Detection of food pathogens

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History

• Early 1900’s
  • Contaminated food, milk and water caused many foodborne illnesses

• Sanitary revolution
  • Sewage and water treatment
  • Hand-washing, sanitation
  • Pasteurization of milk- 1908
  • Refrigeration in homes- 1913
History

- Animals identified as a source of foodborne pathogens
  - Improved animal care and feeding
  - Improved carcass processing
- Surveillance and research
- Outbreak investigations
- Laws and policies regarding food handling
Bacterial Diseases

1. Airborne Bacterial Diseases
2. Foodborne & Waterborne Bacterial Diseases
3. Soilborne Bacterial Diseases
4. Arthropodborne Bacterial Diseases
5. Sexually Transmitted Bacterial Diseases
6. Miscellaneous Bacterial Diseases
Foodborne Intoxications vs Infections

Foodborne intoxications: Caused by the exotoxin secreted by bacteria in contaminated food.

Foodborne infections: Caused by the ingestion of live bacteria that colonize the digestive tract.
Epidemiology

- Foodborne diseases each year in US
  - Affects 1 in 4 Americans
  - 76 million illnesses
  - 325,000 hospitalizations
  - 5,000 deaths
    - 1,500 of those deaths caused by *Salmonella, Listeria, and Toxoplasma*
Epidemiology

• Many unrecognized or unreported
  • Mild disease undetected
  • Same pathogens in water and person to person
  • Emerging pathogens unidentifiable
• Greatest risk
  • Elderly
  • Children
  • Immunocompromised
Foodborne & Waterborne Bacterial Diseases

- Botulism
- Staphylococcal Food Poisoning
- Clostridial Food Poisoning
- Typhoid Fever
- Salmonellosis
- Shigellosis
- Cholera
- Diseases associated with Escherichia coli
- Camphylobacteriosis and Helicobacteriosis
Foodborne diseases

- **Botulism**
  - *Clostridium botulinum*
  - *Clostridium perfringins*
  - *Clostridium tetani*

- **Staphylococcal Food Poisoning**
  - *Staph. aureus*
  - *Staph epidermidis*

- **Clostridial Food Poisoning**
  - *Clostridium perfringins*

- **Typhoid Fever**
  - *Salmonella typhi*

- **Salmonellosis**
  - *Salmonella enteriditis*
  - *Salmonella gallinarum*
  - *Salmonella typhimurium*
Foodborne diseases

• **Shigellosis**
  - *Shigella sonnei*
  - *Shigella dysenteriae*
  - *Shigella flexneri*
  - *Shigella boydii*

• **Cholera**
  - *Vibrio cholerae*
  - *Vibrio parahaemolyticus*

• **Diseases associated with *Escherichia coli***
  - *E. coli strain O157:H7*

• **Camphylobacteriosis and Helicobacteriosis**
  - *Camphylobacter jejuni*
  - *Helicobacter pylori*
Soilborne Bacterial Diseases

- Anthrax
- Tetanus
- Gas Gangrene
- Leptospirosis
- Listeriosis (*Listeria monocytogenes*)
Incubation period of foodborne diseases

**Figure 1**
Usual incubation period ranges for select foodborne diseases

- **Hepatitis A**
- **Listeria monocytogenes**
- **Yersinia enterocolitica**
- **E. coli O157:H7**
- **Campylobacter**
- **Shigella**
- **Cyclospora cayetanensis**
- **Clostridium botulinum**
- **Salmonella, nontyphoidal**
- **Clostridium perfringens**
- **Vibrio parahaemolyticus**
- **Staphylococcus aureus**

