

Rapid Detection of Pathogenic Free-Living Amoebae in Water

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Key words: pathogenic, free-living amoebae, morphology, electrophoresis, antibody.

Introduction

There are plenty of microorganisms in water which could be potential health hazards including free-living amoebae (FLA) such as *Naegleria fowleri* and *Acanthamoeba castellanii*. Although in water these amoebae feed mainly on bacteria, some of them became tissue feeders and facultative pathogen of human brains and eyes. *Acanthamoeba* spp. and *Hartmannella vermiformis* have been reported to be associated with keratitis in man (Govinda et al. & Jeanette, 1990; John et al., 1991; Devonshire et al., 1993; Mohamed Kamel et al. & Norazah, 1995; Nakisah et al., 1995; 1998; Burnakis et al., 1997; Lam et al., 1997). Since FLA are abundant in nature and are of different types, development of techniques on exact and quick identification of these pathogenic species is required.

Material and Methods

In this study, samples from keratitis patient, obtained from Department of Ophthalmology, Hospital Kuala Lumpur, Malaysia were used to isolate and identify the amoeba species. The amoeba isolates were grown on non-nutrient agar (NNA) seeded with heat-killed *Escherichia coli* and were incubated at 30° C for 3 to 4 days. Clonal isolation of each isolate was done by subpassing the isolate on fresh NNA agar plate seeded with *E. coli* until a single species of amoeba was obtained. The amoebae obtained in this study were identified based on morphology of trophozoites and cysts, both by light and electron microscopy (SEM and TEM) and locomotion, following the key of Page et al. (1988). In some cases, growth temperature tolerance of the amoebae were included to identify the species. Other than these techniques, the identification of amoebae was also done by electrophoresis

analyses for its protein profile, proteinases profile and DNA profile by RFLP-PCR. In this study, the morphology and electrophoresis analyses of *Acanthamoeba castellanii*, obtained from Institute of Medical Research, Kuala Lumpur were also examined in comparison for identifying the amoebae when necessary.

Results and Discussion

The results obtained in this study indicated that only one species of amoeba was isolated from human's corneal scrapings. The trophozoite form of amoeba is flattened, broad and irregular outline and producing a number of slender, spine-like processes around the cell, suggesting the amoeba belongs to the genus of *Acanthamoeba*. The measurement of the trophozoites ranging from 20 µm to 40 µm. The cysts of this amoeba consist of two walls; the outer wall was wrinkled and thick, and the inner wall was either triangular, or quadrangular. The mean of cyst diameter was about 16 µm. The size and morphology of both trophozoites and cysts of this amoeba are similar to control species indicating that the isolated amoeba could be *A. castellanii*. Detail morphological (as observed under light and electron microscopy (SEM and TEM) and non-morphological characteristics for both species, however will be further looked into. The result of this study also indicated that the isolate exhibits seven major protein bands on SDS-PAGE gel. The protein will be identified further using Gelatin-SDS-PAGE gels for obtaining its proteinases profile. The future work also will include DNA profile analyses by RFLP-PCR. The results from these analyses will determine which protein will be extracted from the amoeba (immunogenic protein) to be used in raising monoclonal

antibody in order to develop a diagnostic kit for detecting this amoeba in water.

We are hoping to get more samples of corneal scrapings and aqueous humor from Hospital Kuala Lumpur in order to be able to list several species of pathogenic amoebae isolated from humans, especially those that are associated with keratitis in man. The failure of getting constant supply of such samples delayed the progress of this study.

Conclusions

The result obtained from this study so far indicated that only one amoeba species was isolated from human's corneal scrapings. Based on its morphology of trophozoites and cysts, this species can be identified tentatively as *Acanthamoeba castellanii*.

Benefits from the study

A diagnostic kit for quick and reliable method of identifying pathogenic free-living amoebae will be invented.

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Project Publications in Refereed Journals

None.

Project Publications in Conference Proceedings

None.

Graduate Research

None.