Nucleotide sequence of the small subunit rRNA of *Septata intestinalis*

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Microsporidia are obligate intracellular protozoon parasites that have been reported to infect every major animal group. Intestinal microsporidiosis was first reported in 1985 in patients with human immunodeficiency virus infection (AIDS) and chronic diarrhea (1, 2). The associated microsporidian was identified as *Enterocytozoon bieneusi* (1, 2). Recently, a second microsporidian has been identified as causing diarrhea in AIDS patients (3). This organism not only infects enterocytes like *E.bieneusi* but also cells in the lamina propria. On transmission electron microscopy (TEM) it is distinct from other microsporidia and thus it has been named *Septata intestinalis* (3). The latter organism is probably most closely aligned with the family *Encephalitozoonidae*, which contains only the genus *Encephalitozoon*. This alignment, however, required a modification of the family definition to include only the more general features (3). In order to obtain more information on this newly discovered microsporidian human pathogen and its relationship to other microsporidia we cloned and sequenced the small subunit rRNA gene (SSU-rRNA) of *Septata intestinalis* from infected human intestinal biopsies.

DNA was extracted from human intestinal biopsies from HIV patients with diarrhea who had electron microscopic (TEM) confirmed *S.intestinalis* infection. SSU rRNA gene amplification was performed as described previously (4) using primers that are conserved in SSU-rRNA (5′ end: 5′-CACCAGGTTGATTCTGCCTGAC-3′; 3′ end: 5′-GGTTTACCTTGTTACGACTT-3′) located at both ends of the SSU-rRNA gene. An amplification product of a single band of about 1300 Bp was obtained. Subcloning, purification and sequencing were performed as previously descibed (4). In addition, a conserved primer set located on the SSU-rRNA gene (530f: 51 (530f: 5′-GTGCCATCCAGCCGCGG-3′, (5)) and on the large subunit rRNA (580r: 5′-GGTCCGTGTTTCAAGACGG-3′, (5)) was also employed to amplify material from *S.intestinalis*. These primers amplified a 1350 Bp region containing the 3′ end of the SSU-rRNA gene, the spacer region and the 5′ end of the large subunit rRNA gene. The sequence at the 3′ end of the SSU-rRNA was obtained by sequencing from the large subunit rRNA sequence into the SSU-rRNA sequence using primer 228r (5′-GTTAGTTTCTTTTCCTCC-3′, (5)).

COMMENTS

The *S.intestinalis* SSU-rRNA sequence obtained by direct sequencing was 1283-bp in length with 321 A, 218 C, 430 G, and 314 T. Based on the alignment with *E.cuniculi* an additional 5 Bp is present at the 5′ end giving a length of 1288 bp for the SSU-rRNA.

This rRNA sequence displays a high similarity with that of *Encephalitozoon cuniculi* (4, 6). The similarity was shown to be 87.8% by Bestfit program. Even at the loop region 590−650 (*Escherichia coli* numbering), the two species are very similar.

The SSU-rRNA sequenced obtained for *Septata intestinalis* is distinct from that reported for *Encephalitozoon hellem* (5) confirming these are distinct organisms.

It has been reported that *Encephalitozoon hellem* and *Encephalitozoon cuniculi* on SSU-rRNA analysis display a greater pair-wise difference in sequence (0.231) than is seen between *Vairimorpha necatrix* and *Vairimorpha lymantriae* (0.097) (5). The pair-wise difference in sequence at family level using this analysis was 0.5−0.6 (5). Preliminary analysis of the SSU-rRNA sequence using this apporach supports the modification of the family *Encephalitozoonidae* (3) to include *Septata intestinalis*.

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REFERENCES

1. Desportes,I., Le Charpentier,Y., Galian,A.B.F., Cochand-Priollet,B., Lavergne,A. Ravisse,P. and Modigliani,R. (1985) *J.Protozool.* **32**, 250−254.
2. Modigliani,R., Bories,C., Le Charpentier,Y., Salmeron,M., Messing,B., Galian,A., Rambaud,J.C. and Desportes,I. (1985) **Gut 26**, 179−187.
3. Cali,A., Kotler,D.P. and Orenstein,J.M. (1993) *J. Euk. Microbiol.* **40**, 101−112.
4. Zhu,X., Tanowitz,H.B., Wittner,M., Cali,A., Weiss,L.M. (1993) *Nucleic Acid Res.* **21**, 1315.
5. Vossbrink,C.R., Baker,M.D., Didier,E.S., Vossbrinck,B.A.D., Shadduck,J.A. (1993) *J. Euk. Microbiol.* **40**, 354−362.
6. Hartskeerl,R.A., Schuitema,A.R. and deWachter,R. (1993) *Nucleic Acid Res.* **21**, 1489.
7. Vossbrink,C.R., Maddox,J.V., Friedman,D., Debrunner-Vossbrink,B.A. and Woese,C.R. (1987) *Nature* **326**, 411−414.

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