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ORAL MICROBIOLOGY
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Analysis of the strain relatedness of oral *Candida albicans* in patients with diabetes mellitus using polymerase chain reaction-fingerprinting

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To increase our understanding of *Candida* pathogenicity, the identification of those strains most frequently associated with infections is of paramount importance. Polymerase chain reaction (PCR)-based methods are extremely effective in differentiating and determining reproducibility, they require minimum starting material and are rapid and simple to perform. In this study, the genetic relatedness of *Candida albicans* was assessed for two geographically different patient groups (London, UK and Parma, Italy) affected by diabetes mellitus. *C. albicans* samples from the oral cavities of non-diabetic healthy subjects were also examined by PCR fingerprinting to evaluate the possible genetic differences among endogenous strains in individuals with and without diabetes mellitus. PCR fingerprinting, with subsequent phylogenetic analysis of *C. albicans* isolates from the diabetic patients from London and Italy and from the non-diabetic subjects, revealed that there were significant differences ($P < 0.0001$) between *C. albicans* isolates indicative of the distinct ecological niches that occur in the oral cavities of these patient cohorts. The most diverse group comprised the isolates from the diabetic patients in the UK, possibly reflecting the antifungal treatment that these patients had received. Further studies that include isolates from patient cohorts with systemic diseases other than diabetes mellitus, and from more diverse geographic localities are required to explain the relatedness of *C. albicans* isolates in the mouth.

Key words: *Candida albicans* isolates; diabetes mellitus; polymerase chain reaction fingerprinting

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The increasing incidence of oral candidosis, particularly in immunocompromised patients, and the emergence of strains resistant to conventional antifungal therapies have led to the implementation of laboratory techniques that allow the rapid identification of causative organisms and the study of *Candida* strains at species levels. To increase our understanding of *Candida* pathogenicity, the identification of those strains most frequently associated with infections is of paramount importance.

Candida spp., and *Candida albicans* in particular, are ubiquitous organisms that can be acquired endogenously from the host's normal flora or exogenously from the environment, from infected patients or from health-care workers. The ability to identify particularly infective strains allows outbreaks of infections to be monitored and cross-infection controls to be assessed (24).

In addition, strain identification can establish whether relapses of infection are

the result of a novel infecting organism or of persistence/re-infection by the original strain. Furthermore, it is important to remember that more than one strain can be involved in an infection (23, 24).

Specific techniques can also determine the dynamics of yeast populations and allow the study of the association between virulence or drug resistance and specific clones or clusters of related strains.

Traditionally, the methods used to characterize and speciate *Candida* strains were based on the analysis of phenotypic traits, such as colony morphology (and color indicator media), biochemical and serological tests, characteristic carbohydrate assimilation profiles, and chemical resistance patterns. These methods are easy to perform, but they are not particularly specific because unrelated strains of *Candida*, and sometimes even different species, often share phenotypic characteristics (24).

For this reason, strain differentiation methods have been developed based on the analysis of genotypic differences. Genotypic methods based on nucleic acid analysis offer greater differentiation and reproducibility for strain delineation than phenotyping (2, 12).

Strategies based on polymerase chain reactions (PCR), such as PCR fingerprinting and randomly amplified polymorphic DNA methods have been used in particular to investigate cluster infections caused by identical or similar *Candida* strains (22), the emergence of resistant strains during antifungal therapy (15), the colonization pattern of strains in different clinical situations and the microevolution of strains within a particular species (5, 7, 9, 19). These methods are extremely effective in differentiating and determining reproducibility; they require minimum starting material and are rapid and simple to perform (1, 3, 4, 6, 8, 16).

Different PCR fingerprinting studies have been used to investigate *C. albicans* types associated with particular disease conditions, anatomical sites or geographical areas (2, 23). In particular, it has recently been shown that certain related groups of *C. albicans* clones may be endemic in specific hospitals and geographic areas (4, 13, 18, 21). Furthermore, a discrete genetic cluster of fluconazole-resistant *C. albicans* strains recovered from separate human-immunodeficiency-virus-infected patients has recently been observed (24, 25). This finding supports the hypothesis that resistance occurred independently or that a resistant strain was spread by horizontal transmission among patients and highlights the potential value of strain characterization in elucidating pathogenic and epidemiological traits.

