# Detection and Prevention of Foodborne Pathogens

METHODS FOR PREVENTING ILLNESS AND OUTBREAKS

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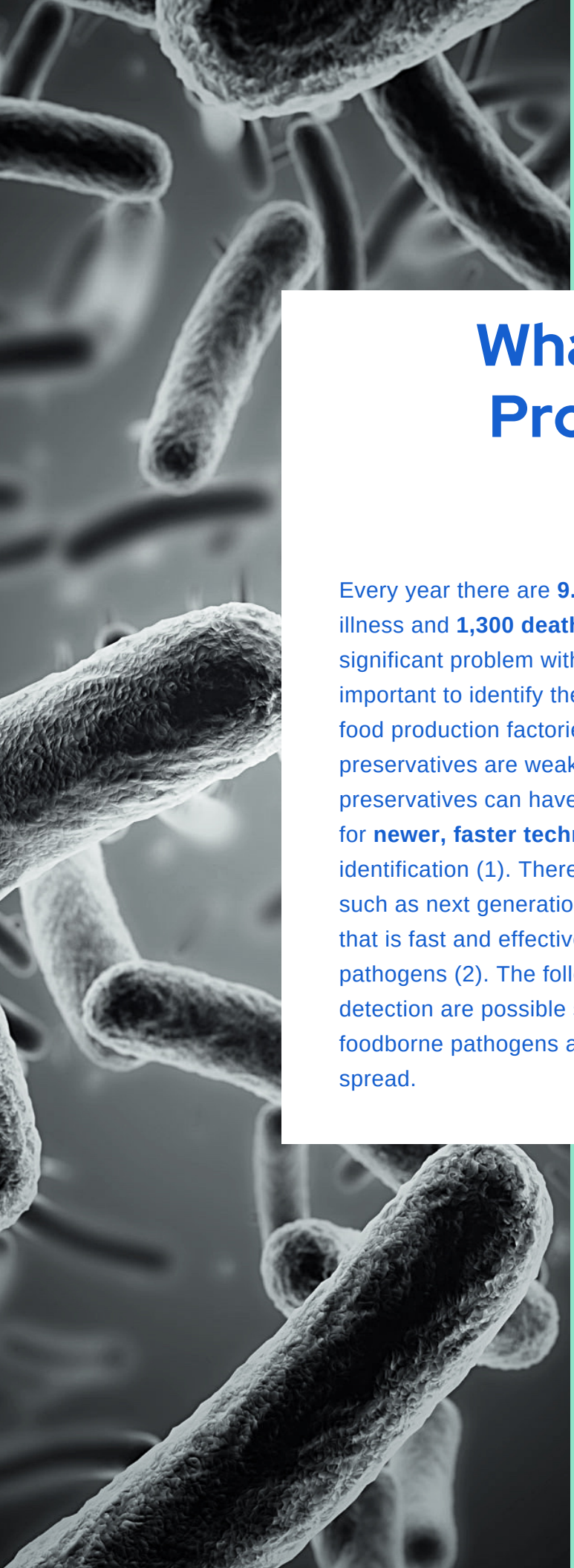
BIOL 370
FINAL PROJECT



# What is the Problem?

Every year there are **9.4 million cases** of foodborne illness and **1,300 deaths** (1). These numbers point to a significant problem with foodborne pathogens, so it is important to identify these pathogens at their sources– food production factories and farms. Because natural preservatives are weak antimicrobials and chemical preservatives can have harmful side effects, the need for **newer, faster technology** is pivotal for pathogen identification (1). There is new **genomic** technology, such as next generation sequencing and phage therapy, that is fast and effective for identifying foodborne pathogens (2). The following descriptions of methods for detection are possible solutions for both identifying foodborne pathogens and preventing their further spread.

# Amplicon Sequencing with Illumina

This new technology is a form of Next Generation Sequencing that includes developing a set of primers called the "food-borne pathogen panel" (FPP) to **identify multiple bacteria at once** through **multiplex PCR** and **Illumina MiSeq** Sequencing. Common bacteria that can be identified include: *Listeria monocytogenes, Escherichia coli, Salmonella enterica, Staphylococcus aureus, and Yersinia enterocolitica.* Using the protocol below, this technology can be implemented in food factories and farms to identify foodborne pathogens in common food sources, such as seafood, raw meat, eggs, shellfish, raw milk and fruits and vegetables. The following **method** was used for detection in a simulated food matrix, created by inoculating poultry, caciotta cheese and swordfish with specific pathogen strains (3):

Highlights (3):

- Illumina generated sequences ranging from 95,059 to 422,836 bases
- Successfully detected 3 out of 3 pathogen strains in **poultry**
- Successfully detected 3 out of 3 pathogen strains in caciotta **cheese**
- Successfully detected 2 out of 2 pathogen strains in **swordfish**
- When tested on **naturally contaminated** food samples, **high levels** of *E. coli* and *S. enterica* were detected and **lower levels** of *S. aureus* and *Y. enterocolitica* were detected (possible differences in DNA extraction)
- Real world application as "quality checks" in food factories with the proper machinery on site (3)

