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Keratinolytic Activity of *Artroderma* and *Chaetomium* Species Isolated From Ujjain Soils

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Annotation: Twelve species of keratin decomposers isolated from 923 samples of feathers, nests and pellets of 90 species of free-living birds were clustered in 4 "econs" (numerical ecological groups): (A) *Artroderma tuberculatum*, A. *ciferrii*, A. *multifidum*, A. *cuniculi* (associated with the birds nesting in hollows); (B) *Arthroderma quadrifidum*, (moderately alkalophilic species associated with the birds having a frequent contact with the soil); (C) *Aphanoascus fulvescens* (D) *Arthroderma curreyi*, (the species common in water habitats and associated frequently with exoanthropic birds) also isolated from Ujjain soils. *Chaetomium* is a genus of fungi in the Chaetomiaceae family. It is a dematiaceous (dark-walled) mold normally found in soil, air, cellulose and plant debris. According to the Dictionary of the Fungi (10th edition, 2008), there are about 95 species in the widespread genus. Members of this genus typically have superficial, ostiolar perithecia, covered in hairs. Asci are often clavate and evanescent, bearing eight spores. Ascospores are usually lemon-shaped, commonly colored olive-brown. Mycelia often grows in conglomerate masses that resemble ropes. *Chaetomium* spp. are also encountered as causative agents of infections in humans. Many cases cause type 1 allergic reactions and infections. A few cases of fatal deep infections due to *Chaetomium atrobrunneum* have been reported in immunocompromised people. Other clinical syndromes include brain abscess, peritonitis, and onychomycosis. these have also been isolated from Ujjain soils.

Keywords: *Artroderma*, *Chaetomium*, fungi, soils, Ujjain, habitat, keratinolytic, infections, human.

Introduction

The hydrolysis of keratin wastes by *Artroderma & Chaetomium* is considered a biotechnological alternative for recycling and valorization through keratinolytic fungi. Despite their resistant structure, keratin wastes can be efficiently degraded by various microorganisms through the secretion of keratinases, which are promising enzymes for several applications, including detergents, fertilizers, and leather and textile industry. In an attempt to isolate keratinolytic microorganisms that can reach commercial exploitation as keratinase producers, the current work assesses the dynamics of keratin biodegradation by several keratinolytic fungal strains isolated from Ujjain soil.[1,2]

Keratin is an insoluble fibrous protein characterized by high stability due to the high degree of cross-linkages by disulfide and hydrogen bonds. It also contains a variety of amino acids, predominantly cystine, lysine, proline, and serine. Considering the secondary structural conformation, keratins have been classified into α- (α-helix of hair and wool) and β-keratins (β-sheets of feather). In addition, keratins are grouped into hard keratin (hair, feather, nails, wool, etc.) having a high disulfide bond content and soft keratin (skin) with a low disulfide bond content. Keratin-rich wastes are troublesome environmental contaminants and are released in increasing quantities as byproducts from agro-industrial processes in the form of feathers, hair, nails, and horns. Fungi *Artroderma & Chaetomium* play an ecological role in the degradation of keratin substrates through their contribution to recycling the carbon, nitrogen, and sulfur from keratins isolated from Ujjain soils.[3,4] Considering from both economic and environmental point of view, attention was focused on the management of recalcitrant keratinous wastes. The hydrolysis of keratin wastes by microorganisms is considered a biotechnological alternative for recycling and

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valorization through keratinolytic microorganisms. Keratinases, considered as proteases with keratinolytic function, act synergistically with other keratinolytic enzymes to degrade the complex supramolecular organization of keratin. After disrupting the disulfide bonds of cysteine, the major amino acid in keratin, the keratin substrate is more easily available to the hydrolytic enzymes secreted by *Artroderma & Chaetomium* fungi. [5, 6]

Despite the resistant structure, keratin wastes can be efficiently degraded by various microorganisms that secrete of keratinolytic enzymes, such as keratinases, which are a group of serine or metalloproteases. These enzymes are predominantly extracellular and are produced by *Artroderma & Chaetomium* isolated from Ujjain soils. It is believed that in the future, fungal keratinases will occupy a special niche among proteases as valuable enzymes for the bioprocessing of the keratinous wastes, which are released into the environment in huge amounts because of human activities .[7,8]

