Antibiotic alternatives for controlling foodborne pathogens and disease in poultry

Abhi Upadhyay, Ph.D.

Dr. Annie Donoghue, Dr. Narayan Rath, 2 Vacant SY

Poultry Production and Product Safety Research,

Fayetteville, AR

United States Department of Agriculture

Agricultural Research Service

Project goals

To provide the poultry industry with practical antibiotic alternatives for the control of bacterial foodborne pathogens in conventional and organic poultry sectors.

CRIS project

- **Mucin adapted probiotic bacteria**
- **Mucin adapted bacteriophages**
- ***Campylobacter* specific CRISPR-Cas system**
- **Plant-derived compounds**
- **Egg-shell Membrane modulator**

Project plan

Pre-harvest control of *Campylobacter* and *Salmonella*

Reduce the incidence of *Campylobacter* in poultry using
(i) Mucin adapted probiotics
(ii) Plant-derived compounds
(iii) Mucin adapted bacteriophage with or without CRISPR-Cas system
(iv) Egg shell membrane modulator

Reduce the incidence of *Salmonella* in poultry using
(i) Egg shell membrane modulator

Investigate the mechanisms of action of probiotic bacteria, bacteriophage and plant-derived compounds for anti-*Campylobacter/Salmonella* efficacy using phenotypic assay, gene expression analysis and RNA transcriptomics

Post-harvest control of *Campylobacter* and *Salmonella*

Reduce the incidence of *Campylobacter* and *Salmonella* on poultry carcass using
(i) Plant-derived compounds
(ii) Mucin adapted bacteriophage with or without CRISPR-Cas system

Plant-derived compounds

- **Major groups:** Polyphenols, Flavonoids, Alkaloids, Lectins, Tannins

- **Advantages:** Multiple mechanisms of action

Phytochemicals	Source
Trans-cinnamaldehyde (TC)	Cinnamon trees
Carvacrol (CR)	Oregano oil
Eugenol (EG)	Clove
β-resorcylic acid (BR)	Resins, Brazilian wood

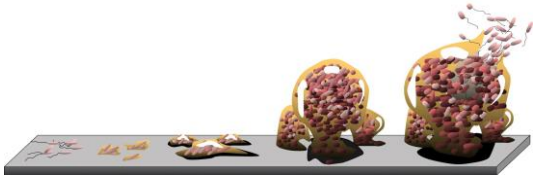



FDA approved- GRAS status compounds

Strategies to control foodborne pathogens

Pre-harvest persistence

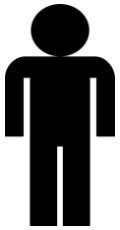
Campylobacter spp.
Salmonella spp.



Persistence in processing environment

Listeria monocytogenes
Campylobacter spp.
Salmonella spp.

Post-harvest contamination

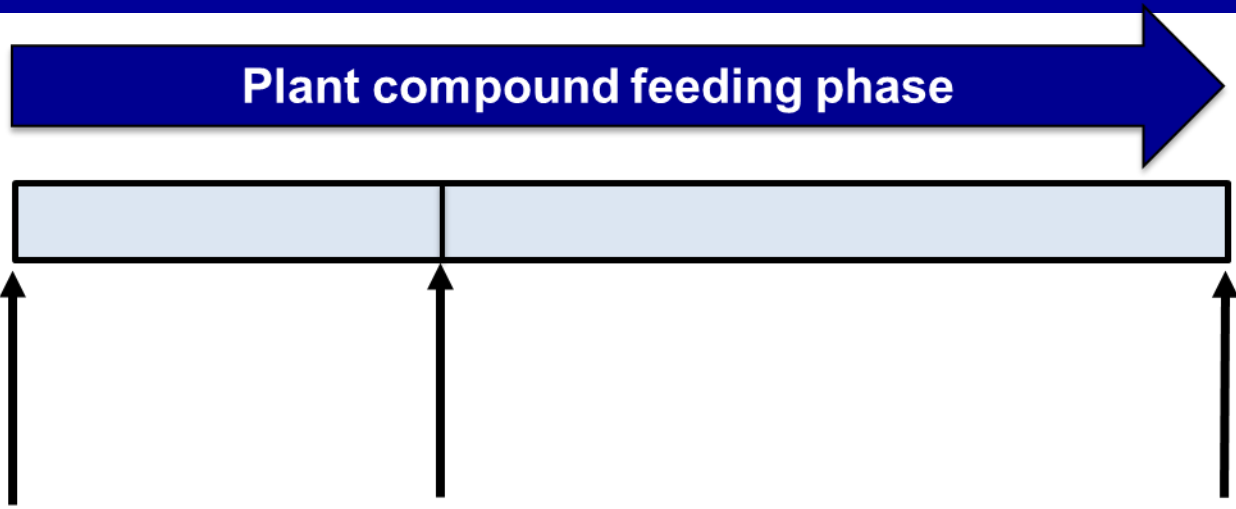


Anti-virulence strategies

(1) Reducing pathogen colonization in food animals

Efficacy of Beta-Resorcylic acid in reducing *Campylobacter jejuni* and *Salmonella* Enteritidis colonization in broiler chicken

Experimental Approach 1



Day 0
Grouping of birds

(Cobb500)

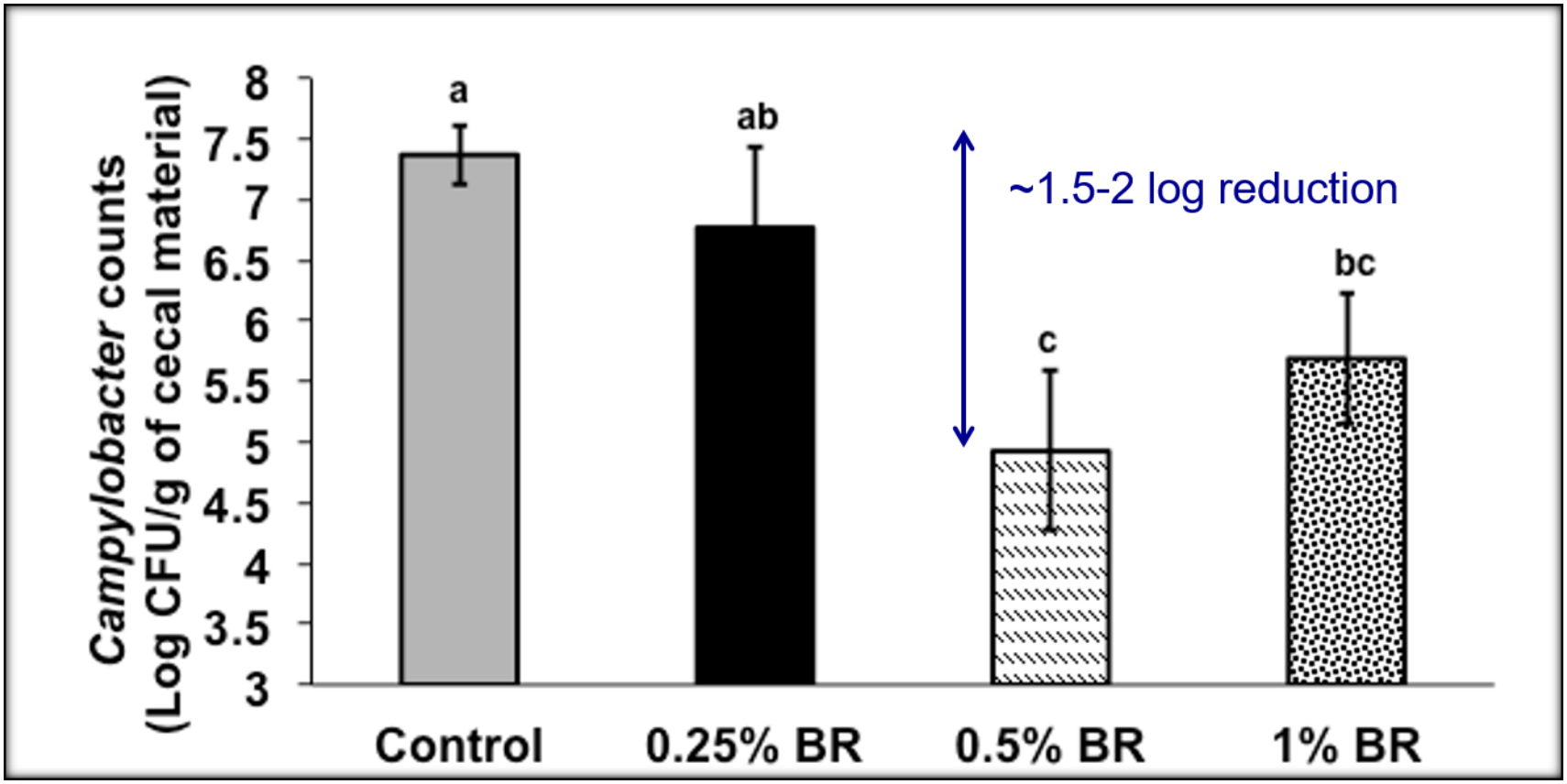
Control
0.25% BR
0.5% BR
1% BR

Day 7
Orally challenged with *C. jejuni*
(7 log CFU/bird)

Day 14
C. jejuni enumeration in cecal contents

10 birds/treatment/trial

EFFECT OF BR ON CAMPYLOBACTER JEJUNI CECAL COLONIZATION

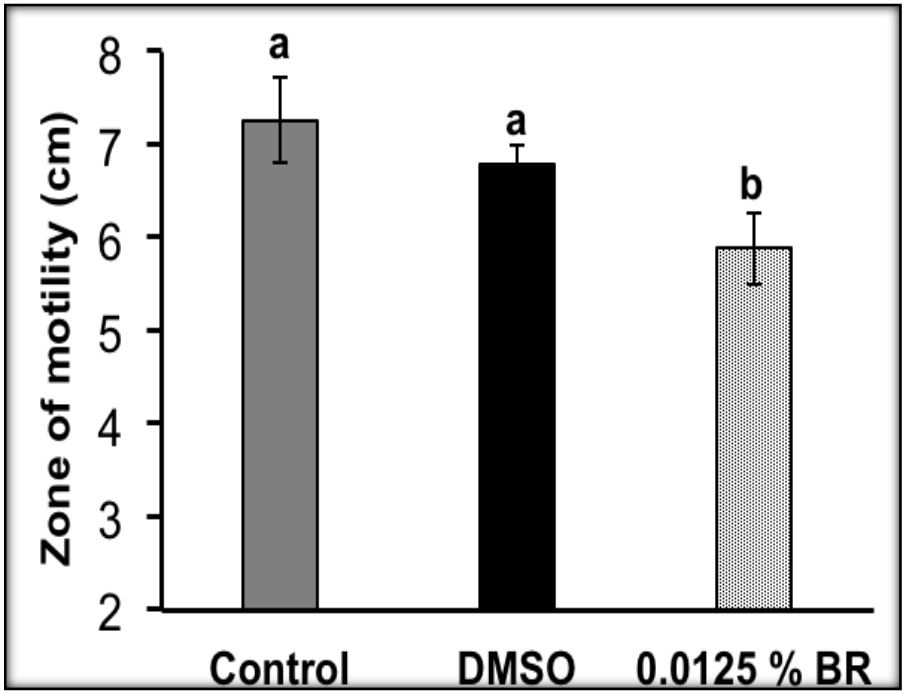


Wagle,B.R., Upadhyay, A., et al., 2017, *Under review Frontiers in Microbiology*

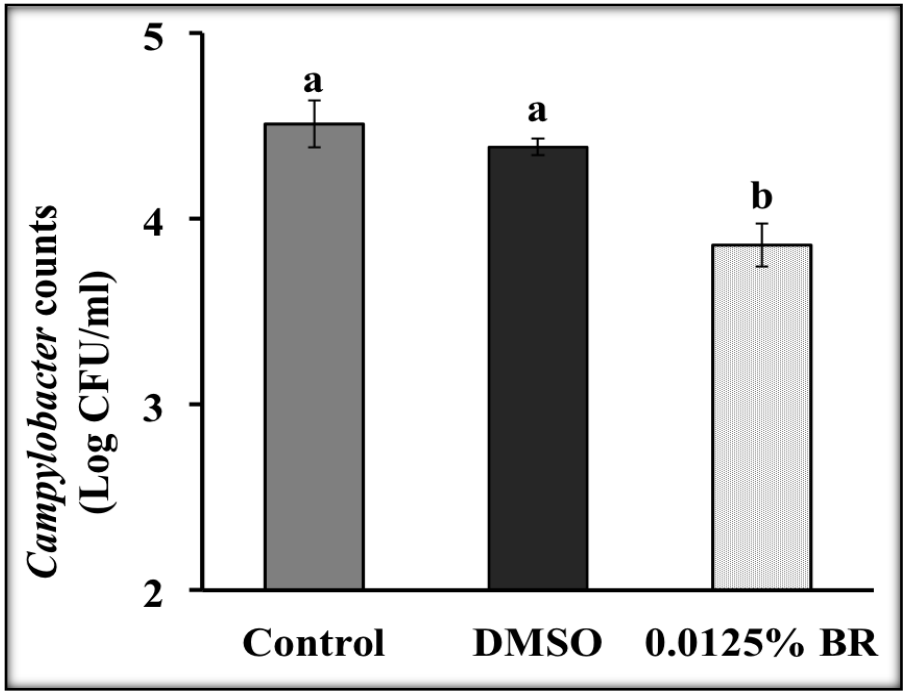
$P<0.05$

Effect of BR on *Campylobacter jejuni* motility and attachment to epithelial cells

