



Isolation and Identification of Fungi from Spoiled food samples and testing their Antibiotic efficacy

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Abstract:

Spoilage is the process in which food deteriorates to the point where in it is not edible to humans. Microorganisms are mainly responsible for food spoilage which includes bacteria and fungi. A study was carried out on the fruits that were spoiled due to the fungal infection. *Rhizopus*, *Trichoderma*, *Penicillium* and *Aspergillus* were isolated from these foods and were cultured. As fungal species are well known for their antibacterial nature the focus of the work was to use these fungi for the production of antibiotics that are of huge industrial importance. Pure cultures of various bacterial pathogens like *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Edwardella* were obtained and were tested for their sensitivity towards the identified fungus using kirby bauer method. The sensitivity test results showed that several fungi isolated from the spoiled food had the potential to produce the antibiotics that could inhibit the growth of the human bacterial pathogens. These results can be employed for the industrial scale production of antibiotics from fungus and the use of spoiled food substances as the source for the isolation of useful Fungus. The secondary metabolite extract of *Trichoderma* showed good zones of inhibition against the test bacterial samples with the range of 24mm, 28mm and 9mm against *Staphylococcus aureus*, *Streptococcus agalactiae* and *Edwardella* respectively. *Rhizopus* could not exhibit the antibacterial activity against the test bacteria. The metabolites of *Penicillium* showed the zones ranging from 17mm and 7mm against *Streptococcus* and *agalactiae* respectively. *Aspergillus* metabolites exhibited the inhibition zones ranging 14mm and 18mm against *Pseudomonas* and *Edwardella* respectively. From the results it can be concluded that the spoiled food can be used as a source for the isolation of the fungal species capable of producing potential antibiotics.

Keywords: Antibacterial, *Rhizopus*, *Trichoderma*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Edwardella*, *Pseudomonas*, Sensitivity Test

Introduction:

Food microbiology is the study of the microorganisms that inhabit, create, or contaminate food. In food microbiology one of the major importance is the study of microorganisms causing food spoilage ^[1]. Food borne pathogens are the leading causes of illness and death in undeveloped countries, killing approximately 1.8 million people annually ^[2]. In developed countries, food borne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars in medical care and lost productivity ^[3]. New food borne pathogens and food borne diseases are likely to emerge, driven by factors such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status. There are growing concerns that terrorists could use pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth ^[4]. Food contamination and spoilage is most commonly seen, which can give rise to many health hazards. Thus, to overcome these problems the present study was undertaken. The main principle behind the focus of the study is to analyze various fungus infected fruits and to check the antimicrobial activity by extracting fungal secondary metabolites. The study is to find out whether the fungal metabolites have the capability to inhibit bacterial pathogens. Human pathogens that cause food born infections or food poisoning can be prevented by the use of these antibiotics or their modified forms either as food preservatives or as a normal antibiotic to cure food borne bacteria infections.

Materials and methods:

For the isolation of human pathogenic bacteria samples were collected from throat swab, pimples, rashes and urine. Methods used for identification of

bacteria were Gram's staining and Bio-chemical tests as per Bergey's Manual.

Isolation of fungal cultures:

Six fruit samples infected with fungus were collected (jackfruit, papaya, sugarcane, coconut, banana and corn) from different regions of Hyderabad, into sterile polythene bags and brought to laboratory. Fungus was cultured on Czapekdox agar medium and identified by staining them with lacto phenol cotton blue.

Preparation of fungal extracts:

The identified fungus were cultured in the Czapekdox broth and incubated for 5 days. After incubation the broth was observed. The broth should contain fungal mat like growth and the secondary metabolites produced in the 5 days of incubation. To separate the fungal mat and the spores the broth was filtered by Whatman's no: 1 filter paper and the solution was collected in falcon tubes and then centrifuged the extract for 5 minutes at 3000rpm. The supernatant was collected and preserved.

Identification of various compounds in the fungal extracts [qualitative analysis]

The fungal extracts were subjected to qualitative analysis for the identification of compounds following the methodology of ^[4] Harbone [1998] and Kokate [2001].

Test for alkaloids:

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solutions produce white yellowish precipitate with the addition of few drops of Mayer's reagent. Most of the alkaloids are precipitated from neutral or slightly acidic solution by Mayer reagent.

