Evaluation of Infrequent-Restriction-Site PCR for Epidemiological Typing of *Candida tropicalis*

Hu-Lin Han[1], Sook-Jin Jang[1,2], Geon Park[2], Jong-Hee Shin[3], Sung-Heui Shin[1], Young-Lae Moon[4], Dae-Soo Moon[2], Young-Jin Park[2]

[1]Research Center for Resistant Cells, Departments of [2]Laboratory Medicine and [4]Orthopaedic Surgery, College of Medicine, Chosun University, [3]Department of Laboratory Medicine, College of Medicine, Chonnam National University, Gwangju, Korea

Background: We evaluated the usefulness of a newly developed molecular typing method of infrequent restriction site polymerase chain reaction (IRS-PCR) as an epidemiological DNA fingerprinting tool for *Candida tropicalis*.
Methods: Thirty-two strains of *C. tropicalis* comprising eight sporadic strains and 24 clonal strains belonging to six clones, of which clonal type were previously confirmed by pulsed-field gel electrophoresis (PFGE), were tested by IRS-PCR to evaluate the usefulness of this technique. Twenty strains of *Candida* species, including *C. glabrata*, *C. krusei*, *C. albicans*, and *C. parapsilosis*, were also tested to assess the ability of IRS-PCR to discriminate among species of *Candida*.
Results: Using the IRS-PCR assay, sporadic strains of *C. tropicalis* could not be differentiated from clonal strains. Most strains belonging to the same clones were classified as different IRS-PCR types or clusters, and some different sporadic strains were classified as the same IRS-PCR types. When pattern variation was examined for different strains of *C. tropicalis* using IRS-PCR, pairwise similarity measured by the Dice coefficient was 75.4～100%. In contrast, pairwise similarity among isolates of five different species of *Candida* was 25～69.2%. Therefore, five different species of *Candida* were easily differentiated.
Conclusion: The IRS-PCR typing assay appears to be an inadequate tool for the epidemiological typing of *C. tropicalis*, because the typing result of IRS-PCR is not comparable to that of PFGE. To our knowledge, this is the first evaluation study for IRS-PCR as an epidemiological typing tool for *C. tropicalis*. **(Korean J Clin Microbiol 2007;10:96-101)**

Key Words: Infrequent-restriction-site polymerase chain reaction, *Candida tropicalis,* Technology assessment, Epidemiologic study

INTRODUCTION

Many *Candida* species have emerged as common pathogens in a variety of infections. Nosocomial candidemia has also increased rapidly, and nosocomial outbreaks of candidiasis caused by several species of *Candida* have been reported[1-3]. *Candida tropicalis* is a major cause of invasive candidal infections. It is widely recognized as a leading cause of fungemia among oncology patients and other seriously ill hospitalized individuals. The importance of *C. tropicalis* infection is further highlighted by reports of high mortality associated with hematogenous infections caused by this species[4].

Molecular epidemiological studies have indicated the possible nosocomial transmission of *Candida*[5], and continued surveillance for the presence of non-*Candida albicans* species in hospitalized patients is recommended[6]. Most molecular epidemiological assays are complicated and time-consuming and are therefore not well suited for use in a routine diagnostic setting. Rapid typing methods are essential for a prompt and sensitive evaluation of *Candida* outbreaks in hospitals so that effective measures can be instituted when common sources are identified.

Recently, the new typing method of infrequent restriction site polymerase chain reaction (IRS-PCR) was introduced as a rapid, sensitive, and widely applicable fingerprinting tool[7]. The method is easy to implement and has good reproducibility[8]. However, no results of the application of this new technology in relation to the epidemiological typing of *C. tropicalis* have been reported. Therefore, we used the IRS-PCR technique to evaluate *C. tropicalis* to assess the technique's suitability for epidemiological typing.

