# *Salmonella* Wins the War with Prebiotics and Probiotics during Infection


**Narine Arabyan[1]**, Prerak Desai[1†], Jigna Shah[1£], Reed N. Gann[1‡], Hai Yu[2], Thao Ly Yang[2], Yanhong Li[2], Nguyet Kong[1], Mai Lee Yang[1§], Janneth Pinzon[1‡], Xi Chen[2], and Bart C. Weimer[1]

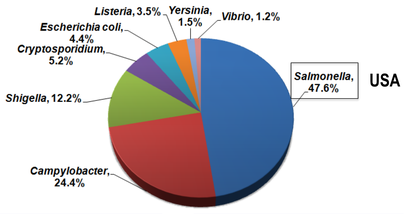
[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.; [2]Department of Chemistry, University of California, Davis, CA 95616, USA.; [†]Present address: Zoetis, 333 Portage Street, Kalamazoo, MI 49007, USA.; [£]Present address: MPI Research, 54943 North Main Street, Mattawan, MI 49071, USA.; [‡]Present address: Air University, 501 LeMay Plaza North, Maxwell AFB, AL 36112, USA.; [§]Present address: Department of Clinical Laboratory and Pathology, Sutter Medical Center, 2825 Capitol Ave. Sacramento, CA 95816, USA.; [ǂ]Present address: Department of Plant Sciences, University of California, Davis, CA 95616, USA.


## ABSTRACT


Prebiotics and probiotics reduce and prevent GI infections by modulating the gut microbiome. Prebiotics proliferate beneficial gut bacteria while probiotics prevent the attachment and invasion of pathogens. However, the mechanisms of these intricate processes are inadequately understood that alter *Salmonella* infection dynamics. We hypothesized that *Salmonella* digests prebiotics via glycosyl hydrolases (GHs) and defeats *Bifidobacterium infantis* by reducing inflammatory signal transduction pathways and mediating host cell death. The effect of prebiotics and probiotics were determined during *Salmonella* infection in presence or absence of *B. infantis*. Differential gene expression and small metabolite profiles of *Salmonella*, *B. infantis* and colonic epithelial cells (Caco-2) were determined. Extrinsic and intrinsic cell death pathways were characterized by measuring caspase activity. This study determined that prebiotics modified *Salmonella* adhesion and invasion during infection. *Salmonella* degraded human milk oligosaccharides (HMO) using GHs. The resulting non-digested HMO fragments did not inhibit *Salmonella* infection. Pathogen exclusion potential of *B. infantis* was tested to determine that *Salmonella* infection was not altered, but inflammatory signal transduction pathways were reduced. *B. infantis* reduced caspase-9 and caspase-3/7 activities in Caco-2 leading to induction of Akt phosphorylation in absence of *Salmonella*. Arginine catabolism, required for virulence, was repressed in *Salmonella* when *B. infantis* was present. *B. infantis* also reduced carbon flux of *Salmonella* through repression of genes in glycolysis and TCA cycle during co-infection corresponding with significant ($p<0.05$) accumulation of glucose, fructose, trehalose, maltose, glucose-6P, pyruvate and citrate while the remaining TCA metabolites (α-ketoglutarate, malate, fumarate and succinate) were depleted. However, when infected with *Salmonella* alone, glucose metabolism in *Salmonella* was induced ($q\leq0.01$). In presence of *Salmonella*, *B. infantis* was unable to rescue the host cell death, indicating that *Salmonella* induced cytotoxicity. This study established that *B. infantis* altered cell signaling during infection by repressing caspase-9 and caspase-3/7 activities in intrinsic death pathways, repressed arginine catabolism, glycolysis and TCA cycle; however, *Salmonella* still mediated host cell death via mitochondrial dysfunction. These data indicate that *Salmonella* defeats both prebiotics and probiotics during infection.


## INTRODUCTION

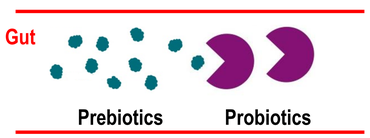
### *Salmonella* tops GI illnesses associated with food