**Hours**

**Days**

1. Invasive form, incubation period for diarrheal disease unknown.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Associated Foods</th>
<th>Infective Dose (no. of organisms)</th>
<th>Incubation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Raw milk, and raw or under-cooked meat, poultry &amp; shellfish</td>
<td>400-500</td>
<td>2 to 5 days</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Raw/undercooked eggs, poultry, and meat; raw milk and dairy products; seafood; chocolate; salad and spices</td>
<td>15–20</td>
<td>12 to 24 h</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Raw/undercooked eggs, poultry, and meat; raw milk and dairy products;</td>
<td>&lt;10</td>
<td>2 to 4 days</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Soft cheese, raw milk, improperly processed ice cream, raw leafy vegetables; raw meat and poultry</td>
<td>&lt; 1000</td>
<td>2 days to 3 weeks</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>Improperly canned foods and vacuum packaged and tightly wrapped food</td>
<td>&lt; nano grams</td>
<td>12-36 h</td>
</tr>
<tr>
<td><em>Hepatitis A virus</em></td>
<td>Sandwiches, fruits and fruit juices, milk and milk products, vegetables, salads, shellfish, and iced drinks</td>
<td>10-100</td>
<td>Unknown</td>
</tr>
<tr>
<td><em>Norwalk virus/Norovirus</em></td>
<td>Raw oysters/shellfish, water and ice, salads, and frosting</td>
<td>Presumed to be low</td>
<td>1-2 days</td>
</tr>
</tbody>
</table>
At the Slaughter Plant

- FSIS target organisms
  - *Salmonella* and *E. coli*
- Control points
  - Removal of internal organs
  - Minimize contact between carcasses
  - Proper movement through facilities
  - Chilling
  - Cooking processes (time, temperature)
Irradiation

- Used since 1986 for *Trichina* control in pork
- Gamma rays
  - Poultry in 1990/1992
  - Meat in 1997/1999
  - Reduction of bacterial pathogens
- Kills living cells of organisms
  - Damaged and cannot survive
In the Home

- Drink pasteurized milk and juices
- Wash hands carefully and frequently
  - After using the bathroom
  - Changing infant’s diapers
  - Cleaning up animal feces
- Wash hands before preparing food
In the Home

- Wash raw fruits and vegetables before eating
- After contact with raw meat or poultry
  - Wash hands, utensils and kitchen surfaces
  - Hot soapy water
- Defrost meats in the refrigerator
On Farm Strategies

• Testing and removal for *Salmonella*
  • Serologic, fecal culture, hide culture
• Vaccinating
  • Many serotypes
  • Varying effectiveness
• Minimize rodents, wild birds
• Isolation of new animals
Microbiological testing of foods

• an integral part of food production,
• most often applied for end-product control
  • ineffective because of:
    • logistical complexities in sampling and the heterogeneous
distribution of contamination.
    • The implementation of the hazard analysis and critical control points
      (HACCP)
      • shifted the burden from testing to process control to ensure food safety

• However
  • microbiological testing remains a critical tool in process control
    monitoring, quality control, surveillance, risk assessment.
  • Moreover, environmental sampling and analysis have become
    routine in food production as well as in outbreak tracing and
    tracking
Reasons for Testing

Primary purposes

• to establish the absence of pathogens or their toxins
• to ensure the safety of foods, and to enumerate total or indicator microbial load
• to monitor effectiveness of hygienic processing and to verify product quality and shelf-life stability

Secondary purposes

• Food safety emphasis on the farm-to-fork approach has increased field testing
• increased number of tests for large-scale data collection for risk assessment
• surveillance and monitoring at the primary production levels
• the increasing need of food producers to quantify the level of pathogens in food samples.
Methods

1. Detection method must be rapid
2. The method should detect the desired specific pathogen
3. Method must be sensitive to detect small numbers of pathogens
4. The detection method should produce a quantitative analysis to help determine the severity of the hazard
5. The method should be multiplex (i.e., capable of detecting more than one contaminant simultaneously)
Testing Methods

**SAMPLES**
Environmental / Food / Clinical

**Microscopy**
- Light:
  - Cell counting
  - Cell and colony morphology
- Electron:
  - Subcellular components

**Culture**
- Screening
- Selection
- Enrichment

**Biochemistry**
- Biotyping
- Carbohydrate fermentation
- Enzyme detection

**Immunology**
- Serotyping
- Immunofluorescence
- Enzyme immunoassay (ELISA)

**Molecular biology**
- Genetic typing (ryotyping)
- 16 S DNA Sequencing
- PCR
- DNA-DNA Hybridization

**Biosensor**
- Enzyme
- Antibody
- Cell
- DNA, Aptamer
- Biomimetic
- Phage

**Bioreceptor**
- Optical:
  - Raman and FTIR
  - SPR
  - Fiber optic
- Electrochemical:
  - Amperometry
  - Voltammetry
  - Potentiometry
  - Impedimetry
  - Conductometry
- Mass based:
  - Piezoelectric (QCM, SAW)
  - Magnetoclastic

**Transducers**

Sequenced Genomes
Bar Coding DNA
Barcode searching

Full length barcodes

Microcodes
Summerbell, Seifert, Levesque, et al. (2005)
Phil. Trans. R. Soc. B (360), 1897-1903.
Comparative sequence based identification is only meaningful if:

- well-curated, robust and reliable databases are available
- that are populated with sequence data from:
  - type or reference strains (where possible)
  - a wide range of clinical strains
  - a wide variety of target species
- the strains have been rigorously validated in terms of their nomenclature
Which area of DNA?

