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Keratinophilic Fungi Distribution, Pathogenicity and Biotechnological Potential

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Abstract: This chapter discusses the occurrence of Keratinophilic fungi in different habitats wherein the population of these fungi is expected to occur: air, soil from various environments like gardens, caves, cold and saline habitat. The ability to degrade the keratinic material and mechanism of action is discussed. Importance of these fungi in petroleum hydrocarbon degradation is discussed. Various metabolites with antibacterial, antifungal, antitumor and related compounds produced by this group of fungi are documented. Examples of synthesis of silver nanoparticles are also included. The need for an extensive survey of Keratinophilic fungi from unexplored substrates and habitats and biochemical potentialities is highlighted. It is concluded that fungi other than dermatophytes can be opportunistic pathogens. The need for a culture collection of this group of fungi is also highlighted.

Keywords: Keratinophilic fungi, Extreme climate, Secondary metabolites, Culture collection, Keratin degradation, Silver nanoparticles, Database

Introduction: Keratin is a natural fibrous protein which forms the outermost keratinized layer of the skin and its appendages in man and animals. Keratin contains high sulphur-containing aminoacid, e.g., cysteine and methionine which makes it resistant to microbial degradation. Keratin is colonized and degraded by soil microorganisms, principally keratinophilic fungi. The presence of disulphide bridges makes keratin poorly biodegradable (Gopinath et al. 2015). These fungi have a number of common morphological and physiological characters and are members of the primitive Ascomycetes family, Gymnoascaceae. Within this family, there are number of species which are able to decompose keratin while they are still a part of man and animals. This article covers the different habitats from where these fungi are frequently recorded followed by the pathogenicity of these fungi, their ability to degrade keratin, petroleum products and to produce bioactive metabolites and synthesis of nanoparticles.

Distribution of Keratinophilic Fungi:

1.) Soil in General-The distribution of dermatophytes in soil was reported by Vanbreuseghem (1952). Later on, various researchers isolated these fungi from soils of different countries, i.e. Australia (McAleer 1980), Egypt (Abdel-Hafez and El-Sharouny 1987; Zaki et al. 2005), Nigeria (Ogbonna and Pugh 1987), West Bank of Jordan (Ali-Shtayeh 1989), Kuwait (Al-Musallam 1989), Spain (Calvo et al. 1984; Ulfig et al. 1997), New Guinea (Filipello Marchisio et al. 1991), Malaysia (Soon 1991), Antarctica (Mercantini et al. 1993), France (Agut et al. 1995), Italy (Caretta et al. 1990; Papini et al. 1998), Palestine (Ali-Shtayeh et al. 2002), Bahrain (Deshmukh et al. 2008), Iran (Kachuei et al. 2012), West Indies (Gugnani et al.

2012), Jamaica (Gugnani et al. 2014), Argentina (Sarmiento et al. 2015) and Tunisia (Anane 2012; Anane et al. 2015).

Dey and Kakoti (1955) reported Microsporum gypseum for the first time from the soils of Dibrugarh district of Assam, India. Later on these fungi were isolated from various Indian states which included Delhi (Randhawa and Sandhu 1965), Madhya Pradesh (Kushwaha and Agrawal 1976; Deshmukh and Agrawal 1983a), Bihar (Verma et al. 1982), Jharkhand (Kumar et al. 2012), Uttar Pradesh (Nigam 1987; Deshmukh and Verekar 2011a), Jammu and Kashmir (Kaul and Sumbali 2000a; Deshmukh 2002a), Rajasthan (Jain and Sharma 2011), Karnataka (Deshmukh et al. 2000; Vidyasagar et al. 2005), Orissa (Roy et al. 1972; Ghosh and Bhatt 2000), Goa (Deshmukh and Agrawal 1983b), Maharashtra (Padhye et al. 1966, 1967; Deshmukh 1999; Deshmukh and Verekar 2014a), Madras (Ramesh and Hilda 1998–99; Deshmukh and Verekar 2011b), Kerala (Deshmukh 2002b), the Andamans (Dixit and Kushwaha 1990), Himachal Pradesh (Deshmukh and Verekar 2006a), 76 S. A. Verekar and S. K. Deshmukh Ladakh (Deshmukh et al. 2010; Kotwal and Sumbali 2014), Gujarat (Deshmukh and Verekar 2014b), Assam (Deshmukh et al. 2017) and Chhattisgarh (Deshmukh and Shukala 2000). Some of the dermatophytes and other keratinophilic fungi are responsible for superficial infections in human and animals and are therefore of possible significance in human welfare (Emmons et al. 1977; Monga and Mohapatra 1980; De Hoog and Guarro 1995).

Various distribution patterns of occurrence of these fungi are reported. These fungi are mainly reported from the locality frequented by man and animals. In most of these studies of keratinophiles, soil samples were taken randomly from the surface and at a depth not exceeding 3–10 cm from cattle farms, gardens, roadsides, forests, watercourse banks, grasslands, animal house floors and poultry farms. These studies established universal distribution pattern of keratinophilic fungi in soils with high frequency in the habitats frequently inhabited by humans and animals. As keratinophilic fungi have been reported from soils of various habitats of different geographical areas of the world, hence of epidemiological significance. Some of the habitats from where these fungi are prevalent, are described below.

