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Mycoflora of Animal and Human Hairs from Riyadh, Saudi Arabia

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Abstract

A total number of 28 species were isolated from different type of hairs including, sheep, goat, cow, rabbit and human hairs, by hair baiting technique. Isolated fungi were tested for hair degradation and also grown on Modified Dermatophyte Test Medium (DTM). A correlation was found between quantity of hair degradation and percent of coloured zone shown on DTM by the fungi tested.

Introduction

Fungi that could degrade hairs are generally termed as keratinophilic-fungi (Carmichael, 1962; Van-Oorschot, 1980). These fungi have the biological ability to metabolize keratinaceous substance from animals such as hairs, nails that constitute the external surfaces of the animal body (Mercantini *et al.*, 1989; Ali-Shtayeh and Arda, 1989). Although some of these fungi metabolize the keratin in a saprobiontic activity therefore only utilize the inert keratinic fragments while, in contrast, others have developed a biochemical activity and become parasites. This latter types of fungi are known as dermatophytes (Frey *et al.*, 1979; Rippon, 1982; Matsumoto and Ajello, 1987). Recent research tends to give more concentration on the keratinolytic capacity of other fungi, although with less frequency, and to recognise their role in causing human and animal diseases (Nigam and Kushwaha, 1990; Abdel-Hafez *et al.*, 1990; Ali-Shtayeh *et al.*, 1988). Epidemiological studies that earlier carried out were aimed at defining the relationships between the above mentioned fungi and the environment like from soils (Marsella and Mercantini, 1986; Nigam and Kushwaha, 1990), air dust (Abdel-Hafez *et al.*, 1989), schools (Morganti and Tampieri, 1985; Abdel-Mallek *et al.*, 1988), public parks (Morganti and Tampieri, 1985), Zoos (Marsella *et al.*, 1985) and also from household environments (Bokhary and Parvez, 1995). In Saudi Arabia, some work have been done on keratinophilic fungi (Bagy and Gohar, 1988) but main work have been concentrated on the distribution of dermatophytes and other human pathogenic fungi (El-Hams, 1989; Al-Sogair *et al.*, 1989; Abdel-Fattah *et al.*, 1972). No work so far has been done on to isolate fungi from animal hairs. The aim of this study was to isolate the fungi from different type of hairs and grow the isolated fungi on modified Dermatophytes test medium to see color-zone formation to observe the co-relation between the percent of color-zone formation and the percent degradation of hair by a particular fungi.

Materials and Methods

Samples Collection: Hair samples of animal were collected in a sterilized polyethylene bags or in sterile petridishes from

animal market in Riyadh while human hair samples were collected from barber shops in Riyadh. Many replicate of each type of hair samples were collected and then sample replicates of one type of hairs were mixed together in the laboratory.

Isolation of Fungi by Hair Baiting Technique: Isolation of keratinophilic fungi was carried out by hair baiting technique (Larone, 1995). Sterilized filter papers (two in each plate) were put in sterilized disposable petridishes. Sterilized water was added to each plate for moisture. Approximately 0.5 gram of hair samples (cut about 1 cm) was distributed over the sterilized filter paper. Ten replicates of each type of sample were prepared in the same way. These plates were then incubated for 3 weeks at room temperature. Sterilized water was added at intervals to keep filter paper wet. After 3 weeks hair samples were examined for presence of fungi under the microscopes. Slides were prepared for identification.

Isolation of Pure Culture of Fungi: Pure Cultures of fungi were obtained on Sabouraud Dextrose agar containing rose bengal (0.33 g/L) and streptomycin sulphate (0.3 g/L). Later on isolated cultures were maintained on Sabouraud Dextrose agar plates and in slants.

Percent Weight Loss Test for Hairs: Sabouraud dextrose broth containing 1 gram of human child hair (cut into small pieces) was used for weight loss test. In a 100 ml conical flask, 50 ml of Sabouraud broth medium and 1 gram of hair pieces (oven dried at 170°C for 24 hrs) was added and then autoclaved at 121°C for 15 minutes for sterilisation. Five replicates were prepared in the same way for each type of fungus. This medium was then inoculated with fungal spore suspension which was prepared from fresh culture of fungus by adding 5 ml of sterilized water and shaking vigorously to get spore and propogules suspension. A final concentration of 5×10^3 per ml of spore was prepared by serial dilution for inoculation of medium. Inoculated flasks were incubated for a period of 3 weeks at room temperature. Culture medium were then filtered through Whatman No. 1 filter paper and hair were washed throughly

several times with distilled water to get rid of all traces of fungus. Hair samples were then dried up in an oven at 170°C for 24 hrs prior to taking dry weight.

