MOLECULAR CHARACTERIZATION OF FOODBORNE PATHOGENS RESEARCH UNIT

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MOLECULAR CHARACTERIZATION OF FOODBORNE PATHOGEN RESPONSES TO STRESS (PROJECT NO. 8072-42000-082-00D)

- 1) Pina Fratamico (retired in 2018), 2) Yanhong Liu, 3) Nereus "Jack" Gunther, 4) Jacob Elder (post-doc since 2018), 5) Computational Biologist (vacant)
- *Listeria monocytogenes*
  - Molecular characterization (stress responses, virulence); survival with exposure to organic acids and natural antimicrobials
- Extra-intestinal pathogenic *E. coli* (ExPEC) and Shiga toxin-producing *E. coli* (STEC)
  - Molecular characterization (stress responses, virulence, genetic markers; detection and identification)
- *Campylobacter* spp.
  - Biofilms, technologies for inactivation and decreasing survival in poultry

OUTLINE

- Summary of published results
- Ongoing research
- Potential impact
- Future directions

SUMMARY OF RESULTS

- Sixty *L. monocytogenes* isolates from different sources were typed and screened for salt and acid tolerance. Strains with different stress responses have been sequenced.

Microbial & Biochemical Technology

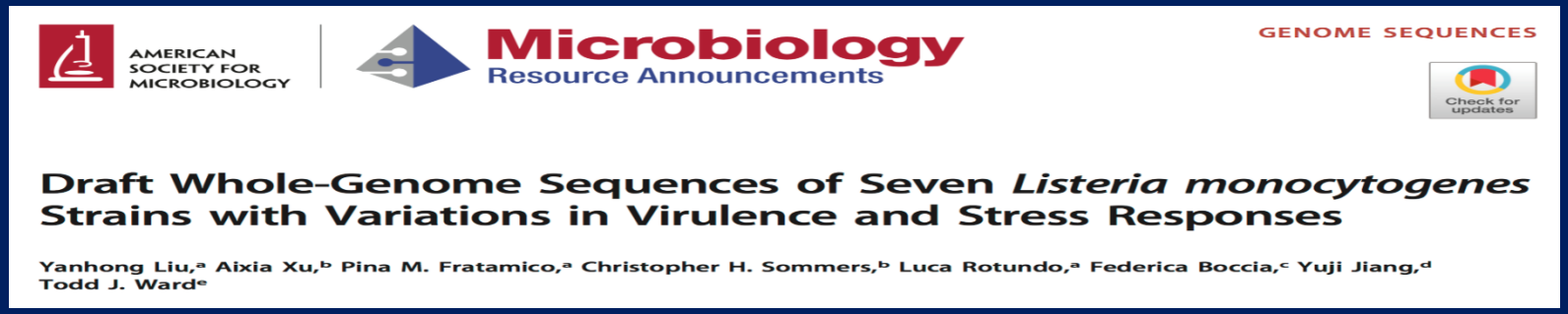
Edlind and Liu, J Microb Biochem Technol 2015, 7:6
http://dx.doi.org/10.4172/1948-5948.1000238

Research Article Open Access

Development and Evaluation of a Commercial Sequence-Based Strain Typing Service for *Listeria monocytogenes*

Tom Edlind[1] and Yanhong Liu[2*]

