Original Article

Typing of Common Dermatophytes by Random Amplification of Polymorphic DNA

Zhihong Zhong^{1,2}, Ruoyu Li^{1,2}, Dongmei Li¹, Duanli Wang¹

¹Systematic Mycology and Lichenology Laboratory, Institute of Microbiology,
Chinese Academy of Sciences, Beijing, 100080, P. R. China.

²Department of Dermatology, The First Hospital of Beijing Medical University,
Beijing, 100034, P. R. China.

[Received: 15, October 1996. Accepted: 10, April 1997]

Abstract

Seventy-six clinical isolates of dermatophytes and 8 preserved strains of *Arthroderma* were investigated by random amplification of polymorphic DNA (RAPD) assay to determine the DNA types of common dermatophytes and find any relationships of DNA-based typing with morphology, teleomorphs, geographic origins and sites of human infection. It was found that each species showed a distinct DNA pattern, which can be used as an identification marker. Forty-two isolates of *Trichophyton mentagrophytes* were classified into 3 main types and intratype polymorphism was revealed with some primers. No simple relationship was found between DNA type and morphology, but the DNA types of strains were closely related to their geographic origins. The RAPD groups of *Arthroderma benhamiae*, *A. vanbreuseghemii*, *A. gypseae* and *A. otae* were distinctly different, whereas the 42 clinical isolates of *T. mentagrophytes* shared similar patterns with *A. vanbreuseghemii*. Among 30 clinical isolates of *T. rubrum*, 22 showed almost identical RAPD type, while the other 8 strains differed slightly in band patterns. *T. rubrum* exhibited great differences from *A. benhamiae* and *A. vanbreuseghemii*. In conclusion, RAPD provides a stable and reliable means of typing common dermatophytes, and is a powerful tool for dermatophyte identification and epidemiological study.

Key words: Dermatophytes, DNA typing, Epidemiology, Random amplification of polymorphic DNA

Introduction

Dermatophytes are a group of fungal pathogens which infect skin, hair and nails. In many countries more than 10% of the population is infected. There are approximately 45 species divided among 3 anamorphic genera. The teleomorphic stage of some species is in the genus *Arthroderma*.^{1–3)} *Trichophyton rubrum* is the most common dermatophyte and causes tinea pedis, tinea cruris, tinea corporis and tinea unguium in humans.³⁾ In China, 60%–80% of dermatophytoses are caused by this organism.⁴⁾ *T. mentagrophytes* is the second most important pathogen in most countries.^{2, 5)}

Phenotype, such as gross microscopic morphology, is the basis of classification and identification of dermatophytes; however, it often varies with culture conditions. Potentially more precise, molecular methods are now available to study these

organisms, e.g., isoelectric focusing,⁶⁾ restriction fragment length polymorphism (RFLP) for total cellular DNA⁷⁾ and mitochondrial DNA (mtDNA) analysis⁸⁾. Since the strains involved in the studies are limited, there are still many unresolved questions. Random amplification of polymorphic DNA (RAPD) was developed by Welsh and McClelland⁹⁾ and Williams et al.¹⁰⁾ in 1990. This has proved to be a valuable assay for DNA typing and epidemiological studies of such human pathogens as *Candida*,^{11, 12)} *Aspergillus*,¹³⁾ and *Cryptococcus*.¹⁴⁾

In this study, 76 clinical isolates of dermatophytes and 8 preserved strains of *Arthroderma* were examined by RAPD. We sought to answer the following questions. Is there geographic variation in RAPD types? Is there more than one DNA type of isolates from the same body location? Is there any relationship between RAPD pattern and anatomical location? What is the relationship between morphology and genotype?