Received 28 August, 2007, Accepted 3 October, 2007
Correspondence: Sook-Jin Jang, Department of Laboratory Medicine, College of Medicine, Chosun University, 588, Seoseok-dong, Dong-gu, Gwangju 501-717, Korea. (Tel) 82-62-220-3259, (Fax) 82-62-232-2063, (E-mail) sjbjang@chosun.ac.kr

MATERIALS AND METHODS

1. Microorganisms

Thirty-two clinical isolates of *C. tropicalis,* of which clonal type were previously confirmed by pulsed-field gel electrophoresis (PFGE)[9] in nearby Chonnam University Hospital, were

used to evaluate IRS-PCR typing efficiency. A total of 20 strains of four species of *Candida* other than *C. tropicalis* were also examined to assess the ability of IRS-PCR for differentiation of commonly isolated *Candida* species: Five strains per each species of *Candida* consisting of one type strain (*Candida glabrata,* ATCC 90030; *Candida krusei,* ATCC 14243; *Candida albicans,* ATCC 64550; *Candida parapsilosis*, ATCC 22019) and four clinical isolates were tested.

The identification of *Candida* species was performed based on morphology and biochemical reaction patterns. The colonial morphology of *Candida* was observed after 48 h of incubation on cornmeal agar and CHROMagar Candida (Hardy Diagnostics, Santa Maria, CA, USA). The API 20C (bioMérieux Inc., Durham, NC, USA) and ATB 32C (bioMérieux) system kits were used for biochemical tests, and the results were interpreted according to the manufacturers' instructions.

2. Preparation of template DNA

DNA was extracted using the proteinase K method. Briefly, 1.5 mL of *Candida* cells was cultured overnight in LB broth and then centrifuged. Lysis buffer (300 μL of 1.0× TEN buffer [1 M Tris, pH 8.0, 5 M NaCl, 0.5 M EDTA], 20 μL of 10% SDS, 20 μL of 20 mg/mL proteinase K) was added to the pellet. The sample was digested at 54°C overnight.

We modified the Mazurek[7] method to prepare the extracted DNA for IRS-PCR. Briefly, 400 ng of genomic DNA was digested with 10 U of *Hha*I (New England Biolabs Inc., Ipswich, MA, USA) and 10 U of *Xba*I (New England Biolabs Inc.) in the appropriate digestion buffer (total volume of 12.5 μL) for 2 h at 37°C. A 7.5 μL aliquot of ligase solution (40 U of T4 DNA ligase containing 12.6 pmol ATP, 20 pmol adaptor AX [AX1-AX2] and 20 pmol adaptor AH [AH1-AH2]) was added to the mixture. The solution was incubated at 16°C for 1.5 h for ligation and at 37°C for 20 min to inactivate the T4 DNA ligase. The sample was re-digested with 5 U of *Xba*I and 5 U of *Hha*I at 37°C for 15 min. The resultant solution was stored at −20°C until use.

3. Primers and adaptors

Table 1 shows the complete sequences of each adaptor and corresponding primers used[7]. To prepare the adaptors, equimolar amounts of individual oligonucleotide adaptor pairs were combined in 1× PCR buffer (10 mM Tris-Cl, pH 8.3, 50 mM KCl, 0.1% Triton X-100) with 1.5 mM $MgCl_2$, and the mixture was allowed to anneal as it cooled from 80 to 4°C over 1 h in a thermal cycler. The stock adaptor was stored at a concentration of 14.4 μM at −20℃.

Table 1. Oligonucleotides used and their sequences

Oligonucleotide	Sequence
AH1	5'-AGA ACT GAC CTC GAC TCG CAC G-3'*
AH2	5'-TGC GAG T-3'
AX1	5'-PO_4-CTA GTA CTG GCA GAC TCT-3'
AX2	5'-GCC AGT A-3'
PX-G	5'-AGA GTC TC CAG TAC TAG AG-3'

*This sequence was used to construct the AH adaptor and as a primer in IRS-PCR.

4. IRS-PCR amplification

The PCR mixture contained 1× $MgCl_2$-free PCR buffer (Tris-HCl, pH 8.0, 50 mM KCl, 0.1% Triton X-100), 2.5 mM $MgCl_2$, 200 μM each of deoxynuleoside triphosphates, 1.0 M oligonucleotide primers AH1 and PX-G, 4 μL of template DNA (one- fifth of the ligation-redigestion product), and 0.1 U of *Taq* DNA polymerase (Promega Corp., Madison, WI, USA). Distilled water was added for a total volume of 50 μL. Amplification was performed in a GeneAmp PCR System 2400 (Applied Biosystems, Foster, CA, USA) using: initial denaturation at 94℃

