Dermatophytes: Their taxonomy, ecology and pathogenicity

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Summary Current concepts of anamorphic and teleomorphic states of dermatophytes, sampling techniques and techniques for mating studies are discussed. Ecological groupings and sources of infection; pathogenicity with emphasis on proteolytic enzymes including its biochemical assays, characterization and molecular weight size are reviewed.

Key words Dermatophytes, Taxonomy, Ecology, Pathogenicity

In culture dermatophyte morphology, for purposes of nomenclature, can be divided into two states on the basis of stages in the life cycle, the anamorphic and the teleomorphic states. The anamorph is the state where asexual or somatic reproduction occurs and has a distinct morphology. The teleomorph, on the other hand, is the sexually reproductive ("perfect") state, morphologically (and/or karyologically) differentiated from the anamorph [1]. Sexual reproduction has been demonstrated in a number of species which requires two compatible isolates ("+"and "-") on a suitable medium. A workable classification of dermatophytes is best based on the macroscopic and microscopic morphology of the asexual state in culture and the Emmons [2] classification system emphasizes these characteristics.

Definition

Dermatophytes are a group of morphologically and physiologically related molds some of which cause well-defined infections: dermatophytoses (tineas or ringworm) [3]. They possess two important properties: they are keratinophilic and keratinolytic. This means they have the ability to digest keratin *in vitro* in their saprophytic state and utilize it as a substrate and some may invade tissues *in vivo* and provoke tineas. However, their morphology in the parasitic growth phase is different from the morphology exhibited in culture or *in vitro.*

Anamorphic states

Dermatophytes as saprophytes reproduce asexually by simple sporulation of arthro-, micro- and macroconidia produced from specialised conidiogenous cells. Dermatophyte species also exhibit a range of vegetative structures with typical arrangement on hyphae, chlamydospores, spirals, antler-shaped hyphae (chandeliers), nodular organs, pectinate organs and racquet hyphae [2,4]. In addition, some physiological characteristics based on nutritional requirements [4-8]such as vitamin deficiency can be used to identify some dermatophytes.

Most dermatophyte colonies develop forms and pigmentation which can allow a presumptive identification of that dermatophyte species. The appearance of a fungus colony depends on the medium used, but for comparative purposes Sabouraud dextrose agar (SDA) medium is conventionally used to obtain colonies which can be compared to others reported in the literature [4]. Ajello [4] lists five important colony characteristics to look for in presumptive identification of a dermatophyte culture when it is one to three weeks old: (1) rate of growth (2) general topography (flat, heaped, regularly or irregularly folded) (3) texture (yeast-like, glabrous, powdery, granular, velvety or cottony) (4) surface pigmentation and (5) reverse pigmentation.

Based on the above criteria, particularly on differences in conidial morphology, dermatophyte species can be classified into three genera within the Fungi Imperfecti (or Deuteromycotina) namely: *Epidermophyton*, *Microsporum*, and *Trichophyton* [2]. The studies of Cole and Samson [9] have shown that the ontogeny of the holothallic conidia of *Microsporum* and *Trichophyton* is essentially the same. Their only difference is the macroconidial cell-wall thickness and presence of echinulations in *Microsporum* species which are absent in *Trichophyton* species [10-11].

However, there has been some controversy in the broad classification of some dermatophytes. Benedek [12] felt that the genus *Achorion* should have been retained [2] as was *Epidermophyton* simply because of its "established usage" and that Emmon's proposal cannot be considered a natural classification. The proposed system [13,14] distinguished the genera *Epidermophyton*, *Microsporum*, *Trichophyton*, *Microides* and *Keratinomyces*. Ajello [15] rejected Vanbreuseghem's genus *Microides* based on the similarity in morphology of *M. interdigitalis* to *T. mentagrophytes*. He proposed that *M. interdigitalis* should be considered a variety of *T. mentagrophytes*, to be known as *T. mentagrophytes var. interdigitale* while Emmons [2] considered *T. interdigitale* to be a synonym of *T. mentagrophytes*.

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One particularly controversial genus is *Keratinomyces*. This genus, with its species *K. ajelloi*, was established [16] but was modified [17] to *T. ajelloi* due to the earlier inadequate treatment of the genus by not providing essential facts that differentiate *Keratinomyces* from the genus *Trichophyton*. An important reason for the transfer was the observation that *K. ajelloi* apparently produces microconidia [18]. The transfer was further supported by the fact that *K. ajelloi* produced cleistothecia of the genus *Arthroderma* in which the sexual states of *Trichophyton* were classified [19].

However, antigenic studies have shown that *T. ajelloi* has little similarity to *Trichophyton* species, emphasing the need to retain its original designation. Additionally *K. ajelloi* has thick smooth cell walls while *Trichophyton* species have thin walls [13,14,16,20].

There are also disagreements of the species concept in certain groups. For example, Vanbreuseghem *et al*., [14] separated *Microsporum langeroni* [22] and *M. rivalieri* [21] from the classic *M. audouinii* Gruby, 1843, a move which has not been favoured by several American and European workers.

The principal classification systems of dermatophytes, compared to that of Sabouraud is presented below in Table 1.

Table 1. Generic subdivisions of dermatophytes.

Sabouraud	Vanbreuseghem *et al.*	Emmons, Ajello *et al.*
Epidermophyton Lang, 1879	*Epidermophyton*	*Epidermophyton*
Microsporum Gruby, 1843	*Microsporum* (+ *Achorion gypseum*)	*Microsporum* (+ *A. gypseum*)
Achorion Remak, 1845	*Trichophyton* (- *A. gypseum*)	*Trichophyton* (- *A. gypseum*)
Trichophyton Malmsten, 1845		
endothrix	*Trichophyton*	*Trichophyton*
ectothrix megaspore	*Trichophyton*	*Trichophyton*
ectothrix microides	*Microides*	*Trichophyton*
Unknown	*Keratinomyces*	*Trichophyton*

(- = excluding A. *gypseum*; + = including A. *gypseum*)
Modified from Vanbreuseghem *et al.* [14]

Besides the traditional mycological criteria of identification, other techniques applied more recently have been based on analyses of serological antigens [23], comparison of DNA base compositions [24,25] fatty acid composition [26] and enzyme isoelectric focusing [27,28]. Such studies and others have supported the differentiation of morphologically and physiologically similar species e.g. *M. canis*, *M. equinum*, *M. distortum*, *T. kuryangei* and *T. megninii* [27,28]. Radiolabelled DNA hybridization techniques employed [25] as a taxonomic tool, supported the present mycological grouping of dermatophytes based on the limited number of species examined.

Additional developments include the use of electrophoretic protein patterns used in the study of *M. canis* and reported to be able to distinguish between genetically similar strains of *M. canis* [29,30]. Also, Mochizuki *et al*., [31] used mitochondrial DNA restriction analysis to investigate the taxonomical relationship between *T. interdigitale* (*T. mentagrophytes var. interdigitale*) and other members of the *T. mentagrophytes* complex.

Current concepts of the anamorphic state

On the basis of anamorph morphology, two species of *Epidermophyton*, approximately 18 species of *Microsporum* (Table 2) and 25 species of *Trichophyton* (Table 3) are considered valid members of these genera.

Table 2. The principal members of the genera *Epidermophyton* and *Microsporum.*

Epidermophyton Sabouraud, 1910	*M. fulvum* Uriburu, 1909
E. floccosum (Harz, 1870), Langeron & Milochevitch, 1930	*M. gallinae* (Megnin, 1881), Grigorakis, 1929
E. stockdaleae, Prochaki & Engelhardt-Zasada, 1974	*M. gypseum* (Bodin, 1902), Guiart & Grigorakis, 1928
Microsporum Gruby, 1843	*M. magellanicum* Coretta & Piontelli, 1977
M. amazonicum Moraes, Borelli & Feo, 1967	*M. nanum* Fuentes, 1956
M. audouinii Gruby, 1843	*M. persicolor* (Sabouraud, 1910), Guiart & Grigorakis, 1928
M. boullardii Dominik & Majchrowicz, 1965	*M. praecox* Rivalieri, 1954
M. canis Bodin, 1902	*M. racemosum* Borelli, 1965
M. cookei Ajello, 1959	*M. ripariae* Hubalek & Rush-Munro, 1973
M. equinum (Bodin, 1902), Guegen 1904	*M. vanbreuseghemii* Georg, Ajello, Friedman & Brinkman, 1962
M. distortum Di Menna & Marples, 1954	*M. ferrugineum* Ota, 1921

Modified from Ajello [17,32], Vanbreuseghem *et al.* [14], Howard [33] and Rippon [34].

Table 3. Members of the genus *Trichophyton*[+] (Malmsten, 1845).

T. ajelloi (Vanbreuseghem, 1952), Ajello, 1968	*T. phaseoliforme* Borelli & Feo, 1966
T. concentricum Blanchard, 1895	*T. rubrum* (Castellani, 1910)
T. equinum (Matruchot & Dassonvile, 1898), Gedoelst, 1902	*T. schöenleinii* (Lebert, Gedoelst, 1902), Langeroni & Milochevitch, 1930
T. flavescens Padhye & Carmichael, 1971	*T. simii* (Pinoy, 1912), Stockdale, Mackenzie & Austwick, 1965
T. georgiae Varsavsky & Ajello, 1964	*T. soudanense* Joyeux, 1912
T. gloriae Ajello, 1967	*T. terrestre* Durie & Frey, 1957
T. gourvilii Catanei, 1933	*T. tonsurans* Malmsten, 1845
T. longifusus (Florian & Galgoczy, 1964), Ajello, 1968	*T. vanbreuseghemii* Rious, Jarry & Juminer, 1964
T. mariatii Ajello & Cheng, 1967	*T. verrucosum* Bodin, 1902
T. megninii Blanchard, 1896	*T. yaoundei* Cochet & Doby-Dubois, 1957
T. mentagrophytes (Robin, 1853), Blanchard, 1896	
var. *interdigitale* Priestley, 1917	
var. erinacei Smith & Marples, 1963	
var. quinckeanum (Zopf, 1890), Macleod & Muende, 1940	

[+] Various authors differ in their treatment of certain of the species.
Modified from Ajello [17,32], Vanbreuseghem *et al.* [14], Howard [33] and Rippon [34].

The main features distinguishing the three dermatophyte genera are:

Epidermophyton

The genus is characterised by large macroconidia which are thin-walled, multicellular, club-shaped and clustered in bunches. Microconidia are not produced. The genus' features are based on *E. floccosum* [2].

