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Research Article

FUNGAL GROWTH ON LEATHER SURFACE AND IDENTIFICATION OF THEIR GENERA BASED ON MACROSCOPIC APPEARANCES AND MICROSCOPIC MORPHOLOGY

Atul Adhikari *, Arabinda Changmai, Koushik Sen Gupta, Biplab Kumar Dey
Faculty of Pharmaceutical Science, Assam down town University, Guwahati, Assam, India
*Corresponding Author Email: adhikari.atul5566@gmail.com

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ABSTRACT

Leather and goods made out of it are susceptible to microbial growth when it gets suitable temperature and humidity condition as it has a nutrient medium of protein and lipids. In this study, leather samples were collected from the market and stored in 28±2 °C temperature and high humidity to allow microbial growth. The microbes visible on the surface were cultured on the nutrient medium of Potato Dextrose Agar acidified with tartaric acid so that bacterial growth could be inhibited. The distinctive fungi colonies were then subcultured and observed under the microscopic magnifications to identify the fungi genera. Seven visually distinctive fungi colonies were identified as *Mucor* and *Aspergillus* genera based on macroscopic appearances and microscopic morphology.

Keywords: Fungal growth, Fungi identification, Leather, Nutrient medium.

INTRODUCTION

Leather being a biological product is rich in protein and lipids which acts as a suitable nutrient medium for the growth of microorganisms. Similar environments for the growth of microorganisms are available in tanned products of leather as well. The proteins, fats in the form of glycerides that are present in the leather is an ideal source of nutrients having a pH value around 4 that is required for fungal growth. In presence of suitable source of nutrient and high humidity conditions, the spores and hyphae germinate and grow.[1]

Leathers such as picked pelts, wet-blues and vegetable tanned moist leathers are susceptible for fungal attack. The risk of fungal growth is increased mostly during storage and shipping. Some common species of microorganisms found responsible for damage of leather by previous studies were *Mucor*, *Aspergillus*, *Paecilomyces*, *Rhizopus*, *Trichoderma*, *Penicillium*, etc.[2]

In 2004, sufficient evidence was found by the Institute of Medicine (IOM) which linked exposure to molds with upper respiratory tract infections such as cough and wheeze in healthy condition. And, in people with asthma and immune-mediated conditions, asthma symptoms and hypersensitivity pneumonitis were observed. In 2009, World Health Organization (WHO) published guidance, "WHO Guidelines for Indoor Air Quality: Dampness and Mould" which also suggests the effect of indoor dampness on the growth of fungi typically molds and indoor air quality.

Leather and products made out of leather are very susceptible to undesirable, aggressive actions of fungi during manufacture, storage, and shipping and in use. Fungi, present as spores and hyphae has become leading cause for biodeterioration of raw leather as well as leather products.[3, 4]

MATERIALS AND METHODS

Materials

Chemicals and reagent used for the preparation of media and culture were of analytical grade. Deionized sterilized water was used throughout the experiment.

Methods

Collection of leather

Collection of leather samples was carried out randomly from market irrespective of type and source of leather.

Tropical Chamber

Humidity required for fungal growth was maintained by preparing a tropical chamber. A closed desiccator was used as tropical chamber and the temperature was maintained at 28±2 °C. Water free from microorganisms was poured inside the desiccator to maintain high humidity.[3]

Growth of fungus on the leather surface

The samples were kept in open air for 7 days so that they can get exposure to microorganisms. The leather samples were then stored in the tropical chamber. Any visible fungal growth on the leather samples were checked regularly after 7 days.[3]

Preparation of Nutrient Medium

Potato Dextrose Agar (PDA) acidified with 10% tartaric acid was used as a nutrient medium for the culture of fungi on sterilized Petri plates. PDA solution was sterilized in an autoclave at 15 lbs pressure (121°C) for 30 minutes. The medium was acidified with sterile 10% tartaric acid. Approximately 1 ml of tartaric acid was added to previously sterilized PDA medium to prevent bacterial growth in low pH condition. Additionally, 10 mg Chloramphenicol per 100 ml of medium was added to further prevent bacterial growth. Approximately 20 ml of prepared media were poured in each sterilized Petri plates and left to cool and solidify under aseptic condition.[5, 6]

