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FUNGAL STRAINS ISOLATED FROM SEVERAL CASES OF HUMAN DERMATOPHYTOSES

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Abstract

Dermatophytosis has become one of the most common human infectious diseases in the world so it is of interest to dedicate more studies to its etiological agent's dermatophytes. These keratinophilic and keratinolytic filamentous fungi have the ability to invade and colonize keratinized layers of the skin and their appendages. Dermatophytes fungi group include three anamorphic genera namely Epidermophyton, Microsporum and Trichophyton. These three genera include geophilic, zoophilic and anthropophilic species. Usually these filamentous fungi are identified on the basis of conidia morphology and sometimes with specific physiological characters, such as the hair strand perforation and urea hydrolysis. The objective of present study was to isolate and to identify some filamentous dermatophytes fungi from human superficial mycoses. Isolated samples (scales, fragments of nails and subungual debris) were cultured on specific culture media during four months. After incubation time, morphological characters of cultured fungal were observed macroscopically and microscopically. The following cultural characteristics were analyzed: texture, surface and reverse color of the colony, the presence of pigmentation. Microscopic examination offered data on specific characters such as, the presence/absence of macroconidia and microconidia, theirs shape, their septa number, the presence of chlamydoconidia, spiral hyphae and nodular organs. The strains identification was completed with in vitro hair strand perforation and urea hydrolysis. The isolated microbial strains were identified as belonging to Trichophyton and Microsporum genera.

Keywords: *dermatophytosis, dermatophytes fungi, Microsporum, Trichophyton.*

INTRODUCTION

Dermatophytosis are superficial skin infections confined to the stratum corneum, produced by filamentous fungi called dermatophytes. In a case of dermatophytosis the lesions are very characteristic and can affect different areas of the body surface. Depending on the area of the body affected by fungal infection, dermatophytosis (ringworm or *tinea*) are classified in several type, namely: *tinea barbae* (chin, mustache), *tinea capitis* (scalp, eyebrows, eyelashes), *tinea corporis* (glabrous skin), *tinea cruris* (groin), *tinea faciei* (on the face), *tinea favosa* (favus), *tinea imbricata*, *tinea manuum* (on hand), *tinea unghium* (on nails), *tinea pedis* (at legs) (Hainer, 2003; Vander Straten et al., 2003; Gupta et al., 2008).
In the dermatophytes group are included three anamorphic genera of hyaline filamentous fungi namely *Epidermophyton, Microsporum* and *Trichophyton* (Weeks, 2003; Kayzer, 2005; http://www.doctorfungus.org/). They have the ability to invade and colonize keratinized layers (Sharma et al., 2011) and to produce enzymes (keratinases), endoproteases and exoproteases (Monod, 2008). Dermatophytes include geophilic, zoophilic and anthropofilic species, with a restricted or a worldwide geographical distribution (Achterman, 2012).
Dermatophytosis can vary from acute to chronic forms, depending on many factors, including the host, species of fungus involved or lesions location on the body surface (Vermout et al., 2008, Bramono, 2012).
Dermatophytosis can be transmitted directly by contact with infected person or indirectly through contact with infected products or objects (Gupta et al., 2003).

In recent years, the risk of fungal infections has been increasing drastically so it is of interest to solve these diseases. The objective of present study was to isolate and to identify some filamentous dermatophytes fungi from human superficial mycoses. Isolated samples (scales, fragments of nails and subungual debris) were cultured on specific culture media during four months.

MATERIALS AND METHODS

Samples

A number of 34 samples were used in this study, represented by skin scales, hair strands, nails fragments and subungual debris. Samples used in study were collected in 2008-2009 in the Mycotic Infections laboratory of the National Institute of Research & Development for Microbiology and Immunology Cantacuzino from apparently healthy people.

Sampling method

A classic protocol was used for samples collection (Coman and Mares, 2000; Mares and Bazgan, 2008). The samples were collected after cleaning the affected area with 70% alcohol. From the skin surface, the samples were collected by scraping the lesion from the center to its edge using a sterile scalpel. The hair samples were plucked or shave (where the hair could not be plucked) using a sterile tuck. The nail fragments were collected using a sterile scissors, while the nail bed was scraped and the subungual debris have been collected.