Table 2. Results of the IRS-PCR typing assay of 32 clinical isolates of *Candida tropicalis* arranged by PFGE type

Character	PFGE type*	N	IRS-PCR type	N
Clonal	α7	2	A10	1
			A12	1
	α8	9	A2	1
			A4	1
			A6	2
			A8	1
			A11	1
			A14	1
			B1	1
			D2	1
	α10	3	A1	1
			A12	1
			C3	1
	α11	2	A8	1
			C1	1
	α12	3	A5	1
			A12	1
			A13	1
	β4	5	A1	2
			A4	1
			A8	1
			A9	1
Sporadic	α3	1	A3	1
	α14	1	A8	1
	α16	1	A1	1
	α17	1	A12	1
	β5	1	B2	1
	β6	1	A1	1
	γ	1	D1	1
	ε	1	A12	1

*Determined previously by Rho et al.[9].

for 5 min; 30 cycles of denaturation at 94°C for 5 min, primer annealing at 60°C for 30 s, and extension at 72°C for 90 s; and a final extension at 72°C for 7 min.

5. Pattern visualization and data analysis

Aliquots of 20 μL of PCR product were loaded into the wells (1.0 cm×5 mm) of 6.5% polyacrylamide gel prepared with 30% acrylamide-bisacrylamide (29 : 1) solution in 0.5× TBE buffer (22.5 mM Tris-borate, 0.5 mM EDTA). After electrophoresis for 1.75 h at 100 V, the gel was stained with ethidium bromide (0.5 mg/mL) for 8 min, destained with water for 25 min, and photographed under UV illumination.

Pattern clustering on a matrix of the Dice coefficient[10,11] was based on the unweighted pair group method using arithmetic averages (UPGAMA). Dendrograms were constructed using diversity database software 2.2.0 (Bio-Rad Labs, Hercules, CA, USA).

6. Assessment of reproducibility

To determine the reproducibility of the IRS-PCR method for the epidemiological study of *C. tropicalis*, we undertook run-to-run correlation of the IRS-PCR for the index strain selected from the epidemiologically related strains. Five sample aliquots obtained from the index strain were studied in four independent experiments to assess intra- and interexperimental variability.

7. Assessment of performance of IRS-PCR typing based on the PFGE type previously determined

The IRS-PCR types were compared with the macrorestriction types that were previously obtained using PFGE[9] to assess the adequacy of the performance of IRS-PCR. These isolates comprised of eight sporadic strains and 24 strains of six clones. On the PFGE analysis, the clonal strains consisted of two clusters, α and β: cluster α contained five α clones and cluster β contained one β clone in the PFGE typing (Table 2).

RESULT

1. Typeability of strains

All 52 *Candida* isolates were typed using IRS-PCR, giving a typeability of 100%.

2. Reproducibility of IRS-PCR assay

The analysis of gels from the reproducibility tests indicated that fragments between 150 and 800 bp were most reproducible. Fragments above and below this range were ignored in further analyses. An examination of the pattern reproducibility showed that the run-to-run correlation of IRS-PCR for the index strain of *C. tropicalis* varied from 92.9～100%, based on band matching using the Dice coefficient. Five sample aliquots of the index strain tested in the same day to assess intraexperimental variability showed the same pattern (Fig. 1).

3. Performance of IRS-PCR assay

Fig. 2 shows result of several IRS-PCR type from eight strains of *C. tropicalis*. Computerized gel analysis of IRS-PCR profiles divided the 32 *C. tropicalis* strains into four clusters (A through D) using a similarity coefficients (S_{AB}) threshold of 0.90 (Fig. 3).

