Trichophyton mentagrophytes a keratinophilic fungus

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Summary

Trichophyton mentagrophytes is a keratinophylic fungus belonging to a homogeneous group of fungi called the dermatophytes. The dermatophytes cause a variety of cutaneous infections in humans and animals. *T. mentagrophytes* has at least five different variants which make up the *Trichophyton mentagrophytes* complex. Two perfect states *Arthroderma benhamiae* and *Arthroderma vanbreuseghemii* have been ascribed for *T. mentagrophytes*. The organism has variable characteristics with anthropophilic form producing sparse aerial mycelium with numerous spores. The zoophilic isolate produces powdery or granular colonies. Microscopically the most consistent feature of *T. mentagrophytes* is the production of globose microaleuriospores arranged in grape-like clusters.
T. mentagrophytes assimilates phosphorus, potassium, sodium and calcium. It utilizes methionine but is inhibited by folic acid. The organism has been recovered from a variety of sources such as soil, floor of swimming pools, hairs of wild boar, cats and dogs, farm animals, foot wears, shower stalls and from human toewebs without clinical lesions.
T. mentagrophytes breaks down keratinous substrates by both chemical and mechanical ways. Five different keratinolytic enzymes from ten strains of *T. mentagrophytes* have been isolated. These enzymes are known to play a role in pathogenesis of infections caused by this organisms in both humans and animals.

Key words *Trichophyton mentagrophytes*, Keratinophilic fungi, Dermatophytes

Trichophyton mentagrophytes is a member of a homogenous group of fungi called dermatophytes. Dermatophytes form a group of pathogenic fungi which have a marked affinity for keratin, attacking skin, hair and nails. *T. mentagrophytes* like other dermatophytes has the ability to break down or digest keratinized areas in humans, birds and other mammals. Keratin is a highly insoluble scleroprotein rarely used as a substrate in nature. Certain insects such as the clothes moth (*Tinea*), carpet beetles (*Dermestes*) and the biting lice (*Mallphyaga*) utilize keratin [1,2].

The molecular structure of keratin varies from species to species and therefore different enzyme keratinases have evolved relatively specificaly to digest a variety of keratin in different human and animal hosts. Dermatophytes are divided into three ecological groups depending on their natural, animal or environmental host.

(i) Anthropophilic species which have humans as the natural host.
(ii) Zoophilic species which normally live on other animals.
(iii) Geophilic species whose natural habitat is still the soil. Under appropriate conditions zoophilic and geophilic species are transmitted to humans [3].

Three genera of dermatophytes are recognized as human pathogens, viz *Trichophyton, Microsporum* and *Epidermophyton. Trichophyton mentagrophytes* a zoophilic dermatophyte has at least five different variants, *T. mentagrophytes* var *interdigitale* is anthropophilic, *T. mentagrophytes* var *nodulare* is a rare anthropophilic form occasionally isolated from cases of *tinea pedis*, *T. mentagrophytes* var *mentagrophytes, T. mentagrophytes* var *quinckeanum* and *T. mentagrophytes* var *erinacei* are zoophilic dermatophytes. All these variants make up the *T. mentagrophytes* complex, and differentiating these variants is impossible on any one medium [4,5]. In this review *T. mentagrophytes* is used to cover the complex.

Taxonomy and mating behaviour of *Trichophyton mentagrophytes*

The taxonomy of *T. mentagrophytes* has a complicated history. The recent discovery of its sexual or perfect state has made its taxonomy a lot clearer. Two perfect states have been ascribed for *T. mentagrophytes*: *Arthroderma benhamiae* and *Arthroderma vanbreuseghemii* [5-7]. These sexual or perfect states were obtained by crossing "+" and "-" tester strains of these heterothallic *Arthroderma* species. Many isolates do not mate with known tester strains of *Arthroderma benhamiae* and

