Pathogenic properties of dermatophytes and it ' s potential in keratin degradation

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Abstract

Dermatophytes play a major role in the degradation of animal tissues such as keratin. Keratin considered to be main component in the skin. The aim of this research was to study the mechanisms of degradation of keratin via *Trichophyton mentagrophytes* . *T. mentagrophytes* was grown on leqiud media supported by keratin azure as a source of carbon and nitrogen. Keratinolytic activities were observed by measuring the activity of keratinase (release keratin azure).The results showed that the dermatophytic fungus (*T. mentagrophytes*) was able to extract nutrients from the substrate. In addition, the fungus showed the highest ability to release azure in test medium.

Introduction

Keratinolytic fungi have pathogenic properties to animals and human (Dermatophytes) [Gherbawy *et al*, 2006]. Since a long time, the ability of fungal keratinase considered the key of animal dermatopathogenicty. [Scott and Untereiner, 2004]. The term dermatomycosis is includes colonization and infection of keratinous material by fungi (dermatophytes) because they need keratin to grow. Dermatophytes originally are a group of fungi that can in vivo and/or in vitro, colonize and break down the keratinized tissues of human and animals. In addition, they consider keratinolytic fungi that originally grow as saprophytic, but they adapted to become parasitic on human and animal [Sharma and Sharma, 2010].These fungi have drawn the attention in medical study therefore they cause human and animal diseases known as ringworm (clinical name Tinea) which affects the keratin in hair, nails and stratum corneum (top layer) of the skin [Ashbee and Evans, 2002]. In many countries dermatophytes are a major public health problem especially in tropical countries such as India because of their warm, humid, tropical climate, crowded living and relatively poor socio-economic conditions [Singh *et al.*, 2009]. The majority of the fungi producing diseases in human and animals exist freely in nature as soil saprophytes. Dermatophytes are spread by direct contact from infected people, animals and soil. Variety of studies has been reported that soils are important sources of dermatophytes Ringworms which occur in animals may spread to humans. As a result, animals considered as a direct source of human infections. .There are approximately 20 species of dermatophyte fungi from the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are responsible of ringworms [Ashbee and Evans,2002]. These fungi able to colonize the layer corneum first then grow in a radial shape without penetrating practicable tissue (e.g. invasion of hair is non-living tissue). If the fungus enters viable tissue and continues to grow, then infection is present. [Kaul and Sumbali, 1999]. Ringworm has symptoms such as dry scaling, pain, inflammation which result boggy wound. Although organisms are essentially causing diseases, some believe revealed that dermatophytes can be isolated from the feet or scalp without any obvious mark of infection. Some studies reported that lesions can be found as mark of infection [Ashbee and Evans,2002]. Ecologically, dermatophytes classified to three groups which are:

- Anthropophilic (people-loving)
- Geophilic (soil-loving).
- Zoophilic (animal-loving).

Geophilic and Zoophilic dermatophytes usually are more ruthless and self-limiting infection. However,

Anthropophilic species cause little inflammation which continues of infection for long time. Dermatophytes fungi classified in the anamorphic genera *Epidermophyton, Keratinomyces, Microsporum,* and *Trichophyton.* (Kaul and Sumbali, 1999). Majority of dermatophytes belong to families Arthrodermataceae and Onygenaceae in Ascomycetes. Almost of fungi grow on higher plants or their remains, and survive saprophytically. In contrast, Arthrodermataceae and Onygenaceae are unusual thus majority of them are associated with birds and mammals. Supsecuently, these consider true fungi which stongly degrade keratin and involve important pathogens for human and animals [kushwaha and Gupta, 2008].

Aim of the Study

This research aims to study the ability of keratinolytic fungus (*Trichophyton mentagrophytes*) related to dermatophytes.

Materials and Methods

There is one type of keratinous substrate was proposed for this study.

- **Keratinous substrate**

The fungal strain was tested using a commercial keratinous substrate is called Keratin Azure (Sigma) as carbon and nitrogen source(Fig.1) Keratin Azure is a keratinous substrate dyed with Ramazol Brilliant Blue R. The dye is associated with the substrate; the measurement of keratin degradation is expressed by release of the blue colour. If the fungus releases the blue colour into the medium then this can easily measure using a spectrophotometer [Wainwright, 1982].

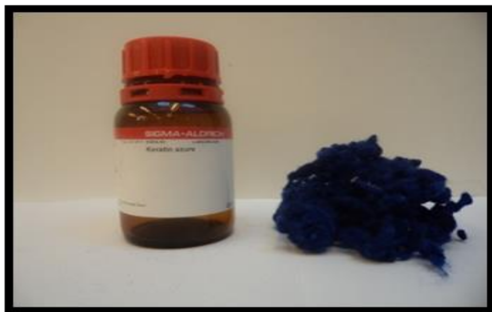

Figure 1: Keratin Azure before cutting.

- **Preparation of keratin azure solution**

Keratin azure was cut into small pieces, 5g keratin azure was added to 300 ml distilled water, put on a shaker overnight and then the blue liquid(Fig.2) was added to test media. Keratinolytic assays were achieved via release of the blue colour in liquid medium-keratin azure [Letourneau *et al*., 1998].