In this study, *C. albicans* genetic relatedness was assessed for two geographically different patient groups (London, UK and Parma, Italy) affected by diabetes mellitus. In this way, it would be possible to establish a geographic variation among oral *C. albicans* strains that may be indicative of local diabetic treatment, including antifungal use. Furthermore, *C. albicans* samples from oral cavities of non-diabetic healthy subjects were also analyzed by PCR fingerprinting to evaluate the possible genetic differences among diabetic endogenous strains and non-diabetic strains. To the best of our knowledge, this is the first study to evaluate the genetic variability of the *C. albicans* strains among diabetic patients and non-diabetic subjects using PCR fingerprinting methods. Furthermore, it is hypothesized that UK diabetics and Italian diabetics will carry genetically distinct *C. albicans* strains arising from the respective different treatment regimens.

Material and methods

Diabetic and non-diabetic subjects

As previously described (10), two geographically different groups of patients affected by diabetes mellitus (137 from London, UK and 105 from Parma, Italy) and a total of 130 healthy, non-diabetic patients (enrolled in London, UK) were investigated in this study. A medical history of each patient was recorded, including tobacco-smoking habits, type of diabetes mellitus (type 1 and 2) and duration of disease (time since diagnosis <10 years or >10 years). The presence of most common long-term complications of diabetes (retinopathy, neuropathy, and nephropathy) was also recorded. Glycemic control, assessed by HbA_1c (glycosylated hemoglobin), was used as a measure of recent diabetic control. Patients were then classified into three comparable groups depending upon the % hemoglobin glycosylation at the time of oral examination (group 1: <7.5%; group 2: >7.5%, <8.5%; group 3: >8.5%). All the patients received a detailed oral examination, with all areas of the oral cavity thoroughly inspected together with the presence or absence of dentures (either partial or full) (Table 1).

None of the diabetic or non-diabetic subjects evaluated in the present study was affected by oral candidosis at the time of the oral examination. In addition none of these patients had received antifungal drugs in the preceding 6 months.

Oral *Candida* isolates

C. albicans isolates were collected as previously described (10). All isolates were well characterized and had been defined at species level as *C. albicans* (10) and tested for their *in vitro* antifungal susceptibility to six antifungal agents at two different concentrations (fluconazole 8 and 64 μg/ml, itraconazole 0.5 and 4 μg/ml, miconazole

Table 1. Comparison of patient gender, age, type, and control of diabetes mellitus (DM) and denture status between patients with diabetes from two different geographic locales

Variables investigated	London DM patients (%)	Parma DM patients (%)	*P*-value
Gender			
Male	70/137 (51.1)	55/105 (52.4)	0.89
Female	67/137 (48.9)	50/105 (47.6)	
Age, mean (SD)	54.8 (16.2)	61.1 (14.1)	0.001
DM type			
Type 1	56/137 (40.9)	19/105 (18.1)	0.0001
Type 2	81/137 (59.1)	86/105 (81.9)	
Time since diagnosis of DM			
<10 years	35/101[1] (34.7)	54/105 (51.4)	0.08
>10 years	66/101[1] (65.3)	51/105 (48.6)	
Complications of DM			
Neuropathy	37/137 (27)	11/105 (10.5)	<0.0001
Retinopathy	41/137 (29.9)	31/105 (29.5)	
Nephropathy	4/137 (2.9)	21/105 (20)	
Diabetic control (HbA_1c)			
HbA_1c <7.5%	52/137 (38)	23/105 (22)	<0.0001
HbA_1c >7.5 to <8.5%	21/137 (15.3)	41/105 (39)	
HbA_1c >8.5%	64/137 (46.7)	41/105 (39)	
Denture status			
Dentate	97/137 (70.8)	45/105 (42.9)	<0.0001
Dentures	40/137 (29.2)	60/105 (57.1)	

Statistical analysis was performed using Fisher's exact and chi-squared parametric tests.
[1]For 36 of the London DM patients it was not possible to establish the date when their DM was diagnosed.

0.5 and 8 μg/ml, ketoconazole 0.5 and 4 μg/ml, amphotericin B 2 and 8 μg/ml and 5-FC 2 and 32 μg/ml) with a commercial kit following the methodology outlined by the manufacturer (Fungitest, Bio-Rad, Manrnes La-Coquette, France), as previously described (11).

PCR fingerprinting analysis

The analysis conducted in this study attempted to evaluate the genetic differences in *C. albicans* isolated from three different groups of patients (diabetic patients from London, UK; diabetic patients from Parma, UK; and non-diabetic control patients from London, UK). The analysis did not include other *Candida* spp., which may present a greater genetic variability. Furthermore, because five different PCR fingerprinting primers were used (Table 2), the analysis was limited to a representative number of *C. albicans* isolates (89 isolates), and a power analysis before this study suggested that this number would be adequate to reveal any genetic differences present.