**USA (CDC/FDA/USDA)**
- 1.4-1.6 million annual cases
- 1000 deaths
- $ 2.3 billion / year

**WHO (Worldwide)**
- 1.5 billion cases

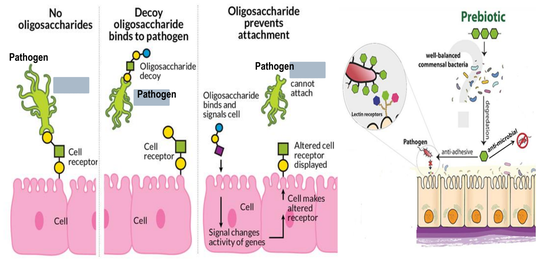
### Prebiotic and Probiotics




**Carbohydrates that cannot be digested by the human body.**

**"Good" bacteria that help keep your digestive system healthy by controlling growth of harmful bacteria.**

### Prebiotics function as decoys, anti-adhesives or anti-microbials ?

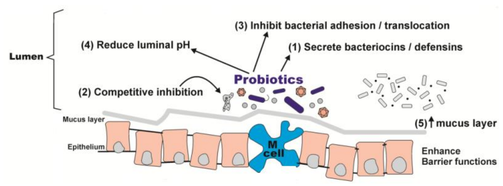






## INTRODUCTION

### The action mechanisms of probiotics




**Prebiotics enable beneficial gut bacteria proliferation. Probiotics prevent the attachment and invasion of pathogens.**

**The mechanisms of these processes that alter *Salmonella* infection are inadequately understood.**

**We hypothesized that *Salmonella* digests prebiotics via its glycosyl hydrolases (GHs) and defeats *Bifidobacterium infantis* by reducing inflammatory signal transduction pathways mediating host cell death.**

## EXPERIMENTAL DESIGN

**Define the ability of pathogen to degrade OS as a carbon source (growth studies)**

↓

**Determine of prebiotic blocking changed the location of the pathogen (*in vitro* Adhesion and Invasion (A/I) of Caco-2 cells)**

↓

**Genomic analysis of GH complement in *Salmonella* and *Bifidobacterium* (CAZy analysis)**

↓

**Construct deletion mutants of GHs in *Salmonella* to examine the role of OS digesting enzymes during host association**

↓

**Investigate pathogen exclusion potential of probiotics**

↓

**Determine host cell survival during A/I of *Salmonella* and *Bifidobacterium* (cytotoxicity assay)**

↓

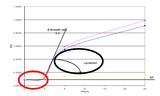
**Investigate how *Salmonella* adapts to the presence of *Bifidobacterium* in the host environment (gene expression)**

↓

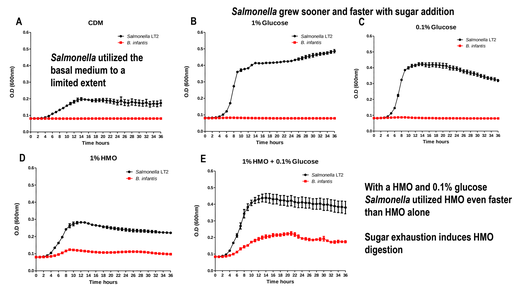
**Investigate extrinsic and intrinsic cell death pathways (caspase-8, caspase-9 and caspase-3/7)**

## RESULTS

### *Salmonella* degrades and digests human milk oligosaccharides (HMOs)



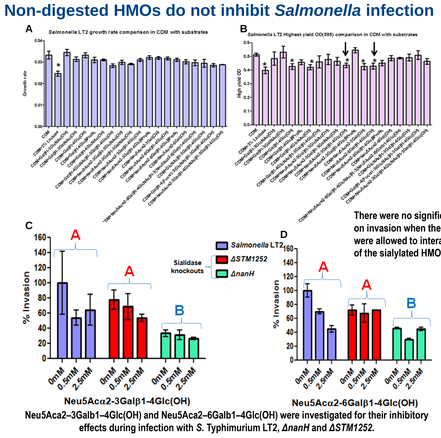
**Growth:**
1). Time of growth (Gt)
2). Growth reduction




With a HMO and 0.1% glucose *Salmonella* utilized HMO even faster than HMO alone

Sugar exhaustion induces HMO digestion

### Non-digested HMOs do not inhibit *Salmonella* infection



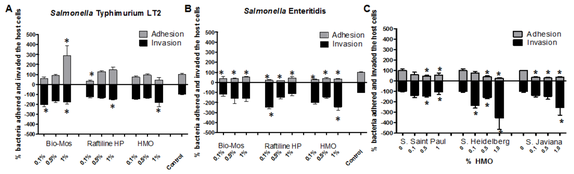




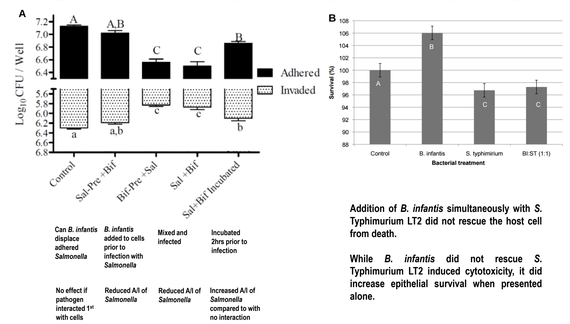
There were no significant effects on invasion when the pathogens were allowed to interact with either of the sialylated HMO.

Neu5Aca2–3Galb1–4Glc(OH) and Neu5Aca2–6Galb1–4Glc(OH) were investigated for their inhibitory effects during infection with *S.* Typhimurium LT2, *ΔnanH* and *ΔSTM1252*.

