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PROJECT

ISOLATION AND IDENTIFICATION OF *PENICILLIUM* 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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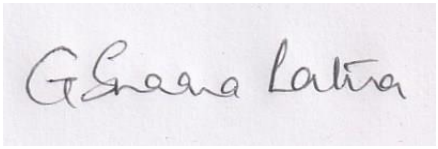
CERTIFICATE

This is to certify that this thesis entitled, **"ISOLATION AND IDENTIFICATION OF *PENICILLIUM* SPECIES"** is a *bona fide* Project work done by **Students of Biomedical sciences**, **Hindu College,** under my guidance. 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.



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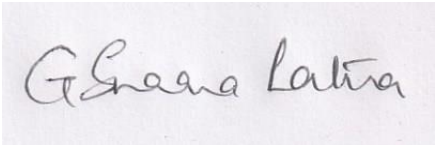

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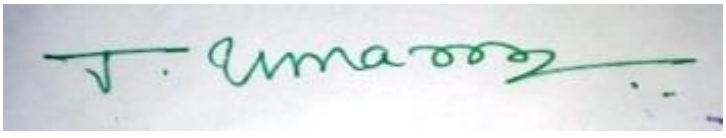

Dr. J. UMA MAHESWARA RAO

DECLARATION

We hereby declare that the thesis entitled "ISOLATION AND IDENTIFICATION OF Penicillium species 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. Swarna Latha, 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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ABSTRACT

The investigation is aimed to find out the fungal diversity, also to isolate and identify the *Penicillium* species in the soil samples collected in the Hindu college campus, Guntur. For isolation and identification of *Penicillium*, 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* 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, mycelium, spores, isolation, staining, PDA medium, Penicillium.

CHAPTER – 01

INTRODUCTION

INTRODUCTION

The soil serves as a reservoir for many microbial communities of plants and herbs which can produce, CO_2 and nitrogen. 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 are present for short periods in a limited zone. 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, bio- remediation, natural cycling, as bio-fertilizers 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. 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 a protective health to supply 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 with microbial expert (Karthikeyan - Optimization of Enzyme Production in *Trichoderma viridae)*.

OBJECTIVES

- To determine variety of fungal organisms from soil.
- Identify antibiotic producing organism from soil (in particular, penicillin producing).

SURVEY OF LITERATURE

Mycology Guidebook was presented by Mycology Guidebook Committee in 1981. Seifert, K.A in 1992 worked on isolation of filamentous Fungi. Maheswari etal worked on Thermophilic fungi: Their physiology and enzymes in 2000. Krik et al in 2004 contributed for methods of studying soil microbial diversity. Mycology in Textbook of Microbiology was published by Ananthanarayana in the year 2005. Optimization of Enzyme Production in Trichoderma viridae using Carbon and Nitrogen source is published by Karthikeya et al in 2014. Ratna Kumar et al worked Isolation and identification of soil mycoflora in agriculturalfields in 2015.

CHAPTER – 02

DISCOVERY, STRUCTURE AND MECHANISM OF PENICILLIN

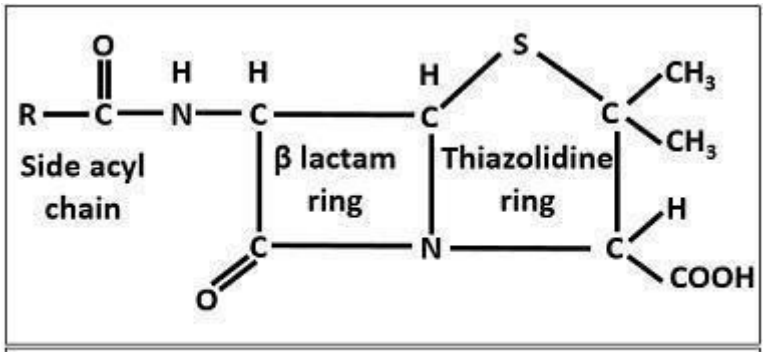
DISCOVERY OF PENICILLIN

The first of the modern antibiotics, and still one of the most useful, penicillin is produced by *Penicillium notatum, Penicillium chrysogenum*, and by other species of moulds. The first penicillin was isolated by Flemming in 1929, when he found it as a contaminanton a culture plate. Florey and his associates at Oxford university isolated the active ingredient and used the crude material clinically in 1940.