Genomic DNA was extracted from all isolates, as previously described (20). Five separate PCR fingerprinting techniques were performed to assess strain similarity genotypically. Each of these techniques used a specific single primer (Table 2) and controlled thermocycler parameters.

In particular, the DNA of the isolates was amplified in a reaction volume of 20 μl containing 1 μM from each primer (0.5 μl), 0.5 U (0.5 μl) of Red *Taq* polymerase (Sigma-Aldrich, Dorset, UK) and Red *Taq* buffer (supplied by the same manufacturer as a 10 × concentrate and used to give a final concentration of 1.1 mM $MgCl_2$, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM GTP and 0.2 mM dTTP).

PCR fingerprinting with the M13 phage core sequence, the intergenic spacer repeat of tRNA T3B, the telomeric core sequence TELO1, the simple repeat sequences (GACA)4 (Table 2) was performed with a heated-lid thermocycle PCR machine (Techne Genius, Jepson Bolton & Co Ltd, Watford, Herts, UK) for 1 cycle at 97°C for 3 min, 40 cycles for 40 s at 93°C, 2 min at 50°C, 40 s at 72°C, and a final step of 10 min at 72°C. PCR fingerprinting with the otigonucleotide OPA-03, which requires lower temperatures, was performed for one cycle at 97°C for 3 min, 45 cycles for 1 min at 93°C, 2 min at 36°C, 2 min at 72°C, and a final step of 10 min at 72°C, as previously reported (25).

All PCR amplicons were separated in 2% agarose gel in TAE buffer for 2 h at 2 V/cm, and visualized by ultraviolet transillumination following ethidium-bromide staining.

The results of the five PCR fingerprinting methods were combined by selecting three to five polymorphic bands for each method, resulting in 17 polymorphic bands that were shown to be widely spread through the genome. Each band was scored as either present or absent. These results were phylogenetically analyzed using the computer program PAUP 4.08. Simple analyses were carried out using the optimal criterion as distance and pairwise distances between each of the isolates measured. To aid visualization of the relationship between isolates based on this analysis, a phylogram was generated by the unweighted pair-group method with arithmetic mean (UPGMA) (17). Ties (if encountered) were broken systematically and the distance measure was set to mean character difference.

Results

Twenty-nine *C. albicans* isolates from the non-diabetic control UK patients, 30 from the diabetic UK patients and 30 from the diabetic Italian patients (for a total of 89 *C. albicans* isolates) were analyzed using the results from all five fingerprinting methods. Each isolate was given a designation consisting of 17 digits. All 17 of the polymorphic bands chosen were parsimony informative. These digits were either 0 or 1, depending on whether the polymorphic band was either absent or present respectively. For example, Table 3 shows the genotypic designation of eight randomly chosen *C. albicans* isolates. The number of differences in the bands between strains was measured.

Figures 1–5 show examples of electrophoretic separation of PCR fingerprinting using the five different primers. Statistical analyses of these PCR fingerprint banding pattern differences (Table 4) showed that there was an average of 3.24 differences between all isolates and genotype B; the C isolate group was significantly more genetically coherent (mean difference of 2.23 bands) than the genotype A *C. albicans* isolate group (mean difference of 3.72 bands, $P < 0.0001$). Moreover, the isolates from the Italian diabetic patients were the most genetically cohesive group, with a mean difference of only 2.93 bands; the isolates from the control patients had 3.09 mean differences and the isolates from the UK diabetic patients had significantly more genetic diversity with a mean number of differences between isolates of 3.65 ($P < 0.0001$; Table 4).

Phylogenetic analysis of these isolates was graphically depicted by a phylogram, shown in Fig. 6. The distance shown at the bottom of this phylogram of 0.1 is the distance within which strains are 90% similar. The 89 *C. albicans* isolates studied can thus be grouped and sub-grouped based on this phylogenetic analysis. Figure 6 (A,B) shows that these isolates can be divided into four large genotypic groups (A to D) that are co-located on the phylogram (Fig. 6) and are more than 90% genotypically similar, while the other 17 genotypic sub-groups (A1–A17) are co-located and more than 95% similar.