Microsporum

The genus produces both micro- and macroconidia. Macroconidia are multiseptate, with a thin or thick echinulate cell wall, spindle shaped and may be numerous or scarce. However, the essential distinguishing feature of this genus is the echinulations on the macroconidial cell wall. The thickness of the cell wall and shape varies depending on the species. Microconidia are pyriform, about 2-3µm. The type species is *M.audouinii* Gruby, 1843.

Trichophyton

This genus produces smooth walled macroconidia and microconidia. Macroconidia are thin walled and cigar-shaped. Microconidia may be pyriform 2-3µm or irregular in form. Some species rarely produce macroconidia. The type species is *T. tonsurans* Malmsten, 1845.

Teleomorphic states

The existence of a sexual phase in the growth cycle of dermatophytes was described when cleistothecia with ascospores were obtained by cultivating *M. gypseum* on soil baited with feathers [35-36]. The fungus was named *Gymnoascus gypseus* [33,37]. This confirmed Nannizzi's work [37-39] and at the sametime Dawson and Gentles [19] demonstrated the existence of a sexual stage of *Trichophyton terrestre* Durie and Frey, which they named *Arthroderma quadrifidum.*

Since then the perfect states of a number of dermatophytes have been discovered and this has resulted in some changes to the classification of these fungi [10,13,32,40]. All are members of the subdivision Ascomycotina which includes all fungi that, after nuclear fusion and chromosomal reduction, proceed to form asci and ascospores. They belong to the Class Plectomycetes, Order Onygenales [41].

The production of asci occurs inside an ascocarp (cleistothecium or gymnothecium [42]. The wall (peridium) of the cleistothecium is composed of loosely interwoven, thin-walled, light coloured hyphae, which is characteristic of the family Arthrodermataceae.

In the genus *Arthroderma* Berkeley, 1860 the outer cells of the peridial hyphae are short and markedly swollen at each end, appearing constricted in the middle. The swelling may be symmetrical or mostly on the outward side of the curved hyphae. The swellings are thick-walled and markedly spiny, while the short intervening constricted portion is thin walled, smooth and inconspicuous [42].

The second genus *Nannizzia* [39] is characterised by the peridial hyphae being branched in a verticillate manner and composed of thick-walled, aseptate, hyaline cells with one or more symmetrical constrictions. There are numerous free ends and various appendages - ring, straight or loosely coiled hyphae and spiral hyphae [39].

It has been proposed that the two genera should be unified into one genus: *Arthroderma* Berkeley, 1860 because the morphological differences are not significant to warrant a new genus [14,43]. But Stockdale [39]had argued that the branching pattern of *Arthroderma* is consistently dichotomous and never verticillate while *Nannizzia* is commonly verticillate, sometimes dichotomous and rarely uncinate. Stockdale [39] considers these differences of generic significance while McGinnis [11] and Weitzman *et al.* [43] regard them as of little significance. Another important difference is the fact that crossmating between the two genera has never been reported to occur.

Mating studies have led to the discovery of the perfect states of a number of dermatophyte species. Some of the anamorphic states have been found to represent a complex of species, e.g. the *M. gypseum-fulvum* complex, the *T. terrestre* complex and the *T. mentagrophytes* complex.

The *M. gypseum-fulvum* complex represents three sexual dermatophyte species, namely *Arthroderma incurvata* [39], *A. gypsea* and *A. fulva* [44]. Similarly, the *T. terrestre* complex represents three sexual states, *A. quadrifidum*, *A. insingulare*, and *A. lenticularum* [42,45]. Both *A. quadrifidum* and *A. insingulare* have been isolated from soil, animal hair and feathers as *T. terrestre* [46-48].

The *T. mentagrophytes* complex represents two sexual dermatophyte species, *A. vanbreuseghemii* [49] and *A. benhamiae* [50]. *T. mentagrophytes var. interdigitale*, one of the most prevalent species and of great importance to public health, is an imperfect species resembling morphologically the conidial state of *A. vanbreuseghemii*. Mitochondrial DNA restriction enzyme mapping has shown that the restriction profiles of strains of *T. mentagrophytes var. interdigitale* and *A. vanbreuseghemii* are identical [31], reinforcing a linkage of the two species, as postulated from its morphology [51]. *T. mentagrophytes var. interdigitale* is therefore considered a member of the *T. mentagrophytes* complex.

Takashio [40,49,52-54] has suggested that the species *A. benhamiae* can be divided into two races, one Americano-European and one African, with two varieties, *var. caviae* and *var. erinacei* respectively. The variety *erinacei* has two mating types: the "+" corresponding to *T. erinacei* [45] or *T. mentagrophytes var. erinacei* [56], the "-" species having been isolated from the African hedgehog [57]. *A. benhamiae var. caviae* has only the "-" mating type.

To date, 11 conidial species of *Trichophyton* (*Arthroderma*, Table 4) and 10 species of *Microsporum* (*Arthroderma*, formerly *Nannizzia*, Table 5) are known to reproduce sexually (De Vroey, personal communication).

Table 4. *Trichophyton* species with a known teleomorphic state.

Teleomorph	Anamorph
Arthroderma Berkeley, 1860; Malmsten, 1845	*Trichophyton*
A. curreyi Berkeley, 1860	*not named*
A. tuberculatum Kuehn, 1960	not named
A. benhamiae Ajello & Cheng, 1967	*T. mentagrophytes* var. *mentagrophytes*
A. ciferrii Varsavsky & Ajello, 1964	*T. georgiae*
A. flavescens Rees, 1967	*T. flavescens*
A. gertleri Böhme, 1967	*T. vanbreuseghemii*
A. gloriae Ajello, 1967	*T. gloriae*
A. insingulare Padhye & Carmichael, 1972	*T. terrestre*
A. lenticularum Pore, Tsao & Plunkett, 1965	*T. terrestre*
A. quadrifidum Dawson & Gentles, 1961	*T. terrestre*
A. simii Stockdale, Mackenzie & Austwick, 1965	*T. simii*
A. uncinatum Dawson & Gentles, 1961	*T. (K.) ajelloi*
A. vanbreuseghemii Takashio, 1973	*T. mentagrophytes* var. *interdigitale*

Modified from Ajello [17,32], Vanbreuseghem *et al.* [14], Howard [33] and De Vroey (personal communication)

Table 5. *Microsporum* species with a known teleomorphic state.

Teleomorph	Anamorph
Arthroderma Stockdale, 1961	*Microsporum* Gruby, 1843
A. borelli Padhye & Ajello, 1975	*M. amazonicum*
A. cajetani Ajello, 1961	*M. cookei*
A. fulva Stockdale, 1963	*M. fulvum*
A. grubyia Georg, Ajello, Friedman & Brinkman, 1962	*M. vanbreuseghemii*
A. gypsea Stockdale, 1963	*M. gypseum*
A. incurvata Stockdale, 1961	*M. gypseum*
A. obtusa Dawson & Gentles, 1961	*M. nanum*
A. otae Hasegawa & Usui, 1975	*M. canis*
A. persicolor Stockdale, 1967	*M. persicolor*
A. racemosa Rush-Munro, Smith & Borelli, 1970	*M. racemosum*
A. corniculata Takashio & De Vroey, 1982	*M. boullardii*
A. cookiella De Clercq, 1983	not named

Modified from Ajello [17,32], Vanbreuseghem *et al.* [14], Howard [33], De Vroey (personal communication)

Techniques for mating studies

All dermatophyte species with a known sexual stage are heterothallic. Matings of compatible strains of the same dermatophyte species are able to produce ascocarps containing ascospores which usually develop in approximately one month. The appropriate *in vitro* techniques have made the identification of dermatophytes more accurate and reliable [58].

In the early studies on sexual reproduction in dermatophytes the substrate used was either sterilised or unsterilised soil with keratin, such as hair or feathers, sprinkled on top [59]. Dawson *et al*. [60] studied species of formerly *Nannizzia* and *Arthroderma*, and found that sterilised soil was unsatisfactory while unsterilised soil sprinkled with horse mane or tail hair is very satisfactory in stimulating sexual reproduction among the dermatophytes. Human hair was found to be a poor keratin bait in mating studies.

Although one can obtain cleistothecia of dermatophytes in soil, it is not a satisfactory medium for mating studies because the keratin becomes colonised by hyphae, making it difficult to observe the reaction between the two colonies. Mating is inhibited on media containing high concentrations of nutrients supporting good vegetative growth [61]. De Vroey [62] devised a niger-seed medium which supported cleistothecium formation by *M. gypseum*. Weitzman and Silva-Hunter [63] formulated an oatmeal agar with salts and with or without tomato paste. Another medium used for mating studies is that of Takashio *et al*. [64] composed of ground *Guizzotia abyssinica* seeds with salts.

The first observation of "sexual stimulation" as opposed to true mating between different dermatophyte species was made following the discovery of *A. simii*, the perfect state of *T. simii* [44]. A mating type can be revealed by the proliferation of white fluffy hyphae, often with the formation of ascocarp initials, which occur when a different dermatophyte species of unknown mating type makes contact with *A. simii* of the opposite mating type [58]. This technique has significantly increased our ability to determine mating types.

Once fertile cleistothecia have been obtained, single ascospore culturing can be performed especially for further mating studies and for taxonomical purposes [55,65,66]. Dermatophyte colonies started from single spores produce a uniform and consistent appearance which is important for identification [4] and the technique can also be used to rejuvenate degenerating sexual strains [55].

To obtain ascospores from an ascus, the standard technique has been the use of a dissecting De Fonbrune micromanipulator [58,65,66]. Another technique is a manual method [65] which employs a fine needle to isolate a mature cleistothecium, after first rolling it on a 4% solid agar surface to remove conidia, soil particles or hair depending on the medium used for mating. The cleaned cleistothecium can be transferred to a second agar plate where it is crushed in a loopful of sterile water. Mature ascospores, as determined by microscopic examination, are subcultured before or after germination [67].

Ecological groupings and sources of infection

The dermatophytes have been divided [67] into three ecological groups: geophiles, zoophiles and anthropophiles. Probably some of these fungal pathogens in evolving from their natural habitat in the soil, have developed host specificity, resulting in these three groups. Individual dermatophytes differ considerably in their host range and importance as agents of disease in man and animals. The differences in host specificity has been attributed to the differences in keratin of the hosts [68].

Geophiles are primarily soil-inhabiting and only rarely encountered as agents of ringworm, with the exception of *M. gypseum*.

Zoophiles are essentially animal pathogens, although they may cause infection in humans.

Anthropophiles are restricted to man, very rarely infecting animals.

Geophiles

Geophiles exist as saprophytes in the soil and have the ability to competitively colonise keratinous substrates successfully. Their distribution appears to relate to the distribution of available keratin [47,69,70]. But the distribution is also influenced by the pH of the soil and generally they prefer a near neutral pH [71].

