

# Spotted Array Identification of *Listeria monocytogenes* 4b and 1/2a Genes Transcriptionally regulated (2-Fold) When Exposed to Human Cells

Eric Miller and Douglas Christensen, Ph. D.; Wayne State College

## Abstract

*Listeria monocytogenes* is a food borne pathogen that causes up to one death for every three people who contract listeriosis. There are serotypes of low (1/2a) and high (4b) virulence to humans. The objective of this research is to study gene regulation variations between the serotypes 4b and 1/2a. Each serotype was exposed to actively growing human kidney 293 cells, colon cancer (CaCO) cells or spent cell-free media (control groups) for 16h. All treatments were carried out in biological triplicate. After incubation, total RNA was isolated from each *L. monocytogenes* serotype, modified and Cy labeled prior to spotted array exposure on a *L. monocytogenes* 4b 3-fold spotted array (>7600 gene fragments). Microarray data were statistically analyzed for genes which underwent a 2-fold change in transcriptional regulation. Sequence analysis of common hits among triplicate samples from both the 4b and 1/2a serotypes resulted in the identification of several genes affected by human cell contact. These included multiple hits from pyruvate phosphate dikinase and Fts Family Proteins, with additional genes not normally associated with virulence to include glyoxalase family protein, CBS domain protein, dihydrolipoamide acetyltransferase, fatty acid/phospholipid synthesis protein PlsX, malonyl CoA-acyl carrier protein transacylase, Phosphoglycerate mutase family protein, cell division ABC transporter, UDP-N-acetyl muramate-alanine ligase, and several conserved hypothetical proteins. This work was funded by the Nebraska IN-BRE grant NIH grant # P20RR16469.

## Introduction

*Listeria monocytogenes* is a gram positive, food-borne pathogen. It is found in many ready-to-eat foods such as processed hotdogs, bologna, and other lunch meats. *Listeria* also inhabits a wide range of environments including soil, plants, and water.<sup>1</sup> Listeriosis, the disease associated with *Listeria*, has shown to have a high mortality rate, approximately 20-25%, in those individuals infected.<sup>2</sup>



Currently, 13 serotypes of *Listeria monocytogenes* have been identified and separated into three distinct lineages. Many clinical isolates reported three main serotypes: 1/2b, 4b (both Lineage I) and 1/2a (Lineage II). In general, it has been found that serotypes of Lineage II are only found in sporadically in human isolates, while Lineage I serotypes are commonly isolated from epidemic cases.<sup>3</sup> Investigation into the whole genome of serotypes 4b and 1/2a revealed both serotypes lack genes which are specific to each lineage. Such variations include surface and sugar metabolism proteins.<sup>4</sup> Little research has been done to find differences in pathogenic potential due to gene regulation between the lineages.

In this study, *Listeria monocytogenes* serotypes 4b and 1/2a were grown and exposed to human cells, 293-Kidney and cancer of the colon (CaCO-2) cells. The total RNA was then extracted from the *Listeria* and mRNA was modified for labeling. Microarray technology was then utilized to identify gene regulation between the serotypes and their respective controls. We only concentrated on genes which were found to be common among both serotypes.

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## Materials and Methods

- 293-Kidney Cells ATCC CRL-1573
- CaCO-2 Cells ATCC HTB-37
- *Listeria monocytogenes* 10403S, serotypes 4b and 1/2a; both obtained from Dept. of Food Science and Technology of University of Nebraska at Lincoln (UN-L)
- *Listeria monocytogenes* (3-fold) 4b DNA library obtained from Dr. Andrew Benson, Dept. of Food Science and Technology at UN-L
- 4b *L. monocytogenes* gene chips obtained by collaboration between Wayne State College and University of Nebraska Medical Center (UNMC)

## 293-Kidney and CaCO-2 Cell Culture

- 293-Kidney cells - 20mL of Dulbecco's Modified Eagle Medium (ATCC) containing 10% fetal bovine serum (FBS)
- CaCO-2 cells - 20mL Minimal Essential Medium (ATCC) with 20% FBS
- Grown in 5% CO<sub>2</sub> and 37 C and maintained in flasks until confluent on bottom of flask.

## Listeria cultures

- Grown overnight in 5 mL LB broth in shaking incubator at 37 C.

## Inoculation of human cells with L. monocytogenes

- Flasks contained either human cells or utilized medium extracted from human cell flask
- Performed in biological triplicates
- *Listeria* exposed to conditions for 16 hours

