

Nucleotide sequence of a small subunit ribosomal RNA (16S-like rRNA) gene from *Entamoeba histolytica*: differentiation of pathogenic from nonpathogenic isolates

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It has been estimated that 10% of the world's population is infected with *Entamoeba histolytica* (1), but only a small minority develop invasive colitis or liver abscesses. A major area of debate has been whether genetically determined strain differences are responsible for the wide spectrum of clinical disease seen with *E. histolytica* infection (2, 3). Strains isolated from patients with invasive disease have distinct isoenzymes (2) and surface antigens (4, 5), and can be differentiated by DNA probes (6, 7). To investigate whether these strains differ in their ribosomal RNA, a small subunit ribosomal RNA (16S-like rRNA) gene was isolated from a clinical pathogenic strain (SD 4) and cloned into a Bluescript plasmid (Stratagene, San Diego, CA) and designated pEH39. The inserted rDNA was sequenced fully on both strands by the Exonuclease III deletion method (ExoMeth II Sequencing Kit, Stratagene) and by the dideoxy-nucleotide chain termination method using Sequenase 2.0 (US Biochemical, Cleveland, Ohio). The complete sequence (1496 bases) of the 16S-like rRNA gene was unambiguous and AT-rich (62%).

Using FASTA to search current data banks (GenBank Release 68 and EMBL Release 27), we found that the 16S-like rRNA gene sequence of *E. histolytica* had 65.8% identity in a 784 nt overlap with a *Plasmodium falciparum* 18S rRNA gene (8), 75.1% identity in a 349 nt overlap with a *Xenopus laevis* 18S rRNA gene (9), and 74.4% identity in a 352 nt overlap with a human 18S rRNA gene (10). These results confirm the findings of Sogin's group (11), placing *E. histolytica* as one of the more recently divergent protist lineages. It had previously been shown that the 25S rRNA genes of *E. histolytica* were located on a 25 kb palindromic circular DNA (12, 13). Whether the 16S-like rRNA genes are also present on the same extrachromosomal DNA remains to be determined.

To compare the 16S-like rRNA sequences of strains isolated from patients with invasive disease (pathogenic) and those from asymptomatic patients (nonpathogenic), Southern blots of *Eco*RI-digested genomic DNA of *E. histolytica* were hybridized with the α-^{32}P-dATP-labeled 16S-like rDNA cloned fragment. The hybridization patterns clearly distinguish pathogenic from nonpathogenic isolates. A pathogenic axenic strain (HM-1:IMSS, ATCC 30459) and 10 pathogenic clinical isolates exhibited a single ~9.0 kb band, while 6 nonpathogenic isolates contained two bands of ~9.0 and 2.8 kb (Figure 1). These results show that pathogenic and nonpathogenic *E. histolytica* differ at the gene level and suggest that the 16S-like rRNA probe could be of value in diagnostic and epidemiological studies.

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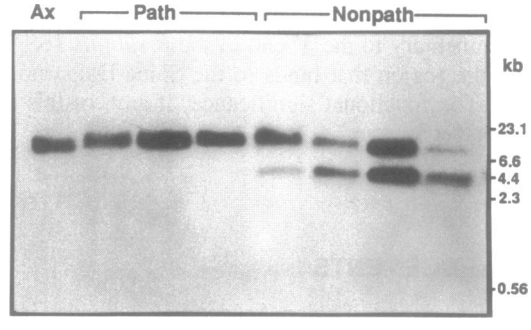

Figure 1. Autoradiograph of Southern blot from 0.8% agarose gel electrophoresis of *Eco*RI-digested genomic DNA from axenic strain HM-1 (Ax), 3 pathogenic clinical isolates (SD 136, SD 143, and FAT 957), and four nonpathogenic strains (SD 11, SD 107, REF 291, and SAW 1734) probed with end-labeled 16S-like rDNA fragment. Identical results were obtained with an additional 6 pathogenic and 2 nonpathogenic strains (data not shown). *Hin*dIII-digested lambda DNA markers are on the right.

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