- **LM Strain typing method has been commercialized by microbiType, LLC**



AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

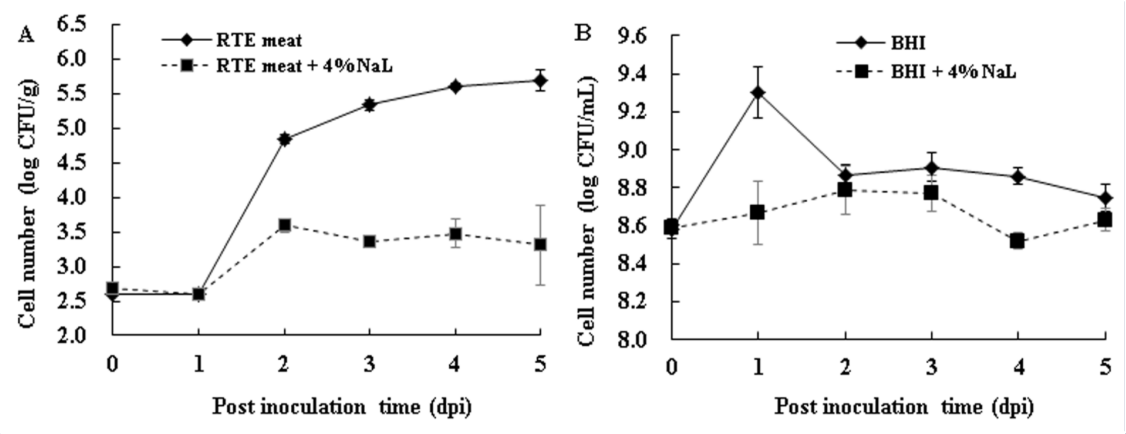
Check for updates

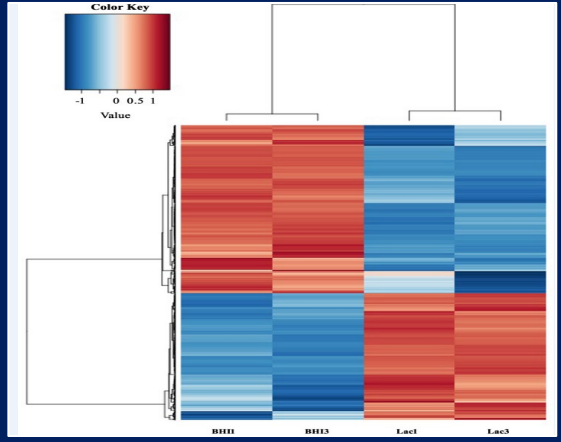
Draft Whole-Genome Sequences of Seven *Listeria monocytogenes* Strains with Variations in Virulence and Stress Responses

Yanhong Liu,[a] Aixia Xu,[b] Pina M. Fratamico,[a] Christopher H. Sommers,[b] Luca Rotundo,[a] Federica Boccia,[c] Yuji Jiang,[d] Todd J. Ward[e]

COMPARATIVE TRANSCRIPTOMICS OF *L. MONOCYTOGENES* EXPOSED TO SODIUM LACTATE

- Sodium lactate used as antimicrobial in RTE meat
- RNA-Seq - transcriptomics of *L. monocytogenes* exposed to sodium lactate (4%)
  - 766 genes differentially expressed in BHI
  - Down-regulated (n=437): chemotaxis, flagella assembly
  - Up-regulated (n=329): virulence-related genes – *hly, inlA, inlE, actA, plcA, plcB, iap*, and others
  - Confirmed by qRT-PCR (92 genes)





Food Control 91 (2018) 193–201

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Food Control

journal homepage: www.elsevier.com/locate/foodcont

Comparative transcriptome RNA-Seq analysis of *Listeria monocytogenes* with sodium lactate adaptation

Yujuan Suo [a, 1], Shigang Gao [a, 1], Gian Marco Baranzoni [b], Yanping Xie [b], Yanhong Liu [b, *]

Olive leaf extract: natural antimicrobial with health benefit

frontiers in Microbiology

Front Microbiol. 2017; 8: 113.
Published online 2017 Feb 2. doi: 10.3389/fmicb.2017.00113

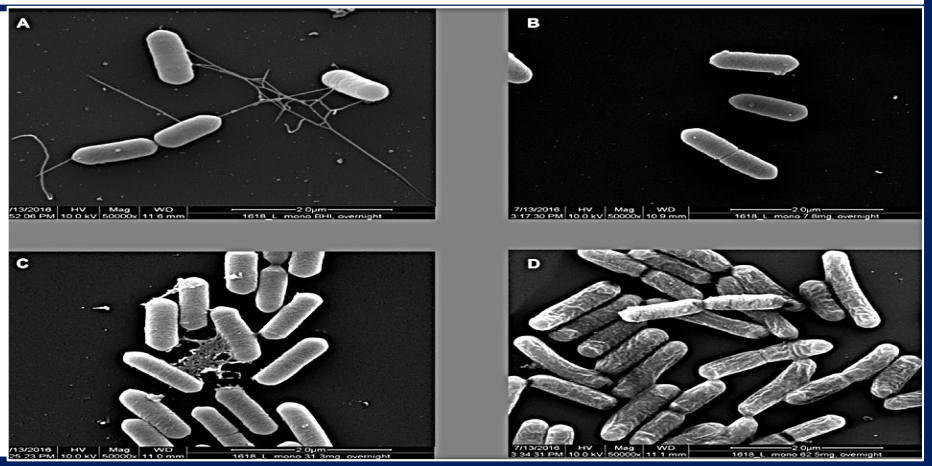
PMCID: PMC5288333

Assessment of the Antimicrobial Activity of Olive Leaf Extract Against Foodborne Bacterial Pathogens

