Vannellid Species Isolated from Freshwater Source in a Park in Jamaica, West Indies

Supplementary Issue: Microbial Diversity

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ABSTRACT: Free-living amoebae (FLA) occupy a wide range of freshwater, marine, and soil habitats, and are opportunistic pathogens in human beings. While Acanthamoeba spp., Naegleria fowleri, and Balamuthia mandrillaris are well-known opportunistic organisms, Vannella epipetala is nonpathogenic. Sediments were collected from a freshwater source from a park in Jamaica to investigate the presence of FLA. Acanthamoeba and Naegleria spp. were not recovered; however, a Vannellid species identified by microscopy and PCR analysis as V. epipetala was isolated. These nonpathogens pose a threat to human beings as they may act as Trojan horses for microsporidian parasites and other pathogens, thereby facilitating their transmission to human beings.

KEYWORDS: Free-living amoebae (FLA), Vannella epipetala, microsporidian parasites, Jamaica, West Indies

Introduction

Free-living amoebae (FLA) are widely distributed in the environment and are normally harmless to human beings; however, Acanthamoeba spp., Naegleria fowleri, Sappinia spp., and Balamuthia mandrillaris are opportunistic pathogens causing severe CNS, eye, skin, colon, and liver diseases.1 Vannella spp. are commonly found in fresh and salt water bodies and are more recently found in tap water, soil, gills and organs of fishes, biofilm, sewer outflows, municipal waste water treatment plant release sites, and the leaf surface of plants (Spondias mombin [Anacardiaceae]) found in Costa Rica.2–7 FLA are increasingly recognized as important pathogens with increased reports of human cases and exposure to infection through antibody titer studies.1,3,4 Vannella spp. isolated from soil are not pathogenic but can act as reservoirs for pathogenic microsporidian parasites. While there are over 40 aquatic species of Vannella, only some are pathogenic to vertebrates, in particular fishes.2,7 The SSU rRNA gene of Vannellids are constantly mutating and are linked to increased pathogenicity in fishes. It was established that some members of the genus cause diseases of the gills and organs in fishes, but no data exist on the potential to cause disease in human beings.

The fact that this gene is rapidly evolving should raise interests to understand its evolutionary behavior, its pathogenicity in fishes, their ability to transmit other pathogens, and the possibility of causing disease in human beings and other animals.6 Since Vannella epipetala is not only found to inhabit soil and fresh and salt water bodies but also the surface of leaves, the diversity of habitat types highlights its survival versatility.6 V. epipetala was not reported from previous studies of FLA in Jamaica,10,11 and the occurrence of this organism in the Caribbean is unknown. The aim of the study was to investigate the presence of FLA types in river sediments and to characterize these organisms and investigate their pathogenic potential.

Materials and Methods

Soil sediments were collected from the bank of the Castleton River in a 50 mL sterile centrifuge tube (Corning Incorporated). Approximately 10 g of soil was inoculated onto 2% non-nutrient agar (NNA) plates seeded with heat-killed Escherichia coli and incubated at room temperature (~30°C) for seven days. NNA plates were examined using an inverted microscope for the growth of amoebae. Plates containing amoebae were scraped, and the material was

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centrifuged after addition of phosphate-buffered saline. DNA was extracted by placing 1–2 mL of amoebic cultures directly into the Maxwell® 16 Tissue DNA Purification Kit sample cartridge (Promega Corporation). Amebic genomic DNA was purified using the Maxwell® 16 Instrument as described in the Maxwell® 16 DNA Purification Kits Technical Manual #TM284 (Promega Corporation). DNA yield and purity were determined using the NanoDrop® 1000 spectrophotometer (Fisher Scientific) as previously described. DNA amplification reactions were performed using universal markers for FLA and specific markers for *V. epipetala*. Amplification products were fractionated using 2% agarose electrophoresis gel stained with a solution of 20,000× of REALSAFE Nucleic Acid Staining Solution (Durviz) and visualized under UV light. PCR products were purified using the QIAquick® PCR Purification kit (QIAGEN), according to the manufacturer’s instructions, and sequenced in both directions. The sequencing was done in a MegaBACE 1000 automatic sequencer (Healthcare Biosciences) using the University of La Laguna sequencing services (Servicio de Secuenciación SEGAI, University of La Laguna). Homology analyses of the obtained DNA sequences were performed using BLAST analysis and compared to the sequences available at the GenBank database.

**Results and Discussion**

A single isolate of *Vannella* was recovered from the NNA plate. This was identified as *V. epipetala* using PCR and DNA sequencing. BLAST analysis revealed a 99% homology with the available sequences of other *V. epipetala* strains deposited in the GenBank database. This is the first report of *V. epipetala* from the Caribbean and expands the geographic location from where this species has been recovered. The Castleton River from which the organism was recovered is associated with a botanical garden, which is a heavily used recreational site. The public heath significance cannot be established on a single isolate, although this finding has some significance. *Vannella* spp. are not harmful to human beings but are hosts and Trojan horses of microsporidian parasites, bacteria, and other pathogens. Amaral-Zettler et al.16 reported that the SSU ribosomal ribonucleic acid (SS rRNA) gene sequence could be used to differentiate between species of *Vannella*, the presence of pentagonal glycostyles in this genus was the main characteristic used to separate it from *Platyamoeba*. Contradictory to this, molecular work established the unreliability of relying on morphological features for the differentiation of organisms at the genus or species level.6,16 Further, Page18 designated cyst formation as a morphological characteristic that could be used for the identification of *Platyamoeba* spp.; however, the cystic form was later observed in *Vannellids*.3,19 Despite the efforts to differentiate the genera *Vannella* and *Platyamoeba*, morphological, molecular, and phylogenetic studies performed by Sims et al.,17 Dyková et al.,5 and Amaral-Zettler et al.,6 respectively, proved that both genera were very closely related. Similar to the observation noted by Amaral-Zettler et al.,6 the floating form remained contracted with short pseudopodia-like protrusions (Fig. 1). Amebic growth was achieved at room temperature (~30°C), which differed significantly to the findings of Amaral-Zettler et al.,6 who reported optimal growth at temperatures of 20°C and 25°C, no growth and a low survival rate at 30°C. The variation in growth patterns might be associated with the isolate type.

*Acanthamoeba* spp. and *B. mandrillaris* have been isolated from soil from recreational sites in Jamaica; however, this was the first report of *V. epipetala*.10,11 The isolation of *V. epipetala* from soil in this study is the second report of the isolation of
Vannellid species isolated from freshwater source in Jamaica

Figure 1. Stages of *V. epipetala* from river sediment. (A) trophozoite (Tr) and floating form (Fl) and (B) cyst (cys) (40×).

this amoeba from an environmental source and the first report of its isolation in the Caribbean. Further work should be done to investigate the possibility of *V. epipetala* hosting pathogens that may be potentially harmful to human beings.

Author Contributions
Conceived and designed the experiments: CDT, and JLM. Analyzed the data: CDT, JLM, MRB, BV and JFL. Wrote the first draft of the manuscript: CDT, JLM, JFL. Contributed to the writing of the manuscript: CDT, MRB, BV, JFL and JLM. Agree with manuscript results and conclusions: CDT, JLM, BV, MRB and JFL. All authors reviewed and approved of the final manuscript.

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