2.) Alkaline Soil- Usar soil is an alkaline soil and cover barren lands in India. Usar soil is commonly found in the area with poor/no drainage and less percolation. The soil is white alkaline type with pH of 7.5–11.0. Areas with alkaline soils show prominent whitish salt excess, with low moisture content, high temperature, drought and intense solar radiations for the most of the months of the year. These soils have scanty vegetation in the form of patches of poor grass growth. Deshmukh and Verekar (2011a) reported Chrysosporium indicum, C. lucknowense, C. pannicola, C. tropicum, Chr. state of Ctenomyces serratus, Gymnascella dankailensis, Gymnoascus reessii, Microsporum gypseum, Trichophyton mentagrophytes and T. terrestre from Usar soils of Lucknow, Bareilly, Azamgarh, Balia and Pratapgarh districts of Uttar Pradesh. Similarly, Trichophyton verrucosum, T. mentagrophytes, Microsporum audouinii, M. canis and Chrysosporium tropicum were isolated from the soils collected from the vicinity of Jaipur where pH ranges from 6.5 to 8.5 (Jain and Sharma 2011).

3.)Public Parks-The local residents frequently visit public parks especially for morning/evening walks. Many of them bring their pets along with them. Part of the park is completely dedicated to children having swings, slides, small playground, etc. Due to lack of proper fencing these parks are some time invaded by animals such as buffalo's, cows, bullocks, horses and pigs. The rat's borrows are also seen in the 6 Keratinophilic Fungi Distribution, Pathogenicity … 77 parks. The common birds are also seen in this park. Due to human and animal activity, the keratinous material is added to the park soil. The organic matters like keratin, faeces of animals and other organic matters residues provide suitable environment for

the growth of these fungi with resultant health risk. There are reports of these fungi from public parks of Agra, Kanpur and Lucknow, and the list includes C. carmichaelii, C. georgii, C. indicum, C. keratinophilum, C. queenslandicum, C. tropicum, C. xerophilum, C. zonatum, Ctenomyces serratus, Geomyces pannorum, Gymnoascus hyalinospora, M. gypseum, M. fulvum, M. vanbreuseghmii, M. nanum, Malbranchea pulchella, Myceliophthora vellera, Nannizzia gypsea, T. verrucosum and T. mentagrophytes (Singh and Kushwaha 2010). While surveying keratinophilic fungi from the five public parks of Mumbai 11 species of 8 genera were recorded, viz. Arthrographis kalrae, Auxarthron conjugatum, C. indicum, C. queenslandicum, C. zonatum, Gymnascella dankaliensis, G. hyalinospora, M. gypseum, Myriodontium keratinophilum, Trichophyton mentagrophytes and Uncinocarpus reesii. Occurrence of keratinophilic fungi in public parks of Mumbai is of public health concern (Deshmukh and Verekar 2012).

More recently Pakshir et al. (2013) isolated keratinophilic fungi from soils of number of parks in Shiraz, and the list includes Chrysosporium sp., Microsporum gypseum, M. fulvum, Malbranchea sp. Phialophora reptans, Bipolaris spicifera, Bionectria ochroleuca, Ochroconis constricta, Scedosporium dehoogii, Cephalosporium curtipes, Nectria mauritiicola, Scedosporium apiospermum, along with the species of Fusarium, Acremonium, Penicillium, Chaetomium Scopulariopsis and Tritirachium sp. Majority of these fungi were recovered from the soils where pH ranges from 7 to 8. These reports highlighted that the soils of public parks are heavily contaminated with dermatophytes and other keratinophilic fungi.

4.)Grazed Pastures- Arthroderma multifidum, A. uncinatum, Clonostachys rosea, Engyodontium album, Chrysosporium sp., Chrysosporium keratinophilum, Malbranchea sp., Microsporum sp., M. vallerea, Paecilomyces carneus, P. lilacinus, Pochonia chlamydosporia, Trichophyton ajelloi, T. terrestre and Verticillium sp. were recovered from the soils of long-term fold-grazed pastures in national parks of Slovakia and non-fold-grazed pasture in sierra Stolicke vrchy. Trichophyton ajelloi and P. lilacinus were prevalent in all the soil samples. In the fold-grazed pasture, keratinophilic fungi were more abundant in compared to non-fold-grazed pasture. Substantially lower presence of the other keratinophilic fungi in non-fold-grazed pasture may be because of low pH of these soils (Javoreková et al. 2012).

5.) Potted Plants -Hedayati et al. (2004) isolated Microsporum gypseum, M. cookei and Chrysosporium sp. from the soils samples of potted plants from hospitals in Sari, Iran. The presence of these fungi in potted plants in hospitals could be a potential source of infection. Similarly, the soil samples of potted plants inside houses, hotels and offices from 15 localities of Kanpur, India, yielded Acremonium sp., Acremonium implicatum, A. hennbertii, Aphanoascus terreus, A. keratinophilic, Arthroderma cunniculi, Botryotrichum piluliferum, Chrysosporium indicum, C. keratinophilum, C. queenslandicum, C. pannicola, C. sulfureum, C. merdarium, C. zonatum, C. tropicum, Ctenomyces serratus, Malbranchea pulchella, Microsporum gypseum, Trichophyton vanbreseghmii and Verticillium tenuipes. Occurrence of these fungi in soils of indoor plants may be the source of dermatomycosis and other diseases in human (Singh et al. 2009a, b).