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Arthroderma vanbreuseghemii. According to Hironaga and Watanabe [8] Japanese isolates belonged to three categories (i) compatible with *A. vanbreuseghemii* (ii) incompatible with known tester "-" type *Trichophyton interdigitale* and (iii) incompatible with "-" *granulosum asteroides* form. Takashio [9] divided *A. benhamiae* into an American-European race and an African race and observed that intra-racial matings were very fertile whereas inter-racial matings were only moderately or poorly fertile or completely sterile. He also observed that the variety *erinacei* belonging to the African race and var *caviae* belonging to the American-European race were sexually degenerate in comparison to the normal strain of *A. benhamiae*. The Finnish and Swedish strains of *T. mentagrophytes* were compatible with *A. benhamiae* (+ type) and support the European dominance of *A. benhamiae*.

Recent studies on genetic homogeneity of *T. mentagrophytes* var *interdigitale* shows that this species is a genetically homogenous taxon derived from *A. vanbreuseghemii* and not to be considered as a morphologically and sexually degenerated isolate of *A. benhamiae* [10]. As for the taxonomy of *T. mentagrophytes* var *interdigitale*, a lineage to *A. vanbreuseghemii* has been postulated from its Morphology [11] mating behaviour and pathogenicity [12] and isoelectric focusing pattern analysis of somatic extracts [13]. Recent studies support the hypothesis that *T. mentagrophytes* is the most anthropophilic and sexually degenerated end of a generic taxon represented by *A. vanbreuseghemii* which causes infection in animals.

Traditionally, dermatophytes that exhibit teleomorphs (perfect stage) are classified as members of the family Gymnoascaceae in the class Ascomycetes and phylum Ascomycota. The family is characterized by small and mostly spherical ascomata with loose basket-like walls. Recently some authors have combined Gymnoascaceae with related family Onygenaceae using the older name Onygenaceae for the new acronym. The two families are related in several aspects such as (1) lack of phialides (2) lack of melanin-like pigments (3) possession of spherical asci (4) presence of colourless to brightly coloured one celled ascospores and (5) almost universal keratinophilous nutrition [5].

Morphology and physiology

Trichophyton mentagrophytes is a very variable organism and many characteristics are not consistent. On Sabouraud dextrose agar (SDA), the anthropophilic form grows as a flat downy thallus with white edges and a cream-tinted central area. On potato dextrose agar (PDA) a colony with sparse aerial mycelium with numerous spores is observed. Zoophilic isolates produce a flat rapidly-growing granular cream, yellowish, buff to tan colony. Sometimes the colony may be reddish-brown in colour. Mycelium is usually sparse and the powdery or granular appearance is due to the production of numerous microaleuriospores. Some strains produce powdery lavender-tinged surface. Numerous variations of colony morphology are seen among the *Trichophyton mentagrophytes* complex [1,14].

Microscopically, the most consistent feature of *T. mentagrophytes* is the production of globose microaleuriospores arranged in grape-like clusters (engrappe). These are most abundant in zoophilic strains. The spores are more clavate-shaped and resemble those of *Trichophyton rubrum*. Macroaleuriospores are thin walled, smooth and variable in shape. They are generally cigar-shaped with a narrow attachment at the base. These spores measure 20-50 x 4-8 µm and they have three to five cells. The typical features of *T. mentagrophytes* are numerous microaleuriospores, some macroaleuriospores, and several spiral or coiled hyphae. Nodular bodies, chlamydospores, racquet mycelium, antler-like hyphae may also be seen. Hair perforating organs and a lack of pigment on cormeal agar and potato dextrose agar separate *T. mentagrophytes* from *T. rubrum* [15].