Address corresponding to: Zhihong Zhong
Apt. 2-1003, Nan San Jie Zhong Guan Cun
Beijing, 100080, P. R. China

Materials and Methods

Isolates and identification: Seventy-six clinical isolates of dermatophytes which included 42 strains of *T. mentagrophytes* (M1~M42), 30 *T. rubrum* (R1~R30), and each of *Microsporum gypseum*, *M. canis*, *T. tonsurans*, *T. violaceum* were used in this study. All isolates were obtained from our outpatient department, except 5 from Yinchuan (north-west China) provided by the Department of Dermatology, Affiliated Hospital of Ningxia Medical University and 5 from the First Hospital of Wuhan (south China), respectively. The preserved strains included 1 '+' and 1 '-' strains of *Arthroderma benhamiae* (*A.b.*), *A. vanbreuseghemii* (*A.v.*), *A. gypsea* (*A.g.*) and *A. otae* (*A.o.*), provided by the Research Center for Pathogenic Fungi &

Microbiol Toxicoses of Chiba University in Japan. The strains examined are listed in Table 1.

Clinical isolates were obtained from patients with tinea capitis, tinea pedis, tinea manuum, tinea corporis, tinea cruris, and tinea unguium. They were identified by culture characteristics and microscopic appearance. Biochemical and physiological methods such as cultures on cornmeal agar and urea agar were also used.

DNA extraction: Each isolate was inoculated on petri dishes of Sabouraud's dextrose agar for 2 weeks at 25°C. Mycelium approximately 0.1~0.3g in weight was harvested into an Eppendorf tube, and DNA extraction was carried out using benzyl chloride, similar to the method described previously.¹⁵⁾

RAPD analysis: PCR was performed in a total

Table 1-1. Examined strains of *Trichophyton mentagrophytes* (M1→M42)

Test code	Original code	DNA type	Lesion site	Test code	Original code	DNA type	Lesion site
M1	Y-WPE	I	Unrecorded	M22	11470	II	Tinea corporis
M2	Y-DFL	I	Unrecorded	M23	12779	II	Tinea corporis
M3	Y-QKJ	I	Unrecorded	M24	12559	II	Tinea pedis
M4	Y-LGW	II	Unrecorded	M25	12910	II	Tinea pedis
M5	Y-WJT	I	Unrecorded	M26	12973	II	Tinea pedis
M6	10516	II	Tinea capitis	M27	12961	II	Tinea pedis
M7	10187	III	Tinea corporis	M28	Z-249	II	Tinea pedis
M8	10208	II	Unrecorded	M29	Z-281	II	Tinea pedis
M9	10484	II	Tinea corporis	M30	Z-229	II	Tinea pedis
M10	10397	II	Tinea corporis	M31	Z-234	II	Tinea pedis
M11	W-FOR	III	Unrecorded	M32	Z-155	II	Tinea pedis
M12	W-THR	III	Unrecorded	M33	Z-265	II	Tinea pedis
M13	W-ONE	III	Unrecorded	M34	Z-133	II	Tinea pedis
M14	W-WCG	III	Unrecorded	M35	Z-240	II	Tinea pedis
M15	W-LLS	III	Unrecorded	M36	Z-215	II	Tinea pedis
M16	10209	II	Tinea capitis	M37	Z-159	II	Tinea pedis
M17	12541	II	Tinea capitis	M38	Z-225	II	Tinea pedis
M18	11759	III	Tinea capitis	M39	Z-135	II	Tinea pedis
M19	11878	III	Tinea capitis	M40	Z-238	II	Tinea pedis
M20	12542	I	Tinea capitis	M41	Z-275	II	Tinea pedis
M21	10515	II	Tinea corporis	M42	Z-132	II	Tinea pedis

Table 1-2. Examined strains of *Trichophyton rubrum* (R1→R30)

