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ISOLATION OF KERATINOLYTIC FUNGI FROM SOIL SAMPLES OF POULTRY FARMS

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ABSTRACT

Keratinophilic fungi are those which exhibit affinity to keratin and capable of growing on hair, skin, feathers, or horns etc. Keratinolytic fungi are specialized in decomposition of keratin, being the main component of these substrata. Keratinophillic fungi accompany keratinolytic fungi, utilizing non-protein components of the substrata or the products of keratin decomposition. Environmental factors play an important role in the growth and sporulation of Keratinophilic fungi. The soil represents the main reservoir of fungi and rich in keratinous material is most conducive for the growth and occurrence of keratinophilic fungi. In the present study, the keratinophillic fungal cultures were isolated from soil samples of poultry farm area. The isolation of fungal cultures was performed on Keratin based potato dextrose agar. The total of 20 pure fungal isolates was obtained, out of which 05 showed positive results for keratinase production. The keratinase producers were stained and identified via lacto phenol cotton blue staining. The fungal strains were confirmed as *Trichoderma atroviride, Aspergillus niger, Fusarium verticillioides, Alternaria alternata, and Microsporum gypseum.* These isolates were maintained in pure form on potato dextrose agar based slants and kept at 4^0C till further use.

KEYWORDS: Keratinase producing fungi, keratinophillic, keratinolytic, soil samples, poultry farm area.

1. INTRODUCTION

Soils that are rich in keratinous material are most conductive for the growth and occurrence of keratinophillic fungi. The potentially pathogenic keratinophilic fungi and allied geophilic dermatophytes species are widespread worldwide. The forest, farmyard, park soil as well as sediments of the rivers and oceans contain humus and organic material which are the best

candidate for growth of keratinolytic and saprophytic fungi keratinolytic fungi are ecologically important and recently have attracted the attention throughout the world. They play a significant role in the natural degradation of keratinized residue. specific class of proteolytic enzymes includes the keratinases which catalyse the hydrolysis of keratins. They are the key enzymes in fungal invasion of skin and skin formations.[1] The keratinolytic enzymes are significantly involved in microbial bioconversion of keratinous wastes. For this purpose, enzymes mainly of bacteria, such as *Streptomyces* and *Bacillus* spp.[2-5] have been investigated. Keratin fungi act as good bio-indicators of environmental waste pollution. These indicators display the level of environmental contamination with human or animals faces and allow evaluating the risk of infectious disease resulting from the contamination. Keratin is considered as the main natural substratum for keratinolytic fungi. Keratinolytic fungi are associated with human and or animal activities. In particular these microorganisms commonly occur within highly populated areas to which keratin remnants are continuously delivered.[6,7] The present study is about the isolation and screening of keratino-phillic/keratinolytic fungi from soil samples of poultry waste dumped sites.

2. METHODOLOGY

2.1 Collection of soil samples

The soil samples were collected from poultry farm areas. About 500 g of soil was collected from each site by using sterilized spatula and kept in sterilized sealed polythene bags duly labeled. Further samples were brought to the laboratory, stored in refrigerator till the final analysis.

2.2 Isolation of keratinophillic/keratinolytic fungi

Isolation of keratinolytic fungi from each of the soil samples was done by using the modified technique using the hair bait.[8]

2.2.1 Hair Baiting Technique

The sterilized petriplates were half filled with different soil samples separately and moistened with distilled water, baited with sterile human hair. Antibacterial agent (Erythromycin, 1 mg/ml) was added to prevent bacterial growth. These dishes were incubated at 25^{o}C -27^{o}C, examined for fungal growth over a period of weeks. Hair baits observed with fungal spores were separately inoculated on Potato dextrose agar medium (PDA) supplemented with mineral salts, Erythromycin (100 mg/L) and actidione (500-mg/L) and incubated for 3-4

weeks at 25^{o}C.[9] The soil plates were inoculated with hair baits and observed weekly at room temperature and after one month of inoculation.

2.3 Evaluation of *in vitro* keratinolytic activity of isolated keratinophillic fungi

The isolates were screened for keratinase activity. The method was modified.[10] The feather powder enriched agar medium was further poured in the sterilized petriplates. Further, the broth (supernatant) cultures of different fungal strains were introduced in separate wells pre-punched after settling of agar medium. The clear zone indicates the promising keratinase producer fungal cultures. The similar technique was adopted for screening of keratinolytic bacterial cultures.[11]

2.4 Identification of isolated promising keratinophillic/keratinolytic fungal cultures

The positive keratinophillic cultures were identified to species or genera level based on macro- and microbiological characteristics and using selected taxonomic monographs. The identification was done on the basis of colonial and morphological characters using monographic descriptions and other available literature.[12]