In an ultrastructural study of *T. mentagrophytes* using neutral red stain, Naka [16] observed that vacuoles were the only ultrastructural components to be associated with neutral red particles. The vacuoles are considered to be counterparts of mammalian lysosomes and play a role in hydrolysis of old intracellular organelles and in storage of amino acids, minerals and other metabolites [17]. In ascospore cell wall formation in *A. vanbreuseghemii*, using freeze substitution method, the mature ascospore cell wall was found to consist of an inner electron-translucent layer and an outer electron-dense layer, both originate as precipitates of electron-dense granules between the double membrane system of the ascospore. The outer wall layer showed a mottled appearance between two electron-dense boundaries of different thickness [18]. Tanaka *et al.* [18] further studied electron-microscopic localization of sugar residues by post-embedding method using wheat germ agglutinin colloidal gold complex. The authors visualized the localization of glycan containing N-acetyl glucosamine such as chitin molecules in the inner layer of the cell wall.

The physiology of dermatophytes has always been studied for a better understanding of their mode of infection, host-parasite relationship and specialized nature of parasitism to evolve better therapeutic measures and improved diagnostic approaches [19]. With respect to utilization of carbon, nitrogen and vitamins, dermatophytes present a versatile nutritional range. In a study on physiological prerequisites for the transition to parasitism of ancestor dermatophytes, *T. mentagrophytes* assimilated phosphorus, potassium, sodium and calcium and utilized glucose and amine nitrogen during morpho-physiological reduction and differentiation [20].

Dermatophytes have a common preference for carbohydrates among carbon sources, growing best in the presence of glucose [20,21]. Although they can well utilize carbon from other sources, they are not able to utilize the trisaccharide melezitose probably due to lack of specific enzymes. Specificity to particular amino acids as a source of nitrogen has been observed in some dermatophytes. *T. tonsurans* is specific for ornithine, citrulline or arginine and *T. mentagrophytes* is specific for methionine [20,22]. The majority of the dermatophytes are autotrophic for vitamins. Pereiro-Miguens [23] demonstrated the growth inhibition of *T. rubrum* by vitamin C and of *T. quinckeanum* and *T. mentagrophytes* by folic acid. Specificity to nutritional characteristics in certain species of dermatophytes has led to suggestions by some authors on the use of nutritional properties for the identification of certain species of dermatophytes [15,24]. There is no specific differentiation among dermatophytes with respect to metal ion utilization [25].

Ecology of *T. mentagrophytes*

Using hair baiting techniques Vanbreuseghem [26] and later workers found a rich flora of keratinophilic fungi including *T.mentagrophytes* in the soil medium. This indicates that soil harbours a variety of keratinophilic fungi, both dermatophytes and non-dermatophytes, most of which are seldom involved in infections but have the potential to cause infection. There is a natural evolution

from keratin-utilizing soil saprophytes (geophilic species) to association with and final invasion of thickly cornified substrate in living animals (zoophilic species) and man (anthropophilic species). Adaptation to parasitic existence has resulted in a reduced ability to produce spores which is abundant in soil inhabiting species.

T. mentagrophytes has been recovered from soil from many countries for example India [27,28] and Italy [29,30]. In Belgium, *T.mentagrophytes* was the predominant keratinophilic fungus recovered from the floors of both traditional and sub tropical swimming paradises. Subtropical swimming pool is a new type of indoor swimming pool introduced in Belgium in 1981. The floors of the subtropical swimming pools were found to be the more contaminated. Detandt and Nolard [31] attributed the higher rate of contamination to the huge number of visitors, the complexity of construction, the choice of materials and the long opening hours. Mercantini *et al.* [32] recovered *T. mentagrophytes* from soil in Antarctic environment and from dust samples at an Italian science base.

This organism has also been isolated from hairs of wild boars (*Sus scoria*), although there was no evidence of infection in these boars [33]. In Austria, Breuer-Strosberg [34] recovered *T. mentagrophytes* in 5.2% of the cases of infection from cats and 9% from dogs. In western Australia. *T. mentagrophytes* was recovered from domestic pets, farm animals, laboratory animals and wild animals as well as humans. Of all the dermatophytes recovered from western Australia, *T. mentagrophytes* has the widest host range. A kangaroo was found to be carrying *T. mentagrophytes* which it transferred to a laboratory employee who developed a skin lesion [35].

