Short Communication

URA5* GENE OF *CRYPTOCOCCUS NEOFORMANS* VAR. *GATTII*: EVIDENCE FOR A CLOSE PHYLOGENETIC RELATIONSHIP BETWEEN *C. NEOFORMANS* VAR. *GATTII* AND *C. NEOFORMANS* VAR. *NEOFORMANS

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Cryptococcus neoformans causes life-threatening meningoencephalitis in about 10% of patients with AIDS in the United States (21). *C. neoformans* (strains) have been divided into two varieties known as *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* (10–12,19). The two varieties manifest differences in biochemical (2,15,17), morphological (14,19), serological (9,11), and epidemiological characteristics (6,7,13). The exact phylogenetic relationship between *C. neoformans* var. *gattii* and *C. neoformans* var. *neoformans* is unclear. DNA hybridization studies indicate that their DNAs are substantially different with only 55–63% homology (1). However, analysis of restriction fragment length polymorphisms (RFLPs) of polymerase chain reaction-amplified segments of the rRNA genes revealed no differences between the two varieties (20).

To better understand the genetic relationship between *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* we have determined the nucleotide sequence of the *URA5* protein coding region from two *C. neoformans* var. *gattii* strains. The nucleotide sequences of the several fungal *URA5* genes have been reported (3,5,16,18), and deduced amino acid sequences show conservation of protein structural motifs (16,18). The *URA5* (orotidine monophosphate pyrophosphorylase) locus of *C. neoformans* var. *neoformans* (5) is polymorphic in nucle-

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Abbreviations: PCR, polymerase chain reaction; *URA5*, orotidine monophosphate pyrophosphorylase; RFLP, restriction fragment length polymorphism.

tide sequence with conservation of the enzyme primary structure (4).

C. neoformans strains were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, U.S.A.). Strains ATCC 24065 (Serotype B) and ATCC 24066 (Serotype C) were resistant to L-canavanine consistent with their classification as *C. neoformans* var. *gattii* (*Filobasidiella neoformans* var. *bacillispora*) (15). The *URA5* DNA from the *C. neoformans* var. *gattii* strains ATCC 24065 and 24066 was amplified from genomic DNA by polymerase chain reaction (PCR) (8). The PCR used oligonucleotides TTAAGACCTCTGAACACC and ATGTCCTCCCAAGCCCTC designed from the *C. neoformans* var. *neoformans* *URA5* sequence (5). Amplification conditions were: 92°C for 1 min, 45°C for 1 min, and 72°C for 1.5 min performed for at least 30 cycles in a Perkin-Elmer thermal cycler (Piscataway, New Jersey, U.S.A.).

Amplification of the *URA5* gene from *C. neoformans* var. *gattii* ATCC 24065 and 24066 yielded a single DNA segment which migrated in agarose gels with an apparent size of 0.8 kb. The PCR-amplified DNA segments were cloned into the pCRTM1000 vector (TA Cloning System; Invitrogen, San Diego, U.S.A.). *E. coli* transformants were selected on agar containing kanamycin 50 µg/ml. Plasmid DNA was purified using the Qiagen Maxi-Prep columns (Chatsworth, California, U.S.A.) according to the manufacturer's instructions. The DNA sequence was determined by the dideoxynucleotide-chain termination method utilizing the Sequenase version 1.0 kit (United States Biochemical, Cleveland, Ohio, U.S.A.). For sequencing, the following oligonucleotide primers were used: the amplification primers (see above); M13 reverse AACAGCTATGACCATG; M13 17-mer GTA-AAACGACGGCCAGT; and GGAGGCCTTGAGAATGTCAAT and CAATC-CCCTTACTTCTTCAAT (complementary to internal *URA5* DNA sequence). The *URA5* sequence for ATCC 24065 and 24066 were determined from one and two pCRTM 1000 clones containing amplified *URA5* insert, respectively.

Figure 1 shows nucleotide sequence of the protein coding region of the *URA5* gene from the two strains of *C. neoformans* var. *gattii*. There were three exons and two introns which are identical in size and location to those of the var. *neoformans* strain B-3501 (5). The nucleotide sequences of the ATCC 24065 and 24066 *C. neoformans* var. *gattii* *URA5* genes differ from that of the *C. neoformans* var. *neoformans* B-3501 gene (5) by approximately 8%. However, the majority of the nucleotide differences were silent and the predicted *URA5* amino acid sequences for the ATCC 24065 and 24066 *C. neoformans* var. *gattii* (strains) differ by only 3 and 4 amino acids, respectively, from that of strain B-3501 (5). The two *C. neoformans* var. *gattii* sequences have been deposited in Genbank (Los Alamos, New Mexico, U.S.A.) under accession number M93026.

