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PROJECT

ISOLATION AND IDENTIFICATION OF ASPERGILLUS SPECIES

By

Students of II B. Sc.
Biomedical Sciences

Guided by

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Submitted to

The Research Committee

HINDU COLLEGE, GUNTUR

JUNE - 2022

HINDU COLLEGE, GUNTUR

(Re-accredited by NAAC, a Grade - A)

Department of Biomedical sciences

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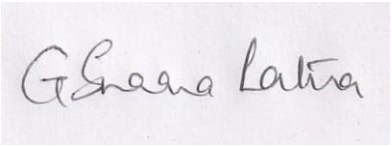
CERTIFICATE

This is to certify that this thesis entitled, **"ISOLATION AND IDENTIFICATION OF *ASPERGILLUS* SPECIES"** is a *bona fide* Project work done by **Students of Biomedical sciences, Hindu College,** under my guidence. This Project work or any part thereof has not been submitted elsewhere for award of any Degree or Diploma. This work is found to be satisfactory.



G. SWARNA LATHA

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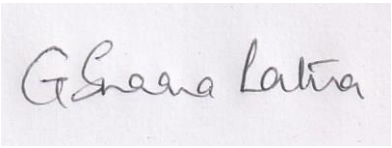

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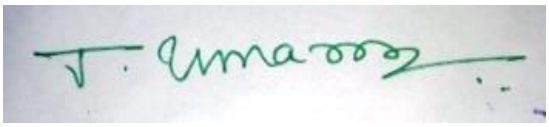

Dr. J. UMA MAHESWARA RAO

DECLARATION

We hereby declare that the thesis entitled "ISOLATION AND IDENTIFICATION OF Aspergillus spp. FROM SOIL AT HINDU COLLEGE, GUNTUR" submitted by us to Acharya Nagarjuna University, Guntur, in partial fulfillment for the award of degree of B.Sc., Biomedical Sciences is a record of our original research work carried at department of Biomedical Sciences, Hindu college, Market center, Guntur. Under the guidance of Smt. G.Swarnalatha, Assistant professor, Department of Microbiology, Hindu college. The thesis are any part there of has not formed the basis for the award of any degree, diploma, associateship, fellowship, or any other similar title of this or any other university, previously.

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INDEX

ABSTRACT

The investigation is aimed to find out the fungal diversity, also to isolate and identify the *Aspergillus* species in the soil samples collected in the Hindu college campus, Guntur. For isolation and identification, plating, pure culturing, incubating, staining, inoculating such techniques are used. The mycelia and cytoplasm are stained using Lactophenol and cotton blue (provides light blue background). The stained specimens are observed under the microscope for identification and photograph is taken. Among the identified species the Keratinophilic fungi *Aspergillus Niger* is found in maximum numbers in the campus and followed by others. All the isolates are identified with standard key and microbial expert.

Key words: Fungi, isolation, magnification, mycelium, Aspergillus.

CHAPTER – 01

INTRODUCTION

INTRODUCTION

The soil serves as a reservoir for many microbial communities of plants and herbs which can be producing, CO_2 and nitrogen cycle. The microorganisms play a major role in soil ecosystem (Text book of Microbiology by Ananthanarayana). Microbial composition and functioning, changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control. Soil is an oligotrophic medium for the growth of fungi because the fungal growths are extremely limited for most of the time and readily available. For most of the time, fungi is either dormant, or they metabolize and grow very slowly utilizing a range of organic molecules. The fungi distribute organic matter away from the roots. In general, the concentration of microbes is greatest close to the surface of roots (rhizosphere) and hyphae of arbuscular mycorrhizal fungi (mycorrhizosphere), where exudates are extraordinarily important source of organic energy entering from soils (Textbook of Microbiology by Surinder Kumar). Genetic studies have shown that fungi is more closely related to animals than to plants. Fungi have 80 percent or more of the same genes as humans (Ratna Kumar, P.K., Hemanth Isolation and identification of soil mycoflora). Fungi is not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as biofertilizers and many other ways. Fungal biotechnology has become an integral part of the human welfare. Fungus benefits most plants by suppressing plant root diseases and fungi promote healthier plants by attacking plant pathogens with fungal enzymes. Fungi also use antagonism to reduce competition by producing antibodies, which suppress other microorganisms from growing (Medical Microbiology by David Greenwood and Mike Barer) They produce many vitamins which promote plant growth. Beneficial fungi also form protective webs and nets around roots and leaves to protect the host plant. Fungus also protects plants by supplying both water and phosphorus to the plant roots during droughts. Different fungal communities are identified from soil samples collected from different locations of Hindu college campus and it is identified and confirmed with microbial expert (Karthikeyan - Optimization of Enzyme Production in *Trichoderma viridae)*.

