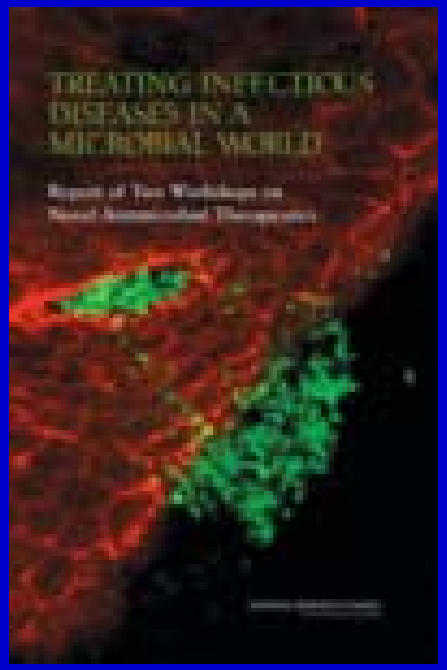
Development of Interventions to Reduce Human Food-borne Bacterial Pathogens
&
Molecular Characterization and Gastrointestinal Tract Ecology of Commensal Human Food-borne Bacterial Pathogens in the Chicken

Bruce S. Seal

(http://www.ars.usda.gov/main/main.htm)

Poultry Microbiological Safety Research Unit
Russell Research Center, ARS, USDA
Athens, GA



Committee on New Directions in The Study of Antimicrobial Therapeutics: New Classes of Antimicrobials

This study was conducted between the National Academy of Sciences and the National Institutes of Health.

Confocal micrograph depicting a common behavior of bacteria (green) gathering in mucus-mucins shed by host epithelial cells (red).

Recommendations of the panel include:

Development of strategies that will selectively target pathogenic organisms while avoiding targeting the host and beneficial or benign organisms.

Characterization and enumeration of the normal resident microbes in the host and further understanding of the relationship among resident microbial populations.

Development of strategies to manipulate chemical signaling in the host microbial population.

Research to determine which bacterial mechanisms should be used in pro/pre-biotic therapies and to identify delivery mechanisms that would be most effective for pro-biotic therapies.

Mining the Natural World: Discover, Diversify, and Deliver or;
Pro/pre-biotics in a Genomics World

Comparison of *Lactobacillus* spp. bacteriocins

```
OR7          KTYYGTNGVHCTKNSLWGKVRLKNM-----KYDQNTTYMGRLQDILLGWATGAFGKTFH
             KTYYGTNGVHCTK   SLWGKVRLKN +           +  +    +  +    ILLGWATGAFGKTFH
acidocin A   KTYYGTNGVHCTKKSLWGKVRLKNVIPGTLCRKQSLPIKQDLKILLGWATGAFGKTFH
```

OR7 (*Lactobacillus salivarius*) vs. acidocin A (*Lactobacillus acidophilus*)

OR7 peptide amino acid composition:
28.4% charged (RKHYCD)
3.3% acidic (DE)
13.3% basic (KR)
31.6% polar (NCQSTY)
23.4% hydrophobic (AILFWV)
no glutamine (E) or proline (P)

The OR-7 amino acid sequence resulted in a peptide with a predicted molecular weight of 6,214 consisting primarily of amphipathic beta-turn regions with a predicted 9.5 isoelectric point

This structure was as predicted for other membrane disrupting cationic Class IIa antibacterial peptides

Salmonella colonization model for testing anti-*Salmonella* bacteriocins

N. J. Stern, G. R. Siragusa and J. E. Line

Bacteriocin S760 inhibits

Bacillus cereus

Bacillus licheniformis

E. coli O157

Salmonella spp.

Clostridium perfringens

Campylobacter jejuni

Objective

Consistently populate mature broilers with *Salmonella* to assess efficacy of bacteriocins.

Bacteriocin from *Streptococcus cricetus* found in chicken intestinal flora

Research Direction

Apply bacteriocins in feed of *Salmonella* colonized broilers several days prior to slaughter.

