FUNGAL SALGINLARIN ARAŞTIRILMASI;

kullanılan yöntemler, RAPD ve rDNA IS1 sekans analizi uygulamaları

Barış Otlu
İnönü Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı, Malatya.

- Yatan hasta popülasyonundaki değişikliklerle birlikte,
  hastanede kazanılan ekzojen veya endojen kaynaklı mantar enfeksiyonlarının görülme sıklığı son yıllarda giderek artmaktadır.
    - Artan cerrahi girişimler
    - Doku ve organ transplantasyonları
    - Kanser tedavilerindeki bağışıklık sistemini baskılayıcı girişimler

Fungal genotiplendirme niçin gerekli ?

- İnfeksiyonun endojen mi yoksa eksojen kaynaklı mı ? olduğunun belirlenmesi
- Salgınların kaynağının belirlenmesi
- Yayılma yollarının belirlenmesi ve korunma
- Rölaps mı yoksa reinfeksiyon mu ? olduğunun belirlenmesi
- Hastane ortamındaki mikroorganizma klonlarının ortaya konması

Fungal genotiplendirme

- Mantarların genotiplendirmesinde kullanılacak tek bir "altın standart" yöntem yok.
- Çalışılacak mikroorganizmaya göre yöntem seçilmeli. Kullanılan yöntemlerin avantajları ve dezavantajları söz konusudur.
- Seçilecek yönteminin başarısı tekrarlanabilirliğine ve ayrım gücünün yüksek olmasına bağlıdır.

Fungal genotiplendirme

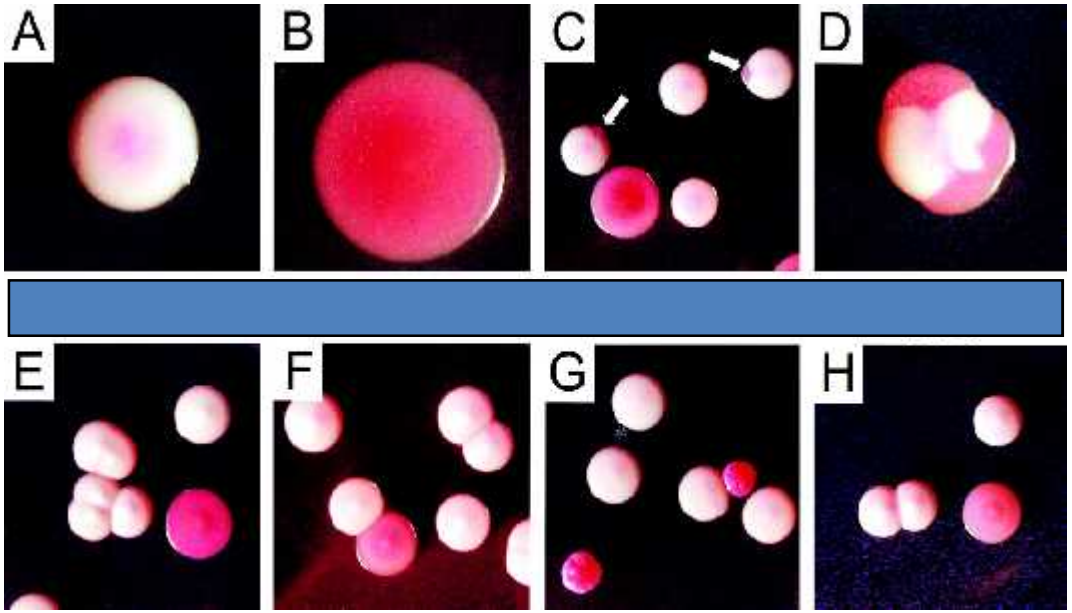
- İlk tiplendirme çalışmalarında fenotipik yöntemler kullanılmıştır.
  - Serotiplendirme
  - Morfotiplendirme
  - Rezistotiplendirme
  - Biyotiplendirme
  - Öldürücü maya tiplendirmesi
  - Hücresel proteinlerin analizi

Fungal genotiplendirme

- Fenotipik yöntemlerin ayrım güçleri çoğunlukla yetersizdir.
- Fenotipik değişim söz konusu;

  C. albicans, C. tropicalis, C. glabrata, Cryptococcus neoformans

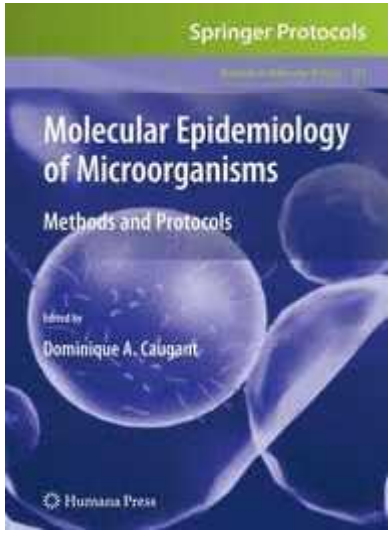
  Bu da hatalı değerlendirmelere neden olabilir.



Örneğin; *C. albicans* WO-1 kökeninde beyaz-opak koloni dönüşümü 10^{-3} kadar sıklıkla geri dönüşümlüdür.

Fungal genotiplendirme

- Moleküler genotiplendirme yöntemlerinin uygulama alanına sokulması ile fenotipik yöntemlerin yetersiz ayrım gücünden kaynaklanan dezavantajları önemli ölçüde ortadan kaldırılmıştır.

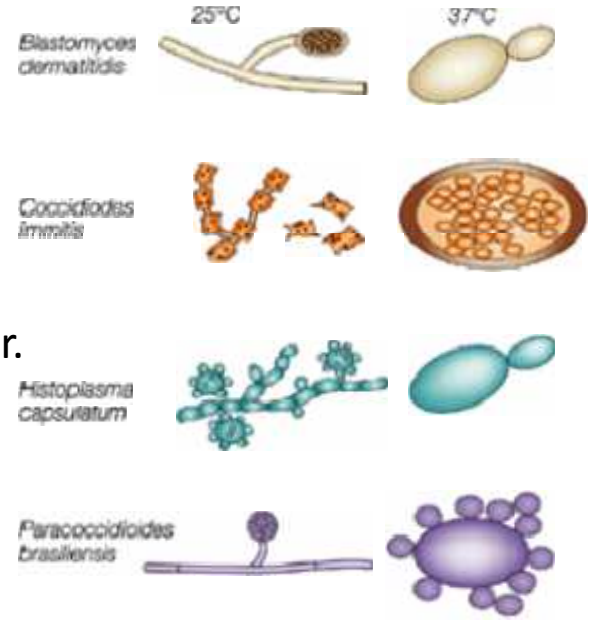


Fungal genotiplendirme

- Genotiplendirilmeye başlarken hatırlanması gerekenler;
  - Mantarların ökaryotik olması genotiplendirilmelerinde çeşitli sorunlar oluşturmaktadır.

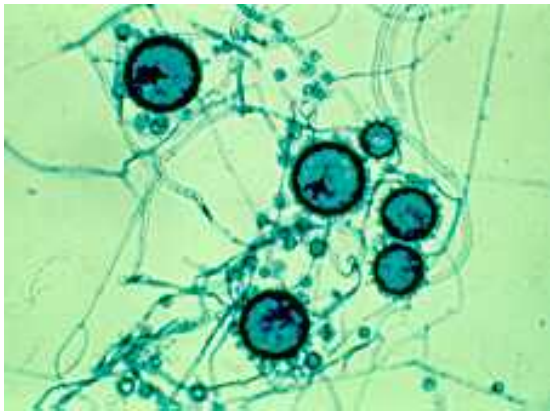
Bu sorunların başında;

- hem seksüel hem de aseksüel üreyebilmeleri
- ökaryotlarda görülen doğal rekombinasyon
- bazı fungal patojenlerin diploid yapı göstermeleri
- bazılarının dimorfik yapıda olabilmeleri gelmektedir.



Fungal genotiplendirme

- Genotiplendirilmeye başlarken hatırlanması gerekenler;
  - Çoğu mantar türleri seksüel üreme gösterirler ve haploid (N) yapıdadırlar.
  - Örneğin;
    - *Histoplasma capsulatum*'un eşeyli formu olan *Ajellomyces capsulatum* haploid (N) yapıdadır.
    - Seksüel üreyen mantar türleri kendi kendileri ile eşleşebilecekleri gibi bir eşeye de ihtiyaç duyabilir yada her iki şekilde de üreme gösterebilirler



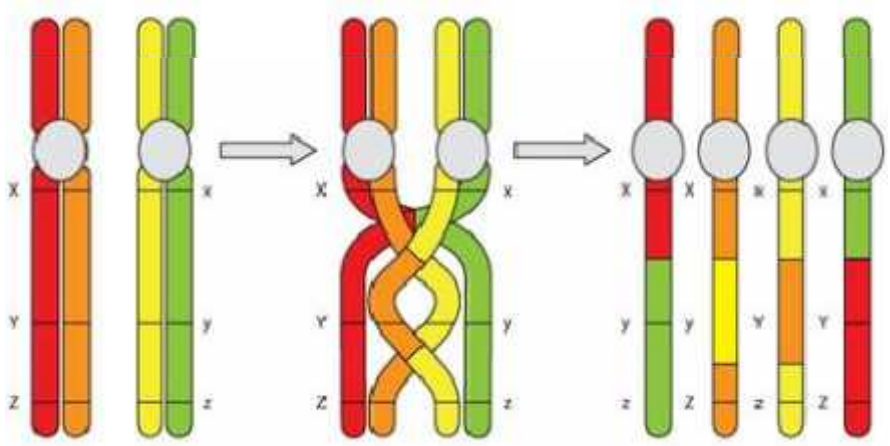
Fungal genotiplendirme

- Genotiplendirilmeye başlarken hatırlanması gerekenler;
  - Bununla birlikte mantarların yaklaşık %25'i aseksüel üreme gösterirler.
  - Tıbbi önemleri gittikçe artan;

    C. albicans, Aspergillus fumigatus, Trichopyton rubrum aseksüel üreme gösterirler.
  - Bazidomiçet sınıfından olan *C. albicans* diploid (2n) yapıdayken bu sınıftaki çoğu tür dikaryotik yapıdadır (N+N).
  - Yine bazidomiçetlerden *Crytococcus neoformans*, *Malessezia spp*., Trichosporon spp. gibi diğer türler konakta dikaryotik yapı gösterebilirler.

Fungal genotiplendirme

- Genotiplendirilmeye başlarken hatırlanması gerekenler;
  - Mantarlarda genetik çeşitlilik;
    - mayoz sırasında “reassorment” veya “crossing over”
    - mayoz dışı rekombinasyon veya paraseksüalite
    - aseksüel olarak sporlar ile üreme



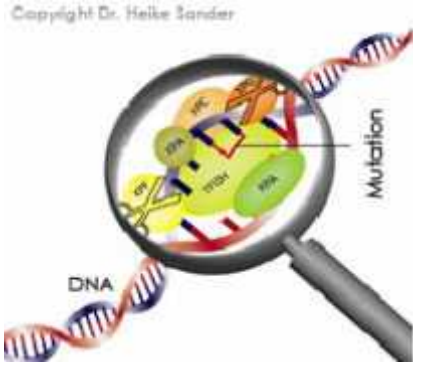
Fungal genotiplendirme

- Genotiplendirilmeye başlarken hatırlanması gerekenler;
  - Aseksüel üreme gösteren mantarlar genotiplendirilirken,
    - çoğalma klonal olacaktır
    - bu yüzden değişkenliği fazla olan tek bir lokusun kullanılması yeterli epidemiyolojik bilgiyi sağlayabilir.
  - Seksüel üreme gösterenlerde;
    - genomun her bölgesi farklı evrimsel değişimi yansıtabilir
    - bu durumda tek bir değişken lokus genetik bireylerdeki farklılığı göstermez, çok sayıda lokusun birlikte değerlendirilmesi gereklidir.

Fungal genotiplendirme

- Genotiplendirilmeye başlarken hatırlanması gerekenler;
  - Bu sebeplerle mantarlarla ilgili moleküler epidemiyolojik çalışmalara başlamadan önce incelenecek olan organizmanın, üreme ve genomik özelliklerinin bilinmesi gereklidir.