Yanhong Liu,* Lindsay C. McKeever, and Nasir S. A. Malik

TABLE 1 | Growth inhibition of OLE to *Escherichia coli O157:H7*, *Salmonella* Enteritidis, and *Listeria monocytogenes*.

	Bacterial growth inhibition (%)		
	L. monocytogenes F2365	*S.* Enteritidis	*E. coli* O157:H7
Olive leaf extract (OLE) (62.5 mg/ml)	100	100	95
Oleuropein (25 mg/ml)	94	36	58
Verbascoside (25 mg/ml)	100	65	82



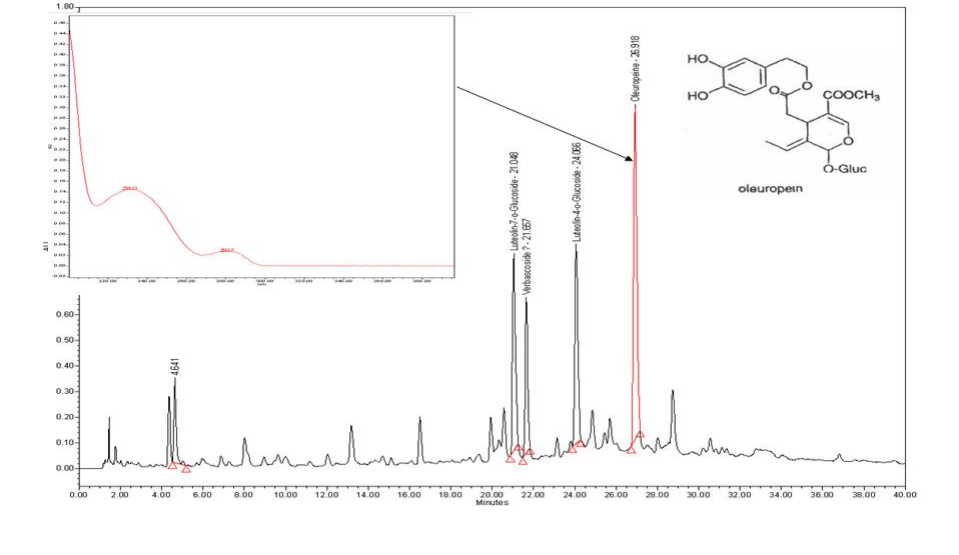
Decreased cell mobility, flagella, biofilm formation

Attracted attention of Euromed, Inc. provide free olive leaf extract

ACS Book chapter

Liu, Y., McKeever, L. C., Suo, Y. Jin, T. Z. and Malik, NS. Antimicrobial activities of olive leaf extract and its potential use in food industry. Natural and Bio-Based Antimicrobials for FooACSd Applications, Chapter 6, 2018, pp 119-132. *ACS Symposium Series*, Volume 1287. Publication Date (Web): September 04, 2018 (Chapter). DOI: 10.1021/bk-2018-1287.ch006.

Systems biology for oleuropein (major component of olive extract)



