Taiwania, 42(3): 201-206, 1997

Use of Random Amplified Polymorphic DNAs Markers to Distinguish Temperature Isolates of *Aspergillus fumigatus* of Taiwan

Mao-Yen Chen[(1)], Kuei-Yu Chen[(2)], San-San Tsay[(1)] and Zuei-Ching Chen[(1,3)]

(Manuscript received 28 July 1997; accepted 30 August 1997)

ABSTRACT : Four temperature isolates of *Aspergillus fumigatus* Fres. from Taiwan and six isolates from several culture collection centers (CBS, ATCC and IMI) were analyzed by random amplified polymorphic DNAs (RAPDs). Ten oligonucleotide decamers were used to generate selective markers from genomic DNAs and five primers gave adequate discrimination between isolates. All isolates were classified into four groups by RAPDs that consistent to their physiological characteristics. The results indicate that RAPDs analysis is useful to assess the genetic diversity and intraspecies temperature variation among different temperature strains of *Aspergillus fumigatus*.

KEY WORDS : ***Aspergillus fumigatus*, RAPDs, Intraspecies temperature variation.**

INTRODUCTION

Aspergillus fumigatus Fres., a cosmopolitan and saprophytic fungus, is recoverable from air, soil, water, decaying vegetations and organic debris. It is also known as an opportunistic fungus which invades skins, nails, lungs and central nerval system, particularly in lungs and respiratory system, cause aspergillosis, and form fungus balls in its infection site (Kown-Chung and Bennett, 1992).

Fungi have been classified according to their temperature profiles as psychrophiles, mesophiles and thermophiles. Not all fungi lend themselves to such a rigid classification. Thus *A. fumigatus*, which has a temperature range of 12-52 ℃ might be called a thermotolerant mesophile (Conney and Emerson, 1964). *A. fumigatus* contains many strains that vary their response to temperature and biosynthesis of secondary metabolites. As a pathogen, most studies about *A. fumigatus* focus on the pathogenicity of different strains (Mondon *et al.*, 1995; Rinyu *et al.*, 1995) and the host's immune responses (Kobayashi *et al.* 1993). DNA typing of *A. fumigatus* can provide the means to match isolates from linked sources and distinguish isolates from diverse origins (Birch *et al.*, 1995). And other methods like random amplified polymorphic DNAs (RAPDs) analysis, isozyme analysis (IEA), and restriction fragment length polymorphisms (RFLPs) were involved. Either IEA or RFLPs

1. Department of Botany, National Taiwan University, Taipei 106, Taiwan, Republic of China.
2. Department of Biology, Chinese Culture University, Taipei 111, Taiwan, Republic of China.
3. Corresponding author. (Fax: 886-2-3625372. E-mail: chenrc@ccms.ntu.edu.tw)

has the disadvantage of being laborious and time-consuming. RAPDs analysis is consequently regarded as a time-saving, cost-effective and laborsaving technique for studies of genetics diversity, genetic relationship and population genetics (Vierling and Nguyen, 1993).

The purpose of this study is to assess the genetic diversity among different temperature strains of *Aspergillus fumigatus* by RAPDs.

MATERIALS AND METHODS

Fungal isolates

Four isolates of *Aspergillus fumigatus* from Taiwan (Chen, 1992) and six strains obtained from several culture collection centers (CBS, IMI, ATCC) (Table 1) were used in this study. All strains were selected by their responses to temperature (based on the data provided by culture collection centers), and were divided into four groups: transitional mesophile, mesophile, thermotolerant mesophile and theromophile. The definition of each group was according to Udagawa *et al.*(1986).

Table 1. Source and physiological characteristics of *Aspergillus fumigatus*.

No.	Culture source and isolation source	Physiological characteristics
1	CBS-192.65 (1)	Thermotolerant mesophile
2	ATCC-9197 (2)	Mesophile
3	ATCC-10894	Mesophile
4	ATCC-32722	Theromophiles
5	IMI-809353 (3)	Transitional mesophile
6	IMI-016030	Theromophiles
7	NTU-4T51 (4)	Thermotolerant mesophile
8	NTU-6T53	Thermotolerant mesophile
9	NTU-2T56	Thermotolerant mesophile
10	NTU-13T51	Thermotolerant mesophile

(1) CBS: Centraalbureau voor Schimmelcultures, Barrn, Netherlands.
(2) ATCC: American Type Culture Collection, Washington, D. C., USA.
(3) IMI: International Mycology Institue, Egham, UK.
(4) NTU: National Taiwan University, Department of Botany, Mycology laboratory.