Fungal genotiplendirme



- Mantarların moleküler tiplendirilmelerinde seçilecek genom bölgesi
  - Seçilecek genom bölgesi orta derecede mutasyona açık olmalıdır
  - Genom üzerinde seçilen bölgenin evrimsel baskı altında olması, yanlış yorumlara yol açabilir.
  - Seçilen bölgede çeşitliliği oluşturan mutasyonların geri dönüşümlü olmaması gerekir.

Fungal genotiplendirme

- Kullanılan genotiplendirme yöntemleri
  - Pulsed field gel electrophoresis (PFGE)
    - Electrophoretic karyotyping (EK)
  - Arbitrarily Primed PCR (AP-PCR)
  - Amplified fragment lenght polymorphism (AFLP)
  - Multilocus sequence typing (MLST)
  - Restriction fragment lenght polymorphisim (RFLP)
  - Repetitive-sequence-based PCR (rep-PCR)
  - Variable number of short tandem repeat (VNTR)

Fungal genotiplendirme

- Hangi yöntem ?
  - çalışılacak mikroorganizmanın türü
  - yöntemin ayrım gücü
  - tekrarlanabilirliliği
  - kullanım kolaylığı
  - kurulum maliyeti
  - testin süresi
  - yorumlama kolaylığı

Fungal genotiplendirme

- *Electrophoretic karyotyping*
  - *"Pulsed Field Gel Electrophoresis"*
  - Fungal epidemiyoloji için ideal olarak kabul edilmiştir.

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3756–3760, June 1985
Genetics

An electrophoretic karyotype for yeast

(DNA/chromosomes/orthogonal-field-alternation gel electrophoresis)

GEORGES F. CARLE AND MAYNARD V. OLSON

Department of Genetics, Box 8031, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Herschel L. Roman, January 28, 1985

ABSTRACT The chromosomal DNA molecules of a standard laboratory strain of *Saccharomyces cerevisiae* have been separated into 12 well-resolved bands by orthogonal-field-alternation gel electrophoresis. DNA·DNA hybridization probes derived from cloned genes have been used to correlate this banding pattern with yeast's genetically defined chromosomes. The 12 bands are shown to represent 9 singlets and 3 comigrating doublets, thereby accounting for 15 chromosomes that were identified as I–XI and XIII–XVI. Because the three comigrating doublets could be readily resolved in certain laboratory yeast strains that contain chromosome-length polymorphisms relative to our standard strain, all 15 of these chromosomes could be displayed as a single band in at least one of four strains that were studied. A 16th chromosome (number XII), which is known to contain the genes for rRNA, does not reproducibly enter the gels. By making use of the band identifications, the previously unmapped fragment F8 was assigned to chromosome XIII. With the possible exception of chromosomes that differ greatly in size or electrophoretic behavior from all the known chromosomes, the results appear

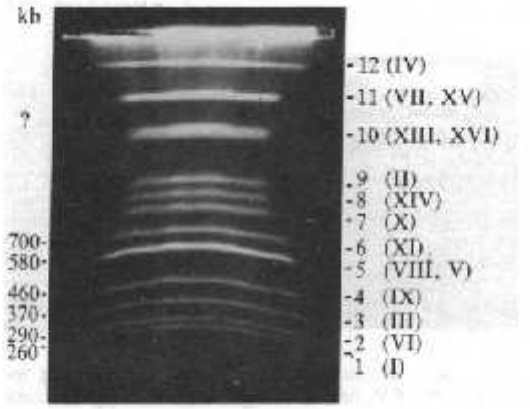


FIG. 1. Ethidium bromide stained agarose gel on which the chromosomal DNA molecules of yeast (strain AB972) have been resolved. The size estimates on the left and the band-numbering system on the right are from ref. 4, while the chromosome assignments summarize the conclusions of the present study.

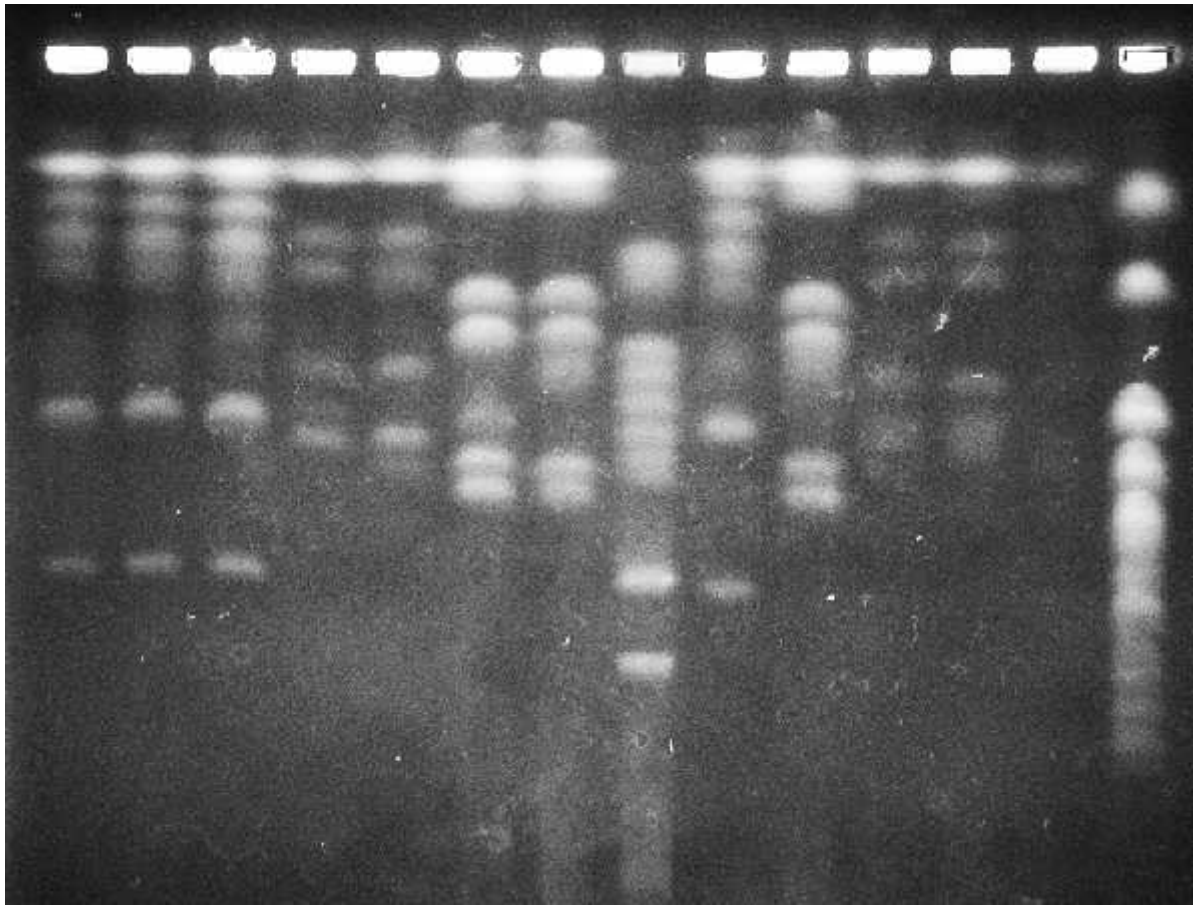
Fungal genotiplendirme

- *Electrophoretic karyotyping*

Organism	Genome size (Mb)	Chromosome size range (Mb)	No. of chromosomes (haploid)	Confirmation	CLPs reported[b]
Acremonium chrysogenum	33	2.6–6.4	8		No
Agaricus bisporus	34	1.2–4	13		No
Aspergillus nidulans	31	0.4–4	10–15	Genetic map, cytology	Yes
Aspergillus niger	35.5–38.5	3.5–6.6	8	Genetic map	No
Aspergillus oryzae	35	2.8–7	8		No
Beauveria nivea	26–33	0.9–6.6	8–10[c]		Yes
Candida utilis	Not estimated	0.4 >3.5	≥8[c]		Yes
Candida albicans	16 17	0.66 4.3	8 9	Physical map	Yes
Cercospora kikuchii	28.4	2.0 5.5	8		No
Cladosporium fulvum	44	1.9–5.4	11		Yes
Cochliobolus heterostrophus	35	1.3–3.7	15 or 16	RFLP map	Yes
Colletotrichum gloeosporioides					
Type A	Not estimated	0.270–6	13–15		Yes
Type B	Not estimated	0.330–>6	6–8		Yes
Coprinus cinereus	37.5	1–5	13	Microscopy	Yes
Cryptococcus neoformans var. *neoformans*	21–24.5	0.770–3.9	12–13		Yes
Fusarium oxysporum	41–51.5	0.8–6.7	11–14		Yes
Fusarium oxysporum f. sp. *cubense*	32.1–58.9	0.6–7.5	9–14		Yes
Fusarium solani	40	0.4–6	13		No
Histoplasma capsulatum	Not estimated	0.5–>5.7	≥7[c]		Yes
Leptosphaeria maculans	17.5–23	0.7–3.7	6–14		Yes
Magnaporthe grisea	40	3–10 and 0.47–2.2	7 + 1–4 minichromosomes	RFLP map	Yes
Mucor circinelloides	39	2.3–8.1	≥8		Yes
Nectria haematococca	8.5	<0.6–7	10–15		Yes
Neurospora crassa	47	4–12.6	7	Microscopy, genetic maps	Yes
Phanerochaete chrysosporium	15	1.8 5	7		Yes
Pneumocystis carinii	7 8	0.3 0.7	14 16		Yes
Pythium sylvaticum	37 38	1.9 5.1	≥13		Yes
Pythium ultimum	23.7 37.6	1.28 4.6	9 14		Yes
Saccharomyces cerevisiae	13.5 14.5[d]	0.24 3	16	Physical map, microscopy, genetic map	Yes

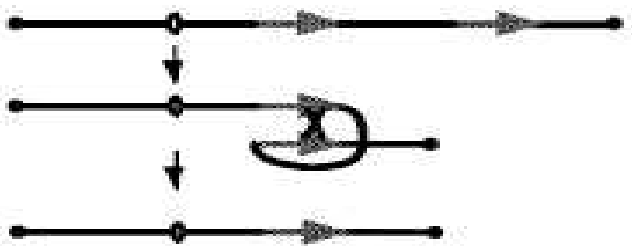
Fungal genotiplendirme

- *Electrophoretic karyotyping*
  - Kromozamal yeniden düzenlenmeler



Fungal genotiplendirme

- *Electrophoretic karyotyping*
  - Kromozamal yeniden düzenlenmeler



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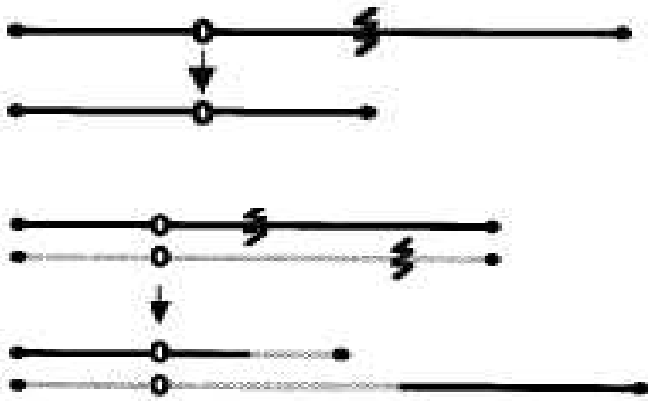
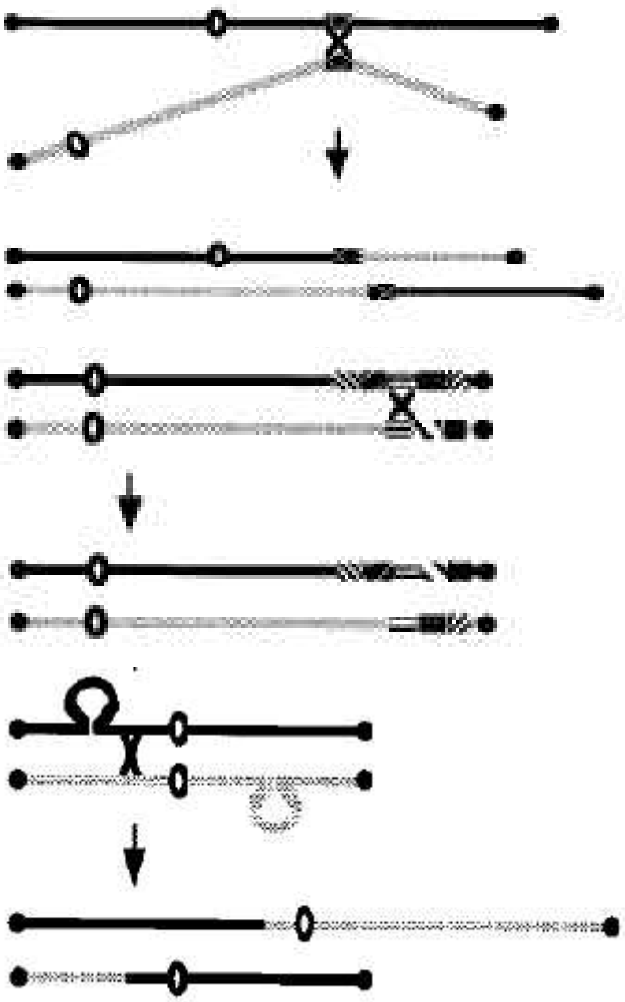


kırılma

Fungal genotiplendirme

- *Electrophoretic karyotyping*
  - Kromozamal yeniden düzenlenmeler



Homolog olmayan kromozonlar arasında normal olmayan rekobinasyonlar sonucu tranlokasyonlar meydana gelir.

