Pitfalls in Acanthamoeba genotyping

Acanthamoeba is a protozoan (a eukaryotic microorganism), widespread in several natural environments such as soil and water as a free-living microbial predator. However, it may occasionally infect humans and other animals causing diseases, like keratitis, a sight-threatening eye infection often associated with the use of contact lenses, and granulomatous encephalitis, a chronic and often fatal brain infection, as well as disseminated tissue and multiorgan infections. Furthermore, Acanthamoeba can also harbor various microbial pathogens, facilitating their transmission.

Acanthamoeba includes numerous species, some of which are associated with different diseases. However, several of the described species, based mainly on the morphology of the cysts, have been shown to be invalid. Today Acanthamoeba strains are identified by molecular methods, usually by analysing the nucleotide sequences of the gene for the small subunit of the ribosomal RNA (18S rDNA). In this way, the Acanthamoeba strains sharing high similarity in their 18S rDNA sequences are grouped into the same genetic type (genotype), and the relationships among them may be inferred by using phylogenetic trees. Currently, at least 20 genotypes (named T1 to T20) have been established.

In practice, however, when a large number of strains should be analyzed, obtaining the full 18S sequence of Acanthamoeba is expensive and time-consuming because it is relatively long (more than 2200 base pairs). Therefore, a rapid and economical identification is usually carried out, by getting shorter fragments of the gene (less than 500 bp), but which contain variable nucleotides that are specific for each genotype, called diagnostic fragment. The identification is thus possible by comparing the shorter fragment with sequences of reference.

To be really efficient, this approach requires that the reference sequence for each genotype is well defined. For this, these sequences must be almost entirely known, in order to identify all the regions with variable nucleotides and to build reliable phylogenetic trees, as relationships inferred by using short portions are often incorrect.

Confusion and even diagnostic errors emerged in recent studies, mainly because short 18S sequences were used as references for new genotypes. An example is T16, which appeared distinct from the same genotype correctly defined on the basis of the entire 18S sequence. With the aim to clarify this situation and to suggest minimal standards to identify genotypes, the entire 18S sequence was obtained from one of these putative misidentified strains, and molecular phylogenetic analyses were carried out on both full and partial 18S sequences. The results showed that various putative misidentified strains are indeed distinct, not only from the correct (complete 18S) reference genotype T16, which is mainly environmental, but also between them. Most of them, labeled first on the basis of short sequences as T16 or as T4, belong to a new genotype T20, comprising various highly pathogenic strains for both humans and animals. Another environmental isolate proved to be a new species, Acanthamoeba micheli, in the genotype T19, while an

additional strain infecting a dog could represent another new genotype.

In conclusion, while the use of short diagnostic fragments having almost 100% matches with reference sequences, remains a valid option for rapid and economic tests of a large number of strains, identification errors occur when matches are low and/or short sequences are used also as references. In these cases, strain identification should be performed by accurate phylogenetic analyses based on the full 18S sequence.

Publication

Acanthamoeba misidentification and multiple labels: redefining genotypes T16, T19, and T20 and proposal for Acanthamoeba micheli sp. nov. (genotype T19).
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