Motility

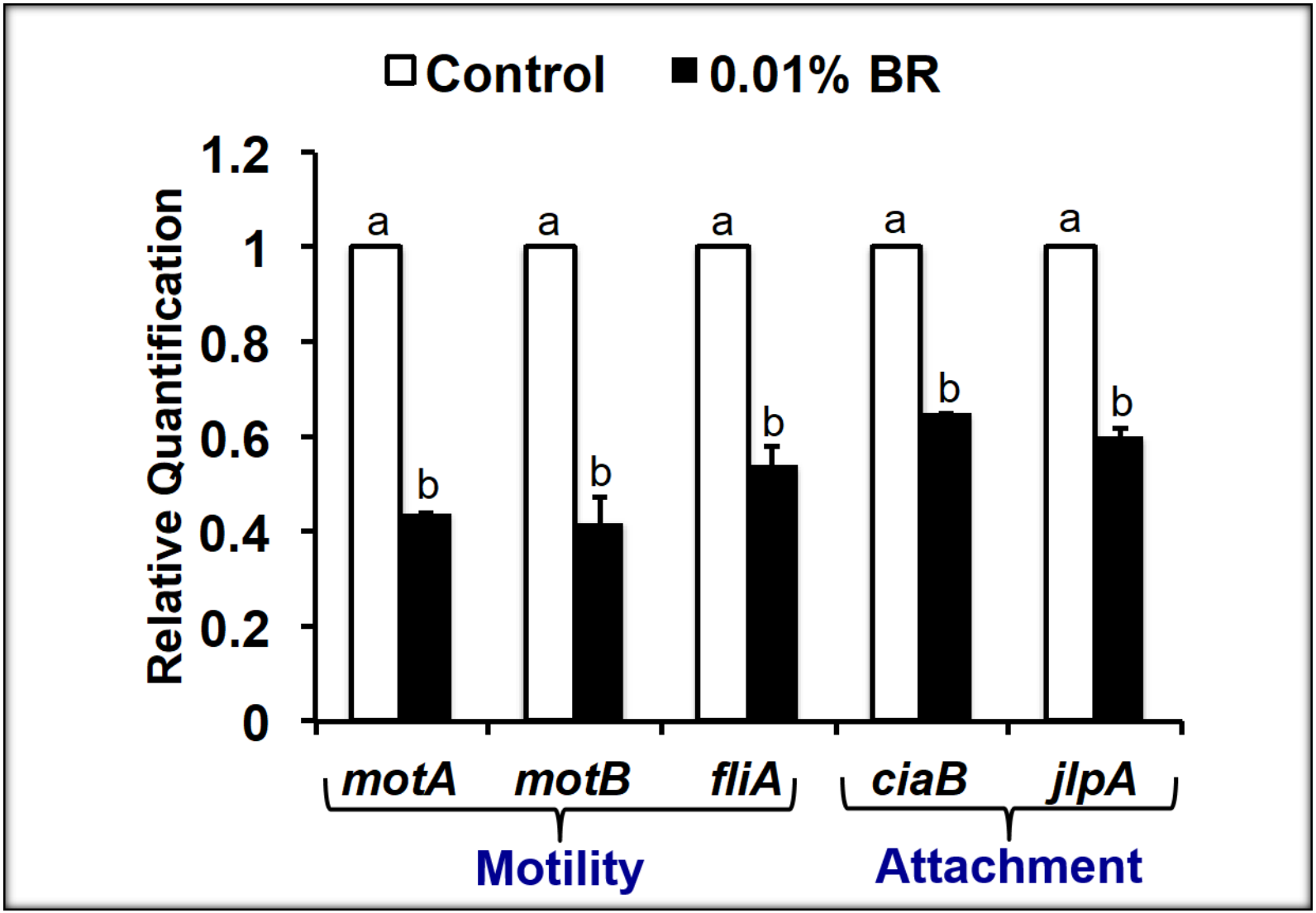


Attachment to epithelial cells



P<0.05

Effect of BR on *C. jejuni* chicken colonization genes



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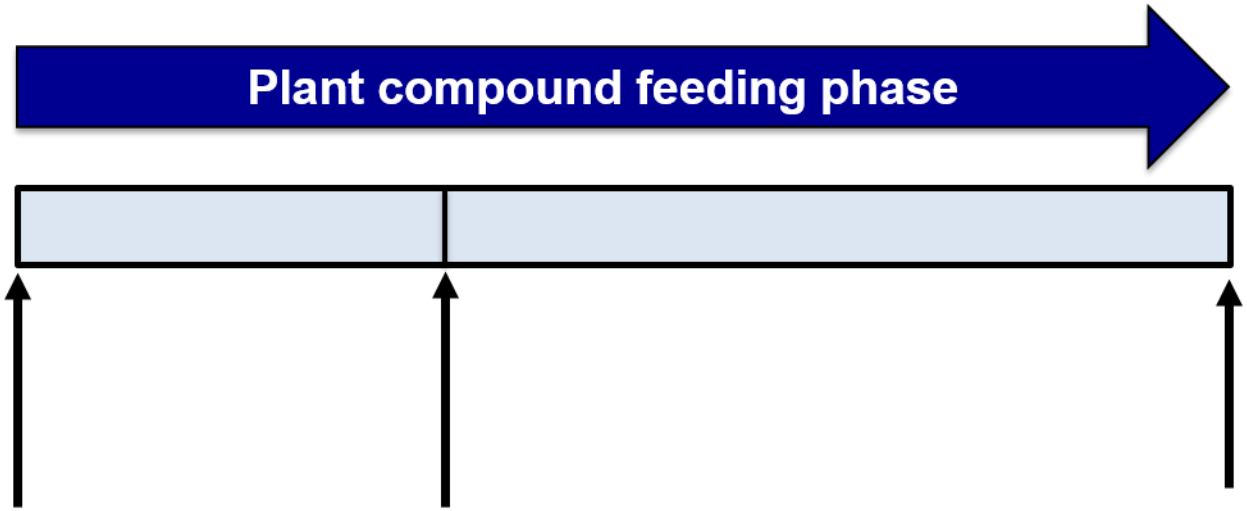
Experimental Approach 2



Day 0
Grouping of birds

(Cobb500)

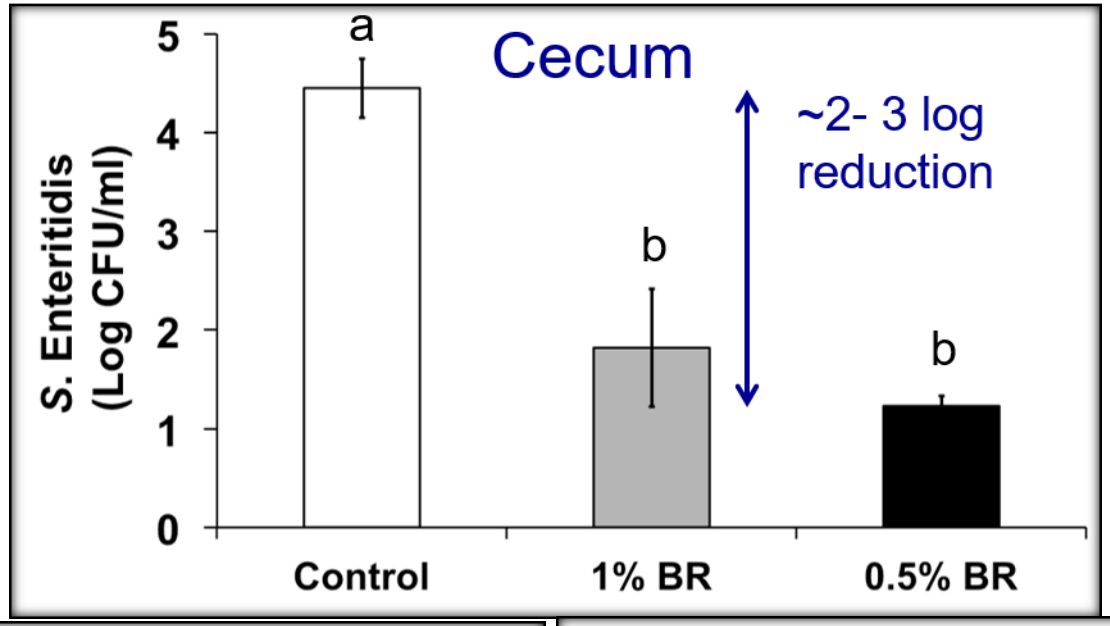
Control
0.5% BR
1% BR

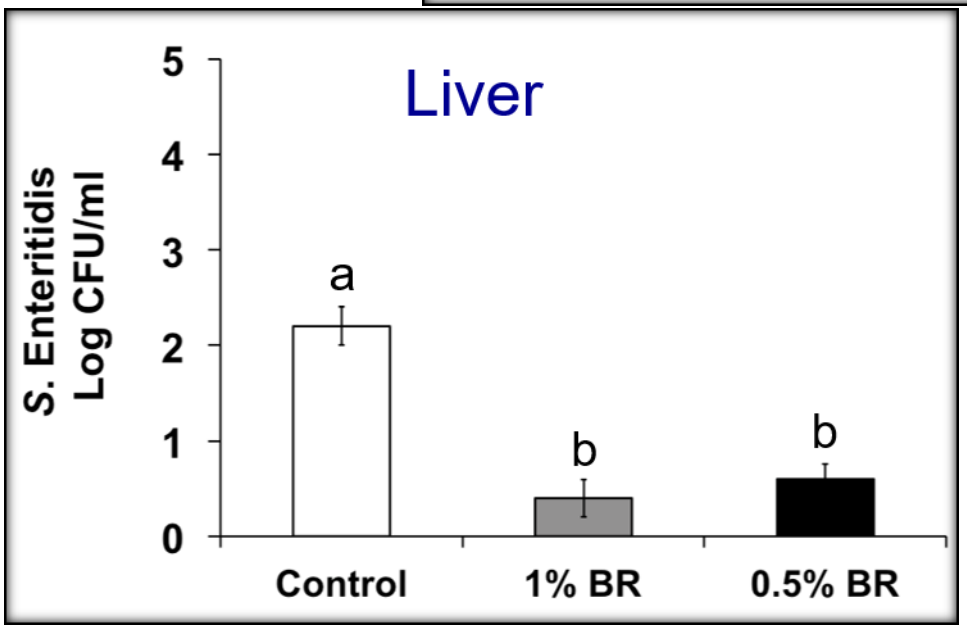
Day 7
Orally challenged with *S.* Enteritidis (7 log CFU/bird)

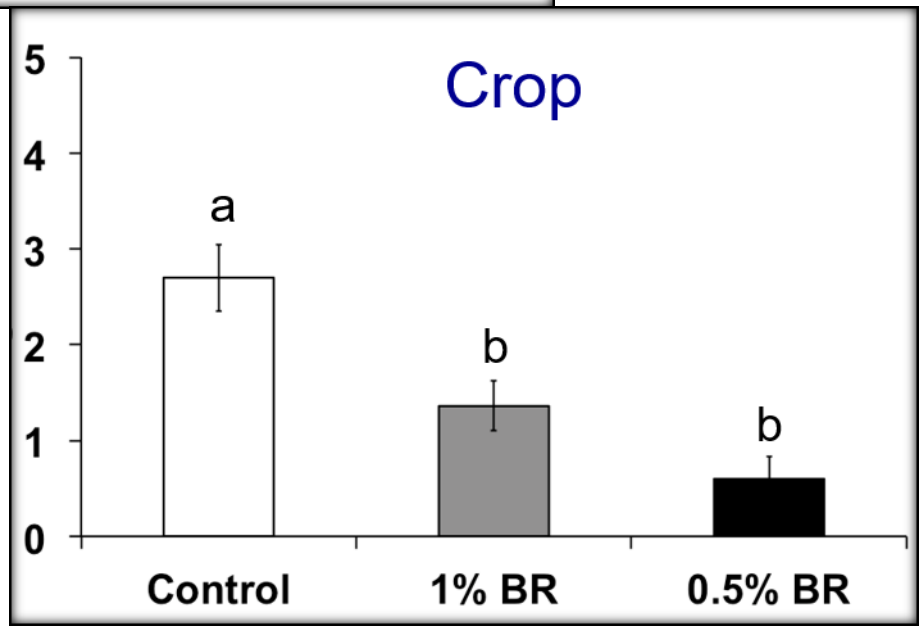
Day 21
S. Enteritidis enumeration in cecum liver crop

10 birds/treatment/trial

Effect of BR on *S.* Enteritidis cecal, crop, and liver colonization in 21 day old broiler chickens

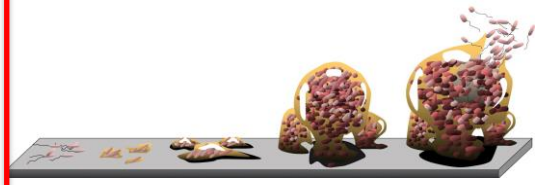






Strategies to control foodborne pathogens (1)