Test code	Original code	Lesion site	Test code	Original code	Lesion site
R1	12437	Tinea unguium	R16	12534	Tinea unguium
R2	12533	Tinea unguium	R17	12535	Tinea unguium
R3	12750	Tinea unguium	R18	12405	Tinea unguium
R4	12557	Tinea cruris	R19	12678	Tinea unguium
R5	12595	Tinea cruris	R20	12616	Tinea unguium
R6	12592	Tinea cruris	R21	12466	Tinea unguium
R7	12706	Tinea corporis	R22	12691	Tinea unguium
R8	12585	Tinea corporis	R23	12653	Tinea unguium
R9	12511	Tinea corporis	R24	12590	Tinea cruris
R10	12532	Tinea pedis	R25	12598	Tinea cruris
R11	12706	Tinea pedis	R26	12567	Tinea cruris
R12	12526	Tinea pedis	R27	12625	Tinea cruris
R13	12658	Tinea manuum	R28	12576	Tinea cruris
R14	12547	Tinea manuum	R29	12495	Tinea cruris
R15	12517	Tinea manuum	R30	12491	Tinea cruris

Table 1-3. Other dermatophytes and preserved strains

Species	Test code	Original code	Lesion	Source
<i>M. gypseum</i>	G1	12056	Tinea corporis	Beijing
<i>M. canis</i>	C1	12630	Tinea capitis	Beijing
<i>T. tonsurans</i>	T1	12524	Tinea corporis	Beijing
<i>T. violaceum</i>	V1	12624	Tinea capitis	Beijing
1. <i>Arthroderma</i>	<i>A.v.</i> (+)	Unrecorded	Research Center for Pathogenic	
<i>benhamiae</i> (+)(-)	<i>A.v.</i> (-)	Unrecorded	Fungi & Microbiol Toxicoses of Chiba University	
2. <i>Arthroderma</i>	<i>A.v.</i> (+)	IFM41287		
<i>vanbreuseghemii</i> (+)(-)	<i>A.v.</i> (-)	IFM41288	Chiba University	
3. <i>Arthroderma</i>	<i>A.g.</i> (+)	IFM5310		
<i>gypsea</i> (+)(-)	<i>A.g.</i> (-)	IFM5309	Chiba University	
4. <i>Arthroderma</i>	<i>A.o.</i> (+)	IFM5288		
<i>otae</i> (+)(-)	<i>A.o.</i> (-)	IFM5289	Chiba University	

Table 2. Primers used

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
OPI-03	CAGAAGCCCA	OPI-07	CAGCGACAAG
OPI-17	GGTGGTGATG	OPI-20	AAAGTGCGGG
OPK-01	CATTCGAGCC	OPK-08	GAACACTGGG
OPK-09	CCCTACCGAC	OPK-17	CCCAGCTGTG
OPK-19	CACAGGCGGA	OPK-20	GTGTCGCGAG

volume of 25 μ l, containing 2.5 μ l of 10 $\times$ buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 22 mM MgCl₂, 0.001% gelatin), 1.25 μ l of dNTP, 2 units of Taq DNA polymerase, all provided by the Genetics Institute of Academia Sinica, and 1 μ l of template DNA solution. The RAPD primer kit was obtained from Operon Technologies Inc. (Alameda, Calif., USA). The primers used in this experiment are shown in Table 2.

The PCR was conducted on a minicycler PTC-150 from MJ Research Corporation, USA programmed for one cycle of 40 sec at 94°C, 45 cycles of 20 sec at 94°C, 1 min at 35°C, 1 min at 72°C, and finally, extension for 10 min at 72°C. The PCR products were separated by electrophoresis on 1.5% agarose gel with 1 $\times$ TBE as running buffer, stained with ethidium bromide and photographed under UV light. The image was stored in UVP's GDS 5000 gel recording system, and the molecular weights of the fragments were determined with the aid of Gelbase software in this system. Single matching coefficient (SM) was calculated and a dendrogram was generated automatically by UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis with the SAHN program in NTSYS-pc software.

