BRIEF COMMUNICATION

IDENTIFICATION OF Pseudomonas spp. AS AMOEBA-RESISTANT MICROORGANISMS IN ISOLATES OF Acanthamoeba

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SUMMARY

Acanthamoeba is a “Trojan horse” of the microbial world. The aim of this study was to identify the presence of Pseudomonas as an amoeba-resistant microorganism in 12 isolates of Acanthamoeba. All isolates showed the genus Pseudomonas spp. as amoeba-resistant microorganisms. Thus, one can see that the Acanthamoeba isolates studied are hosts of Pseudomonas.

KEYWORDS: Acanthamoeba; Pseudomonas; Amoeba-resistant microorganism.

Acanthamoeba is an opportunistic human pathogen that is ubiquitously distributed in the environment. It is a causative agent of cutaneous lesions, sinus infections, vision threatening keratitis and rare but fatal encephalitis, known as granulomatous amoebic encephalitis. In addition, it has the ability to act as a host/reservoir for microbial pathogens.

Free-living amoebae feed by phagocytosis mainly on bacteria, fungi, and algae, and digestion occurs within phagolysosomes. Some microorganisms have evolved and have become resistant to predation by protists, since they are not internalized or are able to survive, grow, and exit free-living amoebae after internalization. Acanthamoeba is shown to be host/reservoir for numerous bacteria, including the genus Pseudomonas spp., among other bacterial pathogens.

Pseudomonas spp. are highly adaptable bacteria that can colonize various environmental niches, including soil and marine habitats, plants and animals. Pseudomonas spp. are also opportunistic human pathogens, causing infection of the eyes, ears, skin, urethra and respiratory tract in cystic fibrosis (CF) in burned patients, as well as other immunocompromised individuals.

In nature, free-living amoebae of the genus Acanthamoeba feed by Pseudomonas spp., which are widely distributed in the environment. Their encounter may be facilitated through better adherence of Pseudomonas (than E. coli) to Acanthamoeba. However, some Pseudomonas spp. have evolved to become resistant to predation by amoebae, as demonstrated by the isolation of Acanthamoeba naturally infected with P. aeruginosa. Hence, free-living amoebae might also play a role as a reservoir for some amoeba-resistant strains of Pseudomonas, similar to what was shown for Legionella spp. This is important, given the role of Pseudomonas aeruginosa as a causative agent of pneumonia. Acanthamoeba has been isolated from contact lens care systems contaminated with Gram-negative bacteria, including Pseudomonas aeruginosa.

Many studies have evaluated the interaction between Acanthamoeba spp. and Pseudomonas spp., as well as investigated the presence of these bacterial genera as amoeba-resistant bacteria.

In this study, the conventional technique of Polymerase Chain Reaction (PCR) was used, in order to identify the presence of the genus Pseudomonas spp. as amoeba-resistant microorganisms in isolates of Acanthamoeba.

A total of 12 environmental samples existing in the laboratory were used in this study: seven isolates from air-conditioning units identified as Acanthamoeba A2, A3, A4, A5, A7, A8 and A10, and five isolates from contact lens cases, Acanthamoeba A1, A6, A9, A11 and A12. The isolates were cultured in PYG media at 30 °C (2% protease peptone, 0.2% yeast extract, and 1.5% glucose) supplemented with penicillin and streptomycin (Life Technologies). The total DNA in the sample was extracted, as described by ALJANABI & MARTINEZ. The fresh culture containing 10⁶ trophozoites was homogenized in 400 µL of sterile salt homogenizing buffer (0.4 M NaCl 10 mM Tris–HCl pH 8.0 and 2 mM EDTA pH 8.0), then, 40 µL of 20% SDS (2% final concentration) and 8 µL of 20 mg/mL proteinase K (400 µg/mL final concentration) were added and mixed well. The samples were incubated at 65 °C for, at
least, one h, after which 300 µL of 6 M NaCl (NaCl saturated H2O) was added to each sample. Samples were vortexed for 30s at maximum speed, and tubes spun down for 30 min at 10,000 x g. The supernatant was transferred to fresh tubes. An equal volume of isopropanol was added to each sample and samples were incubated at -20 °C for one h. Samples were then centrifuged for 20 min, at 4 °C and at 10,000 x g. The pellet was washed with 70% ethanol, dried and finally resuspended in 100 µL sterile H2O.