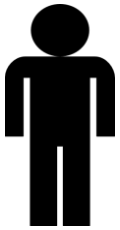
Pre-harvest persistence



Persistence in processing environment

C. jejuni

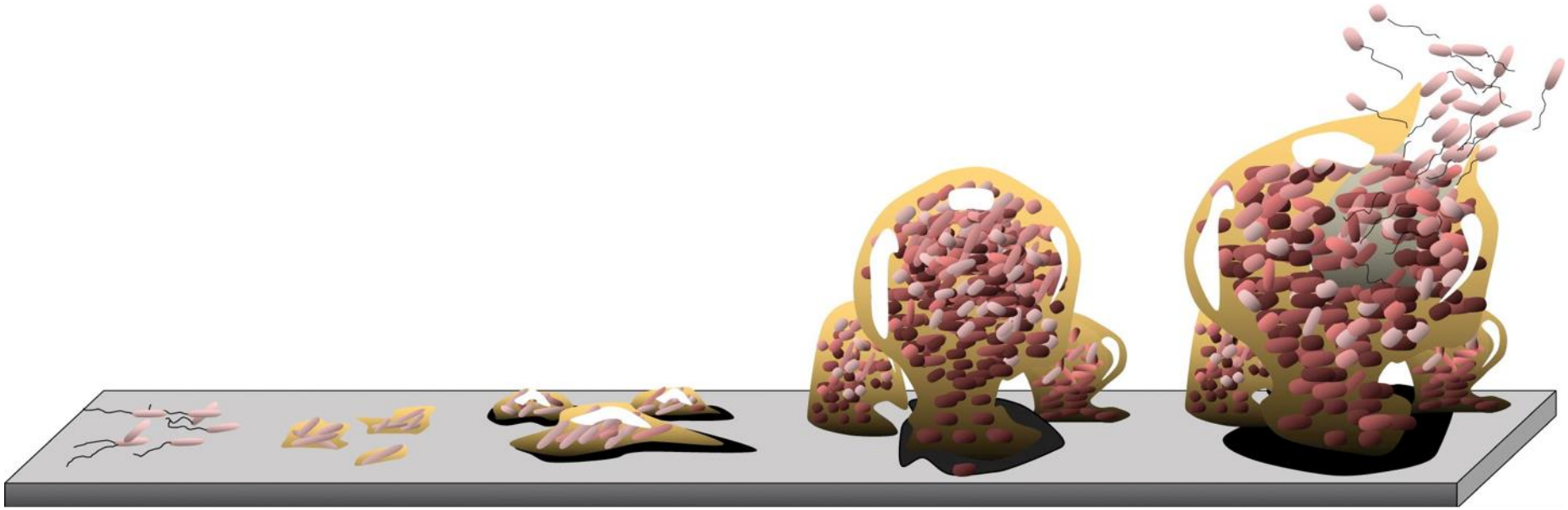
Post-harvest contamination



Anti-virulence strategies

Bacterial biofilms

Food products

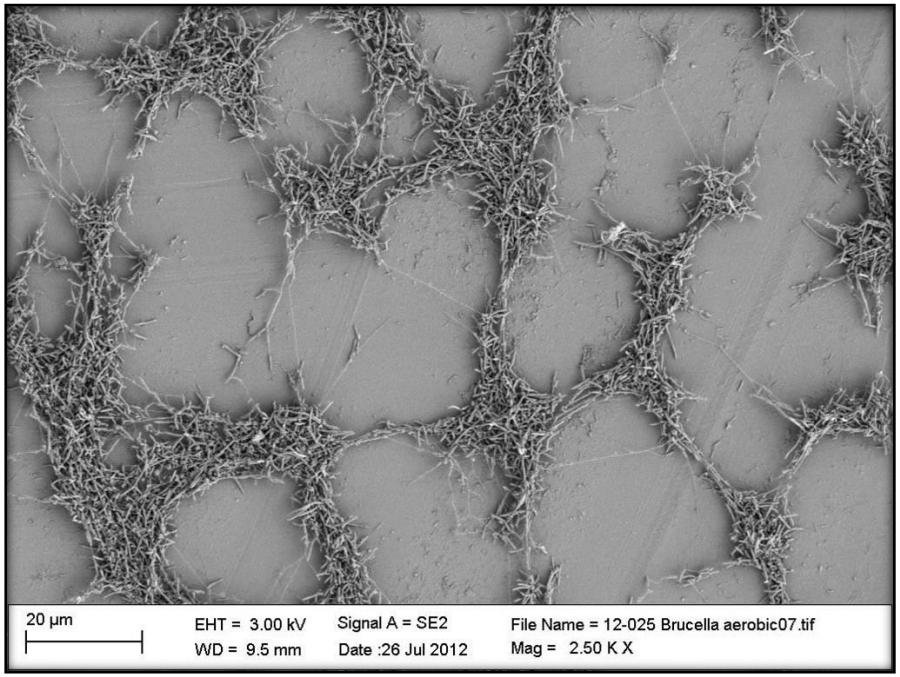


Micro-colonies

Mature biofilm

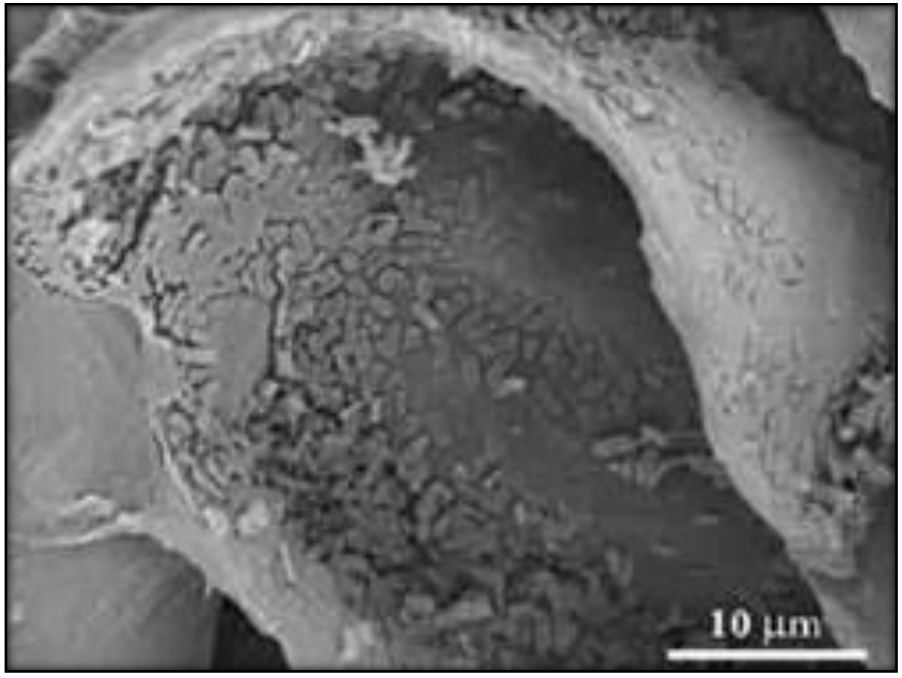
Campylobacter and *Salmonella* biofilms

Campylobacter



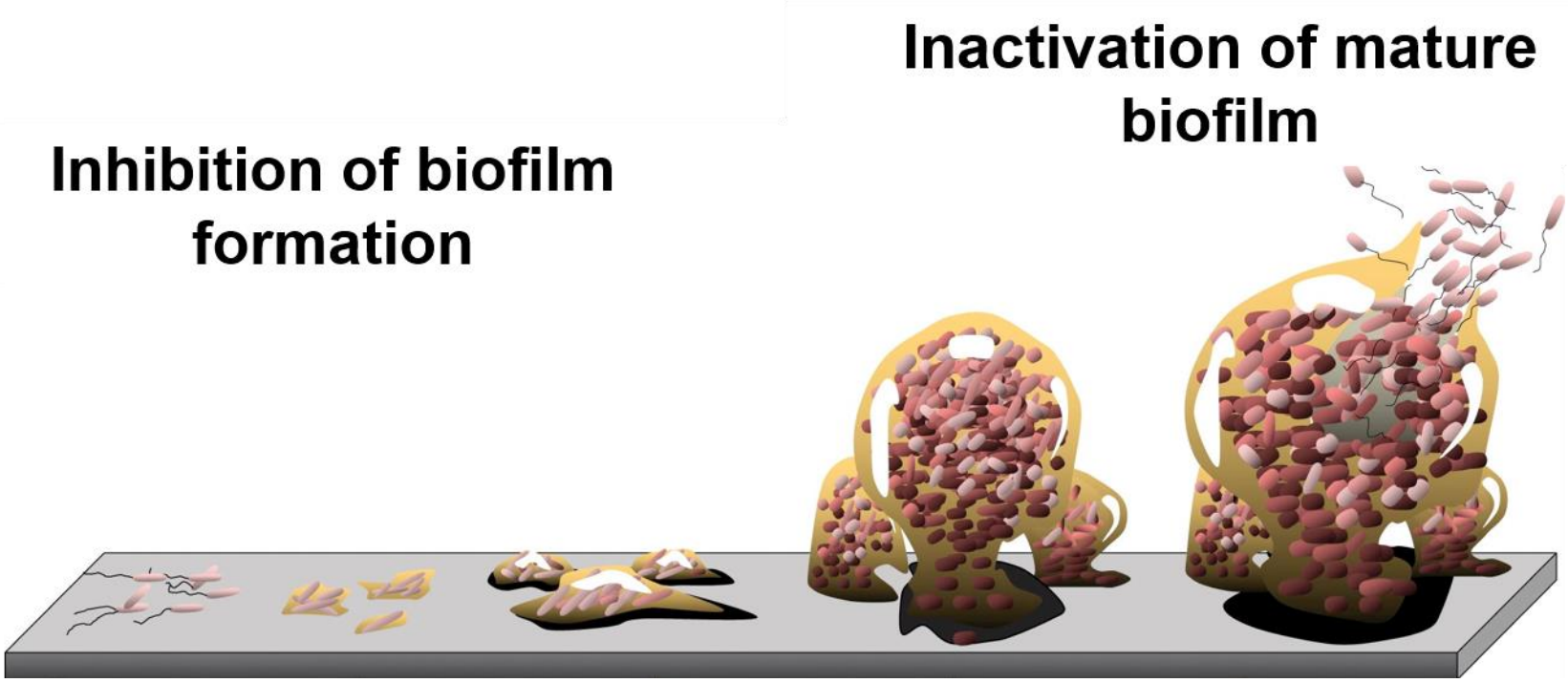
Brown et al., 2013. Journal of Applied Microbiology, 115, 1212-1221

Salmonella spp.



Annous et al., 2004, 2005, Journal of Food Science, 74(1), R24–R37

Antibiofilm strategy



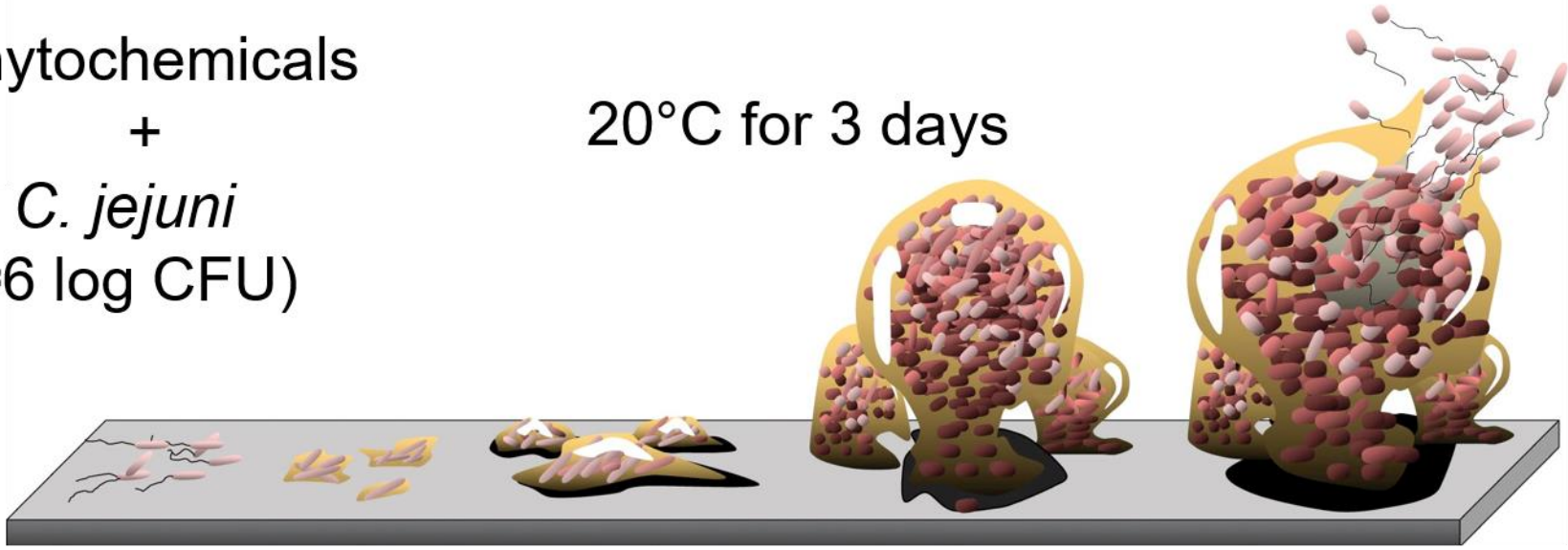
(2) CONTROLLING PATHOGENS IN FOOD PROCESSING ENVIRONMENT

The efficacy of phytochemicals in controlling *C. jejuni* biofilms

Inhibition of *C. jejuni* biofilm formation by phytochemicals

Phytochemicals
+
C. jejuni
(≈6 log CFU)

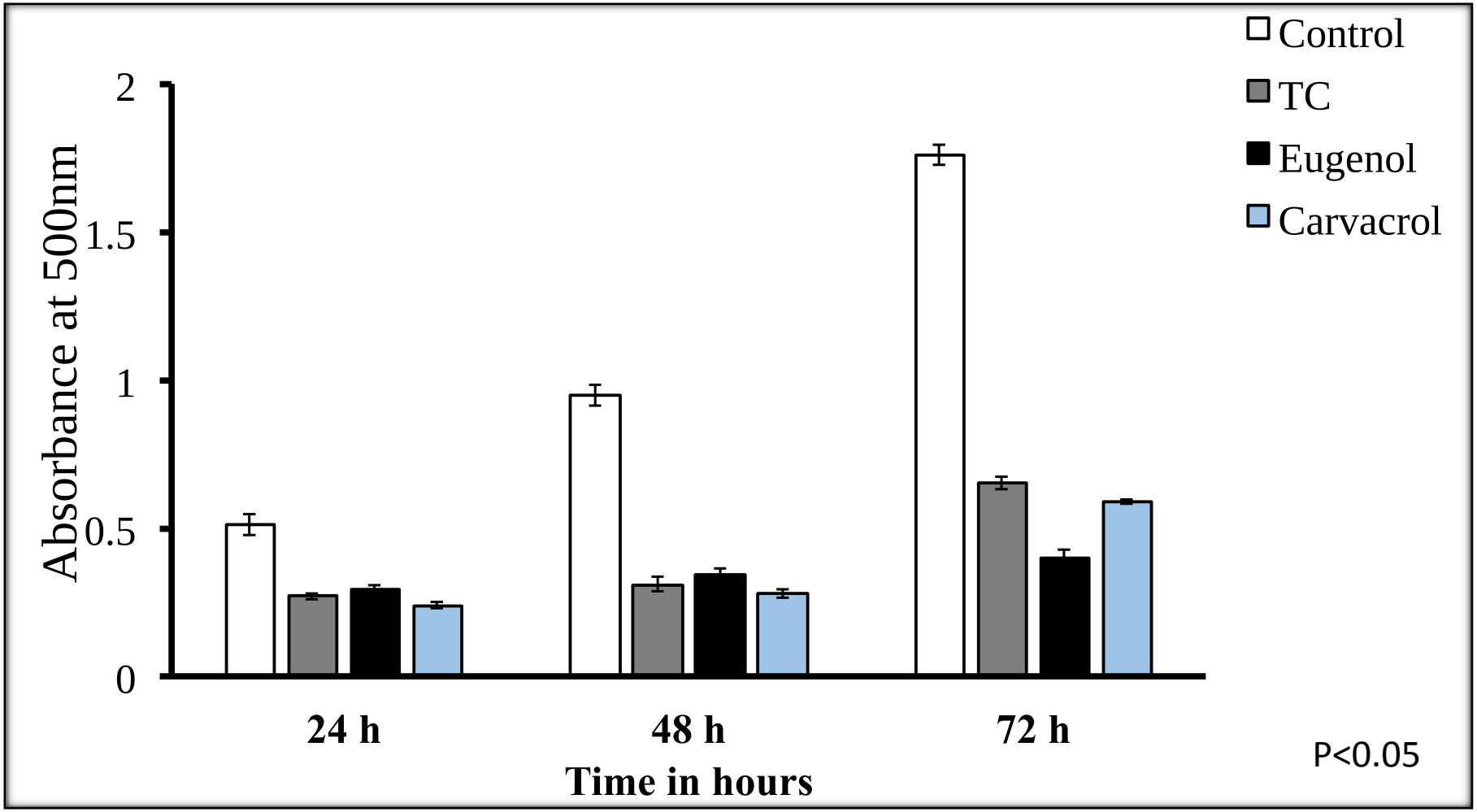
20°C for 3 days



Quantification of *C. jejuni* biofilm
(TTC staining)
(24, 48, 72 h)

Brown et al., 2013, J Appl Microbiol; Brown et al., 2014 AEM

Inhibition of *C. jejuni* biofilm formation by phytochemicals



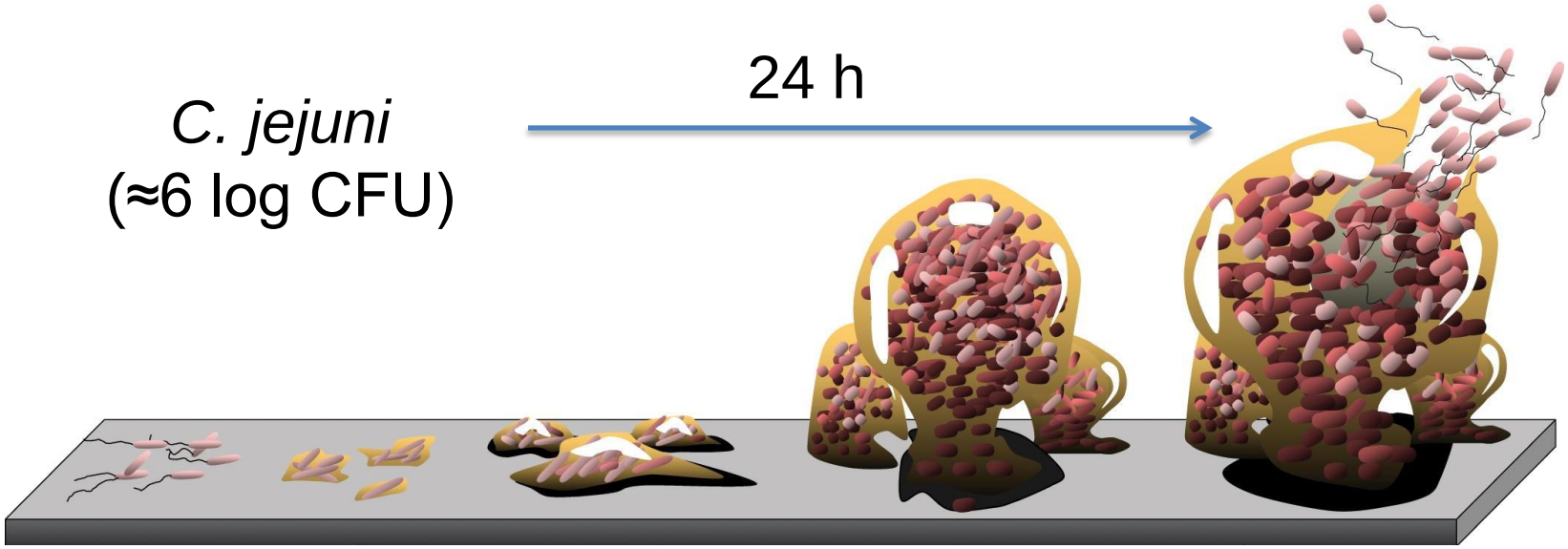
Inactivation of *C. jejuni* biofilm by phytochemicals

