"Interaction between zoonotic bacteria and Free Living Amoebae (FLA): a possible relevant aspect of public health importance?"

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BACKGROUND

The association between **free-living amoebae (FLA)** and **pathogenic bacteria** is an issue that has gained great importance due to the environmental and health consequences that it implies



Representative micrograph of *Acanthamoeba castellanii* interactions with *Enterobacter aerogenes;* from Yousuf et al., 2013.

Bacteria can benefit from interactions with FLA

- 1) their ability to escape predation and to resist intracellular digestion (cyst form)
- 2) their ability to resist digestion but also to grow within the protozoan vegetative form





FREE-LIVING AMOEBAE

Life Cycle of FLA



FLA life cycle modified picture from: https://www.cdc.gov/dpdx/freelivingamebic/index.html Ubiquitous protozoa widely distributed in nature do not require a host organism survival (facultative parasites)

FLA have dual biological characteristics

(i) an exozoic, non parasitic phase (**CYST**), capable of transformation into (ii) endozoic parasitic stage (**TROPHOZOITE**)





TROPHOZOITE

on CYSTIC STAGE, amoebae

are tolerant from:

Dessication Starvation Chemical Physical agents

FREE-LIVING AMOEBAE



FLA (amoebal cysts) highly resistant to physical and chemical stresses and protect any intracellular microorganism from deleterious environmental conditions that would normally kill them





FLA AND PATHOGENIC *E. COLI*

The role of **protozoa in the environmental life cycle of pathogenic** *E. coli* **is much less examined** compared to classical obligatory or facultative intracellular bacteria



Investigate the potential for ubiquitous free-living amoebae (*Acanthamoeba*) to support the growth of **different intestinal pathogenic** *E. coli* and their interactions





STRAINS

BACTERIA

E. coli O157:H7 obtained from the collection of EURL-VTEC

E. coli K-12 laboratory strain (BL21) used as non-pathogen

AMOEBAE HOST

Acanthamoeba sp. (wild type from thermal water $T = 40^{\circ}C$) grown in PYG medium in tissue culture flasks at 37°C (min. cell. confluence 20%)

INVASION ASSAY

Gentamicin (150ug/ml) for 1h Wash 3 times with PBS Acanthamoeba solubilization Acanthamoeba lysis with 0,5% SDS *E. coli* 0157:H7 / *E. coli* K12 counting by plating

* * * * EU-RL * * VTEC * * * *



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INTRACELLULAR SURVIVAL ASSAY



counting by plating

* * * * ^{EU-RL} * * ^{VTEC} * * * *



PRELIMINARY RESULTS Invasion Assay







PRELIMINARY RESULTS INTRACELLULAR SURVIVAL ASSAY







PRELIMINARY RESULTS INTRACELLULAR SURVIVAL ASSAY







CONCLUSIONS

✓ Acanthamoeba sp. can internalize pathogenic and non pathogenic E. coli

- ✓ internalized bacteria (pathogenic and non-pathogenic) are able to survive within amoebae for 48h (with low confluence of amoebae!)
- ✓ our preliminary results are compatible with a possible role of free living amoebae hosting and contributing to the environmental persistence of EHEC



- investigate the potential for free-living amoebae to support the growth of other intestinal pathogenic *E. coli*
- identify the bacterial determinants responsible for EHEC interactions with amoebae





Thanks for your Attention