Table 3. Genetic strain designation of eight randomly selected isolates based on the 17 polymorphic PCR fingerprinting methods

Strain designation	TELO	GACA	M13	OPA-03	T3B
A	0100	010	011	11	10100
B	0100	110	011	11	11101
C	0010	110	011	10	00001
D	0010	110	011	01	00011
E	0101	110	011	11	11101
F	0100	110	011	11	11101
G	0100	010	011	11	11101
H	0101	110	111	11	11101

Table 2. Designation and sequence of the five primers used in each of the five distinct PCR fingerprinting techniques for phylogenetic analysis

Designation	Sequence
TELO	TGG GTG TGT GGG TGT GTG GGT GTG
GACA	GAC AGA CAG ACA GAC A
M13	GAC GGT GGC GGT TCT
OPA-03	AGT CAG CCA C
T3B	AGG TCG CGG GTT CGA ATC

Discussion

The observed variation in genotypic diversity of the *C. albicans* isolates in the present study may be indicative of the distinct ecological niches that occur in the oral cavities of these patient cohorts. The statistical correlation of this genotypic diversity, as assessed in the present study, shows that the group of *C. albicans* strains isolated from the diabetic patients in the UK were the most diverse (mean diversity 3.65, Table 4). This diversity was statisti-

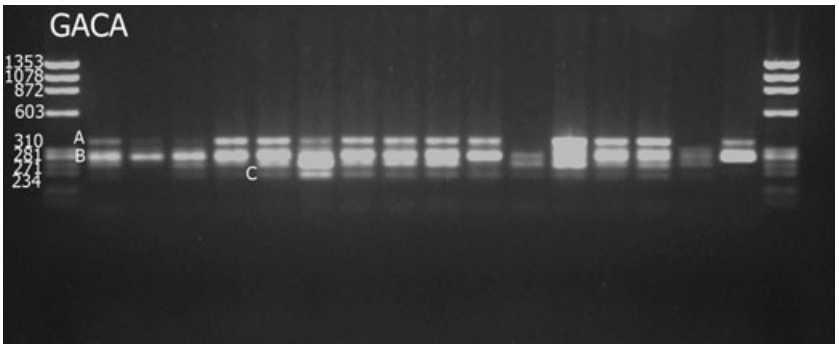

Fig. 1. Examples of electrophoretic separation of PCR fingerprinting using $(GACA)_4$ sequence. Three bands were selected for evaluation in this study: (A) *c.* 310 bp, (B) *c.* 281 bp; (C) *c.* 234 bp.

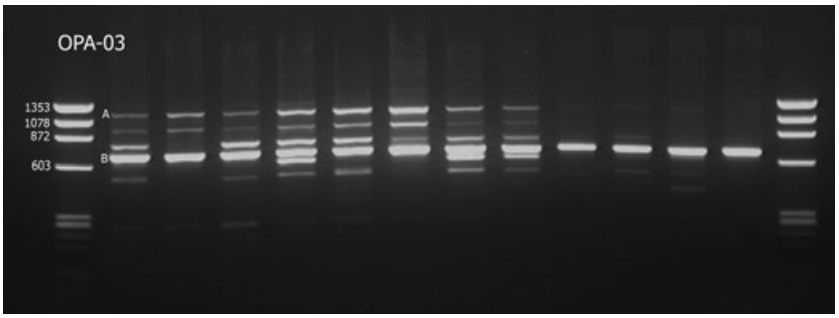

Fig. 2. Examples of electrophoretic separation of PCR fingerprinting using OPA-03 sequence. Two bands were selected for evaluation in this study: (A) *c.* 1053 bp; (B) above 603 bp.

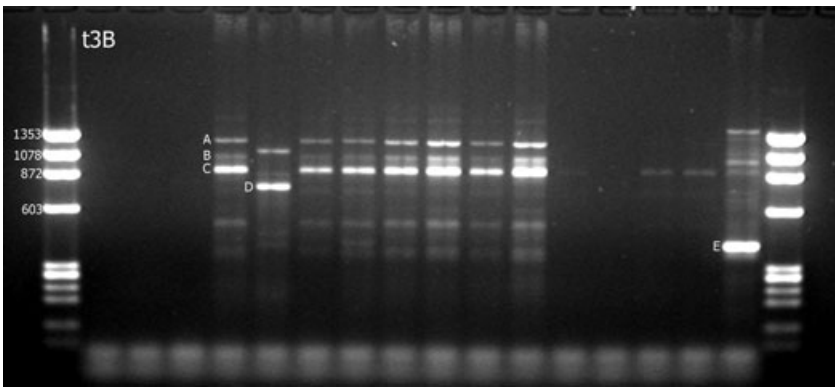

Fig. 3. Examples of electrophoretic separation of PCR fingerprinting using T3 B sequence. Five bands were selected for evaluation in this study: (A) *c.* 1053 bp; (B) *c.* 1078 bp; (C) *c.* 872 bp; (D) below 872 bp; (E) above 310 bp.

cally higher than that of either control patient isolates (mean diversity 3.09, Table 4) or isolates from diabetic patients in Italy (mean diversity 2.93, Table 4). This result may reflect the patient cohort from which these strains were isolated, with diabetic patients in the UK affected by longstanding diabetes mellitus and with more diabetic complications (neuropathies and retinopathies) than patients from Italy. Alternatively, it may reflect the antifungal treatment that these patients received. In particular, more *Candida* isolates from UK patients had increased resistance to azole agents and a trend of increased resistance to amphotericin B than those from diabetic patients from Parma (11).

