



## MORPHOLOGICAL AND BIOCHEMICAL VARIATIONS OF TRICHOPHYTON RUBRUM

by

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### INTRODUCTION

*Trichophyton rubrum* is the most frequently found species that causes Dermatophytosis in Bangkok<sup>(1)</sup>, Chiang Mai<sup>(2)</sup> and other geographic regions<sup>(3,4,5)</sup>. This might be due to anthropophilic and cosmopolitan properties of this organism. As described in Beneke's Medical Mycology Manual<sup>(6)</sup> *T. rubrum* produces velvety to cottony colony with reddish to rose-purple pigment on the reverse side. Microscopic characteristics consist of numerous clavate microconidia, chlamyospores, racquet hyphae and nodular bodies. Macroconidium is thin walled, 3-8 celled. Physiologically *T. rubrum* produces red pigment on potato dextrose agar and cornmeal dextrose agar and does not perforate autoclaved hair. Urease test

is negative in 7 days. The identification of this species, however, is not an easy task. Balabanoff<sup>(7)</sup> pointed out that *T. rubrum* was the markedly variable species which had many morphological forms between granular with complete typical conidia and fluffy with different form of conidia. Numerous variants of *T. rubrum* have been found in many parts of the world as in India<sup>(8)</sup>, Thailand<sup>(9)</sup>, Hongkong<sup>(10)</sup> and New Zealand<sup>(11)</sup>. Besides, biochemical characteristics of this species are not, some what, reliable for identification. Thammayya et al<sup>(12)</sup> made a contradictory report that *T. rubrum* gave positive urease test in 7 days instead of negative one.

This paper reports morphological and biochemical variations of 53 isolates of *T. rubrum* which were isolated

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from patients at Chiang Mai Hospital including their sensitivity to some antifungal drugs and their enzymatic activities.

### Materials and Methods

The fungi were isolated from patients of Dermatophytosis at Chiang Mai Hospital, Chiang Mai, Thailand, from January 1974 to March 1975. Mycosel agar plates (BBL, Division of BioQuest, Cockeysville, Maryland 21030) were used as medium for the isolation. *Trichophyton rubrum* was identified according to Beneke and Rogers<sup>(6)</sup> morphologically and then, transferred onto Mycosel agar slants at room temperature for further experiments as follows.

### Macroscopic observation

Colonial textures, topography and pigmentation were observed and recorded at 4 weeks on Mycosel agar plates which carefully sealed with masking tape.

### Microscopic observation

Slide culture was made for each fungal isolate. All such specific structures as macroconidia, microconidia etc. were examined within a period of 60 days.

### Biochemical tests

#### A. Urease test (13)

Each isolate of *T. rubrum* was grown in modified Christensen's urea medium. The test was positive when the agar medium turning red within 7 days.

#### B. Pigment production in potato dextrose agar

Growth of each isolate on potato dextrose agar plate (Difco Laboratory, Detroit, Michigan) were observed for pigment production within a period of 30 days.

#### C. Hair perforation test (14)

Fragment of infant hair in 25 ml of sterile distilled water with a small amount of 10% sterilized yeast extract solution in a petridish was inoculated with each of the fungi. Hair perforation was observed within 28 days of incubation at room temperature.

#### D. Growth on casein agar (15).

Casein agar (Difco Laboratory, Detroit, Michigan) was used to test the ability of each fungal isolate to grow on this medium in comparison with that on Sabouraud agar

### In vitro drug susceptibility test.

Different concentration (mcg/ml or mcl/ml) of Griseofulvin (Glaxo-

Vidhyasom, Thailand). Ezon T (Yamanouchi Pharm, Japan), Mycosynalar (Protochemie, Switzerland) and Canesten (Bayer Lever Kusen, Germany) were incorporated each into a set of Sabouraud agar slants. A uniform inoculum of the tested fungus about a pin head size was transplanted onto each of this medium and observed for growth within 30 days with a control growth on Sabouraud agar without antifungal drug. Minimum inhibitory concentration of each drug against each isolate of the fungi was recorded.