J Ind Microbiol Biotechnol (2016) 43:1705–1717
DOI 10.1007/s10295-016-1841-8

CrossMark

APPLIED GENOMICS & SYSTEMS BIOTECHNOLOGY - ORIGINAL PAPER

SIMB
Society for Industrial Microbiology and Biotechnology

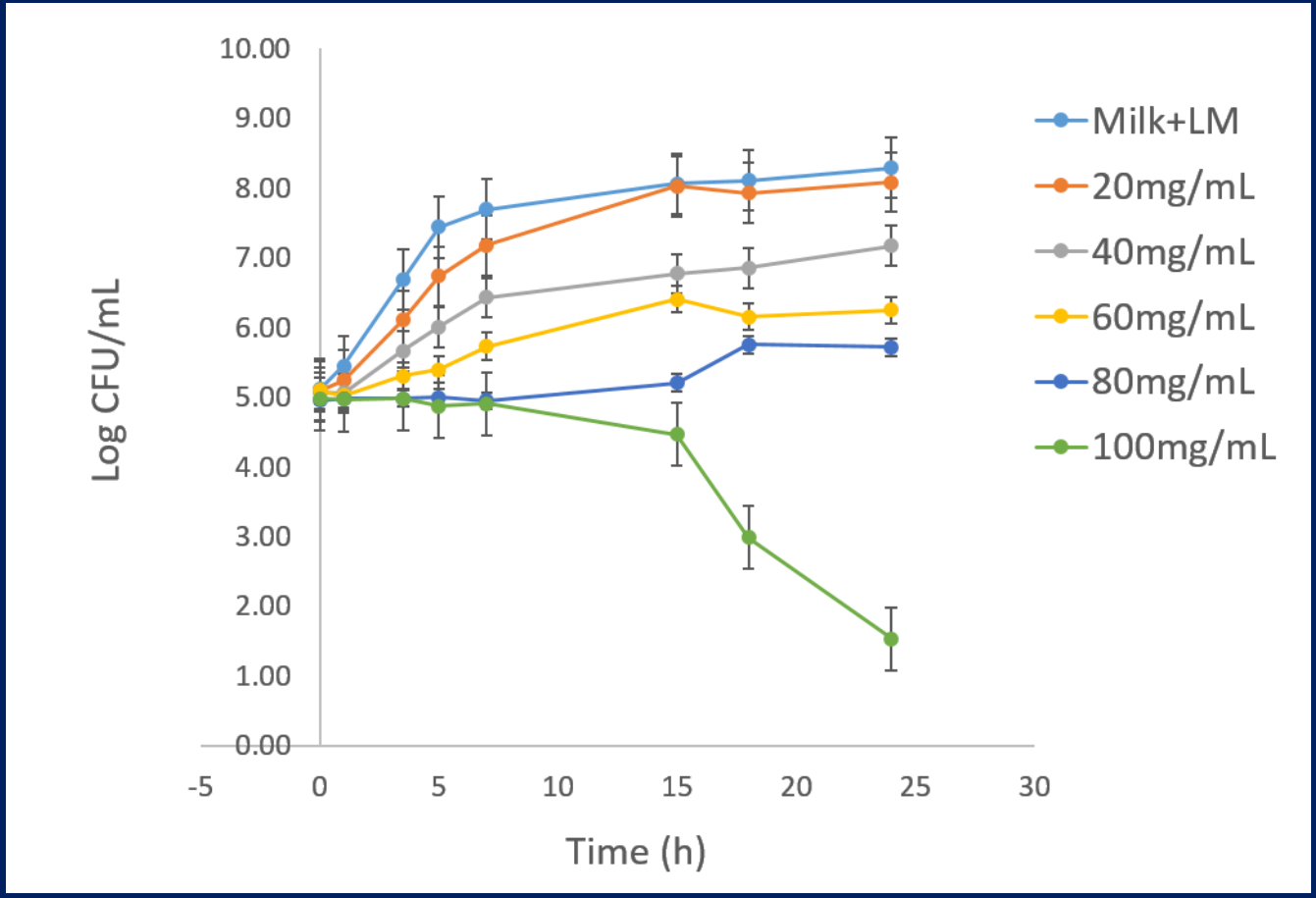
A systems biology approach to investigate the antimicrobial activity of oleuropein

Xianhua Li[1] · Yanhong Liu[2] · Qian Jia[3] · Virginia LaMacchia[1] · Kathryn O'Donoghue[1] · Zuyi Huang[1]