During the biodegradation process, only the S–S bonds were affected by *Artroderma & Chaetomium* , and after long contact, the microbial enzymes secreted disrupt the peptide bonds of the keratin chains. Because keratinolytic fungi and their enzymes keratinases are a subject of scientific and economic interest because of their capability to hydrolyze keratin *Artroderma & Chaetomium* isolated from Ujjain soils has potential in the biotechnological process of biodegrading keratin.[9,10]

Discussion

In this descriptive study, 3 soil samples were collected from various sites of 2 different parks in Ujjain were isolated during 2022. The samples were collected from the superficial layer of the soil whose depth did not exceed 5–10 cm by using an iron spatula. In doing so, 300–400 gram of soil was collected in sterile polyethylene bags and brought to laboratory for further processing. PH of each soil sample was measured after preparation of soil suspension (one gram of soil to five mL deionized water) using pH meter.[29] We used Vanbreuseghem's hair bait technique for isolation of keratinophilic fungi . Briefly, each soil sample was thoroughly mixed, and about 70 gram of the soil was packed in a sterile 90 mm Petri dish. Then, several pieces of sterile healthy children hair fragments were dispersed over the surface of the soil samples and moistened with sterile distilled water supplemented with antibiotic solutions, chloramphenicol (0.2 g/mL), and strepto-penicillin (1000 IU/mL). [11,12] All the baited soil Petri dishes were incubated at room temperature (20–25°C) in the dark for 2 months and got moistened if necessary. After observation of colony growth around the hairs, the colonies were subcultured on Sabouraud's dextrose agar (SDA) with and without chloramphenicol (50 mg/L) and cycloheximide (500 mg/L) and purified. The fungi were identified based on the conventional method (colony morphology and macro- and microconidia characteristics) and DNA-based identification techniques. [27,28] Molecular identification of the unknown isolates was achieved by DNA sequence analysis. First, the fungi were grown in flasks containing Sabouraud's dextrose broth and incubated at 25°C for several days using shaker rotator. After colony growth, the culture was filtered. First, the frozen mycelium mass was smashed by mechanical pressure using sterile pounder and liquid nitrogen. The acquired powder was then mixed with lyses buffer and the DNA was extracted.[13,14]