#### Study of enzymatic activities in culture medium

After a pure culture of the fungus had been grown on Brain Heart Infusion agar (Difco Laboratory, Detroit, Michigan) for 3 weeks at 25°C, a small plug of medium 1 mm. from the colony edge was removed with the use of 1 cm. diameter cork borer to obtain a constant amount of agar medium. Two of such agar plugs were put into 1 ml. of each of the substrates. Enzymatic activities were stopped after two hours of incubation in water bath at 37°C by the addition of 1 ml Tris buffer pH 9.8 to each of the substrate tubes. Enzymatic activity was directly proportional to free paranitrophenol released, which was measured with Coleman Spectrophotometer (Model 6/35) at 410 nm, the

activity was expressed as optical density (OD) per two plugs per two hours multiplied by 1000.

The substrates used were:-

Paranitrophenyl A-D glucopyranoside  
(0.5 mg/ml in 0.1 M acetate buffer pH 5.4)

Paranitrophenyl B-D glucopyranoside  
(0.5 mg/ml in 0.1 M acetate buffer pH 5.4)

Orthonitrophenyl B-D galactopyranoside  
(0.5 mg/ml in 0.1 M citrate phosphate buffer pH 7.0)

Paranitrophenyl phosphate  
(0.5 mg/ml in 0.1 M Tris HCl buffer pH 8.6 for alkaline phosphatase and in 0.1 M Sodium acetate buffer pH 5.2 for acid phosphatase).

#### Results

From 108 dermatophytosis suspected cases, 54 were positive for dermatophytes. Fifty five isolates of the fungi were responsible for the infections. Among these isolates, 53 were identified morphologically as *Trichophyton rubrum*, 2 as *T. mentagrophytes*.

Although the 53 isolates of *T. rubrum* possessed the same criteria for the identification of this species, they showed variation in both macroscopic and microscopic appearances. They were roughly divided, according to colonial textures regardless the pigmentation, into 3 forms: Velvety,

Granular and Fluffy. Meanwhile, microscopic structures were classified into 3 types; Type I, Type II and Type III.

A Type I produces numerous microconidia with many chlamyospores. Macroconidia are variable from none to common\*\* in number (Figure 1)

A Type II shows chlamyospores predominantly with rare\* macroconidia and a small number\*\*\* of microconidia (Figure II)

A Type III appears largely as sterile hyphae with a small number of chlamyospores and microconidia. Macroconidia was absent or rare (Figure III)

The majority of *T. rubrum* isolates was Velvety (56.6%) whereas Granular (18.8%) and Fluffy (24.5%) forms were less frequently found. Each form of colonial texture produced mostly Type I microscopic characteristic (Table 1.) *Trichophyton rubrum* isolates showed different degrees of sensitivity to Griseofulvin, Ezon T, Mycosynalar and Canesten. Base on statistical difference ( $P < 0.05$ ), isolates

\* Rare = 1-10 macroconidia per slide Culture

\*\* Common = more Than 10 macroconidia per slide cultuec.

\*\*\* Small number = 1-20% approximately of a slide culture.

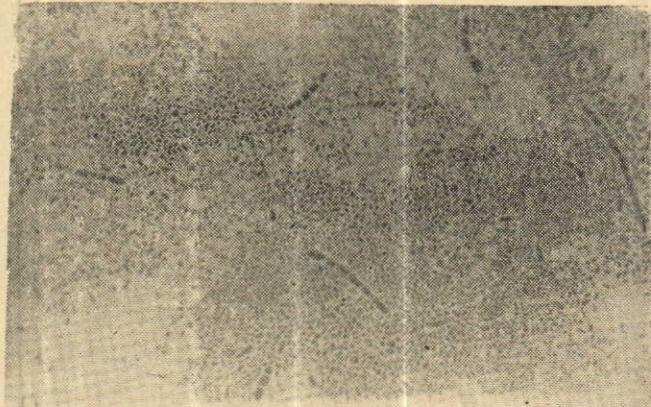


FIGURE. I A TYPE I MICROSCOPIC CHARACTERISTIC.