Percent Coloured-Zone Test: For percent coloured-zone test, a disk from fresh culture of a fungus was inoculated on Modified Dermatophyte Test Medium, which contain (Mycobiotic agar 35.6 /g (DIFCO. USA), 40 ml phenol red solution (0.5 g phenol red dissolved in 15 ml of 0.1 N NaOH made upto 100 ml with distilled water), 6 ml of 0.8 M HCl and 1000 ml water. This medium was autoclaved to sterilize for 10 minutes at 121°C in an autoclave and dispensed into petridishes (approx. 20 ml per plate) for inoculation. After 7 days of inoculation, the diameter of the colony and diameter of the coloured-zone was measured and percent coloured-zone was calculated according to the following formula.

$$\% \text{ coloured zone} = \frac{\text{Diam. of coloured - zone} - \text{Diam. of colony}}{\text{Diam. of colony}}$$

Results and Discussion

A total number of twenty eight species of fungi were isolated from sheep, goat, cow, rabbit and human hairs. Out of these twenty species were found on sheep's hairs, nineteen on human's hair, sixteen on cow's hairs, fifteen on goat's hair, and thirteen on rabbit's hairs. Genus *Aspergillus*

Table 1: Isolation of fungi from different type of hairs

Fungi	Types of hair				
	Sheep	Goat	Cow	Rabbit	Human
<i>A. corymbifera</i>	+	+	+	-	-
<i>A. ramose</i>	+	+	-	-	-
<i>A. alternate</i>	+	+	+	+	+
<i>A. chlamydospora</i>	+	+	+	+	+
<i>A. candidus</i>	-	-	-	-	+
<i>A. ellipticus</i>	+	-	+	-	+
<i>A. flavus</i>	+	+	+	+	+
<i>A. fumigatus</i>	+	+	+	+	+
<i>A. parasiticus</i>	-	-	-	-	+
<i>C. carmichaeli</i>	+	+	+	-	+
<i>C. keratinophilum</i>	+	+	+	+	+
<i>C. tuberculatum</i>	-	-	-	+	-
<i>C. tropicum</i>	+	+	-	-	+
<i>C. carrionii</i>	-	-	+	+	-
<i>C. herbarum</i>	-	-	+	-	-
<i>G. candidum</i>	+	+	+	+	+
<i>M. audouinii</i>	+	-	-	-	+
<i>M. canis</i>	+	+	+	+	+
<i>Monilia</i> sp.	+	-	-	-	-
<i>M. pusilus</i>	+	+	+	+	+
<i>P. chrysogenum</i>	+	+	+	+	+
<i>P. funiculosum</i>	-	-	+	-	-
<i>S. album</i>	-	-	-	+	-
<i>S. aurantiacum</i>	+	-	-	-	+
<i>T. mentagrophytes</i>	+	+	+	+	+
<i>T. rubrum</i>	+	-	-	-	+
<i>T. caligans</i>	-	-	-	-	+
<i>U. chlamydosporum</i>	+	+	-	-	-
Total No. of species	20	15	16	13	19

was the dominating genus represented by five species followed by *Chrysosporium* with four species. *Alternaria alternata*, *A. chlamydospora*, *Aspergillus flavus*, *A. fumigatus*, *Geotrichum candidum*, *Chrysosporium keratinophilum*, *Mucor pusillus*, *Penicillium chrysogenum* and *Trichophyton mentagrophytes*, were found on all type of hairs, while *Aspergillus candidus*, *A. parasiticus*, *Chrysosporium tuberculatum*, *Monilia* sp., *Penicillium funiculosum*, *Scytalidium album* and *Torula caligans* were isolated from a particular type of hair only (Table 1).

Twenty six species were tested for percent degradation of hairs (% dry weight) and percent of coloured-zone on DTM. Two fungus *Cladosporium carrionii* and *Trichophyton rubrum* were failed to grow on Sabouraud Dextrose Agar while isolation. They were seen on hairs only and identified directly from microscopic slides prepared for hairs (Table 2).

Table 2: Percent degradation of hairs (% dry weight) and per cent of colored-zone on DTM shown by isolated fungi

Fungi	% hair weight loss (dry weight)	% colored-zone DTM
<i>A. corymbifera</i>	2.5	11
<i>A. ramose</i>	2.8	12
<i>A. alternate</i>	5.6	18
<i>A. chlamydospora</i>	7.9	24
<i>A. candidus</i>	3.6	13
<i>A. ellipticus</i>	8.5	26
<i>A. flavus</i>	18.6	35
<i>A. fumigatus</i>	23.5	39
<i>A. parasiticus</i>	2.3	9
<i>C. carmichaeli</i>	32.6	56
<i>C. keratinophilum</i>	43.2	64
<i>C. tuberculatum</i>	29.3	43
<i>C. tropicum</i>	26.4	36
<i>C. herbarum</i>	4.8	15
<i>G. candidum</i>	3.2	14
<i>M. audouinii</i>	18.3	36
<i>M. canis</i>	20.5	39
<i>Monilia</i> sp.	15.5	28
<i>M. pusilus</i>	2.9	13
<i>P. chrysogenum</i>	16.8	33
<i>P. funiculosum</i>	11.3	22
<i>S. album</i>	16.5	26
<i>S. aurantiacum</i>	18.4	28
<i>T. mentagrophytes</i>	23.6	39
<i>T. caligans</i>	6.5	22
<i>U. chlamydosporum</i>	18.6	28