Further research is necessary to elucidate the true cause of the observed genotypic diversity, with the inclusion of isolates from patient cohorts with systemic diseases different to diabetes, and from more diverse geographic localities.

The broad genotypic subgroups of isolates designated as subgroups A, B, and C are based on the presence or absence of a transposable intron within the 25S rDNA (14). Genotypic subgroup A does not have this intron, while in subgroup B it is present in all copies of the 25S rDNA (379 nucleotide, named Group I intron) and in subgroup C it is partially present (a mixture of intron-containing and intronless 25S rDNA) (14). The analysis of the genotypic diversity in the present study showed that subgroup A isolates without the intron were less genotypically cohesive than subgroup B or C isolates. It may well be that the ability to acquire this intron within the 25S rDNA is confined to a particularly genetically cohesive sub-group of *C. albicans* that possesses currently unknown phenotypic traits. Interestingly, the present study did not find any association between a particular genotype of *C. albicans* and the type, disease progression and degree of control of diabetes mellitus, although previous studies have shown that these strains exhibit enhanced antifungal susceptibility (13, 14). In particular, these studies showed that genotypes B and C of *C. albicans* showed a greater susceptibility to 5-FC than the intronless *C. albicans* genotype A.

Phylogenetic analysis of these isolates, as presented in the phylogram (Fig. 6 A,B) and the table of clustered isolates (Table 4), shows a different aspect of the genotypic diversity of these isolates from that expressed by the statistical analysis. The statistical analysis describes the similarity that each isolate had with other isolates from patients within the same cohort. The phylogenetic analysis assesses the uniqueness of each isolate and parsimoniously compares this with all isolates studied.

Phylogenetic analysis has revealed several interesting findings. Firstly, this PCR fingerprinting method found genetic identity in eight pairs of isolates (subgroups A2, A5, A10 twice, A14, A16 twice and A17), four triplets (subgroups A3, A4 and A8 twice), a group of seven isolates (subgroup A4), a group of 10 isolates (subgroup A2) and a group of 12 isolates (also in subgroup A2) (Fig. 6). This would indicate that the PCR fingerprinting method is not so overtly sensitive that it discriminates all isolates to the individual level, yet it is able to cluster genetically similar isolates into useful epidemiological subgroups. The ability to discriminate isolates to an individual identity would

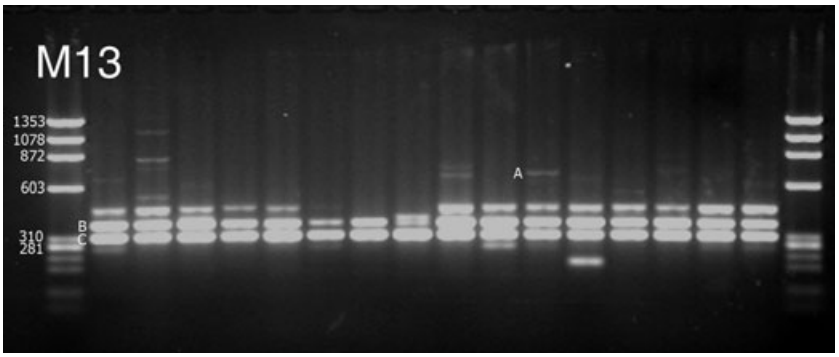

Fig. 4. Examples of electrophoretic separation of PCR fingerprinting using M13 phage core sequence. Three bands were selected for evaluation in this study: (A) below 872 bp; (B) above 310 bp; (C) *c.* 310 bp.

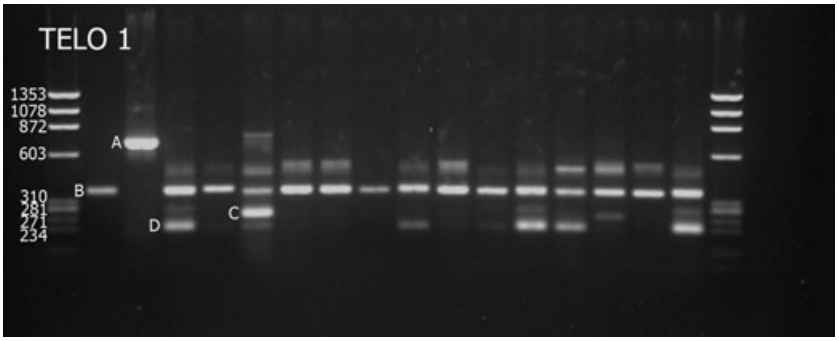

Fig. 5. Examples of electrophoretic separation of PCR fingerprinting using TELO 1. Four bands were selected for evaluation in this study: (A) above 603 bp; (B) above 310 bp; (C) *c.* 271 bp; (D) *c.* 234 bp.