3. RESULTS AND DISCUSSION

The study was performed for isolation and screening of keratinophillic fungal cultures from poultry farm area. The isolation of pure fungal cultures was performed on Keratin based potato dextrose agar. The total of 20 pure fungal isolates was obtained, out of which 05 showed positive results for keratinase production. The keratinase producers were stained and identified via lacto phenol cotton blue staining. The fungal strains were confirmed as *Trichoderma atroviride, Aspergillus niger, Fusarium verticillioides, Alternaria alternata, and Microsporum gypseum.* The isolates were maintained in pure form on potato dextrose agar based slants and kept at 4^{0}C till further use. The microscopic features and identification of strain is recorded in **Table 1**. The promising keratinase producers (supernatant broth) showed zone of clearance on keratin agar plates in comparison to negative control (introduced with sterilized N-saline). The keratinolytic activity of the positive keratinase producers is shown in **Figure 1**.

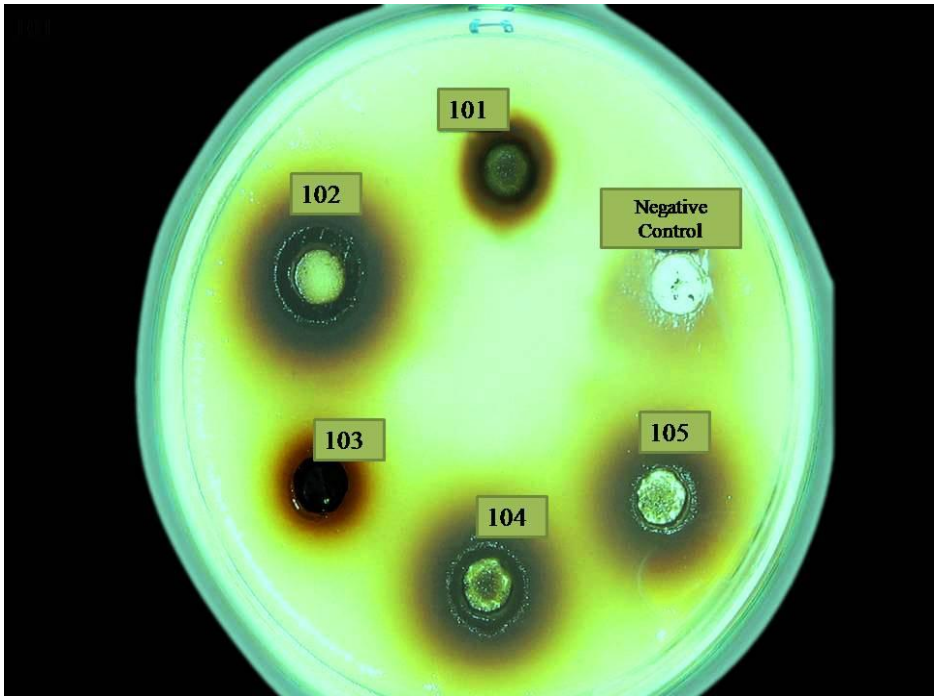
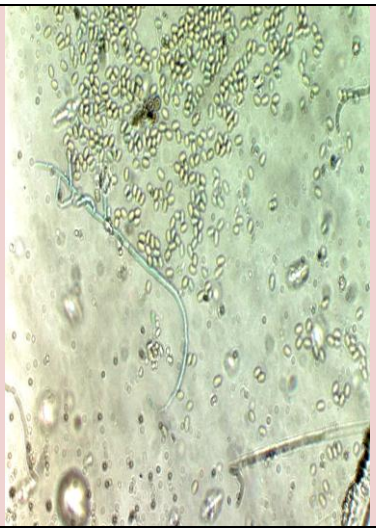
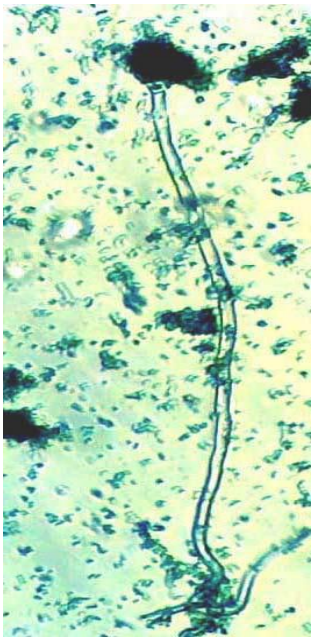
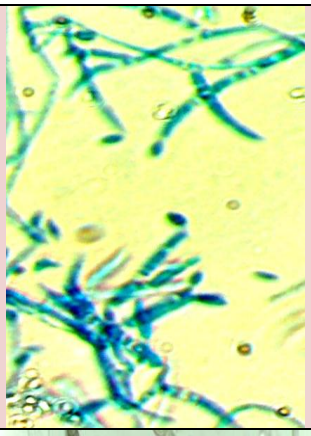
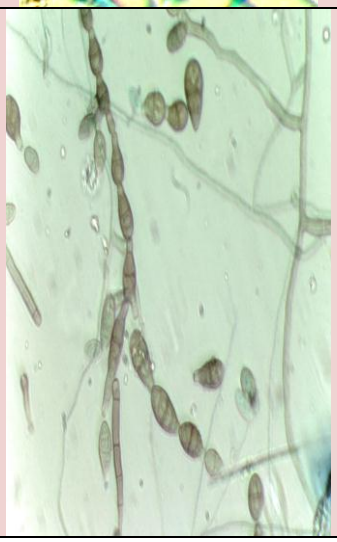