Ongoing Research

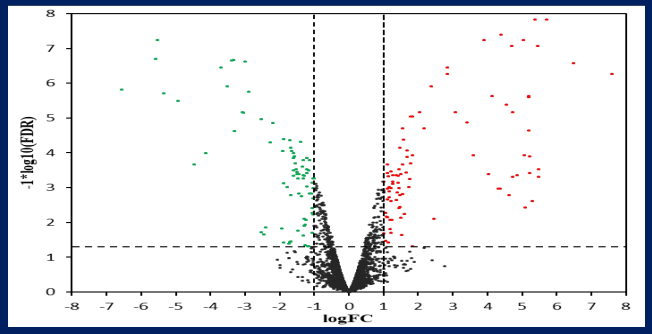
- **Transcriptomics of *L. monocytogenes* treated with olive leaf extract**
- **Screening for antimicrobial compounds from plant extracts**
- **Molecular serotyping of *E. coli* using AgriSeq technology (Pina)**
- **Extraintestinal pathogenic *E. coli* (ExPEC) in collaboration with Dr. Christopher Sommers**

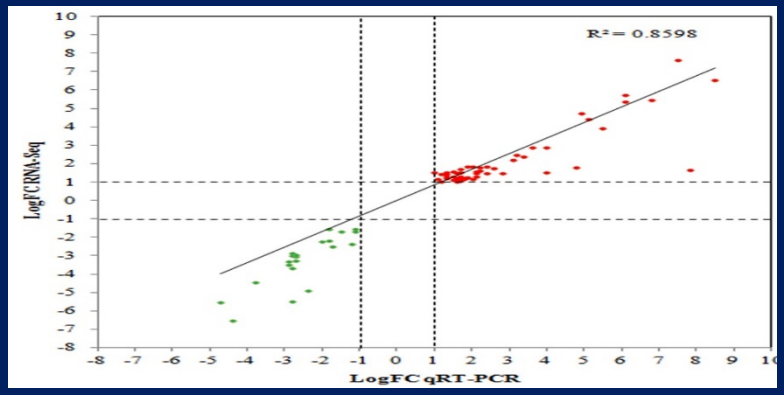
Olive leaf extract inhibited the growth of *L. monocytogenes* in milk

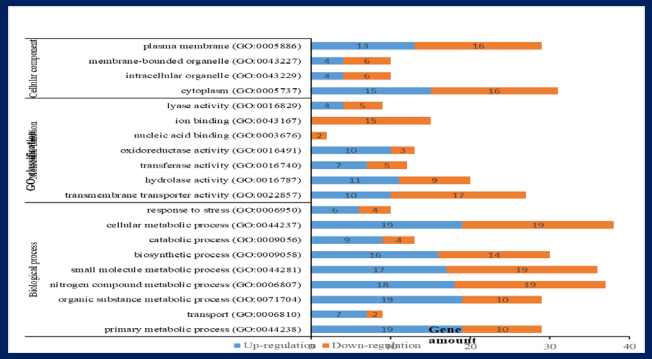


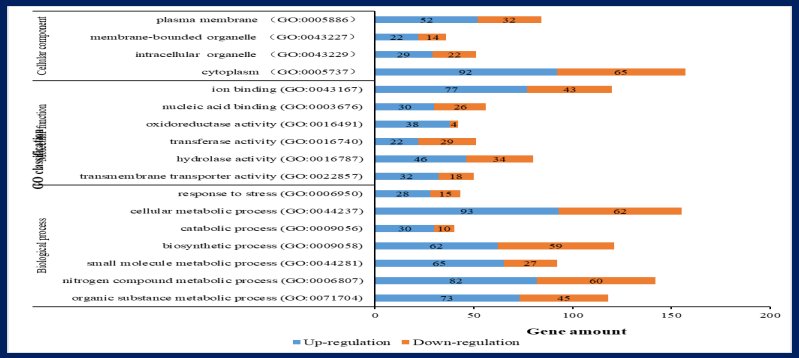
Transcriptomics of *L. monocytogenes* exposed to olive leaf extract

RNA-Seq - transcriptomics of *L. monocytogenes* exposed to sub-lethal dose of olive leaf extract (7.8mg/ml) . Time points: 0, 3.5-hr and 24-hr