The *URA5* nucleotide sequences of the ATCC 24065 and 24066 were identical except for a silent nucleotide difference at the third position of codon 52 and a replacement nucleotide difference at the second position of codon 143 (asparagine to glycine change). The polymorphism at codon 143 was confirmed by hybridizing the γ -[³²P]-ATP labeled oligonucleotide GGAGGCCTTGAGAATGTCAAT to

24065	ATG TCC TCC CAA GCC CTC	GAC TTC GCC AAG GTT GCC TTC ATC GAG GCT	48
24066	---	---	
B3501	---	---	
24065	GCC ATC GAA CAC GGC GTG CTT CTT TTT GGT AAC TTT ACC TTG AAG TCA		96
24066	---	---	
B3501	---	---	
24065	GGC cgggtgagccatatcgaagtaacttccgagtccaatcgcatctgacatgtgttcagt	CAA	157
24066	---	---	
B3501	---	---	
24065	TCC CCT TAC TTC TTC AAT GCC GGT CTC CTT TAT TCC TCA TCC CTT CTC		205
24066	---	---	
B3501	---	---	
24065	TCA ACT ACT GCC CAG GCT TAC GCC AAA ATA CTT TCG TCT TCT AGG ATT		253
24066	---	---	
B3501	---	---	
24065	CCT GAC TTT GAC GTC CTC TTC GGC CCT GCT TAT AAG GGT ATT TCC TTG		301
24066	---	---	
B3501	---	---	
24065	GCT GCT GTC TCC GCT GTA AGC CTT TAC CAG CAA ACT GGC AAA GAC ATC		349
24066	---	---	
B3501	---	---	
24065	GGT TAC TGC TAC AAC AGG AAG GAG AAG AAG GAC gtgagtctgtcctgaccag		401
24066	---	---	
B3501	---	---	
24065	cgcaacagcaatgagctcataagccagtag	CAC GGC GAG GGC GGT ACT ATG GTC	455
24066	---	---	
B3501	---	---	
24065	GGT GCA CCT CTC AAG GGC AGA ATC GTC ATC ATC GAC GAC GTC CTC ACC		503
24066	---	---	
B3501	---	---	
24065	TCT GGC AAG GCC ATT CGT GAA GCC ATT GAC ATT CTC AAG GCC TCT CCT		551
24066	---	---	
B3501	---	---	
24065	GAA GCC AAG CTC GTT GGA ATT GTC CAG CTT GTC GAC AGG CAA GAG AAA		599
24066	---	---	
B3501	---	---	
24065	GGC CAA AGC GGT AGC GGT AAG AGT ACC GTA CAA GAG GTC GAG GAA GAG		647
24066	---	---	
B3501	---	---	
24065	TTC GGC GTA CCC GTC GAG CCT ATC ATT GGT TTG GAC GAT ATC GTG AAG		695
24066	---	---	
B3501	---	---	
24065	TAC TTG GAA AGC TCC GGG AAG TGG GAG AAG GAG TTG CAA GAG GTC AGG		743
24066	---	---	
B3501	---	---	
24065	AAG TAC AGG GAA GAG TAC GGT GTT CAG AGG TCT TAA	var. <i>gattii</i>	779
24066	---	var. <i>gattii</i>	
B3501	---	var. <i>neoformans</i>	

Fig. 1. Nucleotide sequence of the *URA5* protein coding region of *Cryptococcus neoformans* var. *gattii* ATCC 24065 and 24066.

The *URA5* sequence of the *C. neoformans* var. *neoformans* B-3501 (5) is shown for comparison. A hyphen (-) indicates that the base is identical to that in 24065. Replacement nucleotide changes are indicated by capital letters. Silent nucleotide changes are indicated by lowercase letters.

Nylon membranes containing PCR-amplified *URA5* DNA at various temperatures. Briefly, PCR-amplified *URA5* DNA was transferred to a modified Nylon membrane using 1.5 M NaCl and 0.15 M sodium citrate, and probed with γ -[32 P]-ATP labeled oligonucleotide in a solution of 0.75 M NaCl, 0.075 M sodium citrate, 7% sodium dodecyl sulfate, 10 $\times$ Denhardt's reagent, 10% dextran sulfate, and 0.020 M sodium phosphate (pH 7.2) at various temperatures. Above 60°C, this oligonucleotide exhibited markedly decreased hybridization for the *URA5* DNA of strain 24066 relative to that of strain 24065 (data not shown). Thus, two alleles of the *C. neoformans* var. *gattii* *URA5* gene were identified.

In summary, the *C. neoformans* var. *gattii* *URA5* gene encodes a protein of 225 amino acids which shares 98% amino acid sequence homology with that predicted for *Cryptococcus neoformans* var. *neoformans*. Two alleles were identified for the *C. neoformans* var. *gattii* *URA5* gene which differ at amino acid position 143. The nucleotide sequences of the *C. neoformans* var. *gattii* and *C. neoformans* var. *neoformans* *URA5* genes differ by 8%. The paucity of amino acid changes is in contrast to the frequent nucleotide differences and suggests strong selection against changes in protein structure. The fact that the intron size and position are identical and that the predicted protein structures are highly homologous suggests recent evolutionary divergence for the two *C. neoformans* varieties.

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