Culture method

Collected samples were cultured on two solid media, potato extract medium and Sabouraud medium. After inoculation, the Petri plates were incubated at 25-30˚C, for 4 weeks. The microbial growth was monitored daily during the entire period.

Identification methods

Macroscopic observations

Macroscopic observations of isolated strains in solid cultures were carried out weekly, noting the growth rate, colonies morphology and pigment formation in the culture medium.

Microscopic observations

The investigations were done using an Olympus BX51 microscope and consisted in direct microscopy in a drop of 10% potassium hydroxide (KOH) for skin scales examination and 20% for nails examination and in wet mounts mounted in a drop of lactophenol cotton blue and, respectively.

Slide culture technique

The technique was performed in a wet room made into a Petri plate by placing a fragment of sterile filter paper and moistening it with sterile distilled water. Over the filter paper a fragment of sterile glass in U-shape was placed and over which was placed a sterile microscope slide. A block of potato extract medium of about 1 cm^2 was placed over the slide, in the center. The medium was inoculated with a mycelium from the fungal strain thereafter covered by a cover slip. The Petri plate was incubated at 30˚C until fungal growth was observed (Figure 1) Microscopic examination was made by wet mounts prepared in a drop of lactophenol cotton blue.

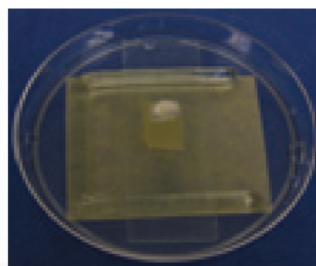

Figure 1. Slide culture technique

Urea hydrolysis test

The isolated fungi were incubated in urea liquid medium for 7 days at 25-30°C. The inoculated test tubes were examined daily to observe a possible color change of the culture medium in case of a positive test from straw to reddish-purple. Urea hydrolysis test was used to distinguish *Trichoplyton mentagrophytes* from *Trichophyton rubrum. Trichophyton mentagrophytes* is usually urease positive in 7 days and *Trichophyton rubrum* is usually urease negative

In vitro hair strand perforation test

The test was performed by placing a few hair strands fragments in a sterile Petri plate and adding 10 ml of sterile distilled water and 0.1 ml of yeast extract 10%. A piece of fungal mycelium was transferred in Petri plate and incubated at 25°C for 21 days. Periodically was done wet mounts, mounted in a drop of lactophenol cotton blue.

RESULTS AND DISCUSSIONS

In the positive skin scraping samples, on direct microscopy (KOH), fungal elements as networks of branching fungal hyphae were observed (Figure 2). For 6 samples, the KOH test was negative and no fungal elements were relieved.

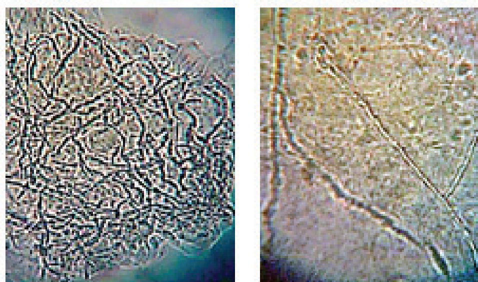

Figure 2. Skin scraping infected with septate, branching fungal hyphae

Collected samples were cultured on potato extract medium and Sabouraud medium in Petri plates. Of the 34 samples were obtained 28 fungal isolates and all were used for subsequent identification. The fungal colonies developed after 14 days of incubation were investigated through macroscopic and microscopic observations. Macroscopic observations of isolated strains offer information about growth rate, colonies morphology (color of colony obverse and reverse, shape of the edges, colony surface appearance, and texture) and pigment formation in the culture medium. Based on these informations, isolated fungi were identified as belonging to *Trichophyton* and *Microsporum* genera. Therefore, 22 strains belong to the genus *Trichophyton*, as *Trichophytoninterdigitale, Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Trichophyton sp.* and 6 strains belong to the genus *Microsporum, as Microsporum canis* and *Microsporum sp.* (Figure 3).