Procedure

1. D.O.H. chicks challenged with 10^8 cfu of 3 poultry-derived salmonellae serotypes and ceca tested at weeks 1, 2, 3, 4.
2. 4 week old chickens administered vancomycin, and three days later challenged with 10^8 cfu and ceca tested at weeks 5 and 6.

Results Summary

1. Populations of Salmonella decreased from ~log 6/g of cecal matter to <log 1-2 at week 4. DOH challenged chicks colonized minimally by wk. 4
2. Post antibiotic gut disruption resulted in levels of ~$\log^6$ and $\log^2$ cfu Salmonella /g at weeks 5 and 6 respectively.

Comparison of Four Sampling Methods for the Detection of *Salmonella* spp. in Broiler Litter

R.J. Buhr[1], N.A. Cox[1],

L.J. Richardson[1] and J.A. Cason[2]

[1]Poultry Microbiological Safety Research Unit and [2]Poultry Processing and Meat Quality Research Unit

RRC, USDA-ARS

FECAL DROPPINGS

LITTER GRAB

DRAG SWAB

SOCK

Broiler Litter Sampling

Method	*Salmonella* (+/-)
Fecal droppings	22%
Litter grab	31%
Drag swab	36%
Sock	59%*

CONCLUSION

- **Results indicate that when sampling material comes in greater contact with broiler litter by stepping on a sample material (sock or drag swab), the samples are more likely to detect *Salmonella*.**

Semi-automated rep-PCR Predicts *Salmonella* serotypes

Gregory R. Siragusa*[1], M.G. Wise[2], J. Levine[3], O. Adeyemo[4], K. Pupedis[3], J.C. Jones[2], D. Abbott[4] and Nathan Bauer[5]

***corresponding author. Phone: (706) 546-3596**
E-mail: siragusa@saa.ars.usda.gov

[1]Poultry Microbiological Safety Research Unit Agricultural Research Service, USDA, Athens, Georgia
[2]Bacterial Barcodes, Athens, GA
[3]Outbreaks Section, Eastern Laboratory, [4]Food Emergency Response Network, FSIS, USDA, Athens, GA and [5]FSIS, USDA, College Station, TX

rep-PCR

rep-PCR is a DNA based typing technique wherein a signature pattern is generated from the PCR amplification of a product off of repetitive extragenic palindromic sequences within a genome