Phytochemicals

20°C for 1, 5, 10 min

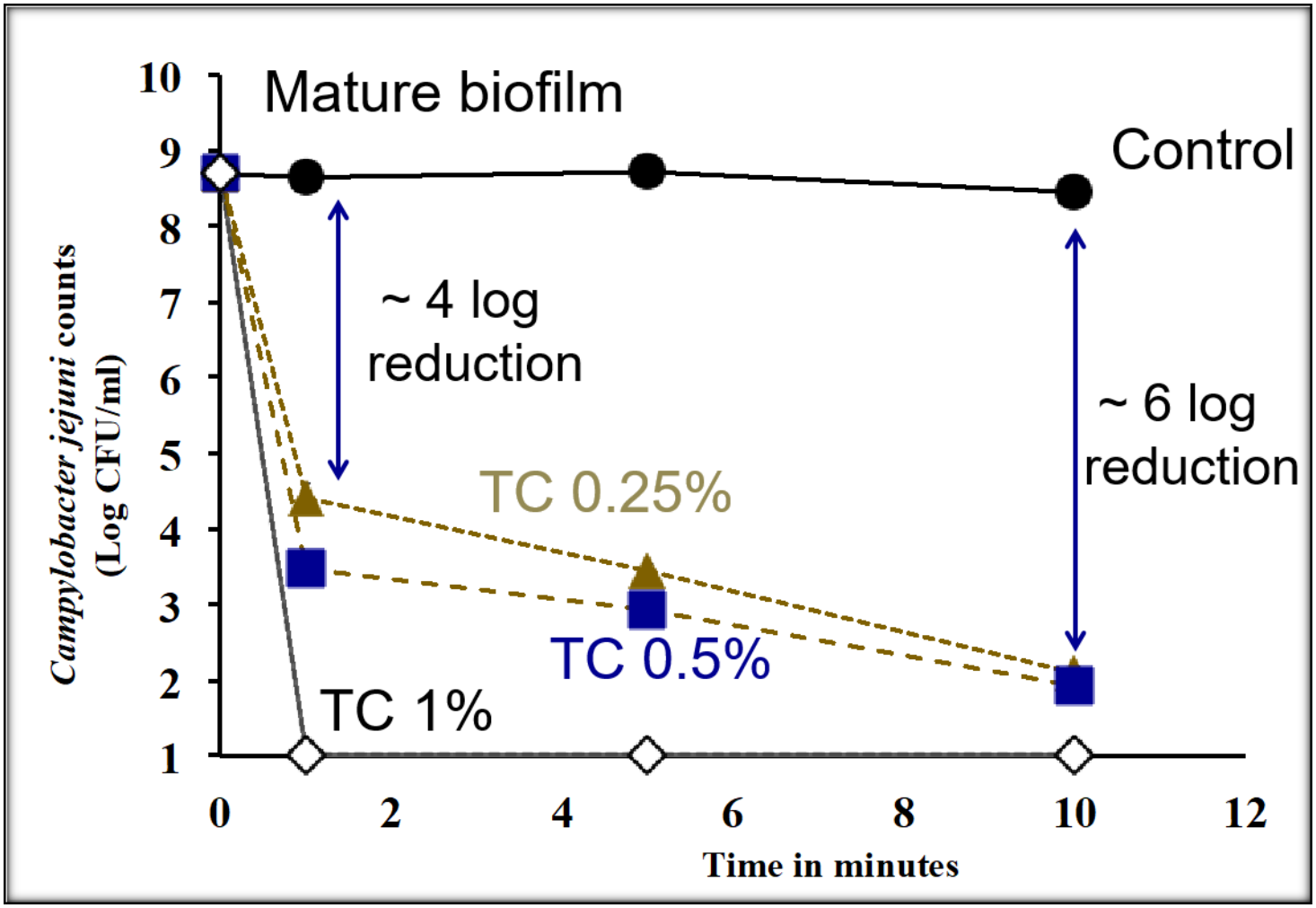
C. jejuni
(≈6 log CFU)

24 h



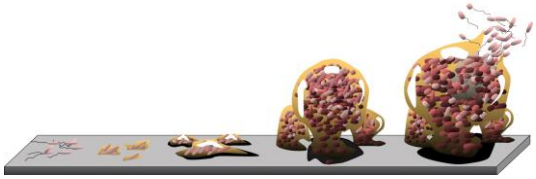
Enumeration of *C. jejuni* in biofilm

Inactivation of mature biofilm by phytochemicals



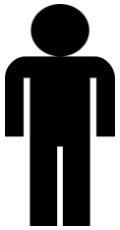
Strategies to control foodborne pathogens (2)

Pre-harvest persistence



Persistence in processing environment

Post-harvest contamination



Anti-virulence strategies

(3) Post-harvest control of foodborne pathogens in food products

Efficacy of Beta-resorcylic acid in reducing *C. jejuni* on chicken skin and meat.

Wagle. B.R., Upadhyay, A., et al., 2017, Journal of Food Protection (under review)

Contamination of Chicken carcass

Contamination during slaughter

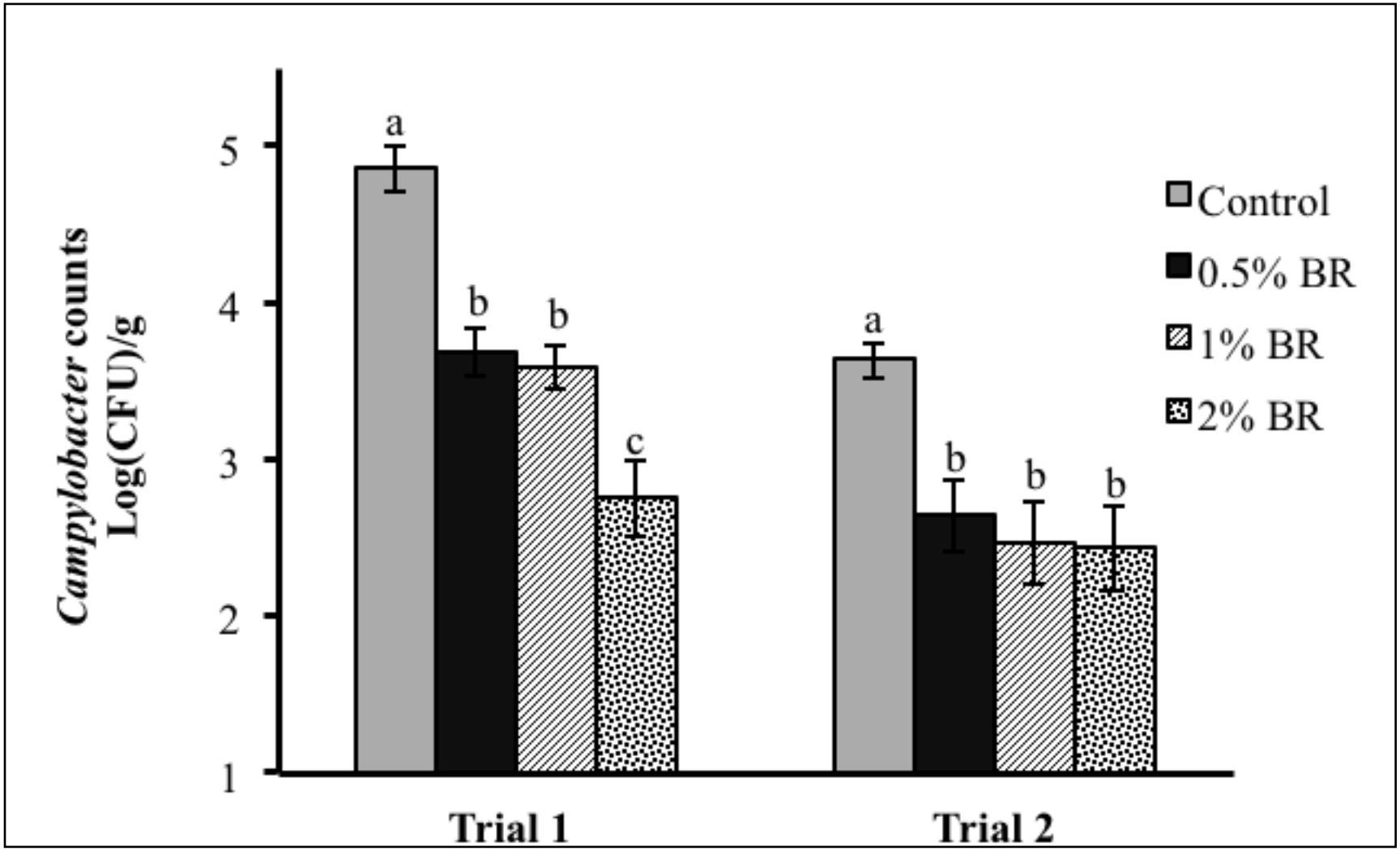
Inactivation of *C. jejuni* on chicken skin and meat by Beta-resorcylic acid

C. jejuni inoculation
(≈6 log CFU/sample)

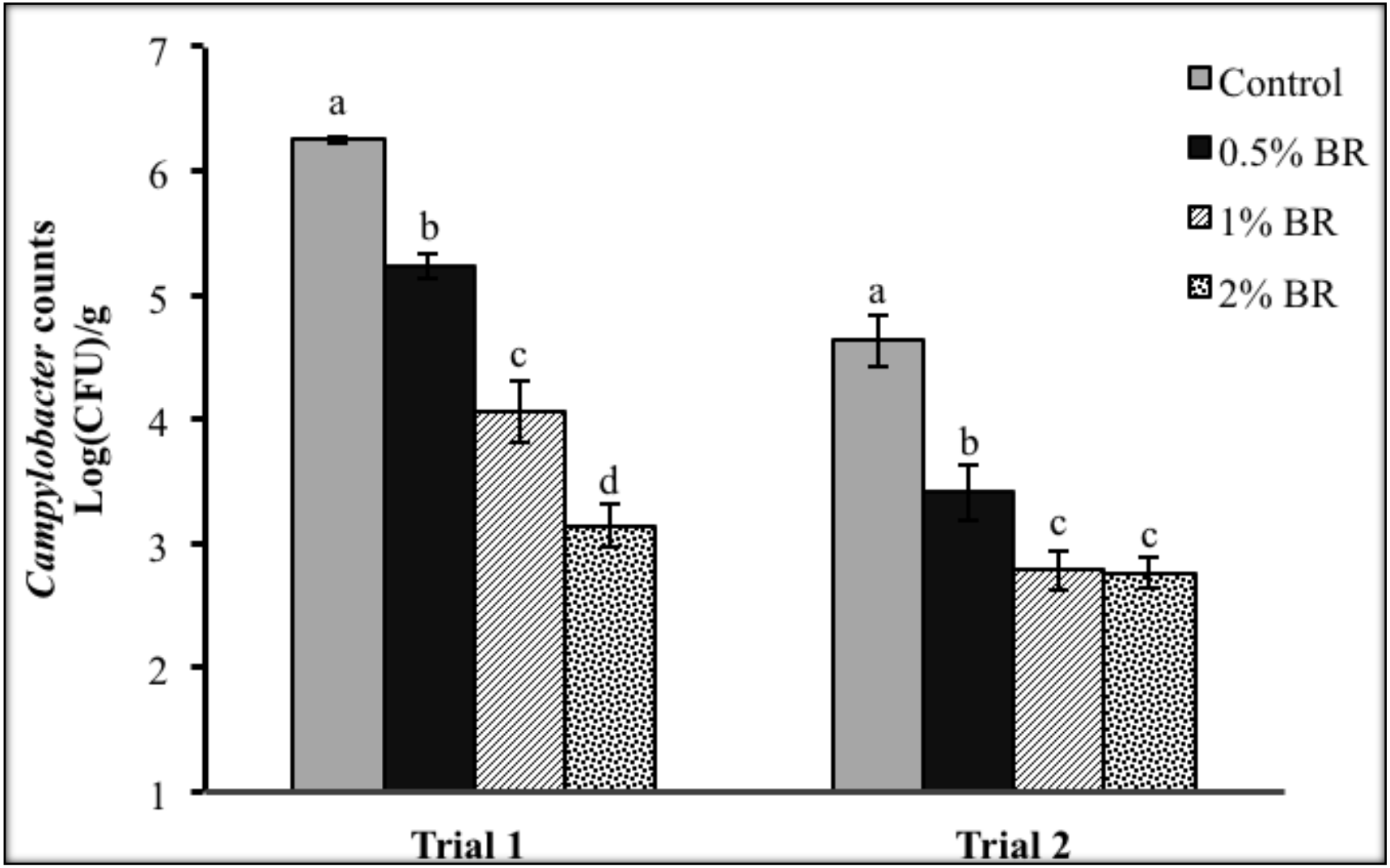
Antimicrobial wash at 25ºC
30 sec

Enumeration of surviving *C. jejuni*

Effect of Beta-resorcylic acid on survival of *C. jejuni* on chicken skin

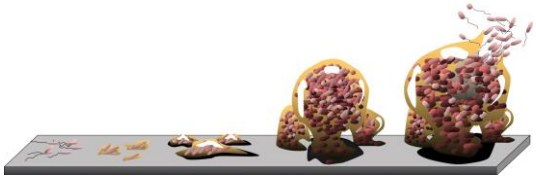


Effect of Beta-resorcylic acid on survival of *C. jejuni* on chicken breast meat



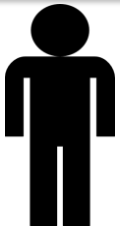
Strategies to control foodborne pathogens (3)