Animals, apparently healthy, serve as a source of infection to humans. In rural environments rabbits were most frequently implicated in the transmission of *T. mentagrophytes* to humans [36]. *T. mentagrophytes* has been recovered from foot wears or shoes using adhesive tape strip technique [37] and from shoes and shower stalls [38].

Keratinolytic capabilities

As pathogens that have a predilection for skin, hair, nails, feathers etc, special interest has focused on their ability to grow on keratin substrates. In the early eighteenth century many workers proved beyond doubt the power of *T. mentagrophytes* and other dermatophytes to grow on keratin substrates such as wool, feather, nails, human and animals hairs [1,39-41]. Vanbreuseghem [2] was so impressed with the specificity of ringworm fungi to the hair that he suggested a classification of various types of parasitic hair invasion. The property was used earlier by Ajello and Georg [43] to distinguish between *T. mentagrophytes* and *T. rubrum*.

In vitro studies of hair invasion by Davidson and Gregory [44] and Vanbreuseghem [2] have shown different ways of perforating the hair, and further demonstrated that:

1. The morphology of the dermatophyte grown on hair *in-vitro* is quite different from that characteristic of parasitic state. In parasitic state few spores are produced.
2. Hair may be invaded *in vitro* either by the penetration of the perforating organ or by the progressive dissolution of the hair from the cortex towards the centre.
3. The breakdown of keratin can be observed not only in humans but in most varieties of animal hair as well as feathers.
4. Dermatophytes are the only fungi that are able to attack hair in this way.
5. Hair invasion in this way provides a means of diagnosing dermatophytic infections.

The current knowledge of the mechanism of the invasion of hair in-situ has originated from the work of Sabouraud [45]. The spores in contact with hair, nails feathers or any keratin substrate, germinate, producing filaments which penetrate the corneal layer into the follicle. Below the infundibulum the corneal epidermis is missing and so the parasite deprived of its only medium for growth can no longer descend further but it is close to the hair which occupies the follicle and the hair is keratinized almost from the base to the neck of the pillar bulb. The parasite then lifts the cuticle of the hair and penetrates it. Having invaded the hair in depth grows down to the level where the hair is no longer keratinized and thus cannot descend further.

Review of literature on the mechanical and chemical breakdown of hairs by dermatophytes showed that in disease, fungi live on non-keratins, having disrupted the keratinous structure by mechanical force. The first proof of the presence of a keratinolytic exo-enzyme in dermatophytes has been demonstrated by Evolceanu and Lazar Maria [46]. They found the final products of breakdown in the presence of this enzyme to be amino acids. Dermatophytes are capable of utilizing keratin as their source of carbon and nitrogen. Kunert [47] observed the decomposition of human hair by dermatophytes *in vitro* and hypothesized that "sulphitolysis" occurred, a process which resulted in the cleaving of disulphide bonds, resulting to the ease with which the denatured keratin was degraded by extracellular proteases produced by the fungus. Using the peptide release assay method and human stratum corneum as a substrate, Samdani *et al.* [48] found that after three weeks culture, *T. mentagrophytes* showed higher proteolytic activity than *T. rubrum* in *tinea pedis* (2044 ± 1067 µg protein released h^{-1} mg^{-1}, n = 13 compared to 828 ± 614 µg protein released h^{-1} mg^{-1}, n = 13). In *tinea unguium*, the values at 3 weeks were generally lower. *T. mentagrophytes* activity was 837 ± 121 µg protein released h^{-1} mg^{-1} compared to 470 ± 271 µg protein released h-1 mg^{-1} for *T. rubrum*. The authors concluded that proteolytic activities varied not only between species but also on the site of attack by the fungus.

Proteases that are produced by dermatophytes *in vitro* may well play an important role in pathogenesis of fungal disease *in vivo*. Many workers have suggested that the pathogenicity of micro-organisms is related to the production of proteases which enable them to parasitize such tissues as stratum corneum [48-50]. Specific enzyme keratinase has been isolated from *T. mentagrophytes* was found to be the most keratinolytic and was seen to breakdown the hair keratin. Large numbers of granules and mitochondria were observed in *T. mentagrophytes* which were not found in other species and the authors suggested that this could be due to higher enzymatic activity of the organism. Further evidence of the enzymatic role in the breakdown of keratin has also been provided by Mercer and Verma [56].

