TELANGANA TRIBAL WELFARE RESIDENTIAL DEGREE COLLEGE (GIRLS)ASIFABAD

Affiliated to Kakatiya University

DEPARTMENT OF MICROBIOLOGY

A REPORT ON STUDENT STUDY PROJECT

TELANGANA TRIBAL WELFARE RESIDENTIAL DEGREE COLLEGE (GIRLS)ASIFABAD

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DEPARTMENT OF MICROBIOLOGY

A REPORT ON STUDENT STUDY PROJECT

ACADEMIC YEAR 2020-2021

ISOLATION AND IDENTIFICATION OF AIR MICROFLORA IN MICROBIOLOGY LABORATORY

Submitted By

1.CH.Akshitha BSc.MB.Z.C III year

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Supervised By : P.Priny Priyanka

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CERTIFICATE

This is to certify that the Study Project,titled "ISOLATION AND IDENTIFICATION OF AIR MICROFLORA IN MICROBIOLOGY LABORATORY" for the academic year 2020-2021 has been successfully completed by CH.Akshitha , R.Sreeja ,J.Akshitha, R.Chayawathi , J.Rajitha , CH.Praveena of BSc.MB.Z.C III year for the fulfilment of Departmental Annual Curricular Plan.

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A REPORT

Title of the Project : Isolation and Identification of air microflora in Microbiology laboratory

Names of the students to whom the work assigned :

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ISOLATION AND IDENTIFICATION OF AIR MICROFLORA IN MICROBIOLOGY LABORATORY

INTRODUCTION

Gases, dust particles, water vapour and air contain microorganisms. There are vegetable cells and spores of bacteria, fungi and algae, viruses and protozoa cysts. Since air is often exposed to sunlight, it has a higher temperature and less moisture. So, if not protected from desiccation. Most of these microbial forms will die. Air serves as a transport or dispersal medium for microorganisms; they occur in relatively small numbers in air when compared with soil or water.

The microflora of air can be studied under two headings: outdoor and indoor microflora.

Outdoor Microflora: -The common genera of fungi found in outdoor air are clostridium and sporobolomyces.Besides these two genera *aspergillus, alternaria, phytophthora* and erysiphe are found. Some forms of yeasts like basidiospores, ascospores are also found. The fragments of mycelium and conidia molds along with bacterial flora like bacillus, clostridium, Sarnia, micrococcus, corynebacterium and achromobacter are widely found.

Indoor Microflora: - Commonly found microorganisms are fungi and bacteria. The comments genera of fungi are *penicillium, aspergillus and staphylococcus, bacillus* and *clostridium* genera of bacteria are found in indoor air in microbiology laboratory.

OBJECTIVES

This research work aimed at identifying the various microorganisms present in the air
The Specific objectives of the study include:

- To isolate the microorganisms present in the air .
- To identify the microorganisms .
- To create awareness on the diseases associated with the organisms present in the air.

METHODS

Some samples were collected from a microbiology laboratory. Those samples were obtained from surfaces of work benches, sinks, windows and doors in four laboratories using sterile cotton swab sticks. These swabs were inoculated on different Medias. Those were selective Medias like macconkey agar, enrichment media ,chocolate agar ,sabouraud dextrose agar. The plates were incubated at 37°c for 24 to 48 hrs. The pure culture was isolated and stored under temperature. The identification was done by gram stating and biochemical tests. Another method used to obtain the indoor micro flora in the microbiology laboratory was the open Petri dish method. The two types of media were sabouraud dextrose agar and pal sunflower seeds medium. The open culture plates were placed inside the microbiology laboratory for 5 to 10 minutes. These plates were then incubated for 24 to 48 hrs. To obtain growth.

DISCUSSION

The commonest microbes were found after both experiments: Bacteria: *Bacillus aureus, Bacillus subtilis, Salmonella typhi* .Commonly found bacteria of Genus Bacilli which are gram positive rod. They have the ability to form heat resistant endospores.

Fungi: Penicillium, aspergillus commonly found in indoor micro flora. *Aspergillus*, *Alternaria* were found in outdoor micro flora.

Yeasts: Basidiospores, ascospores, the fragments of mycelium and conidia molds were commonly found in microbiology laboratories.