A few geophiles do have the additional capacity to cause ringworm in some species of animals, including man. These dermatophytes are generally contracted directly from soil containing a high number of spores and are only rarely transmitted from man to man or lower animals to man [32,70]. For example, *M. nanum*, which causes ringworm in animals, especially pigs, is mainly associated with surroundings having pigs [70,72,73]. The proof for its geophilic existence was provided by observation of macroconidia in soil [32], since it is well known that these spores are not formed on infected animals.

But the principle virulent geophilic dermatophytes are members of the *M. gypseum-fulvum* complex. This complex has been well documented as a pathogen in man and animals. According to Georg [74], soil isolates of *M. gypseum* compared to animal isolates have a low pathogenicity and only very virulent strains are able to establish infection. Alternatively, strains of low infectivity may increase in virulence after "passage" through a host of low resistance. Of the three dermatophyte strains, pathogenicity studies with laboratory animals have shown

A. fulva to be the least pathogenic, while no notable differences have been observed with *A. gypsea* and *A. incurvata* [75,76]. The distribution of the *M. gypseum-fulvum* complex is world-wide [47,77-79].

For pathogenic geophilic dermatophytes, infective propagules originate from saprobic sources, are transmitted either directly or indirectly, and are referred to as saprobic-parasitic (S-P) infections [3,70]. This mode of infection is common for *M. gypseum*, where the source of most infections in man and animals is the soil [4]. In children facial ringworm by *M. gypseum* can follow recreational exposure to soil-borne propagules of this fungus [3]. Occupational exposure is illustrated by reported cases in gardeners [3] and small epidemics observed in, for example, cucumber growers [80,81]. The macro- and microconidia, ascospores and other propagules are produced during the saprophytic growth of dermatophytes on keratin in soil or other biotopes (e.g. birds nests in the case of *M. ripariae*) [70] and it is these which form the potential inoculum.

The *T. terrestre* complex is considered to be non-pathogenic [33], although human infections by *T. terrestre* have been reported [82] and experimental animal infections have also been successfully induced [83]. Other geophilic dermatophytes include *M. cookei* and *T. ajelloi* which are non-pathogenic. *Microsporum cookei* is a geophile with a global distribution, often isolated from soil and also from rodents and other animals not showing any clinical symptoms of ringworm [84,139,159]. Human infections by *M. cookei* have rarely been reported [86,87]. *T. ajelloi* is commonly found in colder climates but is sporadic in hot climates [47], possibly because higher temperatures inhibit its growth. The fungus has been found to be more often associated with acid soils than with alkaline soils [47].

Zoophiles

Zoophilic species are basically animal pathogens, often with a single preferred animal host or very limited host range, outside which they are found only in exceptional circumstances [88]. Zoophilic dermatophytes rarely grow actively as saprophytes but survive in a dormant state on contaminated materials of animal origin.

M. canis, *T. verrucosum* and *T. mentagrophytes* are common agents of ringworm in animals but are also frequently associated with human infection. The amount of literature on human infections due to the three dermatophytes is enough evidence of their human affinity. Of the three, *M. canis* is the best documented [89-92]. This is mainly because it causes a lot of scalp ringworm in children [88]. *M. canis* commonly infects pet animals and especially cats and dogs which shed infective particles into the domestic environment and contact with this results in familial infections [3]. Like other types of ringworm, young children particularly in the age range 5-14 years are more susceptible to infection than adults. Similarly, kittens and puppies are more susceptible to ringworm than adult animals [93]. *M. canis* is also known to cause ringworm in horses, monkeys, apes and chinchillas [14].

Another dermatophyte species closely related to *M. canis* is *M. distortum*, known to cause ringworm infections in monkeys, dogs and cats. It has been reported to occur mainly in New Zealand [94,95], Australia and the United States [14]. It is now regarded as a variety of *M. canis*. *T. verrucosum*, on the other hand, is a common cause of *tinea* in cattle. It has also been reported in donkeys, dogs, goats, sheep and horses [48]. Close contact by man with infected animals and their fomites leads to contracting the fungus. It is also generally accepted that in countries with cold winters where housing of the animals is required, the incidence of *T. verrucosum* rises in both animals and humans at that time of the year [88]. Cattle breeders and veterinarians, occasionally suffer from tineas due to *T. verrucosum*, which is mainly an agent of inflammatory skin and scalp lesions (kerion). Members of the *T. mentagrophytes* complex (with the exception of *T. mentagrophytes var. interdigitale*) are transmitted from wild rodents and the prevalence of human infections due to this fungus is known to be higher in rural areas where there is a reservoir of rodents e.g. North America and Europe [96-98]. *T. mentagrophytes* has occasionally been isolated from the soil [45,99] where it can survive for several months.

Anthropophiles

Anthropophilic species are primarily adapted for parasitism of man, but some species occasionally cause ringworm in animals. For example, *T. rubrum* has been reported to have caused an infection in a dog [74,100]. Anthropophilic dermatophytes are mainly associated with community life. Since transmission is man to man, contracting the disease therefore requires human contact. The spread of anthropophiles is more common in communities like schools, barracks, prisons and the family [93,101]. In concentrated communities, the use of facilities such as shower-rooms, and common headgear leads to rapid spread of infection.

Four of the *Microsporum* species, according to Vanbreuseghem, can be distinguished from each other on clinical, epidemiological and mycological grounds: *M. audouinii*, *M. langeroni*, *M. rivalieri* and *M. ferrugineum*. *M. langeroni* [22,102,103] has been separated from the classic *M. audouinii* by its geographic region (restricted to Central Africa) and unlike *M. audouinii* can cause *tinea corporis* (ringworm of the glabrous skin) and can be inoculated to produce experimental lesions in guinea pigs. However, most mycologists consider *M. langeroni* and *M. rivalieri* as variaties of *M. audouinii*.

Of the anthropophilic *Trichophyton* species *T. rubrum* is a very common cause of *tinea unguium, cruris*, and *pedis* [3,34,101,104]. *T. rubrum* very rarely invades hair *in vivo*. The distribution of *T. rubrum* is global, cutting across all populations and ethnic groups [3,34]. It is a dermatophyte becoming more prevalent among urban populations, especially in developed countries, due mainly to the "modern" way of life such as the wearing of occlusive shoes, which maintain heat and humidity [93]. It is also able to adapt to its environment in a way other species can not emulate [105]. In India, *T. rubrum* causes *tinea corporis* in women and *tinea cruris* in men due to the sari (worn by women around the waist) and the dhobie (loin cloth) worn by men, both of which are tight-fitting [34,68]. *T. rubrum* is also known to cause chronic forms of infections and it has been suggested that the amino acid composition of perspiration may predispose individuals to chronic infection. Certain amino acids are considered "inducers" of *T. rubrum* infections [68]. Pushkarenko and Pushkarenko [106] in their investigations found patients with chronic *T. rubrum* had a higher than normal content of leucine, lysine, asparagine and histidine in their sweat. Rippon and Scherr [107] were able to induce arthroconidia formation in *T. rubrum* at 32°C and 37°C with a medium containing a high amino acid concentration.

T. mentagrophytes var. interdigitale, a member of the *T. mentagrophytes* complex, is essentially a cause of *tinea pedis* and *tinea cruris*, and does not invade hair *in vivo* [108]. The infection of the skin of the foot usually

originates in the interdigital clefts, sometimes spreading to the soles and dorsum and occasionally the ankles and leg and ultimately to the toenails, resulting in *tinea unguium* [109].

E. floccosum, the only pathogenic species in this genus is a common cause of *tinea pedis* and *tinea cruris* (eczema marginatum of Hebrae) affecting inguinal areas, particularly in males, although some infections do occur in females [14,33,101,109]. Sometimes it is also responsible for *tinea unguium* infections. But *E. floccosum* is not known to invade hair [14]. Other anthropophilic dermatophytes are of limited geographical distribution (apart from *M. audouinii var. audouinii*). Humans are the common host. It should be noted that a number of other species have been described but are of very limited distribution [110].

Anthropophilic dermatophytes are commonly transmitted by infectious propagules originating from active lesions of another individual. This mode of transmission is called the parasitic - parasitic (P-P) mode of infection [3,70]. This transmission can be direct or else indirect through an intermediary item such as hairbrushes, combs, clothes, towels and bedding [111] or even from contaminated furniture or dressing rooms. In addition, transmission can occur from carriage of pathogenic dermatophytes on "normal" human scalps or "healthy" animals [92,97]. In the case of "carriers", "carriage" could be of either or both: propagules derived from infected animals or propagules from the saprobic growth of the fungus [112].

The environmental occurence of dermatophytes is well documented. Indirect transmission through the environment for all types of *tinea* infections can be acquired by contact with a significant inoculum. Such transmission has often been reported to play a prominent role [70,111,113,114]. Using various indirect sampling techniques, a number of authors have demonstrated the presence of pathogenic dermatophytes in the environment (e.g. Gentles' "velvetpad", Mackenzie's "hairbrush" and "gauze pad" methods and Mariat's "carpet-square technique"). Examples of dermatophyte species isolated include *M. audouinii* [115], *T. tonsurans* [111], *M. canis* [113,116] and *T. mentagrophytes*, all of which must have been derived from fomites contaminated from active infections.

Sampling techniques for dermatophytes

Keratin-baiting technique

Fungi from different ecological groups require different sampling methods and isolation techniques [117-119]. Prophetically, Sabouraud (1910) expressed an opinion that soil may represent a continuous and inexhaustible reservoir of dermatophytes being a natural habitat for their saprophytic life. The early failure to recognise the existence of soil keratinophilic fungi was due to a lack of an isolation technique for these fungi. The introduction of the hairbaiting technique [16] led the way to many other investigations. The technique has firmly established the source of some infections, but only for some dermatophytes, such as those by *M. gypseum* [81]. Vanbreuseghem's method was essentially that which Nannizzi used when he reported the discovery of a sexual stage of *M. gypseum* in 1927 [81]. The technique has now become a standard method for isolation of keratinophilic fungi from the soil [47,83,84,120-122].

Chmel [121] considers fungi such as dermatophytes to have started as saprophytes on dead decaying parts of plants or other organic substances. Litter, therefore, was and is the main reservoir for keratinophilic fungi. Gordon [123] demonstrated by direct visualisation that *M. gypseum* existed in the soil in the form of spores (macroconidia). The same has also been demonstrated for *M. nanum* [124], *M. cookei*, *T. ajelloi* and *T. terrestre* [125].

