4. (Plate: 5, 6)

**c) Pot culture experiment:** Pearl millet (***Pennisetum glaucum)*** and fenugreek (***Trigonella foenum-graecum)*** seeds and pots (plastic cups) were sterilized. The pots were filled with soil and enzyme treated seeds were sowed on the pots. After 10 days the grown plants were uprooted and parameters like root length, shoot length, amount of chlorophyll and protein content were measured. (Plate: 7, 8) (Table: 3 ,4, 5, 6)

**7) IDENTIFICATION OF BACTERIA:**

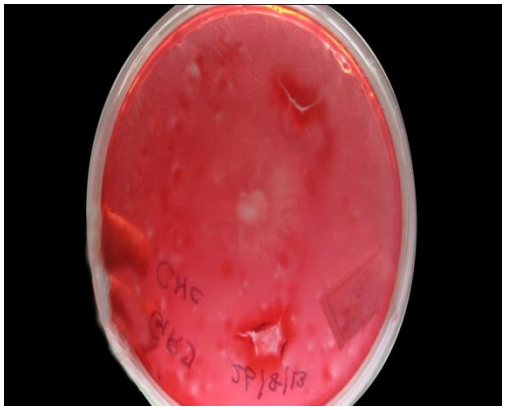
By performing biochemical tests gram staining and by studying colony morphology B5 was identified as ***Flavobacterium odoratum*** and B6 was identified as ***Klebsiella planticola.*** (Table: 7)

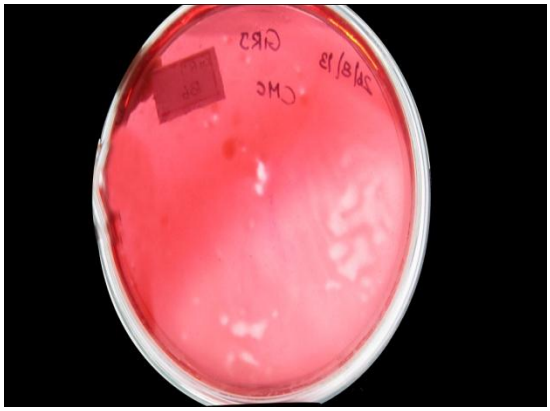
**8) IDENFICATION OF FUNGI:**

By staining the fungal culture F5 was identified as ***Aspergillus niger*** *and* F4 was identified as ***Aspergillus sp(1).***

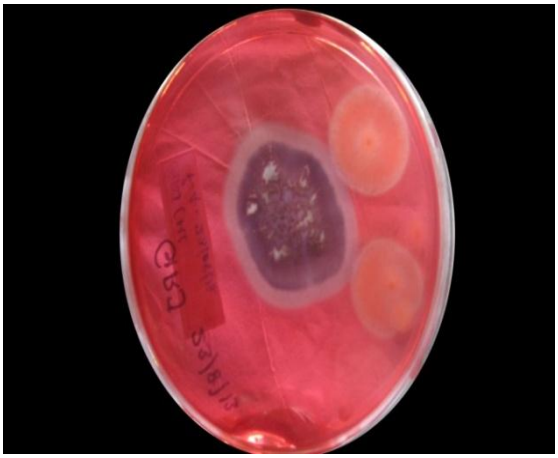
**PLATE: 1**

**B5** **B6**





**F4** **F5**



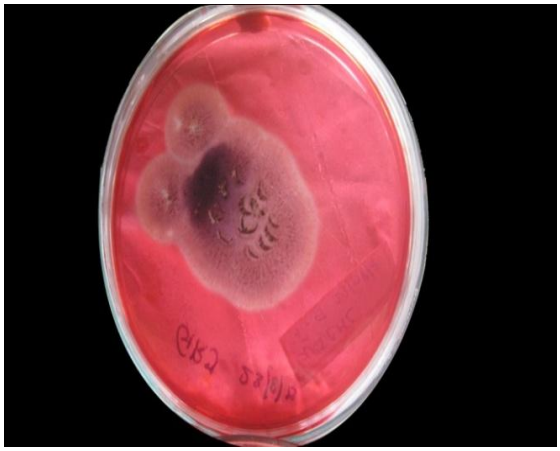


**TABLE: 1**

**ENZYME ASSAY**

**SPECTROPHOTOMETRIC METHOD**

| S.NO | CULTURE | AMOUNT OF CELLULOSE (µg/ml) |
|---|---|---|
| 1 | B5 | 256.8 |
| 2 | B6 | 385.2 |
| 3 | F4 | 192.6 |
| 4 | F5 | 321.0 |

**TABLE: 2**

**ENZYME ASSAY**

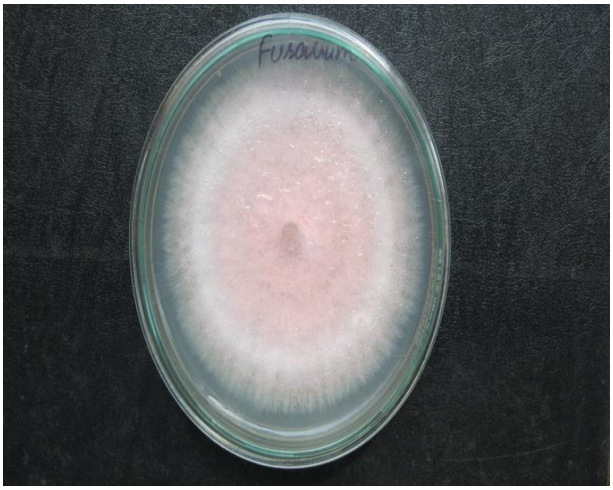
**COTTON ASSAY**

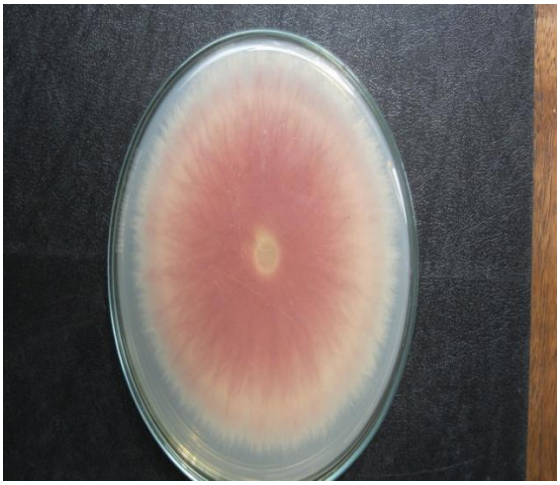
| S.NO | CULTURE | AMOUNT OF CELLULOSE (µg/ml) |
|---|---|---|
| 1 | B5 | 471.0 |
| 2 | B6 | 600.0 |
| 3 | F4 | 406.8 |
| 4 | F5 | 535.2 |