AIMS AND OBJECTIVES

- To determine variety of fungal organisms from soil.
- To isolate industrially important fungi from soil (particularly in production of citric acid).

Survey of Literature

Mycology Guidebook was presented by Mycology Guidebook Committee in 1981. Seifert, K.A in 1992 contributed in isolation of filamentous Fungi. Maheswari et al worked on Thermophilic fungi: Their physiology and enzymes in 2000. In 2004 Methods of studying soil microbial diversity is contributed by Krik et al. Optimization of Enzyme Production in *Trichoderma viridae* using Carbon and Nitrogen source is published by Karthikeya et al in 2014.

CHAPTER – 02

MATERIALS AND METHODS

MATERIALS AND METHODS

The Hindu college campus is divided into 07 zones and in each zone five locations are selected and from each location soil samples are collected, near roots where most of the microbial activity is concentrated. Soil samples (approximately 5g) are collected with clean, dry and sterile polythene bags along with sterile spatula. The collected samples are brought to the laboratory and preserved for further studies. The soil samples are collected from the month of January 2022 to March 2022 in Hindu college campus at various locations. The soil samples collected from seven different zones of Hindu college campus are mentioned in Table 1.

Table:-1 Soil collected from different zones of Hindu college

SL.NO	ZONE
1	Parking area
2	Main entrance
3	Tennis court
4	Cricket ground
5	Saraswathi Vanam
6	Flower garden
7	Nakshtra Vanam

Preparation of soil sample and microbial culture

In systematic screening process for isolation of fungus from07 soil samples are collected at different locations in Hindu college campus. Samples are collected near roots where most of the microbial activity is concentrated. 1gram of collected soil samples are diluted, with 09 ml of sterile distilled water. 0.1ml of suspension is added to sterile petri plates in triplicates containing sterile Potato Dextrose Agar (PDA) medium (fungal medium). The plates are incubated at temperature of 28 ^{0}C for 5 -7 days. A greater number of species are isolated and most of the fungus appear as heavily sporulated on PDA (potato dextrose plates) plates. Pure culturing is done using test tubes containing fresh agar slants of PDA medium. The test tubes are stored in refrigerator. When inoculums are transferred into petri plates containing fungal nutrient media cells are not separated from each other. Therefore, mixed colonies are developed. Hence isolation of pure culture from mixed colonies is rather difficult. Therefore, spread plate technique is employed for pure culturing.

Potato dextrose agar [PDA] medium

Potato dextrose agar [PDA] medium is used for the cultivation of fungi. Potato dextrose agar is a general medium for yeasts and moulds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products and testing cosmetics. PDA can be used for growing clinically significant yeasts and moulds. The nutritionally rich base [potato infusion] encourages mould sporulation and pigment production in some dermatophytes.

Potato dextrose agar is composed of dehydrated potato infusion and dextrose that encourage luxuriant fungal growth. Agar is added as the solidifying agent. In general, specific amount of sterile tartaric acid [10%] is used to lower the pH of the PDA medium to 3.5 ± 0.1, therefore inhibiting bacterial growth. Chloramphenicol acts as a selective agent to inhibit bacterial over growth of competing microbes from mixed specimens, while permitting the selective isolation of fungi.

USES OF POTATO DEXTROSE AGAR [PDA]

- Potato dextrose agar is used for the detection of yeasts and moulds in dairy products and prepared foods.
- Potato dextrose agar with chloramphenicol is recommended for the selective cultivation of fungi from mixed samples.
- It may also be used for cultivation of yeasts and moulds from clinical specimens.

COMPOSITION OF POTATO DEXTROSE AGAR [PDA]

Potato infusion	- 200gm
Dextrose	- 20gm
Agar	- 20gm
Distilled water	- 1liter
pH	- 5.6 ± 0.2

FIGURE-1: COLLECTION OF SOIL SAMPLE

FIGURE - 2:-MEDIUM PREPARATION

Staining of fungi

The fungal propagules are either hyaline (colourless) or different colours. The hyaline mycelia / spores / conidia and cytoplasm can be stained by using Lactophenol (in unavailability, Crystal violet). Cotton blue stains cytoplasm and results in light blue background. Lactophenol acts as a cleaning agent. The stained specimens are observed under the light microscope for identification and microphotograph is taken under 10x × 45x magnification.