The IRS-PCR assay could not differentiate among the eight epidemiologically unrelated sporadic strains of *C. tropicalis* from the 24 clonal strains belonging to the six clones. Fifteen of 19 strains

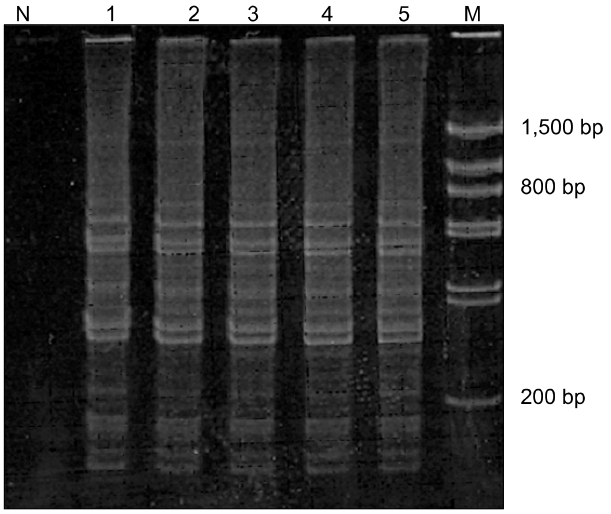

Fig. 1. The result of intraexperimental variability test. Five sample aliquots of the same index strain showed the same pattern each other. N, negative control of IRS-PCR Lane 1 to 5, the index strain M, 100-bp～1.5-kb DNA ladder (M102R-1, BIO BASIC INC., Markham, ON, Canada).

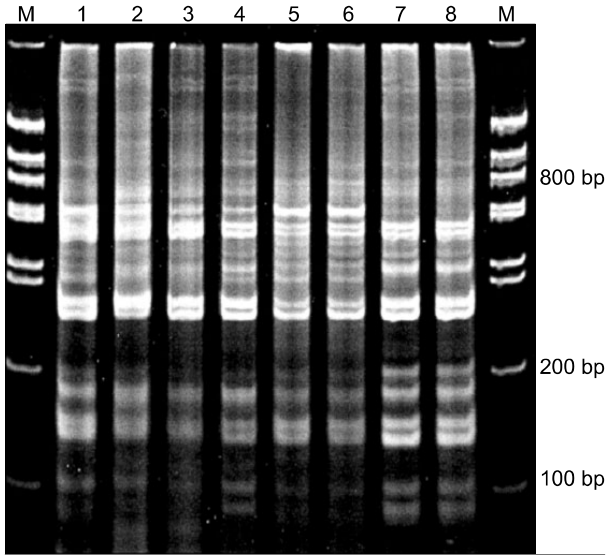

Fig. 2. IRS-PCR typing assay patterns of *Candida tropicalis*. Lanes 1-8 (IRS-PCR type and specimen number [in parentheses]: lane 1, A1 (T40); lane 2, A12 (T18); lane 3, B1 (T19); lane 4, B2 (T33); lane 5, C1 (T29); lane 6, C2 (T50); lane 7, D1 (T31); lane 8, D2 (T16). Lane M, 100-bp～1.5-kb DNA ladder (M102R-1, BIO BASIC INC.).

in cluster α belonged to cluster A by IRS-PCR typing. However, all five strains of cluster β also belonged to cluster A in the IRS-PCR typing. In other words, the two different clusters of clonal strains were not differentiated by the IRS-PCR assay. Generally, each member of the six clones belonged to the same clusters as other members of the respective clone, except for four strains. Two of nine strains of α8, one of three strains of α10, and one of two strains of α12 showed IRS-PCR types that belonged to different clusters from those of other members of each clone. Most strains of the same clonal groups showed diverse IRS-PCR types. Only two (A6) of nine strains of the α8 clone and two (A1) of five strains of the β4 clone showed the same IRS-PCR type. Different sporadic strains were falsely grouped into the same group, e.g., strains α16 and β6 into A1 and strains α17 and ε into A12, in the IRS-PCR typing (Table 1).

Because strains of the same clones showed different types or clusters and different sporadic strains showed the same types, the IRS-PCR typing assay result was not concordant with the results of PFGE. The calculated discriminatory ability of IRS-PCR and PFGE using Simpson's index of diversity[12] were 0.94 and 0.89, respectively.

When the pattern variation was examined for different strains of *C. tropicalis* using IRS-PCR, the pairwise similarity, as measured by the Dice coefficient, was 75.4～100%, which explained the low discriminatory power of IRS-PCR. In contrast, the pairwise similarity among isolates of five different species of *Candida* was 25～69.2%. The five different species of *Candida* were easily differentiated (Fig. 4).