Pre-harvest persistence



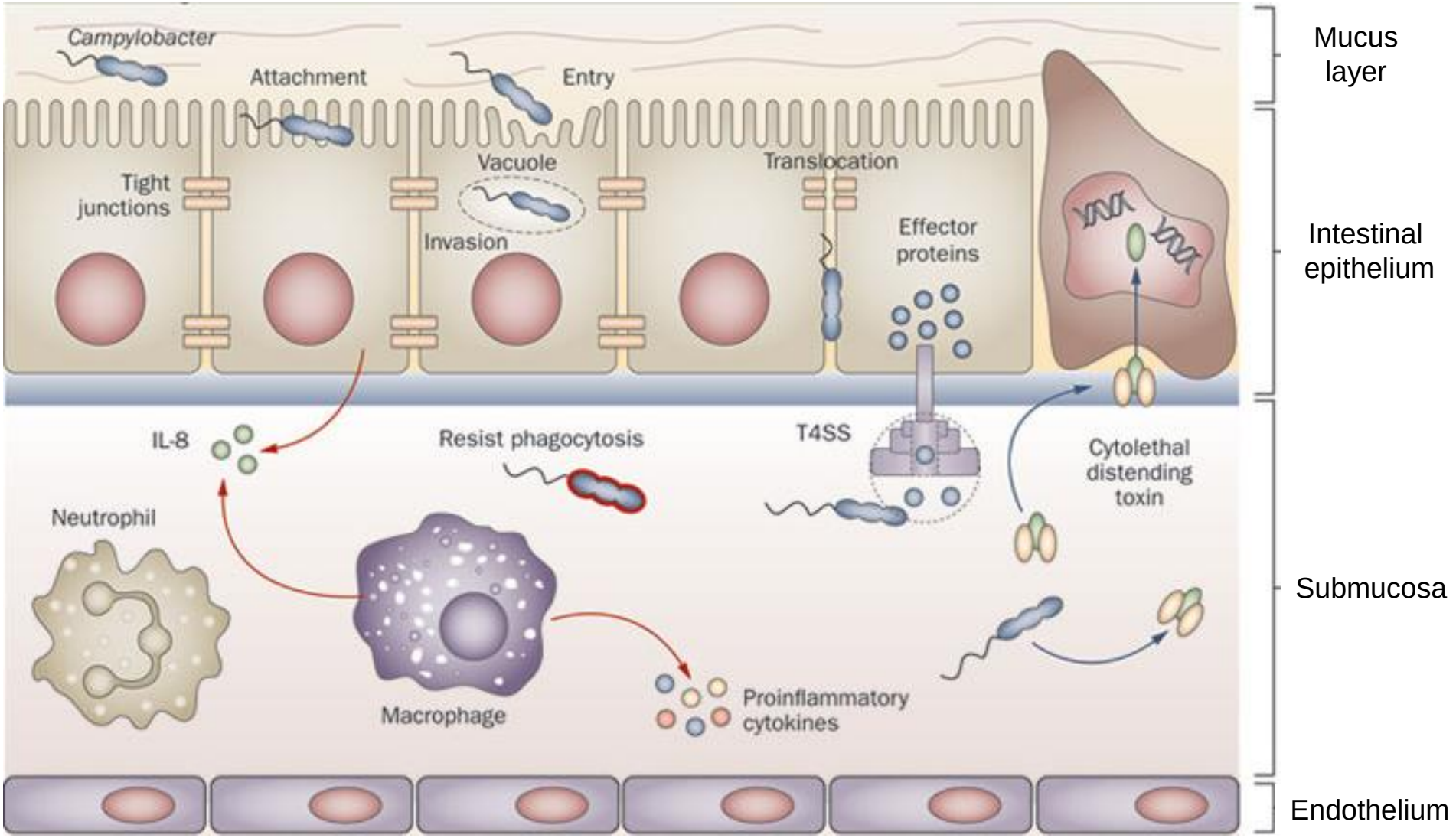
Persistence in processing environment

Post-harvest contamination



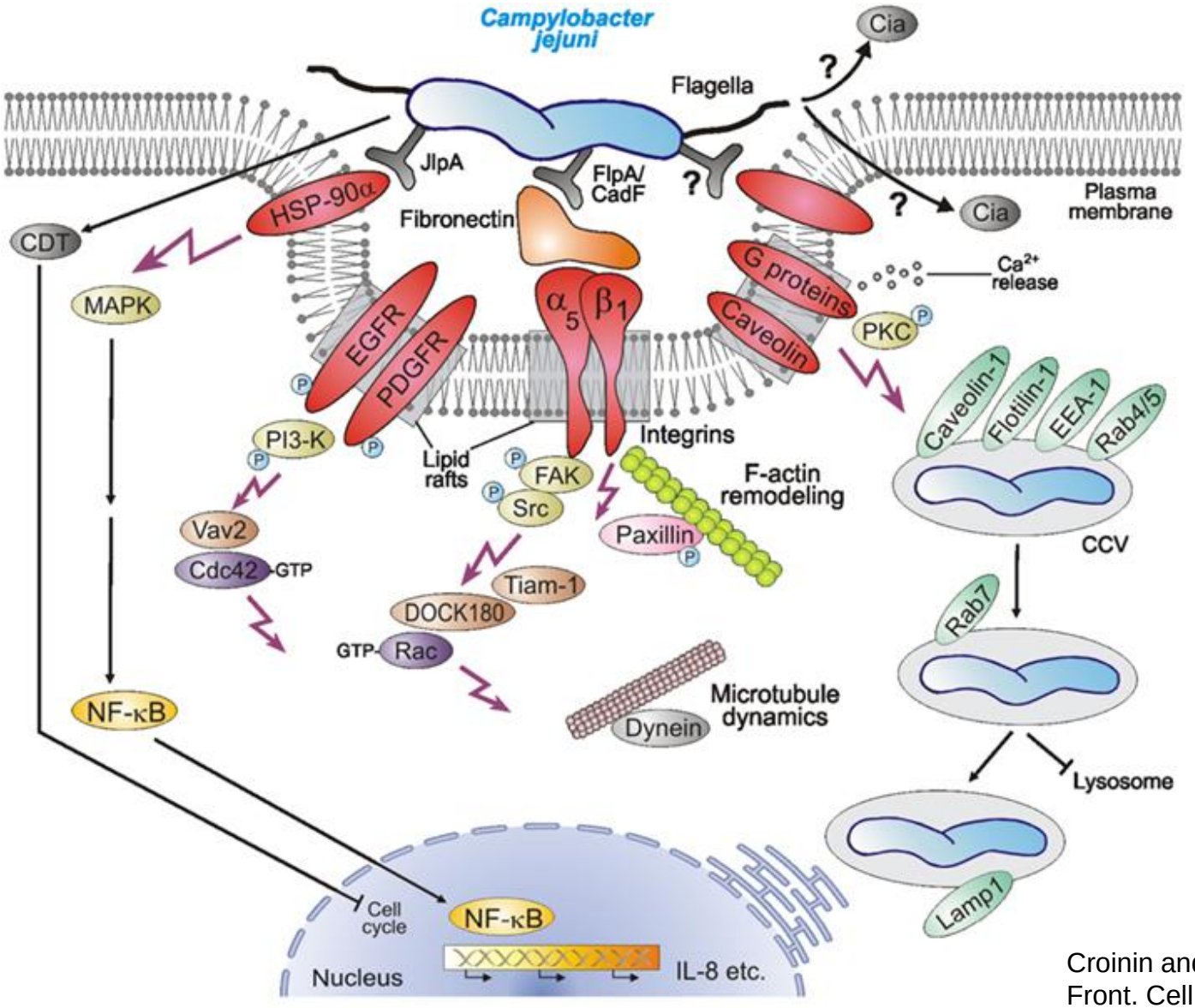
Anti-virulence strategies

Pathophysiology of Campylobacteriosis



Man, S.M., 2011. Nature Reviews Gastroenterology and Hepatology, 8(12), pp.669-685.

Virulence factors in *C. jejuni*



Croinin and Backert, 2012
Front. Cell. Infect. Microbiol

(4) Controlling human infection by modulating pathogen virulence

Efficacy of phytochemicals in reducing *Campylobacter jejuni* virulence attributes *in vitro*

Strains- ATCC-11168, ATCC 81-176, Wild type S-8

Phytochemicals	Source
Trans-cinnamaldehyde (TC)	Cinnamon trees
Carvacrol (CR)	Oregano oil
Eugenol (EG)	Clove

Sub-inhibitory concentrations

- Compound concentrations not inhibiting bacterial growth but modulating virulence

(Zhanel et al., 1992; Fonseca et al., 2004; Johny et al., 2010; Upadhyay et al., 2012)

Hypothesis: Phytochemicals (TC, CR, EG) reduce the expression of critical virulence factors in *Campylobacter jejuni*

Phytochemical concentrations

Phytochemicals	SICs (%)	
Trans-cinnamaldehyde (TC)	0.005	0.01
Carvacrol (CR)	0.001	0.002
Eugenol (EG)	0.005	0.01

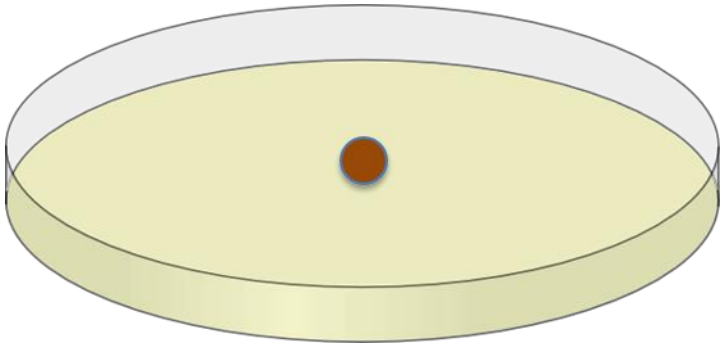
Specific objectives

To investigate the efficacy of TC, CR, EG on *C. jejuni*

- Motility
- Adhesion to and invasion of epithelial cells
- Translocation of epithelial barrier
- Production of CDT
- Expression of virulence genes

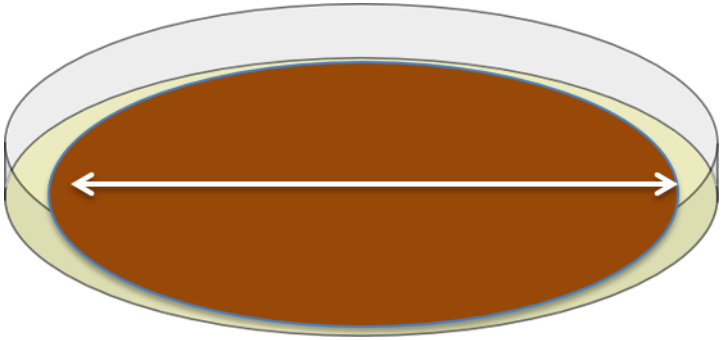
Effect of phytochemicals on *C. jejuni* motility

Motility agar
(0.3%)



5 µl *C. jejuni*
(≈6 log CFU)

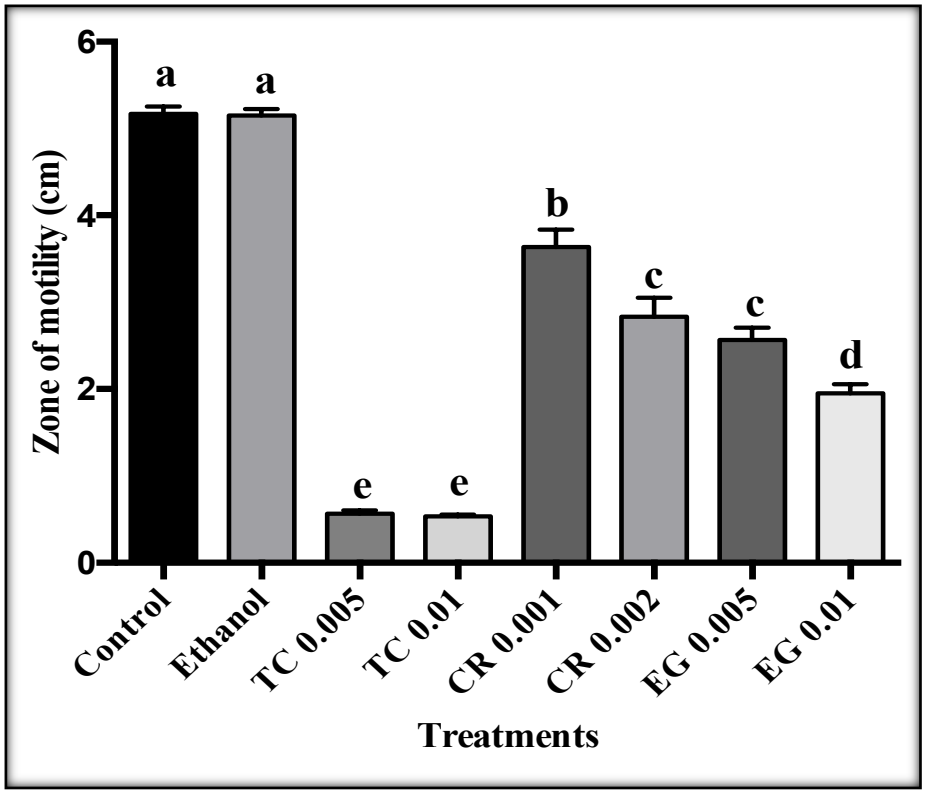
Microaerophilic Incubation
(37°C for 24 h)



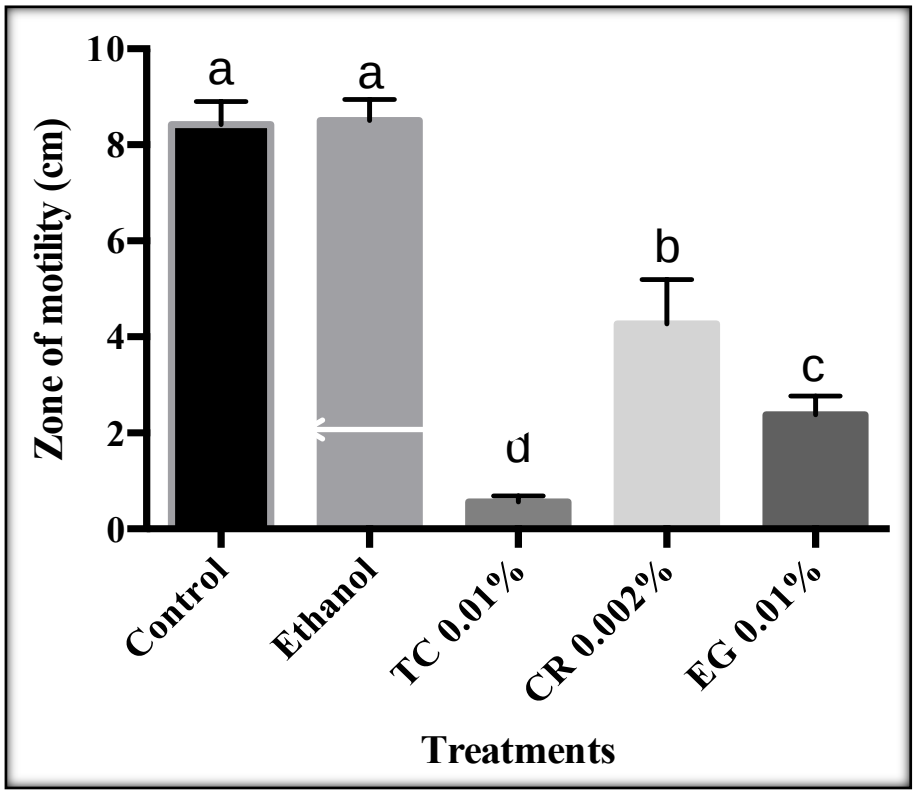
(Niu and Gilbert, 2004)