In an *in vitro* model of dermatophyte invasion of human hair follicles, Rashid *et al.* [57] observed that *T. mentagrophytes* invaded the shaft end of the hair and extended along the hair shaft towards the bulb area. Initially, the cuticles formed a barrier to fungal penetration of hair. After incubation for 4 days, germlings of *T. mentagrophytes* penetrated under the cuticle and in between layers of cuticular cells to invade the cortex. In experimental nail infection, Richardson [58] observed that

in the absence of extraneous nutrients, *T. mentagrophytes* completely degraded healthy nail plate.

Rashid *et al.* [59] studied the early events in the invasion of human nail plate by *T. mentagrophytes* and observed that at about 6 hours after incubation of nail fragments, adherence and germination of arthroconida occurred. By 16 hours small germ tubes with side branches were evident. At about 24 hours micro-colonies became established. At 48 hours, mycelium had formed and at about 72 hours, most of the nail fragments were covered with fungal growth. Nail penetration occurred from the ventral surface through the intercellular spaces and with longer incubation all three layers were invaded by arthroconidia growing through the channel. Nail invasion occurred in the absence of added nutrients. The author further observed that the dermatophyte fungus invaded the nail by a combination of mechanical and chemical factors as found in hair invasion.

Siesenop and Bohm [19] used various keratins as substrates and isolated five different keratinolytic enzymes from ten strains of *T. mentagrophytes*, whose molecular weight ranged from 28 Kda to 65 Kda. The authors further observed that duration and intensity of keratinase secretion were strongly influenced by the keratinous substrate used.

Diseases of keratinized areas of humans and animals

Hydrolysis of keratin by keratinases is an important aspect of fungal pathogenesis. For dermatophytes to induce active infection, the arthroconidia lodging on the skin surface must be able to penetrate the stratum corneum. According to the *in vitro* model presented by Aljabre *et al.* [60] the pattern of dermatophyte growth on stratum corneum occurs in three stages, germination of arthroconidia, penetration of stratum corneum followed by formation of arthroconidia. If the arthroconidia have a fastidious requirement for germination, then the chances for successful penetration and invasion of the skin tissues are drastically reduced. *T. mentagrophytes* is not fastidious and readily germinates in the presence of high humidity and nutrients provided by the stratum corneum. This probably explains why fungal skin infections are higher in warm and humid conditions. Hashimoto and Blumenthal [61] found that activating conidia of *T. mentagrophytes* by holding them in distilled water at 28°C for 24h resulted in a significant increase in germination. Epidemiologically this indicates that activated arthroconidia are highly infectious for normally hydrated skin.

Infections in animals

Apart from zoophilic dermatophytes, animals are also infected by geophilic dermatophytes which they pick up from the soil. *T. verrucosum*, *T. mentagrophytes* and *M. canis* are the only three species of zoophilic dermatophytes that are consistently recovered from human disease. Most infected and/or significantly colonized animals appear clinically normal and so can easily transmit the infecting organisms to humans [62].

In a survey in New Zealand carried out by Baxter [62] 36% of cats and 7.5% of dogs examined routinely by a hair brush technique were found to be carriers of *M. canis* and other dermatophytes. The zoophilic dermatopytes are significant causal agents of human ringworm in many areas of the world. For instance, animal–derived dermatophytes accounted for around 20% of all human dermatophytic infections. Epidemiological features of these infections are associated with the degree of contact between the animal reservoir especially young immature animal and a susceptible human host.