FIGURE – 3:-STAINING

The effect of Lactophenol Cotton Blue

Lactophenol Cotton Blue (LPCB) is a stain used for making semipermanent microscopic preparation of fungi. The LPCB stain has three following components.

- Phenol: kills any organism.
- Lactic acid: preserves fungal structures
- Cotton blue: stains the chitin and cellulose of the fungal cell wall intensely blue.

Identification of Fungi

The isolated fungus is identified to the genus level and to the species when possible on the basis of macro morphological (the colonies are examined for slow or rapid growth) topographical (flat, heaped, regularly or irregularly folded), textural (yeast like, powdery, granular, velvety or cottony), surface pigmentation and reverse pigmentation and micro morphological (Hyphae, macro conidia, micro conidia, chlamydospores and other special fungal structure) characteristics using suitable media, slide cultures and the most updated keys for identifications. The identified fungus is confirmed with microbial expert.

FIGURE–4:-AUTOCLAVING

FIGURE- 5:-PDA MEDIUM PLANTING AND SAMPLING

CHAPTER – 03

RESULT AND DISCUSSION

RESULTS AND DISCUSSION

Our study is aimed for the isolation of soil fungi from different locations of Hindu college campus, Guntur and grown invitro during the period of January 2022 to March 2022. Isolated fungi is identified with help of standard books. In our investigation, isolates are obtained from the soil samples. From the fungal isolates the species belonging to the genera *Aspergillus* and *Mucor* are dominant. The identified soil fungus is *Aspergillus niger, Aspergillus fumigatus, Penicillium chrysogenum, Colletotrichum gloeosporioides, Mucor species Rhizopus stolonifer, Rhizopus oryzae, Cladophialophora species* etc. Among the identified species the *Keratinophilic* fungi *Aspergillus niger* are found in maximum numbers in the campus and followed by Mucor species. Soil is a multi-layered surface complex of mineral and organic constituents are present in solid, liquid and gaseous states. The mineral portion of soil results from the actions of weathering and erosion. Broad soil type and slit or clay is defined as largest to smallest of particle size. These particles pack loosely, and plant roots are particular habitats for microorganisms, often in biofilms. Soil also contains plants, animal carcasses and man-made materials. A gram of garden soil may contain around one million fungi such as yeasts, and moulds. Fungi have no chlorophyll and are not able to photosynthesize. They cannot use atmospheric carbon dioxide as a source of carbon; therefore, they are chemo heterotrophic (meaning that, like animals they require a chemical source of energy rather than being able to use light as an energy source as well as organic substrates to get carbon for growth and development). Many fungi are parasitic, often causing disease to their living host plant although some have beneficial relationship with plants. In terms of soil and humus creation, the most important fungi tend to be saprotrophic (live on dead or decaying organic matter, thus breaking it down and converting it into forms that are available to the higher plants). Fungi is present where adequate moisture, temperature and organic substrates are available. Although we normally think of fungi as growing in warm moist forest, many species occur in habitats that are cold, periodically arid, or otherwise seemingly inhospitable. It is important to recognize the conditions

for growth and reproduction vary widely with fungal species. Diversity of most groups of fungi tend to increase in tropical regions, but detailed studies are only in their infancy. From the mycelia, the fungi is able to throw its fruiting, the visible part above the soils (e.g. mushrooms, toadstools, puffballs), may contain millions of spores.

When the fruiting body bursts, these spores are dispersed through the air to settle in fresh environments and are able to lie dormant for up to years until the right conditions for their activations arise or the right food is available. The soil moisture has a direct effect on the population of fungi positively. Hence, at higher moisture the tolerance and colonization is badly affected.

The soil samples are collected from the month of January 2022 to March 2022 in Hindu college campus at various locations. In the present study, the isolated fungi are identified on the basis of cultural, microscopic and morphological characteristics. It is known that Potato dextrose agar (PDA) is the general medium most widely used in the isolation of fungi, having a complete nutritional basis. This is probably the reason why colony development is faster with respect to other media. In our experiment we used Potato Dextrose Agar (PDA) medium in which the growth of the fungi are maximum. Fungal population dominate the soil food web (although they are less in number than the bacteria). Fungi have 40–55% carbon using efficiency so they store and recycle more carbon (C) compared to bacteria.