Effect of phytochemicals on *C. jejuni* motility

Human body temperature



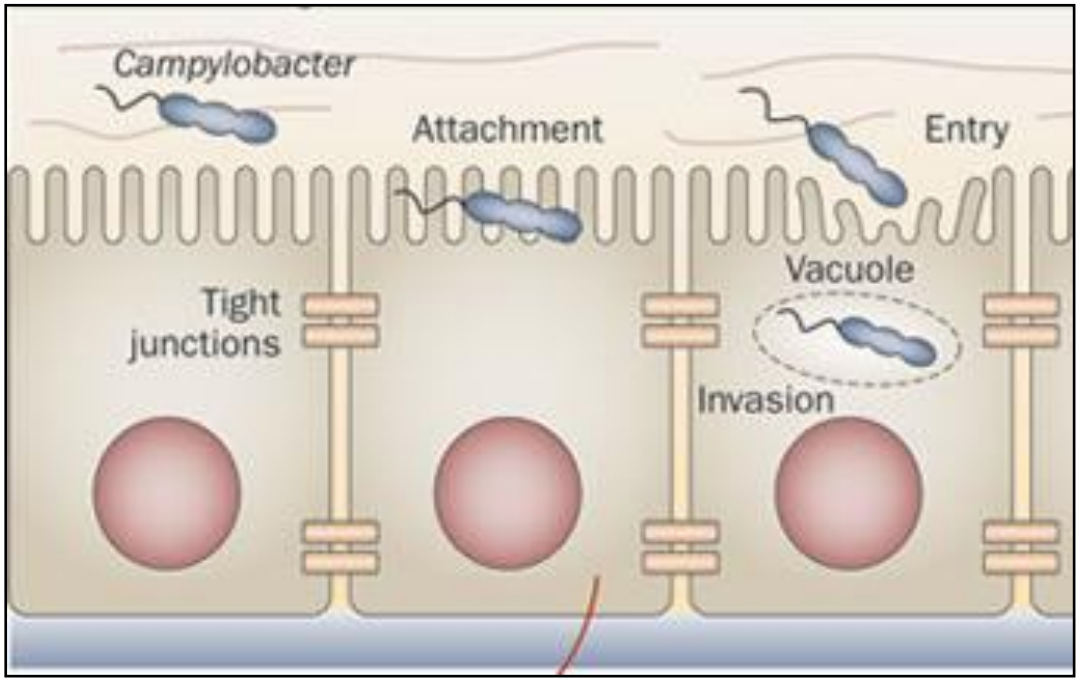
Chicken body temperature



P<0.05

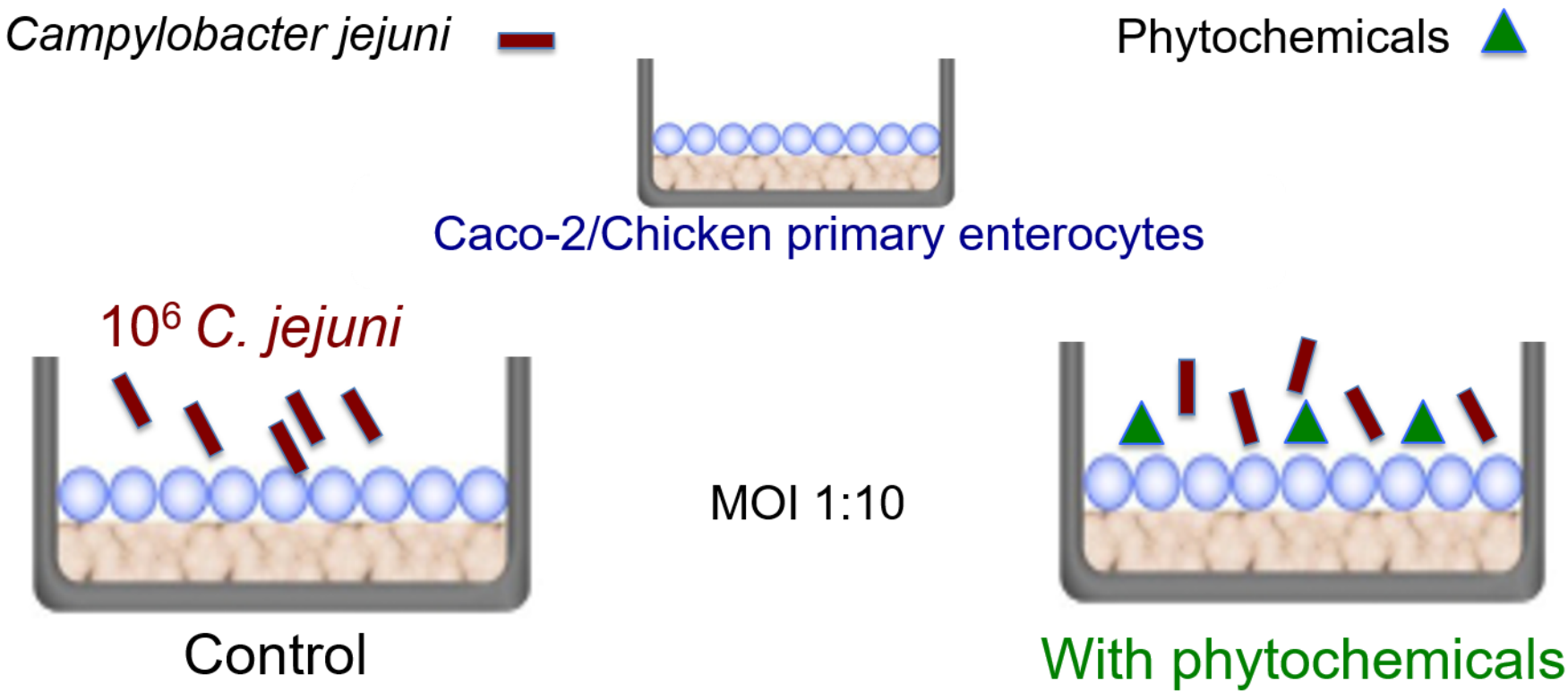
Attachment, invasion and translocation of epithelial barrier by *C. jejuni*

Gut epithelium



Man, S.M., 2011. Nature Reviews Gastroenterology and Hepatology, 8(12), pp.669-685.

Effect of phytochemicals on *C. jejuni* adhesion/invasion to intestinal cells

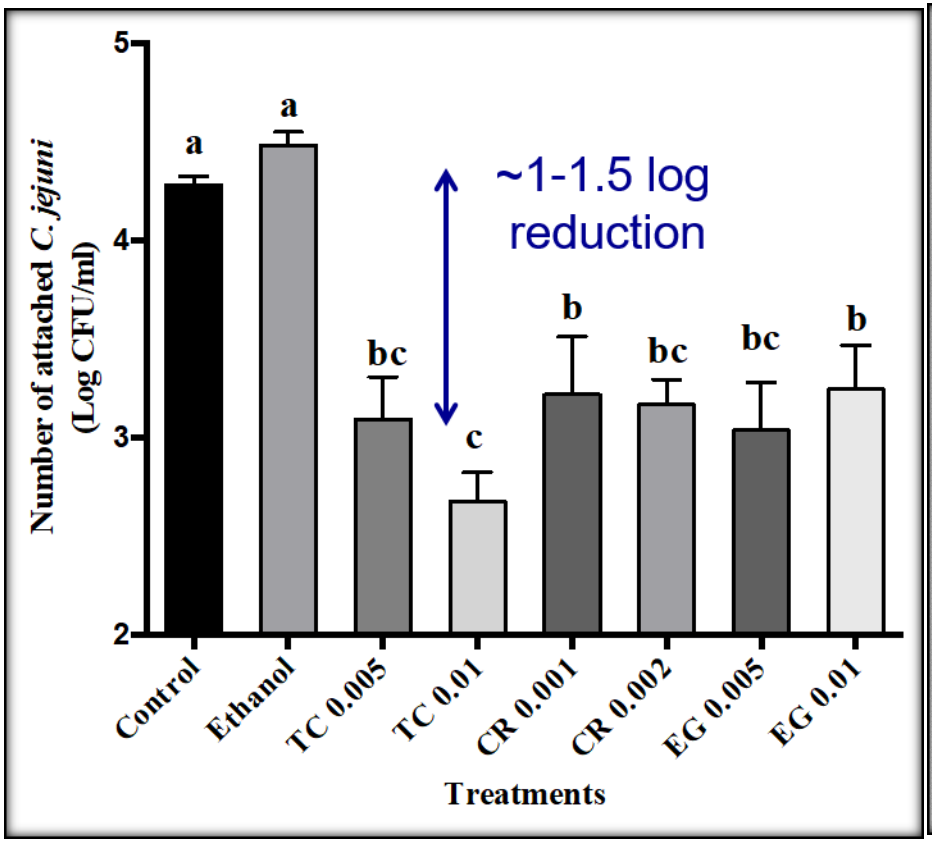


Incubation (37°C, 1.5 h under 5% CO_2)
Bacterial enumeration

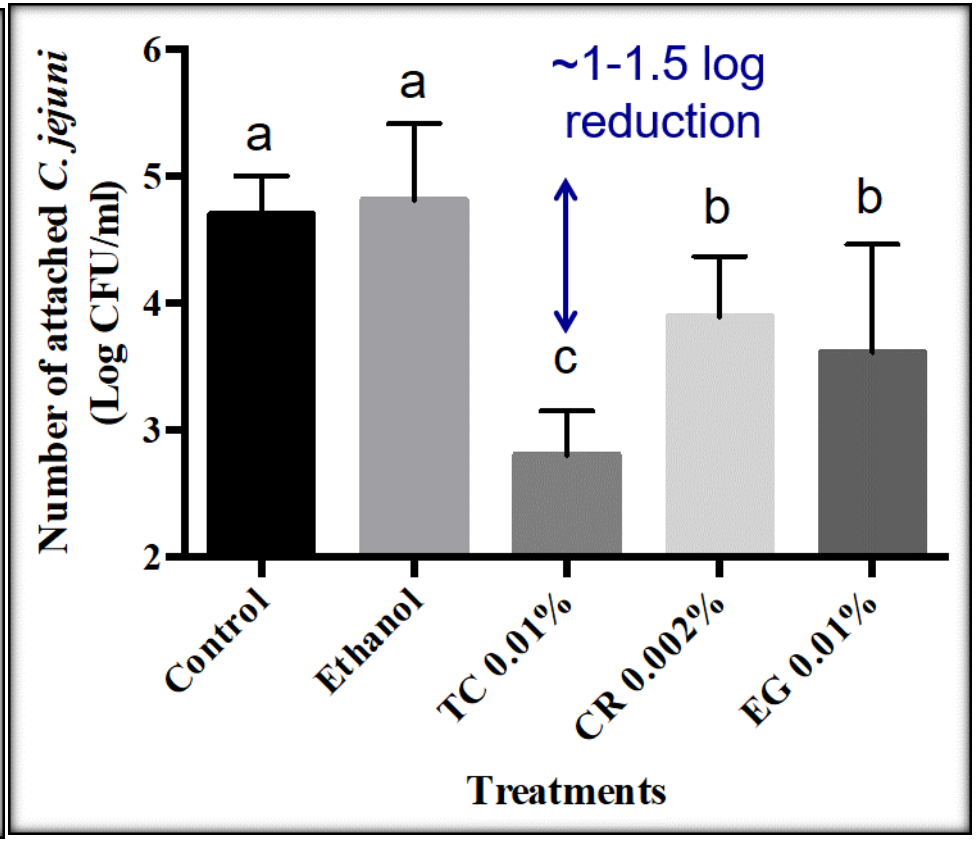
Moroni et al. 2006

Effect of phytochemicals on *C. jejuni* adhesion to intestinal cells

Human intestinal cells (Caco-2)

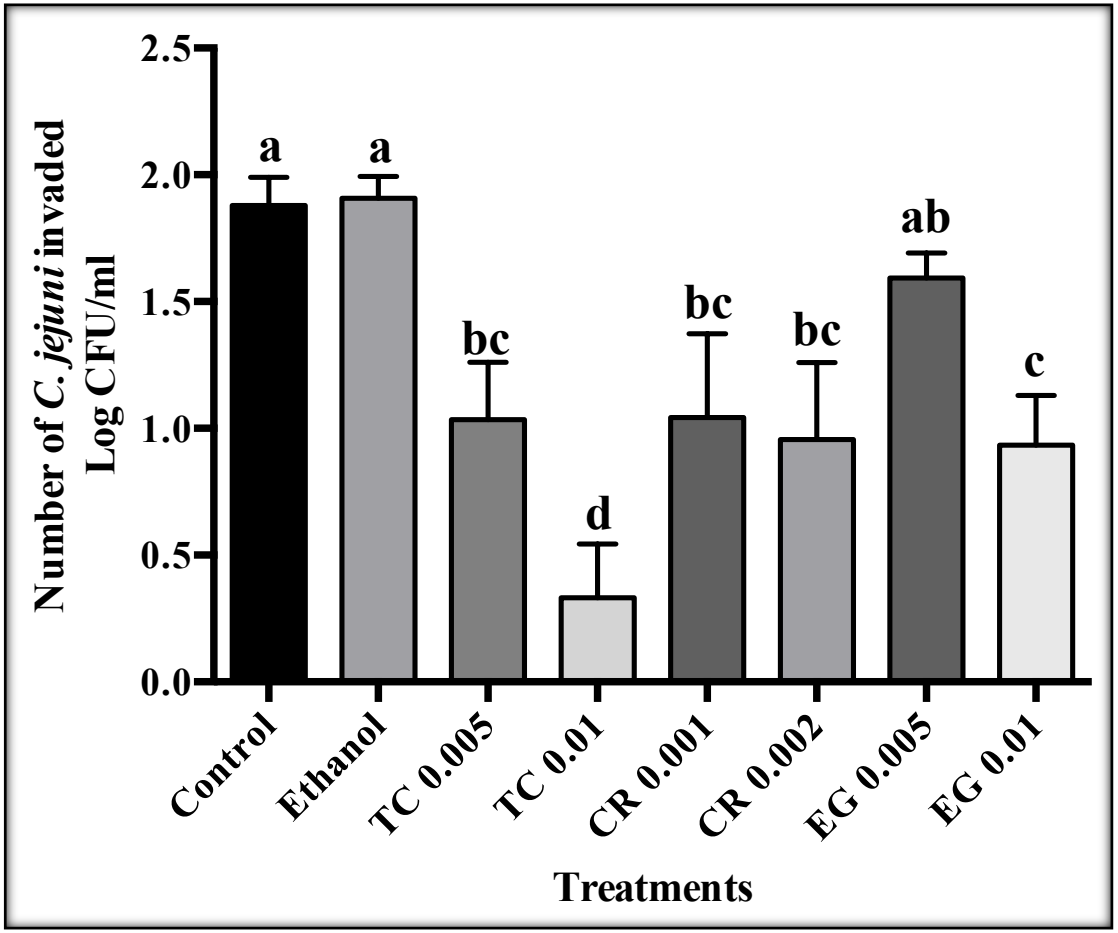


Chicken primary epithelial cells



P<0.05

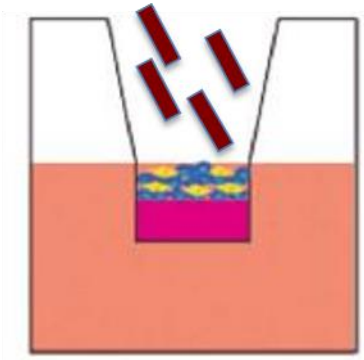
Effect of phytochemicals on *C. jejuni* invasion of intestinal cells* (1)



*P<0.05

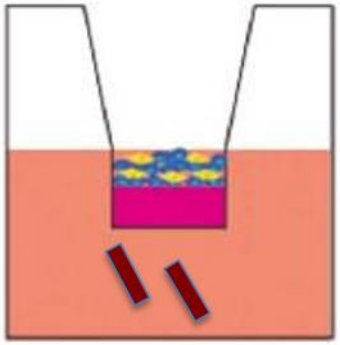
Effect of phytochemicals on *C. jejuni* translocation of intestinal cells (2)

10^6 *C. jejuni*



Caco-2 on
membrane filter (5 um)