Results

RAPD analysis of clinical isolates including *T. mentagrophytes*, *T. rubrum*, *M. gypseum*, *M. canis*, *T. tonsurans* and *T. violaceum* showed that each species had its distinct DNA patterns (Fig. 1). The interspecies difference of *T. mentagrophytes* and

T. rubrum was more pronounced than that of intraspecies when amplified under the same conditions. Genotype relationship of the strains was revealed by multi-primer cluster analysis (Fig. 7).

***T. mentagrophytes*:** Forty-two clinical isolates of *T. mentagrophytes* could be divided into 3 types—types I, II and III, based on RAPD band patterns and multi-primer cluster analysis (inratatype SM>0.90). Five strains (11.9%) were classified as type I, 29 strains (69.0%) as type II, and 8 strains (19.0%) as type III.

When amplified with primer OPI-3, the intertype difference was clearly demonstrated by bands at 1191 bp and 428 bp. Few intratype differences were found; hence the DNA type was clearly determined by this primer (Fig. 2). Amplifications with primers OPI-17, OPI-20 and OPK-20 also showed the same 3 main types. Furthermore, intratype pleomorphism was revealed by OPI-17 and OPK-20. Nine strains of type II were divided into 4 subtypes by primer OPI-17 (Fig. 3), and 8 subtypes by multi-primer cluster analysis.

We observed no simple relationship between DNA type and colony morphology. The strains of types I and II were cottony or powdery, while those of type III were all powdery. The strains with similar DNA patterns, especially in type II, probably had different colonies and microscopic appearances.

The DNA patterns of *A.b.*, *A.v.*, *A.g.* (teleomorph of *M. gypseum*) and *A.o.* (that of *M. canis*) were obviously different from each other (SM= $\leq$ 0.76), but intraspecific strains of '+' and '-' in a species were identical in most primers (SM=0.99). Forty-two clinical isolates of *T. mentagrophytes* shared similar DNA patterns with *A.v.* (SM>0.84), (Fig. 1).

The DNA type of *T. mentagrophytes* was closely related to its geographic origin. Four strains from Yinchuan were type I, while only 1 strain was type II. All five strains from Wuhan belonged to

Fig. 1. RAPD fingerprinting comparison among commonly encountered dermatophytes with primer OPI-3 showing 1-*A. benhamiae* (+), 2-*A. benhamiae* (-), 3-*A. vanbreuseghemii* (+), 4-*A. vanbreuseghemii* (-), 5-*T. mentagrophytes* M9 (II), 6-M12 (III), 7-M5(I), 8-10-*T. rubrum* (R19, R20, R21), 11-*A. gypsea*(+), 12-*M. gypseum*, 13-*A. otae* (+), 14-*M. canis*, 15-*T. tonsurans*, 16-*T. violaceum*. Marker (m) is at left.

Fig. 2. RAPD fingerprinting of *T. mentagrophytes* isolates (M1→M15) from different cities with primer OPI-3 showing lanes 1→5: M1, M2, M3, M5, M4, respectively, which are from Yinchuan; 6→10: M6, M9, M8, M10, M7 from Beijing; 11→15: M11, M12, M13, M14, M15 from Wuhan. Isolates of type I are shown in lanes 1→4, type II in 5→9, type III in 10→15. The size markers (m) are given at left.

Fig. 3. RAPD fingerprinting with primer OPI-17 shows the intratype polymorphism of *T. mentagrophytes* (M16, M17, M21→M27), which are all classified as type II. The isolates are also compared with *A. vanbreuseghemii*. 1-M22, 2-M27, 3-M23, 4-M26, 5-M25, 6-*A. vanbreuseghemii* (+), 7-*A. vanbreuseghemii* (-), 8-M17, 9-M24, 10-M16, 11-M21. Marker (m) is at right.

type III. Among 32 strains from Beijing, 28 (87.5%) were type II, 1 (3.1%) was type I, and 3 (9.4%) were type III. The DNA patterns were almost identical in 15 interdigital isolates from a

group of soldiers in Beijing who suffered from tinea pedis.