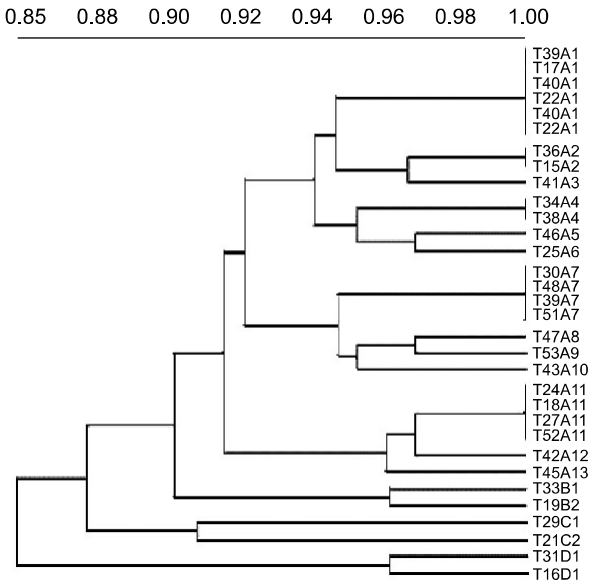

Fig. 3. Dendrogram of the IRS-PCR typing assay of 32 clinical isolates of *Candida tropicalis*. The specimen numbers and corresponding IRS-PCR types are listed at the right. Dendrograms were constructed using diversity database software 2.2.0 (Bio-Rad Labs, Hercules, CA, USA).

DISCUSSION

The results from the IRS-PCR typing assay for *C. tropicalis* were not concordant with those obtained from PFGE. Because the IRS-PCR assay could not differentiate epidemiologically unrelated sporadic strains of *C. tropicalis* from clonal strains, it seems to be inappropriate for use as an epidemiological typing tool for *C. tropicalis*.

The discriminatory power of IRS-PCR appears to vary according to the bacterial species examined. In studying the genetic relationships among *Klebsiella pneumoniae, Acinetobacter baumannii, Serratia marcescens,* and *Legionella pneumophila,* IRS-PCR gave concordant results with PFGE analysis[9,13,14]. The outbreak strains of multi-resistant *A. baumannii* were also clearly differentiated from the epidemiologically unrelated endemic strains by

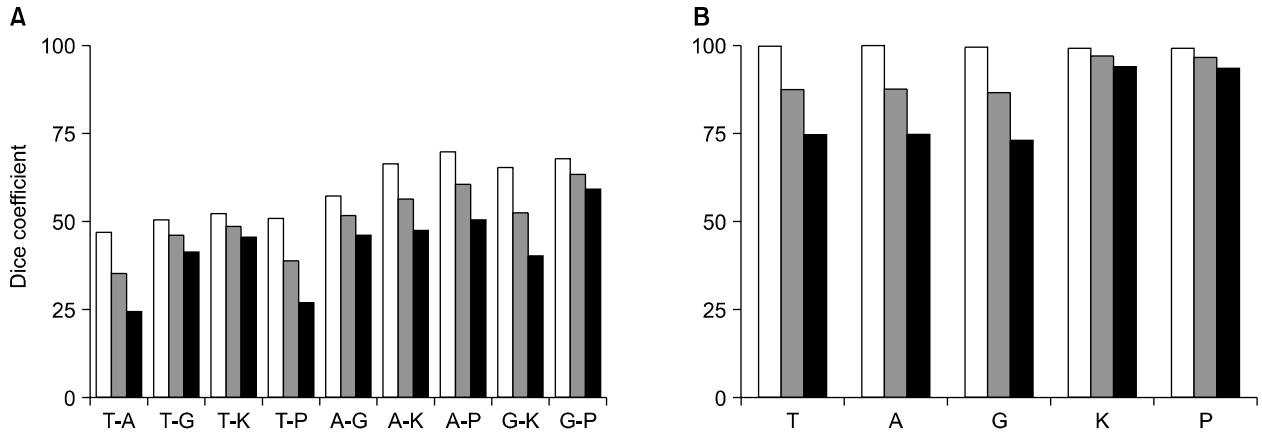

Fig. 4. Histograms showing pairwise similarity as measured by the Dice coefficientfor various (A) species and (B) strains of *Candida*. T, *C. tropicalis;* A, *C. albicans;* G, *C. glabrata;* K, *C. krusei;* P, *C. parapsilosis.* T-A represents pairwise similarity between isolates of *C. tropicalis* and *C. albicans*, etc. Black bar, maximum Dice coefficient; light-gray bar, mean Dice coefficient; dark-gray bar, minimum Dice coefficient.