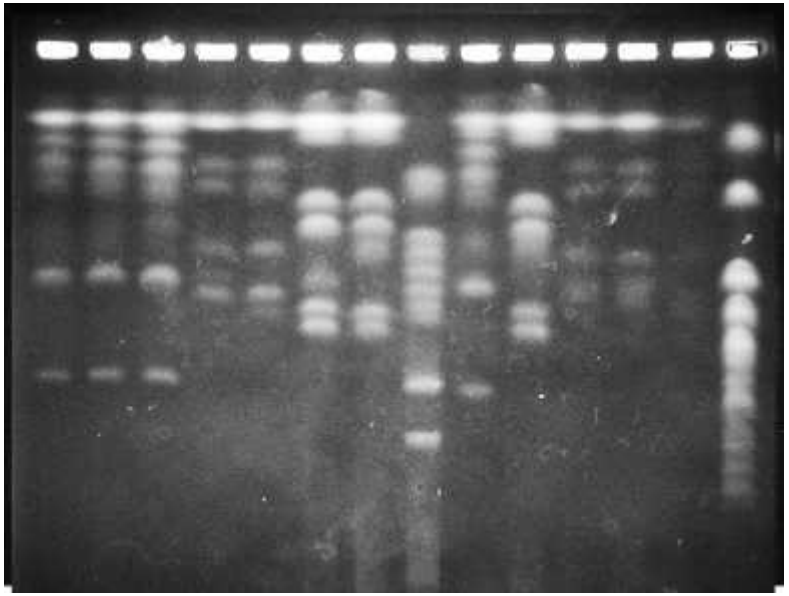
Homolog olmayan krmozomlar arasındaki polimorfizmler.

Kardeş kromotitlerin homolog olmayan lokuslarındaki kopmalar.

Fungal genotiplendirme

- *Electrophoretic karyotyping*
  - Homoplasi görülme sıklığı fazladır.
  - İki izolat aynı bant profilini gösterse bile örnekler aynı olmayabilir.
  - Pahalı ekipman gerektirir
  - Uzun zaman alır.

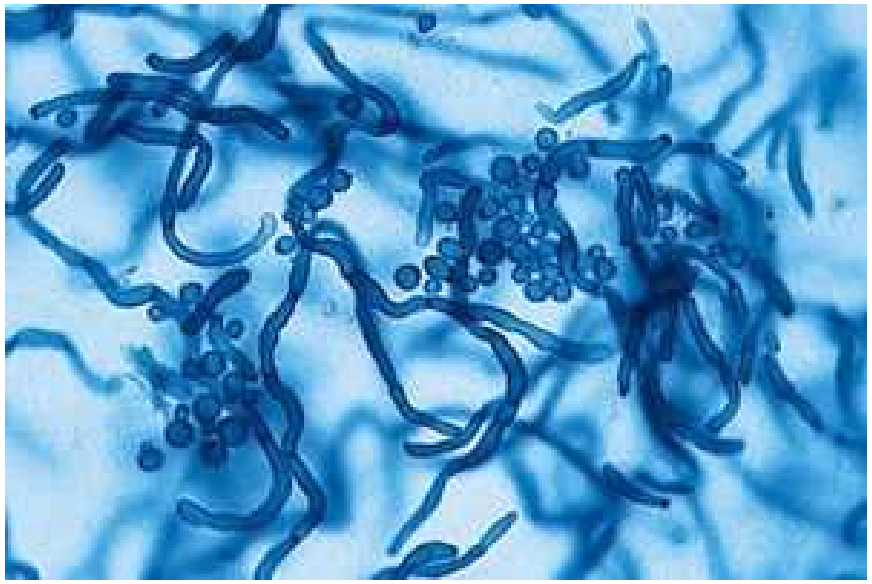
Tek bir bant farkı önmeli



36 saat, 90-360 saniye, 3.5 V/cm2, 12 °C

Fungal genotiplendirme

- *Electrophoretic karyotyping*
  - Yapılan bir çalışmada bu yöntemin *Malassezia spp.* türleri için yeterli çeşitliliği sağlayamadığı bildirilmişitir.



Fungal genotiplendirme

- *Electrophoretic karyotyping*

ORIGINAL ARTICLE

Genetic relationships between *Candida albicans* strains isolated from dental plaque, trachea, and bronchoalveolar lavage fluid from mechanically ventilated intensive care unit patients

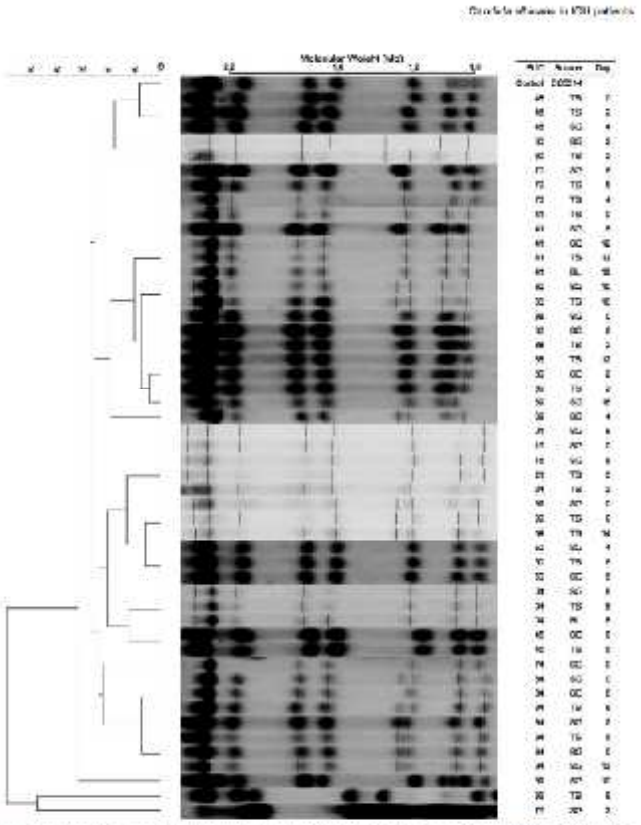
Seok-Mo Heo[1,2], Robert S. Sung[1], Frank A. Scannapieco[1] and Elaine M. Haase[1]*

[1]Department of Oral Biology, University at Buffalo, The State University of New York, Buffalo, NY, USA, [2]Periodontics and Endodontics, University at Buffalo, The State University of New York, Buffalo, NY, USA

Candida albicans often resides in the oral cavity of healthy humans as a harmless commensal organism. This opportunistic fungus can cause significant disease in critically ill patients, such as those undergoing mechanical ventilation in the intensive care unit (ICU) having compromised local airway defense mechanisms. The goal of this study was to determine the intra- and inter-patient genetic relationship between strains of *C. albicans* recovered from dental plaque, tracheal secretions, and the lower airway by bronchoalveolar lavage of patients undergoing mechanical ventilation. Three pulsed-field gel electrophoresis (PFGE) typing methods were used to determine the genetic relatedness of the *C. albicans* strains, including electrophoretic karyotyping (EK) and restriction endonuclease analysis of the genome using *Sfi*I (REAG-S) and *Bss*HII (REAG-B). The *C. albicans* isolates from dental plaque and tracheo-bronchial sites from the same patient were genetically indistinguishable and retained over time, whereas strains from different patients usually separated into different genotypes. Among the three methods, REAG-B proved to be the most discriminatory method to differentiate isolates. The finding of genetically similar strains from the oral and tracheo-bronchial sites from the same patient supports the notion that the oral cavity may serve as an important source for *C. albicans* spread to the trachea and lung of mechanically ventilated patients.

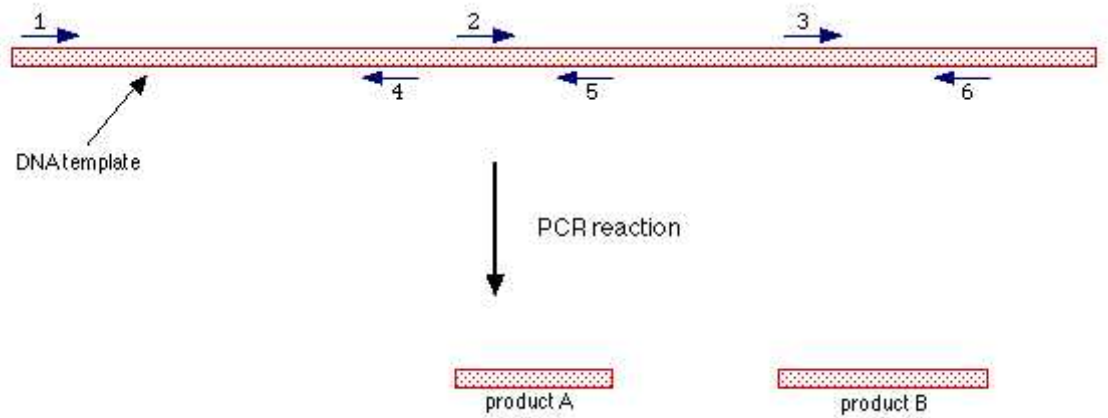
Keywords: yeast, pulsed-field gel electrophoresis (PFGE), molecular epidemiology, mechanical ventilation, oral cavity

Received: 15 February 2011; Revised: 19 May 2011; Accepted: 20 May 2011; Published: 20 June 2011



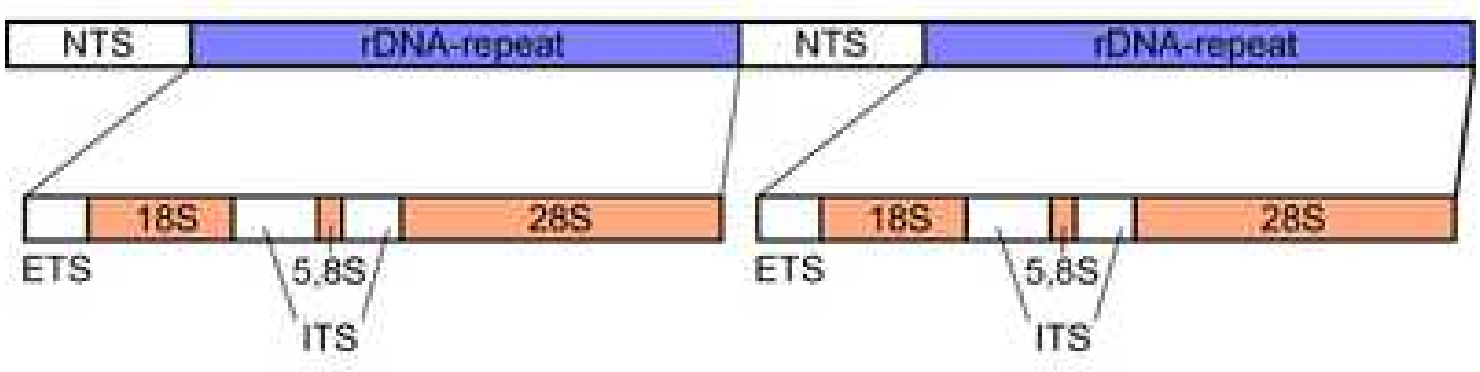
Fungal genotiplendirme

- *Arbitrarily Primed PCR* (AP-PCR)
  - Mantarların genotiplendirmesinde sık başvurulan yöntemlerden biridir.
  - Bir veya daha fazla primerle rastgele çoğaltılır ve ortaya çıkan patern farklılıkları değerlendirilir.
  - Kullanılan primerler genellikle 9-10 bazlık kısa primerler olup G-C'ce zengindir.