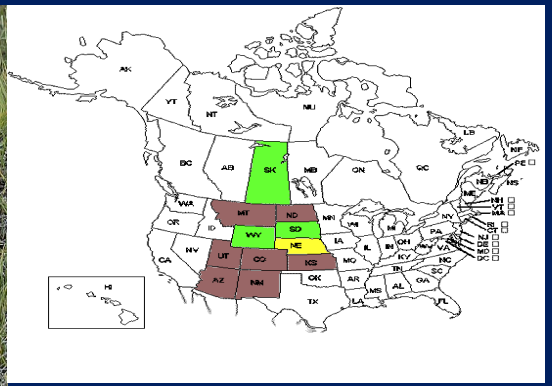
171 and 490 differentially expressed genes (DGEs) in 3.5-hr and 24-hr treatments

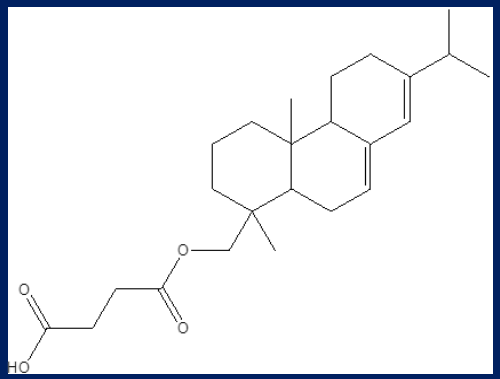
37 DEGs in both 3.5-hr and 24-hr treatments

Very good correlation of RNA-Seq with qRT-PCR data (R^2=0.86)

SCREENING PLANT EXTRACTS FOR ANTIMICROBIAL ACTIVITY

- 1000 extracts tested (weeds, trees, grasses, etc.)
  - 4 extracts with antimicrobial activity against *L. monocytogenes*
  - 1 extract from a weed – potent compound (ng/ml-complete inactivation) was purified, retested, and structure determined





CHARACTERIZATION OF THE PURIFIED COMPOUND FROM THRIFT MOCK GOLDEN WEED

- **The compound was synthesized in the lab the anti-listeria activity of the compound was confirmed (MIC: 7.8 µg/ml).**
- **Known to have anti-inflammatory function, first to find anti-bacterial activity**
- **Only inhibited *L. monocytogenes*, not Gram (-) pathogens including *E. coli* and *Salmonella***
- **Testing more G(+) and G(-) pathogens for specificity: Specific to L. monocytogenes**
- **Ames test for mutagenic potential of this compound: negative**

MOLECULAR SEROTYPING OF *E. COLI*

PLoS ONE. 2016. 11(1):e0147434

RESEARCH ARTICLE

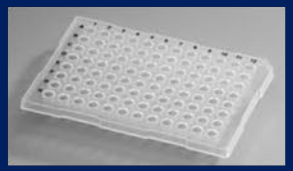
Comparison of O-Antigen Gene Clusters of All O-Serogroups of *Escherichia coli* and Proposal for Adopting a New Nomenclature for O-Typing

Chitrita DebRoy[1]*, Pina M. Fratamico[2], Xianghe Yan[2], GianMarco Baranzoni[2], Yanhong Liu[2], David S. Needleman[2], Robert Tebbs[3], Catherine D. O'Connell[3], Adam Allred[3], Michelle Swimley[3], Michael Mwangi[1], Vivek Kapur[1], Juan A. Raygoza Garay[1], Elisabeth L. Roberts[1], Robab Katani[1]

- **Meetings at Penn State Univ. 2015 and 2017**
  - **Chitrita DebRoy and Ed Dudley (PSU), Flemming Scheutz (Denmark), Stefano Morabito (Italy), Claire Jenkins (England), Peter Reeves (Australia), Atsushi Iguchi (Japan), Nancy Strockbine (CDC), Sara Christianson and Linda Chui (Canada), Adrian Cookson (New Zealand), Peter Feng, Dave Lacher et al. (FDA), Jim Johnson (UMinn), Mark Eppinger (UTexas), Patrick Fach (France), Janssen Vaccines, and others**
- **IonAmpliSeq™ - AgriSeq – multiplexed PCR chemistry**
  - **Designed primers specific for all O-groups and H-types, all *stx* subtypes, *eae*, and control (*Thermotoga maritima*) --- Total 241 primer sets**