STRUCTURE AND CHARACTERISTICS OF PENICILLIN

Most penicillins are derivatives of 6-aminopenicillanic acid and differ from one another only with respect to the side chain attached to its amino group. The most crucial feature of the molecule is the β-lactam ring, which appears to be essential for activity. Penicillinase, the enzyme synthesized by many penicillin-resistant bacteria that destroys the penicillin activity by hydrolyzing a bond in the β-lactam ring.

Several chemically different penicillins are produced bybiosynthesis in a single fermentation. These differ from each other in various ways. Penicillin-G or benzylpenicillin, the first antibiotic to be widely used in medicine, is effective against *Gonococci, Meningococci,* and several gram-positive pathogens such as *Streptococci* and *Staphylococci*. But it must be administered parenterally because it is destroyed by stomach acid (HCl). Penicillin-*V* is similar to penicillin-*G* but it is more acid resistant and can be given orally. Ampicillin can be administered orally because it is an acid stable and has a broader spectrum of activity as it is effective against gram-negative bacteria such as *Hemophilus, Salmonella and Shigella*. Carbenicillin is acid stable and active against gram-negative bacteria like *Pseudomonas and Proteus*. It is not well absorbed by small intestine. Ticarcillin is also a broad spectrum and particularly potent *against Pseudomonas and Proteus*. Methicillin is acid- labile, penicillinase- resistant; and less active than Penicillin-G.



STRUCTURE OF PENICILLIN

MECHANISM OF ACTION OF PENICILLIN

The mechanism of action of penicillins is still not completely known. It has been proposed that penicillins inhibit the enzyme catalyzing the transpeptidation reaction, which results in the blockage of the synthesis of a complete, fully cross-linked peptidoglycan and leads to osmotic lysis. The mechanism is consistent with the observation that penicillins act only on growing bacteria that are synthesizing new peptidoglycan, and the fully grown bacterial cells remain unaffected. However, more recently it has been discovered that penicillins bind to several penicillin-binding proteins and may destroy bacteria by activating their own autolytic enzymes. There now is some evidence that penicillins kill bacteria even in the absence of autolysins or murein hydrolyses. Lysis could occur after bacterial viability has alreadybeen lost. Penicillin may stimulate special proteins called bacterial holins to form holes or lesionsin the plasma membrane. This could lead directly to membrane leakage and death. Murein hydrolyses through the holes, disrupt the peptidoglycan and lyse the cell. In the light of above, the mechanism of penicillin action appears more complex than previously imagined and needs thorough investigation.

Although penicillins are rarely toxic in human patients, it may give rise to sensitivity reactions which vary from a mild skin reaction to severe anaphylaxis.Therefore, patients should be questioned about penicillin allergies before treatment.

CHAPTER – 03

MATERIALS AND METHODS

MATERIALS AND METHODS

The Hindu college campus is divided into 7 zones and in each zone five locations are selected and from each location soil samples are collected near roots where, most ofthe 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 thelaboratory and preserved for further studies. The soil samples are collected from the month of January 2022 to March 2022 in Hindu college at various locations. The soil samples collected fromseven 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	Main entrance
2	Cricket ground
3	Tennis court
4	Parking area
5	Saraswathi Vanam
6	Nakshatra Vanam
7	Canteen

Preparation of soil sample and microbial culture

In systematic screening process for isolation of fungus from 07 soil samples are collected at different locations in Hindu college campus. Samples are collectednear 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 platesin 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 sporulated on PDA plates. Pure culturing is done using test tube containing fresh agar slants of PDA medium. The test tubes are stored in refrigerator. When inoculums are transferred into petri plates containing nutrient media cells are not separated from each other. Therefore, mixed colonies are developed. Hence isolation of pure culture from mixedcolonies is rather difficult. Therefore, spread plate technique is employed for pure culturing.