**PLATE: 2**

**PURE CULTURE OF THE PATHOGENS AGAINST WHICH THE DUAL CULTRE WAS DONE**

***Fusarium oxysporum***

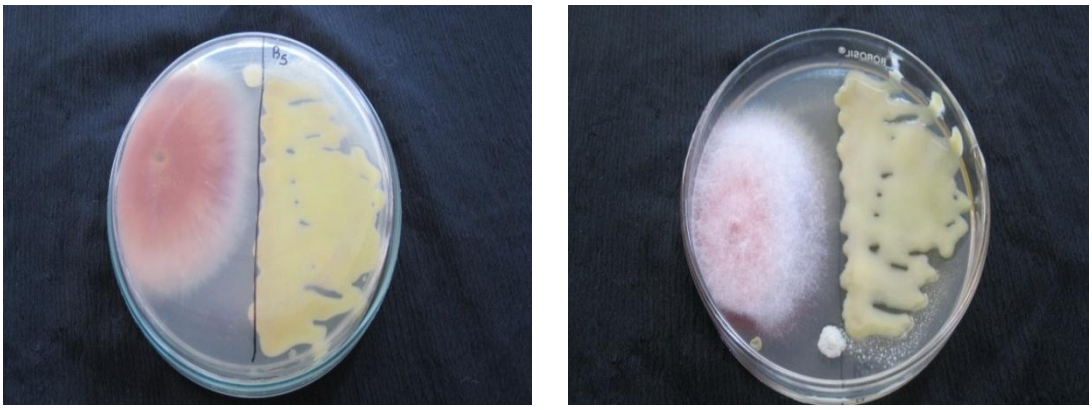


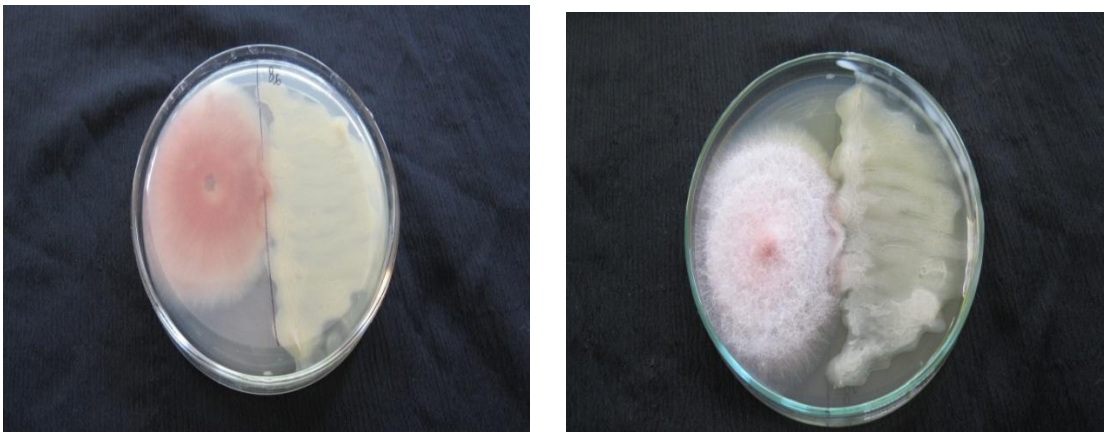
***Rhizoctona solani***

**PLATE: 3**

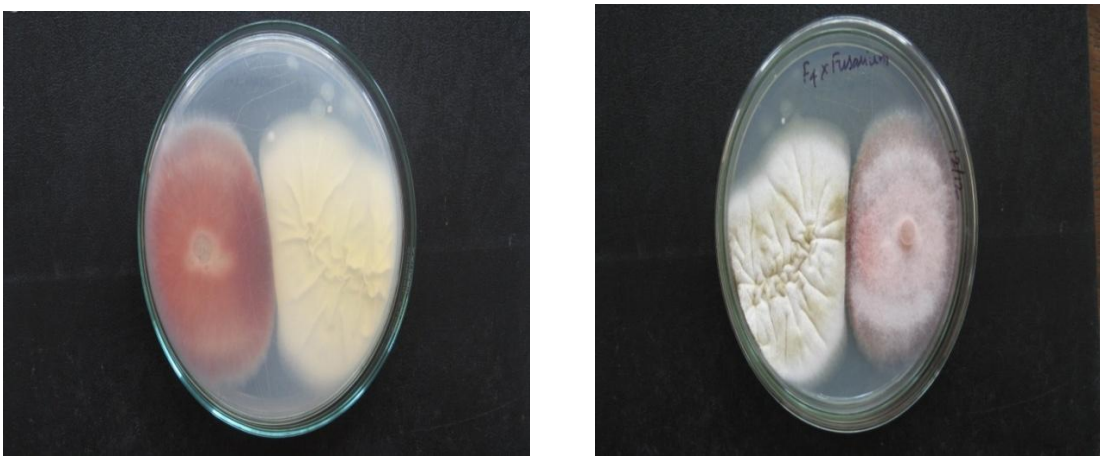
## ACTIVITY OF THE ISOLATED ORGANISM AGAIST *Fusarium oxysporum*



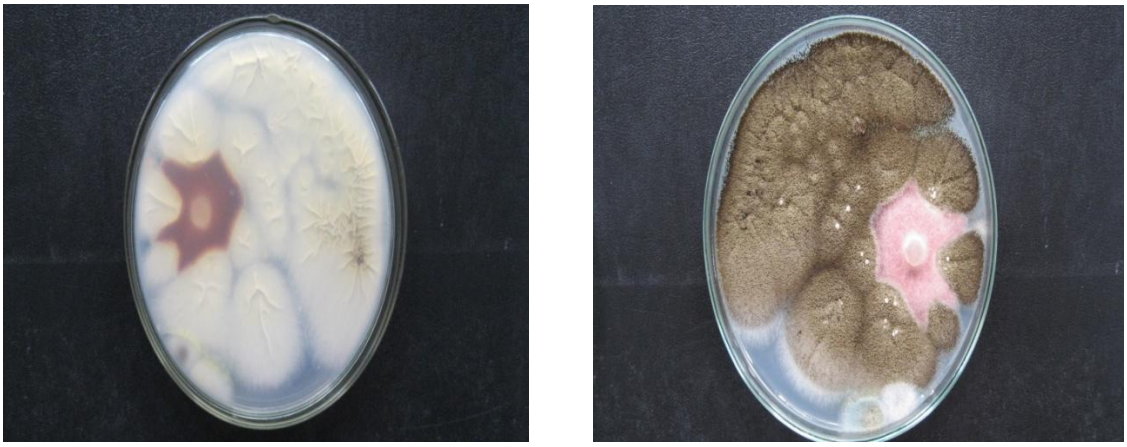
**B5 AGAINST *Fusarium oxysporum (partial inhibition)***



