SUMMARY

1. A close scrutiny of the superficial and cutaneous human dermatophytic incitants by the investigators here at Rourkela, Orissa, India during their round the year study helped to identify the only species of *T. rubrum* with their variants.

2. Despite the climatic barrier, with the result of great population shifts, deterioration of general hygiene and other such factors the dermatophyte *T. rubrum* became worldwide in distribution. They are more frequently reported in warmer and humid weather conditions with the temperature ranging from 30°-37°C.

3. The common clinical types of this dermatophyte were identified to be *tinea corporis, tinea cruris, tinea pedis, tinea manuum, tinea unguium*, causing irritations at the sites skin, groin, feet, hand & palm and nails respectively.

4. The isolation and identification of the three variants of the dermatophyte, *T. rubrum* viz Avariant, Granular and Dysgonic type was done on the basis of certain confirmed tests performed. They are hair perforation, pigment production, macro and micro-morphological studies etc.

5. The three variants of *T. rubrum* were maintained in Sabouraud's Dextrose Agar medium which promoted maximum growth of the organism. The enzymes secreted by the by three variants of *T. rubrum* were screened qualitatively and quantitatively following the assay method of (Hankin and Anagnostakis, 1975).

6. The variants of *T. rubrum* being pathogenic, the production of extracellular enzymes like protease, lipase were detected. Positive urease production was determined in case of granular and avariant type. No L-amylase and L-asparaginase activity and a very feeble keratinolytic activity were obtained in all strains of *T. rubrum*. (Table-1)

7. Although more than one enzyme was detected in the medium but protein being an important constituent of the skin and building block molecule of the cell- the ultimate unit of life, it was felt necessary to study the enzyme which hydrolyses such macromolecules. Hence the study of the enzyme, protease was taken in succeeding chapters.

8. The three variants of the dermatophyte, *T. rubrum* were grown in different synthetic, semisynthetic and natural media upto 14 days and growth and protease activity under such conditions examined. All the variants grew best in basal...
synthetic medium and showed a high proteolytic activity in this medium. However, Lilly & Barnett’s modified synthetic medium and SD broth supported good growth but both the media could not support for the good protease production. Natural malt extract medium showed complete inhibition in the growth of the variants, (Table 2a). Hence the basal synthetic medium was taken for standardization of various parameters like pH, temperature, substrate concentration, aeration, total protein content etc. and for carbon, nitrogen assimilation and vitamin requirement studies.

9. The organism grew well at a range of 28°C to 37°C (Fig. 2). pH 7.0 supported the best growth of all the three variants (Fig. 1).

10. Citrate phosphate buffer with pH 7.0 supported for maximum enzyme production (Fig. 3).

11. Temperature ranging from 35°C-37°C, substrate concentration of 1% and incubation time of 1 hour was accounted to be maximum for protease production (Fig. 4, 5, 6).

12. The growth and enzyme activity was seen to be maximum on 10th day of incubation both under static and shaking cultures but aeration favoured the maximum activity as against the static cultures (Fig. 7a, b).

13. Substrates like gelatin, egg albumin and casein were used to study the extent of protease production. Protease activity was found to be maximum in casein both in static condition and under aeration as against other two substrates. The protease activity was maximum on 10th day and aeration gave better results than static cultures (Fig. 7b, 8, 9).

14. In the basal synthetic medium, the effect of substitution of carbon sources on growth and protease activity at different days of incubation of the three variants were tested. This included mono, di, polysaccharides and two polyols such as glycerol and mannitol.

15. Hexose sugar like glucose supported for the best growth of the variants of the organism but a significant decline in protease activity was seen even at low concentration of 0.5%. Disaccharide’s like sucrose and lactose were the least favourable for the growth and protease activity. Mannitol and glycerol promoted good growth but could not equally support for better protease activity. Starch promoted moderate growth in all the variants and induced a better protease activity as compared to other carbohydrates. All the three variants grew well and
showed high enzyme activity in the control medium with casein as a sole source of carbon and nitrogen. (Fig. 10a, b).

16. In comparison to the control sets taken both inorganic and organic nitrogen compounds did not help the organism in their growth and protease production. In inorganic nitrogen compounds KNO₃ promoted good growth and maximum activity as compared to other inorganic compounds in the 10th day of incubation (Fig. 11a, b). NH₄Cl also supported for a better protease activity on the same day (Fig. 11b).

17. Among the organic nitrogen sources, Asparagine favoured for the best growth of all the three variants of *T. rubrum* followed by lysine, valine, histidine, methionine, glutamine, aspartic acid and glutamic acid but there was a decline in protease activity in all the amino acids against the control media which showed that these amino acids did not have inducing effect on protease production (Fig. 12a, b).

18. In the study of the effect of vitamin on growth and protease production it was observed that they had little or no effect on the organism. Out of the 4 vitamins tested pyridoxine and thiamine had little effect. However inhibitory effect was seen in ascorbic acid and combination of all the vitamins tested (Fig. 13a, b).

19. Further the enzyme extruded in the culture medium of *T. rubrum* was purified by Sephadex G-100 gel filtration and preparative polyacrylamide gel electrophoresis. The enzyme that has been purified here accounted for serine-proteinase like activity. The three strains showed similar peaks of protease activity (Table-3).

20. The study of effect of antimycotic antifungals showed azoles to be more effective than polyene antibiotic in inhibiting the growth of *T. rubrum*. Among azoles tested, clotrimazole and ketoconazole showed maximum fungistatic effect followed by griseofulvin. Growth pattern of the variants of organisms with all antimycotics were seen to decline and finally became static at high concentration (Table- 4-9) but there was no significant decline in protease activity (Fig. 14-18).

21. The plant extracts of various types obtained from different plants were found to have pharmaceutical properties. The results of present study with *Azadirachta, Pongamia, Madhuca oil, Ricinus, Eucalyptus oil* and *Turmeric* extract showed that there was a decline in growth (Table- 10-15) and protease activity when *Eucalyptus, Azadirachta, Pongamia oils* were used (Fig. 19-24).