### Prebiotics modify *Salmonella* adhesion and invasion during infection


### Effect of *B. infantis* on adhesion and invasion of *Salmonella*




Can *B. infantis* displace adhered *Salmonella*

*B. infantis* added to cells prior to infection with *Salmonella*

Mixed and infected

Incubated 2hrs prior to infection

No effect if pathogen interacted 1st with cells

Reduced A/I of *Salmonella*

Reduced A/I of *Salmonella*

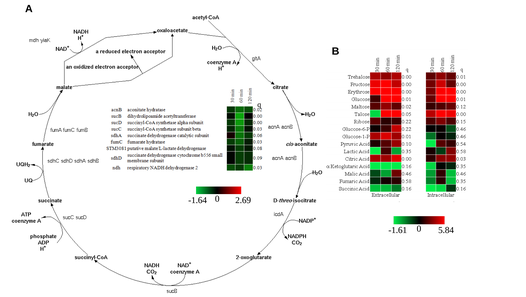
Increased A/I of *Salmonella* compared to with no interaction

Addition of *B. infantis* simultaneously with *S.* Typhimurium LT2 did not rescue the host cell from death.

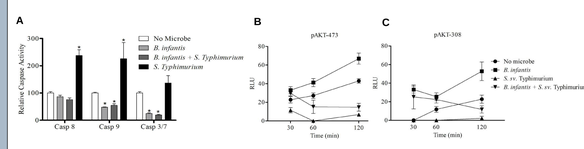
While *B. infantis* did not rescue *S.* Typhimurium LT2 induced cytotoxicity, it did increase epithelial survival when presented alone.

## RESULTS

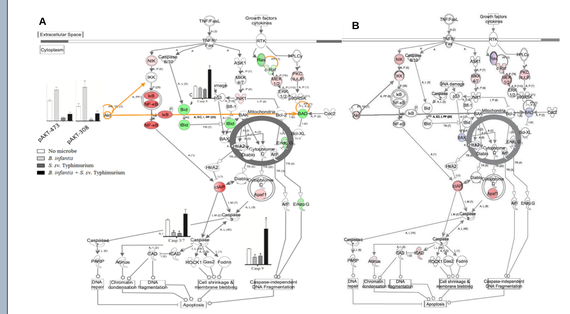
### *B. infantis* reduces carbon flux of *S.* Typhimurium LT2 through glycolysis and the TCA cycle during co-infection




### *B. infantis* alters cell signaling during infection; however, it did not inhibit *Salmonella* mediated host cell death




### Host cell death signaling pathway during infection of Caco-2 cells following microbial association




## DISCUSSION

The aim of this study was to define specific oligosaccharides involved in protecting the gut and the inhibition of pathogens with probiotics.

- *S.* Typhimurium LT2 degrades and digests HMOs as a carbon source.
- *S.* Typhimurium LT2 heavily relies on its GH enzymes for the degradation of dietary carbohydrate based substrates.
  - Analysis of *Salmonella* CAZome represented by genes encoding GHs reveal *Salmonella* is able to recognize, bind and digest more carbohydrates than *B. infantis* (data not shown), further implicating that *Salmonella* is equipped with unique GHs necessary to digest HMOs and other complex oligosaccharides during infection before these prebiotics can act as decoys.
- There were no significant effects on invasion when the pathogens were allowed to interact with non-digested HMOs.
- The pathogen exclusion potential of probiotics was tested to determine if *B. infantis* inhibits infection by *Salmonella*.
- *B. infantis* altered cell signaling during infection by repressing caspase-9 and caspase-3/7 activities in intrinsic death pathways, repressed arginine catabolism, glycolysis and TCA cycle; however, *Salmonella* still mediated host cell death via mitochondrial dysfunction.
- By combining microbial genomics, oligosaccharide utilization and infection analyses, this study provides unprecedented details how *Salmonella* defeats both prebiotics and probiotics during infection of Caco-2 cells.
- This study further expands our understanding of infection characteristics between a pathogen and a probiotic.

This study showed that *Salmonella* digests prebiotics via its GHs and *B. infantis* was unable to rescue the host cell death in presence of *Salmonella*, indicating that *Salmonella* defeats both prebiotics and probiotics during infection.

## CONTACT INFORMATION AND FUNDING


**Bart C. Weimer, Ph.D.** (bcweimer@ucdavis.edu)
**Narine Arabyan** (narabyan@ucdavis.edu)
UC Davis (VM:PHR) VetMed3B - Room 4016
1089 Veterinary Medicine Dr. Davis, CA 95616
(530)752-6426
http://weimermicrolab.org
http://www.100kgenomes.org


Weimer Lab

100K Pathogen Genome Project


**NIH**
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