

Molecular and Physiological Evaluation of Subtropical Environmental Isolates of *Acanthamoeba* spp., Causal Agent of *Acanthamoeba* Keratitis

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Abstract

Purpose: Molecular examination of *Acanthamoeba* has identified thirteen rDNA genotypes within the genus, but one genotype (T4) accounts for almost all cases of *Acanthamoeba* keratitis (AK). Here, we begin investigating whether T4 might be the most abundant genotype in nature, possibly explaining its prevalence in AK. We also examine physiological characteristics of acquired strains including salt tolerance, which has been hypothesized as a factor in *Acanthamoeba* pathogenicity.

Methods: Twenty-four *Acanthamoeba* strains were isolated from beach sand (N=20), soil (N=3) and tap water (N=1) in south Florida. For comparison, five corneal scrape isolates from Hong Kong, one beach strain from Scotland, and a freshwater pond strain from the USA also were studied. Salinity tolerance was determined by measurement of migration rates at salt concentrations ranging from freshwater (0 parts-per-thousand [ppt]) to saltwater (32ppt). Genotype determination was done by sequence analysis of a highly informative region of the 18S rRNA gene. Phylogenetic analysis determined strain relatedness and was used to examine any correlation between genotype, location, and physiological tolerance.

Results: All amoebae grew at salt concentrations of 0, 10, and 20 ppt. Overall, beach and corneal isolates 'preferred' 10 ppt. Despite this 10 ppt 'preference' eighteen of twenty beach isolates also grew at 32 ppt and interestingly, four of the five corneal isolates also grew at 32 ppt. None of the soil or tap water amoebae grew at 32 ppt, instead growing best at 0 ppt. Phylogenetic analysis resulted in 20 of 24 samples being designated T4, the AK-associated genotype. The remaining isolates were genotype T5. Nine T4 and two T5 unique sequences were obtained. Little correlation was observed between beach location, salt tolerance, and genetic relatedness.

Conclusions: T4 was the dominant genotype in our study. This is consistent with the assumption that the relative abundance of T4 is a factor in pathogenicity. Most beach sand and AK isolates had T4 genotypes, while the tap water isolate and soil amoebae were all T5 genotypes. However, we have never discovered any cases of AK caused by T5 strains and, thus, the data also are consistent with the possible importance of other factors for pathogenicity such as the ability to grow at higher salt concentration.

Key Words: *Acanthamoeba* • keratitis • ribosomal RNA