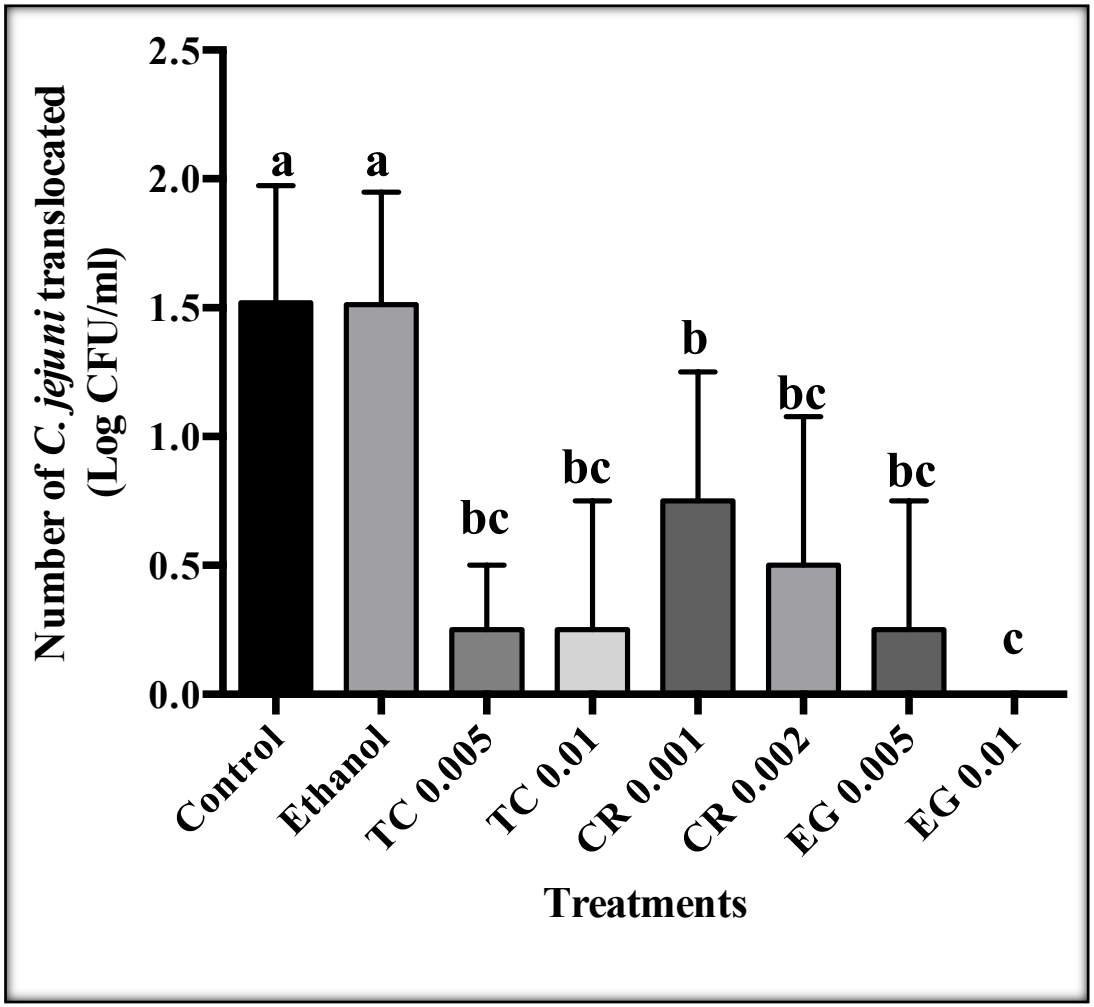
Incubation (37°C 3h under 5% CO_2)



Bacterial enumeration

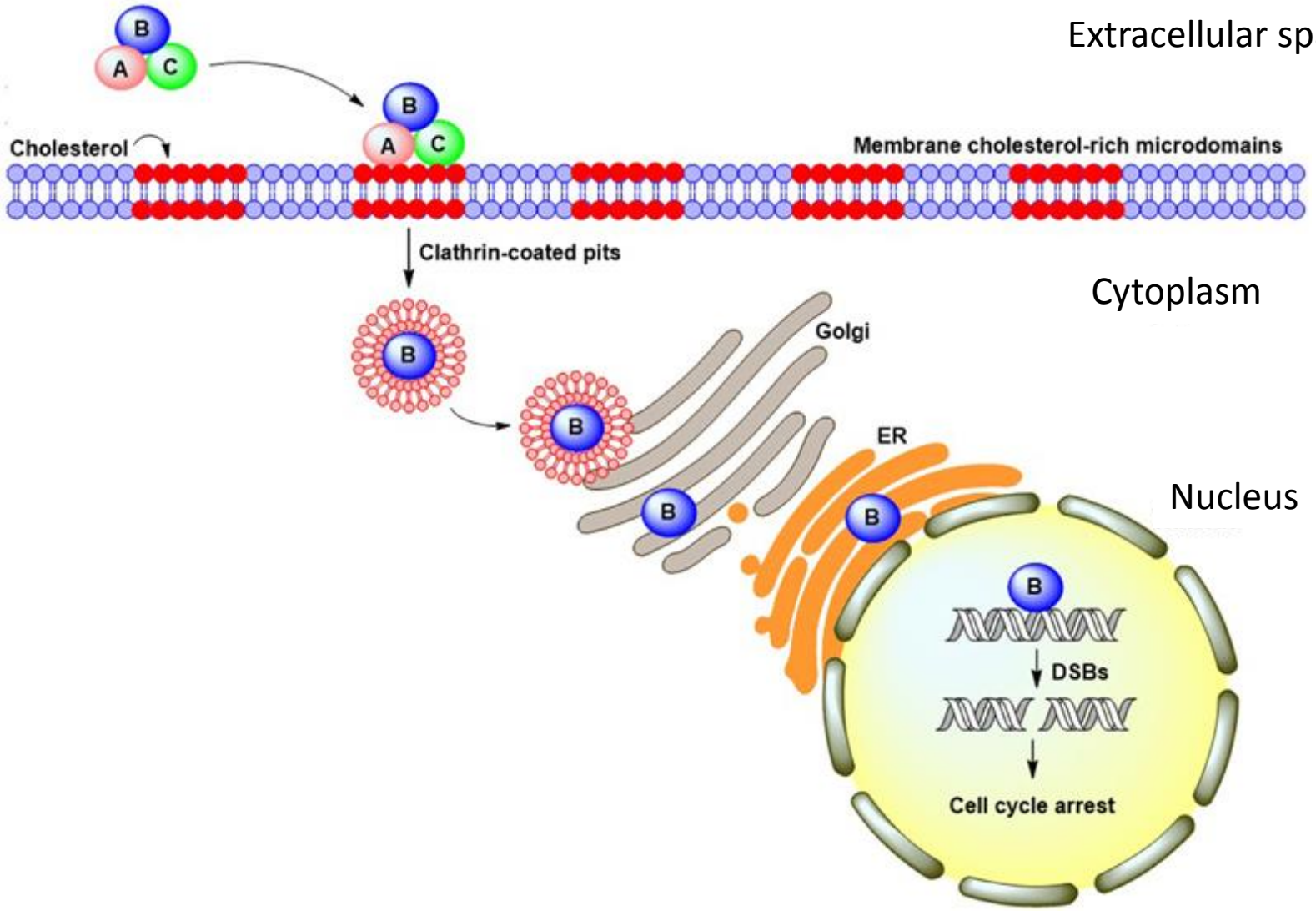
Koo, Bhunia et al., 2012. PloS One

Effect of phytochemicals on *C. jejuni* translocation of intestinal cells (3)



P<0.05

C. jejuni Cytolethal distending toxin



Lai et al., 2016; Front. Cell. Infect. Microbiol.,

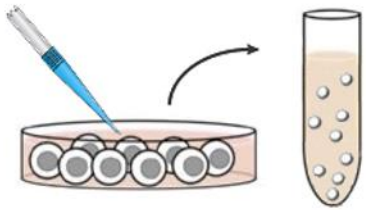
Effect of phytochemicals on *C. jejuni* CDT production (1)

C. jejuni (37°C for 24 h)
With or without phytochemicals

↓

Supernatant (CDT) collected

↓

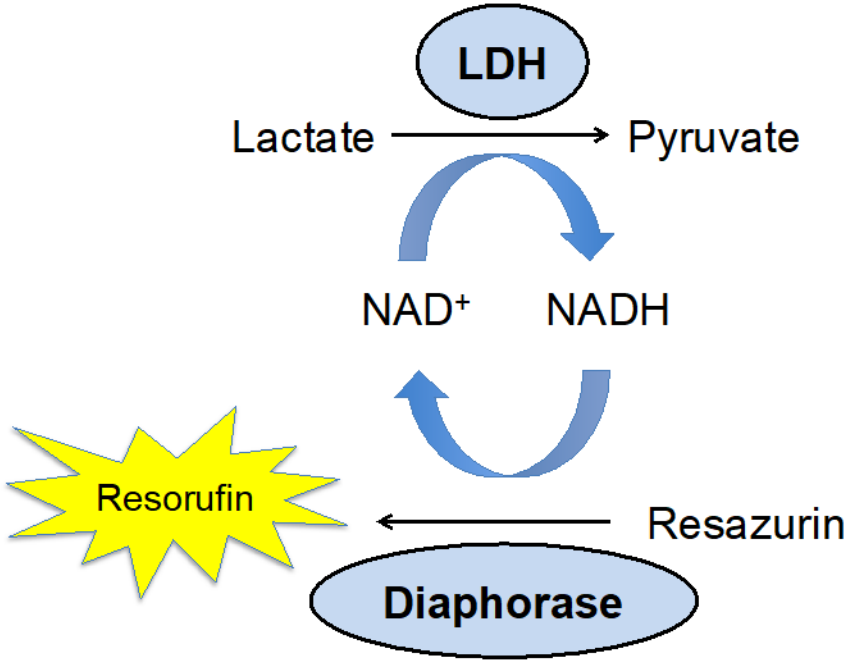


Incubation 9 days, 37°C

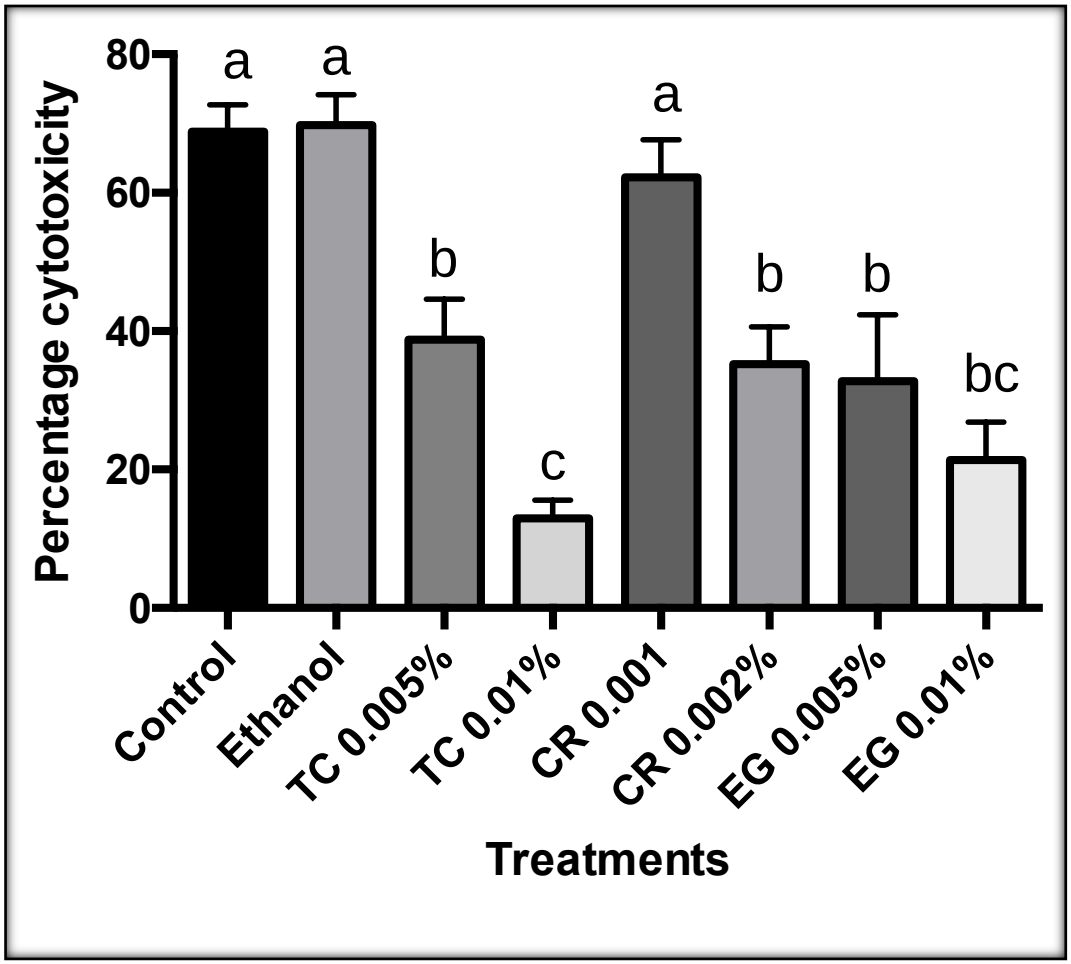
CDT quantification

CytoTox-ONE™ Membrane integrity kit

LDH based fluorescence assay



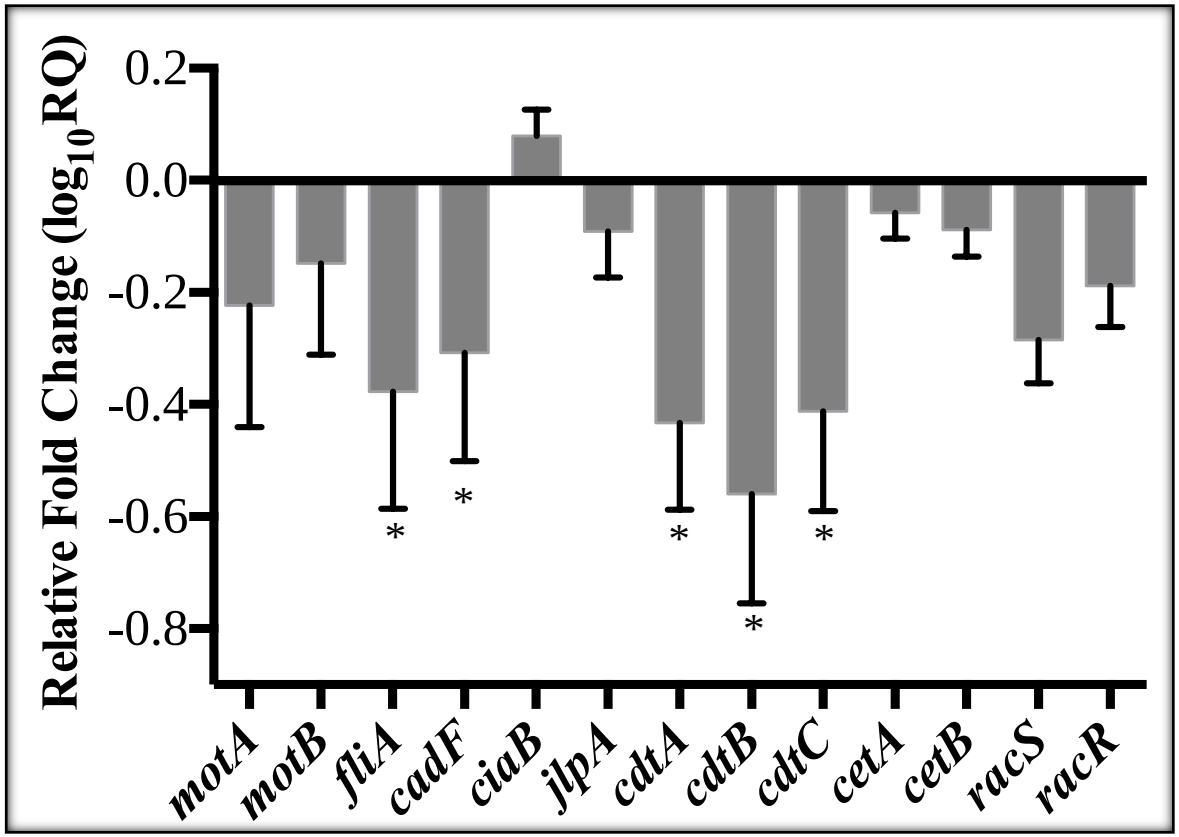
Effect of phytochemicals on *C. jejuni* CDT production (2)