Direct agar inoculation method

Binocular microscope was used to observe and pick fungal spores from leather surface using inoculating needle. Spores from distinct colonies were inoculated into nutrient medium and incubated at 28 ± 1°C for 7 days. Aseptic conditions were maintained during inoculation process. Visually distinctive fungi on main culture plate were subcultured on similar conditions.[7]

Preparation and observation of microscopic slides

Microscopic slides of fungi were prepared using Lactophenol Cotton Blue (Himedia Ltd) as a mounting medium under aseptic conditions. Sterilized needles were used to select fungi under the binocular microscope and placed over a glass slide. A drop of Lactophenol Cotton Blue was placed above the fungi on the slide and a coverslip was placed carefully over it. The glass slides were then observed under different microscopic magnifications (up to 40X).[8]

Figure 1: Fungal growth observed on the leather surface

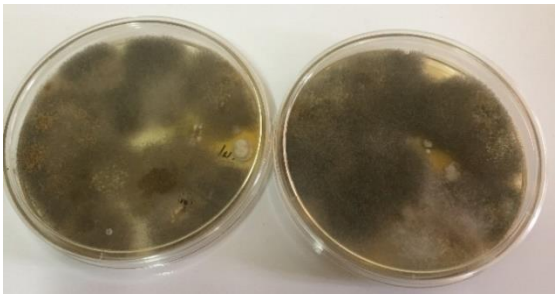

Figure 2: Culture of fungus obtained from leather on Potato Dextrose medium

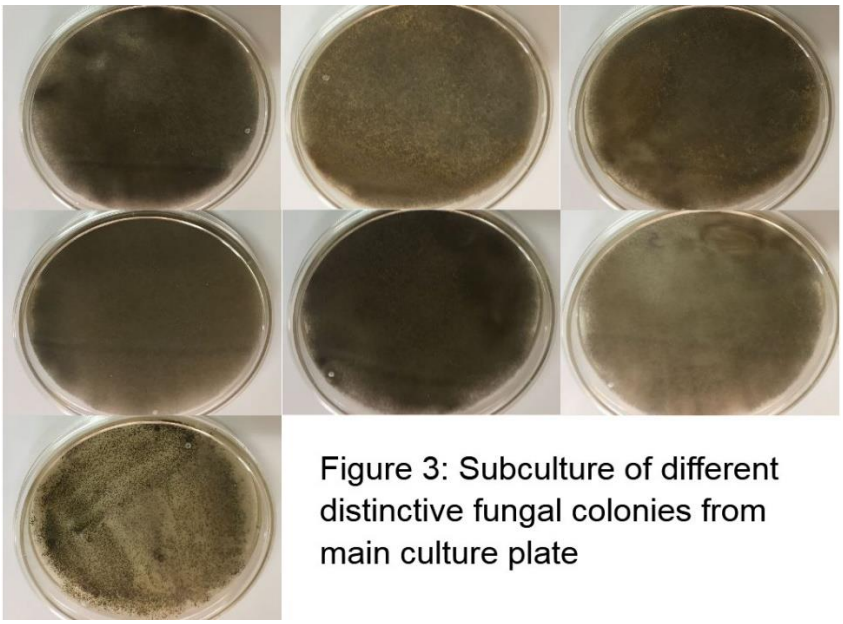

Figure 3: Subculture of different fungal colonies from the main culture plate

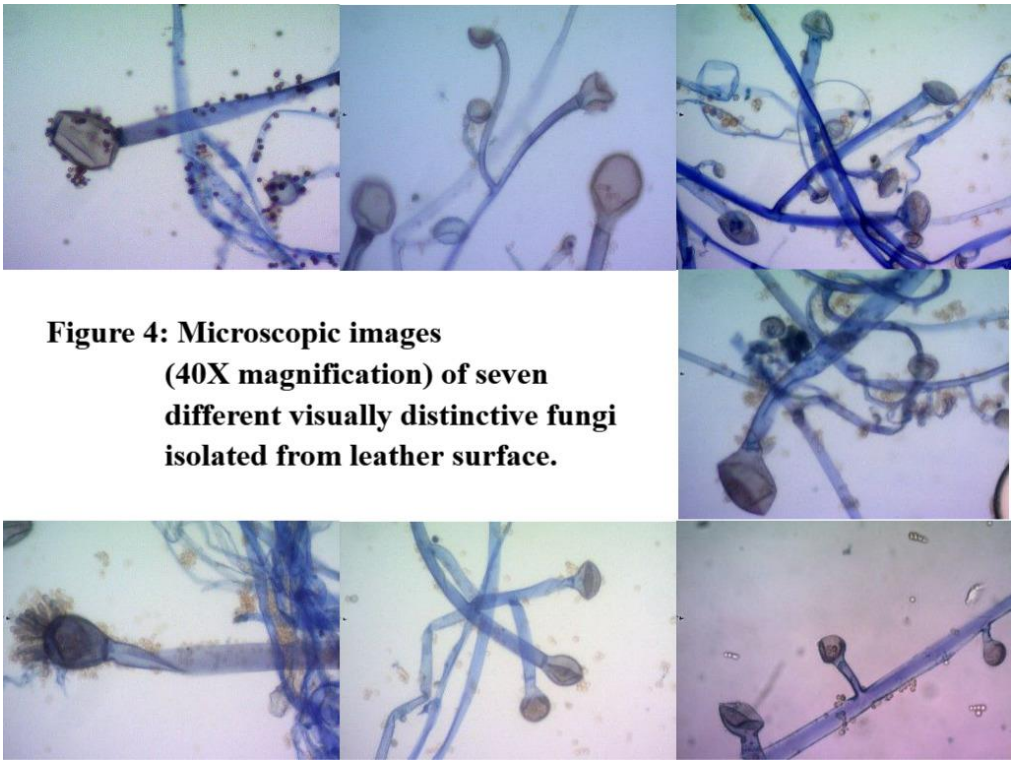

Figure 4: Microscopic images of seven different visually distinctive fungi isolated from the leather surface

RESULTS AND DISCUSSION

The leather samples were exposed to air and dust for 1 week to get them charged with microorganisms and spores in the open air. Microbial growth was visually observed after 7 days regularly. After 4 weeks fungus growth was clearly visible through naked eyes. Those fungi from the surface of leather were randomly selected and cultured on PDA media. After 7 days of fungus growth on nutrient media, when observed visually and under the microscope seven distinct different kinds of colonies were observed. These all distinctive colonies were then subcultured over the Petri plates using the same type of nutrient medium.