Several loci have been suggested, a common set of standardized regions were selected by the respective communities:

- For **animals** and many other eukaryotes, the mitochondrial COI gene
- For **land plants**, the concatenation of the rbcL and matK chloroplast genes
- For **fungi**, the internal transcribed spacer (ITS) region
- For **bacteria**, the 16S ribosomal genes
Barcode databases

BOLD is a web based workbench and database supporting the acquisition, storage, analysis, and publication of DNA barcode records. By assembling molecular, morphological, and distributional data, it bridges a traditional bioinformatics chasm. BOLD is the most prominently used barcoding software and is freely available to any researcher with interests in DNA barcoding.

www.boldsystems.org
Molecular Biology Methods

Nucleic acid based

- Next Generation Sequencing
- Oligonucleotide DNA microarrays
- Nucleic acid sequence based amplification (NASBA)
- Ligation detection reaction - universal arrays (LDR-UA)
- Fragment analysis (PFGE, SSR, RAPD, iSSR etc)
- Polymerase chain reaction methods
- Real Time Quantitative PCR (TaqMan probes, HRM etc)
- loop-mediated isothermal amplification (LAMP)
Sequencing Platforms

- Roche/454 FLX: 2004
- Illumina Solexa Genome Analyzer: 2006
- Applied Biosystems SOLiD™ System: 2007
- Helicos Heliscope™: 2009
- Pacific Biosciences SMRT: 2010
- Ion Torrent: 2011
- Ion Proton 2012
Sequencing Costs

High cost, time consuming, high level of expertise
DNA microarrays

Labeling needed, high costs, time consuming, high level of expertise

Polymerase Chain Reaction (PCR)

- **Target DNA**
  - Denaturation: $T = 94 - 95^\circ C$
  - $t = 30 - 60$ s

- **Primer Annealing**:
  - $T$ = about 5°C below the lowest $T_m$ of the primers to be used
  - $t = 30 - 60$ s

- **Primer Extension**:
  - $T = 72^\circ C$
  - $t$ in min = equal to the number of kb of the product to be amplified

- **Number of cycles**: max. 45

<table>
<thead>
<tr>
<th>cycle number</th>
<th>target copies</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
</tr>
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<td>6</td>
<td>64</td>
</tr>
<tr>
<td>20</td>
<td>1,048,576</td>
</tr>
<tr>
<td>30</td>
<td>1,073,741,824</td>
</tr>
</tbody>
</table>

Contamination prone
Digital PCR

a) Conventional PCR

b) Digital PCR

Split sample by dilution
PCR/OLA

A  Synthesize a pair of oligonucleotide probes

B  Hybridize probes to PCR-amplified DNA

C  Add ligase to hybridized DNA
TaqMan Assay

Labeling needed, close tube approach
High Resolution Melting (HRM)

Results: 1 to 20 of 1722


Related citations
High Resolution Melting (HRM) principle

![Diagram showing normalized melting curves with temperature on the x-axis and fluorescence (normalized) on the y-axis, illustrating the melting of double-stranded DNA and single-stranded DNA.](image-url)

**B. Normalized Melting Curves**

- **Fluorescence (Normalized)**
- **Temperature [°C]**

- Double-stranded DNA (ds DNA)
- Single-stranded DNA (random coils)
loop mediated isothermal amplification
loop mediated isothermal amplification
## DNA Extraction