CONCLUSION

Majority number of bacterial and fungal flora found. · These air microbes are capable of causing various infections like eye infection, food poisoning, skin lesions, and diarrhea. · Some of bacterias can cause conjunctivitis.

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A REPORT ON STUDENT STUDY PROJECT

ACADEMIC YEAR 2021-2022

ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH SPOILED TOMATOES

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CERTIFICATE

This is to certify that the Study Project,titled "ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH SPOILED TOMATOES "for the academic year 2021-2022 has been successfully completed by U.Pavithra, R.Susma bai, T.Srilatha, M.Madhuri, D.Akanksha , M.Sindhuja of BSc.MB.Z.C III year for the fulfilment of Departmental Annual Curricular Plan.

A REPORT

Title of the Project : Isolation and characterization of fungi associated with spoiled tomatoes

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ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH SPOILED TOMATOES

INTRODUCTION

Tomatoes (*Solanum lycopersicum*) is a perishable vegetable widely cultivated and consumed worldwide.Tomatoes is an annual plant which belongs to the Solanaceae family. Tomatoes are essential mainly for its dietary needs and can be consumed in diverse ways. It can be cooked as a vegetable, as an ingredient in many dishes and sauces; in the making of stew, fruit and can be eaten raw in salad. It is rich in nutrients, vitamin, dietary They are good source of natural antioxidant which include carotenoids, vitamin phenolic compound Flavonoid which have shown to eliminate free radicals.Tomatoes has a much lower sugar content than other fruits and is therefore not as sweet and their low pH values make them particularly desirable to fungal decay. Pathogenic fungi, such as *Aspergillus, Fusarium, Mucor, Penicillium*, *Rhizopus*, *cladosporium* species have been implicated in some crop spoilage . Fungi contamination in many Agricultural Products including tomatoes starts in the field.

Both biological and physical damage during the harvest and transportation phases, coupled with large amount of water and soft endocarp makes tomatoes more susceptible to spoilage by fungi .Spoilage of tomatoes are those adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors. These may be changes in taste, smell, appearance or texture of the fruit. Spoiled tomatoes can lead to food borne illness and damage the health of the consumers. Proper handling, transportation and thorough washing with clean or chlorinated water will go a long way in reducing the risk of tomato spoilage by fungi. Therefore, there is a need to assess those fungi associated with spoiled tomato in order to solve problem of food borne illness which can be pathogenic to human.Routine microbiological examination of tomatoes is very crucial as it contributes to a large extent to economic development.

OBJECTIVES

This research work aimed at identifying the various fungi organisms associated with the spoilage of fresh tomatoes.
The Specific objectives of the study include:

- To isolate fungi associated with spoiled tomatoes .
- To determine the prevalence of fungal infection on spoiled tomatoes.
- To identify and characterize different fungal infections associated with spoiled tomatoes.
- To create public health awareness about the excess of fungal in spoiled tomatoes.

MATERIALS AND METHODS

Unwashed and unprocessed spoiled tomatoes were collected from the kitchen at Tribal welfare residential degree college,Asifabad and brought to the laboratory for further analysis.

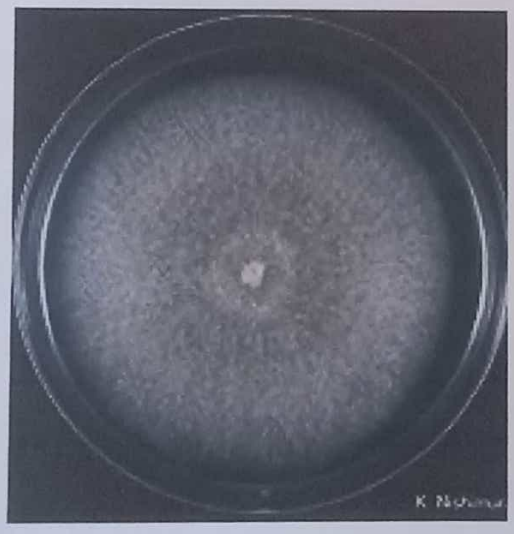
ISOLATION OF FUNGI

The spoiled tomatoes were surface sterilized with the help of 1% $Ca(ocl_2)$ and were tissues adjacent were cut with the help of razor and inoculated into PDA media which are supplemented with penicillin and streptomycin .The plates were incubated for a week at 28°C.Fungi growing out from the the tissues were identified following standard identification manual.

