Chapter 1

GENERAL INTRODUCTION
INTRODUCTION

Keratin is a substance of animal origin which consists of folded polypeptide chains joined by hydrogen bonds, salt cross bridges and disulphide bridges. These are difficult to break, and consequently keratin is a substrate which can only be decomposed slowly by a relatively limited number of fungi. Keratin occurs in nature in the form of various animal appendages like hair, wool, feathers, nails, hooves and horns, and also in the outer keratinized layers of the skin. However, these are not pure keratin. They consist of keratin cells which are held together by a "cement" which is more susceptible to decomposition by microorganisms.

The majority of the fungi which are able to decompose keratin form a group which have a number of morphological and physiological characters in common, and are the members of the primitive ascomycete family, the Gymnoascaceae. Within this family are a number of species which are able to decompose keratin while it is still part of man and animal which bears it. English (1969) categorized these fungi in three groups:

(1) Keratinophilic - Fungus may or may not be able to digest keratinized substrate completely.
(ii) Keratinolytic - Fungus which can digest keratinized substrate completely.

(iii) Dermatophytes - Fungus belonging to the genera Epidermophyton, Keratinomyces, Microsporum or Trichophyton, whether pathogenic to man or animal or not. All the dermatophytes are keratinolytic in nature.

The dermatophytes are pathogenic fungi causing diseases such as ringworm and athlete's foot etc. On the basis of natural habitat and epidemiological standpoint Ajello (1960) classified dermatophytes as anthropophilic, dermatophytes which occur primarily on man and transmitted to animals causing dermatophytoses; zoophilic, dermatophytes which are mainly parasitic to lower animals and transmitted to man through contact; geophilic, species which are predominantly saprobic occur mainly in the soil and rarely associated with pathogenic attack.

Although dermatophytes undoubtedly existed in prehistoric times and have plagued lower animals and man for millions of years, the infections were long endured before their true nature was realized. The first recorded reference to a dermatophyte infection is attributed to Aulus Cornelius Celsus, the Roman encyclopedist, who in his 'De Re Medicina' written around 30 A.D., described a suppurative infection of
the scalp that came to be known as the kerion of Celsus (Rosenthal, 1961). Not until the 19th century was the mycotic etiology of these skin infections discovered. In 1837 Robert Remak, noted hyphae in the crusts of the disease known as favus (Kisch, 1954; Alkiewicz, 1967). This was an epochal discovery since for the first time a microorganism was incriminated as being the cause of a human disease. In 1841 Remak's discovery was independently confirmed by David Gruby (1841a), who carefully but succinctly went on to describe several types of dermatophyte infections: tinea favosa, ectothrix and endothrix trichophytosis and microsporiosis (Gruby, 1841b, 1842, 1843, 1844). Gruby (1843) also discovered the genus Microsporum in 1843 and described M. audouinii on the basis of the appearance of the fungus in clinical materials. The genus Microsporum is one of the three genera of the Fungi Imperfecti in which the dermatophytes are classified today. Malmsten (1945), described Trichophyton the second genus, Epidermophyton the third and last one, was established by Raymond Sabouraud in the year 1910. The classical studied of Sabouraud (1910) opened up new vistas in the realm of medical mycology. A number of reviews have been published dealing with dermatophytes (Ajello, 1960, 1974; Das Gupta et al., 1960; Dey, 1959; Sanyal, Maya, 1969; Taplin, 1976).

