Cell Biology of the Interaction between *Listeria monocytogenes* and *Colpoda* spp.

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Abstract

Intracellular bacterial pathogens, such as *Listeria monocytogenes,* survive and multiply within mammalian cells. However, interaction with protozoans in the external environment may protect this pathogen from harsh conditions. In particular, if *L. monocytogenes* is able to survive within these protozoans, these cellular hosts may act as a vehicle that links contamination of food processing environments to contamination of foods. Though *Colpoda* ciliates are a very common type of protozoa, current knowledge on the interaction between intracellular bacterial pathogens and *Colpoda* at cellular level is limited. The interaction of *L. monocytogenes* and *Colpoda* ciliates was investigated in the present study.

Co-cultures of planktonic and biofilm *L. monocytogenes* DRDC8 with *Colpoda* sp. strains RR (isolate of natural environment) and strain MLS-5 (isolate of food processing environment) at 25^{o}C were used to examine this interaction. Bacteriological counts and microscopy (fluorescence and transmission electron microscopy (TEM)) were used to track the fate of internalized *L. monocytogenes* within the ciliates. TEA chloride was used to inhibit phagocytosis to determine if *L. monocytogenes* induce its own uptake into *Colpoda*. Grazing of *Colpoda* on *L. monocytogenes* biofilms and changes in biofilm structures were evaluated by crystal violet assay and scanning electron microscopy (SEM). Mechanisms utilized by *Colpoda* RR in killing and degradation of DRDC8 were investigated by using chemical inhibitors of phagosome-lysosome fusion (NH_4Cl), vacuolar acidification (bafilomycin A1 and monensin), proteases (protease inhibitor cocktail) and nitric oxide (L-NMMA). In addition, the ability of *Colpoda* to secrete faecal pellets containing bacteria following co-cultures with DRDC8 was examined.

Co-culture of DRDC8 with *Colpoda* RR and MLS-5 provided direct evidence that these ciliates were able to actively phagocytose and kill planktonic and biofilm forms of DRDC8. *L. monocytogenes* was unable to initiate its own uptake into either *Colpoda* RR or MLS-5 and the level of expression of Listeriolysin O did not influence the outcome of co-culture.. The increase in viable counts of *Colpoda* following feeding with DRDC8 together with a concomitant reduction in viable counts of intra-ciliate DRDC8 within a 4 h period, indicated *Colpoda* used *L. monocytogenes* as a food source. This was confirmed by observations that internalized DRDC8 were confined within tight vacuoles the presence of

large food vacuoles containing many electron-dense bacteria-sized particulates within *Colpoda* cytosol. *Colpoda* RR also effectively phagocytosed and degraded *S.* Typhimurium C5, as well as several non-pathogenic bacteria such as *B. subtilis* and *E. coli* DH5α.

An important and novel outcome was the observation that induction of encystment of DRDC8-fed *Colpoda* RR, lead to the entrapment of bacterial cells within cyst outer walls and the cytosol. Furthermore, co-cultures of DRDC8 with *Colpoda* RR and MLS-5 resulted in the secretion of faecal pellets containing intact, viable and respiring DRDC8 cells. Bacteriological counts confirmed that faecal pellet-located DRDC8 were resistant to concentrations of gentamycin (up to 100 μg mL^{-1}) and sodium hypochlorite (up to 10%), that were well above concentrations that are otherwise lethal to suspensions of DRDC8.

Fluorescence microscopy of acidotrophic stains Lysosensor^{TM} Blue DND 167 and acridine orange treated Colpoda cells showed lysosomes fused with DRDC8-containing vacuoles within *Colpoda* RR. Following treatment of co-cultures with NH_4Cl, bafilomycin A1 and protease inhibitor cocktail, viable intra-ciliate bacterial counts and TEM showed evidence of survival without replication of DRDC8 within inhibitor-treated *Colpoda* RR for up to at 24 h post feeding. These outcomes indicated that *Colpoda* RR employs phagosome-lysosome fusion, vacuole acidification and proteases as mechanisms to kill intra-ciliate *L. monocytogenes*. However, nitric oxide was not involved in the killing of DRDC8 within *Colpoda*.

Both *Colpoda* strains used, actively phagocytosed and killed *L. monocytogenes*. *L. monocytogenes* were unable to escape the ciliate phagocytic vacuole and establish an intracellular lifestyle within *Colpoda*. This conclusion is in stark contrast to observations of the fate of *L. monocytogenes* cells within mammalian cells in which these bacteria escape into the cytosol in a Listeriolysin O dependent manner and then spread from cell to cell. However, the release of *Colpoda*-derived faecal pellets containing viable *L. monocytogenes*, indicated that these encapsulated forms of bacteria may provide a reservoir and a mechanism for transmission of pathogens. Considering that a faecal pellet-location endows bacteria with resistance to disinfectants and cleaning agents used in food manufacturing and preparation facilities, this may explain why *L. monocytogenes* is difficult to eradicate from food processing facilities.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Rethish Raghu Nadhanan and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Rethish Raghu Nadhanan

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Abbreviations

A_{570}	absorbance at 570 nm
bp	base pairs
°C	degrees Celsius
CFU	colony forming units
d	days
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
eDNA	extracellular DNA
g	relative centrifugal force
g L^{-1}	grams per litre
h	hour/s
Kb	kilobase/s
kg	kilogram/s
L	litre/s
LB	Luria Bertani broth
LEM	Listeria Enrichment Medium
L-NMMA	N(G)-monomethyl-L-arginine
M	molar
μg	microgram/s
μL	microlitre/s
mg	milligram/s
min	minute/s
mL	millilitre/s
mM	millimolar
moi	multiplicity of infection
MQ	Milli-Q water
NaOCl	sodium hypochlorite
NBT	nitro blue tetrazolium
OD	optical density

O/N	overnight
PBS	phosphate buffered saline
PCR	polymerase chain reaction
RT	room temperature
SEM	scanning electron microscopy / micrograph
sp. / spp.	species
TAE	tris-acetate EDTA buffer
TEA chloride	tetraethylammonium chloride
TEM	transmission electron microscopy / micrograph
TTSS	type three secretion system
vol	volume/s
v/v	volume per volume
w/v	weight per volume
XTT	2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

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Let us be grateful to people who make us happy; they are the charming gardeners who make our souls blossom.

Marcel Proust

Praise the bridge that carried you over.

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