Table 4. Statistical analysis of the PCR fingerprint banding pattern

Variable	Isolates	*n*	Mean	*P*-value
All isolates	89	3916	3.24 (2.06)	<0.0001[1]
C. albicans genotype A	58	1653	3.72 (2.25)	
C. albicans genotypes B and C	31	465	2.23 (1.17)	
Non-diabetic control subjects	29	406	3.09 (1.79)	<0.0001[2]
UK diabetic patients	30	435	3.65 (2.28)	
Italian diabetic patients	30	435	2.93 (1.92)	

Statistical analysis was performed using [1]*t*-test and [2]ANOVA.
n is the number of comparisons made between the isolates within the cohort of patients resulting in the given mean (SD) genotypic diversity for each of the groups. For example, the mean genotypic diversity observed within the Italian diabetic patients' *Candida albicans* isolates was 2.93 (SD 1.92) genetic characters which was significantly lower than that observed in the UK diabetic patients ($P < 0.0001$). Thus, the Italian diabetic patients' *C. albicans* isolates were more genotypically homogeneous than those from the UK diabetic patients.

require methods of much higher sensitivity that the present study, such as DNA sequencing or micro-array technology, and was not the purpose of the present study. Furthermore, the fact that all 17 PCR fingerprinting bands chosen for inclusion in the analysis were parsimony informative reflects the genetic diversity of these codominant markers.

Second, out of the 15 groups of isolates outlined in the preceding paragraph that showed genotypic identity with this method, in only two of these groups did all isolates come from the same patient cohort. This occurred with two pairs of isolates in the subgroups A2 and A16. The fact that this PCR fingerprinting method found isolate identities between isolates from all three patient cohorts reflects the genetic similarity that exists within this fungal species. A more interesting observation is the extreme genetic diversity seen in isolates within subgroup D (Fig. 6). These isolates had very little genetic relationship with either the other *C. albicans* isolates or other members of the same subgroup. The presence of these isolates did not skew the statistical analysis (Fig. 6) as only one of these seven isolates (DL126) came from the most genotypically diverse patient cohort (the strains isolated from the diabetic patients in London, UK). Therefore, as indicated above there must be other reasons for the genotypic diversity observed in the diabetic London patient isolates. Nevertheless, the extremely genetically diverse isolates that make up this subgroup D (Fig. 6) warrant further study. It could be hypothesized that this subgroup represents a unique non-clonal expansion of *C. albicans* strains that may indicate either sexual or some other method of rapid transfer of genetic information.

Finally, and as indicated above, the present analysis was only conducted with patients from two localities, who were either deemed suitable to represent a 'normal' control population, or who had diabetes.

Further studies that include isolates from patient cohorts with systemic diseases other than diabetes, and from more diverse geographic localities are needed to compare the results obtained in these population groups.

References

1. Barchiesi F, Di Francesco LF, Compagnucci P, Arzeni D, Cirioni O, Scalise G. Genotypic identification of sequential *Candida albicans* isolates from AIDS patients by polymerase chain reaction techniques. Eur J Clin Microbiol Infect Dis 1997: **16**: 601–605.
2. Bartie KL, Williams DW, Wilson MJ, Potts AJ, Lewis MA. PCR fingerprinting of *Candida albicans* associated with chronic hyperplastic candidosis and other oral conditions. J Clin Microbiol 2001: **39**: 4066–4075.
3. Carlotti A, Chaib F, Couble A, Bourgeois N, Blanchard V, Villard J. Rapid identification and fingerprinting of *Candida krusei* by PCR-based amplification of the species-specific repetitive polymorphic sequence CKRS-1. J Clin Microbiol 1997: **35**: 1337–1343.
4. Clemons KV, Feroze F, Holmberg K, Stevens DA. Comparative analysis of genetic variability among *Candida albicans* isolates from different geographic locales by three genotypic methods. J Clin Microbiol 1997: **35**: 1332–1336.
5. Dassanayake RS, Samaranayake LP. Randomly amplified polymorphic DNA fingerprinting: the basics. Methods Mol Biol 2003: **226**: 117–122.