AgriSeq workflow

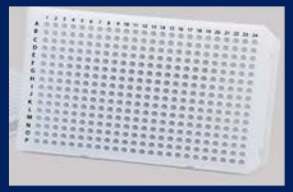
96-well

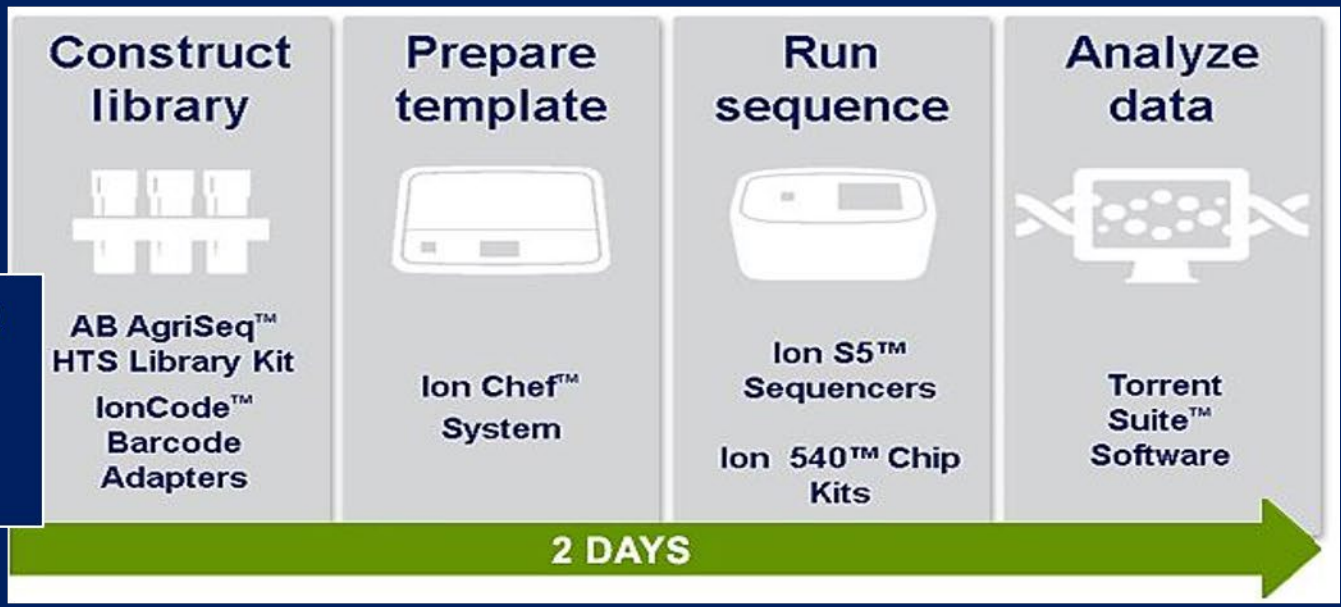


10 ng gDNA input

→ Ultra-high targeted multiplex PCR

384-well





Thousands of primer pairs to amplify specific DNA regions → PCR → digest primers → library of remaining amplicons/ligate barcodes (768 barcodes available) → sequence → analyze – Ion Reporter software

Low cost per sample, results in 2 days, minimal hands on time

AGRISEQ RESULTS

- Positive results with *E. coli* reference strains
  - All O groups and H types
- Field isolates from *E. coli* Reference Center: Positive results with at least 80% of strains
  - 20% likely incorrectly serotyped
- User-friendly analysis software
- To be presented in IAFP annual meeting in 2019

EXTRAINTESTINAL PATHOGENIC *E. COLI* (EXPEC)

- **ExPEC: food and animal source for extraintestinal infections?**
- **Primers for ExPEC-related genes being added to AgriSeq panel**
  - **Including: *vat, fyuA, chuA, yfcV, fimH, papACEFG, sfa, foc, afa/draBC, lpfA2, flu, upaBGH, fdeC, hdp, hra, iha, tosA, yqi, ibeA, hlyCABD, sat, pic, cnf1, ire, iroN, fecB, iutA,* antibiotic resistance genes**
- **Collaboration with Dr. Christopher Sommers**
  - **Analysis of ExPEC isolated from chicken and produce: biofilm assays, virulence gene profiles, stress responses**
  - **Comparison to strains isolated from human ExPEC infections (strains being obtained from a hospital in PA)**