Test for glycosides:

To the extract in glacial acid 0.5 ml of acetic anhydride and few drops of ferric chloride were added. Then concentrated sulfuric acid was added slowly. The formation of reddish brown color at the junction of two layers and bluish green color in the upper layer would indicate the presence of Glycosides.

Test for terpenoids and steroids:

4mg of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulfuric acid was added slowly. The formation of red violet color is an indication for the presence of terpenoids and green bluish color for steroids.

Test for flavonoids:

4mg of extract was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution 5-6 drops of concentrated HCL was added and red color was observed for flavonoids and orange for flavones.

Test for tannins:

To 0.5ml of extract 1ml of water and 1-2 drops of ferric chloride solution were added. Blue color was observed for Gallic tannins and green black for catholic tannins ^[5] [Iyengar 1995].

Screening of the fungal extracts for anti-microbial activity ^[6]:

Muller-Hinton agar medium was prepared as per the composition and sterilized. The test organisms *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Edwardella* were spread plated on to the plates. Wells were punched with a sterile borer and were filled with 50µl of fungal extracts. The plates were incubated and the results were analyzed after 24hrs.

Results and Discussion:

Isolation of pathogenic bacteria from collected samples:

The collected samples like skin sample, Acne (pimples), urine and throat swab were used to isolate causative bacteria which are pathogenic. Nutrient agar medium was used as a nutritional source and spread plate method was used for initial isolation of organisms. The isolated colonies of the bacteria were observed on the plates which were further used for the isolation of bacteria.

Identification of bacteria:

Based on the results of Gram's staining and biochemical tests as per the Bergey's manual the isolated organisms were found to be *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus agalactiae* and

Edwardella whose sensitivity to the fungal extracts was later tested in the study.

Identification of fungus:

Based on the results of microscopic observation and Lacto phenol cotton blue staining the isolated organisms were found to be *Rizopus*, *Trichoderma*, *Pencillium* and *Aspergillus* respectively. These fungi were cultured on czapekdox broth and after incubation and separation of fungus the extract was analyze for the presence of different compounds followed by the antibacterial ability check using zones of inhibition.

Identification of compounds:

In alkaloids test, turbidity and yellow precipitate was not observed in any of the fungal extracts, indicating the presence of neutral compounds. In glycosides test the results observed were, *Trichoderma* – no formation of reddish brown, bluish green colouration at the junction. For the samples of *Rhizopus*, *Pencillium* and *Aspergillus* - colouration at the junction of the 2 layers were observed. In terpenoids and steroids test, red violet colour for terpenoids and green bluish colour for steroids was not observed. In flavonoids test, red or orange colour was not observed. In tannins test, blue colour for gallic tannins or green black for catholic tannins was not seen, hence the compounds are identified to be neutral compounds.

Antimicrobial test:

The zone of inhibition was measured and results were tabulated (**Table:1**)

Fungal extracts	Zone diameter of inhibition (mm)			
	<i>Pseudomonas</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i>	<i>Edwardella</i>
<i>Red rot of sugarcane</i>	18	--	26	25
<i>Trichoderma</i>	--	24	28	9
<i>Rhizopus</i>	--	--	--	--
<i>Penicillium</i>	--	--	17	7
<i>Aspergillus</i>	14	--	--	18

From the above table it can be inferred that except *Rhizopus* all the other fungal extracts were showing some or the other degree of bacterial inhibition to the tested strains.

From the present study it was concluded that the different fungi isolated from the infected fruits have the capability to inhibit the growth of the pathogenic bacteria as identified by the antibacterial activity check using Kirby bauer method. Each fungal extract showed a specific range of inhibition against

Conclusion:

pathogenic bacteria. These results indicate that the secondary metabolites of fungus have an antibacterial activity that can inhibit the pathogenic strains of bacteria that are usually responsible for various diseases. Based on the results it can be inferred that the fungi isolated from the spoiled food would possess the inherent potential to inhibit the growth of the bacteria involved in various pathogenic processes. Thus the fungi can be used in the preparation of extracts that can be modified (so as to make it non non toxic for human consumption) to be used as food preservatives. The fungi can also be used for the preparation of antibiotics that can act against the food borne bacterial infections.

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