Fungal genotiplendirme

- *Arbitrarily Primed PCR* (AP-PCR)
  - Kullanılan primerler tiplendirilecek mikroorganizmanın genom bilgisine gerek olmadan rastgele seçilebileceği gibi, genom üzerindeki belli bölgelere yönelik bilinçli olarak da seçilebilir.
  - rDNA yer alan ITS bölgeleri bu amaçla seçilebilir.



Fungal genotiplendirme

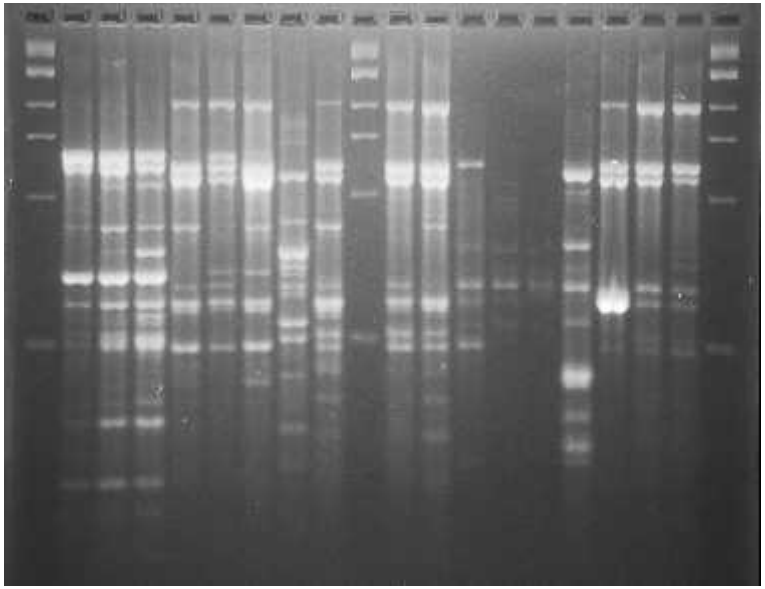
- *Arbitrarily Primed PCR* (AP-PCR)
  - Mantarların tiplendirilmesinde AP-PCR yöntemi;
    - kolay uygulanabilir olması
    - kısa sürede sonuç vermesi
    - nispeten daha ucuz olması

      gibi özellikleri nedeniyle diğer yöntemlere göre daha avantajlıdır.

Fungal genotiplendirme

- *Arbitrarily Primed PCR* (AP-PCR)
  - Özellikle RFLP ile değişiklik göstermeyen izolatlar arasındaki varyasyonları bile tespit edebildiği bildirilmiştir.
  - Örneğin;

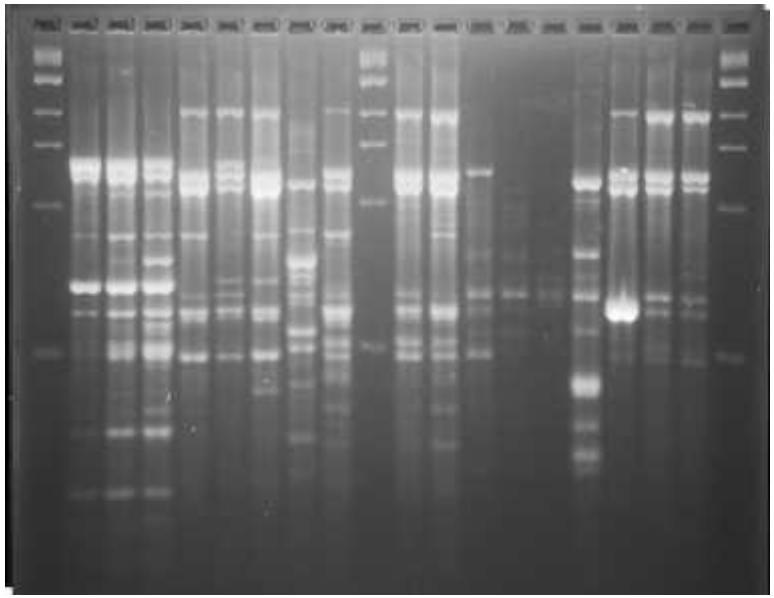
    C. lusitaniae ve *A. fumigatus* için uygulanan çeşitli DNA temelli tiplendirme metotlarının karşılaştırılmasında, AP-PCR ile RFLP'nin kaçırdığı varyasyonları tespit etmiştir.



C. albicans
M13 primer AP-PCR

Fungal genotiplendirme

- *Arbitrarily Primed PCR* (AP-PCR)
  - AP-PCR ile tiplendirmede yöntemin ayrım gücü, kullanılan primerlere göre değişmektedir.
    - OBU1-OBU-2-OBU3 - RSD11-RSD-12
    - ERIC1-ERIC2 - OPBO1-OPBO20-OPG10
    - PC1-PC2 - M13



Fungal genotiplendirme

- *Arbitrarily Primed PCR* (AP-PCR)
  - Tekrarlanabilirliği aynı laboratuvarda bile düşük
  - Başlangıç DNA konsantrasyonları önemli.
  - PCR reaksiyonunu etkileyecek birçok faktörden etkilenebilir.

Fungal genotiplendirme

- *Arbitrarily Primed PCR* (AP-PCR)

Jpn. J. Infect. Dis., 61, 130-132, 2008

Short Communication

Two Possible Cases of *Trichosporon* Infections in Bone-Marrow-Transplanted Children: the First Case of *T. japonicum* Isolated from Clinical Specimens

Handan Ağırbaşlı*, Hulya Bilgen[1], Serra Keceli Özcan[2], Baris Otlu[3], Gulce Sinik[4], Nilgün Çerikçioğlu[4], Riza Durmaz[3], Emine Can, Nevin Yalman, Gunduz Gedikoğlu and Takashi Sugita[5]

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SUMMARY: *Trichosporon* spp. are emerging as opportunistic agents that cause systemic diseases in immunocompromised hosts. Trichosporonosis carries a poor prognosis in neutropenic patients. *Trichosporon japonicum* was isolated from the air and named by Sugita et al. Here we present the first case of *T. japonicum* isolated from a clinical specimen. Two cases of acute myeloid leukemia who had *Trichosporon* isolates are discussed because of their rarity and growing importance. *T. asahii* was isolated from the throat, feces and urine of the first patient. *T. japonicum* was isolated from the sputum of the second patient. Both cases produced high MICs to itraconazole, and low MICs to fluconazole and voriconazole. In virulance factor investigations there was (++) biofilm formation in *T. japonicum* but not in *T. asahii*. Conventional mycological studies were not adequate for the identification of the isolate at the species level. In our second case as in the first one, the isolate was identified as *T. asahii* with 99.9% accuracy by API 20CAUX. Although two *T. asahii* isolates from the same patient yielded identical typing profiles by arbitrary primed-PCR, the isolates of the two different patients showed different arbitrary primed-PCR typing profiles. However, the genetic identification of the other patient's strain gave the result of *T. japonicum*.

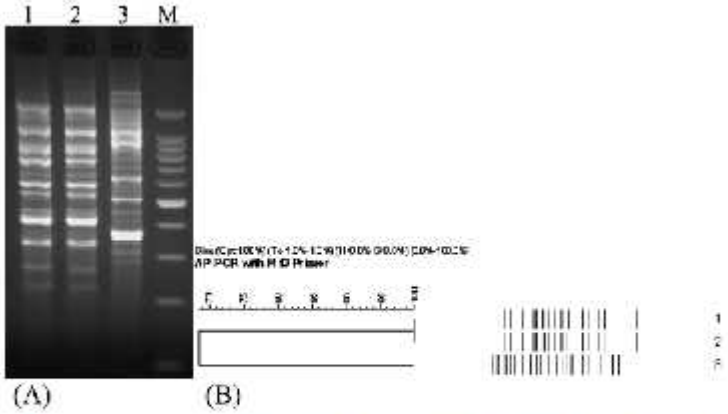

Fig. 1. (A) Agarose gel electrophoresis of the amplified products in AP-PCR with M13 primers. Lane1, Case 1 throat; lane 2, Case 1 stool; lane 3, Case 2 sputum isolates. Lane M is 100-bp DNA ladder. (B) A dendrogram of the DNA fingerprinting patterns of two *T. asahii* isolates (no. 1 and 2) and one *T. japonicum* isolate (no. 3).

Fungal genotiplendirme

New Microbiologica, 31, 491-498, 2008

Species distribution, antifungal susceptibility and clonal relatedness of Candida isolates from patients in neonatal and pediatric intensive care units at a medical center in Turkey

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SUMMARY

The aim of this study was to assess species distribution and antifungal susceptibility and clonal relationships among Candida strains isolated from a group of pediatric/neonatal intensive care (PICU/NICU) patients that had a very high mortality rate (76%). The cases of 21 patients (19 with candidemia, 2 with Candida meningitides) treated over a 1-year period in a Turkish hospital PICU and NICU were retrospectively analyzed. Twenty-eight Candida isolates were detected from blood (20), cerebrospinal fluid (CSF) (2) and other specimens (6). Candida species were identified using the API ID 32C System. Susceptibility testing was done (all 28 isolates) for amphotericin B, fluconazole and itraconazole using the broth microdilution method. Arbitrarily primed polymerase chain reaction (AP PCR) was used for molecular typing of the 3 most common ones: C. albicans (15), C. parapsilosis (6), and C. pelliculosa (4). Electrophoretic karyotyping (EK) was done to check clonal identity obtained by AP-PCR. Of the 20 blood isolates, 8 (40%) were C. albicans, 12 (60%) were non-albicans Candida, and one of the 2 CSF isolates was C. albicans. The overall species distribution was as follows: 15 C. albicans isolates, 6 C. parapsilosis isolates, 4 C. pelliculosa isolates, 2 C. famata isolates and 1 C. tropicalis isolate. Amphotericin B had the best antifungal activity with a MIC_{90} of 0.125 µg/mL, and the rates of susceptibility to fluconazole and itraconazole were 93% and 82%, respectively. AP PCR revealed 11 genotypes (4 were identical pairs, 7 were distinct) among the 15 C. albicans isolates, 2 genotypes (5 were classified in the same type) among the 6 C. parapsilosis isolates, and 4 separate genotypes for the 4 C. pelliculosa isolates. Karyotyping results correlated well with the AP-PCR findings. As indicated in the previous research, our results confirmed that non-albicans Candida species have become more frequently causative agents for invasive fungal infections in the ICU. Transmission of C. albicans and C. pelliculosa was relatively low, but transmission of C. parapsilosis was high, suggesting that more effective control and very strict treatment protocols are needed for patients having high mortality and invasive fungal infection in ICU.