The isolates from different sites of infection showed their genotypic characteristics. In 6 isolates

Fig. 4. RAPD fingerprinting of *T. mentagrophytes* isolates from different sites of infection with primer OPI-3 showing 1-*A. vanbreuseghemii* (+), 2-*A. vanbreuseghemii* (-), 3-7: M16-M20, which were from a lesion of tinea capitis, 8-10: M21-M23, from tinea corporis, 11-14: M24-M27, from tinea pedis. Marker (m) is at left.

Fig. 5. RAPD fingerprinting of *T. rubrum* with primer OPK-17. Lanes 1-15 indicate strains R16-R30. Most strains show a relatively identical DNA pattern, only R19, R20, R22 and R29 are different, and differ from one another. 'm' indicates marker.

Fig. 6. RAPD fingerprinting of *T. rubrum* with primer OPK-19. Lanes 1-2 indicate strains 1-*A.b.* (+), 2-*A.v.* (+), and lanes 3-16 indicate strains R2-R15. In this amplification, the main band of *T. rubrum* is in the same position as *A.b.*. 'm' indicates marker.

from patients with tinea capitis, 1 strain (M20) was type I, 3 strains (M6, M16, M17) were type II and 2 (M18, M19) were type III. In 6 isolates from patients with tinea corporis, 5 (M9, M10, M21, M22, M23) were type II and 1 (M7) was type III. Nineteen strains related to tinea pedis (M24-M42) were all type II (Fig. 4).

***T. rubrum*:** Every clinical isolate identified as

T. rubrum produced red pigmentation on cornmeal agar. Twenty-two of 30 strains of *T. rubrum* showed almost identical RAPD band patterns (Fig. 5), and the other 8 strains had slight differences.

RAPD band pattern and cluster analysis showed that *T. rubrum* were greatly different from *A. benhamiae* and *A. vanbreuseghemii* in most amplifica-

Fig. 7. Dendrogram for *T. mentagrophytes* and other dermatophytes using UPGMA cluster analysis with primers OPI-3, OPI-17, OPI-20 and OPK-20.

tions. Only in primer OPK-19 were the main bands of *T. rubrum* and *A. benhamiae* the same molecular weight (Fig. 6).

A completely identical RAPD band pattern was revealed in strains from different sites of infection in one patient, and the DNA types of isolates from different patients were sometimes identical.

Discussion

The dermatophytes are a group of fungi with many common characteristics, although at times classification can be a problem. For example, it is sometimes difficult to distinguish *T. mentagrophytes* from *T. tonsurans* and *T. m. var. interdigitale* from *T. rubrum* morphologically. In such circumstances, DNA patterns of the isolates become an important distinguishing tool.

In this study, distinct and relatively stable band patterns were seen among different species of dermatophytes (*T. mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. violaceum*, *M. gypseum* and *M. canis*). The interspecies differences of *T. mentagrophytes* and *T. rubrum* were more pronounced than that of intraspecies. The results generally agree with the findings of *Trichophyton spp.* by RAPD in Australia,¹⁶⁾ in which the distinct band patterns among three dermatophytes, *T. mentagrophytes*, *T. rubrum*, *T. tonsurans*, were revealed by primer 5'-ACCCGACCTG-3'. In Japan, Mochizuki and Uehara successfully differentiated *T. mentagrophytes* var. *interdigitale* from *T. rubrum* by RAPD, and found that 6 out of 9 primers were most useful.¹⁷⁾

Therefore, RAPD band patterns provide genetic markers for classification and identification. They show a great potential to correct the mistakes made by phenotypic methods. RAPD not only allows the rapid and precise speciation of the dermatophytes, but also provides insight on build-

ing genetic maps of relatedness by multiprimer cluster analysis. This has important phylogenetic implications. For example, in the present study, dendrograms generated by NTSYS-pc software showed a relatively close relationship between *A. otae* and *M. canis*, but a remote relationship between *A. gypsea* and *M. gypseum*, indicating the complexity in typing of dermatophytes.