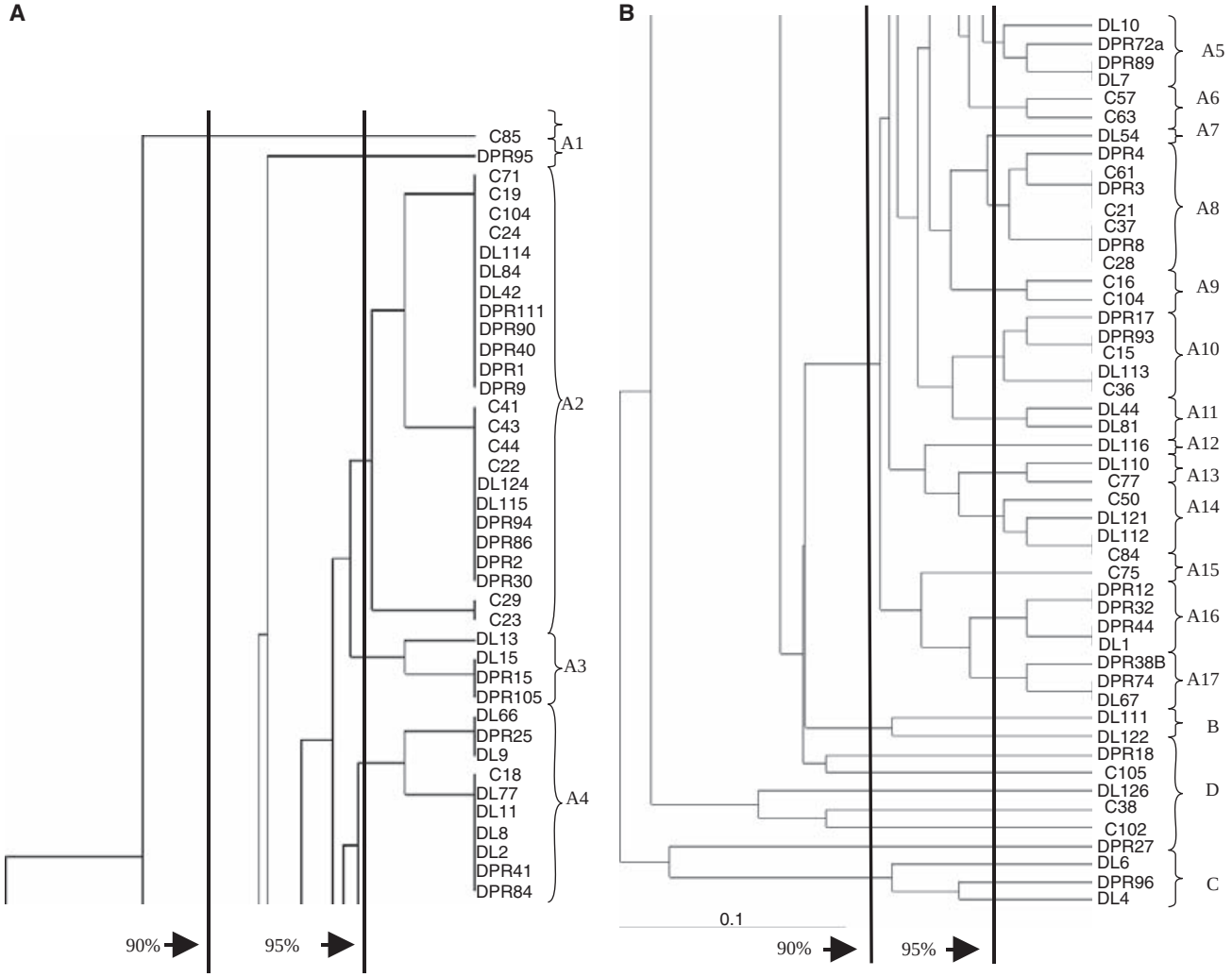

Fig. 6. (A) First part (of two) of the phylogram of the 89 *C. albicans* isolates based on the analysis of the PCR fingerprinting results. To the right of the vertical lines strains are 90% and 95% similar. Phylogenetic grouping and designations shown on the right margin and are based on these levels of similarity. (B) Second part (of two) of the phylogram of the 89 *C. albicans* isolates based on the analysis of the PCR fingerprinting results. To the right of the vertical lines strains are 90% and 95% similar. Phylogenetic grouping and designations shown on the right margin and are based on these levels of similarity.