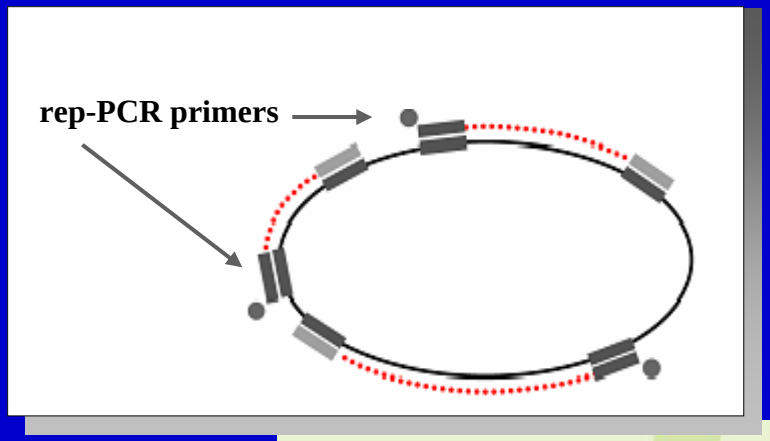
Hence the term rep-PCR

rep elements are found in prokaryotic and eukaryotic genomes

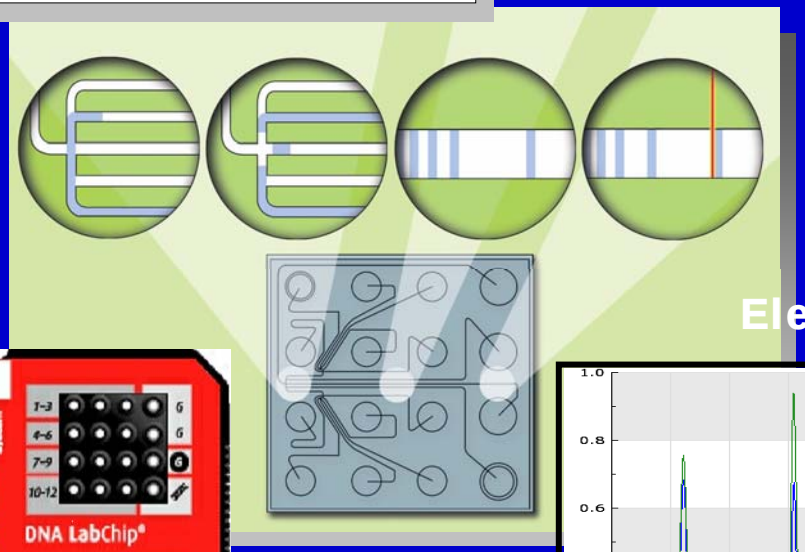
Although the function of these elements is unclear the patterns obtained by resolving the amplification products off the genome result in reproducible data for typing of host DNA

See: Healy et al. *J. Clin. Microbiol*. 43:199-207, 2005

rep-PCR Detection using the LabChip® Device

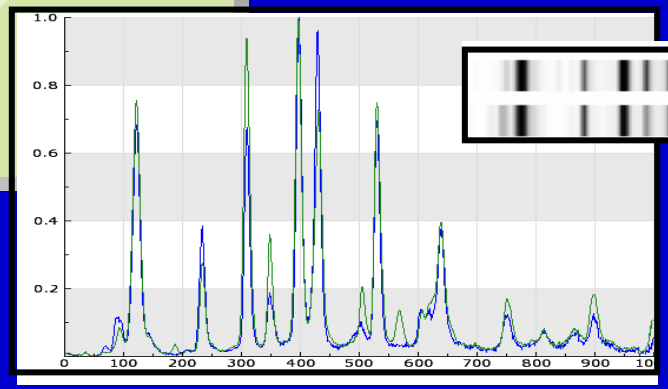


rep-PCR primers bind to many specific repetitive sequences interspersed throughout the genome

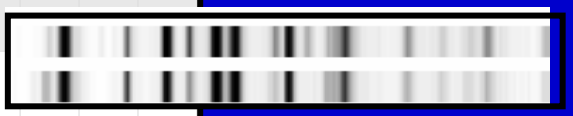


Amplicon separation and detection using lab-on-a-chip microfluidics technology

Electropherogram

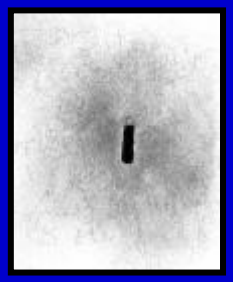


Virtual gel image

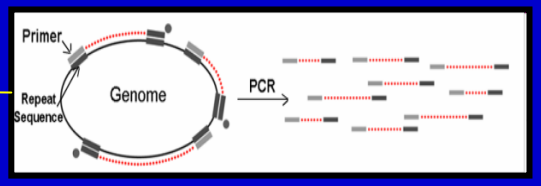


The rep-PCR workflow

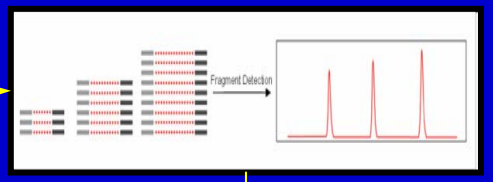
Pure Culture (overnight)



Isolate DNA (0.5 -1 hr)

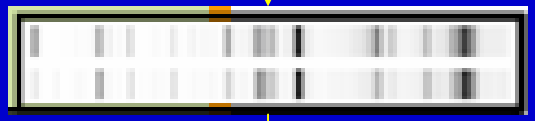


Amplify rep elements (3 hr)

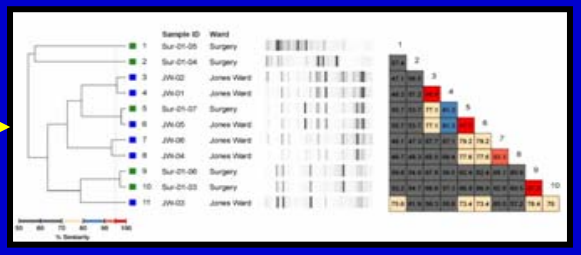


Separate rep PCR amplicons (1.25 hr)

Data streams directly to DiversiLab database via web.