Since fungal communities exist in different forms in the environment, and because of the wide range of litter components, different methods are used for sampling and processing of soil for different fungal groups. The aim of an investigation and the type of litter are important in the choice of sampling procedures. In the case of keratinophilic fungi, surface sampling seems to be preferred. Some investigators before collecting the soil remove the top layer and then representative samples are scooped from the lower layers of soil [121,126].

Sub-sampling may be necessary as it may not be possible to process the entire sample. The size of the subsample is variable, as there is no recommended standard amount or method. This may further reduce the chances of isolating keratinophilic fungi from the soil. Some authors have used 50g per Petri dish without replication [47]. Soil is best processed on the day of collection. If storage is necessary, temperatures of 0-4°C are adequate.

However, there is currently no clear-cut standard on a permissible storage time for different soils, but according to Parkinson *et al*. [127]"most investigators tend to have a practical outlook on this problem". Somerville and Marples [128] stored unenriched soil samples in plastic bags at laboratory temperature for 11 months before reprocessing. They found that storage alone did not significantly affect recovery rate, at least of *M. gypseum*. Marples [47] processed soil samples immediately following collection. Studies by Somerville and Marples [128] using an enrichment technique in which sterilised cowhorn was incorporated with the bulk soil sample, kept the samples for 14 days before processing to isolate keratinophilic fungi. This selectively increased recovery of *M. gypseum* compared to the controls originating from the same sampling sites. Rebell and Taplin [58] consider the enrichment technique to favour some keratinophilic fungi more than others and therefore leads to a false impression of the balance of keratinophilic fungi initially present in the soil. Bakerspigel [129] found that many fungi remain viable in sterilised soil for 1 to 4 years. Loam soil was the most satisfactory and was far superior to Sabouraud's agar for storage of some fungi e.g. *E. floccosum*.

While the technique for the isolation of keratinophilic fungi has remained largely the same, there are variations in the use of the baiting material. Chicken feathers, sheep's wool [72], human hair [4,47] horse hair [83,121,128], hedgehog quill, guinea pig hair [47,128] or a mixture of human and horse hair and chicken feathers [122] have all been used. According to Otcenasek [130], differences in host specificity in spontaneous infections may be due to differences in the affinity of the dermatophytes for the various kinds of keratin. Keratin substances in the feathers of birds and hair of animals are known to be different in their biochemical composition, e.g. in their content of nicotinic acid, cystine, arginine, and tryptophane [131]. The classical work [7] expanded by Philpot [8] and Shadomy and Philpot [132] also demonstrated that some dermatophytes of the *Trichophyton* and *Microsporum* genera have specific nutritional requirements. It is not known whether differences in affinity for keratin bait *in vitro* is related to preferential stimulation of dermatophytes with a specific nutritional requirement present in the type of hair provided.

Hairbrush technique

Sampling of animal coats which may carry keratinophilic fungi is usually based on a modification of the "hairbrush technique"[111,133]. This technique has been used for studying the carrier state of both animals and humans for keratinophilic fungi [114,134-137]. The animals are brushed over the back, shoulders, sides, hindquarters and legs and the brushes transported to the laboratory in brown paper [135] or plastic bags [92,138,139]. The brushes are pressed into a suitable medium.

Gentles *et al*. [91], Katoh *et al*. [138] and Simpanya and Baxter [139] have successfully used the technique for isolation of keratinophilic fungi from cats and dogs. After use the brushes are sterilised in 0.1% chlorhexidine ("Hibitane") solution. Connole [135] immersed the brushes for 30 minutes while Simpanya and Baxter [139] immersed them for 24 hours before cleaning with soap and hot running water. Any residual hair was then picked off with forceps. The brushes with hair samples are normally cultured the same day. If storage of samples is necessary, they can be kept at laboratory temperature until the next day.

Sampling of clinical material

If a Wood's lamp (an ultraviolet light which emits light at 360nm) is available, hair may first be examined in a darkened room before sampling. Hair infected by some species of *Microsporum* particularly *M. canis*, even when no clinical symptoms are apparent, emit a greenish yellow fluorescence [4,14,33,67,109] However, the Wood's lamp is of no value for detecting infection in hairs parasitised by *Trichophyton* species which do not produce fluorescence, except *T. schöenleinii* infected hairs which may emit a dull greenish fluorescence [14,33]. *E. floccosum* never invades hair *in vivo* [14], nor do infected skin scales and nails fluoresce. Medication, artificial fibres and natural secretions can also obscure true fluorescence. However, mycologically positive hair for *M. canis* have been reported in the USA and UK as not producing fluorescence [109]. Ajello *et al*. [4] have also reported that hairs from animals infected with *M. gypseum* do not produce fluorescence.

For suspected cases of skin or hair infection, direct microscopy can provide a first indication of infection. It allows direct examination for fungal elements, septate hyphae and spores. To clear the keratin and expose the fungal elements, 10-20% KOH is used [4,14,67,101], although chlorallactophenol can also be used alone or with a dye. Microscopic examination using a low intensity light source ensures a good contrast between the fungus and the keratinous material. Fungi from infected materials can then be isolated using SDA containing penicillin/streptomycin or chloramphenicol and actidione incubated in the dark at 25-30°C [4,67]. The incubation period generally varies from 1 to 4 weeks on SDA medium.

Pathogenicity

The dermatophyte species within the three genera *Epidermophyton*, *Microsporum* and *Trichophyton* differ in their pathogenicity *in vivo*. While all species invade the stratum corneum of the epidermis and the follicular ostium of hairs, different species vary widely in their capacity to invade hair and nail. The reasons for this observed tissue specificity are unknown, but are thought to be related to specific nutritional requirements or the enzyme production of individual organisms.

Role of proteolytic enzymes in pathogenicity

Self synthesised enzymes serve fungi in a number of ways. They enhance survival in tissues by chemically or physically altering the immediate environment and they act directly by digesting host proteins, thus providing a source of nutrition. Therefore the pathogenic potential of a fungal agent depends on its ability to produce enzymes. In turn variations in enzymatic potential of a fungus may be responsible for differences in the pathogenic effects of various strains [140].

Studies by Rippon and Varadi [141] demonstrated that certain strains of *Microsporum* and *Trichophyton* species produce enzymes able to solubilise the keratin and related fibrous proteins found in skin, hair, claws and hoof. Keratin, elastin and collagen make up 25% of the body weight of mammals. The role of enzymes as virulence factors has also been inferred as they are often found in the tissues of infected animals [142,143]. It has been suggested that they may play a role in breaking down part of the infected tissue(s). Other studies [144,145] have suggested that differences in virulence of mating types may be related to differences in their ability to produce extracellular enzymes such as elastase. However more studies are needed to test the hypothesis [59,146].

Biochemical enzyme assays

The proteinase assays performed in pathogenicity studies are primarily aimed at determining if relative enzyme activities revealed by microorganisms can serve as indicators of virulence. The underlying assumption made in determining enzymatic activity by biochemical assays is that the quantitative variation in, for example, proteolytic and/or elastinolytic activity, is responsible for differences in virulence between strains [147,148]. The identification of such virulence factors has been dependent on, and limited by, the ability to mimic host environmental factors in the laboratory. It is assumed that the expression of most virulence factors is regulated by environmental conditions *in vitro* that presumably reflect similar cues present in the host tissue.

Enzymes as virulence factors are hypothesized to act (a) when the pathogen comes into contact with the target cells and/or (b) at a distance as diffusible soluble product(s) of the pathogen, which may enhance its survival in tissues by chemically or physically altering the immediate environment, or by directly digesting host proteins [85].

Studies employing saprophytic and pseudo-parasitic morphologies have revealed similar keratinase expression for *M. canis* and *M. cookei* isolates [149]. This lends support to the hypothesis [150] that keratolysis probably does not play a critical role once the mycelium is established in the epidermis. Other authors have reported similar observations and suggested a restricted substrate specificity as a possible reason, or the removal of some accessory proteins capable of splitting disulfide bonds present in keratinized proteins [151,152]. This theory has been partly supported by Kunert's studies suggesting that proteolytic enzymes are more active in the presence of reducing agents and of sulphite in particular. He proposed the reduction of sulfide as the reason, since the reduction of 20% of disulphide bonds resulted in enhanced wool hydrolysis.

It should be noted that the capacity of microorganisms to produce enzymes (i.e. proteinases, elastases and keratinases) is normally estimated under controlled conditions of laboratory culture. It is therefore quite likely that some strains may be able to produce varying amounts of enzyme(s) under growth conditions available on the skin of a patient. For example, studies have demonstrated that

leucyl-aminopeptidase, proteinase and keratinase activity of some dermatophyte strains are markedly enhanced when they are grown on keratin *in vitro* as compared to the activity demonstrated during growth on ordinary media. Similar results of proteolytic activity have been reported in *Entamoeba histolytica* trophozoites of two strains known to differ in their virulence [147]. However, the differences were dependent on the substrate used.

Thus the difficulty in using biochemical assays *in vitro* to correlate pathogenicity is that pathogenicity occurs only *in vivo*, under nutritional and environmental conditions which may be different from those provided in laboratory cultures. For this reason, Mahan *et al.* [153] have developed a genetic system, termed "*in vivo* expression technology" (IVET), that does not rely on reproduction of the environmental signals but depends on the induction of genes in the host. This technique uses an avirulent *Salmonella* strain which lacks purA. Functional copies from other bacteria joined to lacZ (a gene that makes an enzyme easily detectable by a colour assay: a reporter gene) are cut and inserted in front of the two gene combinations to make DNA constructs. These are transformed into mutant bacteria lacking purA and used to infect mice. Some of the bacteria that survive after a few days in mice are considered to contain host specific *Salmonella* gene fragments which allow them to survive in the host. Similarly, dermatophytes may not express some of the virulent genes *in vitro* and may only be turned on *in vivo*. Some enzyme assays therefore may be useful only in detecting enzyme production by an organism, but may not necessarily relate to the organism's ability to cause disease.

Characterization of proteinases

The use of proteinase inhibitors by various studies has revealed production of multiple proteinases by dermatophyte species. The use of α-proteinase and phenylmethylsulfonyl fluoride (PMSF) inhibitors using an azocollytic assay has demonstrated the presence of serine-catalysed proteinases from different dermatophyte species [151,154-157]. Takiuchi *et al*. [155,156] estimated a *M. canis* enzyme to be a 45 000-Mr proteinase. The antiserum raised against it cross-reacted with material in the culture filtrates of *M. gypseum*, *T. mentagrophytes* and *T. rubrum*. This result suggests that structurally similar proteinases may be expressed by some or most of the dermatophytes. A similar finding was reported for elastinolytic serine proteinase from *Aspergillus flavus* which had antibodies immunologically related to *A. fumigatus* [158]. These findings together emphasize that structurally similar enzymes may be expressed by closely related species.