These fungal isolates are known to be involved in the etiology of human dermatophytosis (Jackson, 2006; Mahmoudabadi and Yaghoobi, 2008).

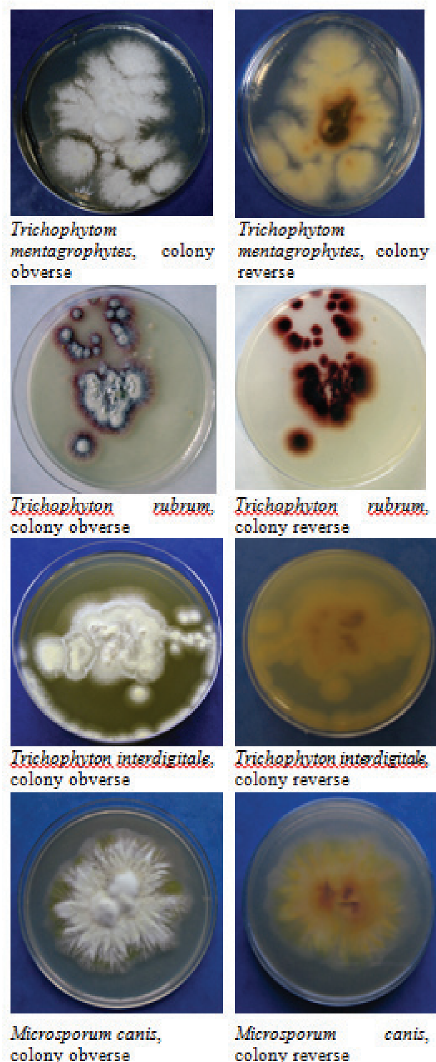

Figure 3. Macroscopic characteristics of isolated Trichophyton sp. and Microsporum sp. strains, on PDA medium at after 14 days of incubation

The preparations with lacthophenol cotton blue relieved some important characters for fungal strains identification, as shape and dimensions of macroconidia and microconidia, macro-conidial septa number, presence of chlamy-doconidia, spiral or raquet hyphae and nodular organs. The results of this investigation are presented in Figure 4.

Characteristic of *Trichophyton mentagrophytes* culture are: rapid growth, granular surface, flat colonies, white to cream colour on obverse, irregular edge, brownish yellow to reddish-brown on reverse.

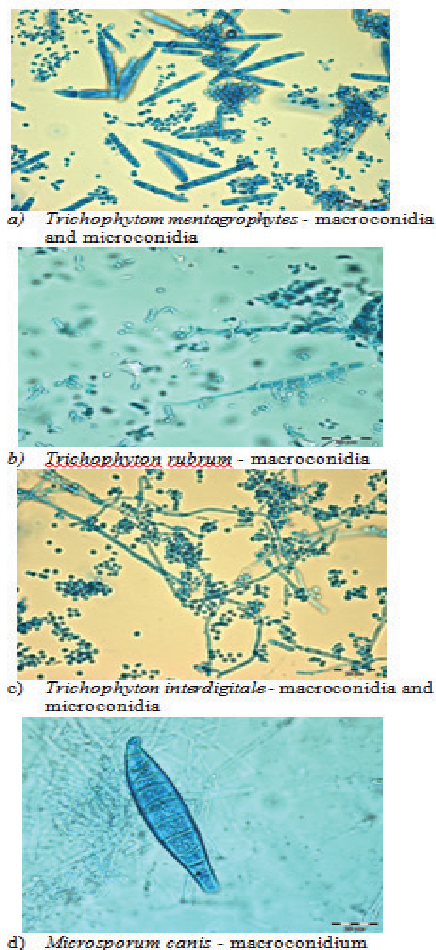

Figure 4. Microscopic characteristics of isolated Trichophyton sp. and Microsporum sp. strains in lacthophenol cotton blue-slide culture technique (400x)

Microscopic observation showed smooth macroconidia (4-6 septa), numerous spherical or pyriform, microconidia, in clusters and spiral hyphae (Figure 4a).

