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Molecular Typing and Phylogenetic Analysis of *Candida albicans* Isolates from Different Patient Populations

A thesis presented in partial fulfilment of the requirements for the degree of PhD
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Nicole Beate Freifrau von Maltzahn

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ABSTRACT

An important question in understanding the epidemiology of *Candida albicans* is to determine whether certain strains, e.g. HSP strains (highly successful strains), more prevalent in Candidosis patients than in healthy individuals, replace commensal strains under certain conditions and whether the replacing strains may cause Candidosis. This question was investigated in experiments which monitored the genetic diversity of commensal *C. albicans* isolates obtained from individuals before and after they were exposed to conditions that may predispose them to the development of Candidosis. The distinctiveness of *C. albicans* strains isolated from individuals was analysed using the phylogenetic method of split decomposition. The method was found to provide a good representation of the phylogenetic information in strain replacement Ca3 data.

Our study highlighted difficulties in monitoring of strain replacement with Ca3 methodology. An indication for strain replacement was observed in one patient at low risk to acquire Candidosis. However, the observations that cancer patients, who were at a high risk of developing Candidosis, were colonised with diverse strains and that healthy individuals could be colonised with different commensal *C. albicans* strains within one body location, cautioned against overinterpretation of this finding. These results demonstrated the need for extensive sampling of larger numbers of isolates from different body locations when evaluating replacement hypotheses.

In investigating potential sources of *C. albicans* infections we successfully isolated this fungus from the hospital environment of high risk patients, demonstrating the potential of the hospital environments as a source for infection causing strains. In characterising strains, a nonradioactive fingerprinting protocol was developed for a more convenient use of Ca3 fingerprinting.

Until now the existence of the HSP group has been based entirely on Ca3 fingerprinting data. To test for the existence of this group we have analysed amplified fragment length polymorphisms (AFLP) of 36 *C. albicans* isolates from different geographical regions. Phylogenetic reconstruction from both data forms (Ca3 and

AFLP data) were highly congruent and suggest a worldwide distribution of HSP strains. Study of the tree building properties of AFLP and Ca3 data using Quartet Puzzling and a tree comparison metric showed that AFLP data were more treelike than the Ca3 data.

However, whilst both AFLP and Ca3 methods provided high resolution data to identify strains and substrains of *C. albicans*, the need for population based studies to test for strain replacement makes the use of either method limited. For this reason, both nuclear ITS and AFLP derived PCR markers were investigated for their potential use in such studies. In particular, one AFLP derived PCR marker that was partially characterised appears very promising for future strain replacement studies. It is likely to provide a simple diagnostic test for rapid identification of HSP strains.

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ABBREVIATIONS

A	adriamycin, immunosuppressive anticancer drug
ABI	Applied Biosystems
ABCM	combination therapy of four immunosuppressive anticancer drugs: adriamycin, BCNU (cisplatin), cyclophosphamide, melphalan
AFLP	amplified fragment length polymorphism
ALL	acute lymphoid leukaemia
AML	acute myeloid leukaemia
APS	ammonium persulphate
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BLAST	basic local alignment search tool
bp	base pair
BSA	bovine serum albumine
C	cyclophosphamide, immunosuppressive anticancer drug
°C	degree Celsius
Ca3	moderately repetitive sequence of the <i>Candida albicans</i> genome
CBP1	corticosteroid binding protein gene
CHOP	combination therapy of three immunosuppressive anticancer drugs: cyclophosphamide, doxorubicin, vincristine, and the adrenocorticoid prednisone
CLL	chronic lymphoid leukaemia
CML	chronic myeloid leukaemia
CMI	cell-mediated immunity
COP	combination therapy of two immunosuppressive anticancer drugs: cyclophosphamide, vincristine, and the adrenocorticoid prednisone
CSPD	disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1.]decan}-4-yl)phenyl phosphate
C1-C3	cleavage sites
DIG	digoxigenin
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dATP	2' deoxyadenosine triphosphate
dCTP	2' deoxycytidine triphosphate
dGTP	2' deoxyguanosine triphosphate
dNTP	deoxynucleoside triphosphate
dTTP	2' deoxythymidine triphosphate
dUTP	2' deoxyuridine triphosphate
EDTA	ethylenediaminetetraacetic acid
ESR	Environmental Science Research, New Zealand
FA	folinic acid, immunosuppressive anticancer drug
F_{ab}	variable sequence fragment of immunoglobulin
g	gram
GC	content of deoxyguanylate and deoxycytidylate in DNA

h	hour
HAS	hospitalisation for at least 7 days, surgery (hip- or knee replacement), and antibiotic treatment
HSP	highly successful pathogens
IPTG	isopropyl-1-thio- β -D-galactoside
ITS	internal transcribed spacer
kb	kilo base
l	liter
LB	Luria-Bertani broth
LTR	long terminal repeats
M	mole
m	milli
min	minute
MLEE	multilocus enzyme electrophoresis
μ	micro
n.a.	not available
NBT	4-nitro blue tetrazolium chloride
NJ	neighbour-joining
OD	optical density
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
RCF	relative centrifugal force
rDNA	DNA that encodes for ribosomal ribonucleic acid
RFLP	restriction fragment length polymorphism
rRNA	ribosomal ribonucleic acid
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	room temperature
SAB	Sabouraud
S_{AB}	Similarity value
SDS	sodium dodecyl sulphate
subsp.	sub species
TBS	tris-buffered saline
TEMED	NNN'N' tetramethylethylenediamine
T_m	melting temperature
Tris	tris(hydroxymethyl)methylamine
U	unit
UPGMA	unweighted pairgroup method with algorithmic means
URS	upstream regulatory sequence
USA	United States of America
V	volt
Vol	volume
vs.	versus

w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactoside
YPD	Yeast-peptone-dextrose medium
5FU	5-fluorouacil, immunosuppressive anticancer drug

In addition, the conventional one-letter codes for deoxyribonucleosides was applied:

deoxyribonucleosides: A, C, G, T for deoxyadenylate, deoxycytidylate, deoxyguanylate and deoxythymidylate, respectively.

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