Fungal cultures and total DNA extraction

For DNA preparation, all tested fungi were grown in Czapek Dox broth (CZB, Difco Laboratories, Detroit, Michigan) at 30°C on a rotary shaker (150 rpm) for 72 h. Genomic DNA was extracted and purified from 0.5 g frozen mycelium according to the protocol of Zhu *et al.* (1993).

DNA amplification (RAPDs)

Ten of the "1" set of random primers obtained from UBC (University of British Columbia, Canada) were tested at least three times for all isolates. Amplification reactions were performed in a volume of 25μl containing 10mM Tris-Cl, pH 8.8, 50mM KCl, 2.5 mM

$MgCl_2$, 0.1% Triton X-100, 200 μM each of dATP, dCTP, dGTP and dTTP (Flowgen), 0.2 μM primer (UBC, set 1), 100 ng of genomic DNA and 1 unit of DynaZyme (Flowgen). Amplification was performed in a MJ Research DNA Thermal Cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C, using the fastest available transitions between each temperature. Amplified products were analyzed by electrophoresis on 1.4% agarose gels, stained with 0.5μg/ml ethidium bromide, and visualized by a UV transilluminator.

Data analysis

RAPDs patterns were compared in pairwise manner using the similarity coefficient (SI) calculated with the formula SI=2C/(A+B), where A and B are the total number of bands generated by every isolates, and C is the number of common bands shared by the compared isolates (Nei and Li, 1979). The dendrogram showing the relationship among the RAPD patterns was constructed based on these coefficients, using the unweighted paired group method of analysis (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Out of 10 primers tested and three time repeats, five (Table 2) produced distinct amplified products ranging in size from 500 to approximately 2000 bps. The choice of primers with different G+C contents range from 50% to 70% provide same quality of RAPD profiles. The other primers did not produce clear amplification products. The banding patterns obtained with primers 20, 40, 48, 80, 90 showed polymorphic banding patterns useful in identifying different thermal isolates (Fig. 1). Genetic diversity among strains was determined with banding patterns from the multiplex RAPD reactions. The data produced, comprising all selective RAPD markers from 10 isolates of *A. fumigatus*, were used to calculate genetic distances, and by pairwise comparisons the phenogram of each primer was constructed (Fig. 2).

Table 2. The decamer primers that producing distinct amplified products.

Primer (UBC set 1)	Sequence 5'→3'	% G + C
20	TCC GGG TTT G	60
40	TTA CCT GGG C	50
48	TTA ACG GGG A	50
80	GTG CTC TAG A	50
90	GGG GGT TAG G	70

To make meaningful pairwise comparisons of amplified DNA bands by scoring them as present or absent, a decision must be made whether to include or exclude the faint bands. The RAPD-derived dendrogram does clearly discriminate among the strains of *A. fumigatus*. All the strains that have different responses to temperature as shown from the present investigation can be divided into 4 groups: No.1, No.7, No. 8, No. 9 and No. 10 (CBS-192.65, NTU-4T51, NTU-6T53, NTU-2T56 and NTU-13T51) were designated as Group I, No. 4 and No. 6 (ATCC-32722 and IMI-016030) as Group II, No. 2 and No. 3 (ATCC-9197 and ATCC-32722) as Group III, and No.5 (IMI-809353) as Group IV.