FIGURE: 1 - COLLECTION OF SAMPLES

Potato dextrose agar [PDA]

Potato dextrose agar [PDA] 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 antibioticsto 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, specified amount of sterile tartaric acid [10%] is used to lower the pH of the medium to
3.5 ± 0.1, inhibiting bacterial growth. Chloramphenicol acts as a selective agent to inhibit bacterialover growth of competing microbes from mixed specimens, while permitting the selective isolationof 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: 2 - SERIAL DILUTION

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 semi-permanent 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 fungi is identified to the genus level and to the species when possible on the basis of macro morphological (the colonies were examined for slow or for 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: PDA MEDIUM PLATING AND SAMPLING

FIGURE – 5: INCUBATION

CHAPTER – 04

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 the 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 namely *Penicillium chrysogenum, Penicillium notatum, Penicillium oxalicum, Penicillium funicolosum*. The mineral portion of soil results from the actions of weathering and erosion. Broad soil type and, slit or clayis 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 organic component of soil, formed by the decomposition of leaves and other plant material by soilmicrobes] the most important fungi tend to be saprotrophic (live on dead or decaying organic matter, thus breaking it down and converting it to forms that are available to the higher plants) . Fungi are 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 fungus is able to throw its fruiting, the visible part above the soil (e.g., mushrooms, toadstools, puffballs), which 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 on of cultural, microscopic and morphological characteristics. It is known that Potato dextrose agar (PDA) medium (fungal medium) 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. Earlierwork reported that maximum growth of fungi, potato dextrose is most favorable. In our experiment we used Potato Dextrose Agar (PDA) medium, the growth of the fungi are maximum. Fungal population dominates 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: *Penicillium* COLONY

FIGURE-7: OBSERVING *Penicillium species* UNDER BINOCULAR MICROSCOPE

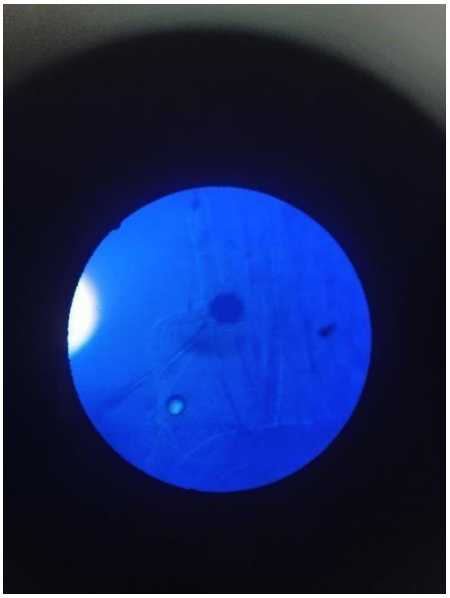


FIGURE – 8 : *Penicillium* UNDER MICROSCOPE

CHAPTER – 05

APPLICATIONS

APPLICATIONS

Penicillium refers to one of the most important antibiotics, which requires fungal strains (*Penicillium notatum* or *Penicillium chrysogenum*). It acts as a cell wall destroyer of bacteria. Nowadays, many derivatives of penicillin are available to kill the majority of bacteria than penicillin and many other derivatives can be synthesized. The structure of penicillin differs in a **side chain** that is linked to the amino group. The β-lactam ring is responsible for **antimicrobial activity** and useful to destroy the cell wall synthesis of gram-positive bacteria, especially *Staphylococcus, Streptococcus species and many other species*.

- Penicillin V potassium used in treating different kinds of infections like pneumonia, respiratory tract and throat infections.
- Penicillin, methicillin, ampicillin, penicillin V, penicillin G and some other antibiotics are synthesized from penicillium Species.
- Penicillin G potassium used in prevention of rheumatic fever.
- Sometimes, penicillin is used in treating anthrax infections on the skin.
- Penicillium species are very significant economically. They produce many products such as antibiotics e.g., methicillin.
- Some of the Penicillium species are widely used in the production of different types of cheese, e.g., blue cheese.
- Numerous Penicillium species are used in the production of organic acids including the citric acid, gluconic acid, tartaric acid and also some enzymes like amylases, proteases, cellulase, lipase and pectinase.
- Penicillin from penicillium which represses the growth of Gram - positive bacteria.
- Penicillin G is administered by intramuscular injection and Penicillin V are given orally to treat various infections.
- Penicillium species are also made use in the production of antifungal drug (useful in treating actinomycosis) and tumor suppressing compounds.

CHAPTER – 06

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