**Foodborne Pathogen Panel** development of primers
(20 base pair oligonucleotides for 25 bacterial species)

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**Extract** DNA from food

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**Multiplex PCR** with Foodborne Pathogen Panel

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Use of primers to **clean up PCR** reaction

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Add **adaptors and barcode**s to PCR sequences

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**Illumina MiSeq**: Insert PCR products into flow cell, and rapid amplification of template will form **monoclonal clusters** for identification

# Whole genome sequencing

Whole genome sequencing (WGS) uses the **entire genetic code** of a species to both identify and allow for evolutionary tracking of a species through phylogenetic trees. WGS can be used to **trace foodborne pathogen outbreaks** to the source, as each pathogen strain shares the same genetic code which allows for the possibility of global databases for sharing real time sequenced data via mobile devices and faster identification (2).

Highlights of WGS to trace a *Salmonella* Typhimurium outbreak in Australia (4):

- Collected food and environmental samples from 7 outbreak areas in different settings (ex. restaurant, bakery, farms supplying eggs, egg grading facility)
- Samples were first sequenced using multi-locus variable-number tandem-repeat analysis (MLVA)
- DNA samples were taken from the isolates and WGS was performed using Illumina NextSeq 500
  - Uses paired end sequencing (2 x 150 base pairs) to assemble genomes
  - Assessed for single nucleotide polymorphisms (SNPs) by comparing the short reads to a reference genome
- **37 whole genome sequences** were generated from the isolates and were determined to be **highly related** with less than 10 SNPs between all isolates
- WGS **successfully traced the outbreak** to eggs from one **specific** facility

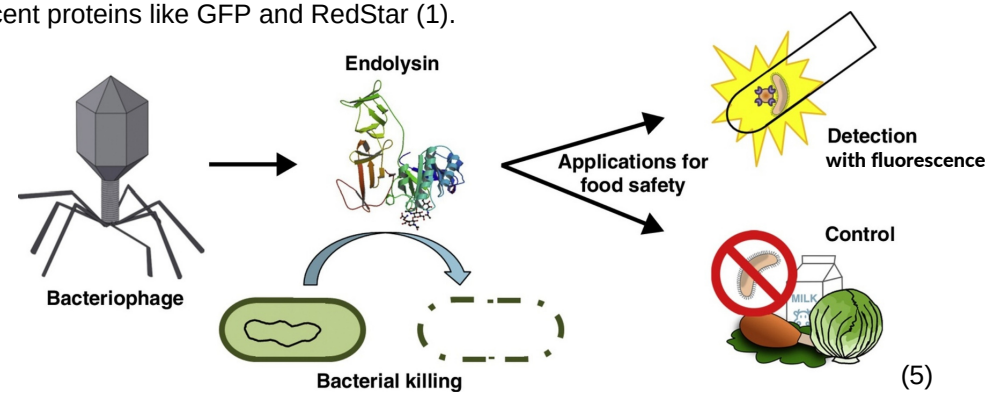
Whole genome sequencing provides **a potential use** for sampling foods and materials used to process foods to generate detailed sequences that can be **linked** and **traced** to common sources to prevent outbreaks from spreading (4). Implementing **real time sequencing technology**, such as minION, can change how the food industry screens for foodborne pathogens and looks for **patterns** in the genetic code across geographical areas (2).

# CBD Phages

Bacteriophage structure consists of: a **head** that contains the genetic material and a **tail** that assists with host recognition and injection of genetic material into the host. After the phage has injected its genetic material and replicated inside the host, phages use an enzyme named **endolysin** to lyse the host. Endolysins contains two domains: EAD (enzymatic activity domain) and CBD (cell wall binding domain), which can be used to develop foodborne pathogen detection technology (1).

Highlights of **CBD phage detection** (1):

- New CBD phage detection technology has been suggested due to the **high specificity** of CBD for specific hosts
- It works by attaching a **fluorescent protein to CBD**, which allows the phage to detect gram positive bacteria, such as *L. monocytogenes, S. aureus, and Bacillus cereus*, in infected foods
- Contaminated food will be indicated by **fluorescence** (can then be removed from food production factories to prevent foodborne illness outbreaks)
- **Phage cocktails** can also be developed to identify multiple pathogens at once using a **multiplex** platform with different fluorescent proteins like GFP and RedStar (1).




(5)

| | Pros | Cons |
|---|---|---|
| **Illumina MiSeq** | • Faster alternative to culture based methods (3)<br>• Large numbers of reads (3)<br>• Can be done in same lane which is cheaper (3) | • Short read length (6)<br>• High cost for machine (6) |
| **WGS** | • More detailed than MLVA (4)<br>• Can indicate common sources (4) | • Short reads can be difficult to connect (7)<br>• May have repeats (7) |
| **CBD Phages** | • Does not lyse human cells (1)<br>• High host specificity (1) | • Can't differentiate between live and dead cells (1)<br>• Possibility of resistance (8)<br>• Can't detect gram negative bacteria (1) |

# References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