6.) Waterlogged Conditions -Paddy fields are in waterlogged condition during different stages of cultivation and personnel involved in planting rice sapling spend 8–10 h per day in warm and wet conditions in the field that favours growth of these fungi. Sundaram (1987) reported Microsporum gypseum, M. canis, Trichophyton terrestre, Trichophyton sp., T. ajelloi, Chrysosporiurn keratinophilum, Aspergillus fumigatus, Penicillium sp. and Mycelia sterilia from the rice field near Madras. Chrysosporium anamorph of Arthroderma cuniculi, C. anamorph of Arthroderma curreyi, C. carmichaeli, C. georgii, C. gourii, C. indicum, C. keratinophilum, C. lobatum, C. merdarium, C. pannicola, C.

queenslandicum, C. tropicum, C. anamorph of Pechtinotricum illiase, C. anamorph of Rollandina vriesii were isolated from paddy fields during different stages of cultivation, eg., transplanting, tillering, milking and maturation (Shrivastava et al. 2008). Chrysosporium keratinophilum followed by C. tropicum was the most dominating geophilic species. These fungi were most prevalent during the milking stage (100%), followed by the maturation stage (89.47%) of paddy cultivation.

7.)Sediments- Abdel-Hafez and el-Sharouny (1990) isolated Chrysosporium state of Arthroderma tuberculatum, C. asperatum, C. georgii, C. indicum, C. keratinophilum, C. pseudomerdarium, C. queenslandicum, Chrysosporium state of Thielavia sepedonium, C. tropicum, Microsporum cookei, M. gypseum, Myceliophthora anamorph of Corynascus novoguineensis, M. vellera and Trichophyton terrestre from sewage sludge samples from Upper Egypt. Similarly, from the sediment samples collected from the Shatt Al-Arab River, and its creeks yielded Aphanoascus fulvescens, A. durus, Chrysosporium crassitunicatum, C. keratinophilum, C. tropicum, M. fulvum, 6 Keratinophilic Fungi Distribution, Pathogenicity … 79 M. gypseum and T. verrucosum (Abdullah and Hassan 1995). In other study, sewage sludge samples collected from seven wastewater treatment plants in Sari city, Mazandaran Province, Islamic Republic of Iran yielded Microsporum gypseum, Chrysosporium spp. and Geotrichum spp. using the hair-baiting technique (Hedayati and Mirzakhani 2009). Ulfig et al. (2006) observed that there was a significant increase in the population of actidione-resistant keratinophilic fungi in the sludge during open-air drying and change in the numbers. Occurrence of these fungi in sewage slug/sediments can be a source of infection to man and animals (Kushwaha 2014).

8.) Cold Desert- Ladakh “land of high passes” in Karakoram mountain ranges in the Indian state of Jammu and Kashmir is the highest plateau with height of 3000 ft and is situated between latitudes 30°N–36°N and longitudes 76°E–79°E. The temperature in this area ranges from 3–35 °C in summer to −20 to −45 °C in winter. Amauroascus kuehnii, Aphanoascus keratinophilus, A. terreus, Auxarthron alboluteum, A. conjugatum, Chrysosporium articulatum, C. minutisporosum, C. mephiticum, C. siglerae, Chrysosporium sp. C. tropicum, C. submersum, C. state of Ctenomyces serratus, Geomyces pannorum were isolated from the soils of cold desert of Ladakh. The samples were collected from places like Pangong Tso, Chang La, Durbuk, Khardung La, Tangste, Lukung, Magnetic Hill, Phey village, Leh and Nimmu and were from pastures, glacier, roadside, bank of lake, uncultivated soil, cultivated soil, river bank and roadside (Deshmukh et al. 2010). Chrysosporium inops, C. merdarium, C. queenslandicum, Chrysosporium anamorph of Gymnoascus demonbreunii along with other fungi were isolated from the soils collected from Khardung (14,738 ft) (Kotwal and Sumbali 2011, 2014).

9.) Antarctic Environment -Antarctica is the coldest, windiest and the driest continent of the Earth, located at the South Pole. In Antarctic environment, the temperature in winter is as low as −63 °C and winter mean temperatures are −57 °C and lower. The temperature in summer is unlikely to be warmer than −18 °C. Low temperatures, high aridity, low and sporadic availability of nutrients are the major stress factors. In such extreme environment, fungi and bacteria can grow because of their oligotrophic nature (Parkinson et al. 1989). Geomyces pannorum, Malbranchea gypsea, Malbranchea sp, Microsporum gypseum, M. megellanicum, Trichophyton mentagrophytes and T. terrestre along with other fungi have been reported from Antarctica (Caretta and Piontelli 1977; Mercantini et al. 1989b; Mario Comerio and Mac Cormack 2004). Keratinophilic fungi were reported from the dust samples collected in various rooms on the Italian scientist-base and from soil in close proximity to the base of Antarctica. The keratinophilic fungi isolated were Malbranchea gypsea, 80 S. A. Verekar and S. K. Deshmukh T. mentagrophytes, Aphanoascus fulvescens, C. carmichaelii, Geomyces pannorum var pannorum and G. pannorum var vinaceus (Mercantini et al. 1993). Keratinophilic fungi found in polar region might have

been tuned to the environmental conditions and adapted to lifecycle, genetic and physiological modifications.