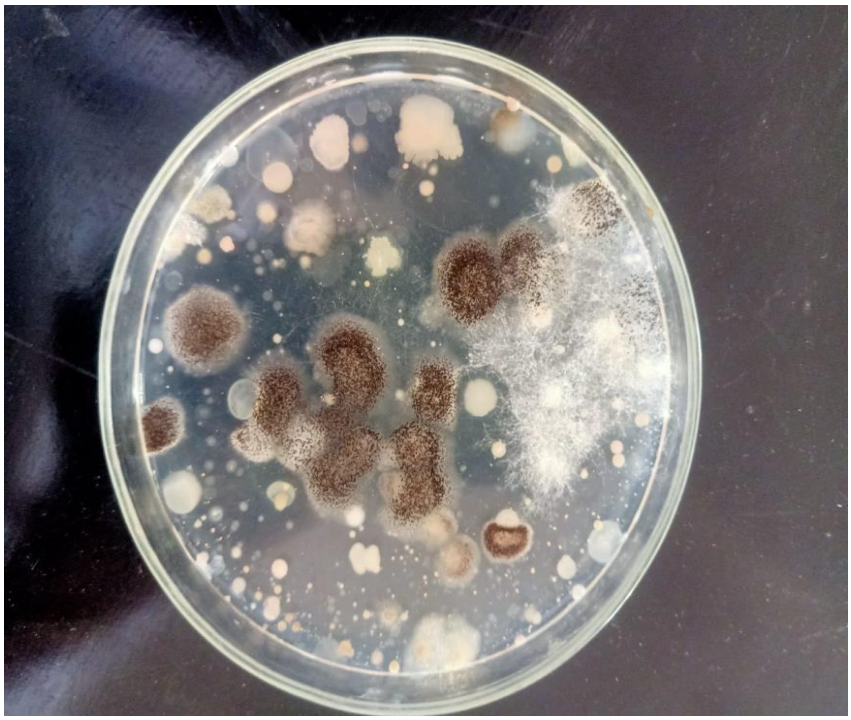

FIGURE – 6 :-*Aspergillus* COLONIES

FIGURE-7:-OBSERVING *Aspergillus niger* UNDER MICROSCOPE

FIGURE-8:-*Aspergillus niger* UNDER MICROSCOPE

CHAPTER – 04

APPLICATIONS

APPLICATIONS

- *Aspergillus niger* is cultured for the industrial production process of various substances. We use various strains of *Aspergillus niger* in the industrial preparation of the citric acid and gluconic acid. These are acceptable for day-to-day intake by the World Health Organization. *Aspergillus niger* fermentation is GRAS (Generally Recognized As Safe) by the US food and drug administration under the Federal Food, Drug, and cosmetic act.

- *Aspergillus niger* inoculation increases seedling root growth of lettuce, pepper, scarlet eggplant, watermelon, and tomato.

- Production of many useful enzymes occurs with the use of industrial fermentation of *Aspergillus niger* (in food industry)

- *Aspergillus niger* is also useful for the extraction of the enzyme, glucose oxidase, we use it in the design of glucose biosensors.

- *Aspergillus niger*, is the source of anti-cancer compounds such as Nigerapyrone -B, Asnipyrone- A, Nigerasterol- A and L- asparginase (Aspergillus Applications in Cancer Research V.K. Nadumane, B. Gajaraj).

CHAPTER – 05

REFERENCES

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1. Ratna Kumar, P.K., Hemanth, G.P. Shiny Niharika and Samuel, K. Kolli. 2015. Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal Srikakulam District. Int. J. Adv. Pharmacol., l4(2): 484-490
2. Maheshwari, R., Bhardwaj, G. and Bhat, M.K. 2000. Thermophilic fungi: Their physiology and enzymes, Microbiology Mol. Biol. Rev., 63: 461-488.
3. Mycology Guidebook Committee, Mycological Society of America, Stevens, R.B., ed. 1981. Mycology Guidebook. University of Washington Press, Seattle.
4. Karthikeyan, P., Kanimozhi, K., Senthilkumar, G., Panneerselvam, A. and Ashok, G. 2014. Optimization of Enzyme Production in Trichodermaviride using Carbon and Nitrogen source. Int. J. Curr. Microbiol. App. Sci., 3(1): 88-95.
5. Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.M., Lee, H. and Trevor, J.T. 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods, 58:169–188.
6. Text book of Microbiology, Surinder Kumar, First edition
7. Seifert, K.A. 1992. Isolation of filamentous fungi, In: D.P. Labeda (ed.) Isolation of Biotechnological Organisms from Nature. Mc-Graw-Hill, New York. pp. 21-51.
8. Aspergillus Applications in Cancer Research, V.K. Nadumane, B. Gajaraj, in New and Future Developments in Microbial Biotechnology and Bioengineering, 2016
9. Medical Microbiology by David Greenwood and Mike Barer, 17th edition.