Genomic and Proteomic Technologies for Analysis and Detection of Food-Borne Pathogens

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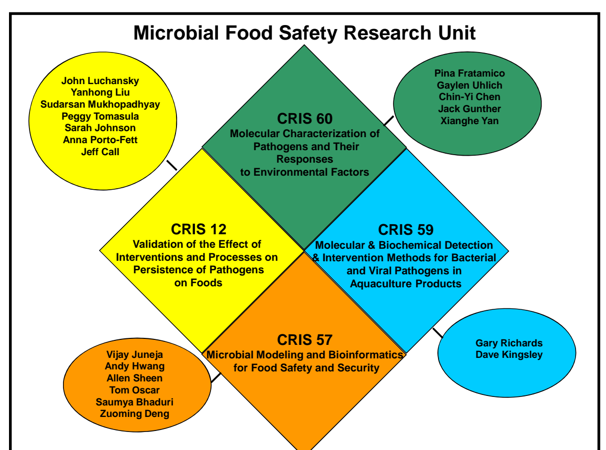
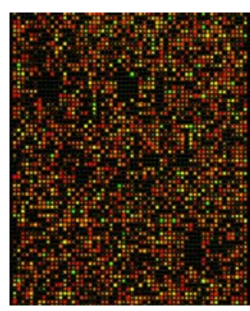


Yanhong Liu, Research Microbiologist
Current Genomic Research on *Listeria monocytogenes*

Genomic evaluation of *L. monocytogenes* in food related environments

- Determine the genes responsible for survival and growth of *L. monocytogenes* in food environments
  - Identify gene targets for improved detection and interventions
- Comparison of proteomic and microarray data
  - Determine the genetics and physiology of food-borne pathogens in foods

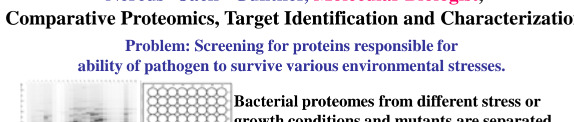
Microarray Results: Differential Gene Expression of *Listeria monocytogenes* in Milk and BHI Medium



- 35-mer oligonucleotides made by Combimatrix.
- 2847 genes on the chips. 2 probes per gene, each probe was spotted in duplicate.
- 26 up-regulated genes: transporters, RNA polymerase sigma-B factors, hypothetical proteins and structural genes.
- 31 down-regulated genes: cold-shock proteins, ribosomal proteins, transcriptional factors, hypothetical proteins, membrane proteins, and transporters.
- Gene-knockouts are being constructed.

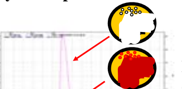
Nereus "Jack" Gunther, Molecular Biologist, Comparative Proteomics, Target Identification and Characterization

Problem: Screening for proteins responsible for ability of pathogen to survive various environmental stresses.



Bacterial proteomes from different stress or growth conditions and mutants are separated, quantitated, and isolated (2-D LC and 2-D gel)

Proteins of interest are identified by mass spectrometer analysis



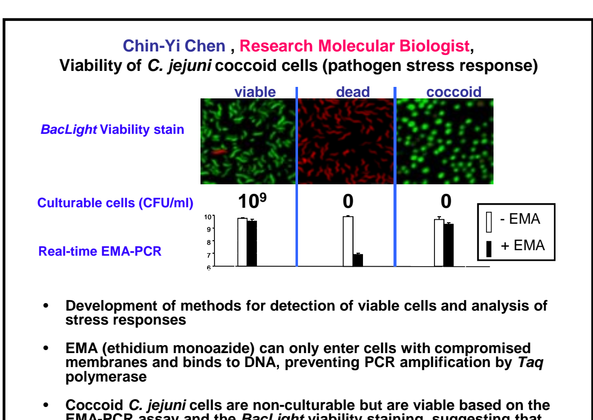
Protein function determined by comparing the expression patterns and characteristics of relevant bacterial phenotypes

Result: Potential biomarkers for increased bacterial virulence identified.