**B6 AGAINST *Fusarium oxysporum (partial inhibition)***



**F4 AGAINST *Fusarium oxysporum (inhibition)***

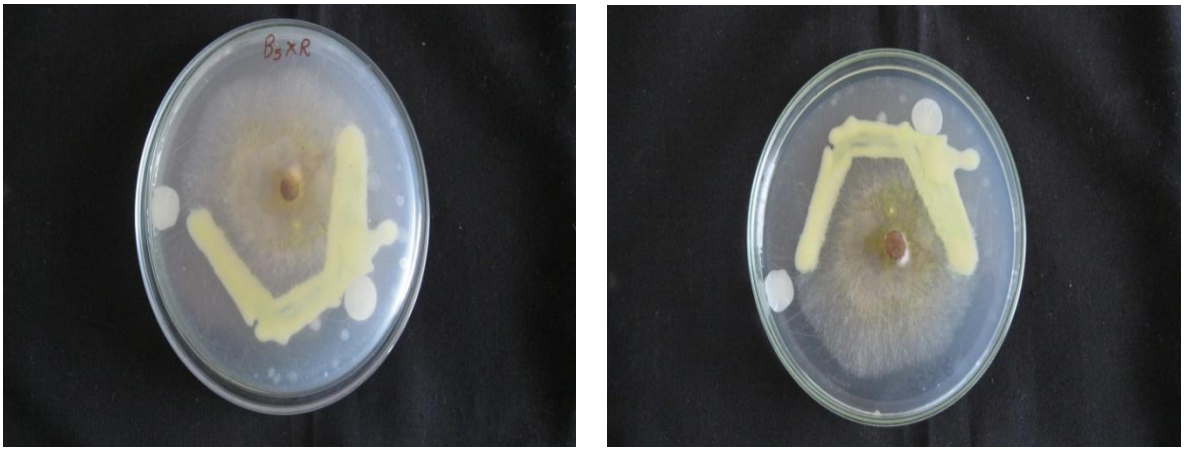


**F5 AGAINST *Fusarium oxysporum (Inhibition)***

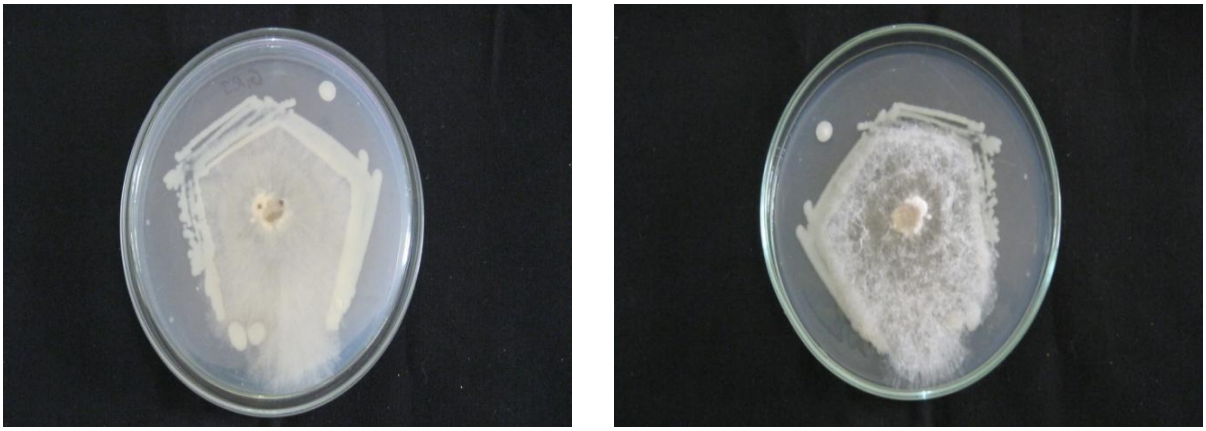
**PLATE: 4**

## ACTIVITY OF THE ISOLATED ORGANISMS AGAIST

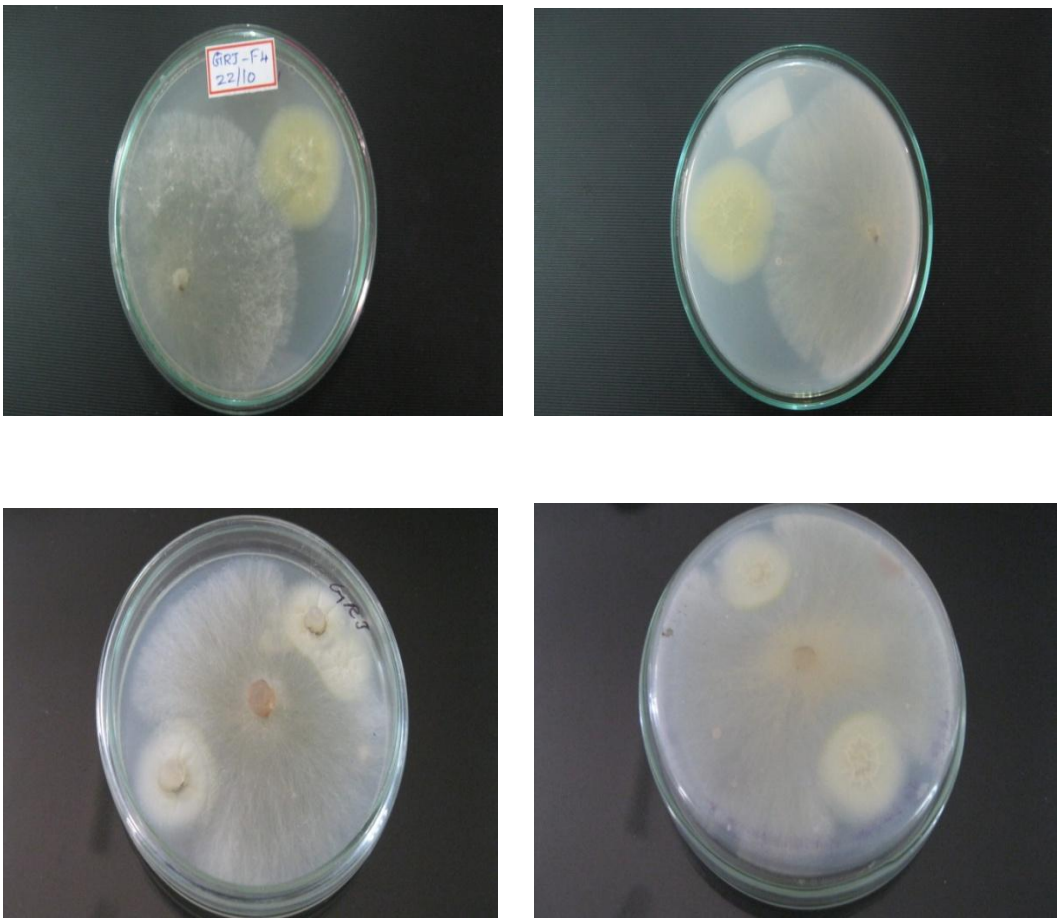
### *Rhizoctona solani*



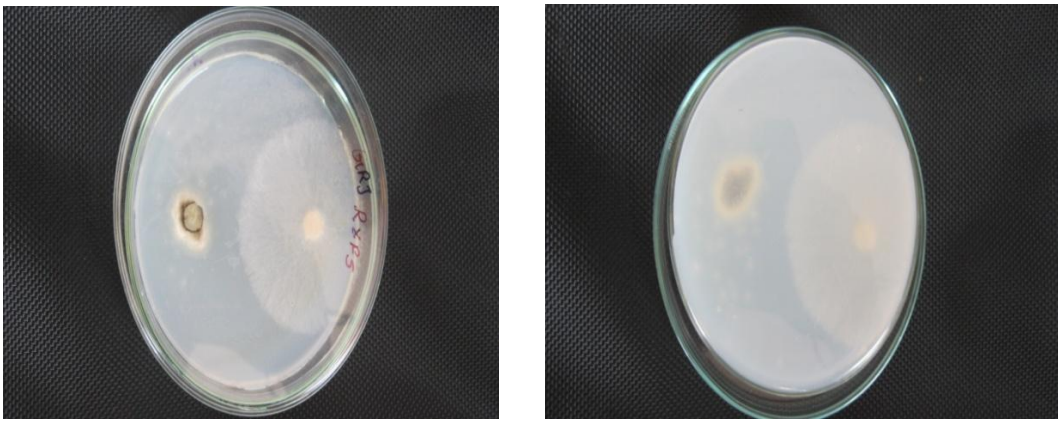