## RNA isolation and modification

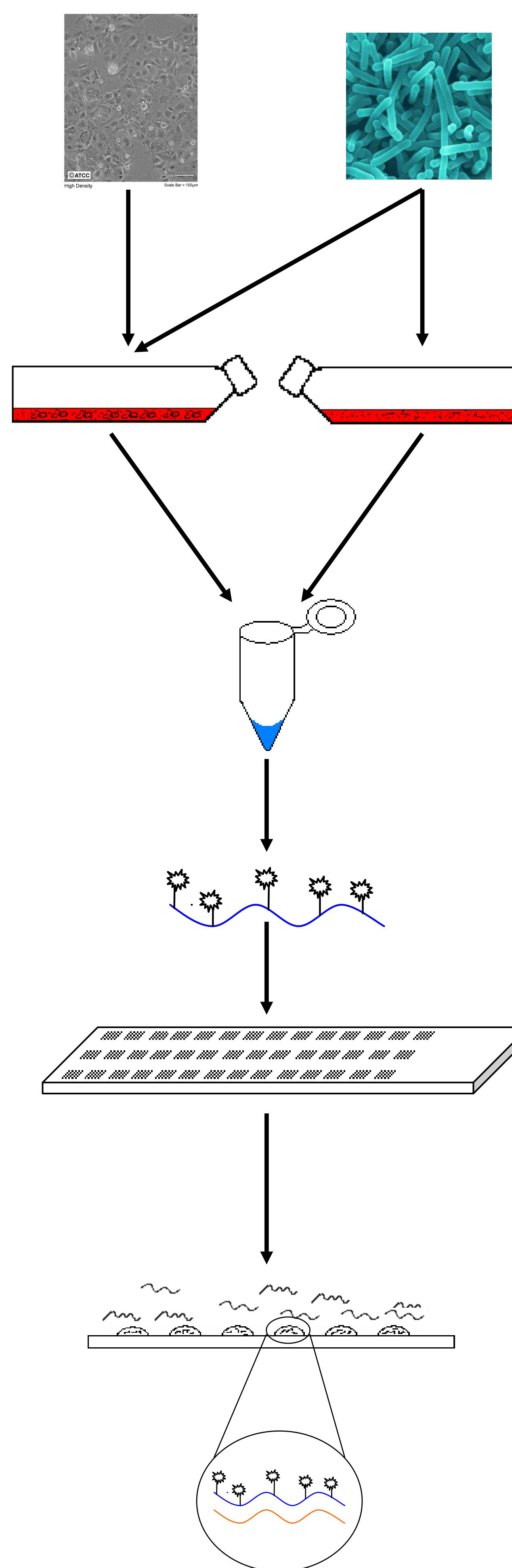
- Isolation according to FastRNA® Pro Blue Kit (QBio gene).
- Modifications
  - Mini Beadbeater™ Biospec Products
  - RNA modification according to MessageAmp™ II-Bacteria *Prokaryotic RNA Amplification Kit* (Ambion)
- Modifications
  - Incubation times
  - Labeling nucleotide 5-3 amino-acytyl UTP was used as the label marker in aRNA instead of biotin 11-CTP or biotin 16-UTP.

## Spotted Array analysis and statistics

- Microarray analysis performed by J. Eudy, Ph.D. UNMC
- Labeled with either Cy3 or Cy5 dye
- Statistical analysis carried out by Lynette Smith, MS of UNMC.
- BRB ArrayTools utilized for statistical analysis

## Sequencing and Gene Determination.

- Plasmid isolation from *Listeria* library using QIAprep® Spin Miniprep Kit (Qiagen)
- Sequencing performed at UNMC
- Forward and reverse M13 primers utilized
- NCBI BLAST utilized for gene identification



## Results

After exposure to 293-kidney cells, serotype 4b only showed a 2-fold increase or decrease in 10 genes, while 1/2a recorded 14 genes up- or down-regulated 2-fold. In cross referencing these genes, only one hit was represented in both findings. Sequencing of this region provided the following gene:

Table 1: Common gene identified after sequencing and proposed function.

Name of Gene:	Function:
UDP-N-acetylmuramate--alanine ligase	forms peptide bonds with hydrolysis of ATP <sup>5</sup>

After exposure to CaCO-2 cells, serotype 4b showed a 2-fold increase or decrease in 31 genes, while 1/2a recorded 103 genes up- or down-regulated 2-fold. Cross referencing these genes resulted in 12 common hits. Sequencing of these regions provided the following genes:

Table 2: Common genes identified after sequencing and proposed function.

Name of Gene:	Function:
Pyruvate, Phosphate Dikinase	catalyzes reversible conversion of AMP, phosphoenolpyruvate (PEP) and pyrophosphate (PPi) to ATP, pyruvate (Pyr) and inorganic phosphate (Pi) <sup>6</sup>
Glyoxalase Family Protein	GLXI converts cytotoxic methylglyoxal S-D-lactoylglutathione and D-lactate by GLXII <sup>7</sup>
FtsK/SpoIIIE Family Protein	chromosome partitioning during cell division and conjugal transfer of DNA <sup>8</sup>
Dihydrolipoamide Acetyltransferase	transfers acetyl group to CoA <sup>9</sup>
Dihydrolipoamide Dehydrogenase	oxidizes the reduced lipoyl moieties through reduction of NAD+ to NADH <sup>9</sup>
CBS Domain Protein	themselves possibly serve as adenosine derivative binding sites <sup>10</sup>
ABC Transporter, Permease Protein FtsX	localize the septic ring <sup>11</sup>
ATP-Binding Protein FtsE	localize the septic ring <sup>11</sup>
Malonyl CoA-acyl Carrier Protein Transacylase	transacylation of malonate from malonyl-CoA to activated holo-ACP, to generate malonyl-ACP, which is an elongation substrate in fatty acid biosynthesis <sup>12</sup>
Acid/Phospholipid Synthesis Protein PlsX	acetyl-ACP converted to acetyl-phosphate intermediate by PlsX then G3P by PlsY <sup>13</sup>
Phosphoglycerate Mutase Family Protein	catalyze the isomerization of 2- and 3-phosphoglycerates and are essential for glucose metabolism in most organisms <sup>14</sup>
Conserved Hypothetical Proteins	Unknown functions

## Conclusions

The purpose of this study was to determine if there was differential regulation of genes in virulent and non-virulent serotypes of *L. monocytogenes*. While the genes identified do not directly play a role in virulence of human cells, they are important for other dogmatic processes. Indirectly, the ability for bacteria to grow and divide rapidly while in the presence of human cells gives rise to one explanation of virulence, competition. *Listeria* would then out compete the human cells for needed nutrients and space. However, this conclusion is pure speculation at this time. More research including Northern and dot blots as well as knock-out or knock-down strains of the reported genes is needed for and truly conclusive data. The knock-out strains pose a problem as many of these genes are required for basic needs of the cell to proliferate.

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