

16th Annual Workshop of the National Reference Laboratories for *E. coli* in the EU

Internalization of STEC into protozoa: Impact on the food testing?

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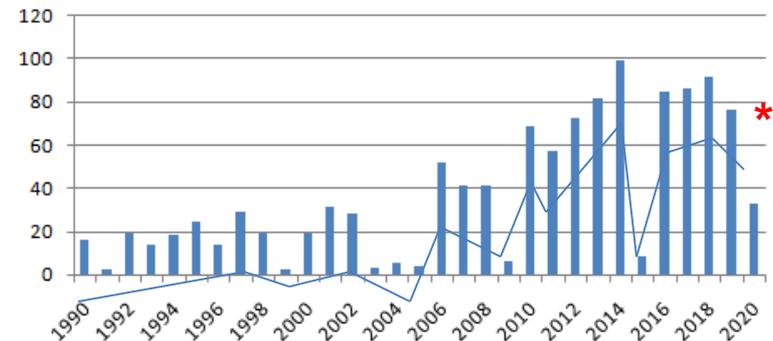


BACKGROUND

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:

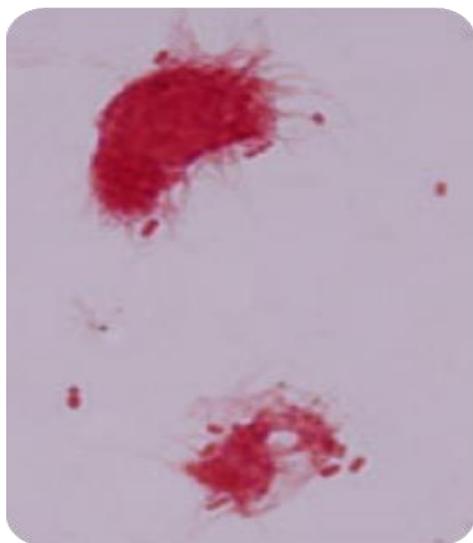
- (i) shape human pathogens from the evolutionary point of view
- (ii) comprise a protective niche and vector for pathogen transmission

Pubmed reports: Interaction FLA and bacteria (1990 - 2020)



* **Update on 2021:** published only one article – «Paradigms of Protist/Bacteria Symbioses Affecting Human Health: *Acanthamoeba* species and *Trichomonas vaginalis*, Henriquez et al. 2021.

BACKGROUND



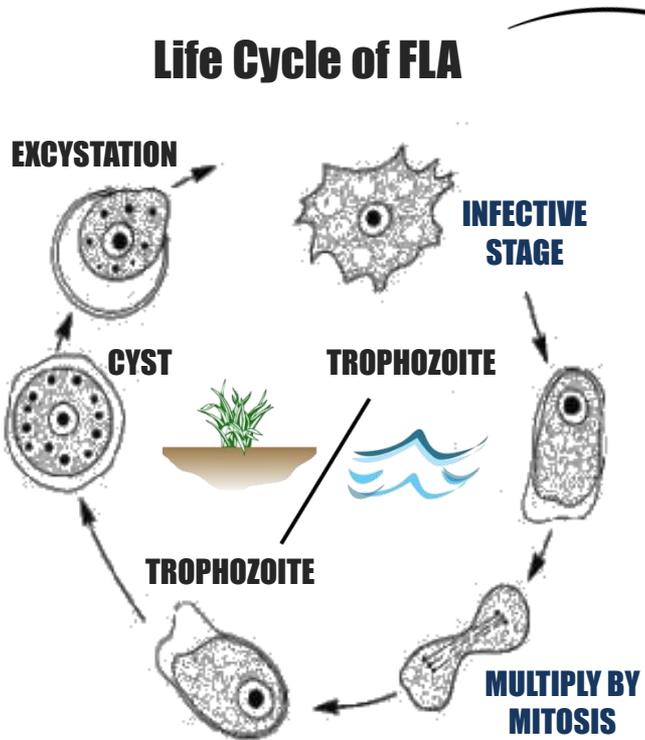
Representative micrograph of *Acanthamoeba castellanii* interactions with *Enterobacter aerogenes*; from Yousuf et al., 2013.