10.) Wildlife Sanctuary -In the wildlife sanctuary, the keratinous material, viz. hair and feathers, are added to the environment and keratinophiles survive on these substrates. Deshmukh (2002a) recorded Arthroderma simii, Chrysosporium indicum, C. keratinophilum C. pannicola, Gymnoascella hylinospora, Malbranchea aurantiaca, Microsporum gypseum complex and Trichophyton terrestre in the soils collected from "The Periyar Wildlife Sanctuary" (Kerala, India). The sanctuary is known for rich fauna including elephants, tigers, bisons and spotted deer. The occurrence of these fungi in "The Periyar Wildlife Sanctuary" can be due to the presence of animals and birds with less human interference (Deshmukh 2002b). Deshmukh and Verekar (2006a) isolated Chrysosporium queenslandicum, C. xerophilum and Malbranchea gypsea from the soils of Pin Valley National Park (Himachal Pradesh, India) famous for herds of Asiatic ibex (Capra ibex), Snow Leopard (Uncia uncia) and Himalayan blue sheep (Pseudois nayaur). Their occurrence is an indication of their adaptation to cold climate. While screening soils of Sanjay Gandhi National Park Mumbai, Deshmukh and Verekar (2014a) reported the presence Aphanoascus durus, Arthroderma corniculatum, Auxarthron umbrinum, Chrysosporium evolceanui, C. indicum, C. tropicum, C. zonatum, Chrysosporium state of Arthroderma tuberculatum, Chrysosporium state of Ctenomyces serratus, Gymnascella dankaliensis, Microsporum gypseum, Myriodontium keratinophilum and Trichophyton mentagrophytes. Deshmukh and Verekar (2014b) also reported Aphanoascus durus, A. fulvuscence, Arthrographis kale, Auxarthron conjugatum, Chrysosporium indicum, C. tropicum, C. zonatum, Chrysosporium state of Ctenomyces serratus, Microsporum gypseum and Trichophyton mentagrophytes from the soils of Gir Forest National Park and Wildlife Sanctuary, Gujarat (India), known for Asiatic Lions (Panthera leo persica). Similarly, a new fungus Auxarthronopsis bandhavgarhensis was isolated from Bandhavgarh National Park situated in the central India state Madhya Pradesh (Sharma et al. 2013). The occurrence of these fungi in these national parks indicates that these fungi are associated with the birds and animals of the national parks.

11.) Poultry Farm- Poultry farm serves as a reservoir of keratinophilic and toxigenic fungi. There are reports on the occurrence of these fungi in poultry environment which include birds, cages, transporting trucks and dumping grounds. Various researchers have reported elevated occurrence of keratinophilic fungi in poultry farm soils (Jain et al. 1985; Deshmukh 1999; Kaul and Sumbali 2000a; Anbu et al. 2004), which are neutral to weakly alkaline in nature and rich in organic matter. A high content of organic carbon, nitrogen, phosphorus, potassium, magnesium, calcium and iron is present in this habitat (Kaul and Sumbali 1998–99). Deshmukh (1999) recovered C. indicum, C. lobatum and Microsporum gypseum from poultry farm soil while surveying keratinophilic fungi from Mumbai and its vicinity. Chrysosporium indicum, C. lucknowense, C. pannicola, C. queenslandicum, Chrysosporium I, Chrysosporium II, C. tropicum, C. zonatum, C. state of Arthroderma tuberculatum, Geomyces pannorum, Malbranchea pulchella, Microsporum gallinae, Trichophyton mentagrophytes and T. simii were isolated from poultry farm soils from Bilaspur district of Chhattisgarh (India) (Deshmukh and Shukala 2000). Kaul and Sumbali (2000a) recorded very high per cent of keratinophilic fungi which were attributed to higher influx of poultry droppings and other remains (feathers and claws) in this soil. Keratinophilic fungi were also recorded from poultry farm from Namakkal and feather dumping sites from Chennai. The isolated fungi were M. gypseum, Trichophyton mentagrophytes, Chrysosporium keratinophilum, Chrysosporium state of Arthroderma tuberculatum, Geomyces pannorum (Anbu et al. 2004). Kaul and Sumbali (2000a, b) isolated C. keratinophilum, C. queenslandicum, C. tropicum, C. indicum and Malbranchea chrysosporoidea from feathers of poultry birds (Gallus domesticus). They also found C. keratinophilum, C. queenslandicum, C. tropicum, C. pannorum and Malbranchea flava from soils of poultry farm. The

study indicates that colonization of these fungi on bird feathers is because they spent more time on ground.

12.) Piggery Soils- Fifteen different keratinophilic fungi were reported from the soil samples collected from the piggeries of Ranchi, Jharkhand (India) (Kumar et al. 2012). The reported fungi were Aspergillus niger, A. terreus A. flavus, Penicillium chrysogenum, Penicillium sp., Alternaria alternata, Fusarium oxysporum, F. solani, Trichoderma harzianum, T. reesei, T. viride, Curvularia lunata, Chrysosporium sp. Mucor pusillus and Rhizopus stolonifer. The majority of them were secondary colonizer on keratin baits. More attention is needed to isolate the true species of keratinophiles from this environment.