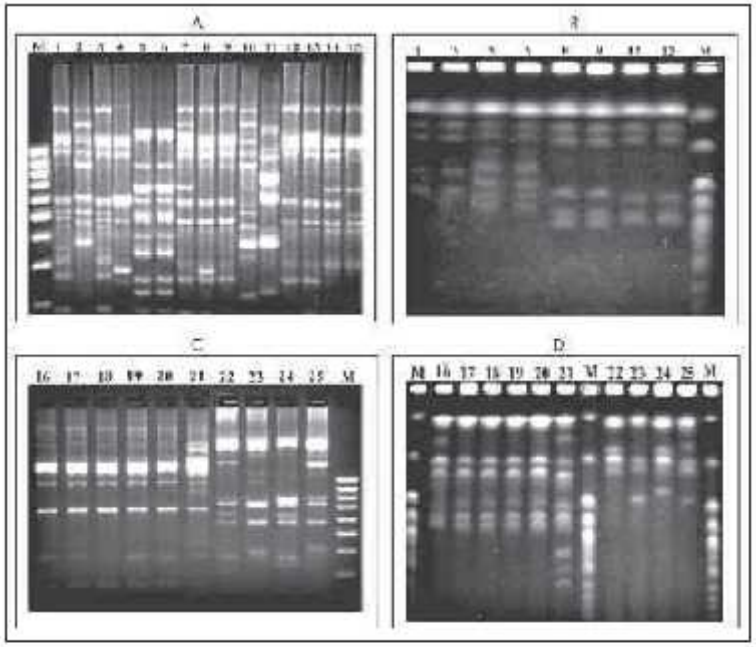

FIGURE 1 *A (Top left): AP PCR results for C. albicans. Lines 1-3: Patient 1 blood, urine and dialysis fluid isolates; Lines 4-6: Patient 2 urine, tracheal aspirate and blood isolates; Line 7: Patient 3 blood isolate; Line 8: Patient 4 blood isolate; Lines 9 and 10: Patient 5 blood and intravenous catheter isolates; Line 11: Patient 6 CSF isolate; Lines 12 and 13: Patient 7 blood and tracheal aspirate isolates; Line 14: Patient 8 blood isolate; Line 15: Patient 9 blood isolate.* **B** *(Top right): EK results for C. albicans. Lines 1 and 3: Patient 1 blood and dialysis fluid isolates; Lines 5 and 6: Patient 2 tracheal aspirate and blood isolates; Lines 8: Patient 4 blood isolate; Line 9: Patient 5 blood isolate; Lines 12 and 13: Patient 7 blood and tracheal aerosol isolates.* **C** *(Bottom left): AP-PCR results for C. parapsilosis (lines 16 23) and C. pelliculosa (lines 22 25). Line 16: Patient 10 blood isolate; Line 17: Patient 11 blood isolate; Line 18: Patient 12 blood isolate; Line 19: Patient 13 blood isolate; Line 20: Patient 14 blood isolate; Line 21: Patient 5 blood isolate; Line 22: Patient 15 blood isolate; Line 23: Patient 16 blood isolate; Line 24: Patient 17 blood isolate; Line 25: Patient 18 blood isolate.* **D** *(Bottom right): EK results for C. parapsilosis (lines 16 23) and C. pelliculosa (lines 22 25). Line 16: Patient 10 blood isolate; Line 17: Patient 11 blood isolate; Line 18: Patient 12 blood isolate; Line 19: Patient 13 blood isolate; Line 20: Patient 14 blood isolate; Line 21: Patient 5 blood isolate; Line 22: Patient 15 blood isolate; Line 23: Patient 16 blood isolate; Line 24: Patient 17 blood isolate; Line 25: Patient 18 blood isolate.*

Fungal genotiplendirme

- *Restriction Fragment Length Polymorphism* (RFLP)
  - RFLP tekniği mantarlarda sıklıkla kullanılır.
  - En sık kullanılan restriksiyon endonükleazlar; *EcoR*I, *Hinf*I, *Sfi*I
  - İlişkisiz kökenlerin araştırılmasında yeterli ayrımı sağlarken muhtemel ilişkili kökenlerin analizlerinde başarılı değildir.

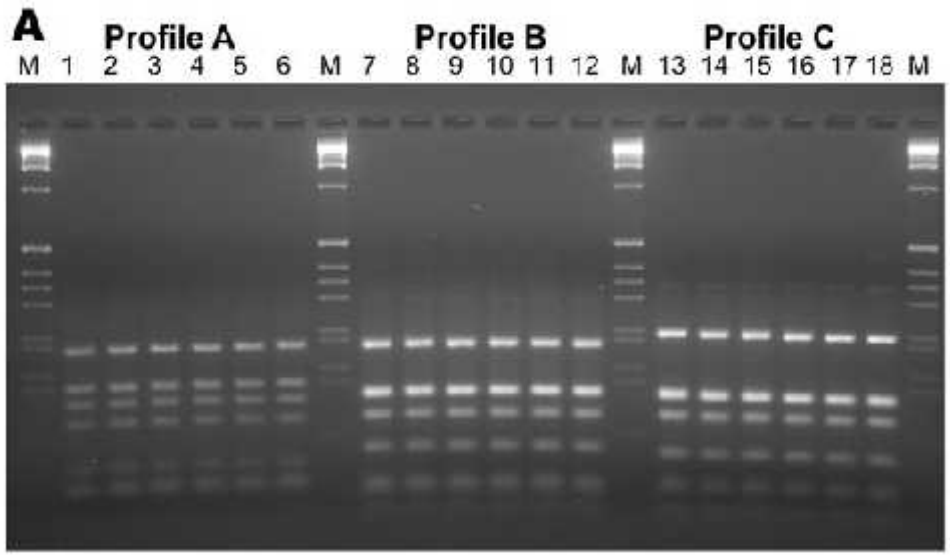

Figure 1. Internal transcribed spacer–restriction fragment length polymorphism (ITS-RFLP) patterns obtained by double digestion with the enzymes *Sau*96I and *Hha*I (A) and of the PCR fingerprinting profiles obtained with the microsatellite specific primer M13 (B) for *Scedosporium prolificans*:

Emerg Infect Dis. 2008 Feb;14(2):282-90.

Fungal genotiplendirme

- *Restriction Fragment Length Polymorphism* (RFLP)

Epidemiological Characteristics of Fatal *Candida krusei* Fungemia in Immunocompromised Febrile Neutropenic Children

In early 1996, fluconazole was introduced as a prophylactic antifungal agent after stem cell transplantation (SCT). At the same time, *Candida krusei* emerged as a chief fungal pathogen among the patients with SCT [1, 2]. In our SCT center, which has a 15-bed capacity unit dedicated for children with malignant diseases and 5-bed unit for SCT patients, low-dose fluconazole is routinely included in the posttransplant prophylaxis regimen (starting at day 7). In recent years, we observed mortality due to fungal sepsis in patients undergoing allogenic SCT and chemotherapy. Here, we report the characteristics of the patients having mortal *C. krusei* fungemia and the molecular typing results of the *C. krusei* strains in order to develop more effective policies for treatment and prevention of fungal infections.

The febrile neutropenic episodes of the 47 children (median age: 9.13 ± 4.73 years) were prospectively evaluated from March 2001 to January 2003. For each patient, daily leukocyte counts and body temperatures were measured. Fever was defined as a single oral temperature of ≥ 38.3°C or a temperature of ≥ 38.0°C for ≥ 1 h. Neutropenia was defined as a neutrophil count of < 500 cells/mm^3, or a count of < 1,000 cells/mm^3 with a predicted decrease to < 500 cells/mm^3 (Guidelines from the Infectious Disease Society of America, 1997). Patients who had both fever and neutropenia were recognized as febrile neutropenic. Nine patients developed severe fungal infection during neutropenia. Three of these having bloodstream infection with the *C. krusei* were analyzed in detail in this study.

Surveillance cultures were taken from blood, throat, stool, urine and catheter exit sites of the patients in the SCT unit when they had febrile episode and/or weekly on a routine basis. Automated blood culture system (Becton Dickinson, pediatric vials, USA) and BACTEC MYCO/F LYTIC (Becton Dickinson) were used for blood cultures. To search exogenous sources of *C. krusei* sepsis, 51 specimens from the hands of 30 health care workers (9 doctors, 11 nurses, 4 laboratory workers, and 6 cleaning staff) and 19 environmental swabs from commonly used areas around the patients (nurse desk, room and toilet doors, refrigerator doors, telephones, etc) were analyzed. antifungal susceptibility. Molecular typing of the nine *C. krusei* strains of the three patients (three blood, two stool, one urine, one throat, one vagina, and one sputum isolates) was performed by pulsed field gel electrophoresis (PFGE) following the protocol described previously [3].

A total of 156 febrile episodes were recorded in the 47 patients within the 312 febrile neutropenic days. Bacteria were isolated in 28 (72%) and fungi in 26 (48%) of the 54 clinical specimens. It was noted that the patients with prolonged and deep neutropenia (hematological malignancy patients) are under an increased risk for fungal infections [1, 4, 5]. In agreement with these findings, we observed systemic fungal infections with high mortality in recent years. Of the 47 patients with febrile neutropenia, 14 had fungal isolation from various body sites, and 9 (19%) died due to fungal infection. Eight of these fatal cases had non-*Candida albicans* infection (Table 1). Distribution of *Candida* spp. among the five non-fatal cases was as follows: *C. krusei* (1), *C. kefyr* (1), *C. albicans* (2), *C. famata* (1). In an Italian tertiary hospital, it was found that 81% of the candidemia episodes were related to non-albicans *Candida* and high mortality rates were observed particularly for hematological (71%) and transplant patients (50%) [6]. In another tertiary care hospital in

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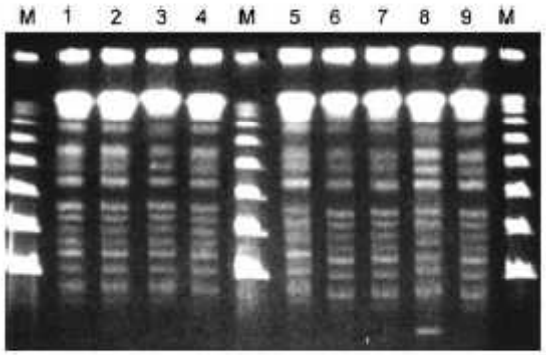


Figure 1. PFGE profile of the 9 *C. krusei* strains. *M* molecular weight marker. *Lanes 1 and 2* patient 1's blood and stool isolates; *lanes 3–6*, patient 2's throat, stool, blood, and sputum isolates, respectively; *lanes 7–9* patient 3's blood, vagina, and throat isolates, respectively.

breaks lasting 1 or 2 days or ineffective doses due to difficulties to obtain liposomal amphotericin B. Amphotericin B treatment was started at tenth day of the febrile neutropenia for two patients and at the first day of the febrile neutropenia and sepsis for the third patient. These patients died on days 8, 21, and 23 after the diagnosis of sepsis. Since there were no concomitant bacterial infections in these patients having many risk factors, the mortality was attributed due to *C. krusei* sepsis. However, it is possible that the high mortality in these patients was

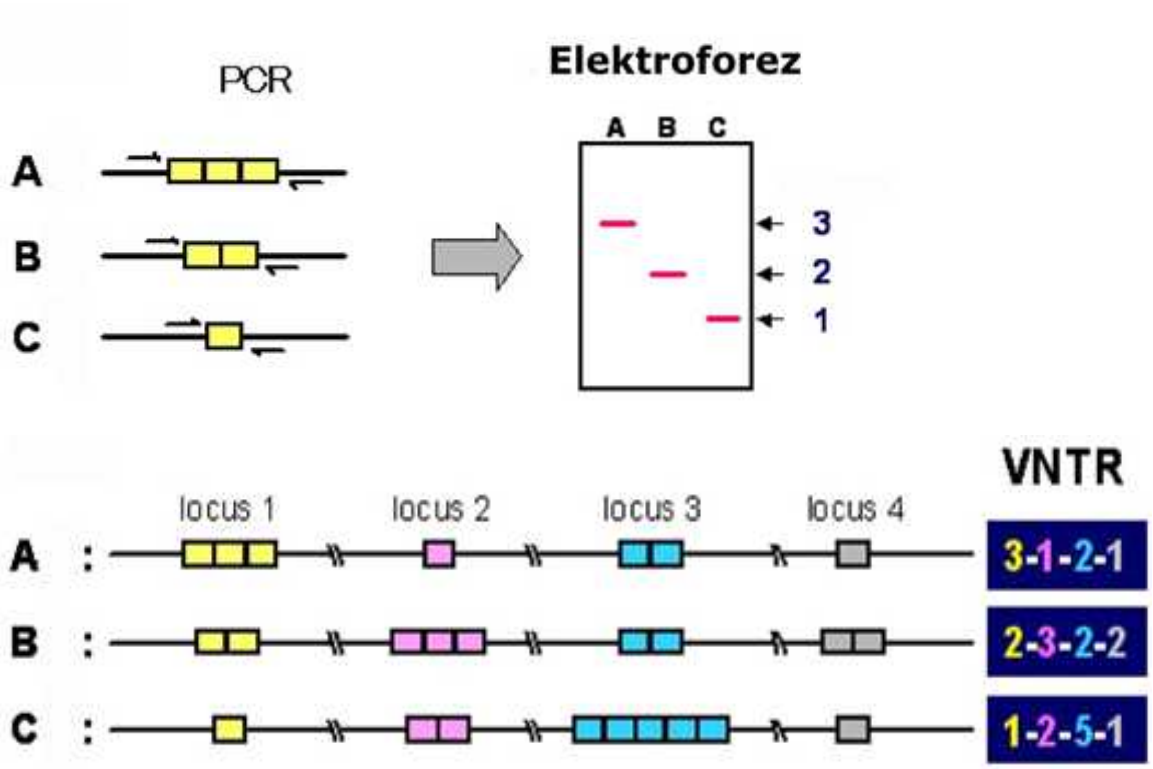
Fungal genotiplendirme

- *Variable number of short tandem repeat (VNTR)*
  - Kromozom üzerindeki art arda tekrarlanan DNA dizilerinin gösterilmesi esasına dayanır..
  - Tekrarlanan ünitelerdeki insersiyon veya delesyona bağlı olarak; tekrar bölgesi uzayıp, kısalabilir.