The occurrence of keratinophilic fungi in soil is known since Vanbreuseghem (1952). A good piece of work in
the field of distribution of keratinophilic fungi was done in all the five continents of the world (Ajello, 1953; Frey and Durie, 1956; Pugh and Mathison, 1962; Bohme, 1964; Frey, 1965; Gip and Paldrok, 1966; Al-Doory, 1969; Knudtson and Robertstad, 1970; Masih Mohammad et al., 1971; Karatygina, 1971; Ajello and Padhye, 1974; De-Bracalenti et al., 1975; Pugh and Hughes, 1975; Chmel and Vlacilikova, 1977; Mercantin et al., 1980). In India the first report of the occurrence of Microsporum gypseum in soil was from a vicinity of Dibrugarh district of Assam in 1955 by Dey and Kakoti. Further work was contributed by Sarkar (1962a, b); Randhawa and Sandhu (1965); Garg (1966); Padhye et al. (1966a, 1967); Roy et al. (1972); Kushwaha and Agrawal (1976a), Jain and Agrawal (1977); Sur and Ghosh (1980a) and Verma et al. (1982). The occurrence of these molds also varies in hill and plain soils (Garg, 1966). Chmel et al. (1972) have stressed the association between the humus content and presence of keratinophilic fungi. Chmel and Vlacilikova (1975) have reported the influence of soil depth and humus content of soil on the presence of these molds.

Numerous investigations of geophilic fungi have shown that they are more prevalent in habitats that have become enriched with humus or with the faeces of birds or bats. As far as geophilic dermatophytes are concerned, however, this finding has not been positively confirmed in that it is only
generally known that they seem to occur in highest number in soils frequented by human and animals. However, it can be seen from the plethora of literature that soil serves as a natural reservoir for fungi pathogenic or potentially pathogenic to man. Because of this it is important to determine more about the ecology of keratinophilic species in general.

Our knowledge regarding the distribution of keratinophilic fungi and their variants is still meagre and fragmentary. It is not known how many new keratinophilic fungi are hidden in the soil and how many of them will be pathogenic to man and animals. Hence it seems essential to have a systematic survey of different type of soils collected from different habitats, distribution pattern in relation to various seasons and to determine their pathogenicity on different animals. Because of this it will be of academic importance to determine more about the ecology of keratinophilic fungi with special reference to those species which are of common occurrence and pathogenic too. This will also help in the study of the epidemiology of dermatophyte infections.

To understand the mode of infection of pathogenic molds, host and parasite relationship, and nature of parasite, it is essential to study the various aspects of the physiology
of different parasites which could be of some help for a scientific approach towards the eradication of a particular disease. Before going in detail we must have an idea about the suitable media, temperature and incubation period for growth and sporulation of a particular pathogen.

The hair possesses a covering of oil and fats secreted by sebaceous glands of the animal skin. In feathers, the production of oil and fats takes place by preening glands. Some workers have reported that the presence of oil and fats, in combination with the keratin protein, hinder the colonization of keratin substrates (Pugh and Evans, 1970; Pugh, 1971, 1972). These reports are insufficient to establish any correlation between the colonizing capacity of keratinophilic fungi and presence of natural oil and fats on the keratin.

The agrochemicals are known to be used for the control of the plant diseases. Undoubtedly these chemicals control the plant diseases but they also give an adverse effect to soil mycoflora (Domsch, 1964, 1970; Midha and Nandwana, 1974; Wainwright, 1977; Kuthubutheen and Pugh, 1979; Jain and Sehgal, 1980a; Pugh and Agrawal, 1982). The keratin is added to the soil during animalization and decomposed by keratinophilic fungi. It is essential to determine the effect of these common agrochemicals on the saprophytic survival of keratinophilic fungi.
During microbial degradation of organic waste a number of volatiles have been produced. These volatiles are either produced by the microbes or by decaying organic matter and play an important role in fungistasis (Hora and Baker, 1970; Dennis and Webster, 1971; Hutchinson, 1973; Lockwood, 1977). These volatiles belong to higher series of alcohols, aldehydes and acetylenic products (Robinson and Garrett, 1969; Smith and Cook, 1975). Inhibitory effects of some organic volatile compounds on the mycelial growth and sporulation of some fungi have been reported by Pathak and Agrawal (1977), Thind and Agrawal (1978) and Singh and Agrawal (1981). Keratinophilic fungi can also survive and grow luxuriantly in association with several other microbes. We have no idea about the sporostatic effects of the volatiles produced by other microbes and also the effective nature of different organic volatiles produced by other soil organisms and their effect on the mycelial growth and sporulation of keratinophilic fungi.