**B5 AGAIST *Rhizoctonia solani (no inhibition)***



**B6 AGAIST *Rhizoctonia solani (inhibition)***




**F4 AGAIST *Rhizoctonia solani (no inhibition)***

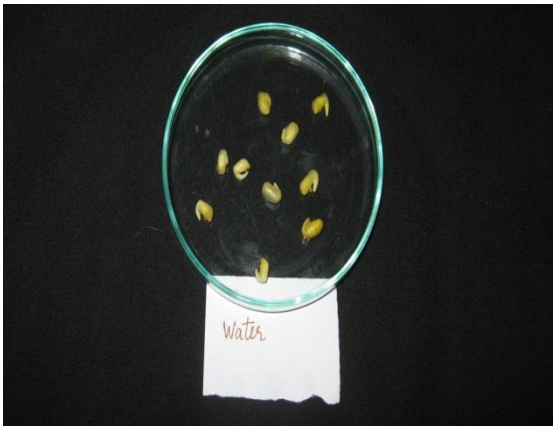


**F5 AGAIST *Rhizoctonia solani (partial inhibition)***

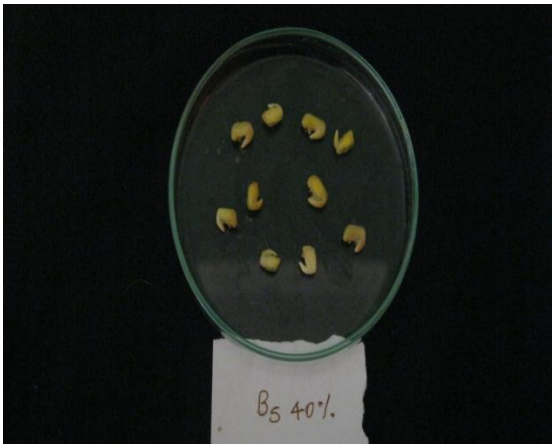
**PLATE: 5**

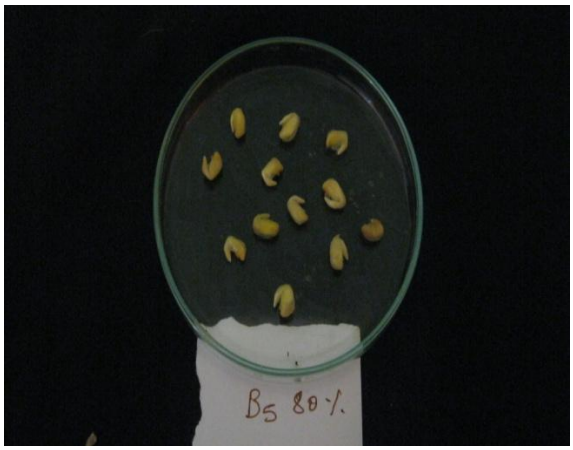
## SEED GERMINATION TEST

## FENUGREK







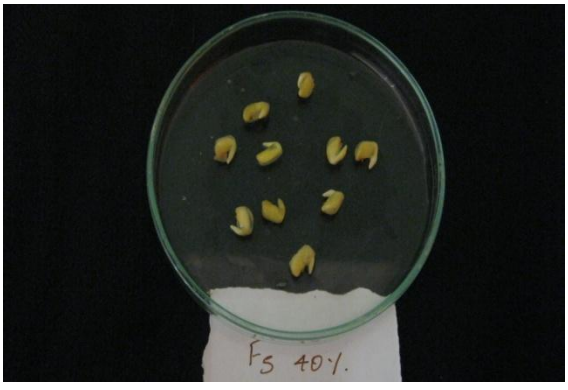




B6 40%


B6 80%.