Chrysosporium species in general caused the highest percent hair weight loss (degradation), while in particular *C. keratinophilum* caused the highest percent of degradation of hairs (43.2%) among all species followed by *C. carmichaeli* (32.6%). The least degradation of hairs was caused by *Aspergillus parasiticus* (2.3%) followed by *Absidia corymbifera* (2.5%). Among *Aspergillus* species, *A.*

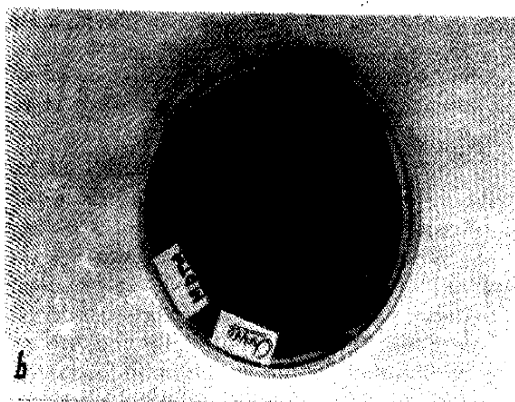
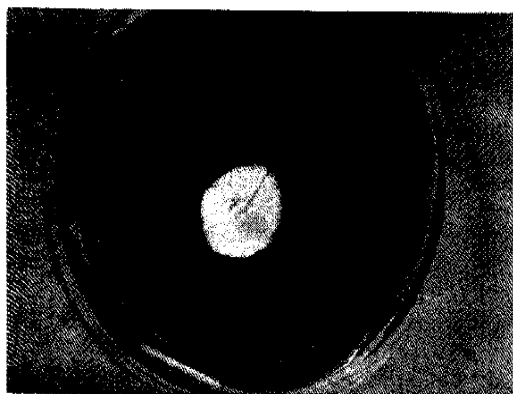


Plate 1: Coloured-zone shown by *Chrysosporium keratinophilum* on Modified Dermatophyte Test Medium (MDTM)

fumigatus (23.5%) was the leading species for hair degradation followed by *A. flavus* (18.6%). Other fungal species which cause considerable loss of hair's weight are *Microsporum canis* (20.5%), *Trichophyton mentagrophytes* (123.6%), *Ulocladium chlamydosporum* (18.6%), *Scytalidium auranticum* (18.4), *Microsporum audouinii* (18.3%), *Penicillium chrysogenum* (16.8%) and *Scytalidium album* (16.5).

Almost same pattern was also found for percent coloured-zone on DTM medium. The fungus which cause the highest degradation of hairs showed the highest percent of colored-zone on DIM. Like *Chrysosporium keratinophilum* caused the highest percent of hair weight loss also showed the highest percent of colored-zone on DTM (Plate 1). This

showed a correlation of amount of keratin degradation by a particular fungus and colored-zone formation on DTM medium. This means the fungus which cause more keratin degradation will show more percent of colored-zone on DTM. Therefore the activity of colored-zone formation by a particular fungus on DTM could be considered as a parameter of keratin degradation by such fungus, although more work are needed in support of this findings.

Among the fungi isolated, *Microsporum audouinii*, *M. canis*, *Trichophyton mentagrophytes* and *T. rubrum* are well known true dermatophytes causing infections in human and animals (Frey *et al.*, 1979; Rippon, 1982; Howards, 1983). While species of *Chrysosporium* are well known to be keratinophilic fungi (Nigam and Kushwaha, 1990; Nigam *et al.*, 1987; Morganti and Tampieri, 1985; Ali-Shtayeh and Arda, 1989). *Geotrichum candidum* was also reported as frequent keratinophilic species in the floor dust of schools (Ali-Shtayeh and Arda, 1989). Species of *Trichophyton* and *Microsporum* were also reported as keratinophilic species from soil, house dust and dust from school (Nigam and Kushwaha, 1990; Ali-Shtayeh and Arda, 1989; Ali-Shtayeh and Al-Sheikh 1988). Other species isolated here were also reported earlier as keratin degrading fungi (Bagy and Gohar, 1988; Abdel-Mallek *et al.*, 1988; Abdel-Hafez *et al.*, 1990). *Aspergillus flavus* (green mould) which are commonly found in almost all types of environment and considered to be harmless mould, was becoming a big killer of patient's with AIDS, those having organ transplant and other who had weakened immune systems (Saudi Gazette, 1996).