Bacteria can benefit from interactions with FLA

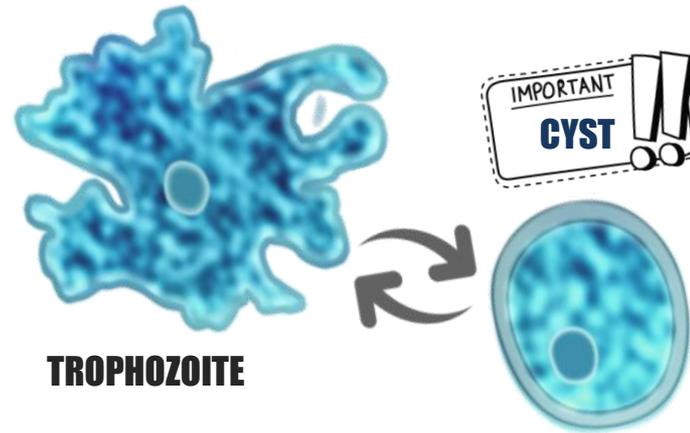
- 1) their ability to **escape predation** and **grow** in the presence of a protozoan
- 2) their ability to **resist intracellular digestion**
- 3) their ability to **resist digestion** but also to **grow within the protozoan vegetative form**

FREE-LIVING AMOEBAE

Life Cycle of FLA



...FROM A PUBLIC HEALTH PERSPECTIVE



CYSTIC STAGE
amoebae are tolerant from **dessication, starvation, chemical and physical agents**

FLA life cycle modified picture from:

<https://www.cdc.gov/dpdx/freelivingamebic/index.html>

RESEARCH OBJECTIVES

Investigate the potential for ubiquitous free-living amoebae (*Acanthamoeba*) to support the growth of **STEC** and their interactions



EXPERIMENTAL PROCEDURES

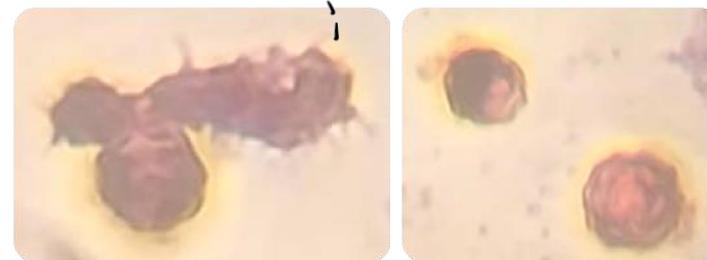


O157:H7	<i>eae+</i> <i>stx1+</i> <i>stx2+</i>	HUS
O157:H7	<i>eae+</i> <i>stx1+</i>	HUS
O157:H7	<i>eae+</i> <i>stx2+</i>	bovine stool
non pathogenic <i>E. coli</i> (ECORI)	-	-