F4 40%.


F4 80%.

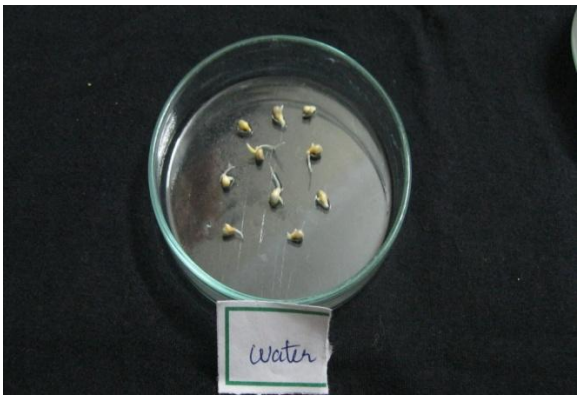

F5 40%.

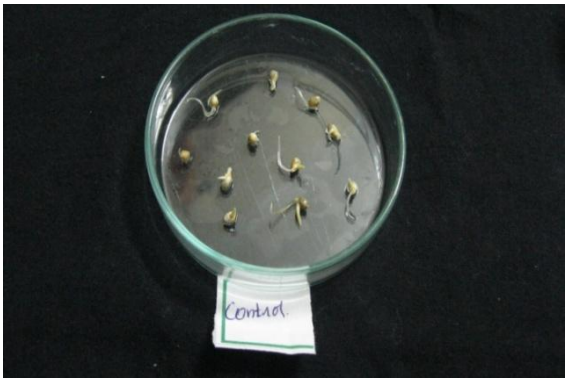

F5 80%.