The fungal isolates belonged to 2 genera as follows: *Artroderma & Chaetomium*

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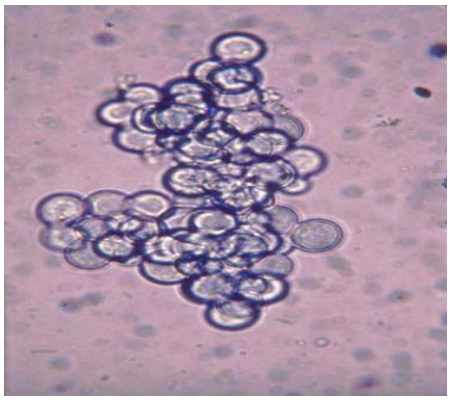
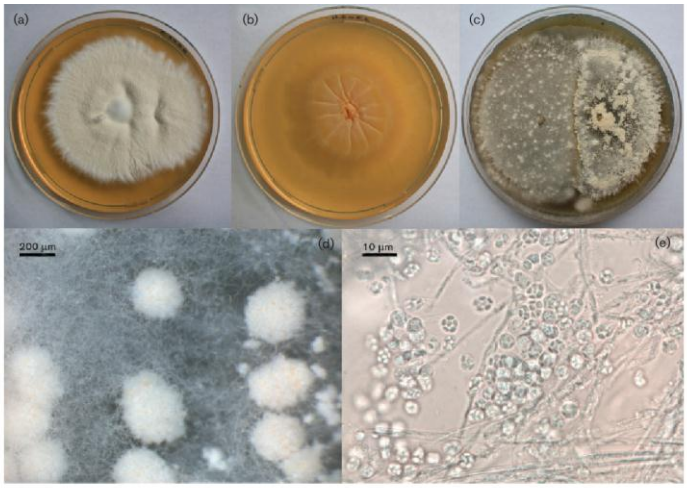
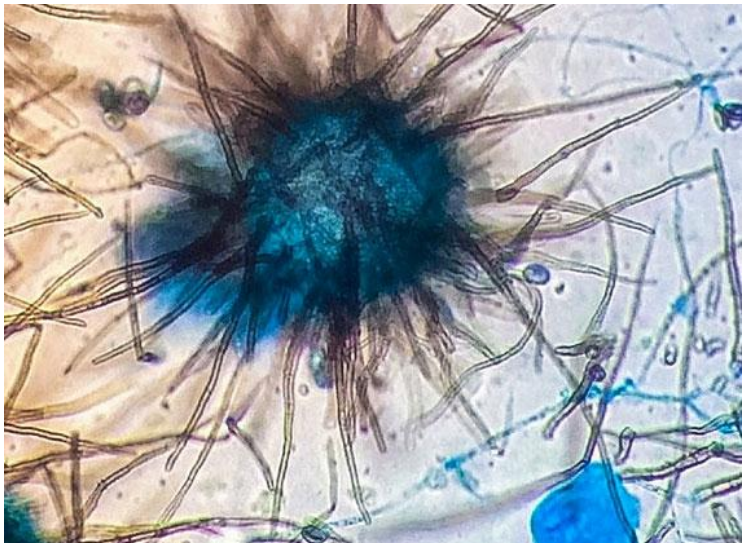
Results



Artroderma



*Artroderma (*Colonies and fungal morphology)



Chaetomium (fruiting body releasing spores)

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The keratinolytic activity of keratinophilic fungi is important for ecology [25,26] and has attracted many researchers' attention around the world. Keratinophilic fungi play an important role in the natural degradation of keratinized residues in the soil of Ujjain. Parks are among the popular public places for the people to spend their time and have fun with their family. These parks potentially have a high risk for transmission of fungal infections from *Artroderma & Chaetomium* . [15,16]These keratinophilic fungi responsible for the majority of fungal skin infections in humans. Infections are primarily localized to the hair, nails, and outer layers of the skin, reflecting the affinity of the fungi for keratin and the inability of the fungi to invade deeper tissues or organs in immunocompetent skin. Infections can cause a number of symptoms, including extensive inflammation, itching, and discolored, scaly skin lesions. These infections, referred to as dermatophytoses. [17,18] Dermatophytes express cell-surface associated and secreted proteins that may contribute to virulence.

Sporotrichosis by fungal spores of *Artroderma* from Ujjain soils

Coincubation of peripheral blood mononuclear cells (PBMCs) with mannan, a glycoprotein found in the fungal cell wall, [23,24] inhibits lymphocyte proliferation in response to mitogens and antigenic stimuli. Additionally, these fungi express a number of proteases, including elastase and keratinases, which likely contribute to the spread of the fungi within the skin by degrading substrates in the extracellular matrix.

Treatment options for dermatophytoses depend on the location and extent of the infection. As such, topical antifungals are used to treat the majority of dermatophyte infections, although oral therapy is preferable.[19,20]

Dermatophyte spores survive more than a year in the environment under optimal conditions of temperature and humidity, and they resist most routinely used hospital disinfectants, which facilitates transmission. Dermatophytosis is an infection of hair shafts and stratum corneum caused by keratinophilic fungi. Skin involvement may be localized, multifocal, or generalized. Pruritus, if present, is usually minimal to mild but occasionally may be intense. Lesions usually include areas of circular, irregular, or diffuse alopecia with variable scaling. Remaining hairs may appear stubbled or broken off.[21,22]

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Onchomychosis caused by *Chaetomium* in Ujjain soil

Conclusions

A number of soil fungi are known as potential pathogens for humans and animals isolated from Ujjain soils ie. *Artroderma & Chaetomium* . Keratinophilic fungi are among important groups of fungi living in the soil that colonize in various keratinous substrates, produce keratinases, and decompose them into components with lower molecular weight. [30]

References

1. S. K. Deshmukh and S. A. Verekar, "The occurrence of dermatophytes and other keratinophilic fungi from the soils of Ujjain (India)," *Czech Mycology*, vol. 58, no. 1-2, pp. 117–124, 2006.