Studies with cysteine inhibitors have demonstrated inhibition of azocollytic activity of *M. canis* but not *M. cookei* using different cysteine inhibitors. Significant inhibition of 73.1% by *p*-chloromercuribenzoic acid (*p*CMB) and 31.2% and 31.6% by L-trans-epoxysuccinyl leucylamido (4-guanidino)-butane (E-64) and iodoacetic acid (IAA) respectively has been demonstrated [85]. This indicates the expression of cysteine proteinase(s) by *M. canis*. Other studies [160,161] found strong inhibition of peptidases of *T.verrucosum var. discoides* and *M. canis* by *p*CMB and 65% inhibition of endogenous respiration in *M. canis* using IAA. The production of cysteine proteinase by a *Micropsorum* species has also been reported [162], although the fungus species was not identified. In addition, *M. fulvum* (*A. fulva*) is also known to produce a cysteine elastinolytic proteinase as characterised by *p*CMB, urea and IAA [163]. However, there is no information on the structural similarity of the cysteine proteinases from different dermatophytes.

All these proteinases reported in dermatophyte species have been implicated in the pathogenicity of a wide range of microorganisms, including *Trypanosoma*, *Aspergillus* and *Serratia* [164]. The presence of more than one type of proteinase has been reported in a number of other species with at least two and sometimes three types of proteinases produced. Multiple forms (isoforms) of a proteinase of the same activity but of different Mr have been reported to be produced by the same organism [164].

Studies of *M. canis* and *M. cookei* for elastase enzyme(s) found that both fungal species express metallo-elastinolytic proteinase(s) [85]. This finding is supported by other workers that microbial elastases are usually metallo-proteinases [165]. But the function of fungal elastases is not well known, although one of their characteristics is a broad substrate specificity. They have the ability to degrade fibronectin, laminin, gamma globulins, α1P1keratin and type IV collagen [166].

Molecular weight (M_r) size of proteinases

Studies with *M. canis* using saprophytic and pseudo-parasitic morphologies revealed six different molecular weight (M_r) proteinases, namely, 122 KDa, 64 KDa, 62 KDa, 45 KDa, 31 KDa, and 25 KDa. Of these, M_r 122 KDa, 62 KDa and 28 KDa were very highly expressed in pseudo-parasitic morphology[167]. The proteinases of M_r 64 KDa, 45 KDa, and 31 KDa were expressed in both saprophytic and pseudo-parasitic morphologies of *M. canis* [149]. This means that some of the enzymes are expressed constitutively appearing in both the saprophytic and pseudo-parasitic forms [168]. A 64 KDa- M_r proteinase was highly expressed in the pseudo-parasitic form, implying that this proteinase is expressed constitutively at low levels and only peaks when induced by a change in morphology, from the saprophytic to the parasitic form.

The M_rs of 122 KDa, 62 KDa, and 25 KDa are induced by a change in morphology from saprophytic to parasitic form. These proteinases must play a critical role in the pathogenicity of *M. canis*, such as causing inflammatory reactions. Studies showed a positive correlation of high proteolytic activity and acute inflammatory infections of *T. mentagrophytes* [169]. Therefore, when keratolysis is considered as distinct from proteolysis of the less hardened epidermal proteins, keratolysis probably plays a less critical role once the mycelium is established in the epidermis [150]. Maeda and Molla [170], studying the role of proteinases in the pathogenicity of bacterial pathogens, including *Serratia marcescens* 56 KDa, 60 KDa, and 73 KDa proteinases and *Pseudomonas aeruginosa* alkaline proteinase and elastase (and also a proteinase from *Aspergillus melleus*), have suggested the involvement of the activation of the Hageman factor and/or prekallikrein of the complement system resulting in enhanced vascular permeability. All the proteinases are reported to degrade immunoglobulins like IgG and IgA and cause destruction of structural matrices like fibronectin. The bacterial proteinases are also reported to inactivate *in vitro* the complement system, e.g. C3 and C5 in human serum.

Takiuchi *et al*. [155,156] using stationary culture, detected a 45 KDa extracellular proteinase using SDS-PAGE of *M. canis* filtrates. This is the same size as that reported later[167]. O'Sullivan and Mathison [150] using shake cultures found that *M. canis* synthesized a complex of proteolytic enzymes with pH optima at pH 6.6, 8.0, and 9.5 to 10.0. Production of multiple proteinases has also been reported in other dermatophytes, such as *T. rubrum* and *T. mentagrophytes* [151,154,157,166,168,171].

A common class of proteinases reported from a wide range of pathogenic organisms which has been subjected to detailed characterisation is serine proteinases [164]. Most of the serine proteinases are generally of a low M_r, in a range of 18.5 to 35 KDa and usually around 25 KDa, although larger enzymes have also been reported [164]. Assuming serine proteinases are critical in fungal pathogenesis, their small size would allow them to diffuse into the lower epidermis, and dermis to cause inflammation. This view is supported by Minocha *et al*. [169] who demonstrated dermo-epidermal separation and spongiosis when fungal extracts were injected intradermally into excised human skin. They suggested that the changes were due to proteolytic enzymes. Peptidases and aminopeptidases, identified by Simpanya [149] using starch gels, probably cleave the peptides generated by proteinases into amino acids. This finding is also supported by Daniels [172] who demonstrated an accumulation of amino acids when *M. canis* was cultured on human hair as a nutrient source. Elastases of M_r 64 KDa and 62 KDa have also been detected from *M .canis* using elastin SDS/PAGE.

However, postulated roles for secreted proteinases do not prove their association with virulence. The detection of the enzymatic activity in most pathogenic microorganisms, including fungi, has been obtained *in vitro*, so that the role of these enzymes *in vivo* remains hypothetical. The detection of serine proteinase(s) in infected tissues [173-176] only provides circumstantial evidence that they may play a pathogenic role. Further evidence for relevance *in vivo* must be demonstrated by showing that the putative determinant is biologically effective in animal tests by protecting animals against the disease with antibodies to it and/or by showing that strains lacking it are less virulent [177]. Purnell and Martin [178] and Purnell [179] were among the early studies to demonstrate an association between virulence and the enzyme alkaline phosphatase. Mutants defective in alkaline phophastase were avirulent compared to the wild type. The virulence was assayed by the morbidity of mice inoculated intravenously. Both active and inactivated keratinases were able to elicit delayed type cutaneous hypersensitive reactions in guinea pigs [180]. In addition the α-globulin fraction of sera contained an inhibitor of the keratinases. The implication of these findings is that keratinases and other enzymes produced during infection do play a role in the invasiveness of the fungus and in hypersensitive reactions associated with dermatophytosis. Curson [181] reported similar findings for two phospholipases produced by amoebae.

Normally pathogenic fungi undergo a morphological transformation to a parasitic morphology during the process of infection. Studies on *T. rubrum* and *M. audouinii* induced both *in vitro* and *in vivo* to assume a yeast morphology consistent with dimorphic pathogenic fungi resulted in increased pathogenicity as manifested by the invasion of deep tissue[107]. But proteinase expression was not investigated. In studies on *Histoplasma capsulatum*, a dimorphic fungus which requires -SH compounds to transform from the mycelial (saprophytic) to yeast (parasitic) phase, it was demonstrated that in the presence of an -SH blocking agent (p-chloromercuryphenylsulfonic acid, PCMS) which inhibits the formation of the yeast phase, the fungus remains in the mycelial form and is non-pathogenic[182,183]. But normal and PCMS treated yeast cells were equally pathogenic in mice [182]. This finding has provided direct evidence that transformation of *Histoplasma* to yeast is a necessary prerequisite for infection. Furthermore, in the early stages of infection there is an increased expression of heat shock proteins (hsp) required in thermoadaptation, along with the acquisition of the capacity to invade. Similar to *Histoplasma*, dermatophytes undergo a morphological change from conidial to hyphae form for infection. Increased proteinase expression probably occurs *in vivo*. These changes can allow the adaptation of the dermatophyte and initiation of infection of their host.

In contrast to *M. canis* and *M. cookei*, a nonpathogen has been shown to express different M_r proteinases (67 KDa, 64 KDa, 63 KDa, 62 KDa, 54 KDa, 52 KDa, and 42 KDa) in stationary cultures and less number of proteinases (63 KDa, 62 KDa, 54 KDa, 52 KDa and 42 Kda) in shake culture. The M_r of *M. cookei* proteinases were similar to those of *M. canis*. However the ability of *M. cookei* to produce a number of proteinases may mean that its lack of pathogenicity may be due to other factors, such as its inability to tolerate high temperatures (weak thermotolerance) as compared to *M. canis*. Some subcutaneous mycoses, such as sporotrichosis and chromoblastomycosis are known to be sensitive to an increase in temperature, which influences their deep fungus pathology [184]. Studies by Baxter [185] compared *T. ajelloi*, a non-pathogenic dermatophyte "trained" to grow at 35°C, to others maintained at 25°C for their ability to infect guinea pigs. Only strains able to grow at 35°C were able to produce low grade scaling with slight erythematous reactions within five days, but this lasted only for a few days. Therefore the ability to grow at or near the host's skin temperature is an important factor in the pathogenicity of a microorganism.

When pathogenicity is defined as an expression of a two component system, the host-parasite relationship, such a definition emphasizes the host as an environment for the parasite [186]. Pathogenicity therefore, with respect to the parasite, is the capacity to use the host environment as a growth medium and to overcome the defence mechanisms of the host [186]. For dermatophytes, nutritional inhibition is less likely because of the available keratinised tissue for growth. Therefore, host defence mechanisms may be a more important factor. For example, host factors affecting the host-parasite relationship in dermatophyte infections include the effect of higher temperature and long-chain unsaturated fatty acids secreted by sebaceous glands after puberty, which have fungistatic and/or fungicidal activity [184,187,188]. Other methods which may offer a selective advantage to the host include inhibition by serine proteinase inhibitors (serpins) of exogenous proteinases. These are postulated to act as virulence factors produced by infectious agents upon infection [189], but these serpin inhibitors can be inactivated by some proteinases as well [170].

