Microsc. Microanal. 18 (Suppl 5), 2012

doi:10.1017/S1431927612012810

Detection of amoeba and amoeba-associated microorganisms (AAMs) from natural and hospital environments

M. F. Caeiro*, R. Costa *,**,******, A. Amorim**, F. F. Vale***, S. Ferreira****, F. Morgado**** and A. P. Alves de Matos*,*****

* Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências da Universidade de Lisboa (FCUL), Campo Grande, 1749-016, Lisboa
** Centro de Oceanografia, FCUL, Campo Grande, 1149-016, Lisboa
*** Faculdade de Engenharia, Universidade Católica Portuguesa, 2635-631 Rio de Mouro
**** CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro
***** Centro Hospitalar de Lisboa Central - HCC, Anatomia Patológica, R. da Beneficência 8, 1069-166 Lisboa

Amoebae graze on bacteria from the environment. In some cases, bacteria acquire resistance to the destructive action of amoebae and survive inside the host [1]. The resistance of microorganisms to amoebae is often accompanied by resistance to the physiologically related human macrophage and displays increased pathogenic potential to humans [2]. The association of bacteria with amoebae, that are ubiquitous in the environment, protects the bacteria from common disinfection measures. This leads to the creation of reservoirs of pathogenic microorganisms that are difficult to eradicate [3]. This process occurs widely in the environment, but is of special concern in hospitals, where many established and emerging pathogens involved in nosocomial disease, such as *Legionella pneumophila*, are increasingly being identified in association with amoebae [4]. One particularly important amoeba-associated microorganism (AAM) is the newly recognized giant mimivirus [5], a member of the Nucleo-Cytoplasmic Large Deoxyribovirus (NCLDV) group. The virus has been isolated from hospital sources in France and, together with other members of the NCLDV group, is known from metagenomic studies to be widespread in the Oceans, representing one of the main groups of marine viruses [6]. Our project aims to detect amoeba and potentially pathogenic AAMs with particular relevance to the NCLDVs in the environment and in hospital facilities. In the present work we have developed methods for detection, isolation and *in vitro* culture of amoebae from several environmental sources including hospital dust, sea water and estuarine sediments.

Suspensions of samples from hospital dust and biofilms and estuarine sediment and water samples were concentrated by centrifugation at 800rpm and seeded in non-nutrient agar covered with monolayers of live *Lactobacillus* spp. or *E. coli*. Growth of amoeba was detected 3 to 10 days after seeding, by inspection of the agar plates with a light microscope (Figure 1a). For light microscopy studies, amoeba suspensions were allowed to attach to glass slides overnight or centrifuged onto the slides with a cytocentrifuge, fixed in methanol and stained with Giemsa. For transmission electron micoscopy, the pelleted amoebae were fixed sequentially in glutaraldehyde, osmium tetroxide and uranyl acetate, followed by dehydration and embedding in Epon-Araldite.

Presently, we are carrying out the characterization of a number of amoebae isolated from the sources mentioned above. Amoebae have been identified both through morphology, using light and electron microscopy, and by PCR amplification of rRNA coding sequences and further sequencing of the obtained amplicons [7]. Most of the isolated amoebae were *Acantamoebae* with characteristic morphologic features like the acanthopodia (Figure 1b). Some belong to other genera yet to be characterized (Figure 1c).

In one sample of amoeba isolated from hospital dust and identified as *Acanthamoeba castellanii*, transmission electron microscopy allowed the identification of amoeba-associated virus-like particles resembling mimiviruses (Figure 1d). Our work continues in order to characterize and isolate amoeba and AAMs from the studied sources.

References
1. Greub G. and Raoult D., Clin. Microbiol. Rev. 17: 413–433, 2004
2. Adiba S. *et al.*, PLoS One 5: e11882, 2010
3. Evstigneeva A. *et al.*, Microbiology 155: 657–664, 2009
4. O'Brien S.J. and Bhopal R.S., Lancet 342: 5-6, 1993
5. Raoult D. *et al.*, Clin Infect Dis. 45:95-102, 2007
6. Monier A. *et al.*, Genome Biol. 9: R106, 2008
7. Eddyani *et al.*, Appl Environ Microbiol. 74: 6547-53, 2008