GAGCCACGTTGCGCGGTCACCCGCGCCCGCACTCGTTCACCGGGCAACGCATA
GCGGACGAAAACCACCCGGCCCTCGTGGTGCGCCACGCAGCTACCGCCGTTC
GCGGGCGCTCCGGTGACCAACGTCAGATTCACTGCATCGTCGCCGGCGCGGG
1. kopya CTGGCGCCGCTCCTCCCCATCGCTTTGCTCTGCATCGTCGCCGGCGCGGGTCA
2. kopya CTGGCGCCGCTCCTCCCCATCGCTTTGCTCTGCATCGTCGCCGGCGCGGGTCA
3. kopya CTGGCGCCGCTCCTCCCCATCGCTTTGCTCTGCATCGTCGCCGGCGCGGGTCAA
TCGAAGATGCCCCGTCACGTGTCACCGGGAGCCGCGTGCGGCTGTAACGTCTT
GATCCGCTCCGACGACGTCAGTTGCCAAGGCACCGAAGTCACCATCAC

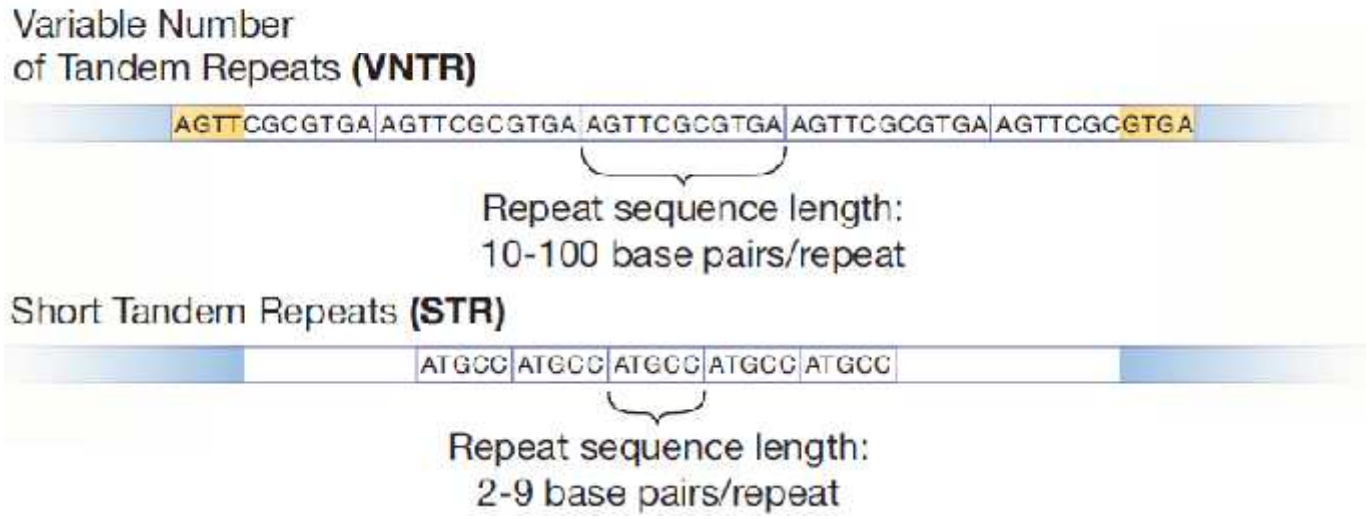
Fungal genotiplendirme

- *Variable number of short tandem repeat (VNTR)*
  - Yöntem basit olarak, tekrarlanan dizilimlerin amplifikasyonu ve oluşan PZR ürünlerinin uzunluk analizi aşamalarından oluşmaktadır.



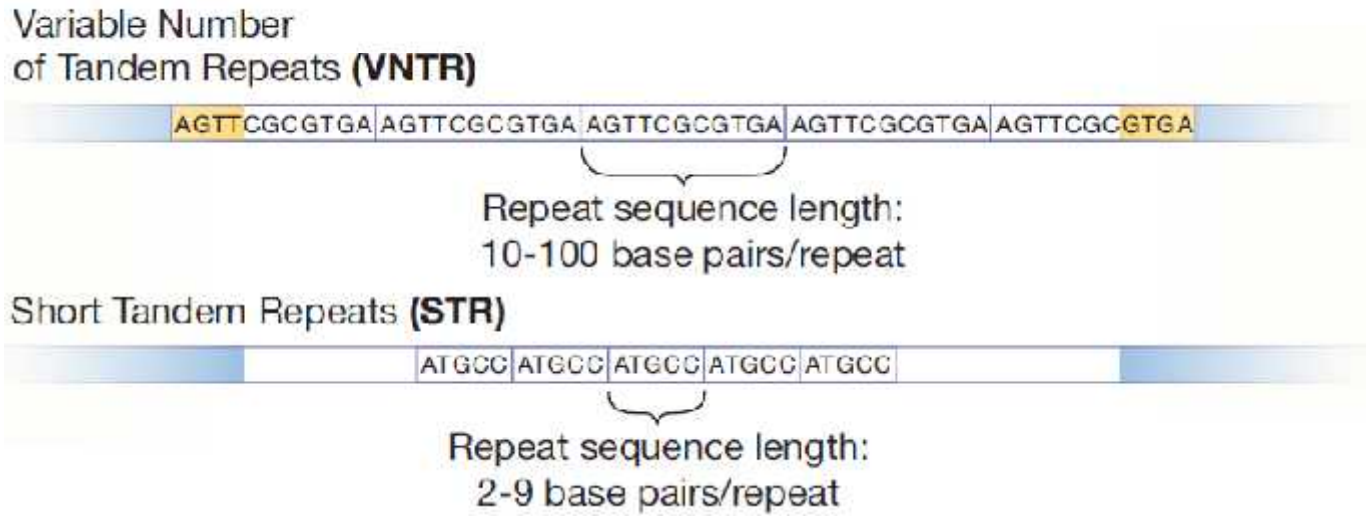
Fungal genotiplendirme

- *Variable number of short tandem repeat (VNTR)*
  - *satellit (megabaz)*
  - *minisatellit (6-100 bp)*
  - *mikrosatellit (1-5 bp)*



Fungal genotiplendirme

- *Variable number of short tandem repeat (VNTR)*
  - *satellit (megabaz)*
  - *minisatellit (6-100 bp)*
  - *mikrosatellit (1-5 bp)*



Fungal genotiplendirme

- *Variable number of short tandem repeat (VNTR)*

Thierry et al. BMC Microbiology 2010, 10:315
http://www.biomedcentral.com/1471-2180/10/315

BMC Microbiology

RESEARCH ARTICLE Open Access

Multiple-locus variable-number tandem repeat analysis for molecular typing of *Aspergillus fumigatus*

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Abstract

Background: Multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) is a prominent subtyping method to resolve closely related microbial isolates to provide information for establishing genetic patterns among isolates and to investigate disease outbreaks. The usefulness of MLVA was recently demonstrated for the avian major pathogen *Chlamydophila psittaci*. In the present study, we developed a similar method for another pathogen of birds: the filamentous fung

Results: We selected 10 VNT markers were tested with 57 species in France, China or M and the reference strain CBS combined loci index calculat markers was used in differen in Guangxi province in Chin from a turkey hatchery in Tr u-pseudtb/MLVAnet/. Three m Minimum Spanning Tree (MS farms in France, the second the third one comprised mo

Conclusions: MLVA displaye analysis revealed an interesti origin rather than their respe

Figure 1 Electrophoretic gel showing VNTR profiles. Lanes 1 to 7 represent the amplification of 7 isolates for marker Asp_330 (11 bp repeat). Samples in lanes 1-2 and 5-7 have 3 repeats and samples in lanes 3-4 have 4 repeats. Lanes 8 to 15 represent the amplification of 8 other isolates for Asp_443 marker (18 bp repeat). Sample in lane 8 has 4 repeats, samples in lanes 9 and 12-14 have 5 repeats, samples in lanes 10-11 and 15 have 7 repeats.

Fungal genotiplendirme

- *Variable number of short tandem repeat (VNTR)*

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0095-1137/07/$08.00+0 doi:10.1128/JCM.01603-07

Multiple-Locus Variable-Number Tandem-Repeat Analysis for Rapid Typing of *Candida glabrata*▽

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A multiple-locus variable-number tandem-repeat analysis (MLVA) using six microsatellite markers was assessed in 127 *Candida glabrata* isolates. Thirty-seven different genotypes, stable both in vitro and in vivo, were observed. The highest discriminatory power ($D = 0.902$) was reached by using only four markers. MLVA seems to be relevant for *C. glabrata* typing.

TABLE 1. Characteristics of microsatellite markers of *C. glabrata*

Marker	GenBank accession no.	Microsatellite sequence[a]	Primer sequence (5′ to 3′)	No. of alleles	Allele size range (bp)	Marker location[b] (noncoding region or name of gene if coding)
Cg4	BZ298249	$(GT)_{19}$	AATGCTGTGTGTGCGTAGT DyeD2 AAAAATTTAGGCCCCATCG	9	228–252	Chromosome L (noncoding)
Cg5	BZ298911	$(GT)_9(CT)_2(GT)_{15}$	CTCAACAGAACCAATTCTGC DyeD3-TCTTCAGGCTGCCAATCTTA	9	122–146	Chromosome M (noncoding)
Cg6	BZ298679	$(CA)_{11}$	DyeD2-AGCAAGAGGGAGGAGGAAACT AAATCCGGGGATAGATGAGG	11	301–342	Chromosome L (noncoding)
Cg7	BZ298409	$BD_2BFBD_2BFB_2D_2B_2DBDB_2D_2B_2$	GATGATTCTGCCCGTTAGGA DyeD3-AAGAGTTCCCTGGTGGAATG	7	179–215	Chromosome J (CAGL0J00595g)
Cg10	BZ297776	$B_3DB_{11}DBD_2B_4DB_2D_2B_{11}D$	TGCCTACGATGAAGAAATCG DyeD4 CTGGTAAGCACCGTTTTGGT	12	249–307	Chromosome E (CAGL0E00561g)
Cg11	BZ298945	$DBD_2B_2D_2B_2D_2(CCA)BD$	CTGGTCTACCCAGCACCAAT DyeD4-TGCTGATACTGGTAGTTTTGTTG	5	141–169	Chromosome A (CAGL0A03872g)

[a] Notation of trinucleotides as used by Steuer et al. (26): B, CAA; D, CAG; F, CTG.
[b] Microsatellite location on *C. glabrata* genome according to the Genolevure database (http://cbi.labri.fr/Genolevures). Microsatellites Cg7, Cg10, and Cg11 were each located in separate genes coding for a protein with no unidentified function.

Fungal genotiplendirme

- *Multi-locus sequence typing (MLST)*
  - 1998 yılında Maiden ve ark. tarafından geliştirildi
  - Metabolik fonksiyonlardan sorumlu korunmuş gen (housekeeping) dizilerinin karşılaştırılması esasına dayanır.