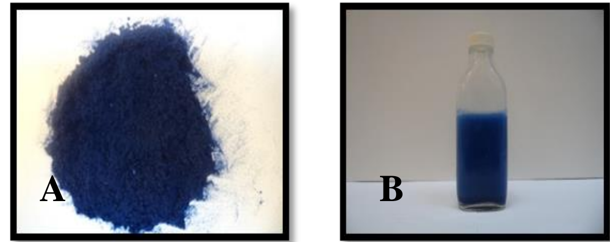

Figure 2: Keratin azure
(A after cutting**, B** Blue liquid)

Isolates

T. mentagrophytes (Dermatophytic fungus) obtained from laboratory (isolated from ringworm) was used in this reaserch (Fig.3). *T. mentagrophytes* was selected for enzyme activity in leqiud medium [Sabouraud Dextrose broth-keratin azure medium (SDB-azure)].

Figure 3: *T. mentagrophytes* in SDA after one week incubation at 25C°.

- **Fungal keratinolytic Assay in Liquid Medium-keratin azure**

The reaction mixture contained 10% (v/v) keratin azure solution in Erlenmeyer flasks (250 ml) added to SD broth (SDB) (Sigma). SDB consisted of 10g peptone, 40g Dextrose in 1L of dH2O. The mixture was sterilized at final pH 6.06 .The medium was then inoculated with 8mm disc of fungus and incubated on revolving shakers (150 rpm) at 25°C for 7,14, 21 and 28 days. A set of uninoculated flasks was included as control. After the end of incubation period the liquid medium was filtered using Whatman filter paper No. 1. The filter paper with biomass was dried in oven over night at 110°C to a stable weight [Jain *et al*., 2012]. The pH was measured during the incubation period using a pH meter. Degradation of keratin azure was determined using the method as described by Letourneau *et al*.,(1998). Keratinolytic activity was determined by measuring the absorbance of keratin azure at 595 nm. The samples after each incubation were filtered and the absorbance of the supernatant was determined at 595 nm.

Results and discussion

keratinolytic Assay in Liquid Medium-keratin azure

T. mentagrophytes showed a rapid response (i.e. dye release at 7 days) (Fig.4). Azure release by the fungus was high at 1 week (0.28), then the proportion decreased after 14 and 21 days incubation (0.042,0.144) which produced weak dye release (Fig.5). After that, the amount of keratinase increased at week 4.

Figure 4: Release of azure in SDB-Azure by *T.mentagrophytes* after 2 weeks incubation**.**

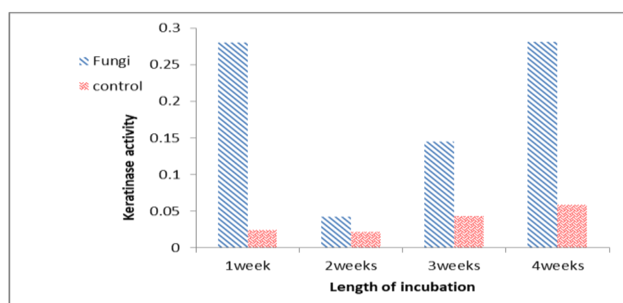

Figure 5: Keratinase activity in SDB-Azure by *T.mentagrophytes* between 7-28 days incubation at 25C°.

Biomass

In Figure 6, it can be clearly seen that the maximum biomass produced by *T. mentagrophytes* occurred after 7 days, the growth then decreased gradually in the days following.

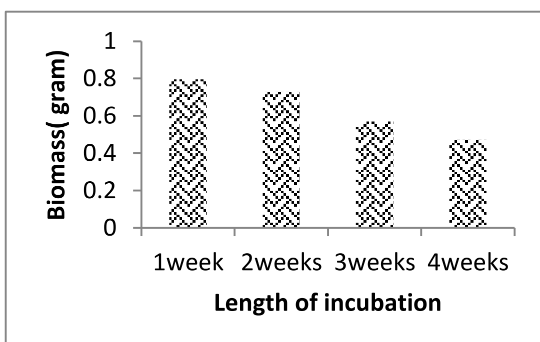

Figure 6: *T. mentagrophytes* biomass production in SDB-Azure medium.

Change in pH

Change in pH of medium was noted after 1 week compared with initial pH(6.06) . The experimental results show that the degradation of keratin azure was accompanied by alkalization of the medium. A maximum pH value was seen in week 4(Fig7).

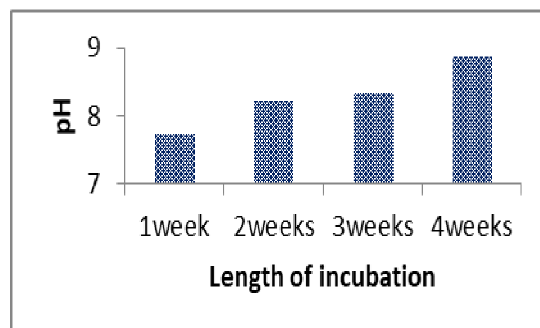

Figure 7: pH values of T. *Mentagrophytes* during incubation period

An Increasing in pH range was observed in all period. The alkalinisation by T. *mentagrophytes* was highly marked in medium contain keratin azure. This may reflect that keratinolytic fungi often alkalinize culture media during growth (Muhsin and Hadi, 2002). Interestingly, this correlation is related to the release of ammonia which raises the alkalinity of the medium. Jain *et al*., (2012) have shown that a change in pH (deamination) toward alkalinity allows the substrate to create an environment for the sulphitolysis and proteolysis. The results also demonstrated that *T. mentagrophytes* which have strong keratinolytic ability increased alkalinity more than those that were less keratinolytic (Kim, 2003). The present data confirms that there is an association between the degree of alkalinisation and the amount of keratin degraded. These results are similar to those reported by Kaul and Sumbali (1999), which confirmed strong keratinolytic activity makes growth medium more alkaline**.**

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