<table>
<thead>
<tr>
<th>Methods</th>
<th>Basis &amp; format</th>
<th>Starting material</th>
<th>Extraction buffer</th>
<th>Elution buffer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicentre</td>
<td>Solution-based; selective precipitation of DNA</td>
<td>5 – 9 mg</td>
<td>300 µL buffer</td>
<td>50 µL TE buffer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Master Pure Purification Kit.</td>
</tr>
<tr>
<td>Modified CTAB</td>
<td>Solution-based; selective precipitation of DNA</td>
<td>100 mg</td>
<td>1000 µL buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl pH 8.0) 550 µL buffer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150 µL TE buffer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Tinker et al., (1993)</td>
</tr>
<tr>
<td>NucleoSpin</td>
<td>Silica membrane binding; spin-column format</td>
<td>120 mg</td>
<td>400 µL buffer</td>
<td>200 µL buffer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Genomic DNA from food</td>
</tr>
<tr>
<td>Qiagen</td>
<td>Silica membrane binding; spin-column format</td>
<td>60 mg</td>
<td>1000 µL buffer</td>
<td>150 µL buffer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DNeasy Plant Handbook</td>
</tr>
<tr>
<td>CTAB</td>
<td>Solution-based; selective precipitation of DNA</td>
<td>100 mg</td>
<td>1000 µL buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl pH 8.0)</td>
<td>150 µL TE buffer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Gryson et al., (2004)</td>
</tr>
<tr>
<td>Roche</td>
<td>Solution-based; magnetic glass particle technology</td>
<td>50 mg</td>
<td>800 µL buffer (10 mM Tris [pH 8.0], 100 mM NaCl, 2 mM EDTA, 1% SDS)</td>
<td>100 µL buffer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sakai et al., (2002); MagNA Pure LC DNA Kit I</td>
</tr>
<tr>
<td>Wizard</td>
<td>Silica resin binding; vacuum manifold format</td>
<td>250 mg</td>
<td>3.0 mL buffer (150 mM NaCl, 2 mM EDTA, 1% SDS, 10 mM Tris base pH 8.0)</td>
<td>100 µL TE buffer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Spoth, and Strauss, (1998)</td>
</tr>
</tbody>
</table>
NEW CULTIVARS

1ST

SEED

HARVESTING

CULTIVATION

2ND

TRANSFORMATION

3RD

Pathogen Detection
Isolation and typing of Lactic acid bacteria

- Number of samples = 100 (300 carcasses)
- Initial characterization by the method of the double layer inhibition
- Selection of psychrotrophic lactic acid bacteria
- Detection for the production of biogenic amines (HPLC)
- Biochemical typing (API 50 CH)
- Molecular typing (sequencing and HRM)
High Resolution Melting analysis (HRM)

- Post PCR closed tube method
- DNA isolation
- PCR (16s rRNA)
- HRM analysis

Controls:
(Leuconostoc lactis, Lactobacillus salivarius, Lactobacillus fermentum, Lactobacillus delbrueckii, Lactobacillus acidophilus, Lactobacillus brevis, Pediococcus acidilactici)
Differences between strains

gb|CP000233.1| Lactobacillus salivarius UCC118, complete genome
Length=1827111
rRNA-23S ribosomal RNA

Identities = 741/743 (99%), Gaps = 0/743 (0%)