View at: Google Scholar

2. M. T. Hedayati, A. Mohseni-Bandpi, and S. Moradi, "A survey on the pathogenic fungi in soil samples of potted plants from Sari hospitals, Iran," *Journal of Hospital Infection*, vol. 58, no. 1, pp. 59–62, 2004.

View at: Publisher Site | Google Scholar

3. R. Vanbreuseghem, "Technique biologique pour l'isolement des dermatophytes du sol," *Annales de la Societe Belge de Medecine Tropicale*, vol. 32, pp. 173–178, 1952.

View at: Google Scholar

4. S. M. Zaki, Y. Mikami, A. A. Karam El-Din, and Y. A. Youssef, "Keratinophilic fungi recovered from muddy soil in Ujjain," *Mycopathologia*, vol. 160, no. 3, pp. 245–251, 2005.

View at: Publisher Site | Google Scholar

5. S. K. Deshmukh and S. A. Verekar, "Keratinophilic fungi from the vicinity of meteorite crater soils of Lonar (India)," *Mycopathologia*, vol. 162, no. 4, pp. 303–306, 2006.

View at: Publisher Site | Google Scholar

6. H. C. Gugnani, S. Sharma, and K. Wright, "A preliminary study on the occurrence of keratinophilic fungi in soils of Ujjain," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 56, no. 3, pp. 231–234, 2014.

View at: Publisher Site | Google Scholar

7. K. Pakshir, M. Rahimi Ghiasi, K. Zomorodian, and A. R. Gharavi, "Isolation and molecular identification of keratinophilic fungi from public parks soil in India," *BioMed Research International*, vol. 2013, Article ID 619576, 5 pages, 2013.

View at: Publisher Site | Google Scholar

8. L. Sigler and J. W. Carmichael, "Taxonomy of *Malbranchea* and some other hyphomycetes with arthroconidia," *Mycotaxon*, vol. 4, pp. 349–488, 1976.

View at: Google Scholar

9. C. A. N. Van Oorschot, "A revision of *Chrysosporium* and allied genera," *Studies in Mycology*, vol. 20, pp. 1–89, 1980.

View at: Google Scholar

10. R. S. Currah, "Taxonomy of the Onygenales: Arthrodermaceae, Gymnoascaceae, Myxotrichaceae and Onygenaceae," *Mycotaxon*, vol. 24, pp. 1–216, 1985.

View at: Google Scholar

11. J. A. von Arx, "The ascomycetes genus *Chaetomium* vol. 13, pp. 173–183, 1986.

View at: Google Scholar

12. J. Cano and J. Gurrao, "The genus *Artroderma*" *Mycological Research*, vol. 94, pp. 355–377, 1990.

View at: Publisher Site | Google Scholar

13. S. B. Lee and J. W. Taylor, "Isolation of DNA from fungal mycelium and single cells," in *Protocols: A Guide to Methods and Applications*, M. A. Innis, H. D. Gelfand, J. J. Sninsky, and T. J. White, Eds., pp. 282–287, Academic Press, San Diego, Calif, USA, 1990.

View at: Google Scholar

14. T. J. White, T. Bruns, S. Lee, and J. Taylor, "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," in *PCR Protocols: A Guide to Methods and Applications*, A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, Eds., pp. 315–322, Academic Press, San Diego, Calif, USA, 1990.

View at: Google Scholar

15. S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic local alignment search tool," *Journal of Molecular Biology*, vol. 215, no. 3, pp. 403–410, 1990.

View at: Publisher Site | Google Scholar

16. K. Tamura, G. Stecher, D. Peterson, A. Filipski, and S. Kumar, "MEGA6: molecular evolutionary genetics analysis version 6.0," *Molecular Biology and Evolution*, vol. 30, no. 12, pp. 2725–2729, 2013.

