Nucleotide sequence of the small ribosomal RNA of *Encephalitozoon cuniculi*

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Microsporidia are very primitive eukaryotic obligate intracellular protozoan parasites that have been implicated as important opportunistic pathogens in patients with HIV-1 infection (1). Very little information is available on the molecular biology of these organisms. The sequence of *Vairomorpha necatrix* rRNA suggests an early branching of microsporidia from the other eukaryotic organisms (2). The DNA of the microsporidian, *Encephalitozoon cuniculi* (Ec), was obtained from rabbit kidney cells (RK-13) infected with the parasite *in vitro*, then amplified by the polymerase chain reaction using a conserved primer set (5′ end: 5′-CACCAGGTTGATTCTGCCTGAC-3′; 3′ end: 5′-GGTTTACCTTGTTACGACTT-3′) located at both ends of the small ribosomal RNA gene. The amplified 1.2 Kb fragment from Ec was inserted into SMAI site of pBluescript II (Strategene, La, CA) in the presence of T4 DNA ligase and SmaI restriction enzyme (3). The resultant positive clones with the correct size were sequenced by double stranded DNA cycle sequencing using Taq polymerase (GIBCO-BRL, Gaithersbutg, MD) with the T7 and T3 promoter primers and other internal primers based on the obtained sequence to walk through the whole gene.

COMMENTS

This rRNA sequence demonstrates a 71.0% similarity to *Vairomorpha necatrix* (2) and 71.7% similarity to *Enterocytozoon bienusi* (4) by the Bestfit program.

The GC content is 52%.

Like other microsporidia *Encephalitozoon cuniculi* lacks eukaryotic small rRNA characteristics: it is shorter in length (only 1,273 nucleotides) and lacks various regions of rRNA considered to be eukaryotic.

Unlike *Vairomorpha necatrix*, *Encephalitozoon cuniculi* has almost the complete sequence at position 484−536 which is aligned to *Escherichia coli* at position 590−650 (2). This region demonstrates little or no homology to other eukaryotes (5, 6), and the sequence is GT-rich, where G accounts for 40%, T accounts for 48%.

rRNA sequence data should prove useful in designing PCR primers for diagnostic tests as has been true for other organisms.

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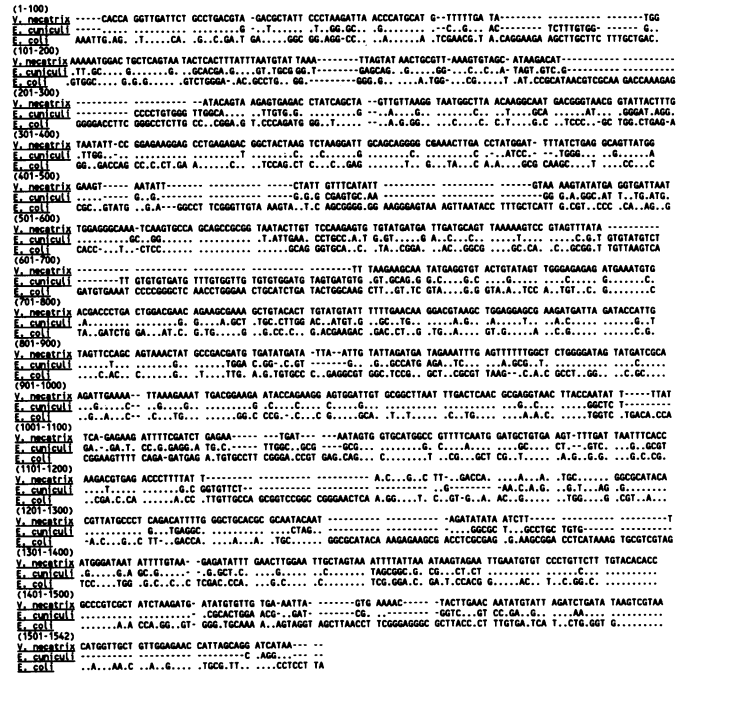

Figure 1. Alignment of the *E.cuniculi* small subunit rRNA sequence with that of *V.necatrix* and *E.coli*. Dots signify the same position; dashes signify that no base occurs at the given position. Numbering, spacing according to the standard *E.coli* sequence.

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