Seven visually distinctive fungi colonies were identified as *Mucor* and *Aspergillus* genera based on macroscopic appearances (Figure 3) and microscopic morphology (Figure 4).

Macroscopic Characteristics *Mucor* had rapid growing fungus which filled the culture plate in a few days with a woolly growth resembling cotton candy. New growth was white in color but turned greyish-brown with aging. The reverse of the culture plate remained a pale white.[8]
Colonies of *Aspergillus* were fast growing. White-yellow to yellow-brown or shades of green was observed in the culture medium.[9]

Microscopic morphology *Mucor* had broad hyphae which were scarcely or non-septate. Sporangiophores were long, branched and terminated in a round spore-filled sporangium.[8]
Conidia of *Aspergillus* were one-celled aggregated in compact columns. This morphology of conidial head made the morphological distinction of *Aspergillus*.[9]

CONCLUSION

Leather from animal sources has been used to manufacture products (Shoes, belts, bag, jackets, etc.) that come in direct contact with the human. Due to the availability of a suitable growth medium, leather has a high risk of microbial growth. This can be linked with both biodeterioration of the product as well as pathogenic risks to humans who come in contact with those products. Some of these microbes can be harm free but some of them can be pathogenic and may cause disease and these microbes may end up in the human system through skin contact. This study provides momentum to take any possible measures to prevent biodeterioration of leather products through microbial growth.

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