Characteristic of *Trichophyton rubrum* culture are**:** moderate growth, flat to slightly raised, white to cream colonies obverse, velvety with an wine red reverse. Microscopic observations showed pencil-shaped abundant macroconidia, often show an terminal appendages and pyriform microconidia (Figure 4b). Characteristic of *Trichophyton interdigitale* culture are**:** moderate growth, flat, white to cream colonies obverse, granular surface, with an yellow reverse. Microscopic observation showed few clavate macroconidia and abundant pyriform microconidia (Figure 4c).

Characteristic of *Microsporum canis* culture are**:** fast growth, flat colonies, white-yellowish surface; golden yellow colony reverse. Microscopic observation showed abundant long, rough, fusiform macroconidia and a few pyriform to clavate microconidia. (Figure 4d).

For a more precise identification, more tests were carried out such as urea hydrolysis test and *in vitro* hair strand perforation test. Urea hydrolysis test was used to distinguish *Trichoplyton mentagrophytes* from *Trichophyton rubrum*. *Trichophyton mentagrophytes* is usually urease positive in 7 days and *Trichophyton rubrum* is usually urease negative.

As it can be shown in Figure 5, the result for *Trichophyton mentagrophytes* is positive (left), the strain producing urease and breaking down urea. Meanwhile, *Trichophyton rubrum* has no hydrolytic activity against urea (right).

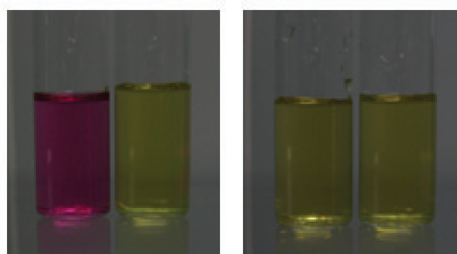

Figure 5. Urea hydrolysis test

The positive result at the *in vitro* hair strand perforation test for *Microsporum canis* is presented in Figure 6.

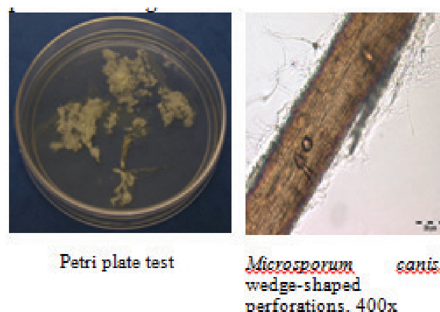

Figure 6. The in vitro hair strand perforation test

Also, *Trichophyton interdigitale*, *Trichophyton mentagrophytes* are able to perforate the hair

strand. *Trichophyton rubrum* concerning the ability to perforate the hair strand in vitro and are negative (data not shown).

The isolation and identification procedure finally provided two predominant fungal strains, namely *Trichophyton rubrum* and *Trichophyton mentagrophytes*.These results are similar to those reported by other authors (Szepietowski et al., 2002; Venkatesan, 2007; Seebacher et al., 2008; Woodfolk, 2012).

CONCLUSIONS

In this study, several dermatophytes fungi were isolated from human infections. From the total number of samples, 82.35% were positive and the isolated strains belonged to dermatophytes fungi. The most frequently isolated strains belong to the *Trichophyton genus*, as *Trichophyton rubrum and Trichophyton mentagrophytes*; our results being in accordance with other similar studies. Of the total of 28 isolated strains *Trichophyton rubrum* reprezents 35,71%; *Trichophyton mentagrophytes* 21,43%; *Trichophyton interdigitale* 7,14%; *Trichophyton sp.* 14,29%; *Microsporum canis* 7,14% and *Microsporum sp*. 14,29%.

The practical importance of the study is that the methods used to identify the fungal strains participate in improvement of dermatophytosis diagnostic algorithm.