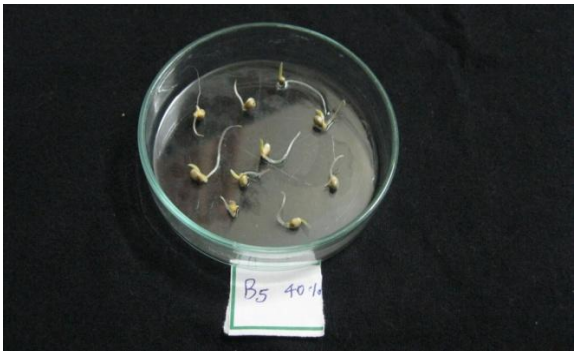
**PLATE: 6**

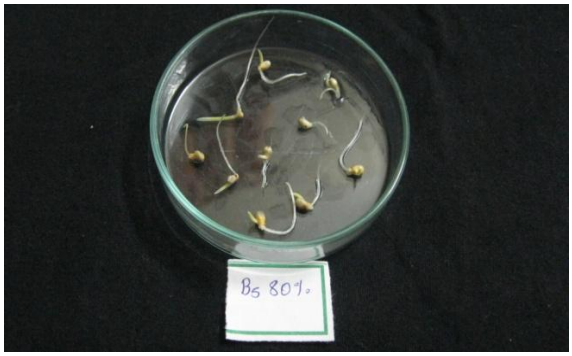
## SEED GERMINATION TEST

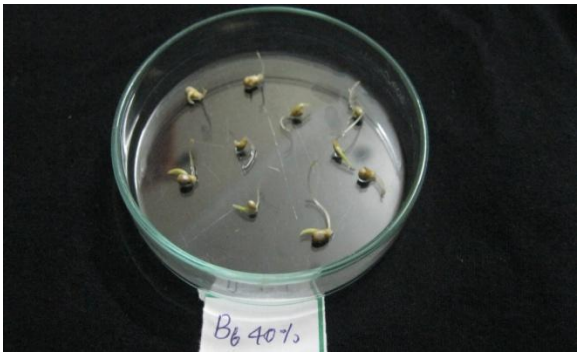
## PEARL MILLET

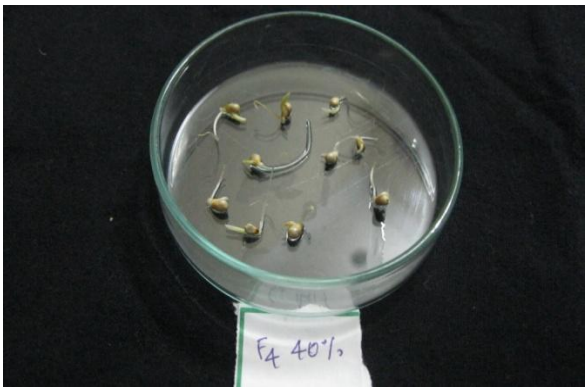


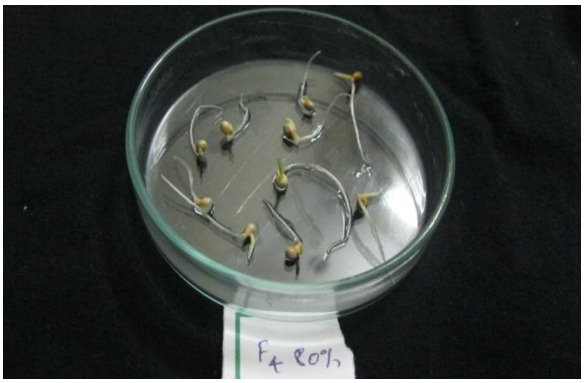



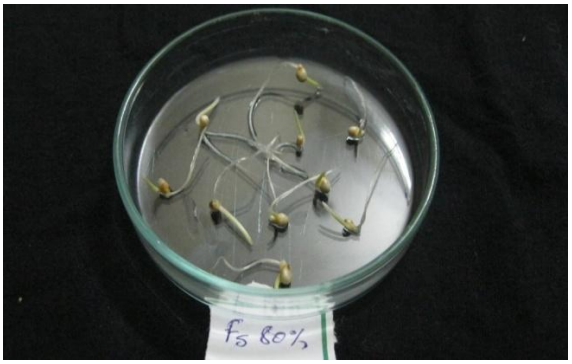





F4 40%


F4 80%


F5 80%

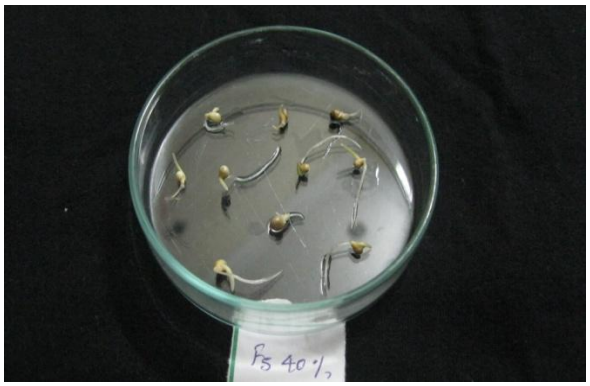

F5 40%

**PLATE: 7**

## POT CULTURE EXPERIMENT

## FENUGREK



**PLATE: 8**

## POT CULTURE EXPERIMENT

## (PEARL MILLET)



B6 40%


B6 - 80%


F4 - 40%


F4 - 80%


Fs - 40%


Fs - 80%

## TABLE: 3

**ROOT LENGTH AND SHOOT LENGTH OF GERMINATED FENUGREK SEEDS AT DIFFERENT CONCENTRATION OF CULTURE FILTRATES**

| ISOLATES | PARAMETERS (cm) | TREATMENTS | |
|---|---|---|---|
| | | CONCENTRATION OF CULTURE FILTRATES | |
| | | 40% | 80% |
| B5 | ROOT LENGTH | 6.5 | 7.0 |
| | SHOOT LENGTH | 5.5 | 5.5 |
| B6 | ROOT LENGTH | 6.0 | 7.5 |
| | SHOOT LENGTH | 5.5 | 6.0 |
| F4 | ROOT LENGTH | 6.0 | 6.0 |
| | SHOOT LENGTH | 4.5 | 5.0 |
| F5 | ROOT LENGTH | 7.0 | 7.5 |
| | SHOOT LENGTH | 6.0 | 6.5 |

| | ROOT LENGTH (cm) | SHOOT LENGTH (cm) |
|---|---|---|
| **WATER** | **5** | **5** |
| **CONTROL** | **5.5** | **5** |

**TABLE: 4**

**ROOT LENGTH AND SHOOT LENGTH OF GERMINATED PEARL MILLET SEEDS AT DIFFERENT CONCENTRATION OF CULTURE FILTRATES**

| ISOLATES | PARAMETERS (cm) | TREATMENTS | |
|---|---|---|---|
| | | CONCENTRATION OF CULTURE FILTRATES | |
| | | 40% | 80% |
| B5 | ROOT LENGTH | 8.5 | 9.0 |
| | SHOOT LENGTH | 10.0 | 11.0 |
| B6 | ROOT LENGTH | 8.0 | 9.5 |
| | SHOOT LENGTH | 13.5 | 14.0 |
| F4 | ROOT LENGTH | 6.5 | 7.0 |
| | SHOOT LENGTH | 9.5 | 10.0 |
| F5 | ROOT LENGTH | 9.0 | 9.5 |
| | SHOOT LENGTH | 14.5 | 15.0 |

| | ROOT LENGTH (cm) | SHOOT LENGTH (cm) |
|---|---|---|
| **WATER** | **8.0** | **9.5** |
| **CONTROL** | **8.0** | **11.0** |

**TABLE: 5**

**ESTIMATION OF PHOTOSYNTHETIC PIGMENTS AND PROTEIN IN CELLULASE TREATED FENUGREK PLANT**

| **TREATMENTS (SEEDS TREATED WITH CULTURE FILTRATES )** | **Concentration of culture filtrates** | **Chl a mg/g** | **Chl b mg/g** | **Total Chl mg/g** | **Protein (µg/g)** |
|---|---|---|---|---|---|
| **B5** | **40%** | **0.625** | **0.655** | **1.28** | **52.23** |
| | **80%** | **0.675** | **0.682** | **1.35** | **55.65** |
| **B6** | **40%** | **0.954** | **0.926** | **1.88** | **72.33** |
| | **80%** | **1.112** | **0.987** | **0.09** | **76.48** |
| **F4** | **40%** | **0.546** | **0.578** | **1.12** | **45.61** |
| | **80%** | **0.549** | **0.567** | **1.12** | **45.86** |
| **F5** | **40%** | **1.135** | **1.118** | **2.25** | **79.52** |
| | **80%** | **1.144** | **1.116** | **2.26** | **79.93** |

**TABLE: 6**

**ESTIMATION OF PHOTOSYNTHETIC PIGMENTS AND PROTEIN IN CELLULASE TREATED PEARL MILLET PLANT**

| TREATMENTS (SEEDS TREATED WITH CULTURE FILTRATES) | Concentration of culture filtrates | Chl a mg/g | Chl b mg/g | Total Chl mg/g | Protein (µg/g) |
|---|---|---|---|---|---|
| B5 | 40% | 0.534 | 0.530 | 1.06 | 72.13 |
| | 80% | 0.568 | 0.572 | 1.14 | 75.56 |
| B6 | 40% | 0.879 | 0.798 | 1.68 | 93.13 |
| | 80% | 0.895 | 0.812 | 1.71 | 97.19 |
| F4 | 40% | 0.438 | 0.451 | 0.89 | 61.84 |
| | 80% | 0.521 | 0.539 | 1.06 | 13.89 |
| F5 | 40% | 0.964 | 0.942 | 1.91 | 94.87 |
| | 80% | 1.113 | 0.986 | 2.10 | 98.26 |

**TABLE: 7**

**IDENTIFICATION OF BACTERIA**

| CULTURE | GRAM NATURE | SHAPE | INDOLE PRODUCTION | METHYL RED | CAESIN DEGRADATION | COLONY COLOR |
|---|---|---|---|---|---|---|
| B5 | - | ROD | - | + | + | YELLOW |
| B6 | - | ROD | - | + | + | WHITE |

**+ positive**

**- negative**

**DISCUSSION:**

The cellulose is world's most abundant organic substance and comprises a major storage form of photosynthesized glucose. It is the major component of biomass energy. Hence cellulolytic enzymes play a vital role in utilizing this biomass energy and in the biological cycle of carbon. Soil is store house of several organisms. Since several microorganisms synthesis cellulase enzyme, these microbes can be employed to degrade cellulose mass efficiently. Cellulolytic enzymes exhibit not only degradative activity but many catalytic activities like seed germination and plant growth promotion.