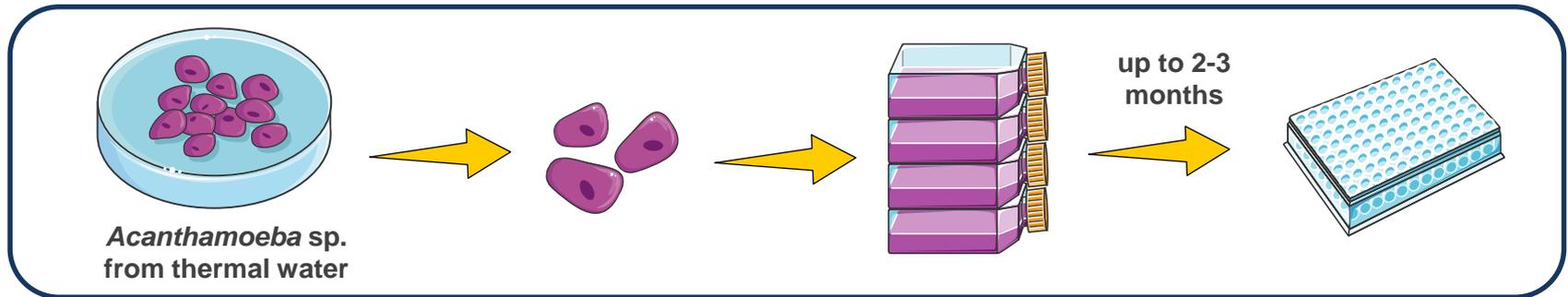
**TROPHOZOITE
EXCYSTING**

CYSTS

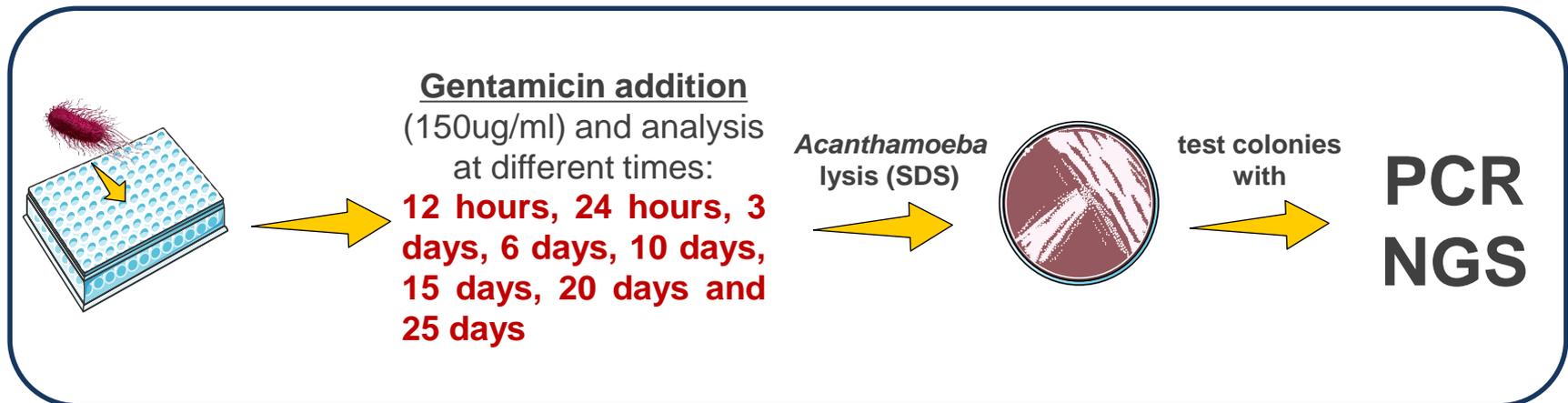


Trophozoite and cysts of *Acanthamoeba* sp. with a stained with Giemsa (40X). Original photos.

PHASE 1 – AXENITATION OF AMOEBIA HOST



PHASE 2 – INTRACELLULAR SURVIVAL ASSAY



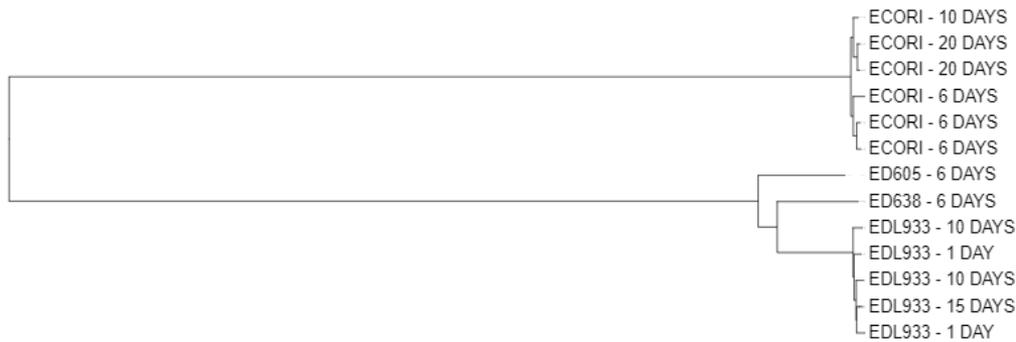
RESULTS

<i>E. coli</i> strains	Virulence genes	Source	12 hours	1 day	3 days	6 days	10 days	15 days	20 days	25 days
O157:H7	<i>eae+</i> <i>stx1+</i> <i>stx2+</i>	HUS	100 cfu	100 cfu	-	-	100 cfu	23 cfu	-	70 cfu
O157:H7	<i>eae+</i> <i>stx1+</i>	HUS	1 cfu	100 cfu	100 cfu	1 cfu	30 cfu	-	-	>100
O157:H7	<i>eae+</i> <i>stx2+</i>	bovine stool	1 cfu	100 cfu	-	14 cfu	100 cfu	-	-	>100
ECORI	-	-	1 cfu	100 cfu	-	100 cfu	>100 cfu	>100 cfu	100 cfu	1 cfu



...STILL IN PROGRESS

Tree scale: 0.001



**TO DATE NO DIFFERENCES
SAME STRAIN AT DIFFERENT
TIMES!!**

chewTree: phylogenetic tree from chewBBACA alleles



NEXT STEPS

??
?? ? ? ? ? ? ?

<i>E. coli</i> strains	Virulence genes	Source	12 hours	1 day	3 days	6 days	10 days	15 days	20 days	25 days
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O157:H7	<i>eae+</i> <i>stx1+</i>	HUS	1 cfu	100 cfu	100 cfu	1 cfu	30 cfu	-	-	>100
O157:H7	<i>eae+</i> <i>stx2+</i>	bovine stool	1 cfu	100 cfu	-	14 cfu	100 cfu	-	-	>100
ECORI	-	-	1 cfu	100 cfu	-	100 cfu	>100 cfu	>100 cfu	100 cfu	1 cfu

use the *rpoS* mRNA as a viability marker!!

CONCLUSIONS

- ✓ ***Acanthamoeba* sp. can internalize pathogenic and non pathogenic *E. coli***
- ✓ **internalized bacteria (pathogenic and non-pathogenic) are able to survive within amoebae up to 25 days!**
- ✓ **our results are compatible with the results of Barker and collaborators (1999) which showed that there is a mutually beneficial interaction between *E. coli* O157 and *Acanthamoeba* trophozoites and that a significant increase in *E. coli* O157 is observed.**
- ✓ **free-living amoebae could play a decisive role in the persistence of pathogenic strains of *E. coli* in the environment favoring their spread with a consequent increase in the risk of infection for humans**



Thanks for your Attention