Also was performed the monitoring of incidence of etiologic agents involved in human dermatophytosis

REFERENCES

1. Achterman R. R., White C. T., 2012. Dermatophyte virulence factors: Identifying and analyzing genes that may contribute to chronic or acute skin infections. International Journal of Microbiology, pp. 1-8.
2. Bramono K., 2012. Chronic Recurrent Dermatophytosis in the Tropics: Studies on Tinea Imbricata in Indonesia. Korean Journal of Medical Mycology,17 (1) :1-7.
3. Coman I., Mares M., 2000. Principalele genuri de micromiceti cu implicatii in patologie, Genul Microsporum, Genul Trichophyton; Diagnosticul bolilor produse de micromiceti. In: Micologie Medicala Aplicata; Editura Junimea, Iasi, 45-70; 154-165; 250-260.
4. Gupta A.K., Chaudhry M., Elewski B., 2003. Tinea corporis, tinea cruris, tinea nigra, and piedra, Dermatologic Clinics, 21: 395–400.
5. Gupta A. K., Cooper E. A., 2008. Dermatophytosis (Tinea) and Other Superficial Fungal Infections. In: Infectious Disease Diagnosis and Treatment of Human Mycoses edited by D. R. Hospenthal, M. G. Rinaldi, Humana Press Inc.Totowa, New Jersey, 355-381.
6. Hainer B. L. 2003. Dermatophyte Infections, American Family Physician, 67 (1) :101-108.
7. Jackson C.J., Takashi Mochizuki T., Barton R.C., 2006. PCR fingerprinting of Trichophyton mentagrophytes var. interdigitale using polymorphic subrepeat loci in the rDNA nontranscribed spacer. Journal of Medical Microbiology, 55, 1349–1355.
8. Kayser F. H., Bienz K.A., Eckert J., Zinkernagel R.M. 2005. Fungi as humans pathogens, Coutaneous mycoses. In: Medical microbiology Thieme, cap. 6, pp. 372-376.
9. Mahmoudabadi A., Z., Yaghoobi R., 2008. Extensive tinea corporis due to Trichophyton rubrum on the trunk, Jundishapur Journal of Microbiology, 1 (1) : 35-37.
10. Mares M., Bazgan O., 2008. Diagnosticul de laborator al infectiilor produse de fungi. In: Tratat de Microbiologie Clinica D. Buiuc, M. Negut, Editia a II-a, Editura Medicala, Bucuresti, 953-1017.
11. Monod M., 2008. Secreted proteases from dermatophytes, Mycopathologia, 166:285-294
12. Seebacher C, Bouchara J-P., Mignon B., 2008. Updates on the epidemiology of dermatophyte infections, Mycopathologia,166:335–352.
13. Sharma M., Sharma M., Rao V.M., 2011. In vitro biodegradation of keratin by dermatophytes and some soil keratinophiles, African Journal of Biochemistry Research, 5 (1) :1-6.
14. Szepietowski J.C., Bielicka E., Maj J., 2002. Inflammatory tinea barbae due to Trichophyton rubrum infection – autoinoculation from fingernail onychomycosis?, Case Reports and Clinical Practice Review, 3 (4) : 254-256.
15. Venkatesan G., Ranjit Singh A.J.A., Murugesan A.G., C. Janaki C., Gokul Shankar S., 2007. Trichophyton rubrum – the predominant etiological agent in human dermatophytoses in Chennai, India. African Journal of Microbiology Research, 009-012.
16. Vander Straten M. R., Hossain M. A., Ghannoum M. A., 2003. Cutaneous infections, Dermatophytosis, onychomycosis and tinea versicolor, Infectious Disease Clinics of North America, 17: 87–112.
17. Vermout S., Tabart J., Baldo A., Mathy A., Losson B., Mignon B., 2008. Pathogenesis of dermatophytosis, Mycopathologia, 166: 267-275.
18. Weeks J., Moser S.A., Elewski B.E., 2003. Superficial cutaneous fungal infection. In: Clinical Mycology, edited by Dismukes W. E., Gappas P. G., Sobel J.D., Oxford University Press, 367-389.
19. Woodfolk J.A., 2005. Allergy and Dermatophytes, Clinical Microbiology Reviews. 18 (1) :30.*** http://www.doctorfungus.org/