Occurrence of Fungi from spoiled Tomatoes

Fungi isolate	**Media**	**Colony Morphology**
Mucor spp	PDA	Blue – Black mold,thread like mycelium
Rhizopus spp	PDA	Black mold like appearance
Fusarium spp	PDA	White cottony type appearance

PDA- Potato Dextrose Agar



Fusarium colony

Rhizopus spps

RESULTS AND DISCUSSION

From the present study,3 isolates *Mucor spp, Rhizopus spp, Fusarium spp* were isolated from the spoiled tomatoes .The fungal isolates were identified on the basis of morphology and colony Characters .

ACKNOWLEDGEMENT

We are very much Thankful to the Department of Microbiology for providing basic requirements for conducting the project work.

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DEPARTMENT OF MICROBIOLOGY

A REPORT ON STUDENT STUDY PROJECT

ACADEMIC YEAR 2022-2023

BACTERIOLOGICAL EXAMINATION OF WATER BY MPN METHOD

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This is to certify that the Study Project,titled "BACTERIOLOGICAL EXAMINATION OF WATER BY MPN METHOD"for the academic year 2022-2023 has been successfully completed by R.Priyanka ,K.Vaishnavi ,HK.Geethanjali, S.Hamksakala , B.Sangeetha , A.Chitti of BSc.MB.Z.C III year for the fulfilment of Departmental Annual Curricular Plan.

A REPORT

Title of the Project : BACTERIOLOGICAL EXAMINATION OF WATER BY MPN METHOD

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ABSTRACT :

The aim of this study is to analyze the microbiological quality of the available drinking water collected directly from various sources, supply outlets, as well as drinking water kept/stored in various containers or storage pots in order to check the drinking water quality and evaluate the awareness of people for maintaining cleanness and hygiene conditions for storage of drinking water.

Hence, the water samples were collected and analyzed for Most Probable Number (MPN) as well as prevalence of various water borne pathogens, indicators organisms to check for presence of fecal contaminants using various media.

Present study indicates that water testing would ensure the supply and availability of contamination – free drinking water and awareness among the people towards sanitation and hygienic conditions for storage of drinking water is needed to keep away the use of contaminated water.

INTRODUCTION :

Drinking water must be virtually acceptable, clear and colourless and without a disagreeable taste and odour .Bacteriological analysis of water supplies should be performed at regular intervals, and not be a random exercise. Frequency of such analysis may range from daily to monthly sampling , depending on the size of the populations served.Drinking water should be free of any pathogenic microorganisms. Ideally , therefore tests should be aimed at detecting such pathogens. However, These are generally present in such small members that they escape detection.

The practice, therefore, is to test for fecal pollution ; if fecal pollution is detected in a water sample, it is inferred that the water from which the sample was drawn may be harboring enteric pathogens. The primary test employed as an indicator of fecal pollution of water is the presence of coliform bacteria because they are invariably present in the feces of human beings and other warm –blooded animals in large numbers and can be easily detected even in high dilutions.

Although coli form bacteria are not exclusively of fecal origin , they serve as presumptive evidence of the presence of bacteria of fecal origin, to be confirmed by the detection of thermo tolerant Escherichia coli, which provides definite proof of fecal pollution.

OBJECTIVES :

- To enumerate the number of bacteria present in drinking water by MPN method.
- To identify the bacteria present in the drinking water.

MATERIALS & METHODS

STUDY AREA

Water samples were collected from five sampling points around Asifabad.

Both raw and water (drinking) from a ground source which is distributed in the city.These sampling points were chosen for study because water is used for human consumption and for agricultural and industrial purpose.It is therefore important to investigate the microbial quality of water at these points.

MPN METHOD FOR ENUMERATION OF BACTERIA

• Accurate and reliable laboratory methods and analyses are a prerequisite to ensure potable drinking water characteristics and to provide an abatement strategy to supply containment free water.

The samples are collected for testing from different areas of ASIFABAD region. The water samples have to be collected in sterile sampling bottles.

• Biological analysis of drinking water samples has to be done within 48 hours of collection.