References

1. De Vroey C, Hennebert, GL. Theoretical and practical implications of the “morph” terminology to the taxonomy and nomenclature of fungi of medical importance. In: Baxter M (Ed.) Proceedings of the Eighth Congress of the International Society for Human and Animal Mycology, Massey University, Palmerston North 1982: 440-445.
2. Emmons CW. Dermatophytes; natural grouping based on the form of the spores and accessory organs. Arch Dermatol Syphil 1934;30:337-362.
3. De Vroey C. Epidemiology of ringworm (dermatophytosis). Seminars in Dermatol 1985; 4:185-200.
4. Ajello L, Georg LK, Kaplan W, Kaufman L. Laboratory Manual for Medical Mycology 1966;US Department of Health, Education and Welfare, Public Health Service, Communicable Disease Centre, Antlanta, Georgia.
5. Stockdale PM. Nutritional requirements of the dermatophytes. Biol Revs1953; 28:84-104.
6. Stockdale PM. Requirements for the growth and sporulation of *Trichophyton* persicolor. J Gen Microbiol 1953; 8:434-441.
7. Georg LK, Camp LB. Routine nutritional tests for the identification of dermatophytes. J Bacteriol 1957; 74:113-121.
8. Philpot CM. The use of nutritional tests for the identification of dermatophytes. Sabouraudia 1977; 15:141-150.
9. Cole GT, Samson RA. Patterns of development in conidial fungi. London, Pittman. 1979:190.
10. Ajello L. Milestones in the history of medical mycology: the dermatophytes. In: Iwata K (Ed.) Recent Advances in Medical and Veterinary Mycology, Proceedings of the sixth Congress of the International Society for Human and Animal Mycology, University of Tokyo, Tokyo 1977: 3-11.
11. McGinnis MR. Recent taxonomic developments and changes in medical mycology. Ann Rev Microbiol 1980; 34:109-135.
12. Benedek T. On a newer trend of classification of dermatophytes. Mycopathol Mycol appl 1948; 4:201-206.
13. Vanbreuseghem R. Classification moderne des dermatophytes. Dermatologica 1977;155:1-6.
14. Vanbreuseghem R, De Vroey C, Takashio M. A Practical guide to medical and veterinary mycology. Masson 1978.
15. Ajello L. Present day concepts of the dermatophytes. Mycopathol Mycol Appl 1962;17:315-324.
16. Vanbreuseghem R. Technique biologique pour l’isolement des dermatophytes du sol. Annls Soc Belge Méd Trop 1952;32:173-177.
17. Ajello L. A taxonomic review of the dermatophytes and related species. Sabouraudia 1968; 6:147-159.
18. Georg LK, Kaplan W, Ajello L, William WM, Tilden Evelyn B. The parasitic nature of the soil fungus *Keratinomyces ajelloi*. J Invest Dermatol 1959;32:539-544.
19. Dawson CO, Gentles JC. The perfect states of *Keratinomyces ajelloi* Vanbreuseghem, *Trichophyton terrestre* Durie and Frey and *Microsporum nanum* Fuentes. Sabouraudia 1961;1:49-57.
20. Mackenzie DWR. Antigenic composition and serology of dermatophytes. In: TumbayE (Ed.) FEMS-Symposium of dermatophytes and Dermatophytoses in Man and Animals. Izmir, Turkey. 1988: 31-38.
21. Vanbreuseghem R. Sur un nouveau dermatophyte isol, au Congo Belge: *Sabouraudites rivalieri* nov. sp. Arch Derm Syphil 1951;7:109-113.
22. Vanbreuseghem R. Dermatomycoses: faut-il distinguer *Microsporum langeroni* et *Microsporum audouini*?. Bull Soc Fr Syphil 1963;70:34-37.
23. Philpot CM. Serological differences among the dermatophytes. Sabouraudia 1978;16:247-256.
24. Davidson FD, Mackenzie DWR, Owen RJ. Deoxyribonucleic acid base composition of dermatophytes. J Gen Microbiol 1980;118:465-470.
25. Davidson FD, Mackenzie DWR. DNA homology studies in the taxonomy of dermatophytes. Sabouraudia 1984;22:117-123.
26. Jones MG, Noble WC. A study of fatty acids as a taxonomic tool for dermatophyte fungi. J Appl Bacteriol 1981;50:577-583.
27. Jeffries CD. Problems and status of the pathogenic fungi. In: Baxter M. (Ed.) Proceedings of the Eighth Congress of the International Society for Human and Animal Mycology, Massey University, Palmerston North 1982: 438-439.
28. Jeffries CD, Reiss Errol, Ajello L. Analytical isoelectric focusing of secreted dermatophyte proteins applied to taxonomic differentiation of *Microsporum* and *Trichophyton* species (preliminary studies). J Med Vet Mycol 1984; 22:369-379.
29. Tucker WDL, Noble WC. The value of electrophoretic protein patterns for the study of *Microsporum canis*. J Med Vet Mycol 1990; 28:117-123.
30. Simpanya MF, Baxter M. Isozyme variation of *Microsporum canis* and *M. cookei* from New Zealand. J Med Mycol 1998;36: 255-262.
31. Mochizuki T, Takada K, Watanabe S, Kawasaki M, Ishizaki H. Taxonomy of *Trichophyton interdigitale* (*Trichophyton mentagrophytes* var. interdigitale) using restriction enzyme analysis of mitochondrial DNA. J Med Vet Mycol 1990;28:191-196.
32. Ajello L. Natural history of the dermatophytes and related species. Mycopathol Mycol appl 1974;53:93-110.
33. Howard DH. Ascomycetes: the Dermatophytes. In: Howard DH (Ed.) Fungi Pathogenic for Humans and Animals. Part A (Biology). Marcel Dekker, Inc. , NY 1983:113-147.
34. Rippon JW. The changing epidemiology and emerging patterns of dermatophyte species. In: McGinnnis MR (Ed.) Current Topics in Medical Mycology (vol. 1). Springer-Verlag 1985: 208-234.
35. Griffin DM. Fungal colonisation of sterile hair in contact with soil. Trans Br Mycol Soc 1960;43:583-596.
36. de Vries GA. Keratinophilic fungi and their action. Antonie van Leeuwenhoek 1962; 28:121-133.
37. Griffin DM. The rediscovery of *Gymnoascus gypseum*, the perfect state of *Microsporum gypseum*, and a note on *Trichophyton terrestre*. Trans Br Mycol Soc 1960; 43:637-642.
38. Griffin DM. Perfect stage of *Microsporum gypseum*. Nature 1960; 186:94-95.
39. Stockdale Phyllis M. *Nannizzia incurvata* gen. nov. , sp. nov. , a perfect state of *Microsporum gypseum* (Bodin) Guiart et Grigorakis. Sabouraudia 1961; 1:41-48.
40. Takashio M. Taxonomy of dermatophytes based on their sexual states. Mycologia 1979;71:968-976.
41. Alexopoulos CJ, Mims CW, Blackwell M. Introductory Mycology (Fourth edition). John Wiley and Sons, Toronto 1996.
42. Padhye AA, Carmichael JW. The genus *Arthroderma* Berkeley. Can J Botany 1971; 49:1525-1540.
43. Weitzman I, McGinnis MR, Padhye AA, Ajello L. The genus *Arthroderma* and its later synonym *Nannizzia*. Mycotaxon 1986; 25:505-518.
44. Stockdale PM. Sexual stimulation between *Arthroderma simii* Stockd. , Mackenzie and Austwick and related species. Sabouraudia1968; 6:176-181.
45. Padhye AA, Carmichael JW. Mating reactions of *Trichophyton simii* and *T. mentagrophytes* strains from poultry farm soil in India. Sabouraudia 1968; 6:238.
46. Marples MJ, Smith JMB. *Trichophyton terrestre* as a resident in hedgehog skin. Sabouraudia 1962; 2:100-107.
47. Marples Mary J. The distribution of keratinophilic fungi in soils from New Zealand and from two Polynesian Islands. Mycopathol Mycol Appl 1965;25:361-372.
48. Beneke ES, Rogers AL. Medical Mycology Manual. Burgess, Minneapolis 1980.
49. Takashio M. Les formes sexuées du complex *Trichophyton mentagrophytes*: *Arthroderma vanbreuseghemii* sp. nov. , *Arthroderma benhamiae* et *Arthroderma simii*. Bull Soc Fr Mycol Méd 1973; 2: 153-156.
50. Ajello L, Cheng Shu-Lan. The perfect state of *Trichophyton mentagrophytes*. Sabouraudia 1976; 5:230-234.
51. Watanabe S, Hiroaga M. Differences or similarities of the clinical lesions produced by “+” and “-” types members of the “*mentagrophytes*” complex in Japan. In: Vanbreuseghem R, De Vroey C (Eds.) Sexuality and Pathogenicity of Fungi. Proc Third Colloquium on Medical Mycol Masson NY 1981: 83-96.
52. Takashio M. Étude des phénomènes de reproduction liés au vieillissement et au rajeunissement des cultures de champignons. Ann Soc Belge Méd Trop 1973; 53:427-580
53. Takashio M. Observations on African and European strains of *Arthroderma benhamiae*. Int J Dermatol 1974; 13:94-101.
54. Takashio M. The *Trichophyton mentagrophytes* complex. The Dermatophytes. In: Iwata K (Ed.) Recent advances in medical and veterinary mycology, Proceedings of the Sixth Congress of the International Society for Human and Animal Mycology. University of Tokyo, Tokyo 1977:271-276.
55. Takashio M. Production, maintenance and utilisation of tester strains by sexual crossings of dermatophytes: apropos of *Nannizzia otae*: In: Vanbreuseghem R, De Vroey C (Eds.) Sexuality and pathogenicity of fungi. Proceedings of the Third Int Colloquium on Med Mycol. Masson NY 1981:69-82.
56. Smith JMB, Marples MJ. *Trichophyton mentagrophytes var. erinacei*. Sabouraudia 1963; 3:1-10.
57. Collinge CA, Stockdale PM, Gregory MW. A mycological study of *Arthroderma benhamiae* from Central African hedgehog. Sabouraudia 1974;12:227-232.
58. Rebell G, Taplin D. Dermatophytes: Their recognition and identification. University of Miami Press, Coral Gables, Florida 1978.
59. Stockdale PM. Sexual stimulation between *Arthroderma simii* and other dermatophytes. In: Vanbreuseghem R, De Vroey C (Eds.) Sexuality and Pathogenicity of Fungi. Proceedings of the Third Inter Colloquium on Med Mycol. Masson NY 1981: 57-68.
60. Dawson C, Gentles JC, Brown EM. Environmental conditions affecting sexual reproduction in species of *Arthroderma* and *Nannizzia*. Sabouraudia 1964; 3:245-250.
61. Davidson M, Unestam T. Factors affecting the sexual reproduction of a dermatophyte, *Arthroderma benhamiae*, in synthetic media. Physiol Plant 1974; 31: 237-244.
62. De Vroey C. Formes sexuées des dermatophytes. Production de cleistothéces de *Microsporum gypseum* (Bodin) Guiart and Grigoraki sur divers milieux steriles. Ann Soc Belge Méd Trop 1964; 44: 831-840.