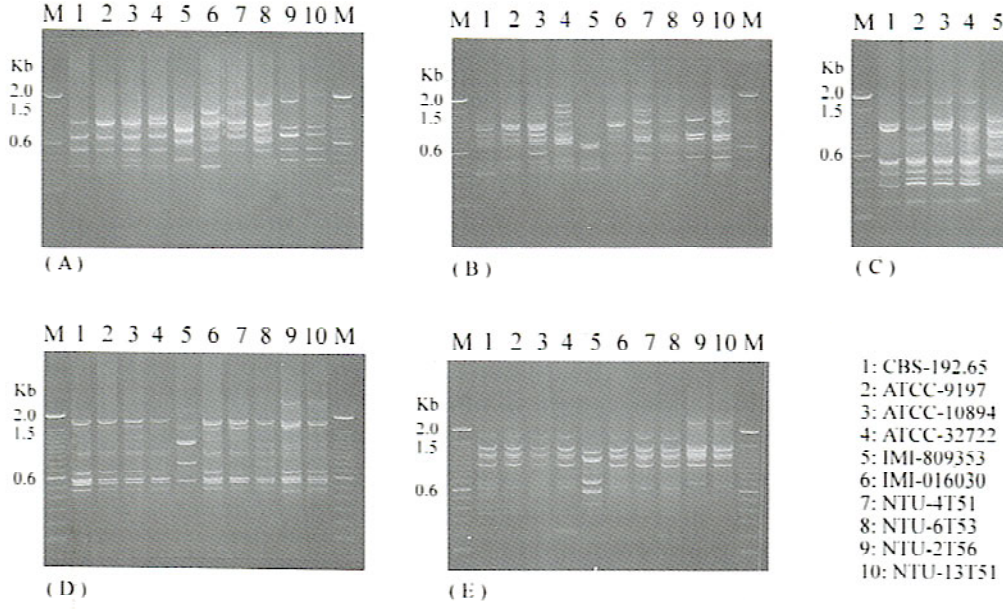

Fig. 1. RAPDs profiles for *A. fumigatus* with different primers. (A) primer-80, (B) primer-90, (C) primer-48, (D) primer-20 and (E) primer-40. On 1.4% agarose gel and staining with 0.5 mg/ml ethidium bromide.

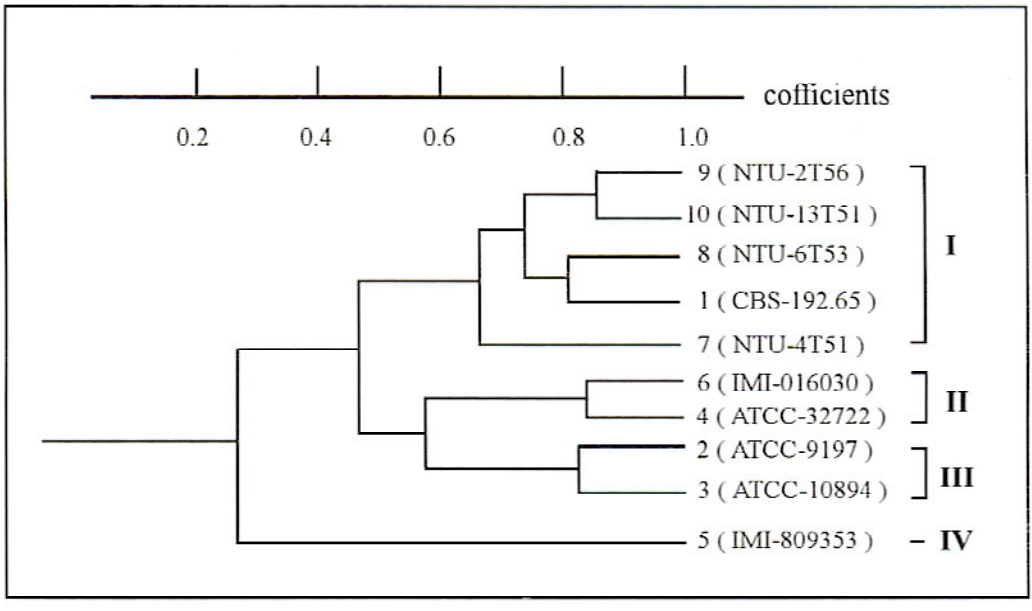

Fig. 2. Phenogram based on RAPD markers amplified from 10 strains of *Aspergillus fumigatus*. The dendrogram showing the relationship among the RAPD patterns were constructed based on these coefficients using the unweighted paired group method of analysis (UPGMA).