• Drinking water samples should be analyzed for Most Probable Number (MPN) using the method given by MC crady in 1915 as well as prevalence of various water borne pathogens and indicator organisms examined using specialized media and biochemical tests for identification of bacteria. MPN is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid brothin ten fold dilutions.It is commonly used in estimating microbial populations in soil,water ,agricultural products and is particularly useful with samples that contain particulate material interferes with plate count enumeration methods.MPN is most commonly applied for quality testing of water i.e.to ensure whether the water is safe or not in terms of bacteria present in it.A group of bacteria commonly referred as fecal coliforms acts as an indicator for fecal contamination of water. The presence of very few fecal coliform bacteria would indicate that a water probably contains no disease causing organisms,while the presence of a large number of fecal coliform bacteria would indicate a very high probability that the water could contain disease producing organisms making the water unsafe for consumption.

PRINCIPLE :

Water to be tested is diluted serially and inoculated in lactose broth, Coliforms if present in water utilize the lactose present in the medium to produce acid and gas. The presence of acid is indicated by colour change of the medium and the presence of gas is diluted as gas bubbles collected in the inverted durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes that give positive results (i.e both colour and gas production) and comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables.

MATERIALS REQUIRED:

- Petri Dishes
- Test tubes
- Sampling bottle (sterile)
- MacConkey or Lactose broth
- EMB agar, Nutrient agar
- Durham's tube
- Test tube stand
- Water sample

PROCEDURE :

MPN method is performed in 3 steps

1. Presumptive test
2. Confirmation test
3. Completed test

PRESUMPTIVE TEST :

The presumptive test is a screening test to sample for the presence of coliform organisms. If the presumptive test is negative, no further testing is performed and the water source is considered microbiologically safe. If however, any tube in the series shows acid and gas ,the water is considered. Unsafe and the confirmed test is performed on the tube displaying positive reactions.

Prepare a macconkey purple media of single and double strength in test tubes with Durham's tube and autoclave it.Take three sets of test tubes containing five tubes in each set ,one set with 10 ml of double strength (DS)other two containing 10 ml of single strength.Using sterile pipettes, transfer 10 ml of water to each of DSbroth tubes. Transfer 1 ml of water sample to each of tubes of one set of SS broth and transfer 0.1ml water to fine tubes of remaining last set of SS broth tubes.Incubated the tubes at 37C for 24hrsAfter incubation, Observed the gas production in Durham's tube and color change of the media.

CONFIRMED TEST:

Confirmed test is done to determine that coliforms are of fecal origin or not and they are E.coli or not.Take the positive tube from the Presumptive test and using EMB in duplicate.Incubate one plate at 37c for 24 hours and another at 44.5for 24 hours

Look for typical colonies in the media ,blue black with green metallic sheen colonies are of E.coli in EMB agar.

COMPLETED TEST:

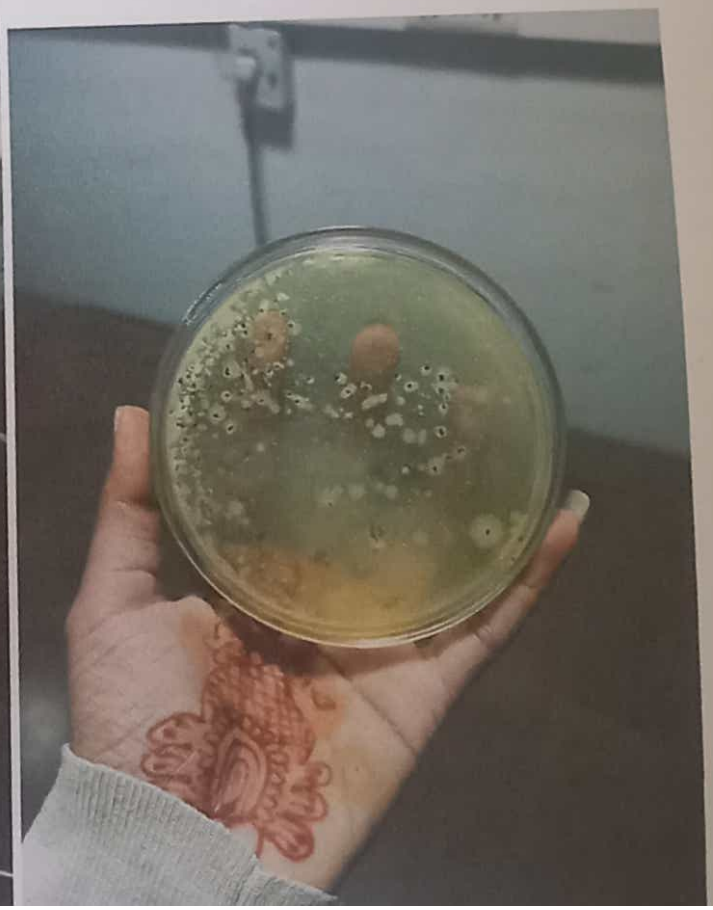
Subculture typical colonies in lactose containing medium and incubated at 37°c and 44.5c.