63. Wietzman I, Silva-Hutner M. Non-keratinous agar media as substrates for the ascigerous state in certain members of the Gymnoascaceae pathogenic for man and animals. Sabouraudia 1967; 5:335-340.
64. Takashio M, De Vroey C. Reproduction sexuelle de certains dermatophytes sur milieu à base de graines de niger (*Guizotia abyssinica*). Bull Soc Fr Mycol Méd 1976;5:141-144.
65. Weitzman I. Variation in *Microsporum gypseum* I. a genetic study of pleomorphism. Sabouraudia 1964; 3:195-204.
66. Weitzman I, Incompatibility in the *Microsporum gypseum* complex. Mycologia 1964; 56:425-435.
67. Georg LK. Animals in public health: Diagnosis and Nature. US Department of Health, Education and Welfare. Public Health Service, Bureau of State Services Communicable Disease Centre. Antlanta, Georgia 1959.
68. Rippon JW. Host specificity in dermatophytoses. In: Baxter M (Ed.) Proceedings of the Eight Congress of the International Society for Human and Animal Mycology. Massey University, Palmerston North 1982:28-33.
69. Mantovani A. The role of animals in the epidemiology of mycoses. Mycopathologia 1978; 65:61-66
70. De Vroey C. Ecological and epidemiological aspects in dermatophytoses. Zbl Bakt Hyg 1984; 257:234-239.
71. Böhme H, Ziegler H. The distribution of geophilic dermatophytes and other keratinophilic fungi in relation to the pH of the soil. Mycopathologia 1969; 38:247-255.
72. Baxter M, Pearson RD. The occurence of *Microsporum nanum* as a human pathogen and animal pathogen in New Zealand. NZ J Med Lab Technol 1969; 23: 87-90.
73. Connole MD. Review of animal mycoses in Australia. Mycopathologia 1990; 111:133-164.
74. Georg LK. Epidemiology of dermatophytes sources of infection, modes of transmission and epidemicity. Ann NY Acad Sci 1960; 89:69-77.
75. Weitzman I, Silva-Hutner M, Kozma I. A comparison of the pathogenicity of three members of the *Microsporum gypseum* complex. Sabouraudia 1967;5:360-365.
76. Gordon MA, Perrin U, Little GN. Differences in pathogenicity between *Microsporum gypseum* and *Microsporum fulvum*. Sabouraudia1967; 5:366-370.
77. Ajello L. The dermatophyte, *Microsporum gypseum*, as a saprophyte and parasite. J Invest Dermatol 1953; 21:157-171.
78. Londero AT, Ramos Cecy D. A twenty-year (1960-1970) survey of dermatophytes in the state of Rio Grande Do Sul, Brazil. In: Proceedings of the 5th International ISHAM Conference on mycoses: superficial, cutaneous, and subcutaneous infections. Scientific Publication N°396, Pan American Health Organisation, Caracas, Venezuela 1985;188-192.
79. Durie EB, Frey Dorothea. The presence of dermatophytes and other keratinophilic fungi in soil. Aust J Dermatol 1962;6:167-171.
80. Alsop J, Prior PP. Ringworm infection in a cucumber green house. Br Med J 1961; 1:1081-1083.
81. Gentles JC. Keratinophilic fungi and diseases caused by them. In: Dalldorf, G. (Ed.) Fungi and fungous diseases. Charles C. Thomas, Illinois 1962:11-21.
82. Marples MJ, Smith JMB. The hedgehog as a source of human ringworm. Nature Lond 1960; 188:867.
83. Bakerspigel A. The keratinophilic fungi of Ontario, Canada. Mycopathol Mycol appl 1974; 53:1-12.
84. Ajello L. A new *Microsporum* and its occurence in soil and on animals. Mycologia 1959; 51:69-76.
85. Simpanya MF, Baxter M. Partial characterisation of proteolytic enzymes of *Microsporum canis* and *Microsporum cookei*. Mycoses 1996; 39: 279-282.
86. Lundell E. *Microsporum cookei* Ajello, An eczematous skin lesion. Mykosen 1965; 12:123-126.
87. Frey D. Isolation of *Microsporum cookei* from a human case. Sabouraudia 1971;9:146-148.
88. English MP. The epidemiology of animal ringworm in man. Brit J Dermatol 1972; 86(suppl):78-87.
89. Marples MJ. The ecology of *Microsporum canis* Bodin in New Zealand. J Hygiene 1956;54:378-387.
90. Klokke AH, de Vries GA. Tinea capitis in chimpanzees caused by *Microsporum canis* Bodin 1902 resembling *M. obesum* Conant 1937. Sabouraudia 1963; 2:268-270.
91. Gentles JC, Dawson C, Christine O, Connole MD. Keratinophilic fungi on cats and dogs II. Sabouraudia 1965; 4:171-175.
92. Baxter M. Ringworm due to *Microsporum canis* in cats and dogs in New Zealand. N Z Vet J 1973;21:33-37.
93. Philpot CM. Some aspects of the epidemiology of tinea. Mycopathol Mycol Appl 1977;62:3-13.
94. di Menna ME, Marples MJ. *Microsporum distortum* sp. nov. from New Zealand. Trans Br Mycol Soc 1954; 37:372-374.
95. Marples MJ, Smith JMB. Further observations on the ecology of *Microsporum distortum*. NZ Med J 1962; 61:539-544.
96. Georg LK. The role of animals as vectors of human fungus diseases. Trans NY Acad Sci 1956;18:639-647.
97. Gentles JC, O'Sullivan JG. Correlation of human and animal ringworm in West of Scotland. Br Med J 1957;ii (5046): 678-682.
98. Kaben U. Verbreitung und epidemiologie der dermatomykosen tierischen ursprung. Mykosen 1967; 10:47-60.
99. Baxter M. Isolation of *Trichophyton mentagrophytes* from British soil. Sabouraudia 1966; 4:207-209.
100. Kaplan W, Gump RH. Ringworm in the dog caused by *Trichophyton rubrum*. Vet Med 1958;53:139-142.
101. Rippon JW. Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes. London, WB Saunders, 1982: 154-248.
102. Vanbreuseghem R. Contribution à l'étude des dermatophytes du Congo Belge: Le *Sabouraudites* (*Microsporum*) *langeroni* nov. sp. Ann Parasit Hum Comp 1950; 25: 509-517.
103. Vanbreuseghem R. Dermatophytes from the Republic of the Congo and Ruanda-Burundi. New description of *Microsporum rivalieri*. Sabouraudia 1963; 2:215-224.
104. Marples MJ. Some problems in the ecology of the dermatophytes. NZ Med J 1959; 58:64-69.
105. English MP. Ecological aspects of dermatophytes regarded essentially as anthropophilic. In: Preusser JH (Ed.) Medical Mycology, Zbl Bkt Suppl 8. Gustav Fisher Verlag, NY 1980:53-59.
106. Pushkarenko VI, Pushkarenko GD. Obobennosti aminokislotnogo sostava poto so stopu litz s klinicheski vyra zhennoi epidermofitiei. Vestn Dermatol Venerol 1969;49:47-49.
107. Rippon JW, Scherr GH. Induced dimorphism in dermatophytes. Mycologia 1959; 51:902-914.
108. De Vroey C. 1988. Dermatophyte, a "nomen dubium"?. In: Tümbay E (Ed) FEMS-Symposium of dermatophytes and dermatophytoses in man and animals. Izmir, Turkey 1988:16-23.
109. Baxter M, Rush-Munro FM. The superficial mycoses of man and animals in New Zealand and their diagnosis (3rd ed). Massey University 1980.
110. Tanaka S, Summerbell RC, Tsuboi R, Kaaman T, Sohnle PG, Matsumoto T, Ray TC. Advances in dermatophytes and dermatophytosis. J Med Vet Mycol 1992;30:29-39.
111. Mackenzie DWR. The extra-human occurence of *Trichophyton tonsurans* var *sulfureum* in a residential school. Sabouraudia 1961;1:58-64.
112. Mariat F, Adan-Campos C. La technique du carr, de tapis, method simple de prelevement dans les mycoses superficielles. Ann Inst Pasteur 1967; 113:666-668.
113. Uscavage JP, Kral F. *Microsporum canis*: isolation from the air. J Small Animal Pract 1961; 1:279-280.
114. Midgley G, Clayton YM. Distribution of dermatophytes and *Candida albicans* spores in the environment. Br J Dermatol 1972; 86:69-77.
115. Friedman L, Derbes VJ, Hodges EP, Sinski JT. The isolation of dermatophytes from the air. J Invest Dermatol 1960;35:3-5.
116. Smith JMB. The pathogenesis of ringworm. In: Baxter M (Ed.) Proceedings of the Eighth Congress of the International Society for Human and Animal Mycology. Massey University Palmerston North 1982: 14-23.
117. Baxter M, Illston Gaylene M. Environmental reservoirs of psychotrophic meat spoilage fungi. NZ Vet J 1977; 25:165-167.
118. Baxter M, Illston Gaylene M. Temperature relationships of fungi isolated at low temperatures from soils and other substrates. Mycopathologia 1980; 72: 21-25.
119. Hussein HM. Fusaria and *Fusarium* toxins in maize. PhD Thesis. Massey Univ New Zealand 1987.
120. Durie EB, Frey D. A new species of *Trichophyton* from New South Wales. Mycologia 1957; 49:401-411.
121. Chmel L, Hasilikova A, Hrasko J, Vlaoilikova. The influence of some ecological factors on keratinophilic fungi in the soil. Sabouraudia 1972; 10:26-34.
122. Carretta G, Ajello L, Padhye AA. Occurence of *Arthroderma gloriae*, a geophile keratinophilic ascomycete, in Italy. J Med Vet Mycol 1990;28:99-102.
123. Gordon MA. The occurence of the dermatophyte *Microsporum gypseum* as a saprophyte in the soil. J Invest Dermatol 1953;20:201-206.
124. Ajello L, Varsavsky E, Ginther, OJ. The natural history of *Microsporum nanum*. Mycologia 1964; 36:873-884.
125. Usunov PY, Detection of soil dermatophytes and other moulds by the float technique. Proc Int Dermatol Symp 1966;10:51-53.
126. Lee Wen Shya. The Isolation of Keratinophilic Fungi from Soil in Palmerston North City and the Surrounding area. B. Sc. Project. 1979; Massey Univ New Zealand.
127. Parkinson D, Gray TRG, Williams ST. Methods for studying the ecology of soil micro-organisms. IBP Handbook, N°19. Blackwell Scientific Publications. Oxford 1971.
128. Somerville DA, Marples Mary J. The effects of soil enrichment on the isolation of keratinophilic fungi from soil samples. Sabouraudia 1967; 6:70-76.
129. Bakerspigel A. Soil as a storage medium for fungi. Mycologia 1953; 45:596-604.
130. Otcenasek M. Ecology of the dermatophytes. Mycopathologia 1978;65:67-72.
131. Bogaty H. Differences between adult and children's hair. J Soc Cosmet Chem 1969;20:159-171.
132. Shadomy HJ, Philpot CM. Utilisation of standard laboratory methods in the laboratory diagnosis of problem dermatophytes. Am J Clin Pathol 1980; 74:197-201.
133. Mackenzie DWR. "Hairbrush diagnosis" in detection and eradication of non-fluorescent scalp ringworm. Br Med J 1963;2:363-365.
134. Rosenthal SA, Wapnick H. The value of MacKenzie's "hairbrush" technic in the isolation of *Trichophyton mentagrophytes* from clinically normal guinea-pigs. J Invest Dermatol 1963;41:5-6.
135. Connole MD. Keratinophilic fungi on cats and dogs. Sabouraudia 1995; 4:45-48.
136. Ive FA. The carrier state of tinea capitis in Nigeria. Br J Dermatol 1966; 78:219-221.
137. Yamamoto I, Kagawa S. Isolation of *Microsporum canis* from the carrier state by hairbrush method. Jap J Med Mycol 1986; 27:27-28.
138. Katoh T, Sano T, Kagawa S. Isolation of dermatophyte from clinically normal scalps in *Microsporum canis* infections using the hairbrush method. Mycopathologia 1990; 112:23-25.