IRS-PCR[15]. In the molecular typing of *Mycobacterium abscessus*, IRS-PCR gave better results than PFGE. Therefore, IRS-PCR may be an efficient substitute for PFGE in analyzing the DNA polymorphism and epidemiology of *M. abscessus*[9]. IRS- PCR was more capable of distinguishing strains from invasive listeriosis than was PCR ribotyping or AP-PCR[16]. Only IRS-PCR succeeded in clearly discriminating the strains related to noninvasive listeriosis from all other strains that were examined[16]. The reproducibility of the IRS-PCR technique in the analysis of *Salmonella enterica* was 100%, but the discrimination was low (D=0.52)[17].

The overall performance of the IRS-PCR typing assay was inadequate and unsatisfactory in grouping *C. tropicalis* strains. However, five *Candida* species were easily differentiated using IRS-PCR. Thus, IRS-PCR may be more useful for the differentiation of *Candida* species, rather than the epidemiological study of *C. tropicalis*. Similarly, arbitrarily primed PCR and *Eag*I-*Hha*I IRS-PCR fingerprinting techniques separated *Bartonella* species effectively, but lacked discriminating power within *Bartonella henselae*[18]. Reference strains of *Mycobacterium tuberculosis* H37Rv, *M. bovis, M. africanum,* and all isolates of *M. tuberculosis* showed similar IRS-PCR patterns[19]. In contrast, electrophoretic DNA patterns obtained from *M. avium, M. intracellulare,* and *M. fortuitum* showed great differences from each other and from those of the reference strains[19]. The authors concluded that IRS-PCR is a useful tool for strain typing of non-tuberculous mycobacteria, but not for *M. tuberculosis*[19]. The reasons for the poor discrimination power for strains of *Bartonella henselae, M. tuberculosis*, and *C. tropicalis* are not clear. However, the similarity in the number and distribution of restriction enzyme recognition sites in the nucleotide sequence among strains of these bacteria may be one possible explanation. If the enzyme combination is changed appropriately, the discrimination power may increase. Handley et al.[8] used three types of restriction enzyme combinations for the identification of human pathogenic *Bartonella* species. These combinations included the frequently cutting endonuclease *Hha*I in conjunction with an infrequently cutting endonuclease *Eag*I, *Sma*I, or *Xba*I. The amplification of fragments flanked by an *Eag*I, *Sma*I, or *Xba*I site in combination with an *Hha*I site produced a series of different-sized amplicons that could be resolved into patterns by polyacrylamide gel electrophoresis (PAGE)[8]. The pattern complexity could be varied by the addition of selective nucleotides to the 3' ends of the *Eag*I-, *Sma*I-, or *Xba*I-specific primers. Because of the flexibility in modulating the pattern complexity and the electrophoretic methods used, these techniques allow for a high level of experimental optimization. If the performance of IRS-PCR for *C. tropicalis* needs to be re-evaluated, it may be helpful to use an enzyme combination other than *Xba*I and *Hha*I.

Some features could be improved to optimize the IRS-PCR procedure. Mazurek[7] reported that even DNA extracted using rapid methods, after preparation of the digestion and ligation step, could be successfully usedin IRS-PCR. However, when we extracted DNA from *C. tropicalis* using a rapid method, only a few bands were visible, and we could not differentiate among strains of *C. tropicalis*. After we used the proteinase K method to extract DNA, an adequate number of bands for analysis were obtained. It is also helpful to adjust the amount of genomic DNA used in IRS-PCR. We adjusted the amount of genomic DNA to 400 ng and used the same amount of DNA for subsequent IRS-PCR. We also included the index strain of *C. tropicalis* in all batches of IRS-PCR and all gel loading procedures, making the comparison of the bands in each lane easier. Although we optimized the procedure for *Candida* using these modifications, the results were somewhat disappointing. The assay reproducibility was relatively high, but the grouping results of IRS-PCR were not comparable with those of PFGE.

