

DERMATOPHYTE GROWTH AND DEGRADATION OF HUMAN STRATUM CORNEUM IN VITRO (PATHOGENESIS OF DERMATOPHYTOSIS).

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Background: This study was carried out to determine growth of dermatophytes using human stratum corneum in vitro and the degrading effect of Keratinases (Proteinases) on stratum corneum for a complete understanding of the host parasite relationship. **Method:** Trichophyton rubrum isolates derived from patients with tinea cruris infections were obtained from the Department of Medical Microbiology, University Hospital of Wales, U.K. Human stratum corneum sterilized with ethylene oxide was used as a nitrogen source in agar culture medium plates. **Result:** Fungal growth took place in plates which contained human stratum corneum particles while there was no growth in the plates without stratum corneum at three weeks after initiation. There was a gradual disappearance of the particles of stratum corneum from the plates at the end of the third week. **Conclusion:** The growth of organisms in plates with human stratum corneum and their disappearance at third week suggested that stratum corneum was not only source of nutrition for the dermatophytes, but also the growing fungal mycelia and the proteinases induced by them were playing a part in the digestion of granules and thus may have an important role in the pathogenesis of dermatophyte infections.

Key-words: Proteinases, Stratum -corneum, Dermatophytes

INTRODUCTION

A large number of fungi, including yeasts, dermatophytes and other moulds, grow on human skin, hair and nails. In UK, Trichophyton rubrum is the most common organism causing human dermatophyte infections involving the toe webs, nails, groin, soles and palms.¹

The main protein constituent of structures in skin susceptible to infection is keratin. It is often assumed that dermatophytes are capable of invading and digesting keratin. When these organisms are cultured in media containing keratin, they grow well and are able to utilize it as a source of carbon and nitrogen.

However, the manner in which these micro-organisms attack and digest horny structures is not clear. Some workers suggest that the mechanical action of the invading mycelium penetrating the stratum corneum is an important part of the destructive process.²⁻⁴ The fungal digestion of animal horn, finger nails and human hairs suspended in agar-based media by dermatophytes such as Microsporum gypseum, Microsporum canis, Trichophyton mentagrophytes and Trichophyton rubrum has been described.⁵ Kunert⁶ observed the decomposition of human hair by dermatophytes in vitro and hypothesized that 'sulphitolysis' occurred, a process which resulted in the cleaving of disulphide bonds.

The denatured keratin was then easily degraded by the extra cellular proteases produced by the fungus. In order to understand the pathogenesis of dermatophyte infections in vivo it was decided to

ascertain the role of fungal growth and the effect of proteinases (Keratinases) released by these organisms on human stratum corneum particles in agar gel medium. The purpose of using human stratum corneum was, that it would provide us with an opportunity to study the effects of dermatophytes quite near to an in vivo situation. The study involved the following steps:

Step 1: To observe the growth of strains of dermatophytes isolated from tinea cruris, using human stratum corneum as the sole source of nitrogen and carbon.

Step 2: To compare the growth of these organisms in the medium containing human stratum corneum with the growth in controls (without stratum corneum).

Step 3: To study the effect of growing fungal mycelia and the proteinases induced by them on the particles of human stratum corneum contained in the agar gel medium.

MATERIAL AND METHODS

Fungal Isolates.

Isolates were derived from five patients suffering from tinea cruris infection with fungal strains of T. rubrum who had been attending the dermatology outpatient clinic at the University Hospital of Wales, Cardiff. Isolates of T. rubrum were identified according to the normal laboratory protocol at the Department of Medical Microbiology, University Hospital of Wales, Cardiff.

Stratum corneum for use as a nitrogen source in the culture plates and as a substrate was

obtained from the heels of normal adult human volunteers who had given informed written consent. The stratum corneum was removed using a commercially available instrument designed to remove callus (Scholl Products, London) and was then sterilized with ethylene oxide. The device produced a fine powder which was used in the growth experiments.

Agar Medium.

The agar medium had the following composition, carbon yeast base, 12 mg/ml (Oxoid Basingstoke, UK); ino-sitol, 0.05 mg/ml (Sigma Chemical Co, Poole, UK); thiamine, 0.01 mg/ml (Sigma); pyrioxidine, 0.01 mg/ml (Sigma); and agar, 20 mg/ml (Sigma). The carbon yeast base and vitamin solutions were all filter sterilized before addition to the distilled water broth. Aliquots (25 ml) of the final solution were poured into Petri dishes containing 100 mg of sterile powdered stratum corneum to give a final concentration of 4 mg/ml. Control plates were prepared containing 25 ml of the above medium without stratum corneum.

Cultivation Method.

Isolates derived from cultures of *T. rubrum* were transferred to the agar plates with and without stratum corneum in duplicate, for each isolate. Each plate was inoculated in the center and then incubated at 30 °C in the dark. The purpose of using human stratum corneum was that it would provide us with an opportunity to study the effects of growing dermatophytes quite near to an in vivo situation. The plates were examined weekly, upto five weeks for the growth of organisms.