References

- Abdel-Fattah, A., M. Hyati and M.A. Abdallah, 1972. Tinea capitis in Riyadh (Saudi Arabia). Mycoses, 15:397-399
- Abdel-Hafez, A.1.1., M.M.K., Bagy and A.A.M. Shoreit, 1989. Keratinophilic fungi in mud of Ibrahimia Canal, Egypt. Cryptogamie Mycol, 10: 275-282.
- Abdel-Hafez, S.I.I., A.H. Moubasher and A. Barakat, 1990. Keratinophilic fungi and sother moulds associated with air dust particles from Egypt, Folia Microbial, 35: 311-325.
- Abdel-Mallek, A.Y., M.M.K. Bagy and A.M. Mcharram, 1988. Fungi of the floor dust in students residential halls of Assiut University, Egypt. Egyptian J. Bot., 31: 69-80.
- Al-Sogair, S., Y. Al-Humaidan and M.K. Moawad, 1989. Scalp fungus infections in the Eastern province of Saudi Arabia. Ann. Saudi Med., 9: 259-262.
- Ali-Shtayeh, M.S. and B.S.A. Al-Sheikh, 1988. Isolation of keratinophilic fungi from the floor dust of Arab kindergarten schools in the West Bank of Jordan. Mycopathologia, 103: 69-73.
- Ali-Shtayeh, M.S. and H.M. Arda, 1989. Isolation of keratinophilic fungi from floor dust in Arab elementary and preparatory schools in the West Bank of Jordan. Mycopathologia, 106: 5-11.
- Ali-Shtayeh, M.S. Arda, M. Hassouna and S.F. Shaheen, 1988. Keratinophilic fungi on the hair of cows, donkeys, rabbits, cats and dogs from West Bank of Jordan. Mycopathologia, 104: 109-121.

- Bagy, M.M.K. and Y.M. Gohar, 1988. Mycoflora of air conditioners dust from Riyadh, Saudi Arabia. *J. Basic Microbial*, 28: 571-577.
- Bokhary, H.A. and S. Parvez, 1995. Fungi inhabiting household environments in Riyadh, Saudi Arabia. *Mycopathologia*, 130: 79-87.
- Carmichael, J.W., 1962. *Chrysosporium* and some other aleurosporic hyphomycetes. *Can.J. Bot.* 40: 1137-1173.
- El-Hams, F.H.M., 1989. Survey on dermatophytes infections in Saudi Arabia. M.Sc. Thesis. Dept. Bot. Microbial. King Saud Univ. Riyadh, Saudi Arabia.
- Frey, D., R.J. Oldfield and R.C. Bridger, 1979. Pathogenic Fungi. Wolfe Medical Pub. Ltd., Holland.
- Howards, H.D., 1983. Fungi Pathogenic for Humans and Animals. Marcel Dekker. Inc.N.Y. U.S.A.
- Larone, D.H., 1995. Medically Important Fungi. ASM Press. Washington, D.C. U.S.A.
- Marsella, R. and R. Mercantini, 1986. Keratinophilic fungi isolated from soil of the Abruzzo National Park, *Mycopathologia*. 94: 97-107.
- Marsella, R., R. Mercantini, P. Spinelli and L. Volterra, 1985. Occurrence of keratinophilic fungi in animals of the Zoological Park of Rome. *Mykosen*, 28: 507-512.
- Matsumoto, T. and L. Ajello, 1987. Current taxonomic concepts pertaining to the dermatophytes and related fungi. *Int. J. Dermatol.*, 26: 490-499.
- Mercantini, R., R. Marsella, G. Prignano, D. Moretto and W. Marmo *et al.*, 1989. Isolation of keratinophilic fungi from the dust of ferry boats and trains in Italy. *Mycoses*, 32: 590-594.
- Morganti, L. and M.P. Tampieri, 1985. The presence of keratinophilic fungi in soil and sand samples from schools and public gardens of the city of Bologna. *Nuovi. Annali. Igiene-e-Microbiologia*, 35: 43-50.
- Nigam, N. and R.K.S. Kushwaha, 1990. Occurrence of keratinophilic fungi with special reference to *Chrysosporium* species in soils of India. *Sydowia*, 42: 200-208.
- Nigam, N., R.K.S. Kushwaha and N. Nigam, 1987. Seven new keratinophilic fungal records from India. *Kavaka*, 15: 29-31.
- Rippon, J.W., 1982. Medical Mycology. The Pathogenic Fungi and the Pathogenic Actinomycetes. W.B. Saunder. Phil. U.S.A.
- Saudi Gazette, 1996. Green Fungus threatens hospital patients-say experts. Friday, May 17, 1996.
- Van-Oorschot, C.A.N., 1980. A revision of *Chrysosporium* and allied genera. *Stud. Mycol.*, 20: 1-89.