POTENTIAL IMPACT

- **The plant extract may have potential to be used as a sanitizer to control *L. monocytogenes* in food processing plants**
- **Patent application for the use of this grass**
- **Development of new natural antimicrobials through screening plant extracts**
- **Olive leaf extract can be used as an antimicrobial and packaging material to control foodborne pathogens**
- **The typing method for *L. monocytogenes* has been commercialized by MicrobiType, LLC.**

FUTURE DIRECTIONS

- **500 additional compounds tested similar to previous potent plant extract**
  - **Many found to have antimicrobial activity**
  - **Fractionation of the extract to identify the active compounds**
  - **Transcriptomics for the active compounds treated with *L. monocytogenes***
  - **Systems biology for active compounds (derivatives) for drug targets**
- **Functional genomics: gene deletion mutants using CRISPR-Cas9 gene editing system**
- **Comparative genomics: whole genome sequencing (WGS) for *L. monocytogenes* strains with differences in stress responses**
- **Intervention: Effect of olive leaf extract in beef inoculated with *L. monocytogenes*, olive leaf extract used as antimicrobial packaging material to control pathogens.**

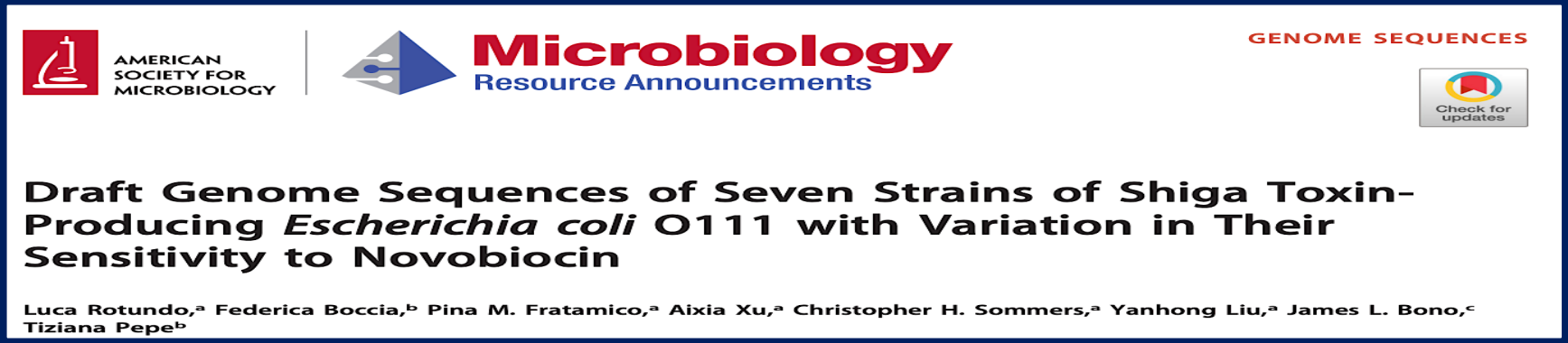
Bioinformatics/Computational needs

More than 90 Shiga toxin-producing *Escherichia coli* isolates from swine, cattle, and humans have been sequenced

Genomic comparison of acid resistant and acid sensitive strains

- 26 strains sequenced, 2 published

Comparison of STEC with variations in resistance to novobiocin



ACKNOWLEDGEMENTS

- USDA-ARS-ERRC (Drs. Jacob Elder, Christopher Sommers, Lihan Huang and Tony Jin)
- USDA-ARS-NCAUR (Dr. Todd Ward)
- Natural Products Discovery Institute (A Division of The Baruch S. Blumberg Institute (Drs. Jason Clement and Mathew Todd)
- FDA (Dr. Atin Datta)
- Villanova University (Fangyuan Zhang and Dr. Jacky Zuyi Huang)
- Shanghai Academy of Agricultural Science (Dr. Yujuan Suo)
- Fujian Agricultural and Forestry University (Drs. Yuji Jiang and Ting Fang)
- E. coli Reference Center at Penn State University (Dr. DebRoy)
- Thermo Fisher Scientific, Inc. (Dr. Tebbs)
- Euromed, Inc. (Dr. Woodman)
- MicrobiType LLC. (Dr. Tom Edlind)