13.) House Dust -Chrysosporium carmichaelii, C. evolceanui, C. indicum, C. keratinophilum, C. merdarium, C. pannicola, C. queenslandicum and C. tropicum and other fungi were isolated from house dust of Kanpur (Nigam and Kushwaha 1990, 1993). From the dust samples collected from hospitals and houses in Kanpur, Acremonium implicatum, A. strictum, Aphanoascus fulvescens, Arthroderma simii, C. queenslandicum, C. indicum, C. pannicola, C. tropicum, Ctenomyces serratus, Gymnoascus reessii, Malbranchea fulva, Malbranchea pulchella, Microsporum gypseum, Microsporum cookie, M. fulvum, Paecilomyces lilacinum, Penicillium expansum, Trichophyton mentagrophytes and T. terrestre were recorded (Singh et al. 2009a, b). In hospitals, C. serratus was less prevalent while the A. simii was the most. In houses, C. queenslandicum was less prevalent and C. tropicum the most (Singh et al. 2009a, b). While surveying keratinophilic fungi from Jaipur, Jain and Sharma (2011) reported Trichophyton terrestre and C. tropicum from house dust. Similarly, Vidyasagar et al. (2005) recovered Microsporum gypseum, Chrysosporium keratinophilum, Trichophyton mentagrophytes, M. nanum and C. tropicum from corridor dust of hospitals and soils of public places from Gulbarga in Karnataka (Vidyasagar et al. 2005). These data show the capability of the long-term existence of pathogenic fungi in the soil of indoor environments and is the main link in the human circulation of these pathogens.

Chrysosporium asperatum, C. state of Arthroderma tuberculatum, C. indicum, C. inops, C. keratinophilum, C. merdarium, C. pannorum, C. queenslandicum, C. tropicum and C. xerophilum along with Trichophyton verrucosum and Trichophyton sp. were isolated from air dust particles from Egypt (Abdel-Hafez et al. 1990a). Moubasher et al. (1990) reported Chrysosporium asperatum, C. indicum, C. keratinophilum, C. merdarium, C. pannorum and C. tropicum from the atmosphere of hay or winnow sites and from combine harvester wheat and sorghum air dust.

Epidermophyton floccosum, M. canis, M. gypseum and T. mentagrophytes along with species of Chrysosporium were isolated from the dust of ferry boats of Italy. C. pannorum was the most prevalent species amongst Chrysosporium. In the same study, E. floccosum, T. mentagrophytes, T. tonsurans, T. ajelloi, Trichophyton spp., M. canis and M. gypseum and seven different species of Chrysosporium were isolated from the dust of railway cars; C. pannorum was the most numerous one amongst the Chrysosporium sp. (Mercantini et al. 1989a).

14.) Meteoritic Crater- Deshmukh and Verekar (2006b) reported Aphanoascus durus, A. punsolae, Auxarthron kuehnii, C. indicum, C. tropicum, Chrysosporium sp., Chrysosporium state of Ctenomyces serratus from Lonar meteorite crater in Maharashtra state (India). Lonar lake water is ten times saltier than drinking water (pH 10.5) and contains salts and minerals of sodium, chloride, carbonates, fluorides and bicarbonates. The area in and around is lush green with dense forest with numerous species of birds and animals. The occurrence of these fungi in such environment shows the addition of keratin material to the crater by birds and animals.

15.) Veterinary Clinic- Microsporum canis, M. gypseum, T. ajelloi, T. mentagrophytes, T. terrestre, C. keratinophilum, C. pannorum, C. tropicum, Chrysosporium sp., C. state of Arthroderma tuberculatum were isolated from the veterinary clinic (Mancianti and Papini 1996). Dermatophytes survives for longer period in dorminant form in the clinic and clinical materials is the matter of concern. The clinical materials can come in the direct contact and be the means of transmission of dermatophytes. The presence of mites on animals can be another source of transmission of these fungi in veterinary clinics. Therefore, such clinics may represents sites where pets and human are exposed to the risk of infection of such pathogenic fungi.

16.) Coastal Habitats- The coastal areas are an environment for migratory birds and burrowing animals. Occurrence of these fungi in such coastal soils supported the view that this habitat is a rich source of keratinophilic fungi. Deshmukh (2002b) reported Chrysosporium indicum, C. keratinophilum, C. tropicum, Chrysosporium state of Ctenomyces serratus, Microsporum gypseum complex, Trichophyton mentragrophytes and T. terrestre from Kovalam beach, Kerala (India). Occurrence of keratinophilic fungi in saline areas may be attributed to the preference of keratin material in such habitats. Many investigators studied the effect of salinity on the growth and survival of fungi and demonstrated that keratinophilic fungi are common in saline environments (Kishimoto and Baker 1969; Deshmukh and Agrawal 1983b; Larrondo and Calvo 1989; Ulfig et al. 1997; Deshmukh 1999). Katiyar and Kushwaha (1997) isolated C. keratinophilum, C. tropicum and Chrysosporium sp. from sand of the Mediterranean Sea, which is poor in organic matter. Pugh and Mathison (1962) found maximum distribution of Chrysosporium in coastal soils due to enrichment by the droppings and fish debris, which favours more the growth of aleurosporic fungi. Relatively high density of these fungi in coastal soils might be due to the human activity and presence of marine birds and burrowing animals.

17.) Salt Pan Soils -Salt pans are very common in the coastal habitats and are part of the salt industry responsible for production of tons of salt around Mumbai and supplied to all over India. Seawater flows into the salt pans and brings keratinic material such as human hair, animal hair and wool fragments from blankets from neighbouring villages during high tide. Moulted feathers from various migrating birds also constitute part of the keratinic material in salt pans. The formation of salt takes 3–4 months. 84 S. A. Verekar and S. K. Deshmukh During that period decomposition of keratin by keratinophilic fungi and related dermatophytes takes place. Deshmukh (2004a) reported Chrysosporium indicum, Microsporum gypseum complex, C. tropicum, Chrysosporium state of Ctenomyces serratus, C. fluviale, Malbranchea aurantiaca, Trichophyton mentagrophytes, T. terrestre, Malbranchea sp. and Uncinocarpus reesii from the soils around salt pans in Mumbai. This investigation indicates that soil of salt pans and surrounding areas provides an unusual ecological environment for keratinophilic fungi and related dermatophytes.