RESULTS

Figure-1 shows the sample and control plates at the start of the experiment. As can be clearly seen in Figure-2 fungal growth took place only in those plates which contained human stratum corneum particles while there was no growth in the control plates three weeks after initiation. There was a gradual disappearance of stratum corneum from the plates over 3 weeks. Particles of stratum corneum were clearly visible at the end of the first week (Figure-3) but were absent by the end of the third week (Figure-4). This disappearance of stratum corneum particles at the third week which corresponded to the maximum proteolytic activity in the growing isolates. In studies carried out earlier it has been shown that the proteolytic activity is maximum at the third week when isolates were grown on glucose peptone agar plates and their proteolytic activity measured using protein assay.⁷



Figure 1: Sample and control plates at the start of the experiment inoculated with an isolate of *Trichophyton rubrum*.

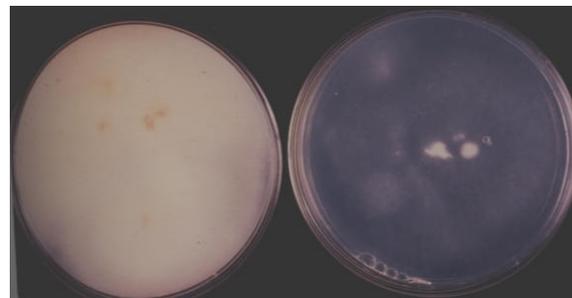


Figure 2: Sample and control plates at the end of the third week. No growth can be seen in the control plates while there is growth in the plate containing human stratum corneum.



Figure 3: Sample plate at the end of first week (close up view) showing some stratum corneum particles



Figure 4: Sample plate (close up view) at the end of the third week demonstrating the disappearance of particles of stratum corneum.

DISCUSSION

Proteinases are produced by dermatophyte in vitro and play an important role in the pathogenesis of fungal infections in vivo. It has been suggested by many workers that the pathogenicity of microorganisms is related to the production of proteinases which enable them to parasitize tissues such as the stratum corneum, nails and hair.^{8,9}

Page, presented convincing photographic evidence that particles of horn which had been suspended in 2 % agar medium in petri dish cultures, were digested by *Microsporum gypseum*. Similar observations were reported with nail particles. It was noted that actual contact of the hyphae with the particle was not a requisite for digestion to occur, although the hyphae had to be close to the fragment or granule.

Similar degradation of hairs, nails and callus by various keratinophilic fungi has already been described.¹⁰ It has been speculated that the degradation of feathers in vitro induced by fungal micro-organisms is due in part to the release of keratinolytic enzymes.^{11,12} Some workers on the other hand suggest that the mechanical action of the invading mycelium penetrating the stratum corneum is an important part of the destructive process.²⁻⁴

On the basis of the findings and observations made by different workers mentioned above and the results obtained in this study, the disappearance of stratum corneum particles could be the result of two factors. Firstly mechanical degradation of the particles could be taking place, as a result of fungal mycelia growing across the agar gel to the particles themselves. Secondly, extra-cellular proteinases might then be induced playing a part in the digestion of granules and also in providing nutrition to the mycelia. These findings suggest that the proteinases may have an important role in the pathogenesis of dermatophytes infections. The significance of this finding in relationship to the disease process itself is unclear but provides further evidence that the production of proteinases may be important in terms of the clinical expression of dermatophytosis.

CONCLUSION

From the results observed in this study it could be suggested that the fungal organism derive their nutrition from the skin. The mechanical action of the invading mycelium penetrating the stratum corneum and the Proteinases (Keratinases) produced by dermatophytes may well play an important role in the pathogenesis of fungal diseases in vivo. This interaction between the fungal hyphae and its products with the host tissue results in the clinical expression of the disease.

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REFERENCES

1. Hay RJ, Brostoff J. Immune responses in patients with chronic *Trichophyton rubrum* infections. *Clin Exp Dermatol* 1977;2:373-80.
2. Baxter M, Mann PR. Electron microscopic studies of the invasion of human hair in vitro by three keratinophilic fungi. *Sabouraudia* 1969;7:33-7.
3. Raubitschek F. Mechanical versus chemical keratolysis by dermatophytes. *Sabouraudia* 1961;1: 87-91.
4. Raubitschek F, Maoz R. Invasion of nails in vitro by certain dermatophytes. *J Invest Dermatol* 1957;28: 261-7.
5. Page RM. Observations on keratin digestion by *Microsporum gypseum*. *Mycologia* 1950;42:591-602.
6. Kunert J. The digestion of human hair by the dermatophyte *Microsporum gypseum* in a submerged culture. *Mykosen* 1972;15:59-71.
7. Samdani AJ, Dykes PJ, Marks R. The proteolytic activity of strains of *T. mentagrophytes* and *T. rubrum* isolated from tinea pedis and tinea unguium infections. *J Med & Veter Mycol* 1995;33:167-70.
8. Tsuboi R, Ko IJ, Matusda K, Ogawa H. A new keratinolytic proteinase from clinical isolates of *Trichophyton mentagrophytes*. *J Dermatol* 1987; 14: 506-8.
9. Tsuboi R, Ko IJ, Takamori K, Ogawa H. Isolation of a keratinolytic proteinase from *Trichophyton mentagrophytes* with enzymatic activity at acidic pH. *Infect Immun* 1989;57: 3479-83.
10. English MP. The saprophytic growth of keratinophilic fungi on keratin. *Sabouraudia* 1963;2:115-30.
11. Kushwaha RKS. The in vitro degradation of peacock feathers by some fungi. *Mykosen* 26:324-6.
12. Wawrzkiwicz K, Lobarzewski J, Wolski T. Intracellular keratinase of *Trichophyton gallinae*. *J Med Vet Mycol* 1987; 25: 261-8.

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