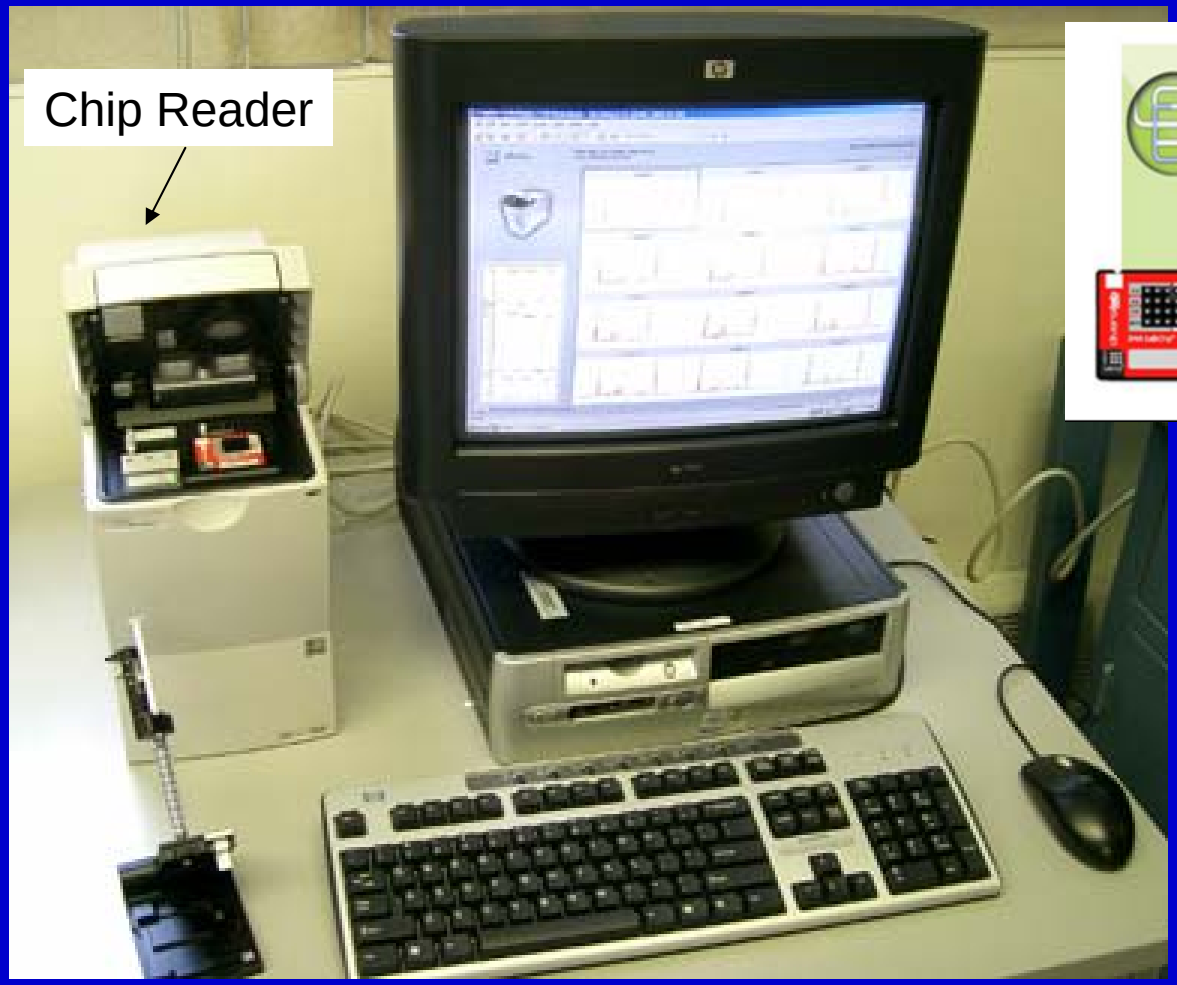
Generate signature

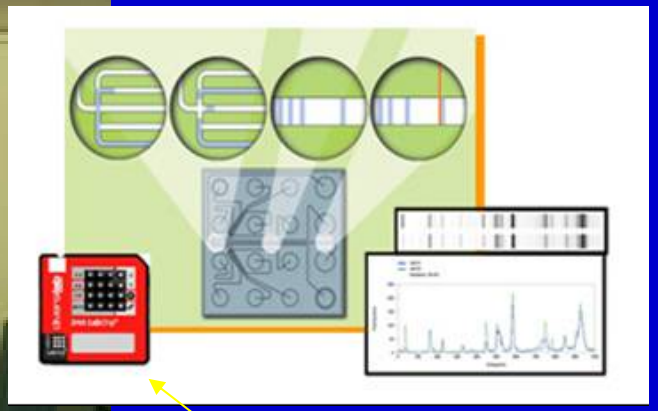


Compare signatures

Results in about 6 hours

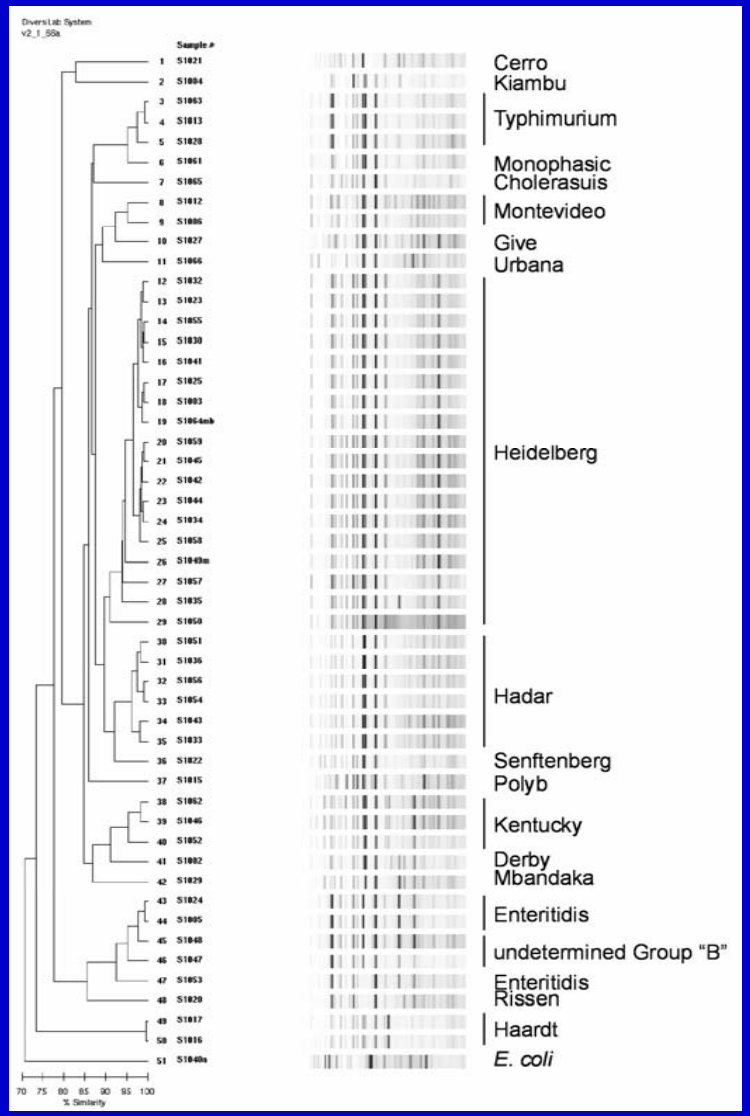
Instrumentation for rep-PCR



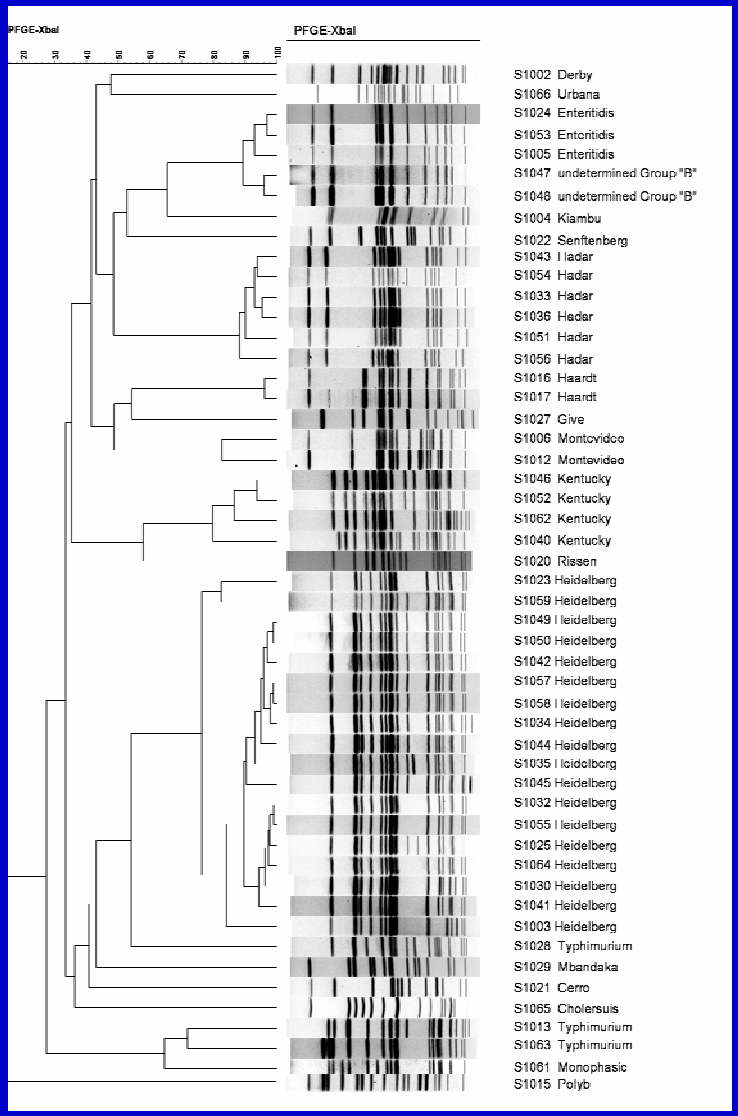


Microfluidic chip

repPCR compared to PFGE



rep-PCR (***Salmonella*** **Primers)**



PFGE (***Xba*I**)

Conclusions

The current level of rep-PCR resolution successfully predicts serotypes within the limited set of *Salmonella* isolates sub-typed, but does not resolve to the extent of PFGE.

Semi-automated rep-PCR is a highly standardized sub-typing method with few hands-on manipulations. For example, no preparation of digestion buffers or enzymes, no camera-based image acquisition.

The rep-PCR process offers checkpoints to assess typing and rep-PCR reproducibility is high across users and labs.

Data acquisition and data mining is web-accessible and user-friendly.

We have begun discussions with Bacterial Barcodes, Inc. scientists to collaborate on developing new generation primers for *Salmonella* to achieve higher levels of resolution at the sub-serotype level.

Ongoing food safety research projects at PMSRU

- Characterization of bacteria
- On-farm intervention strategies
- Alternatives to antibiotic control
- Improving bacterial culture methods
- Reducing pathogen numbers in litter

Slide courtesy of Dr. G. Siragusa

Bacteriophage or viruses of bacteria can also be potentially utilized for control

Poultry Microbiological Safety Research Unit, RRC, ARS, USDA in Athens, GA

Research to protect the public health during poultry production