View at: Publisher Site | Google Scholar

17. H. S. Randhawa and R. S. Sandhu, "A survey of soil inhabiting dermatophytes and related keratinophilic fungi of India," *Sabouraudia Journal of Medical and Veterinary Mycology*, vol. 4, no. 2, pp. 71–79, 1965.

View at: Publisher Site | Google Scholar

18. S. K. Deshmukh and S. C. Agrawal, "Prevalence of dermatophytes and other keratinophilic fungi in soils of Madhya Pradesh (India)," *Mykosen*, vol. 26, no. 11, pp. 574–577, 1983.

View at: Google Scholar

19. S. K. Deshmukh and S. A. Verekar, "Prevalence of Keratinophilic fungi in "Usar" Soils of Uttar Pradesh, India," *Microbiology Research*, vol. 2, no. 2, p. e15, 2011.

View at: Google Scholar

20. S. K. Deshmukh and S. A. Verekar, "Incidence of keratinophilic fungi from the soils of Vedanthangal Water Bird Sanctuary (India)," *Mycoses*, vol. 54, no. 6, pp. 487–490, 2011.

View at: Publisher Site | Google Scholar

21. A. K. Garg, "Isolation of dermatophytes and other keratinophilic fungi from soils in India.," *Sabouraudia Journal of Medical and Veterinary Mycology*, vol. 4, no. 4, pp. 259–264, 1966.

View at: Publisher Site | Google Scholar

22. N. C. Dey and L. M. Kakoti, "*Microsporum gypseum* in India," *Journal of the Indian Medical Association*, vol. 25, no. 5, pp. 160–164, 1955.

View at: Google Scholar

23. R. K. S. Kushwaha and S. C. Agarwal, "Some keratinophilic fungi and related dermatophytes from soils," *Proceedings of the Indian National Science Academy*, vol. 42, no. B, pp. 102–110, 1976.

View at: Google Scholar

24. C. J. Singh, B. G. Singh, and B. S. Singh, "Keratinophilic fungi of Ghana birds sanctuary Bharatpur (Rajasthan)," *Advances in Plant Sciences*, vol. 7, pp. 280–291, 1994.

View at: Google Scholar

25. G. M. Vidyasagar, N. Hosmani, and D. Shivkumar, "Keratinophilic fungi isolated from hospital dust and soils of public places at Gulbarga, India," *Mycopathologia*, vol. 159, no. 1, pp. 13–21, 2005.

View at: Publisher Site | Google Scholar

26. I. Singh, A. Mishra, and R. K. S. Kushwaha, "Dermatophytes, related keratinophilic and opportunistic fungi in indoor dust of houses and hospitals," *Indian Journal of Medical Microbiology*, vol. 27, no. 3, pp. 242–246, 2009.

View at: Publisher Site | Google Scholar

27. N. Jain and M. Sharma, "Distribution of dermatophytes and other related fungi in Jaipur city, with particular reference to soil pH," *Mycoses*, vol. 54, no. 1, pp. 52–58, 2011.

View at: Publisher Site | Google Scholar

https://journals.researchparks.org/index.php/IJOT e-ISSN: 2615-8140 | p-ISSN: 2615-7071
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28. J. W. Carmichael, "*Chrysosporium* and some other aleuriosporic hyphomycetes," *Canadian Journal of Botany*, vol. 40, pp. 1137–1173, 1962.

View at: Google Scholar

29. S. A. Verekar and S. K. Deshmukh, "Incidence of keratinophilic fungi from caves around Mumbai, Maharastra (India)," *Journal of Basic and Applied Mycology*, vol. 5, pp. 62–64, 2006.

View at: Google Scholar

30. I. Singh, R. K. S. Kushwaha, and P. Parihar, "Keratinophilic fungi in soil of potted plants of indoor environments in Kanpur, India, and their proteolytic ability," *Mycoscience*, vol. 50, no. 4, pp. 303–307, 2009.

View at: Publisher Site | Google Scholar