Figure 1: Keratinolytic activity of the promising fungal isolates.

Table 1: Microscopic features and identification of promising keratinophillic fungal strains.

Isolate (NCFT/KF-xxx)	Characteristic features of fungal strains	Staining by Lacto phenol cotton blue	Identification of the fungal strain
101	Colonies grew rapidly up to 9 cm in 4 days at 25° C, smooth surfaced, watery white and sparse mycelia mat but soon develop aerial hyphae on their surface. The color of the colony changes from whitish green to bright green with the development of conidial areas. The reverse of the colony remains uncolored. The hyphae are septate, branched, smooth walled, colorless 15-12 µm wide. Chlamydospores are formed intercalary or terminally. The conidiophores were much branched, form loose tufts, which arise in, distinct and continues ring-like zones.		*Trichoderma atroviride*

102	Colonies were fast growing, mycelium white to yellow, heavily sporulating in black or deep brownish black, reverse colorless or yellow, conidial heads large, globose to radiate in well defined columns, atypical heads also develop, peripheral conidia may not be fully pigmented. Conidiophores walls were smooth, thick, and colorless to brownish. Vesicle was globose, 45 - 75 µm diam. or smaller, fertile all over. Sterigmata were biseriate, primaries 20 - 30 x 5 - 61 µmsometimes septate; secondaries 7-10 x 3.0-3.5 µm. Conidia: globose. 4.0-5.0 µm, brown, walls heavy, irregularly roughened, ridged or echinulate. Sclerotia: cream to vinaceus buff, globose to subglobose		*Aspergillus niger*
103	The fungal isolate forms fast cottony growth on PDA, white with violet shades. The micro-conidia are hyaline, one celled ellipsoidal, cylindrical, usually 3 septate, slightly falcate to straight.		*Fusarium verticillioides*
104	Colonies grew fast and matured within 5 days The mycelium may be either sparse or abundant and variable in color, usually light olive green to brown. Reverse was black Hyphae are dark brown, thick, septate, and branched. Conidiophores are simple, erect, 40-50 µm long, 2-6 µm thick, and often clustered. Conidiophores produce dark pigmented conidia in an acropetal succession of simple or branched chains. These chains normally branch at the beak of a spore, or sometimes from the short lateral projection of the beak.		*Alternaria alternata*

105	Occasionally infects the scalp and skin on various parts of the body: infections are common in lower animals than in humans. Colony surface was flat and spreading and powdery to granular, developing an irregularly fringed border: it is buff at first, then tan to cinnamon brown. Colony often develops a sterile white hyphal border, with cottony white center. Reverse may be yellow, orange-tan, brownish red, or purplish red with septate hyphae. Macroconidia (8-16 x 22--60 urn) appear in enormous numbers, symmetric, rough, and relatively thin walled, with no more than 6septations.		*Microsporum gypseum*

The study thus deciphers that; the soil is a habitat of diverse fungi having keratinophillic and keratinolytic ability. The present study is an indication of dominant biodiversity of such fungi in soil samples where poultry farms are there or the soil is dumped with poultry waste. These keratinophillic fungi are the dominant source of keratinophillic and keratinolytic enzymes having industrial significance. The enzyme's non functionality on collagen enhances its industrial potential.[13] The enzyme can be utilized to prepare soluble crude protein via utilization of feather keratin through microbial fermentation technology.[14]

4. CONCLUSION

The keratinase producers are versatile and had significance importance. The fungal cultures can be easily preserved and stocked for a long period at 28^0C. Keratinase is a proteolytic enzyme which is able to degrade the proteins into peptide fragments ort digested protein moieties. The degraded protein can be utilized as an ingredient in nutritional food items. This enzyme can be utilized as an alternative to sodium sulfide which is the major pollutant obtained from tanneries.

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Conflicts of Interest

There are no conflicts of interest.

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