When the performance of IRS-PCR was compared with electrophoretic karyotyping with PFGE for typing of *C. albicans* and *C. parapsilosis*, genotyping results determined by both methods were generally concordant although some strains showed discordant results[20]. For typing of *C. tropicalis* in this study, the performance of IRS-PCR was not adequate, which was performed by Mazarek's protocol using *Hha*I and *Xba*I. However, IRS-PCR is a rapid and simple method for the discrimination of five species of *Candida*, Thus, IRS-PCR seems to be more useful for the differentiation of *Candida* species, rather than the epidemiological study of *C. tropicalis*. To our knowledge, this is the first evaluation study for IRS-PCR as an epidemiological typing tool for *C. tropicalis*.

Acknewkdgement

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea govemment (MOST)(R13-2003-009).

REFERENCES

1. Costa SF, Marinho I, Araujo EA, Manrique AE, Medeiros EA, Levin AS. Nosocomial fungaemia: a 2-year prospective study. J Hosp Infect 2000;45:69-72.
2. Bukharie HA. Nosocomial candidemia in a tertiary care hospital in Saudi Arabia. Mycopathologia 2002;153:195-8.
3. Chen YC, Chang SC, Luh KT, Hsieh WC. Stable susceptibility of *Candida* blood isolates to fluconazole despite increasing use during the past 10 years. J Antimicrob Chemother 2003;52:71-7.
4. Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin Infect Dis 1997;24: 1122-8.
5. Zhang J, Hollis RJ, Pfaller MA. Variations in DNA subtype and antifungal susceptibility among clinical isolates of *Candida tropicalis*. Diagn Microbiol Infect Dis 1997;27:63-7.
6. Joly S, Pujol C, Schroppel K, Soll DR. Development of two species-specific fingerprinting probes for broad computer-assisted epidemiological studies of *Candida tropicalis*. J Clin Microbiol 1996;34:3063-71.
7. Mazurek GH, Reddy V, Marston BJ, Haas WH, Crawford JT. DNA fingerprinting by infrequent-restriction-site amplification. J Clin Microbiol 1996;34:2386-90.
8. Handley SA and Regnery RL. Differentiation of pathogenic *Bartonella* species by infrequent restriction site PCR. J Clin Microbiol

2000;38:3010-5.
9. Rho J, Kim CS, Jang SJ, Shin JH, Suh SP, Ryang DW. Molecular Typing of Urinary Isolates of *Candida tropicalis* by pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) Analysis. Korean J Infect Dis 2001;33:392-403.
10. Steindel M, Dias Neto E, Pinto CJ, Grisard EC, Menezes CL, Murta SM, et al. Randomly amplified polymorphic DNA (RAPD) and isoenzyme analysis of *Trypanosoma rangeli* strains. J Eukaryot Microbiol 1994;41:261-7.
11. Thong KL, Cheong YM, Puthucheary S, Koh CL, Pang T. Epidemiologic analysis of sporadic *Salmonella typhi* isolates and those from outbreaks by pulsed-field gel electrophoresis. J Clin Microbiol 1994;32:1135-41.
12. Dillon JA, Rahman M, Yeung KH. Discriminatory power of typing schemes based on Simpson's index of diversity for *Neisseria gonorrhoeae*. J Clin Microbiol 1993;31:2831-3.
13. Riffard S, Lo Presti F, Vandenesch F, Forey F, Reyrolle M, Etienne J. Comparative analysis of infrequent-restriction-site PCR and pulsed-field gel electrophoresis for epidemiological typing of *Legionella pneumophila* serogroup 1 strains. J Clin Microbiol 1998;36:161-7.
14. Yoo JH, Choi JH, Shin WS, Huh DH, Cho YK, Kim KM, et al. Application of infrequent-restriction-site PCR to clinical isolates of *Acinetobacter baumannii* and *Serratia marcescens*. J Clin Microbiol 1999;37:3108-12.
15. Wu TL, Su LH, Leu HS, Chiu CH, Chiu YP, Chia JH, et al. Molecular epidemiology of nosocomial infection associated with multi-resistant *Acinetobacter baumannii* by infrequent-restriction-site PCR. J Hosp Infect 2002;51:27-32.
16. Franciosa G, Tartaro S, Wedell-Neergaard C, Aureli P. Characterization of *Listeria monocytogenes* strains involved in invasive and noninvasive listeriosis outbreaks by PCR-based fingerprinting techniques. Appl Environ Microbiol 2001;67:1793-9.
17. Garaizar J, Lopez-Molina N, Laconcha I, Lau Baggesen D, Rementeria A, Vivanco A, et al. Suitability of PCR fingerprinting, infrequent-restriction-site PCR, and pulsed-field gel electrophoresis, combined with computerized gel analysis, in library typing of *Salmonella enterica* serovar enteritidis. Appl Environ Microbiol 2000;66:5273-81.
18. Dillon B, Valenzuela J, Don R, Blanckenberg D, Wigney DI, Malik R, et al. Limited diversity among human isolates of *Bartonella henselae*. J Clin Microbiol 2002;40:4691-9.
19. Choi TY and Kang JO. Application of infrequent-restriction-site amplification for genotyping of *mycobacterium tuberculosis* and non-tuberculous *Mycobacterium*. J Korean Med Sci 2002;17:593-8.
20. Huang YC, Su LH, Wu TL, Lin TY. Genotyping analysis of colonizing candidal isolates from very-low-birthweight infants in a neonatal intensive care unit. J Hosp Infect. 2004 Nov;58(3):200-3.