139. Simpanya MF, Baxter M. Isolation of fungi from the pelage of cats and dogs using the hairbrush technique. Mycopathologia 1996;134: 129-133.
140. Waldorf AR. Host-parasite relationship in opportunistic mycoses. CRC Crit Rev Microbiol 1986;13:133-172.
141. Rippon JW, Varadi DP. The elastases of pathogenic fungi and actinomycetes. J Invest Dermatol 1968;50:54-58.
142. Rippon JW. Extracellular collagenase from *Trichophyton schöenleini*. J Bacteriol 1968; 95:43-46.
143. Rippon JW. Biochemical-genetic determinants of virulence among dermatophytes. In: Mycoses. Proceedings of the Third International Conference on the mycoses. Sao Paulo, Brazil, Washington, Pan American Health Organisation 1975; 88-97.
144. Rippon JW. Elastase production by ringworm fungi. Science 1967; 157:947.
145. Rippon JW. Soo Hoo M. Determination of fungal elastase. Appl Microbiol 1971; 22:471-472.
146. Kwon-Chung Kyung J. Genetics of fungi pathogenic for man. CRC Crit Rev Microbiol 1974;3:115-133.
147. Gadasi Hana, Kobiler D. *Entamoeba histolyitca*: correlation between virulence and content of proteolyitc enzymes. Exp Parasitol 1983;55:105-110.
148. Eakin Ann E, McGrath Mary E, McKerrow JH, Fletterick RJ, Craik CS. Production of crystallizable cruzain, the major cysteine protease from *Trypanosoma cruzi*. J Biol Chem 1993;266:6115-6118.
149. Simpanya MF. Comparative enzyme studies of *Microsporum canis* and *Microsporum cookei* in relation to their pathogenicity and diversity. PhD thesis. 1994; Massey Univ Palmerston North New Zealand.
150. O'Sullivan J, Mathison GE. The localisation and secretion of a proteolytic enzyme complex by the dermatophytic fungus, *Microsporum canis*. J Gen Microbiol 1971;68:319-326.
151. Sanyal AK, Das SK, Banerjee AB. Purification and partial characterisation of an exocellular proteinase from *Trichophyton rubrum*. Sabouraudia 1985; 23:165-178.
152. Kunert J. Effect of reducing agents on proteolytic and keratinolytic activity of enzymes of *Microsporum gypseum*. Mycoses 1992; 35:343-348.
153. Mahan MJ, Slauch JM, Mekalanos JJ. Selection of bacterial virulence genes that are specifically induced in host tissues. Science 1993; 259:686-688.
154. Meevootisom V, Niederpruem DJ. Control of exocellular proteases in dermatophytes and especially *Trichophyton rubrum*. Sabouraudia 1979; 17:91-106.
155. Takiuchi I, Higuchi D, Sei Y, Koga M. Isolation of an extracellular proteinase from *Microsporum canis*. Sabouraudia 1982; 20:281-288.
156. Takiuchi I, Sei Y, Takagi H, Negi M. Partial characterisation of the extracellular keratinase from *Microsporum canis*. Sabouraudia 1984; 22:219-224.
157. Asahi M, Lindquist R, Fukuyama K, Apodaca G, Epstein WL, McKerrow JH. Purification and characterisation of major extracellular proteinases from *Trichophyton rubrum*. Bichem J 1985; 232:139-144.
158. Kolattukudy PE, Lee JD, Rogers LM, *et al.* Evidence for possible involvement of elastolytic serine protease in *Aspergillus*. Infect Immun 1993;61: 2357-2368.
159. Simpanya MF, Baxter M. Isolation of fungi from soil using the keratin baiting technique. Mycopathologia 1996;136: 85-89.
160. Chattaway FW, Thompson CC, Barlow AJE. The action of inhibitors on dermatophytes. Biochem J 1956;63:648-656.
161. Chattaway FW, Ellis DA, Barlow AJE. Peptidases of dermatophytes. J Invest Dermatol 1963;41:31-37.
162. Roberts FF, Doetsch RN. Purification of a highly active protease from a *Microsporum* species. Antonie van Leeuwenhoek 1963;33:145-152.
163. Werb Z, Banda MJ, Mckerrow JH, Sandhaus RA. Elastases and elastin degradation. J Invest Dermatol 1982;79:154-159.
164. North MJ. Comparative biochemistry of the proteinases of eucaryotic microorganisms. Microbiol Rev 1982;46;308-340.
165. McKerrow JH, Pino-Heiss S, Lindquist R, Werb Z. Purification and characterisation of elastinolytic proteinase secreted by cercariae of *Schistosoma mansoni*. J Biol Chem 1985; 260: 3703-3707.
166. Apodaca G, McKerrow JH. Expression of proteolytic activity by cultures of *Trichophyton rubrum*. J Med Vet Mycol 1990;28:159-171.
167. Simpanya MF, Baxter M. Multiple proteinases of two *Microsporum* species. J Med Vet Mycol 1995;34, 31-36.
168. Apodaca G, McKerrow JH. Purification and characterisation of a 27 000-Mr extracellular proteinase from *Trichophyton rubrum*. Infect Immun 1989; 57: 3072-3080.
169. Minocha Y, Pasricha JS, Mohapatra LN, Kandhari KC. Proteolytic activity of dermatophytes and its role in the pathogenesis of skin lesions. Sabouraudia 1972;10:79-85.
170. Maeda H, Molla A. Pathogenic potentials of bacterial proteases. Clin Chim Acta 1989;185:357-368.
171. Apodaca G, McKerrow JH. Regulation of *Trichophyton rubrum* proteolytic activity. Infect Immun 1989;57:3081-3090.
172. Daniels G. The digestion of human hair keratin by *Microsporum canis*. J Gen Microbiol 1953;8:289-294.
173. Olgert RP, Wretlind B. Assessment of protease (elastase) as a *Pseudomonas aeruginosa* virulence factor in experimental mouse burn infection. Infect Immun 1979;24:181-187.
174. Becker I, Prez-Tamayo R, Montfort I, Alvizouri Ana M, Prez-Montfort R. *Entamoeba histolytica*: Role of amebic proteinases and polymorphonuclear leukocytes in acute experimental amebiases in the rat. Exp Parsitol 1988;67:268-280.
175. Reichard U, Büttner S, Eiffert H, Staib F, Rüchel R. Purification and Characterisation of extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue. J Med Microbiol 1990;33:243-251.
176. Gessner AR, Mortensen JE. Pathogenic factors of *Pseudomonas cepacia* isolates from patients with cystic fibrosis. J Med Microbiol 1990;33:115-120.
177. Smith H. The development of studies on the determinants of bacterial pathogenicity. J Comp Pathol 1988;98:253-273.
178. Purnell DM, Martin GM. *Aspergillus nidulans*: association of certain alkaline phosphatase mutations with decreased virulence for mice. J Infect Dis 1971;123:305-306.
179. Purnell DM . Virulence genetics of *Aspergillus nidulans* Eidam: A review. Mycopathologia 1978;65:177-182.
180. Grappel SF, Blank F . Role of keratinases in dermatophytosis, I: Immune responses of guinea pigs infected with *Trichophyton mentagrophytes* and guinea pigs immunized with keratinases. Dermatologica 1972;145:245-255.
181. Curson RTM. Pathogenic Free-Living Amebae - Occurrence in New Zealand Thermal Regions Together with Investigation into their Disinfection, Immunity and Virulence. Ph. D. Thesis. Massey Univ, New Zealand, 1978.
182. Medoff G, Sacco M, Maresca B, Schlessinger D, Painter A, Kobayashi GS, Carrat L. 1986. Irreversible block of the mycelial-to-yeast phase transition of *Histoplasma capsulatum*. Science 1986;231:476-479.
183. Maresca B, Kobayashi GS. Dimorphism in *Histoplasma capsulatum*: a model for the study of cell differentiation in pathogenic fungi. Microbiol Rev 1989;53:186-209.
184. Dei Cas E, Vernes A. Parasitic adaptation of pathogenic fungi to mammalian hosts. CRC Crit Rev Microbiol 1986;13:173-218.
185. Baxter M. Studies on the keratinophilia of dermatophytes. Ph. D. Thesis. Birmingham Univ., England, 1969.
186. Garber ED. A nutrition-inhibition hypothesis of pathogenicity. The Amer Natur 1956;110:183-194.
187. Hajini GH, Kandhari KC, Mohapatra LN, Bhutani LK. Effect of hair oils and fatty acids on the growth of dermatophytes and their in vitro penetration of human scalp hair. Sabouraudia 1970;8:174-176.
188. El-Naghy MA, Maghazy SN, Fadl-Allah EM, El-Gendy ZK. Fungistatic action of natural oils and fatty acids on dermatophytic and saprophytic fungi. Zentralbl Mikrobiol 1992;147:214-220.
189. Hill RE, Hastie ND. Accelerated evolution in reactive centre regions of serine protease inhibitors. Nature 1987; 326:96-99.