The taxonomy of *T. mentagrophytes* has a complicated history. There are many varieties, such as commonly encountered *T. m. var. interdigitale* and *T. m. var. mentagrophytes*. Members of the teleomorph stage include *A. benhamiae*, *A. vanbreuseghemii* and *A. simii*. Therefore, *T. mentagrophytes* is regarded as a 'complex' or 'group'.^{2, 18)}

According to this RAPD analysis, 42 clinical isolates of *T. mentagrophytes* are classified into 3 types: types I, II and III. Intratype polymorphism is also revealed by multi-primer amplification. Intertype differences are more pronounced than those of intratype, but less pronounced than those of interspecies. This implies the great importance of this method in identification and epidemiological studies.

No simple relationship was found between DNA-based typing and morphology in the present study. The colonies of *T. mentagrophytes* had different gross morphological appearances, such as cottony (downy) type and powdery (granular) type. Although five groups, i.e., woolly, downy, cream skin-like, powdery and granular, were defined by Qin Qixian,¹⁹⁾ the morphology of this species has not been universally standardized as yet. Isolates of types I and II could be cottony or powdery, while only powdery isolates were found in type III. The DNA types of 15 interdigital isolates are identical type II in spite of the morphological pleomorphism. This is consistent with the identical mtDNA pattern of different strains of *T. m. var. interdigitale* despite a diversity of appearances.²⁰⁾ The phenotype of strains can be easily affected by such culture conditions as medium and temperature, while DNA type is relatively stable and reliable and shows great significance in this aspect.

The RAPD band pattern of *A.b.* differed greatly from that of *A.v.*. The dendrogram generated by UPGMA cluster analysis (Fig. 5) showed that clinical isolates of *T. mentagrophytes* (types I, II, III) and *A.v.* were in the same group at SM=0.84, while they had little similarity with *A.b.* (SM<0.69). This finding is consistent with the results of mtDNA analysis and isoelectric focusing,^{6, 8)} which can distinguish *A.b.* from *A.v.*. Clinical isolates of the three types of *T. mentagrophytes* shared a pattern similar to that of *A.v.*, and were different from *A.b.*. The strains

examined were identified as *T. m.* var. *interdigitale* and *T. m.* var. *mentagrophytes*, and the mtDNA also showed the same restriction profile among the two varieties and *A.v.*, indicating that they are closely related. According to previous studies the differences among various strains of *T. m.* var. *interdigitale* or *T. m.* var. *mentagrophytes* and the differences between *A.b.* (+) and *A.b.* (−), or between *A.v.* (+) and *A.v.* (−) can hardly be detected by mtDNA analysis and isoelectric focusing,^{6,8)} while in this study the slight differences can be detected by RAPD with proper primers. The teleomorph of the strains we studied is probably *A.v.* based on the similar patterns. As reported previously, in the region of Galicia the species most frequently isolated was also *A.v.*¹⁾ Further study is necessary to clarify their mating types.

It appears that the DNA types of *T. mentagrophytes* are closely related to its geographic origin. The dominant types of the isolates from Yinchuan, Beijing and Wuhan are type I, II and III, respectively, and there are slight overlaps among them. Overall this reflects the trend of strain distribution. This unique distribution might be concerned with geographic positions, population migration patterns, transmission characteristics, people's living conditions, living habits and even clothing styles.

The DNA types of 15 interdigital isolates (M28 ~M42) from a group of soldiers in Beijing were almost identical (type II), indicating that RAPD may provide clues to the source of infection.

No direct relationship was found between DNA type and the infection site of the strains. The isolates from tinea capitis can be type I, II or III. Most strains from patients with tinea corporis were classified as type II and only 1 as type III. Only type II was found in strains related to tinea pedis. Because the number of strains involved is limited, we still do not know whether any group has selectivity when it invades a certain part of the body. Further investigation is needed.