18.) Animals- Keratinophilic fungi are reported from hairs of various animals which include cows, donkeys, rabbits, cats, dogs, goats and wild boars. The most frequently isolated species are Trichophyton mentagrophytes, T. verrucosum, T. ajelloi, T. terrestre, Microsporum gypseum, M. nanum, M. canis, Arthroderma cuniculi, A. curreyi and a number of species of Chrysosporium (Ali-Shtayeh et al. 1988, 1989; Abdel-Hafez et al. 1990b; Al-Musallam 1990; Mancianti et al. 1997; Guzman-Chavez et al. 2000). Mite Tyrophagus putrescentiae was found associated with M. canis and may be responsible for transmitting fungi amongst animals (Caretta et al. 1989). The occurrence of these fungi on these animals can be a risk of dermatophytic infections to man and animals.

19.) Birds and Its Environment -The birds act as reservoirs of zoophilic keratinophiles and transport them from one place to another. It is thought that some fungal association with birds are species specific. These fungi occur in nests and can be transmitted from parents to off springs (Pugh 1972). There are

several reports of occurrence of these fungi from birds and its environment (Sur and Ghosh 1980; Dixit and Kushwaha 1991; Sarangi and Ghosh 1991; Deshmukh 2004b). The most frequently isolated species include Chrysosporium evolceanui, C. indicum, C. tropicum, Microsporum gypseum, M. fulvum, Microsporum sp, Trichophyton mentagrophytes, Malbranchea pulchella, Aphanoascus reticulisporus, Gymnoascoideus petalosporus, Malbranchea fulva, Pseudoarachniotus flavoluteus, P. roseus, R. hyalinospora, Rollandina capitata, Myriodontium keratinophilum. While surveying keratinophilic fungi from different migratory bird Chrysosporium keratinophilum, C. tropicum, C. merdarium, C. luteum, C. parvum, C. pruinosum, C. asperatum, Scopulariopsis sp., Trichophyton terrestre, Arthroderma tuberculatum, Sepedonium spp. were isolated from 10 birds (Budgerigar, Ring neck, Lovebird, Pigeon, Alexandrian parrot, Amazon parrot, African grey, Quail, Duck and Chicken) from different countries coming to Bahrain (Mandeel et al. 2011). Similarly, Auxarthron conjugatum, Chrysosporium fluviale, C. indicum, C. tropicum, Chrysosporium state of Ctenomyces serratus, Gymnoascus petalosporus and Microsporum gypseum complex 6 Keratinophilic Fungi Distribution, Pathogenicity … 85 were recorded from various sites in the vicinity of Vedanthangal Water Bird Sanctuary. From this study, we can conclude that migratory birds can be a mode of transport of variety of keratinophiles from one place to another (Deshmukh and Verekar 2011b).

Pathogenicity:

Members of the genus Chrysosporium have weak pathogenic potential, with human and animal infection reported for only a few taxa. Experimental studies have shown inoculation of this fungus on guinea pig skin to produce erythematous scaling lesions which disappear after 3–5 weeks; however, no apparent invasion of the hair shaft occurs. In white mice, after inoculation, granulomas with necrotic centers can be observed, although conidia of the fungus appear to remain intact. Chrysosporium keratinophilum is one of several soil organisms that is occasionally isolated from skin and nails. Isolation of this species from clinical specimens is generally from human onychomycoses, the mycotic superficial invasion of keratinized tissue of the nail plate. In practice, C. keratinophilum is interpreted to be an infrequent contaminant of keratinaceous clinical specimens, such as hair, skin and nails, with no clinical significance. A pathogenic role for C. keratinophilum is unlikely; however, its ability to remain viable for weeks on skin may suggest pathogenic potential. However, a critical component in limiting its pathogenic potential is its inability to grow at 37 °C, which is the human body temperature[1] discouraging the possibility for this fungi to be infectious to humans. The first report of onychomycosis caused by C. keratinophilum in animals was reported by Pin and his colleagues. The seven Bennett's wallabies (Macropus rufogriseus rufogriseus) they observed had swollen, abnormal claws from which Chrysosporium keratinophilum was repeatedly identified in culture, suggesting that the fungus may factor in disease. In another experimental study, C. keratinophilum showed pathogenic potential in the white mouse, remaining viable in the peritoneal cavity for up to two months. It is possible that C. keratinophilum can cause more generalized infections in a weakened mammalian host.