Gaylen Uhlich, Research Microbiologist, Characterization of proteins up-regulated in a high biofilm forming strain of *E. coli* serotype O157:H7

- Based on proteomics data, a specific lipoprotein is highly expressed in a high biofilm forming strain of *E. coli* O157:H7 (confirmed by real-time PCR)
- Deletion of the lipoprotein results in increased swarming compared to the wild-type strain
- Minor differences in biofilm formation

Chin-Yi Chen, Research Molecular Biologist,
Viability of *C. jejuni* coccoid cells (pathogen stress response)



- Development of methods for detection of viable cells and analysis of stress responses
- EMA (ethidium monoazide) can only enter cells with compromised membranes and binds to DNA, preventing PCR amplification by *Taq* polymerase
- Coccoid *C. jejuni* cells are non-culturable but are viable based on the EMA-PCR assay and the *BacLight* viability staining, suggesting that coccoid cells have intact membranes and either of these methods is sufficient for detection of stressed cells. - He and Chen

Chin-Yi Chen, Research Molecular Biologist,
Sequence and characterization of multi-antibiotic resistant plasmids in *Salmonella* spp.

- Two plasmids, 3.2- and 84.5-kb in size have been sequenced.
- The 3.2-kb plasmid, pU302S, belongs to the ColE1 family and carries the *aph(3)-I* gene (Kan[R]). The 84.5-kb plasmid, pU302L, is an F-like plasmid and contains 14 complete IS elements and multiple resistance genes including *aac3*, *aph(3)-I, sulII, tetA/R, strA/B, blaTEM-1, mph*, and the *mer* operon.
- pU302L carries several intact or partial replicons, and does not contain *oriT* and the *tra* genes necessary for conjugal transfer. This mosaic pattern of sequence similarities suggests that pU302L acquired the resistance genes from a variety of enteric bacteria.

Chen *et. al.* (2007) Plasmid 57:29-43

Pina Fratamico, Research Microbiologist/Lead Scientist
Xianghe Yan, Molecular/Computational Biologist
Select Research Projects

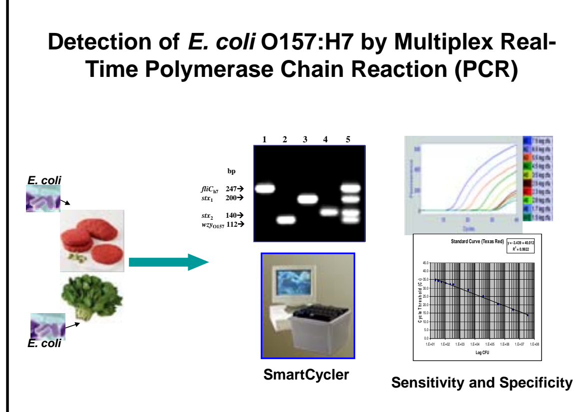
- PCR and PCR-RFLP for speciating *Campylobacter*
- Detection of *E. coli* O157:H7 by multiplex real-time PCR
- Sequencing of *E. coli* O antigen gene clusters and detection of STEC
- Biomarker gene discovery: for speciating, serotyping, antibiotic resistance determination, virulence determination, identification of stress response markers
- DNA sequence of the large virulence plasmid of an enterohemorrhagic *E. coli* serotype O26:H11 strain
- Understanding biofilm formation and quorum sensing through bioinformatics/molecular research

PCR and PCR-RFLP Using *Dde*I to Speciate *Campylobacter*

A: PCR identification

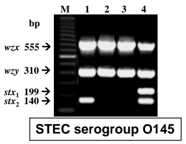
B: PCR-RFLP identification

Detection of *E. coli* O157:H7 by Multiplex Real-Time Polymerase Chain Reaction (PCR)



Sequencing of *E. coli* O Antigen Gene Clusters and Detection of STEC

- Detection of STEC
  - Development of multiplex PCR assays targeting virulence genes and genes in the O antigen gene clusters (e.g., *wzx, wzy*) of specific *E. coli* serogroups
  - Multiplex PCR for detection of *E. coli* O145 in food