Among the organisms studied ***Klebsiella planticola*** and ***Aspergillus niger*** produced considerably high amount of cellulase when compared to the other two organisms. According to a recent study, in-vitro optimization of microorganisms would yield higher amount of cellulase **(Sonia *et al.*,).** Cellulase production with ***Aspergillus niger*** was highest at temperature 45°C, pH 5.0, incubation time (7 days) and in the presence of substrates (rice straw).

A wide range of *Aspergillus* species have been identified to posses all component of cellulase enzyme system **.** In the present study ***Aspergillus niger*** produced enzyme in 7 days at room temperature and with cellulose as substrate. In the millet part the following work has been done in 2008 the fertility status of *Setaria* infecting *Magnaporthe grisea* isolates with Standard Testers were identified by Karthikeyan, and Gnanamanickam. Biological control of *Setaria* blast (*Magnaporthe grisea*) with selected bacterial strains of Bacillus and Pseudomonas species were reported by Karthikeyan, and Gnanamanickam in 2008.

Using PCR method the blast disease caused by *Magnaporthe grisea* in *Setaria italica* was identified rapidly by Karthikeyan, and Gnanamanickam in 2005.

The organism that produced high amount of cellulase enabled fast germination and prompted plant growth. To some extent cellulase enzyme help the organism to defend itself from other pathogens. The study concentrates on organisms that produce cellulase. There are several studies on the potentiality of *Fusarium verticillioides* to produce fumonisin and its responsible gene detection assay was reported by Karthikeyan et al., in 2008. Bakanae disease of rice in North-Western Italy and its PCR based detection of Fusarium species was carried out by **Karthikeyan**, et al., in 2009. The past work on ISR proved induction of systemic resistance in rice to bacterial

blight by 1,2,3-Benzothiadiazole 7-carbothioic acid-S-methyl ester (BTH) treatments was done by **Karthikeyan** and Gnanamanickam in 2011.

The modern technologies based on DNA research paved the better way of understanding the disease like an efficient method for Fungal DNA Extraction highly Suitable for PCR Based Molecular Methods was reported by Karthikeyan et al., in 2010. Molecular breeding of Indica rices for blast and bacterial blight resistance was reviewed by Gnanamanickam, and Karthikeyan in 2002

And followed by PCR based detection of fumonisin producing strains of *Fusarium verticillioides* and gene related to toxin Production was reported by **Karthikeyan**, et al., in 2011. Every pathogen gains virulence with in five years of time so importance have to be given on this aspect of study like Virulence Characteristic analysis and Identification of new Pathotypes of Rice Blast fungus from India. Were reported by **Karthikeyan**, et al. in 2013

From the study it is clear that cellulase producing organisms could control the activity of pathogens and induce seed germination and plant growth. Apart from these applications these organism can degrade cellulose containing biomass and helps maintain biogeochemical cycles particularly carbon cycle. Since the number of industries like paper and pulp industries is continuously increasing, there is a need to study the microorganisms that produce cellulase to promote bioremedy and to make our environment a sustainable one.

**REFERENCES:**


Alexander, M. 1961. Microbiology of cellulose. In: Introduction to Soil Microbiology (2nd Ed.). Johnwiley and Son, Inc. New York and London.

Apun.K, B. C. Jong, and M. A. Salleh, (2000) "Screening and isolation of a cellulolytic and amylolytic *Bacillus* from sago pith waste, "Journal of General and Applied Microbiology, vol. 46, no. 5, pp.263–267.

Bell**, A.** (1981). Biochemical mechanisms of disease resistance. Annu. Rev. Plant Physiology 32:pp. 21-29.

Bhat, M. and Bhat, S. (**1997**) Biotechnology Advances 15, pp583- 620.

Brzobohaty B, Moore I, Kristoffersen P, Bako L, Campos N, Schell J, Palme K**,** (1993). Release of active cytokinin by a ß-glucosidase localized to the maize root meristem. Science 262: pp.1051-1054.

Camassola.M and A. J. P. Dillon, (2007) "Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation, "Journal of Applied Microbiology, vol. 103, no. 6, pp. 2196–2204.

Dharmawardhana, D P, Ellis B E, Carlson J E**,** (1995). A ß-glucosidase from lodgpole pine xylem specific for the lignin precursor coniferin. Plant Physiol. 107: pp331-339.

Fenwick G R, Heaney R K, Mullin W J**,** (1983). Glucosinolates and their breakdown products in food and food plants. CRC Crit. Rev Food Sci. Nutr. 18: 123-201.

Gnanamanickam, S.S, **Karthikeyan, V.** Review article on Molecular breeding of Indica rices for blast and bacterial blight resistance.- Published in Vistas in Botany Co-memmorative volume of Prof. Sekar shetty.37

Hruska A J**,** (1988). Cyanogenic glucosides as defense compounds. A review of the evidence. J Chem Eco 14: pp.2213-2217.

Immanuel.G, R.Dhanusha, P. Prema, and A.Palavesam, (2006) "Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment," *International Journal of Environmental Science and Technology*, vol. 3, no. 1, pp. 25–34.

Jarvis.M, (2003) "Cellulose stacks up," *Nature*, vol. 426, no. 6967, pp.611–612.

Jones D A, (1988). Cyanogenesis in animal-plant interactions. In: D. Evered and S.Harnett (Editors), Cyanide Compounds in Biology. Ciba Foundation Symposia 140:pp.151-170.

**Karthikeyan, V, Rajarajan, R. and Gnanamanickam, S. S.** Virulence Characteristic analysis and Identification of new Pathotypes of Rice Blast fungus from India. **Life Sciences Feed,** vol, 2, Pg. 13-17, 2013.

Karthikeyan, V, R. Rajarajan, S. Patharjan, P. Karthikeyan, P. Saravanakumar, M. Siva, P.S. Aruna Bhavani , P. Palani. PCR based detection of fumonisin producing strains of Fusarium verticillioides and gene related to toxin Production. - Current Botany. Vol 2 (3), Pg. 34-37, 2011.

**Karthikeyan**, **V.** Patharajan, S. Palani, P. Spadaro, D. Gullino, M. L. and Garibaldi, A. Modified Simple Protocol for Efficient Fungal DNA Extraction Highly Suitable for PCR Based Molecular Methods**.** - ***Global journal of Molecular Sciences***- Vol 1 (5): 37-42, 2010.