The identification of *T. rubrum* is usually based on microscopic and gross colonial morphology and physiology typing. Lectin typing is a histochemical method which has occasionally been applied to this species. In the study by Gruseck et al.,²¹⁾ a patient with primary onychomycosis developed generalized lesions after steroid treatment for lung fibrosis. This method found identical lectin patterns for all the isolates, thus recognizing the toenails as the source of reinfection. The technique could not, however, provide sufficient intraspecific differences, which are important to basic pathogenic and epidemiological study.

The present study showed that 30 clinical isolates of *T. rubrum* had relatively high similarity; only 8 strains differed slightly from one another, while the others were almost identical. Identical DNA patterns were revealed in strains from different sites of infection in one patient. The DNA types of isolates from different patients could also be identical, indicating that self-inoculation and transmission may occur among individuals.

According to RAPD band pattern and cluster analysis results, *T. rubrum* is different from other dermatophytes, *A.b.* and *A.v.* in most amplifications. Nishio et al. analyzed the mtDNA of *T. rubrum* by RFLP and found that it can be divided into two types.⁸⁾ The profile of type I was identical with that of *A.b.*, and the strains of type I produced red pigmentation on cornmeal agar. However, the strains of type II which did not produce red pigmentation shared a pattern similar to that of *A.v.*. Our results differ from those of mtDNA analysis, probably due to the different types of DNA studied. MtDNA is relatively conservative when compared with genomic DNA in which RAPD involved, so it may show a genetic relationship between *T. rubrum* and *T. mentagrophytes*, while RAPD reveals the differences between species. In this study, only when amplified with primer OPK-19 was the main band of *T. rubrum* and *A.b.* identical, and these strains developed red pigmentation. These findings are generally consistent with the results of mtDNA analysis.

RAPD provides a fast and economical means of typing for common dermatophytes, and it is relatively stable, reliable, and highly typeable. It is also widely used in other areas of biological science. In recent years, the reproducibility of results has become a big issue with this typing method. In our observation, the results are generally reproducible when a proper primer is used. The main bands are relatively stable, while the weak bands which are less important often show poor reproducibility. However, a comparison of tested strains and standard strains under the same conditions is still necessary when the RAPD band pattern is used as a genetic marker for strain identification.

Every species of dermatophyte can be identified by RAPD, and the species *T. mentagrophytes* can be classified into 3 main types. The relationship of DNA types and morphology, teleomorph species and geographic origins was also shown by this method. In summary, RAPD analysis can be used as an adjunct to phenotyping, and shows great promise in dermatophyte identification and epidemiological studies.

Acknowledgments

We are grateful to Dr. Wenying Zhuang, Professor of Mycology in the Institute of Microbiology Chinese Academy of Science for supervising the experiments, and to Miss Xiaohong Wang and Mrs. Zhe Wan for isolate collection and technical assistance. We acknowledge the Research Center for Pathogenic Fungi and Microbiol Toxicoses of Chiba University in Japan for kindly providing us examined strains. We also thank Mr. Hong Chen and Miss Zhiyu Zhong for data processing, and Dr. Glenn S. Bulmer, Dr. Rytas Vilgalys and Dr. Richard Barton for kind help in process of revision.