=국문초록=

*Candida tropicalis*의 역학적 형별 검사를 위한 Infrequent-Restriction-Site PCR법의 평가

[1]조선대학교 의과대학 내성세포연구센터, [2]진단검사의학교실, [4]정형외과학교실, [3]전남대학교 의과대학 진단검사의학교실

한호림[1], 장숙진[1,2], 박 건[2], 신종희[3], 신성희[1], 문영래[4], 문대수[2], 박영진[2]

배경: *Candida tropicalis*의 역학적 형별검사로 분자생물학적 형별검사인 infrequent-restriction-site PCR (IRS-PCR)법의 유용성을 평가하였다.

방법: IRS-PCR법의 유용성을 평가하기 위해 pulsed-field gel electrophoresis (PFGE)를 통해 형별이 판별된 산발적으로 분리된 8균주와 6가지 클론에 속해 있는 클론성 24균주로 구성된 총 32주의 *C. tropicalis* 균주를 IRS-PCR법으로 분석하였다. *Candida* 균종에 대한 IRS-PCR 방법의 종간 분별력을 평가하기 위해 *Candida glabrata*와 *Candida krusei*, *Candida albicans*, *Candida parapsilosis*의 4균종으로 구성된 20균주를 함께 검사하였다.

결과: IRS-PCR법으로는 산발적인 균주들과 클론성 균주들을 감별할 수 없었다. 동일한 클론에 속해 있는 대부분의 균주들이 서로 다른 IRS-PCR형이나 군집으로 분류되었고 일부의 서로 다른 산발적인 균주들이 동일한 IRS-PCR형으로 분류되었다. *C. tropicalis* 분리주들을 대상으로 각 균주들의 양상간 변이도를 Dice 계수로 측정한 결과 쌍간 유사도는 75.4~100%이었다. 반면 *Candida*의 5균종들을 대상으로 각 균종들의 양상간 변이도를 측정한 Dice 계수는 25~69.2%였다. 그래서 다섯 가지 *Candida* 균종을 서로 쉽게 구별할 수 있었다.

결론: IRS-PCR의 형별 결과는 PFGE에 비견할 만하지 못하여 *C. tropicalis*의 역학적 형별검사 도구로 쓰기에는 부적합한 것으로 여겨졌다. 저자들이 아는 바로는 본고가 *C. tropicalis*의 역학적 형별검사법으로서 IRS-PCR법의 유용성을 살펴본 첫 번째 평가 논문이다. **[대한임상미생물학회지 2007;10:96-101]**

교신저자 : 장숙진, 501-717, 광주광역시 동구 서석동 588
조선대학교 의과대학 진단검사의학교실
Tel: 062-220-3259, Fax: 062-232-2063
E-mail: sjbjang@chosun.ac.kr