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 3140–3145, March 1998
Microbiology

Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms

(molecular typing/*Neisseria meningitidis*/housekeeping genes/World-Wide Web/hyper-virulent clones)

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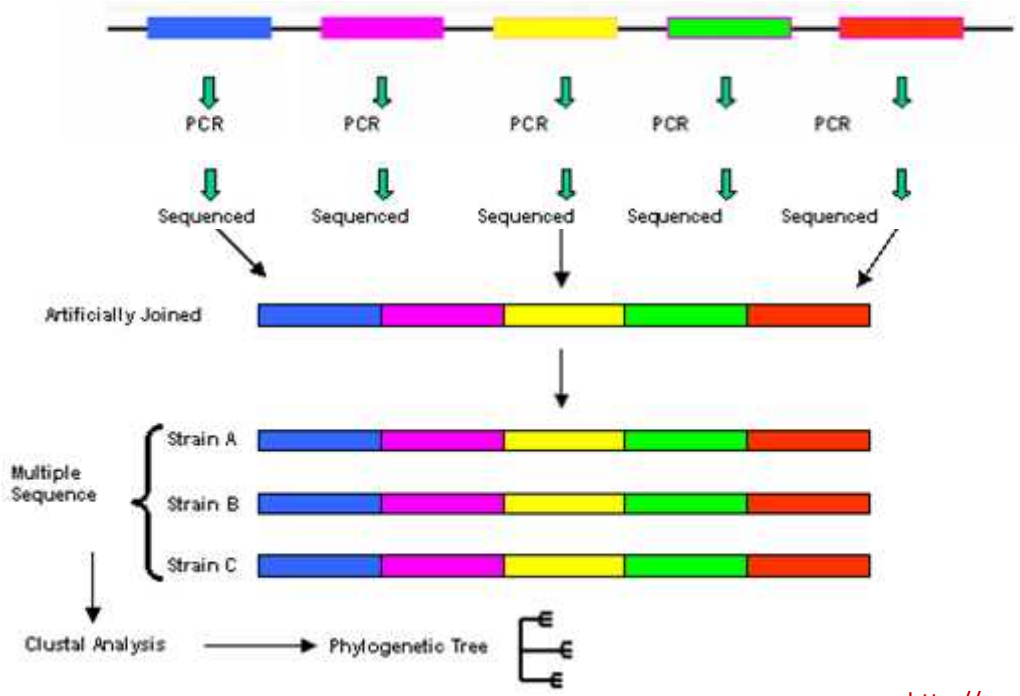
Edited by John Maynard Smith, University of Sussex, Brighton, United Kingdom, and approved January 6, 1998 (received for review October 16, 1997)

Fungal genotiplendirme

- *Multi-locus sequence typing (MLST)*
  - MLST ile housekeeping genlerin yaklaşık 500 bp parçalarının dizi analizi yapılmakta ve elde edilen her bir alele bir kod verilerek, dizi tipi (sequence type, ST) belirlenmektedir.
  - En önemli avantajı matematiksel olarak elde edilen kodların internet ortamında, dünyanın herhangi bir bölgesinden elde edilen diğer kökenlerle karşılaştırılabilmesidir.



http://westnilevirus.okstate.edu/research/2006rr/017.htm

Fungal genotiplendirme

- *Multi-locus sequence typing (MLST)*
  - Çoğunlukla bakterileringenotiplendirilmesinde kullanılan bu yöntem özellikle; *C. albicans , Coccidioides immitis, Histoplasma capsulatum, Cryptococcus neoformans* gibi fungal patojenler için de başarıyla kullanılmaktadır.

Fungal multilocus sequence typing – it's not just for bacteria

John W Taylor* and Matthew C Fisher†

Multilocus sequence typing uses nucleotide sequence from several genes to identify individual microbial pathogens. The data obtained for multilocus sequence typing can be used to recognize fungal species and to determine if the fungi are purely clonal, or if they also recombine. Genetic regions with more polymorphisms and microsatellites might be used to recognize populations within species and are well suited to Bayesian methods of assigning unknown individuals to populations of origin. Knowledge of species, populations and reproductive mode can help answer questions common to all emerging diseases: is the disease due to the recent spread of a pathogen, to the emergence of a virulent strain of an existing pathogen, or to a change in the environment that promotes disease?

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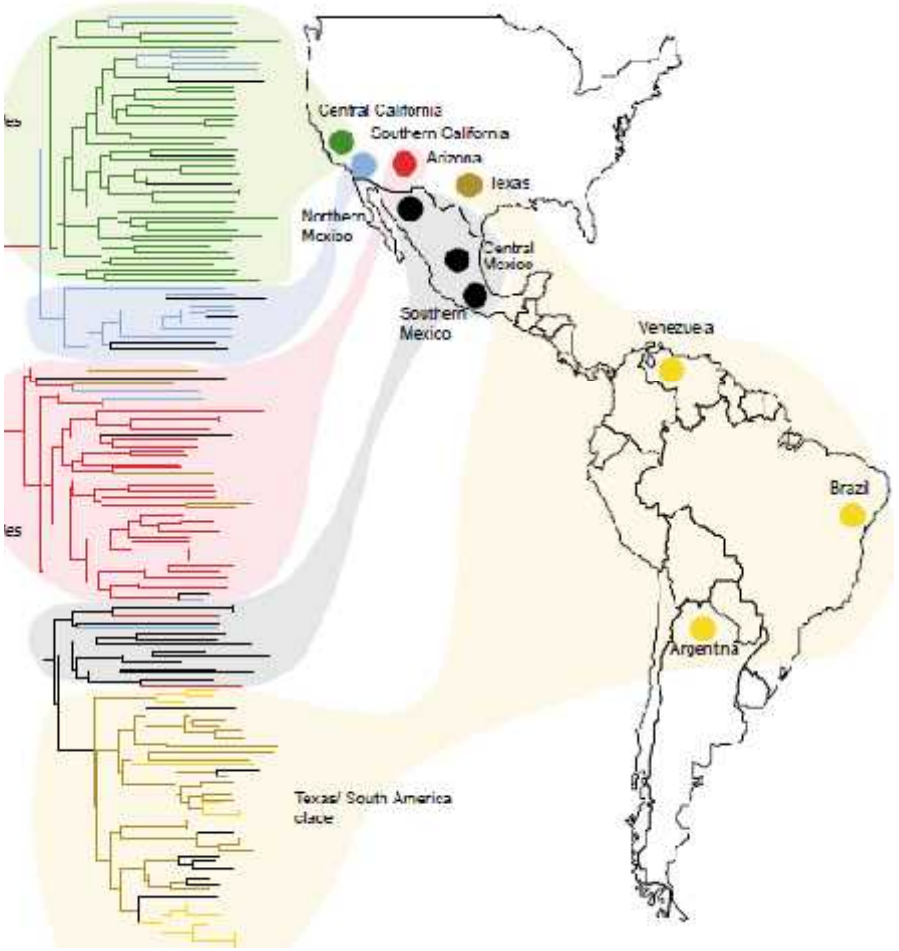
to improve and can be made available to all interested parties from a source on the internet. MLST has replaced older methods that hide variation, such as MLEE (multilocus enzyme electrophoresis), or that required increasing numbers of comparisons among known genotypes as each new genotype was added, such as DNA–DNA hybridization, or that were sensitive to small changes in the laboratory environment, such as electrophoretic karyotyping or randomly amplified polymorphic DNA (RAPDs) [15]. MLST also has the advantage over single nucleotide polymorphism (SNP) analysis that new polymorphic nucleotide positions in any of the gene fragment sequence can be detected and added to the database. This feature makes it possible to add new individuals from new geographic locations to the study without the danger that variation found to be polymorphic in the initial population will be monomorphic in the newly added ones, as can happen with SNPs [16].

Fungal genotiplendirme

- *Multi-locus sequence typing (MLST)*



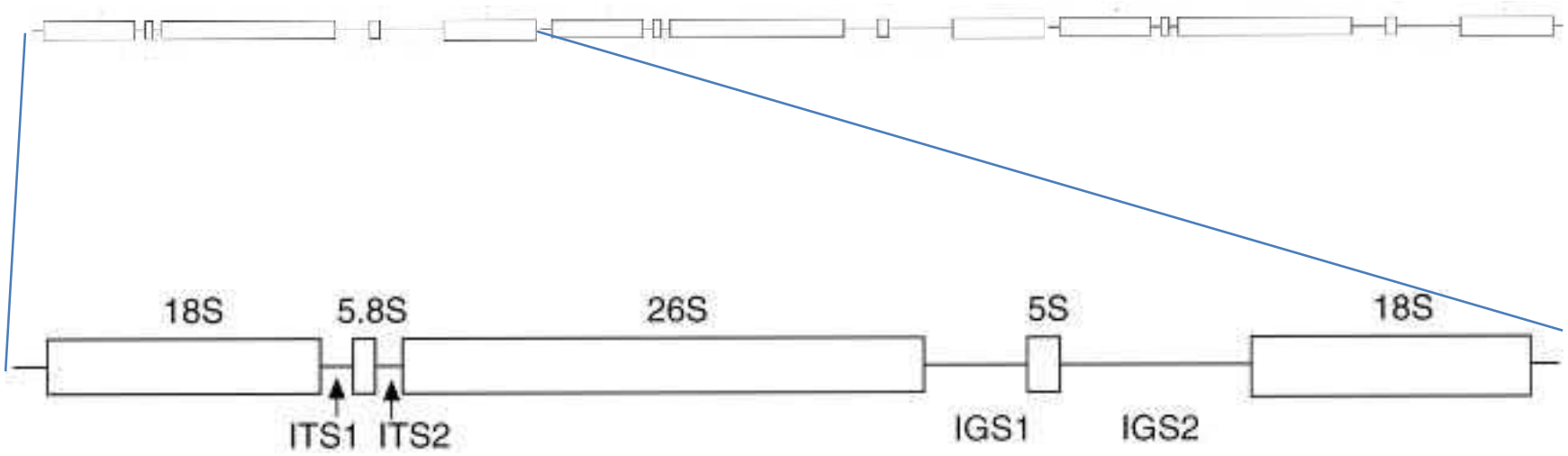
J Clin Microbiol. 2007 May; 45(5): 1469–1477.

Fungal genotiplendirme

- *Multi-locus sequence typing (MLST)*

Fungal genotiplendirme

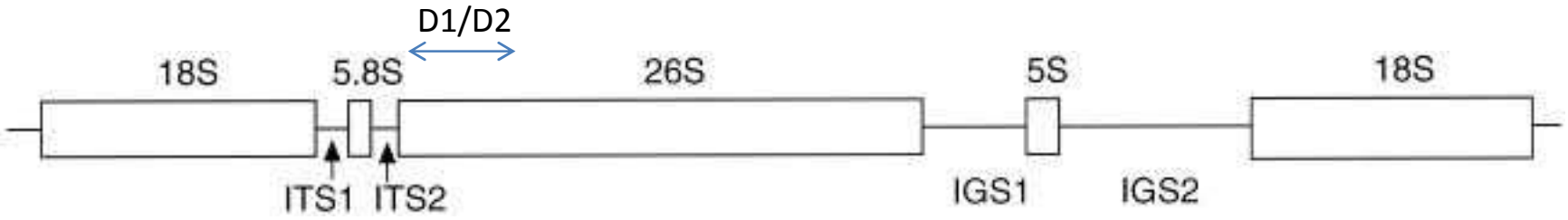
- *Ribosomal DNA ITS ve IGS* bölgelerinin sekans analizi
  - Fungal rRNA genleri; 18S (small-subunit), 5.8S ve 26S (large-subunit) genlerinin kodlandığı sıralı tekrarları içerir.
  - Bu dizinin her tekrarı *internal transcribed spacer* (ITS) ve *intergenic spacer* (IGS) bölgelerini barındırır.