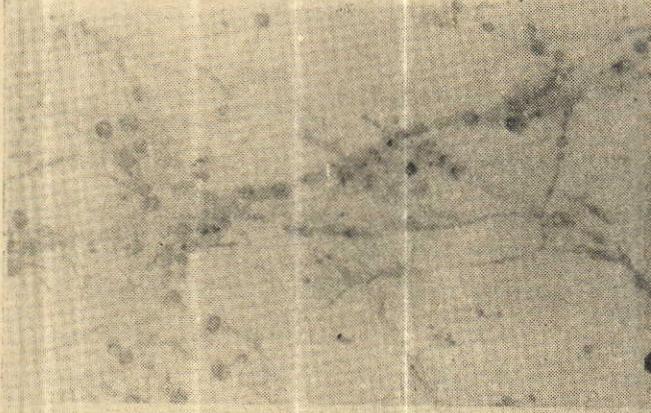


FIGURE. II A TYPE II MICROSCOPIC CHARACTERISTIC.



FIGURE. III A TYPE III MICROSCOPIC CHARACTERISTIC.

**Table 1** Different colonial forms and microscopic characteristics of *Trichophyton rubrum* showing variable degrees of sensitivity to antifungal drugs.

Colonial texture	Number and percentage	No. of isolate showing microscopic characteristics			In vitro drug susceptibility test ( $\bar{x} \pm$ S.D.)				
		Type I	Type II	Type III	Griseofuvin ug/ml	Ezont ul/ml	Mycosynalar ul/ml	Canesten ul/ml	
Velvety (V)	30 56.6%	22	8	0	6.13 $\pm$ 2.47	1.65 $\pm$ 0.52	0.58 $\pm$ 0.41	0.79 $\pm$ 0.62	
Granular (G)	10 18.8%	10	0	0	6.6 $\pm$ 2.37	1.60 $\pm$ 0.48	0.42 $\pm$ 0.16	0.48 $\pm$ 0.31	
Fluffy (F)	13 24.5%	7	2	4	5.93 $\pm$ 2.49	1.35 $\pm$ 0.47	0.5 $\pm$ 0.17	0.77 $\pm$ 0.69	
					P < 0.05 (G/F)	P < 0.05 (G/F) P < 0.01 (V/F)	P < 0.01 (V/G)		P < 0.01 (F/G) P < 0.01 (V/G)

**Table 2** Different biochemical reactions of *Trichophyton rubrum* in connection with colonial forms and microscopic characteristics.

Urease Test	Pigment production in potato-dextrose agar	Number and percentage	No. of isolate giving microscopic characters			No. of isolate showing Colonial Texture		Enzyme activities (- $\pm$ S.D.) Unit (u) = OD/2 plugs/ 2 hours					
			Type I	Type II	Type III	Velvety	Granular	1*	2*	3*	4*	5*	
+	-	12 (22.6%)	6	6	0	11	1	0	81.1 $\pm$ 52.0	240.14 $\pm$ 200.7	41.42 $\pm$ 21.5	172.08 $\pm$ 127.35	195.28 $\pm$ 159.04
+	+	2 (3.7%)	0	2	0	2	0	0					
-	-	5 (9.4%)	4	0	1	3	0	2	60.76 $\pm$ 53.74	206.58 $\pm$ 130.87	48.97 $\pm$ 28.77	261.92 $\pm$ 138.76	363.68 $\pm$ 202.31
-	+	34 (64.1%)	29	2	3	14	9	11					

1\* = Paranitrophenyl A-D glucosidase

2\* = Paranitrophenyl B-D glucosidase

3\* = Orthonitrophenyl B-D galactosidase

4\* = Paranitrophenyl acid phosphatase

5\* = Paranitrophenyl alkaline phosphatase.

P < 0.05

P < 0.01

a of the Fluffy form were more sensitive to Griseofulvin than those of the Granular form and more sensitive to Ezon T than those of the Granular and Velvety forms. However, the Granular form was more sensitive to Mycosynalar than the velvety form and more sensitive to Canesten than the Fluffy and Velvety forms.

