Genotyping of *Acanthamoeba* strains based on mitochondrial DNA RFLPs and 18S rDNA sequences

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SUMMARY

Recent molecular studies of the genus *Acanthamoeba* have revealed that DNA analysis is more reliable than morphology of the cyst or the trophozoites to identify a genus. We extensively studied the relationships between mitochondrial (mt) DNA RFLP types and 18S rDNA sequence types for genotypic identification of *Acanthamoeba.* We examined 99 isolates from keratitis patients and some authentic strains. Mt RFLPs were obtained by BglII and EcoRI digestion of whole mt DNA extracted from cultures. A partial 18S rDNA region (ASA.S1) that contains a diagnostic fragment was sequenced using extracted genomic DNA. The 7 most common mt RFLP types (which include most of the keratitis isolates) had T4 sequences, while the other less common mt RFLP types (found in Japanese isolates) had T3 and T5 sequences. All the major mt RFLP types, except for JAC/E4, were distributed in different sub-clusters in the T4 clade. This means that most of the 18S rDNA sub-clusters consist of multiple mt RFLP types. Interestingly, the isolates of the JAC/E4 type shared a single 18S rDNA sequence. In conclusion, it seems that both the major mt RFLPs and the genotype T4 of 18S rDNA sequence types are able to distinguish the specific clades within *Acanthamoeba* that are associated with human keratitis. This suggests that *Acanthamoeba* strains with any of the 7 major mt DNA types can be classified as T4 in the absence of sequencing data.