Comparing the RAPDs results with physiological responses, four isolates from Taiwan (NTU-4T51, NTU-6T53, NTU-2T56 and NTU-13T51) and CBS-192.65 were classified into Group I, and all strains in Group I are thermotolerant mesophile. Other strains like ATCC-9197 and ATCC-10894 were classified into groups II, the mesophile; ATCC-32722 and IMI-016030 were classified into group III, the thermophile, and IMI-809353, the transitional

mesophile strain was classified to a unique group (Group IV). All isolates classified by RAPDs analysis are useful to assess the genetic diversity among different temperature strains of *A. fumigatus*.

Many studies that involved RAPDs analysis have used the presence vs. absence of band polymorphism to calculate genetic parameters such as Nei's genetic distance to estimate genetic relatedness among individuals. In fact, the RAPDs technique would be more reliable than the similar molecular weight bands decision after combining with Southern transfer and DNA hybridization (Pillay and Kenny, 1995).

LITERATURE CITED

Birch, M., N. Nolard, G. S. Shankland and D. W. Denning. 1995. DNA typing of epidemiologically-related isolates of *Aspergillus fumigatus*. Epidemiol. Infect. **114**: 161-168.

Chen, G. Y. 1992. Taxonomical study of thermophilic and thermotolerant fungi in Taiwan. Ph. D. Dissertation, Institute of Botany, National Taiwan University.

Conney, D. G. and R. Emerson. 1964. Thermophilic Fungi. Freeman, San Francisco.

Kobayashi, M. and I. Miyosh. 1993. Immunoblot analysis of *Aspergillus fumigatus* antigen with human antibodies and lectin probes. Internal Medicine **32**: 98-105

Kwon-Chung, K. J. and J. E. Bennett. 1992. Medical mycology. Lea and Febiger, Philadelphia, PA.

Lin, D., P. F. Lehmann, B. H. Hamory, A. A. Padhye, E. Durry, R. W. Pinner and B. A. Lasker. 1995. Comparison of three typing methods for clinical and environmental isolates of *Aspergillus fumigatus*. J. Med. Microbiol. **33**: 1596-1601.

Mondon, P., J. Thelu, B. Lebeau, P. T. Ambroise and R. Grillot. 1995. Virulence of *Aspergillus fumigatus* strains investigated by random amplified polymorphic DNA analysis. J. Med. Microbiol. **42**: 299-303.

Nei, M. and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76**: 5269-5273.

Pillay, M. and S. T. Kenny. 1995. Anomalies in direct pairwise comparsions of RAPD fragments for genetic analysis. BioTechniques **19**: 694-698.

Rinyu, E., J. Varga and L. Ferenczy. 1995. Phenotypic and genotypic analysis of variability in *Aspergillus fumigatus*. J. Clin. Microbiol. **33**: 2567-2575.

Sneath, P. H. A. and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco.

Udagawa, S. I., T. Awao and S. K. Abdullah. 1986. *Thermophymatospora*, a new thermotolerant genus of Basidiomycetous hyphomycetes. Mycotaxon. **27**: 99-106.

Vierling, R. A. and H. T. Nguyen. 1992. Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. Thero. Appl. Genet. **86**: 497-504.

Zhu, H., F. Qu and L.-H. Zhu. 1993. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. Nucleic Acids Res. **21**: 5279-5280.

利用隨機複製 DNA 標誌分析台灣產煙麴黴之不同溫度品系

陳懋彥[1]、陳桂玉[2]、蔡珊珊[1]、陳瑞青[1,3]

（收稿日期：1997年7月28日；接受日期：1997年8月30日）

摘　　要

煙麴黴種內之十株溫度品系，四株分離自台灣，及六株來自各菌種中心 (CBS，ATCC 及 IMI) 所收集對溫度反應各異的菌株, 以隨機複製 DNA 標誌 (RAPDs) 做種內變異之分析。在 10 組的引子中，有5組引子顯示明顯的差異。實驗的結果將所有的煙麴黴品系區分成四群，且與其生理特性相吻合。以 RAPDs 的分析方式，可以區分出煙麴黴種內溫度變異之品系，並可以將其區分成不同的溫度群。

關鍵詞：煙麴黴，隨機複製 DNA，種內溫度變異。

1. 國立臺灣大學植物學系，臺北市106，臺灣，中華民國。
2. 中國文化大學生物學系，臺北市111，臺灣，中華民國。
3. 通信聯絡員。