Thirty four isolates 64.1% of *T.rubrum* had physiologically typical characteristics; they are negative urease test in 7 days, produce red pigment in potato dextrose agar and do not perforate hair in vitro (Table II). The remaining ones had variable properties i.e. positive urease test, no pigmentation in potato dextrose agar (22.6%); positive urease test with pigmentation in potato dextrose agar (3.7%) and negative urease test without pigmentation in potato dextrose agar (9.4%). *Trichophyton rubrum* isolates seemed to have the same pattern of enzymatic activities for the five substrates tested. They yielded high activities (over 100 u.) of Paranitrophenyl B-D glucosidase, Paranitrophenyl acid and alkaline phosphatases. The negative urease test group, however, yielded significantly higher activities of Paranitrophenyl acid and alkaline phosphatases with  $P < 0.05$  and  $P < 0.01$  respectively.

### Discussion

Several factors influencing the dermatophytes morphologically and physiologically have been found by many authors. Hexose and the related structural formula of monosaccharide support pigment production (16,17). Gross structure of a colony, pigmentation and microscopic appearance are influenced by amino acid and the medium used (18,19). In this study, the same lot of medium was used to ensure the identity of nutrients for growth. The incubation temperature was kept between 25°C-28°C. Subculturing was made at an interval of 15 days with typical portion of the colony to retain the original characteristics. Variation in morphology and biochemical reactions of *T.rubrum* isolates might be, in part, the results of antifungal drugs used during the treatment prior to isolation. Another possibility is the occurrence of unstable intermediate mutants (20). Some isolates, therefore, represent only temporary characteristics until they achieve the constant ones during successive subcultures.

The Velvety and Granular forms of colonial texture mostly produce numerous microconidia while macroconidia are variable. The Fluffy form has more chlamyospore but less microconidia. Although there are some significance differences in the degree of sensitivity to

particular drug in vitro between isolates of different from of colony, the usual dose when used topically are excess beyond the minimum inhibitory concentrations. The colonial form of a pathogen alone, therefore, is not an indicator of the treatment response.

The patterns of enzymatic activity of various *T. rubrum* isolates against the five paranitrophenol derivatives are almost similar although the negative urease test group has higher activities of paranitrophenyl acid and alkaline phosphatases. This should be a new biochemical characteristic of this species which can differentiate some variants of *T. rubrum* that give unusual pattern. It is of interest that

### ย่อเรื่อง

จากการศึกษารูปร่างลักษณะ และปฏิกิริยาทางชีวเคมีโดยละเอียด ของรา *Trichophyton rubrum* จำนวน 53 ตัว ที่แยกได้จากคนไข้ พบว่าลักษณะ Colony และการให้สีแตกต่างกันไป, Colony ชนิด Velvety พบมากที่สุด 56.6% ชนิด Fluffy 24.5% และ Granular 18.8% ลักษณะที่เห็นได้จากกล้องจุลทรรศน์ สามารถแบ่งออกได้เป็น 3 แบบคือ

แบบที่ 1 มี Microconidia มากมาย เห็น Chlamydo-spores 5 อยู่กระจัดกระจาย อาจไม่พบ Macroconidium<sup>+</sup> หรือพบได้บ้างไม่มากนัก

*T. rubrum* isolates which involved in cutaneous infection produced high activity of paranitrophenyl - B - D - glucosidase as well as *Cryptococcus neoformans* (21) and *Histoplasma duboisii* (22) which cause systemic fungal diseases. Moreover, high alkaline phosphatase activity of *T. rubrum* is similar to those of yeast phase of *H. duboisii* (22) and *Blastomyces dermatitidis* (23) Since dermatophytes are well known for its tendency to alkalize the medium (24) and in the ring worm infection, the area involved shows higher pH than the normal part (25). Whether these enzymatic activities have any connection with pathogenesis is a subject of further investigation.

แบบที่ 2 พบ Chlamydo-spores เป็นส่วนมาก, macroconidium มีน้อย (1-10 ต่อ slide culture) Microconidia มีเป็นส่วนน้อย (1%-20% ของ slide culture)

แบบที่ 3 พบ hyphae เป็นส่วนมากมี Microconidia และ Chlamydo-spores เป็นส่วนน้อย (1%-20% ของ slide culture) Macroconidium อาจไม่พบเลย หรือพบน้อยมาก

Colony ชนิด Velvety และ Granular มักจะให้ลักษณะที่เห็นจากกล้องจุลทรรศน์แบบที่ 1 เป็นส่วนมาก ชนิด Fluffy มีโอกาสพบ Chlamydo-spores มาก และ Microconidium น้อย



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