After extraction, the isolates were screened for the presence of bacterial endosymbiont - Bacteria domain - through the 16S rDNA gene amplified by PCR, using primers ID1 (5'-AGAGTTTGATCCTGCTAG3') and rP2 (5'-ACGGGCTACCTTGGTACGACTT3') that amplify 1500 bp in size, described by WEISBURG et al.15, under the following conditions: five min at 94 °C, followed by 35 cycles of one min at 94 °C, one min at 55 °C and one min at 72 °C.

The identification of the presence of Pseudomonas genus DNA occurred using the primers described by SPILKER et al.14 PA-GS-F (5'- GACGGGGTGAATGCTACTTATA-3') and PA-GS-R (5'-CAGGCTACTTTGTTACGGACTT-3') that amplifies 618 pb in size. Amplification was performed in a total volume of 25 µL containing 30 ng DNA, 10 pmol each primer, 5 pmol dNTP, reaction buffer (50 mM KCl, 10 mM Tris–HCl), 1.5 mM MgCl2, and 1 U of Platinum Taq DNA Polymerase (InvitrogenTM). The amplification reaction was carried out in a PTC-150 Minicycler MJ Research thermocycler, under the following conditions: five min at 94 °C, followed by 35 cycles of one min at 94 °C, one min at 58 °C and one min at 72 °C.

The amplification product was separated in 1% agarose gel, stained with 0.5 µM/mL ethidium bromide and observed under a UV-light transilluminator. PCR products were purified using a QIAquick purification kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions, and resolved with a MegaBace 1000 automated sequencer. Analysis of the DNA sequences was performed with the Chromas Lite program and compared to those present in GenBank (http://blast.ncbi.nlm.nih.gov/).

In the present study, all isolates of Acanthamoeba showed internalized bacteria when primers are used to amplify the Bacteria domain and all isolates showed the genus Pseudomonas spp. as amoeba-resistant microorganisms (Fig. 1). A total of six PCR products (Ap1 to Ap6) were sent for sequencing (Table 1) and all were confirmed as Pseudomonas spp.

### Table 1

| Acanthamoeba | Fragment from the gel (GenBank accession) | Similarity BLAST | Access GenBank (number for access) |
|-------------|---------------------------------------|-----------------|-----------------------------------|
| A1          | Ap1 (KF160336)                        | 98%             | Pseudomonas sp. c145(2012) 16S ribosomal RNA gene, partial sequence (JQ781629.1) |
| A3          | Ap2 (KF160337)                        | 96%             | Uncultured Pseudomonas sp. clone 3F10 16S ribosomal RNA gene, partial sequence (HM438578.1) |
| A4          | Ap3 (KF160338)                        | 99%             | Pseudomonas sp. CJ-S-R2A3 16S ribosomal RNA gene, partial sequence (HMS84286.1) |
| A6          | Ap4 (KF160339)                        | 99%             | Pseudomonas sp. c145(2012) 16S ribosomal RNA gene, partial sequence (JQ781629.1) |
| A10         | Ap5 (KF160340)                        | 99%             | Pseudomonas fluorescens strain C-D-TSA4 16S ribosomal RNA gene, partial sequence (HM755599.1) |
| A12         | Ap6 (KF160341)                        | 97%             | Pseudomonas sp. c145(2012) 16S ribosomal RNA gene, partial sequence (JQ781629.1) |
The possible role of *Acanthamoeba* as an evolutionary precursor of pathogenicity in microbial pathogens has been suggested\(^1\). Bacteria or other microbial endosymbiont may also enhance the pathogenicity of *Acanthamoeba*\(^1\). However, the results have been inconclusive. There are a few reports suggesting that amoeba-resistant microorganisms enhance the virulence of *Acanthamoeba*\(^6\).

In addition to the bacteria identified in this work, the presence of other pathogenic amoeba-resistant microorganisms in the water samples tested cannot be discarded. *Acanthamoeba* spp. are also potential reservoirs of *Mycobacterium* spp.\(^3\) and *Legionella* spp., among others microorganisms\(^3\).

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**RESUMO**

Identificação de *Pseudomonas* spp. como microrganismo resistente a ameba em isolados de *Acanthamoeba*

*Acanthamoeba* é um “Cavalo de Tróia” do mundo microbiano. Este estudo teve como objetivo identificar a presença de *Pseudomonas* como microrganismo resistente a ameba em 12 isolados de *Acanthamoeba*. Todos os isolados apresentaram o gênero *Pseudomonas* spp. como um microrganismo resistente a ameba. Assim, podemos ver que os isolados de *Acanthamoeba* estudados são hospedeiros de *Pseudomonas*. **REFERENCES**

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