The essential oils and related compounds which are mainly the products from above ground parts of the seed plants are insufficiently explored for their antifungal property, probably this may be due to the narrow concept, that microbes can produce more potential antifungal substances like antibiotics. Several workers have reported inhibitory effect of essential oils against keratinophilic
fungi (Kushwaha, 1976; Jain and Agrawal, 1978a; Singh and Agrawal, 1979; Jain et al., 1980; Deshmukh and Jain, 1981).

The occurrence of keratinophilic fungi in various habitats is associated with pathogenic attack indicate the presence of a good enzyme producing equipments, by virtue of which they can survive saprophytically in soil and play an important role in the process of infection. The qualitative screening of some enzymes of Trichophyton mentagrophytes and T. rubrum was done by Das Gupta and Shome (1960). The amylum degrading capabilities of these molds have been reported by Sen, 1964; Ziegler and Bohme, 1970; Kushwaha and Agrawal, 1975; Singh and Agrawal, 1981a and Jain, 1982a. Tate (1929), Das Gupta and Shome (1960) and Bohme (1968) have reported the production of an enzyme lipase by these molds. The proteolytic activity in dermatophytes have been also reported by various workers (Mac Fayden, 1895; Tate, 1929; Kunert, 1970; Meevootisom and Niederpruem, 1979).

Keratin, the natural fibrous protein is a cornified part of the skin of vertebrates, which differ from other proteins in its high cystine content. The cystine molecules with disulphide linkages are thought to be responsible for providing stability of keratin molecule and rendering it more resistant to enzymatic digestion. The mechanisms involved are not fully understood but are said to involve
mechanical disruption (Raubitschek and Maoz, 1957 and English, 1963) and digestion with proteolytic and possibly keratinolytic enzymes (Weary et al., 1965 and Yu et al., 1968). However, in vitro breakdown is characterized by the release of proteins, peptides and amino acids resulting in a marked alkalization of the culture fluid.

Doubt has been expressed that keratinophilic fungi are, in fact, able to digest keratin at all (Raubitschek, 1961) and it must be admitted that conclusive proof of keratin digestion by fungi has been not presented. The purpose of the present investigations was to determine whether or not these keratinophilic fungi, could digest keratin and if so, to look for possible unique features in the mechanism. It has been postulated that the unique mechanism of keratin digestion employed by the larva of the clothes moth, Tineola bisselliella might have a parallel role amongst the keratinophilic fungi. Waterhouse (1952) has further investigated the mechanisms of keratin digestion in Tineola larvae and his co-worker Powning (1953) studied the chemical nature of the excretory products in a search for substances capable of reducing or denaturing the wool. He observed that cystine can be reduced to cysteine in the gut and has subsequently detected the presence of NAD-linked cystine and glutathione reductases in the larval gut (Powning and Irzykiewicz, 1960). It will be of immense value to
evaluate the manner in which the soil inhabiting keratinophilic fungi colonize and digest various keratinic substrates.

The present study was undertaken to know further about the distribution of keratinophilic fungi in the soils of Madhya Pradesh, distribution in relation to various seasons and the pathogenicity tests of some of the isolates. Later on attempts were also made to evaluate their nutritional requirements, role of wax on colonization and agrochemicals in relation to saprophytic survival of these molds. The effect of volatile emanations from microbes on spore germination, mycelial growth, and the effect of some organic volatiles on growth and sporulation have been evaluated. An emphasis was also paid for a search of some antifungal agents from essential oils of medicinal plants.

The present research was undertaken to investigate the distribution and nature of keratinophilic fungi with special reference to areas where biotic factors, which provide a keratin source for these fungi, are present. The enzymological studies and in vitro degradation of human hair have been also determined in detail. The data obtained from these studies will be of immense value for predicting occurrence of any disease in a particular area. These will also be of much use for controlling dermatomycoses caused by soil inhabiting keratinophilic fungi and the data will be of academic interest to mycologist and ornithologist too.