References

- 1) Miguens NP: Cutaneous mycoses: A clinical and mycological study. In Proceedings of the 5th Int. Conf. on mycotic superficial cutaneous and subcutaneous infections (Pan American Health Organization ed), p.12-19, Scientific Pub. N396, Washington DC, 1980.
- 2) Rippon JW: The changing epidemiology and emerging patterns of dermatophytes species. In Current Topics in Medical Mycology Vol (1) (McGinnis MR ed), p.208-234, Springer-Verlag, New York, 1985.
- 3) Aly R: Ecology and epidemiology of dermatophyte infections. J Am Acad Dermatol **31**: S21-S25, 1994.
- 4) Li RY, Wan Z, Zhou ZD, Li DM, Wang DL: Epidemiological analysis of pathogenic fungi in Beijing Medical University from 1992 to 1994. Chinese J Dermatol **28**: 188-190, 1995.
- 5) Zhao JY: Changing patterns of agents of superficial mycoses in China. In Proceedings of the Third China-Japan International Congress of Mycosis (Wang DL, Ogawa H eds.), p.111, International Academic Pub, Beijing, 1995.
- 6) Symoens F, Willenz P, Rouma V, Planard C, Nolard N: Isoelectric focusing applied to taxonomic differentiation of the *Trichophyton mentagrophytes* complex and the related *Trichophyton interdigitale*. Mycoses **32**: 652-663, 1989.
- 7) Mochizuki T, Uehara M: Efficacy of total cellular DNA extracted by minipreparation method for molecular taxonomic marker of dermatophytes. Jpn J Med Mycol **36**: 157-160, 1996.
- 8) Nishio K, Kawasaki M, Ishizaki H: Phylogeny of the genera *Trichophyton* using mitochondrial DNA analysis. Mycopathologia **117**: 127-132, 1990.
- 9) Welsh J, McClelland M: Fingerprinting genomes using PCR with arbitrary primers. Nucl Acid Res **18**: 7213-7218, 1990.
- 10) Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res **18**: 6531-6535, 1990.
- 11) Bostock A, Khattak MN, Matthew R, Burnie J: Comparison of PCR finger-printing, by random amplification of polymorphic DNA, with other molecular typing methods for *Candida albicans*. J Gen Microbiol **139**: 2179-2184, 1993.
- 12) Li DM, Wang DL, Li RY, Zhu H, Zhu LH: Assessment of randomly amplified polymorphic DNA markers in the classification of *Candida albicans*. Acta Mycologica Sinica **14**: 123-129, 1995.
- 13) Loudon KW, Burnie JP, Coke AP et al.: Application of polymerase chain reaction to fingerprinting *Aspergillus fumigatus* by random amplification of polymorphic DNA. J Clin Microbiol **5**: 1117-1121, 1993.
- 14) Haynes KA, Sullivan DJ, Coleman DC et al.: Involvement of multiple *Cryptococcus neoformans* strains in a single episode of cryptococcosis and reinfection with novel strains in recurrent infection demonstrated by random amplification of polymorphic DNA and DNA fingerprinting. J Clin Microbiol **33**: 99-102, 1995.
- 15) Zhu H, Jun F, Zhu LH: Isolation of genomic DNA from plant, fungi and bacteria using benzyle chloride. Nucl Acids Res **21**: 5279-5280, 1993.
- 16) Liu D, Coloe S, Pedersen J, Baird R: Use of arbitrarily primed polymerase chain reaction to differentiate *Trichophyton* dermatophytes. FEMS Microbiol Lett **136**: 147-150, 1996.
- 17) Mochizuki T, Uehara M: Efficacy of random amplification of polymorphic DNA method to differentiate between *Trichophyton mentagrophytes* var. *interdigitale* and *Trichophyton rubrum*. Jpn J Med Mycol **37**: 97-100, 1996.
- 18) Rippon JW: Dermatophytosis and dermatomycosis. In Medical Mycology, 2nd ed (Rippon JW ed), p.154-248, Saunders, Philadelphia, 1982.
- 19) Qin QX: Personal Communication.
- 20) Mochizuki T, Tanaka K, Watanabe S, Kawasaki M, Ishizaki H: Taxonomy of *Trichophyton interdigitale* (*Trichophyton mentagrophytes* var. *interdigitale*) by restriction enzyme analysis of mitochondrial DNA. J Med Vet Mycol **28**: 191-196, 1990.
- 21) Gruseck E, Abeck D, Ring J: Relapsing severe *Trichophyton rubrum* infections in an immunocompromised host; evidence of onychomycosis as a source of reinfection based on lectin typing. Mycoses **36**: 275-277, 1993.