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Isolation and Identification of Keratinophilic Fungi from Poultry Farm Waste

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The poultry waste contaminated soil and feather samples were collected from poultry farm near the Gandhigram Rural University, Dindigul, Tamil Nadu, India during December 2008. Followed by serial dilution and plating was done to calculate the CFU/ml of the sample. Further keratin rich substance was used as baits for the isolation of keratinophilic fungi. Then these baits were used for hair baiting technique. The colonies on the Martin's Rose Bengal agar plates were transferred to Sabouroud's Dextrose agar medium. Then the isolated colonies were identified as *Aspergillus flavus*, *Microsporum gypseum*, and *Trichophyton rubrum*. As a result the study could be concluded by saying that of these three fungal species *Microsporum gypseum* is the most predominant colony in both the samples and so this can be used for effective biodegradation of feather rather than incineration.

Key words: *Microsporum gypseum*, Hair baiting technique, Keratinophilic fungi, Biodegradation.

Pollution is a bigger concern in various parts of the world, especially in developing countries. Pollution is undesirable state of the natural environment being contaminated with harmful substances as a consequence of human activities. The fast pace of industrialization, galloping demand for energy and rescures exploitation of natural resource during the last century have been mainly responsible for aggravating the problem of environmental pollution (current science, 2007).

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Feathers take long time for degradation. Increase of feathers in the environment cause environmental problems, respiratory problems in human. The reason for long time degradation is it contains a protein called Keratin. Feather constitutes over 90% proteins. The main component being beta keratin, a fibrous and insoluble structural animal polysaccharide extensively cross linked by disulphide bonds. (Savitha *et al.,* 2007)

The stability of degradation is mainly due to the composition and molecular configuration of constituent aminoacids in addition to the disulphide bonds and also its insolubility. (Wang and parsons, 1977)

The wastes of poultry are currently disposed of by incineration. This method has ecological disadvantages in terms of an apparent energy loss and the production of a large amount

of CO_2. Thus an innovative solution to these problems is urgently needed (Suzuki *et al.,* 2006)

MATERIAL AND METHODS

The poultry waste contaminated soil and feather sample was collected from poultry farm near Gandhigram Rural University, Dindigul, during December 2008.

Isolation of fungi

Serial dilution technique was carried out for both the samples, followed by the plating technique the dilutions used were 10^{-2} and 10^{-3} on Martin's Rose Bengal Agar medium plates.

Preparation of Keratinic substances

A keratin rich substance such as human hair was used as baits. The baits were cut into small pieces of 2-3 cm and were degreased to remove fatty substances. This baits were first washed in distilled water to remove the dust particles and dried, refluxing of baits was done in diethylether for 24 hours. Baits were dried at room temperature and were sterilized at 15 lbs pressure for 15 minutes. (Khanam and Jain, 2002)

Hair baiting technique

Half fill the sterile petri dishes with soil samples. Spread short (2-3 cms) strands of sterilized defatted human hair or horse hair over the surface of the soil. Add 10-15ml of sterile distilled water to the soil to facilitate germination of fungal spores. Some antibacterial antibiotics was added to prevent the bacterial growth. Incubate the prepared material at room temperature (20-25°C) in dark for 4-6 weeks. Examine the plates periodically for the development of mycelium, followed by a loop full of innoculum was taken from hair with fungal growth and was placed over sabourd's Dextrose agar medium. After incubation the colony formation was observed and the colony was pure cultured for further study. (Rahul and Rajak,2003)

Identification

The pure cultured colonies were identified based on macroscopic appearance on the agar plate and microscopic appearance by Lactophenol cotton blue staining.

RESULTS AND DISCUSSION

Feather waste is generated in large quantity as aby product of commercial poultry processing. Feathers are made up primarily of keratin. These feathers have sizable waste disposal problem (Savitha *et al.,* 2007).

Table 1. Isolation of Keratinophilic fungi

S.No	Organism	Dilution factor	No of Colony	CFU/g
1	Fungi	10^{-2}	18	9×103
		10^{-3}	7	3.5×104

Table 2. Identification of Keratinophilic fungi

Sample	Macroscopic appearance	Microscopic appearance	Result	
Soil	1.	Dark green color colony	Conidio spores	*Aspergillus flavus*
	2.	Cottony wine red color colony	Long pencil shaped macro conidia	*Trichophyton rubrum*
	3.	White color colony	Thin walled macroconidia with 4-6 septate	*Microsporum gypseum*
Feather	4.	White color colony macroconidia with 4-6 septate	Thin walled	*Microsporum gypseum*

Some of the microorganisms have the ability to degrade the keratin especially fungi. The fungal growth in the poultry waste contaminated soil and feather is because of the utilization of keratin as its sole source of carbon and nitrogen. They play a significant role in the natural degradation of keratinized residues (Rahul and Rajak, 2003)

Isolation of fungi was carried out in Martin's Rose Bengal agar medium and the results were tabulated in table 1.

The word Keratinophilic means, keratin loving and is sometimes misleading in the sense that all fungi that can grow on keratin rich substance. Keratinophilic species are those only able to use keratin. Others are utilizing non-keratin lipid fraction of the keratin rich substances (Mohamed *et al.,* 2000)

After isolation, hair baiting technique was carried out, to identify the colonies from both the samples SDA medium was used. The results were tabulated in table 2

Degradation and digestion of human hair by *Microsporum gypseum* invitro and suggested it to be due to the action of enzymes, however mechanical action of he mycelia was observed on the cells of cuticle by (Kunert *et al.,* 1981)

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