STEC serogroup O145

E. coli Serogroups in Which the O-antigen Cluster Has Been Sequenced in the MFSRU or by Others

O104	O15	O38	O28
O121	O26	O177	O52
O103	O151	O174	O22
O45	O148	O7	O174
O55	O66	O149	O118
O55	O145	O127	O117
O114	O59	O172	O126
O111	O155	O91	O146
O157	O52	O79	O91
O106	O139	O53	O151
O77	O138	K12	O2
O73	O86	O101	O63
O145			

Category	*E. coli* O serogroups	Disease
ETEC	O6, O8, O15, O20, O25, O27, O63, O78, O80, O85, O115, O128ac, O139, O148, O153, O159, O167	Traveler's diarrhea, Diarrhea in children
EIEC	O28ac, O29, O124, O136, O143, O144, O152, O164, O167	Bacillary dysentery
EPEC	O55, O86, O111, O114, O125, O126, O127, O128ab, O142	Infant diarrhea
EHEC / STEC	O26, O157, O103, O111, O91	Hemorrhagic colitis, Hemolytic uremic syndrome
EAEC	O3, O15, O44, O51, O77, O86 O92, O111, O113, O126, O141	Infant diarrhea
DAEC	not determined yet	Diarrhea in children

Sequencing of the Virulence Plasmid of EHEC Serotype O26:H11

- *E. coli* O26 is classified as enterohemorrhagic (EHEC) or enteropathogenic (EPEC)
- O26, first reported in 1951. *E. coli* O26:H11 is the most important non-O157 EHEC/STEC serotype
- It is almost impossible to categorize *E. coli* as STEC or EHEC by serotyping since there is no clear correlation between serotype and pathotype. So, a genotypic determination is necessary for the identification of these pathogenic strains
- *E. coli* O157 virulence plasmids have been sequenced, but not the plasmids of O26, O111 and O91 etc
- Determine the DNA sequence and the analysis of the virulence plasmid in *E. coli* O26:H11 will determine the similarity to pO157 and will provide insights on how E. coli O26:H11 causes disease

Sequencing of the Virulence Plasmid of EHEC Serotype O26:H11

- Genetic-based methods to detect/identify pathogenic *E. coli* O26 strains are limited to chromosomal target genes, such as:
  - virulence factors: Shiga toxin (*stx*) and intimin (*eae*)
  - H-antigen: *fliC-fliA*
  - genes in O-antigen gene cluster
- Use of genes found on the virulence plasmid of *E. coli* O26:H11 for detection?
- Improved, rapid, and specific genetic-based food testing and diagnostics for EHEC O26

Six plasmids have been sequenced, five out of 6 are completed, and the largest one is in the assembly stage

	Size (kb)	Virulence genes	Resistance genes
Plasmid 1	1.549	None	None
Plasmid 2	3.174	?	None
Plasmid 3	4.073	?	None
Plasmid 4	6.757	?	None
Plasmid 5	72.384	?	Yes
Plasmid 6	>90.000	Yes	No ?

Virulence Genotype Pattern Comparison between *E. coli* O26:H11:K60 ED21 and O157

Virulence genes	O157	O26:H11:K60 ED21
*eae***	Yes	Yes
stx_1**	Yes	Yes
stx_2**	Yes	No
*toxB****	Yes	Yes
*hlyA****	Yes	Yes
*katP****	Yes	Yes
*espP****	Yes	Yes

**: Chromosomal biomarkers
***: Plasmid biomarkers

Plasmids of *E. coli* O26:H11 ED21

Hypothesis: Function of genes encoded on plasmids 1, 2, 3 and 4 could be related to transportation of toxins and other proteins into the cell

Bacterial proteins must cross the host cell bilayer to then exert their mechanism of action

Biofilm Formation in *E. coli* O26:H11 ED21

O26:H11:K60

PDH1: RM

- Based on gene annotation, there is one gene cluster in the largest plasmid, which encodes genes responsible for the biofilm formation
- A GFP-tagged plasmid gene-knockout mutants will be constructed

Acknowledgements

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- Yanhong Liu, Ph.D., Research Microbiologist
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