Abstract

The polymorphism of five new microsatellite *loci*, located outside and inside known coding regions, in the genome of the pathogenic yeast *C. albicans*, was investigated in order to evaluate their applicability to accurately differentiate strains. The microsatellites were selected so that each was assigned to a different chromosome in order to evenly span them throughout the genome. A multiplex PCR strategy was developed allowing the simultaneous screening of the five markers, followed by GenScan analysis of the products, providing a rapid and accurate methodology for genotyping large numbers of strains. A total of 122 *C. albicans* strains, obtained from 80 patients and collected from three health institutions, were analysed using this multiplex system. Seventy-eight different genotypes were observed resulting in a discriminatory power of 0.98. When applying these microsatellites to the identification of strains isolated from recurrent vulvovaginal infections in eight patients, it was found that 13 out of 15 episodes were due to the same strain. When multiple isolates obtained from the same patient were studied the results showed that in different body sites, patients can harbour distinct clones but the infecting population at each body site is monoclonal. These new microsatellites proved to be a valuable tool to differentiate *C. albicans* strains and when compared to other molecular genotyping techniques, revealed to be simple, efficient and reproducible, being suitable for application in large scale epidemiological studies. Allele nomenclature based on the number of repeat sequences rather than fragment size is proposed for the characterization of each strain and contribute for the construction of a public database in light of what is already in use for other organisms.

DEVELOPMENT OF A MICROSATELLITE MULTIPLEX PCR STRATEGY FOR DIFFERENTIATION OF *CANDIDA ALBICANS* STRAINS

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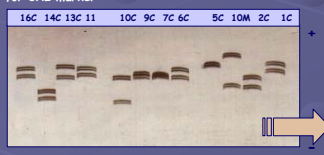
INTRODUCTION

Opportunistic yeast pathogens are common residents of the mucosal surfaces of the gastrointestinal tract, genitourinary system and oral cavity in warm-blooded animals. Although several yeast species can be associated to infection the predominant causal agent of candidiasis is *Candida albicans*. This yeast causes several infections in humans including a wide variety of life threatening conditions triggered by bloodstream infections, especially in immunocompromised patients.
Since pathogenecity and antifungal susceptibility often vary among strains, a rapid and accurate identification of the disease causing strains of *C. albicans* is crucial for clinical treatment and epidemiological studies. Also, if commensal strains can be replaced by certain more pathogenic genotypes, identifying the routes of transmission of the potentially more virulent genotypes could lead to measures to limit their spread.
In the present study, five new microsatellite *loci* were identified and characterised. A PCR multiplex strategy was developed allowing the simultaneous screening of these new markers, followed by GenScan analysis of the products, providing a rapid and accurate methodology for typing large numbers of yeast strains.

RESULTS AND DISCUSSION

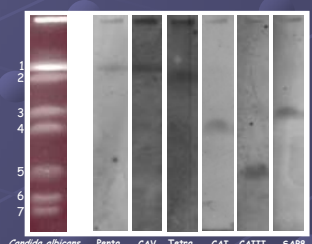
A search in *C.albicans* genome sequences, was conducted for sequences containing short tandem repeats (STRs) or microsatellite. Based on the results of studies on amplification efficiency, species specificity and observed polymorphism, 5 new loci (designated by CAI, CAIII, CAV, Tetra and Penta) were selected for further characterisation.

Denaturing gel electrophoresis of the fragments obtained by PCR of 12 *C. albicans* clinical isolates for CAI marker



All 5 STR *loci* exhibit high number of alleles and genotypes

For allele size determination, the PCR products were run in an ABI 310 Genetic Analyser. Fragment sizes were determined automatically using the GeneScan 3.1 Analysis software. Alleles have been designated by the number of repeats.

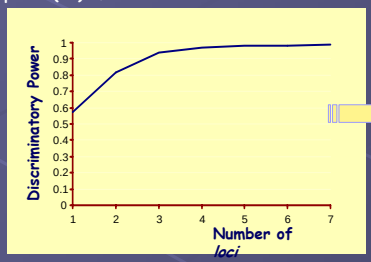


STR selection was made so that each marker was assigned to a different chromosome in order to evenly span them throughout the genome. Separation of *C. albicans* chromosomes was performed by Pulsed Field Gel Electrophoresis (PFGE) followed by hybridisation with the respective probes in high stringency conditions.

Characteristics of the markers involved in the multiplex system

STR *loci*	Repeat sequence	Number of alleles	Number of genotypes	Chromosome location
CAI	$(CAA/G)_n$	27	46	4
CAIII	$(GAA)_n$	6	12	5
CAV	$(ATT)_n$	22	22	1
Tetra	$(TAAA)_n$	30	40	2
Penta	$(CAAAT)_n$	5	8	1
Multiplex	--------	-----	60	--------

The multiplex PCR was applied for genotyping 122 stains collected in three Health Institutions from 69 distinct patients and different body locations. Only strains isolated from non related patients were considered and 78 genotypes were observed resulting in a discriminatory power (DP) of 0.98.



Five STR *loci* are enough to obtain the maximum DP for this multiplex system

Increasing the number of STR *loci* to seven did not improve the discriminatory power of the multiplex system

The multiplex system was applied in the study of multiple isolates from the same patients in order to determine if the infecting yeasts were the same strain or if there were several strains cohabiting in the same local.

Patient	Isolate	Body location	CAI	Tetra	CAIII	CAV	Penta
	1M	Urine	21-25	9-16	6-7	99-99	5-5
A	2M	Urine	21-25	9-16	6-7	99-99	5-5
	31M	Urine	21-25	9-16	6-7	99-99	5-5
	4M	Peritoneal exsudate	26-26	7-7	6-6	99-99	5-5
B	15M	Peritoneal exsudate	26-26	7-7	6-6	99-99	5-5
	17M	Peritoneal exsudate	26-26	7-7	6-6	99-99	5-5
	19M	Peritoneal exsudate	26-26	7-7	6-6	99-99	5-5
C	10M	Upper respiratory tract	17-17	7-14	6-8	99-99	6-6
	12M	Upper respiratory tract	17-17	7-14	6-8	99-99	6-6
	41M	Urine	21-22	7-7	7-7	99-99	6-6
E	43M	Urine	21-22	7-7	7-7	99-99	6-6
	45M	Urine	21-22	7-7	7-7	99-99	6-6
	47M	Urine	21-22	7-7	7-7	99-99	6-6
	48M	Urine	21-22	7-7	7-7	99-99	6-6
I	64M	Upper respiratory tract	22-22	7-7	6-7	99-102	6-6
	67M	Upper respiratory tract	22-22	7-7	6-7	99-102	6-6
	88M	Urine	20-28	42-44	6-9	99-99	5-6
J	69M	Upper respiratory tract	21-25	9-15	6-7	99-99	5-5
	75M	Urine	21-25	9-15	6-7	99-99	5-5
	86M	Urine	21-25	9-15	6-7	99-99	5-5
L	82M	Urine	18-47	9-19	6-11	99-177	5-10
	84M	Urine	18-47	9-19	6-11	99-177	5-10

Conclusions

This multiplex system proved to be a valuable tool to differentiate C. albicans strains being suitable for the study of

Recurrent infections

Nosocomial infections

Large scale epidemiological studies

Taking into account the mode of reproduction of *C. albicans*, the discriminatory power of the multiplex and the results obtained, the infecting strain appears to be the same when considering the same isolation site. In different body locations, the infecting strains are different as it can be seen in the Table above.