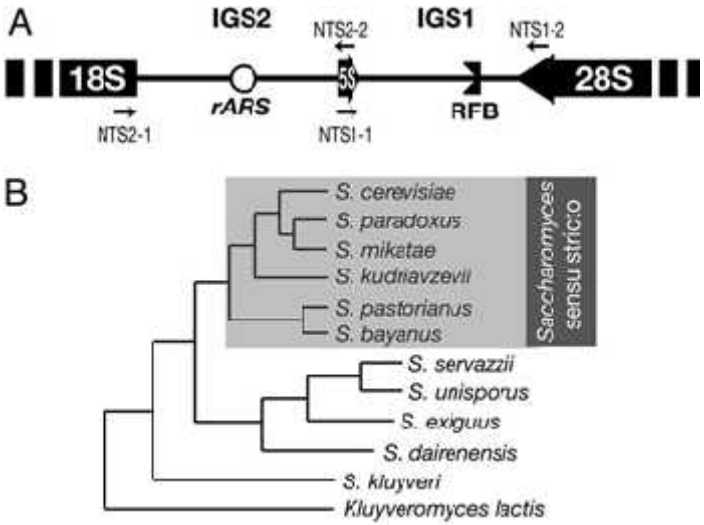


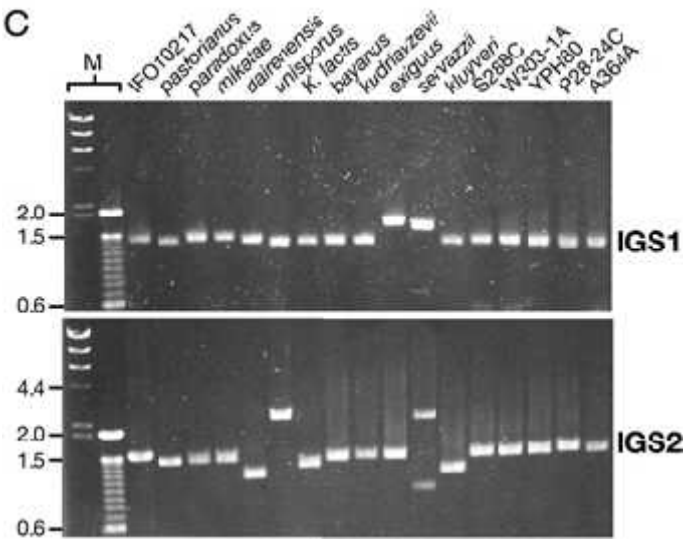
Takashi Sugita ae al. Sequence Analysis of the Ribosomal DNA Intergenic Spacer 1 Regions of *Trichosporon* SpeciesJ Clin Microbiol. 2002 May; 40(5): 1826–1830.

Fungal genotiplendirme

- *Ribosomal DNA ITS ve IGS* bölgelerinin sekans analizi
  - 26S üzerinde yer alan D1-D2 , ITS1/2, IGS 1/2 bölgeleri patojenik mantarların sekans bazlı tanımlamalarında uzun zamandır kullanılmaktadır.



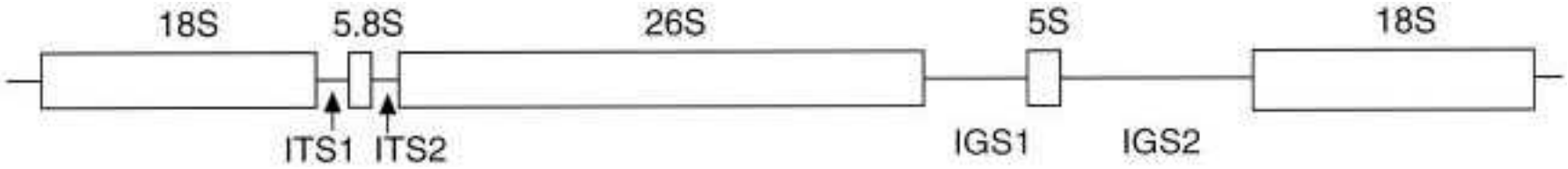




Ganley A R D et al. PNAS 2005;102:11787-11792

Fungal genotiplendirme

- *Ribosomal DNA ITS ve IGS* bölgelerinin sekans analizi
  - Uzunluğu 195-719 bp arasında değişen IGS1 bölgesinin sekans analizi, epidemiyolojik karşılaştırmalar yapmak için diğer bölgelerden daha uygundur.



Takashi Sugita ae al. Sequence Analysis of the Ribosomal DNA Intergenic Spacer 1 Regions of *Trichosporon* SpeciesJ Clin Microbiol. 2002 May; 40(5): 1826–1830.

Fungal genotiplendirme

- *Ribosomal DNA ITS ve IGS* bölgelerinin sekans analizi

Molecular identification, genotyping, and drug susceptibility of the basidiomycetous yeast pathogen *Trichosporon* isolated from Turkish patients

AYSE KALKANCI*†, TAKASHI SUGITA†, SEVTAP ARIKAN‡, MINE YUCESOY§, BEYZA ENER^, FEZA OTAG$, NURI KIRAZ~, SEMRA KUSTIMUR*, BANU SANCAK‡, CANAN EVCI^ & GUROL EMEKTAS$

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Deep-seated infections due to *Trichosporon* species are emerging mycoses that have a very poor prognosis in patients with persistent neutropenia. This study elucidated the mycological characteristics of *Trichosporon* strains obtained from deep-seated infections in Turkish patients and identified by DNA sequence analysis of intergenic spacer (IGS) region 1 of the rDNA locus. In addition, we genotyped the major causative agent, *T. asahii*, and evaluated the *in vitro* drug susceptibility of the isolates. While 87 (81.3%) of the 107 isolates were *T. asahii*, the remaining 20 were *T. faecale* (14.0%), *T. asteroids* (0.9%), *T. coremiiforme* (0.9%), *T. japonicum*, (0.9%), *T. lactis* (0.9%), and a new

Med Mycol. 2010 Feb;48(1):141-6.

Fungal genotiplendirme

- *Ribosomal DNA ITS ve IGS* bölgelerinin sekans analizi

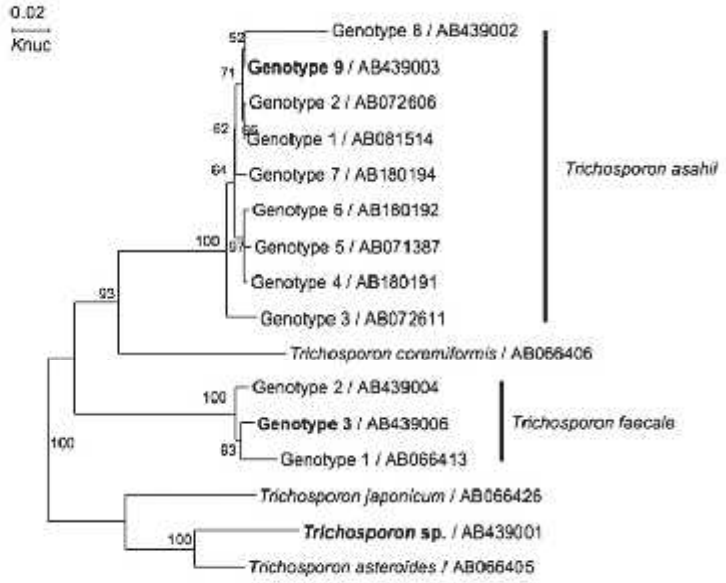

Fig. 2 Molecular phylogenetic trees constructed using the DNA sequence of the IGS 1 region including the new genotype strain from Turkey. The DDBJ/GenBank/EMBL accession numbers are also shown. The numbers indicate the confidence level from 100 replicate bootstrap samplings (frequencies below 50% are not shown). Knuc, Kimura's parameter [19].

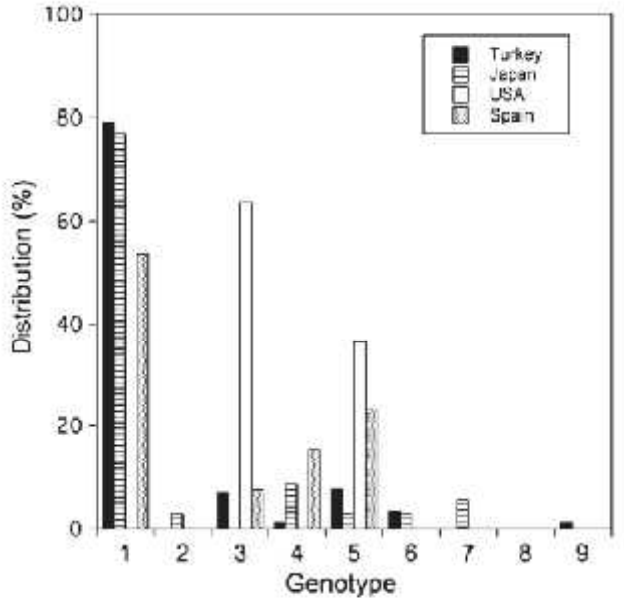

Fig. 3 The distribution of each genotype of *Trichosporon asahii*. Information on the distribution of Japanese, American, and Spanish *T. asahii* isolates is included for comparison [15,28].

Fungal genotiplendirme

- *Ribosomal DNA ITS ve IGS* bölgelerinin sekans analizi

Journal of Medical Microbiology (2009), 58, 745–750 DOI 10.1099/jmm.0.007955-0

Rapid molecular differentiation and genotypic heterogeneity among *Candida parapsilosis* and *Candida orthopsilosis* strains isolated from clinical specimens in Kuwait

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Recent molecular studies have led to the recognition of three distinct species within the *Candida parapsilosis* complex, namely *Candida parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis*. As currently available yeast identification systems fail to differentiate these species, there is a paucity of information on their occurrence in different geographical regions. This study describes a simple PCR-based protocol for rapid discrimination among *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis* strains by using primers derived from unique sequences within the internally transcribed spacer 1 (ITS1)–5.8 rRNA–ITS2 region. Retrospective analysis of 114 *C. parapsilosis*-complex isolates recovered from clinical specimens in Kuwait identified 108 as *C. parapsilosis*, five as *C. orthopsilosis* and none as *C. metapsilosis*. The results were further validated by PCR-RFLP patterns of the secondary alcohol dehydrogenase gene fragment. DNA sequencing of the ITS region and the D1/D2 regions of the 26S rRNA gene confirmed the species-specific identification of all five *C. orthopsilosis* strains. The amplicon length of the intergenic spacer between the 26S and 5S rRNA genes (IGS1) was also species-specific, and PCR-RFLP analyses of the IGS1 region identified two distinct genotypes among the five *C. orthopsilosis* strains, which corresponded with the ITS region sequence data. The three bloodstream *C. orthopsilosis* strains were confined to a single genotype. Among 81 randomly selected *C. parapsilosis* strains, two genotypes were detected by IGS1 region analyses, indicating limited genotypic heterogeneity among *C. parapsilosis sensu stricto* strains. As far as is known, this is the first report on the identification of *C. orthopsilosis* from a bloodstream infection in the Arabian Gulf region.

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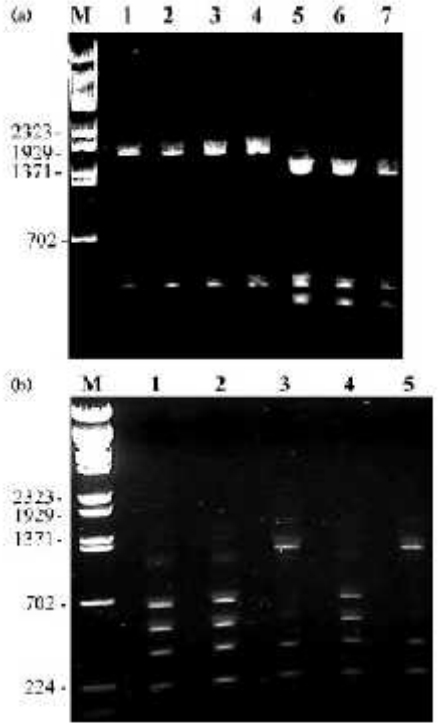

Fig. 3. Representative agarose gel of restriction digestion of IGS1 region amplicons with *Rsa*I from seven selected *C. parapsilosis* strains (*a*) and with *Hin*fI from the five *C. orthopsilosis* strains (*b*). In (a), the RFLP patterns in lanes 1–4 were classified as pattern A, whilst those in lanes 5–7 were classified as pattern B. In (b), the RFLP patterns in lanes 1, 2 and 4 were classified as pattern C, whilst those in lanes 3 and 5 were classified as pattern D. Lane M, *Bst*EII-digested λ DNA ladder, with fragment sizes indicated (bp).