**Karthikeyan** V, Gnanamanickam S.S. Induction of systemic resistance in rice to bacterial blight by 1,2,3-Benzothiadiazole 7-carbothioic acid-S-methyl ester (BTH) treatments. ***Achieves of Phytopathology and Plant Protection***, Vol. 44 (3), 269–281, February 2011.

**Karthikeyan V,** Gnanamanickam S.S. Biological control of *Setaria* blast (*Magnaporthe grisea*) with bacterial strains. ***Crop protection*** . Volume 27, Issue 2, Pg. 263-267. Feb. 2008.

**Karthikeyan V,** Gnanamanickam S.S. Rapid and specific disease detection of blast fungus in *Setaria italica* through PCR method - *Vol. 8. Pg. 349-350.* ***Current Science***. Feb 2005

**Karthikeyan**, V, Davide Spadaro, Maria Lodovica Gullino and Angelo Garibaldi. Bakanae disease of rice in North-Western Italy PCR based detection of Fusarium species ***Journal of Swamy botanical club,*** Vol.23, Pg- 69 January 2009.

**Karthikeyan V,** Gnanamanickam S.S. Determining the Fertility Status of *Setaria* infecting *Magnaporthe grisea* Isolates with Standard Testers and identification of tolerant cultivar of *Setaria italica.* **V.Karthikeyan**, S.S Gnanamanickam, ***Mycopathologia***. Vol. 166(4), Pg. 227-33 Oct 2008.

**Karthikeyan V**, Patrizia Titone, Davide Spadaro, Maria Lodovica Gullino and Angelo Garibaldi. Detection of fumonisin producing strains of *Fusarium verticillioides* and gene related to toxin production. ***Journal of plant pathology***. Vol.90, Pages –S2.32, August 2008.

Koomnok.C, (2005)"Selection of cellulase producing thermophilic fungi," in *Proceedings of the 31st Congress on Science and Technology of Thailand of Technology*, Suranaree University.

Leah R, Kigel J, Svendsen I, Mundy J, (1995). Biochemical and molecular characterization of barley seed ß-glucosidase. J Biol Chem. 270: pp.15789-97.

Lederberg, J. 1992. Cellulases. In: *Encyclopaedia of Microbiology* (Vol. 1; A-C). Academic Press, Inc.

Lee.Y.J, B. K. Kim, B. H. Lee et al., (2008) "Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL-3 utilizing rice hull," *Bioresource Technology*, vol. 99, no. 2, pp.378–386.

Mandels, M., R. Andreotti and R. Roche. 1976. *Biotech. Bioeng. Symp*., 6:pp17-37.

Mandels.M, "Microbial source of cellulose," *Biotechnology and Bioengineering*, vol. 5, pp. 81–105, 1975.

Matsuzaki, T., and A. Koiwai, (1986). Germination inhibition in stigma extracts of tobacco and identification of MeABA, ABA, and ABA-B-D-glucopyranoside. Agric. Biol. Chem. **50**:pp.2193-2199.

Miller.G.L, (1959) "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry*, vol. 31, no. 3, pp.426–428.

Niemeyer H M, (1988). Hydroxamic acid (4-hydroxy-1, 4-benzoxazin-3-ones), defence chemicals in the gramineae. Phytochemistry **27**:pp.3349-3358.

Nishida.Y, K. I. Suzuki, Y. Kumagai, H. Tanaka, A. Inoue, and T.Ojima, (2007)"Isolation and primary structure of a cellulase from the Japanese sea urchin Strongylocentrotus nudus," *Biochimie*, vol.89, no. 8, pp. 1002–1011.

Oxtoby E, Dunn M A, Pancoro A, Hughes M A, (1991). Nucleotide and derived amino acid sequence of the cyanogenic ß-glucosidase (Linamarase) from white clover (*Trifolium repens* L.). Plant Mol Bio. 17:pp.209-219.

Patharajan, S. Karthikeyan, V**.** Integration of Indian traditional herbal medicines with modern technologies - An Overview. ***Pharmacologyonline.*** *vol.* 1: Pg. 876-879. 2010.

Patharajan, S.; Reddy, K.R.N.; Spadaro, D.; Lore, A.; Gullino, M.L.; Garibaldi, A.; Karthikeyan, V.Potential of yeast antagonists on *in vitro* biodegradation of ochratoxin A. *Food Control*, *22*,290–296, 2011.

Philippidis, G.P. 1994. Cellulase Production technology. In: *Enzymatic Conversion of Biomass for Fuel Production.* (Eds): M. E. Himmel *et al*., ACS symposium series 566.

Poulton J E, (1990). Cyanogenesis in Plants. Plant Physiol. 94:pp.401-405.

Saravanakumar P, **Karthikeyan V**, Patharajan S, Kannan T, Sathya K and Kalaichelvan P.T. Effect of 1,3d Glucan of *Ganoderma lucidum* on fibro sarcoma induced mice. - ***Pharmacologyonline*** **Vol.**1: Pg.958-970. 2010.

Saravanakumar, P. Karthikeyan, V**.** and B. Kannabiran. Antifungal activity of Plumbago species against anthracnose fungus *Colletotrichum gloeosporidodes* (Penz) of chilli. ***Achieves of Phytopathology and Plant Protection***, Vol. 44, No. 3, Pg. 287–297 February 2011.

Schliemann W**,** (1984). Hydrolysis of conjugated giberellins by ß-glucosidases from Dwarf Rice (*Oryza sativa* L. cv. Tan-ginbozu). Plant Physiol.116:pp123-132.

Walsh, G. 2002. Industrial enzymes: proteases and carbohydrases. In: *Proteins; Biochemistry and Biotechnology*. John Wiley and Sons. Ltd.

Walter, H.R. 1998. Microcrystalline cellulose technology. In: *Polysaccharide Association Structure in Food*. Marcel Dekker, Inc.

Wiese G, Grambow H, (1986). Indole-3-methanol-ß-D-glucoside and Indole-3-carboxylic acid-ß-D-glucoside are products of indole-3-acetic acid degradation in wheat leaf segments. Phytochemistry 25:pp2451.

Woodward J, Wiseman A, (1982). Fungal and other ß-D-glucosidases, their properties and applications. Enzyme Microb Technol. 4:pp73-93.

Zhang.Y.H.P., and L. R. Lynd, (2004) "Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Non complexed cellulase systems," *Biotechnology and Bioengineering*, vol. 88, no.7, pp. 797–824.