P<0.05

Effect of trans-cinnamaldehyde on expression of *C. jejuni* virulence genes*

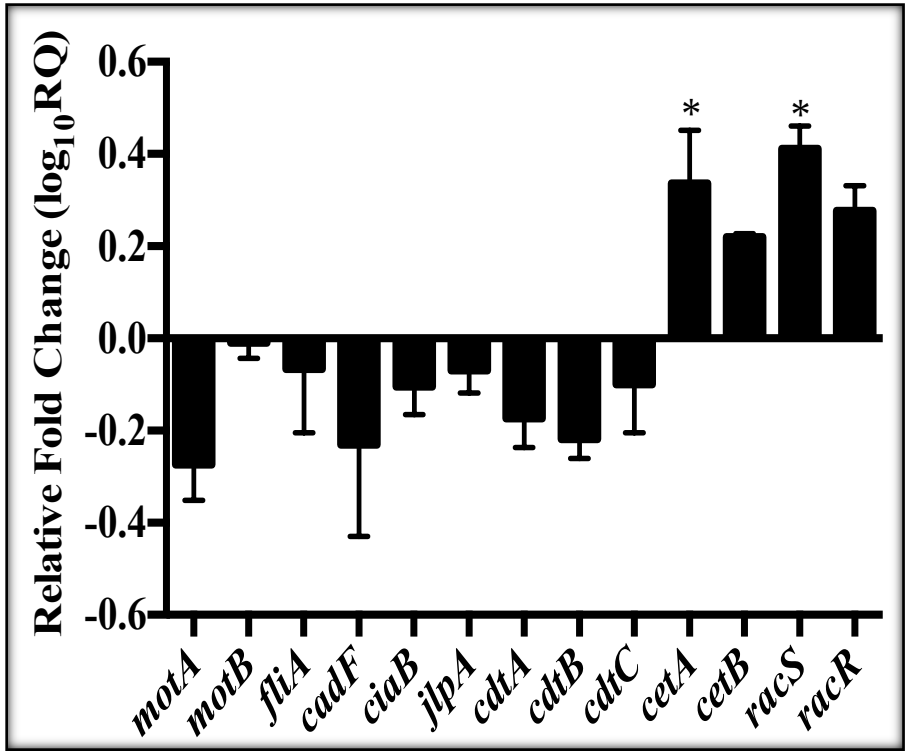
Trans-cinnamaldehyde 0.01%



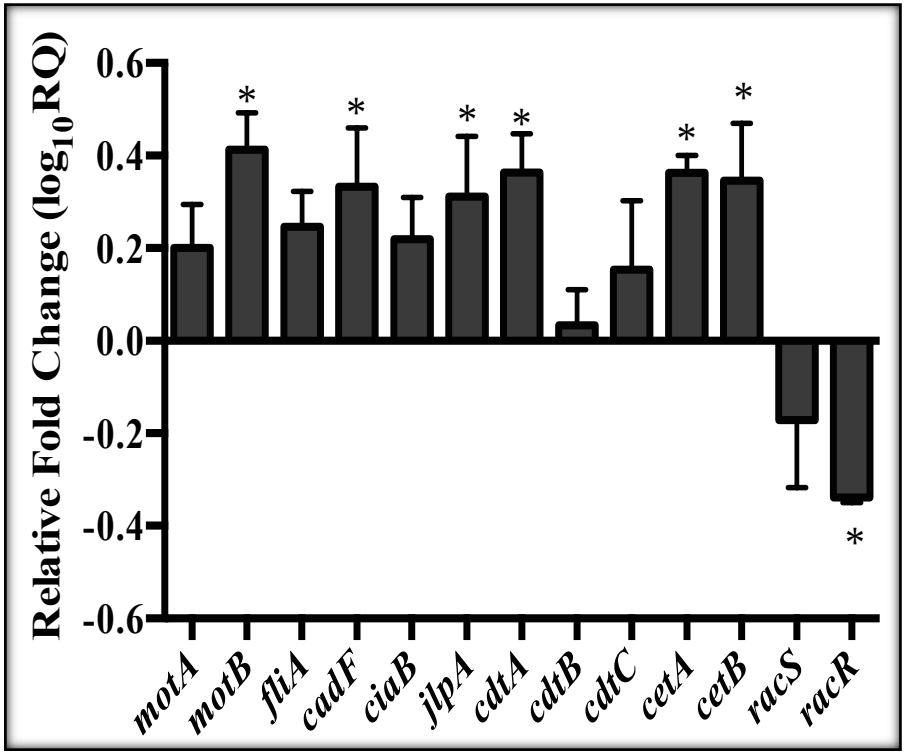
*Treatments significantly different from the control at P<0.05

Effect of carvacrol and eugenol on expression of *C. jejuni* virulence genes*

Carvacrol 0.002%



Eugenol 0.01%

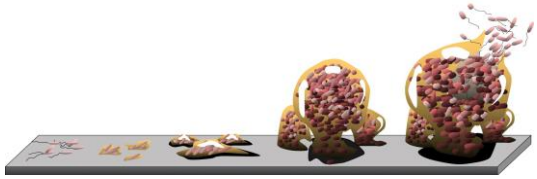


*Treatments significantly different from the control at P<0.05

Pre-harvest persistence	Persistence in processing environment	Post-harvest contamination	Anti-virulence strategies

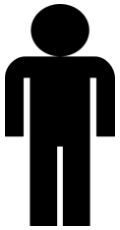
Key takeaways...

Phytochemicals reduced
***C. jejuni and S.* Enteritidis colonization**



Phytochemicals reduced
***C. jejuni* biofilms on food contact surface**

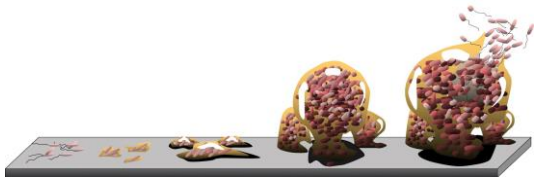
Phytochemicals reduced
***C. jejuni* survival on chicken skin and meat**



Phytochemicals modulate
***C. jejuni* virulence properties**

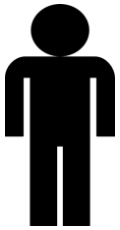
Future research

Effect of phytochemicals on *C. jejuni* transcriptome and cecal microbiome of chickens



Developing novel anti-biofilms strategies for controlling *C. jejuni*

Effect of phytochemical nanoemulsion in reducing *C. jejuni* on poultry products



Developing anti-virulence strategies for controlling *C. jejuni* colonization in chickens and infection in humans

Acknowledgments

Dr. Annie M. Donoghue (USDA-ARS)
Dr. Narayan Rath (USDA-ARS)
Dr. Dan J. Donoghue (University of Arkansas)
Dr. Kumar Venkitanarayanan (University of Connecticut)

Our team

Dr. Pamela J. Blore, Dr. Komala Arsi, Dr. Indu Upadhyaya
Sandip Shrestha, MS; Basanta Wagle, MS; Sarah Wright, MS

Funding

USDA NIFA

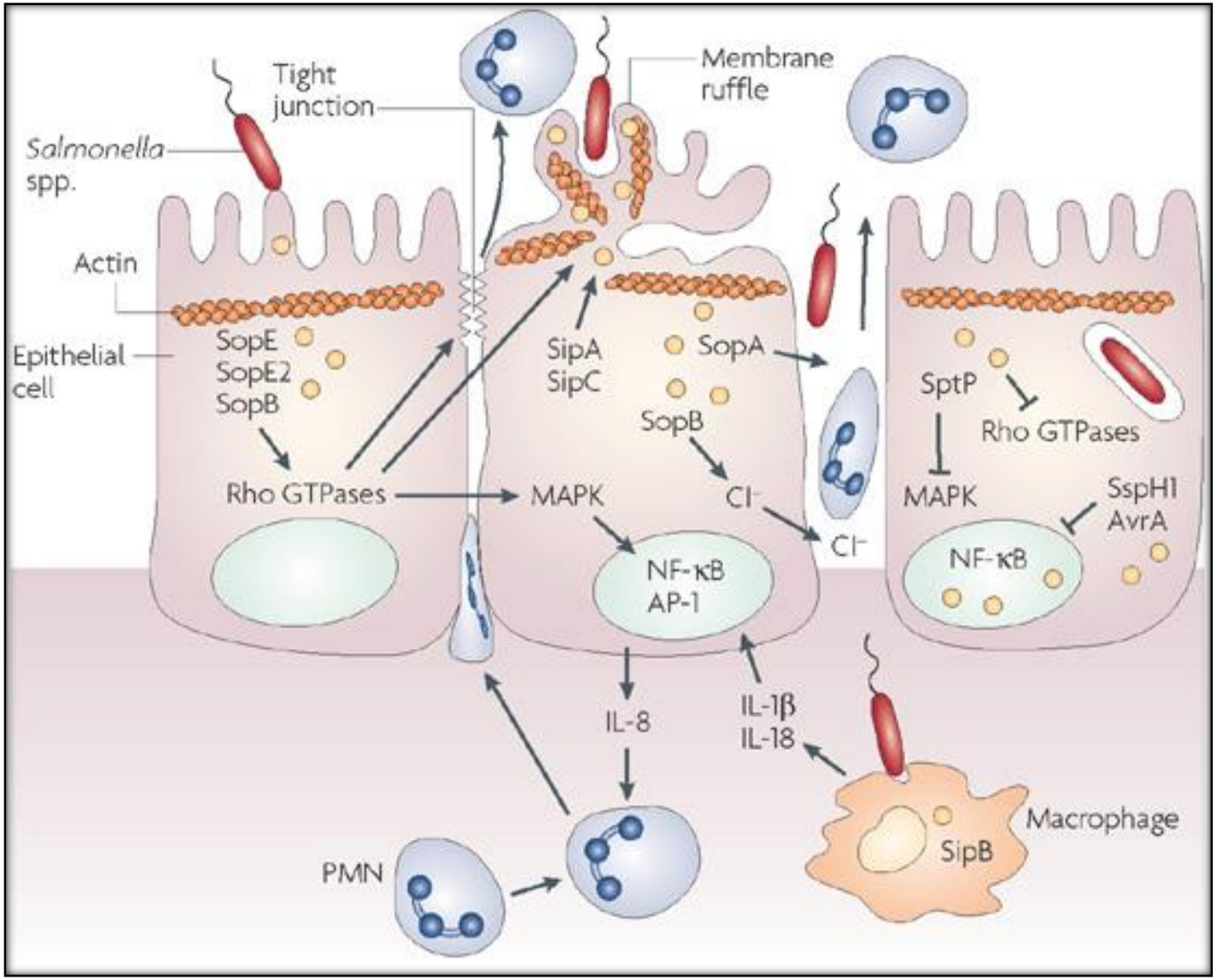
OREI program

Thank You

“Let food be thy medicine and medicine be thy food”

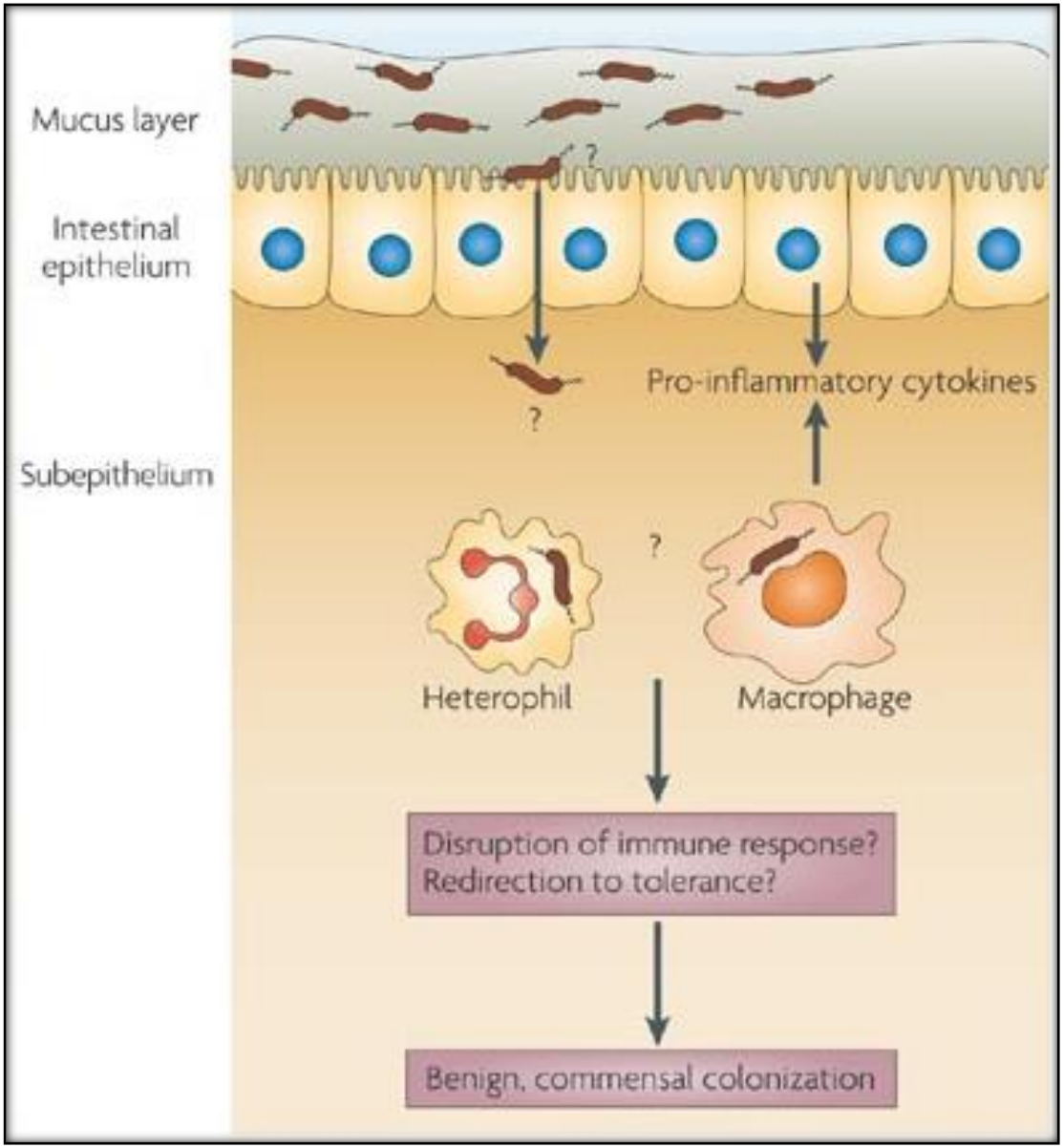
Hippocrates

Salmonella colonization in chicken



Haraga et al., 2008; Nature Reviews Microbiology

Campylobacter colonization in chicken



Young et al., 2007
Nature Reviews
Microbiology