6. Diaz-Guerra TM, Martinez-Suarez JV, Laguna F, Rodriguez-Tudela JL. Comparison of four molecular typing methods for evaluating genetic diversity among *Candida albicans* isolates from human immunodeficiency virus-positive patients with oral candidiasis. J Clin Microbiol 1997: **35**: 856–861.
7. Enger L, Joly S, Pujol C, Simonson P, Pfaller M, Soll DR. Cloning and characterization of a complex DNA fingerprinting probe for *Candida parapsilosis*. J Clin Microbiol 2001: **39**: 658–669.
8. Lischewski A, Ruhnke M, Tennagen I, Schonian G, Morschhauser J, Hacker J. Molecular epidemiology of *Candida* isolates from AIDS patients showing different fluconazole resistance profiles. J Clin Microbiol 1995: **33**: 769–771.
9. Lockhart SR, Joly S, Pujol C, Sobel JD, Pfaller MA, Soll DR. Development and verification of fingerprinting probes for *Candida glabrata*. Microbiology 1997: **143**: 3733–3746.
10. Manfredi M, McCullough MJ, Al-Karaawi ZM, Hurel SJ, Porter SR. The isolation, identification and molecular analysis of *Candida* spp. isolated from the oral cavities of patients with diabetes mellitus. Oral Microbiol Immunol 2002: **17**: 181–185.
11. Manfredi M, McCullough MJ, Polonelli L et al. In vitro antifungal susceptibility to six antifungal agents of 229 *Candida* isolates from patients with diabetes mellitus. Oral Microbiol Immunol 2006: **21**: 177–182.
12. McCullough MJ, Ross BC, Reade PC. *Candida albicans*: a review of its history, taxonomy, epidemiology, virulence attributes, and methods of strain differentiation. Int J Oral Maxillofac Surg 1996: **25**: 136–144.
13. McCullough MJ, Clemons KV, Stevens DA. Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. J Clin Microbiol 1999: **37**: 417–421.
14. Mercure S, Montplaisir S, Lemay G. Correlation between the presence of a self-splicing intron in the 25S rDNA of *C. albicans* and strains susceptibility to 5-fluorocytosine. Nucleic Acids Res 1993: **21**: 6020–6027.
15. Metzgar D, van Belkum A, Field D, Haubrich R, Wills C. Random amplification of polymorphic DNA and microsatellite genotyping of pre- and post-treatment isolates of *Candida* spp. from human immunodeficiency virus-infected patients on different fluconazole regimens. J Clin Microbiol 1998: **36**: 2308–2313.
16. Meyer W, Latouche GN, Daniel HM et al. Identification of pathogenic yeasts of the imperfect genus *Candida* by polymerase chain reaction fingerprinting. Electrophoresis 1997: **18**: 1548–1559.
17. Nei M. *Molecular evolutionary genetics*. New York, NY: Columbia University Press, 1987.

18. Pfaller MA, Lockhart SR, Pujol C et al. Hospital specificity, region specificity, and fluconazole resistance of *Candida albicans* bloodstream isolates. J Clin Microbiol 1998: **36**: 1518–1529.
19. Pujol C, Joly S, Lockhart SR, Noel S, Tibayrenc M, Soll DR. Parity among the randomly amplified polymorphic DNA method, multilocus enzyme electrophoresis, and Southern blot hybridization with the moderately repetitive DNA probe Ca3 for fingerprinting *Candida albicans*. J Clin Microbiol 1997: **35**: 2348–2358.
20. Scherer S, Stevens DA. Application of DNA typing methods to epidemiology and taxonomy of *Candida species*. J Clin Microbiol 1987: **25**: 675–679.
21. Schmid J, Herd S, Hunter PR et al. Evidence for a general-purpose genotype in *Candida albicans*, highly prevalent in multiple geographical regions, patient types and types of infection. Microbiology 1999: **145**: 2405–2413.
22. Shin JH, Kook H, Shin DH et al. Nosocomial cluster of *Candida lipolytica* fungemia in pediatric patients. Eur J Clin Microbiol Infect Dis 2000: **19**: 344–349.
23. Soll DR. The ins and outs of DNA fingerprinting the infectious fungi. Clin Microbiol Rev 2000: **13**: 332–370.
24. Sullivan DJ, Coleman DC. Molecular approaches to identification and typing of *Candida* species. In: Calderone RA, ed. *Candida and candidiasis*. Washington DC, USA: American Society of Microbiology Association (ASM) Press, 2002: 427–441.
25. Xu J, Ramos AR, Vilgalys R, Mitchell TG. Clonal and spontaneous origins of fluconazole resistance in *Candida albicans*. J Clin Microbiol 2000: **38**: 1214–1220.