

REVIEW ARTICLE

Effect of keratin substrates on the growth of keratinophilic fungi

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Abstract

Keratins are the most abundant protein in epithelial cells of vertebrates and represent the major constituents of skin and its appendages such as nail, hair, feather and wool. The protein chains are packed tightly either α -helix (α -keratin) or in β -sheet (β keratin) structure. Therefore, keratins are grouped into hard keratin (feather, hair, hoof and nails) or soft keratin (skin and callus) according to sulphur contents. Due to the strength and stability of keratin very few fungi can utilize keratin and these fungi are called as keratinophilic fungi. Keratinophilic fungi include true fungi which vigorously degrade keratin. These fungi are natural colonizer of keratin substrates like hair, wool and feather. Some of these fungi are well-known dermatophytes and are known to cause superficial cutaneous infections (superficial mycosis). This review will focus on the effect of various keratin substrates on the growth of keratinophilic fungi.

Keywords: Keratins, sulphur, keratinophilic fungi, wool and feather, dermatophytes.

Introduction

Sometime in the early history of vertebrates when they evolved from life in water to life on land, there evolved a specialized protein known as keratin (from the Greek word for horn). This tough, fibrous, insoluble material provided an outer coat that served to prevent the loss of body fluids. Keratin has since proliferated into a wide variety of substances performing many different functions: The claws and armour of reptiles, the feather and beaks of birds, and the hooves, horn, skin, hair and nails of mammals. Keratin is an extremely strong protein which is a major component of skin, hair, hooves, horns and teeth. The amino acids which form keratin have several unique properties, depending on the levels of the various amino acids; keratin can be inflexible and hard, like hooves or soft, as is the case with skin.

Most of the keratin that people interact is actually dead; hair, skin and nails are all formed from dead cells which was shed by the body as new cells push up from underneath. If the dead cells are kept in good condition, they will serve as an insulting layer to protect the delicate new keratin below them. Keratin contains cysteine disulfide, which means that it is able to form a disulfide bridge which is difficult to dissolve. These disulfide bridges create a helical shape that is extremely strong like sulfur atoms which bond to each other from across the helix, creating a fibrous matrix which is not readily soluble. The bond can be extremely strong to make hard cells like those found in hooves, or it can be softer to make flexible keratin like hair and skin which depends upon the amount of cysteine disulfide. Because of the high levels of sulfur in keratin it emits a distinct sulfurous odor which some people find distasteful when it is burnt.

Due to the strength and stability of keratin a very few organisms are able to break it down and utilize it. Only a few actinomycetes and fungi use keratin as a resource. The biggest group of organisms that can utilize keratin as the sole source of carbon and nitrogen are keratinophilic fungi. Keratinophilic fungi occur both in soil and wastewater habitats as saprophytes, some of which are considered to be pathogenic to humans and other organisms (Hoog, 1996). These are minute organism that cannot be seen by the naked eye, unlike macrofungi like mushrooms. The word keratinophilic means 'Keratin loving' and is some misleading in the sense that all fungi that can grow on hair (a common natural keratin substrate) could be considered as keratinophilic. However, hair is not solely made of keratin, and many fungi that grow on natural hair do not actually utilize the keratin, but rather use the non-keratin lipid fraction of the hair. Thus, only fungi which actually degrade keratin should be considered as keratinophilic or keratinolytic fungi. In the kingdom Eumycota (true fungi), two groups namely Deuteromycetes and Ascomycetes are considered as keratinolytic members that occur commonly in soil as keratin decomposers. Some species are potential pathogens which cause infections in the skin and scalp of mammals (The dermatophytes). These dermatophytes include the genera *Microsporium* and *Trichophyton*. It is thought that dermatophytes were initially saprophytes which lived in soil, but due to increasing interaction with animals, they gradually evolved a parasitic lifestyle. Dermatophytes have been classified into three ecological groups based on their habitat preference; they are geophilic (soil loving), zoophilic (animal loving) and anthropophilic (human loving).

The first discovery of keratinophilic fungi from soil was by hair baiting technique, the most common method used for qualitative and quantitative isolation from soil (Vanbreuseghem, 1952). These fungi are natural colonizers of keratin substances. Some are keratinolytic and play an important ecological role in decomposing α -keratins, the insoluble fibrous protein. Because of tight packing of their hair, polypeptide chains in α -helix structures and their linkages by disulphide bonds are considered poorly biodegradable (Kunert *et al.*, 2000). One of the main characteristics of keratins is that they have high mechanical stability and resistance to proteolytic degradation, which depends on the disulphide and hydrogen bonds, salt linkages and other cross-linking (Korkmaz *et al.*, 2004). Therefore, keratinous material is water insoluble and extremely resistant to degradation by common proteolytic enzymes such as trypsin, papain and pepsin (Gupta *et al.*, 2006).

The distribution of these fungi depends on different factors one of which, of vital importance is human and or animal presences. Several studies have been shown that soils are important sources of dermatophytes and keratinophilic fungi. The presence of dermatophytes in soil can be reservoir for infections in human beings. Similarly, polluted water is also a dynamic medium in which a large number of keratinophilic and non-keratinophilic fungus live in close association. Fungi from different ecological groups require different sampling methods and isolation techniques (Baxter and Mann, 1996). A total of 54 soil samples were examined from Jhansi city for isolation and identification of keratinophilic fungi (Chmel and Hasilikowa, 1972). Different sites were selected which include gardens, schools, poultry farms, rivers, hospitals and garbage dumping sites. A total of 23 species belonging to 11 genera were isolated and identified using hair biting technique. The fungi so isolated belong to 5 species from genus *Trichophyton*, 4 species from *Chrysosporium* and 3 species each from *Microsporium* and *Aspergillus*, 2 species from *Fusarium* and one species from each of the following genera: *Rhizopus*, *Alternaria*, *Trichophyton*, *Candida*, *Penicillium* and *Paecilomyces* (Ganaie *et al.*, 2010). The study revealed that among the different sites selected, the soil from gardens (65%) is rich in keratinophilic fungi followed by school (52%), poultry farms (43%), garbage dumping sites (34%), hospitals (30%) and rivers (21%) (Hafeez, 1987).