Martinez Roig and Torres Rodriguez [55] observed from the study on family incidence of dermatophytosis in Barcelona, Spain, that dogs were the most frequent source of infection in towns and rabbits were the most frequent source in rural areas. Ringworm infection in sheep is considered to be rare. In a few reported cases, *T. mentagrophytes* and *T. verrucosum* were the major aetiologic agents encountered [63]. The diagnostic result in animal dermatophytoses obtained by Schmidt [64] showed that *T. mentagrophytes* has the highest prevalence rate in small rodents and *T. equinum* in horses.

Simpanya and Baxter [65] recovered *T. mentagrophytes* and other keratinophilic fungi from the pelage of cats and dogs using hair brush technique from Palmerston North, New Zealand. A study of zoonotic dermatophytosis in an urban population in the Czech-Republic showed that patients infected by *T. mentagrophytes* contacted the organism from guinea pigs. Tanaka *et al.* [42] found serious dermatophytosis caused by *T. mentagrophytes* in a steller sea lion (*Eumetopias jubatus*) at Yomiuri Land Marine Aquarium in Tokyo, Japan.

Infection in humans

In Nigeria, *T. mentagrophytes* has been isolated from cases of foot infections in coal miners [66], form cement factory workers [67] and from school children [66,69]. In Amsterdam, Nieboar *et al.* [30] isolated *T. mentagrophytes* twice as often as *T. rubrum* from industrial workers using common shower rooms. In a study of ringworm in coal miners in Britain, Gentles and Homes [70] recovered *T. mentagrophytes* alone from 224 of 346 clinical specimens, and *T. rubrum* and *T. mentagrophytes* from 13 specimens. In a 12 year study of dermatophytoses in the Gdansk area of Poland, Nowicki [71] recovered *T. mentagrophytes* from 42.1% of cases, *T. rubrum* (14.7%) and *T. tonsurans* (4.6%). Houck *et al.* [4] isolated *T. mentagrophytes*, from an unusual presentation of *tinea capitis* in a 14-year-old girl with a one year history of a painful scalp mass. Debridement or surgical removal of this mass revealed slender papillomatous growth resembling those seen in elephantiasis nostras verrucose. In Cagliari, Italy, Aste *et al.* [72] recovered *T. mentagrophytes* from four out of 17 cases of *tinea capitis* in adults. In epidemiology of dermatophytosis in Rome Italy by Mercantini *et al.* [73] *T. mentagrophytes* was the third most commonly isolated dermatophyte (10.6%). In Northern Finland, Lehenkari and Silvennoinen-Kassinen [74] recovered the organism from 815 (26%) of 3,185 positive cases of dermatophytosis. *T. mentagrophytes* was isolated from 6% of cases of dermatophytosis in different areas of Iran by Khosravi *et al.* [75]. An unusual case of vulvar kerion caused by *T. mentagrophytes*, initially mistaken for a bacterial infection, was reported in a 43 year old woman by Pinto *et al.* [76]. The authors observed that the peculiarity of this case lies in the fact that the kerion is rarely located in the vulva and has not yet been described in literature. On the contrary, according to the authors, kerion by this organism has been found in the scalp, beard, eye-brow etc.

Ungar and Laude [77] recovered *T. mentagrophytes* and *T. rubum* from a case of *tinea capitis.* In a study of *tinea pedis* in marathon runners in Montreal, Canada by Auger *et al.* [78] *T. rubrum* and *T. mentagrophytes* were the most common dermatophytes recovered from both the occult or subclinical athletes's foot and clinical *tinea*

pedis. Infection was more common in men (24.2%) than in women (6.1%). The authors concluded that marathon runners represent a population at risk of occurrence of both clinical and subclinical *tinea pedis* infection.

In summary, *T. mentagrophytes*, representing the complex, is a highly keratinolytic fungus. It secretes a variety of proteases which enable it to attack different karatinous substrates. It is a non-fastidious fungus. The morphology is varied and many characteristics are not consistent. The organism causes a variety of infections in both animals and humans. It is a zoophilic fungus and most human diseases are contacted from animals. It is of worldwide distribution and has a very wide range of hosts. It has never been implicated in infection of deeper tissues or organs.

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