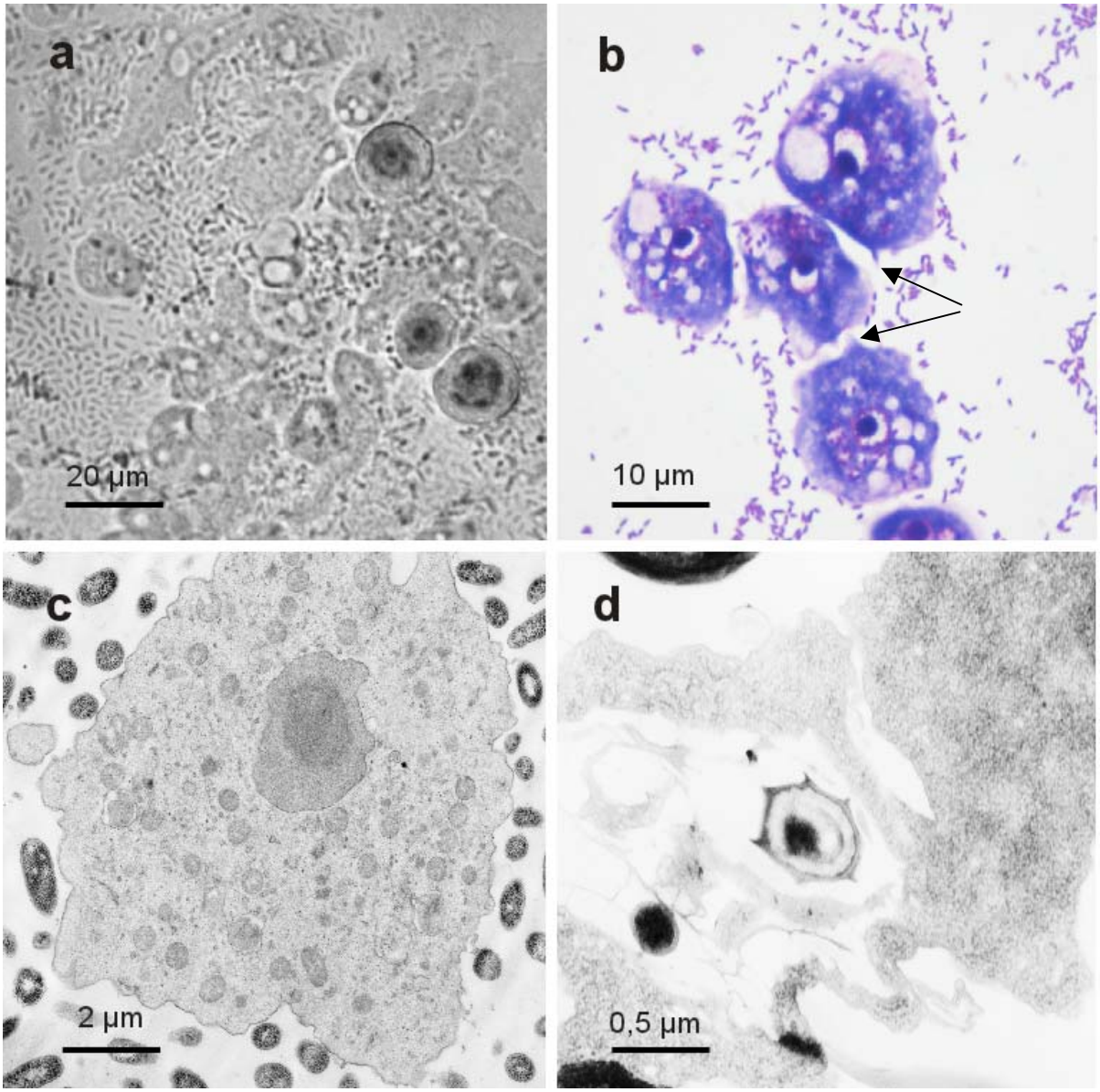

Figure 1. a) Culture of of amoebae recovered from dust in the Curry Cabral Hospital, fed with bacteria. b) Giemsa stained *Acantamoeba* spp. from the same source showing the characteristic acanthopodia (arrows). c) Transmission electron microscopy of an unidentified marine amoeba, from the Tagus estuary. d) Mimivirus-like particle present in primary cultures of *Acantamoeba castellanii* from an Hospital sample.

https://doi.org/10.1017/S1431927612012810 Published online by Cambridge University Press