Query 1  CTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGGGGTAAC  60
Sbjct 75921  CTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGGGGTAAC  75980
Query 61  CGCAAGGAGCCACCGGCTACACTGCACACTCAGAGTTGGGTAACACCCAAAGCCGGTGGGGTAAC  120
Sbjct 75981  CGCAAGGAGCCACCGGCTACACTGCACACTCAGAGTTGGGTAACACCCAAAGCCGGTGGGGTAAC  76040
Query 121  CCGTAGGAGAACCTGCGGCTGGATCACCTCCTTTCTAAGGAATAATTACGGAACCTGTAC  180
Sbjct 76041  CCGTAGGAGAACCTGCGGCTGGATCACCTCCTTTCTAAGGAATAATTACGGAACCTGTAC  76100
Query 181  ATTTATCGGATACCTTTGCTAGTTTTGAGAGGTCATATCTCTCAAGATTTTGTTCTTTGA  240
Sbjct 76101  ATTTATCGGATACCTTTGCTAGTTTTGAGAGGTCATATCTCTCAAGATTTTGTTCTTTGA  76160
Query 241  AAACTAGATATTGATTCTTAATCGCTAAACTCATAACCTATTATCGTTAGATAATATT  300
Sbjct 76161  AAACTAGATATTGATTCTTAATCGCTAAACTCATAACCTATTATCGTTAGATAATATT  76220
Query 301  AAAAAAGAATTATAGTTCTTAATCGCTAAACTCATAACCTATTATCGTTAGATAATATT  360
Sbjct 76221  AAAAAAGAATTATAGTTCTTAATCGCTAAACTCATAACCTATTATCGTTAGATAATATT  76280
Query 361  AGGTTAAGTTATTAAGGGCGTATGGTGGATGCCTTGGCACTAGGAGCCGATGAAGGACGT  420
Sbjct 76281  AGGTTAAGTTATTAAGGGCGTATGGTGGATGCCTTGGCACTAGGAGCCGATGAAGGACGT  76340
Query 421  GACTAACTCGGATACTGGGAGGGTTGTAAGTAACACTATGACCTGGAGATTTCCGAGAT  480
Sbjct 76341  GACTAACTCGGATACTGGGAGGGTTGTAAGTAACACTATGACCTGGAGATTTCCGAGAT  76400
Query 481  GGGGAAACCTAACAGGTTTTACCGCCTGTTATCACTAAGTGAATTCATAGCTTAGTTGAA  540
Sbjct 76401  GGGGAAACCTAACAGGTTTTACCGCCTGTTATCACTAAGTGAATTCATAGCTTAGTTGAA  76460
Query 541  GGTAGACCTGGGGAACTGGAACCGCAGAATCTAGATTCGAGTTACTTATACCAAACTAAGGAGAAGAAGAAGAAGAAATTCGATTTCC  600
Sbjct 76461  GGTAGACCTGGGGAACTGGAACCGCAGAATCTAGATTCGAGTTACTTATACCAAACTAAGGAGAAGAAGAAGAAGAAATTCGATTTCC  76520
Query 601  CTCACTAGGCGGCGAGCCCGAAGCCGGAAGAGCCCAAATAGAAGCTCTTCTTTAGGGTTG  660
Sbjct 76521  CTCACTAGGCGGCGAGCCCGAAGCCGGAAGAGCCCAAATAGAAGCTCTTCTTTAGGGTTG  76580
Query 661  TAGGACTGAACTTTGAGTTTACCAAGAAATGAGTAGTGTACATCTTGGAAGATTAGC  720
Sbjct 76581  TAGGACTGAACTTTGAGTTTACCAAGAAATGAGTAGTGTACATCTTGGAAGATTAGC  76640
Query 721  CAAAGAGATGATAGCTCCGTAA  743
Sbjct 76641  CAAAGAGATGATAGCTCCGTAA  76663
HRM on Lactic Acid Bacteria
Genotyping Listeria strains using HRM

Genotyping of *Listeria monocytogenes* isolates from poultry carcasses using High Resolution Melting (HRM) analysis
Sakaridis, Ganopoulos, Madesis, Tsaftaris and Argiriou
*Biotechnology and Biotechnological Equipment, 2013*
Detection of *Listeria monocytogenes* using LAMP
Traceability
GMO DETECTION
BarCode chips

High-Density Microarray of Small-Subunit Ribosomal DNA Probes
Kenneth H. Wilson,1 Wendy J. Wilson,2 Jennifer L. Radosevich,2 Todd Z. DeSantis,2 Vijay S. Viswanathan,2 Thomas A. Kuczmarski,2 and Gary L. Andersen2*
Some Examples

• Certifying products made from specific plant species or cultivars and animal species or races using DNA Barcoding techniques
  • Unique signature to be used in all the production chain – traceability
  • Protect from fraudulent products the consumers
  • Maintain the added value of traditional products
  • Protect investments made to develop new food products

• Obtained Results: Certification of Mozzarella di buffala, Feta cheese, Hammon from Greek black pig, PDO legumes etc.
Detection of Horse DNA in Meat products

Available also for:
- Donkey
- Mule
- Chicken
- Turkey
- Pig (Halal and Kosher certification)
Biosensors
Figure 6. Electron microscopy zoom of an interdigitated microelectrode (A) and of the channels of a biochip belonging to a microfluid device (B).
Conclusions

• Many DNA methods available for Food pathogen detection and Food Traceability
• Most of them require high cost equipment, high expertise and are time consuming
• Most promising (in our lab):
  • HRM (requires RT-PCR system with HRM capabilities) and LAMP (in principle is enough a water bath)
• Future trends:
  • Low cost NGS
  • Biosensors
Thank you for your attention

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