Biotechnological Potential:

1.) Microbial enzymes- These enzymes have been studied for de-haring processes in the leather industry and hydrolysis of feather and keratin. Samples from poultry wastes, soil, water, and feather were collected from different places in Parbhani. Each sample was placed on feather meal agar plates containing 5 g LG1 feather as the sole carbon and nitrogen source and the obtained colonies were selected, purified and their growth were detected on casein agar medium The well grown isolates on casein agar medium which

producing the largest clearing zone on casein plate were selected for keratinase assays. Out of 16 bacterial isolates, 5 isolates were selected. The best keratinase producing bacterium kea MS21 was selected and identified based on morphological, physiological and some biochemical characteristics. It was recorded as a species belonging to the genus Pseudomonas and identified as Pseudomonas sp. Precipitation and purification of the keratinase enzyme in addition to factors affecting enzyme activity (pH & temp.) were studied. The enzyme molecular weight was determined to be of 30 KD a using sodium dodecyl sulphate polyacryla mide gel electrophoresis analyses. The optimum temperature and pH were determined to be 35°C and pH 8.0, respectively. The effect of some proteases inhibitors and activators were also studied (Bhausaneb, et al., 2011). Dermatophytes are a group of closely related fungi that have the capacity of invading the keratinized tissue (skin, hairs and nails) of human and other animals to produce infections known as dermatophytosis, which are commonly referred to as ringworm (Gugnani, 2000). Dermatophytes can digest keratin and other proteinaceous substrates present in Skin and its appendages, such as nail, hair, and feather, and use it as its sole source of carbon and nitrogen.

Proteolytic and keratinolytic activities of dermatophytes have been a subject of interest for several years to understand the pathogenicity of infection (Venkatesan, 2010). These dermatophytes are also called keretinophilic fungi because of their high affinity for keratin. Keratin is a refractory protein polymer only produced by man and animals; it is the main constituent of epidermal skins, hairs, feathers, reptilian scales, quills, horns, hooves and nails. When these materials are shed into the soil and other potentially moist substrata such as disused nest, they are principally degraded by keratinophilic fungi (Summerbell, et al., 2000). The process of degradation of keratin contained in its natural forms such as hair or feathers seem to be a result of both the mechanical action of the fungus and the activity of enzymes. The invasion of hair by anthropophilic dermatophytes has been investigated with light and electron microscopy under natural and experimental conditions. Studies by scanning electron microscopy of scalp hair from subjects infected with Trichophyton violaceum (Tosti, et al., 1970). Guinea pig skin infected experimentally with T. mentagrophytes (Heath, et al., 1970) showed the hypha growth during in vivo infection. These morphological observations indicate that dermatophytes grow in filamentous form during in vivo infection of hair following the same pattern as on or binary laboratory media. Since mycelium and the remaining insoluble substrate cannot be separated, quantitative measurement of keratin degradation poses a problem and hence only the loss of total dry weight (keratin + mycelium) were calculated (Ziegler, et al., 1963). Calculated the net loss of weight of the substrate by evaluating the 'economic coefficient'. It is believed that the rate and completeness of the keratin degradation is, dependent on the kind of substrate and correspond roughly to its hardness, viz., cystinecontent (Kunert, et al., 2000).

2.) Essential oils for the treatment of mycoses- The Oils from medicinal plants were screened for their activity against A. fumigates and A. nigerby disc diffusion method. Minimum inhibitory concentrations (MIC) of oils against Aspergillus fumigates and Aspergillus niger done by agar dilution method and Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentration (MCCs) data (%v/v) obtained by the broth micro dilution method. The maximum antimitotic activity was demonstrated by oils of Cymbopogon martini, Eucalyptus globules and Cinnamomum zylenicumas compared to control, followed by Cymbopogon citratus which showed activity similar to control (miconazole nitrate). The oils of Menthaspicata, Azadirachtaindica, Eugenia caryophyllata, with aniasomnifera and Zingiber of ficinaleex inhibited moderate activity. The oils of Cuminumcyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculumvulg and Elettaria cardamomum demonstrated comparatively low activity against A. niger and A. fumigatusas compared to control. Mixed oils showed maximum activity as compared to standard. These results support the plant oils can be used to cure mycotic infections and

plant oils may have role as pharmaceutical and preservatives (Bansod, et al., 2008). Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals (Sokmen, et al., 1999). Previous in vitro and in vivo investigations suggested that the essential oils could be used as effective antifungal agents (Adam, et al., 1998). The selection of plants for evaluation was based on traditional usage for treatment of infectious diseases (Janssen, et al., 1986; Crespo, et al., 1990) However; there are only limited data available on the antifungal activity of essential oils against human and plant fungal pathogens. Fungal species of the genera Aspergillus, Fusarium and Alternaria have been considered to be major plant pathogens Worldwide (Ghafoor, et al., 1976). The increasing resistance to antifungal compounds and the reduced number of available drugs led us to search for the new alternatives among aromatic plants and their essential oils, used for their antifungal properties. The antifungal activity can be attributed to the presence of some components such as carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity (Knobloch, et al., 1985; Cimanga, et al., 2002). Plant essential oils are potential source of antimicrobials of natural origin. Essential oils obtained from many plants have recently gained a great popularity and scientific interest. Consumer demand for natural preservatives has increased, whereas the safety aspect of chemical additives has been questioned. The plant oil has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for the development of industrial products. In this review is a compilation of updated information on plant essential oils with antifungal properties (Vidyasaga, et al., 2013). Fungi are a natural part of our environment and play an important role in decomposition of organic matter. They can grow on almost any material if enough moisture is available and cause damage to the structures; decorations are also responsible for the indoor air quality (Verma, et al., 2008). In the antifungal activity of essential oils of selected plant species, viz. Piper nigrum Linn., Ricinuscommunis Linn., Cedrusdeodara Roxb. Loud., Syzygiumaromaticum Linn. Merrill & Perry, Eucalyptus globules Labill., Citrusaurantium Linn., C. limon (Linn.) Burm. F. Oleaeuropaea Linn & Menthapiperita Linn. was assayed for fungi toxicity against two genera, viz. Aspergillusniger and Geotrichum candidum. The highest and broadest activity was shown by the essential oils of S. aromaticum, C. limon, C. aurantiumand M. piperita, while the oils of R. communis, C. deodara and O. europaea demonstrated the lowest level of antifungal activity among the oil tested as compared to standard drug, Ketoconazole. The 5-ppm concentration of essential oils of S. aromaticum, C. Limonand M. piperita completely inhibited the mycelia growth of A. niger and G. Candidum to the same extent as 5 ppm of Ketoconazole. However, the 5-ppm concentration of essential oil of C. aurantium completely inhibited the mycelial growth of G. candidumat 10 ppm concentration to the same extent as 5 ppm concentration of Ketoconazole in positive control (Chaurasia, et al., 2011).