Degradation of various keratin substrates by keratinophilic fungi

Keratinophilic species are able to use materials associated naturally with keratin (Fillipelo *et al.*, 1994). These fungi are natural colonizers of keratin substances. Keratinaceous material such as hair, nail, hair, hoof and horn, contain a large proportion of keratin protein. So keratin is a major component of hair, feather and wool. A distinctive feature of keratin is its relatively high sulfur content due to the presence of sulfur containing amino acids like cysteine, cystine and methionine.

Thus, the disulfide bond is considered to be responsible for the stability of keratin and its resistance to enzymatic degradation (Rawa and Hadi, 2002). Nevertheless, *in vitro* ability of various fungal species to degrade keratin substrates such as hair, wool and feather have been examined (Tawfik, 2002).

Comparative effect of different substrates on the growth of keratinophilic fungi

Four fungal species including two dermatophytes and two saprophytes were isolated from sewage sludge samples at Basrah, Iraq and tested for their degradative ability towards three types of keratin substrates (human hair, chicken feather and wool). The rate of keratin degradation was expressed as weight loss over three week of incubation using a liquid culture medium. Human hair had the highest degradation rate by colonization of *Chrysosporium pannicola* and *Microsporium gypsum* at a rate of 62% and 48% respectively. Chicken feather were highly degraded by *Aspergillus flavus* (32%), while wool degradation was highest by *C. pannicola* (45.5%) and *Trichophyton mentagrophytes var. erincei* (38%). There was a significant difference in keratin substrate degradation rates by the examined fungi.

Keratinase activity was highest for *C. pannicola* and *M. gypsum* in the culture medium baited with human hair. *Aspergillus flavus* revealed highest activity of this enzyme in cultures amended with chicken feather while *T. mentagrophytes var. erincei* showed highest keratinase activity in culture incubated with wool substrate. The amount of protein released into the culture medium varied among the tested fungi. The medium's alkalinity increased over incubation time from 6.5 to 7.8. Microscopic examination showed maceration of the keratin substrates by fungi (Muhsin and Hadi, 2002).

Effect of keratinophilic fungi in degradation on dermal tissue in human beings

Keratinophilic fungi are of prime importance in regard to various skin diseases prevalent in various areas. The majority of the fungi producing diseases in human beings and animals belong to the family of keratinophilic fungi. Some of these fungi are well-known dermatophytes. Dermatophytes are a group of morphologically and physiologically related molds some of which cause well-defined infection: Superficial cutaneous infection (Dermatophytosis) of keratinized tissues (skin, hair and nail) of humans and animals (Vroey, 1985). They have two important properties: keratinophilic and keratinolytic. This means that 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 vitro* and provoke tinea. Mycotic infection is reported throughout the world and is extremely contagious. Both dermatophytes and non-dermatophytes can colonize and invade the keratin of skin, nail, and hair (Deshmukh *et al.*, 2008).

The occurrence of dermatophytes and other keratinophilic fungi enhance the risk of human dermatophytosis. Dermatophytes are spread by direct contact from infected people (anthropophilic), animals (zoophilic) and soil (geophilic) and indirectly from fomites. Other researchers previously reported few cases of tinea due to *M. gypseum* in Ahvaz (Mohmoudabadi, 1997). In superficial fungal infection the pathogenic invading fungus is confined to the horny stratum of the skin and there is no or at least minimal, tissue reaction. This differentiates them from skin mycosis where all the keratinized tissues: skin, hair, fur, feather and nail can be affected. In the latter cases, however, the fungi are usually confined to the horny layers of the skin and its surrounding tissues which cause destruction of these structures and bring an immunological host reaction. This definition disregards fungal infections of the skin and mucous membranes that could occur in the course of a systemic mycosis where there is hematogenous dissemination with skin location, as well as fungal infections that affect the skin as well as subcutaneous tissue or other deeper structures.

Twelve species were dermatophytes or closely related fungi: *Chrysosporium asperatum*, *C. georgii*, *C. indicum*, *C. keratinophilum*, *C. tropicum*, *C. pannorum*, *Microsporium cookie*, *M. ferrugineum*, *M. gypseum*, *Trichophyton ajelloi*, *T. mentagrophytes* and *T. terrestre*. The other 32 species includes member of *Acremonium*, *Alternaria*, *Aspergillus*, *Candida*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Gymnoascus*, *Mucor*, *Paecilomyces*, *Penicillium*, *Scopulariopsis*, *Sporothrix*, *Trichoderma*, *Verticillium* and unidentified yeasts. The fungi isolated are pathogenic or potentially pathogenic to humans and animals and their occurrence in activated sludge poses a health risk to wastewater treatment workers and the environment (Mahmoudabadi and Zarría, 2008).

Conclusion

This review provided information about various keratin substrates namely hair, nail, horn and feather and the effect of these substrates on the growth of the keratinophilic fungi which are pathogenic to humans and other organisms. This review also provided knowledge about the comparative study between various keratinophilic fungi and different keratin substrates. To conclude, different keratinophilic fungi plays an important role in degradation of various keratin substrates and also help in maintaining the environment eco-friendly by minimizing soil and water pollution.

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