Sex has no detectable net benefits for

Candida albicans

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ABSTRACT

Like many other important opportunistic human fungal pathogens, for more than a century *Candida albicans* was thought to be strictly asexual until a parasexual cycle was recently discovered in the laboratory. It is uncertain, however, whether sex is still a viable reproductive strategy for *C. albicans*. In this study I tested whether or not mating enhanced survival of parental genes in this yeast, by mating 10 clinical isolates and testing recombinants' fitness.

Clinical isolates of *C.albicans* usually are diploid, carrying both the *MTL***a** and *MTL*α mating type alleles, each on a different copy of chromosome 5. These strains are apparently incapable of meiosis and cannot mate with each other, because the a1-α2 heterodimer suppresses mating. Through selection on sorbose-containing agar I induced loss of *MTL*-heterozygosity and generated 5 *MTL***a** and 5 *MTL*α derivatives of clinical isolates. Existing mating techniques involve the use of auxotrophic markers, requiring time-consuming sequential disruption of two copies of biosynthetic genes if wild-type clinical isolates are to be mated. Furthermore, auxotrophy affects the virulence of a strain, and this can potentially interfere with comparing the fitness of recombinants with that of their parents. I therefore developed a method for mating clinical isolates marked with two drug resistance markers, the mycophenolic acid (MPA) resistance-conferring allele of *IMH3* and the nourseothricin (NAT) resistance gene *CaNAT1*, allowing selection of recombinants on the basis of resistance to both agents. I marked all *MTL***a** strains

with the MPA resistance gene and all *MTL*α strains with the NAT resistance gene. This allowed 25 combinations for mating. Recombinants were obtained from 15 combinations of 9 strains. It was found that not all *C. albicans* clinical isolates could mate.

Using growth rate as the criterion, I tested the fitness of clinical isolates, *MTL*-homozygous derivatives with and without resistance markers and recombinants during adaptation to a novel environment (YPD medium), maximizing the potential benefits of sex. After computationally correcting for the impact of experimental manipulations, I calculated the net benefit of sex as the difference in the number of offspring from two cells that become mating competent and engage in sex compared to the offspring they could have produced by continued clonal reproduction. My results indicated that, as a rule, engaging in sex reduces the chances of survival of *C. albicans'* genes, in part because *MTL* homozygosis significantly reduced growth rates. Through fitness increase after recombination, sex may eventually confer a net benefit for some strain combinations in the laboratory, but this probably occurs too late to prevent elimination of recombinants by competition and genetic drift in nature. Sex in *C. albicans* therefore diminished parents' chances to pass on their genes to future generations. These findings have a significant impact on the assessment of the role of sex in *C. albicans* and other "asexual" human fungal pathogens. Recent loss of the function of sex and incomplete decay of the sex machinery are the most likely explanation of *C. albicans'*s residual ability to mate, and one that also needs to be considered in other fungal pathogens.

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ABBREVIATIONS

Amp	Ampicillin
aa	amino acid
APS	ammonium persulfate
AWCGS	Alan Wilson Centre Genome services
bp	base pair
^{0}C	degree Celsius
C. albicans	*Candida albicans*
CAIP	Calf intestinal alkaline-phosphate
cDNA	Complementary DNA
Chr (chr)	Chromosome
cm	Centimetre
CSPD	Chloro-5-substituted adamantyl-1,2-dioxetane phosphate
g	gram
DIG	Digoxigenin
DAPI	4', 6'-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
dATP	2'-deoxyadenosine-5'-triphosphate
dCTP	2'-deoxycytidine-5'-triphosphate
dGTP	2'-deoxyguanosine-5'-triphosphate
dTTP	2'-deoxythymidine-5'-triphosphate
dNTP	Deoxynucleoside triphosphate

DTT	Dithiothreitol
DMSO	Dimethyl sulfoxide
E.coli	*Escherichia coli*
EDTA	Ethylenediamine tetra-acetic acid
FACS	Fluorescence Activated Cell Sorting
GPG	General purpose genotype
h	hour
IPTG	Isopropyl-ϒ-D-thiogalactopyranoside
kb	kilobase
L	litre
LB	Luria-Bertaini medium
mg	milligram
μl	microlitre
M	Molar, moles per litre
ml	millilitre
MPA	mycophenolic acid
MM	Minimal medium
MTL	Mating-type like
NAT	nourseothricin
Non-GPG	non-general purpose genotype
OD	Optical Density
ORF	open reading frame
PAGE	Polyacrylamide gel electrophoresis
pBSKS(+)	pBluescript KS(+)
PCR	Polymerase chain reaction

pH	-Log[H^+]
RNase	Ribonuclease
rpm	Revolutions Per Minute
S. cerevisiae	*Saccharomyces cerevisiae*
SDS	sodium dodecyl sulfate
TAE	Tris/ Acetic acid /EDTA
TBE	Tris/Borate/EDTA
TNE	Tris/NaCl/EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
X-gal (BCIG)	bromo-chloro-indolyl-galactopyranoside
YPD	Yeast Extract Peptone Dextrose Medium
w/v	Weight/volume