3.) Green synthesis of nanoparticles-The keratinophilic fungi and related dermatophytes are also known to synthesize silver nanoparticles. It is reported that silver nanoparticles (SNPs) display effective antimicrobial properties (Panacek et al. 2006). The biosynthesis of SNP using three dermatophytic fungi, i.e. Trichophyton rubrum, T. mentagrophytes and Microsporum canis was reported by Moazeni et al. (2012). It was also found that species within the genus of the fungi have the capability to synthesize SNP with different efficiency and physical property (size and shape). Similarly, uniform, spherical SNP in the range of 20–50 nm was synthesized by C. tropicum (Soni and Prakash 2011).

4.) Leather tanning- Chrysosporium keratinophilum produces a thermostable, keratinolytic alkaline protease when grown in medium containing keratin. When grown in a medium that lacked keratin, it had no enzymatic function indicating the inducibility of the enzyme. The keratinolytic protease had maximum activity at pH 9.0 and a temperature maximum of 90 °C, whereas many other fungi, such as T.

mentagraphytes, Microsporum gypseum, T. rubrum, had maximum activity below pH 9.0. Alkaline proteolytic keratinases are important for leather tanning as a ready means of removing hair from hides.

5.) Bioremediation- Waste removal from slaughterhouses is sometimes ploughed into nearby fields becoming a potential health risk since controlled keratin decomposition by anaerobic bacteria produces large quantities of hydrogen sulfide and ammonia. Current studies are demonstrating the usefulness of the proteases produced by C. keratinophilum in bioremediation of this keratinic waste.

6.) Caffeine degradation- In another study comparing caffeine degradation by four different fungi, Nayak and her colleagues found that C, keratinophilum produces the highest rate of caffeine degradation both in the presence and absence of a nitrogen source. This finding suggests that C. keratinophilum may have commercial use for the decaffeination of coffee pulp, and in the process, it could provide nutrient supplement for animal feed or improved substrates for bioethanol production.

7.) Petroleum hydrocarbon degradation- Keratinolytic fungi are found in the biopile at the refinery, with Trichophyton ajelloi as the most dominant species which may be due to the presence of human population in the vicinity and their activity (Ulfig et al. 2003). The keratinolytic and keratinophilic fungi like Microsporum sp., Trichophyton sp. and Chrysosporium sp. are reported in petroleum hydrocarbon degradation (Davies and Westlake 1979; Ulfig 2000). Biopile soil does not favour the growth of dermatophyte and Trichophyton ajelloi, which is a predominating species rarely cause infection (de Hoog and Guarro 1995); therefore, the biopile bioremediation process for petroleum hydrocarbon degradation can be regarded as safe from the fungal epidemiological point of view.

Conclusion: Occurrence of these fungi has been studied in many environments including various types of soil, birds, bird nests and animal farms. Besides, we do not yet know how many novel strains of keratinophilic fungi are hidden in other habitats with varied physical and chemical environmental conditions and how many of them are pathogenic to both man and animals. The ecological factors responsible for the distribution of fungi and various conditions for the formation of anamorph and teleomorph need more attention. Hence, a systemic distribution pattern of dermatophytes and related keratinophilic fungi from unexplored areas such as heated and self-heated materials, environments with low and extreme pH values, high salt and solute concentrations needs attention.

In more than 65 years, scientists are working in this field and isolated keratinophilic fungi from soil, birds and their environment, healthy animal skin and disease specimens but are not available as in India as reference. The availability of these fungi as specimens will definitely be of immense value to younger scientists with new ideas and the ability to get much better results using biotechnological methods. It is of high importance to have culture collections that carry the standard strains and biodiverse isolates. Taxonomic identification by both classical criteria and molecular biological tools is a must in such culture collection centres.

It is evident that keratinophilic fungi have been prolific producers of numbers of important pharmaceutical compounds and novel skeletons. This makes it very important to have a systematic classification of the different active molecules identified from various species related to their ecological prevalence. This group of fungi is known to degrade keratinic material into peptides and amino acids by the action of enzyme keratinase. Keratinase is used industrially to remove hair from a hide in the preparation of quality leather. Certain genetically modified strains are required which can decompose tons of waste keratin that generates into peptides and amino acids. The hydrolytic products can be used as poultry and animal feed whereas the enzyme keratinase can be used in leather industry. The ability of dermatophytes and related keratinophilic fungi can be explored to produce metallic nanoparticles which can have various pharmaceutical and agriculture applications. Keratinophilic fungi

have the ability to remove petroleum hydrocarbons and can be used as cost-effective way to naturally remediate petroleum-contaminated soils.

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