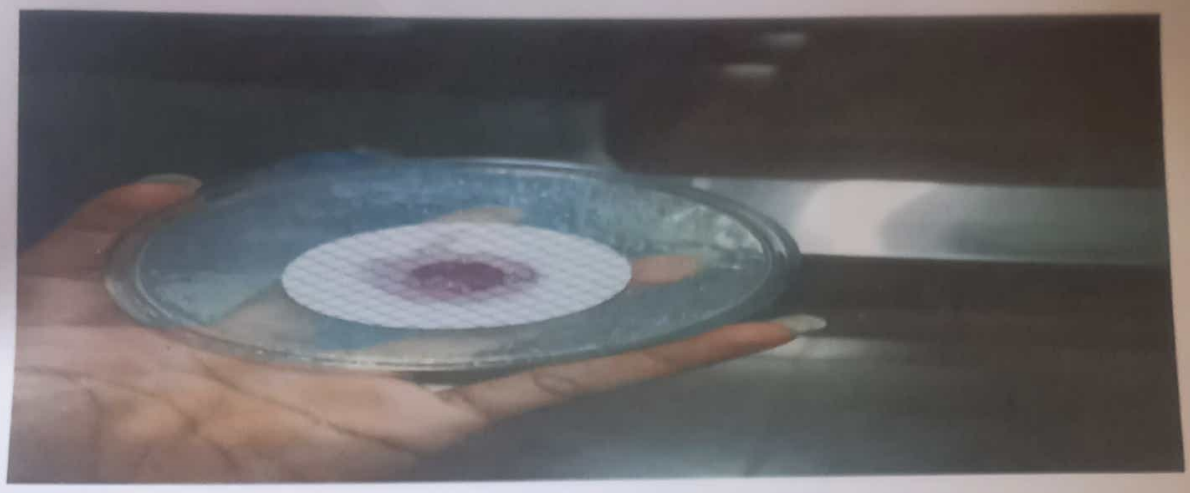
Presence of E.coli is confirmed by the production of gas at 44.5c.Inoculate the colony in a tube of lactose broth with Durham's tube.Subculture the colony on nutrient agar plate .This subculture is considered optimal. Incubate the broth cultures at 37c and 44.5c and nutrient agar at 37c. Examine for acid and gas production in lactose broth .The nutrient agar is used for Gram staining and for IMVIC test.

ADVANTAGES OF MPN METHOD

- Ease of interpretation , either by observation or gas emission.
- Sample toxins are diluted.
- Effective method of analyzing highly turbid samples such as

sediments, sludge, mud etc.





RESULTS AND DISCUSSION

Among all the five samples,the samples in which raw water is taken the positive result in presumptive test and the samples collected from drinking water has showed negative for Presumptive test.The samples with positive result in presumptive test is tested for further confirmatory and completed test .All the samples with raw water have shown positive for confirmatory and completed test.

WHO RECOMMENDED DRINKING WATER STANDARDS:

PARAMETER	UNIT	LIMIT
Calcium	Mgca/l	200.0
chlorides	Mgcl/l	250.0
Fluorides	Mgf/l	1.5
Nitrates	NO3/l	10.0
pH		9.2
Total hardness	Mg/l	500
Alkalinity	Mg/l	500
MICROBIOLOGICAL		
PARAMETERS		
Total bacteria	Count/ml	100
Coliform	Count/100ml	0
E.coli	Count/100ml	0

The above mentioned standards have to be maintained for safe consumption of drinking water.

CONCLUSION:

It is important to assess the quality of water which is being used for consumption whether it is actually reliable and safe for health of the consumers and use with no risk of adverse health effects.Routine basic microbiological analysis of drinking water should be carried out by assessing the presence of E.coli by the culture methods.Microbiological control of drinking water should be the norm everywhere.