Identification of Isolates of Salmonella From Store-Bought Chicken

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Abstract

An attempt to develop a positive control for PCR identification analysis of *Salmonella* Enterica serovars was carried out. Three primer sets representing marker genes *InvA*, *SefA*, and *FimA* were used in three separate cycles of PCR analysis. While *InvA* was indeterminant,*SefA* and *FimA* demonstrated potential utility for future analysis on store-bought chickens.

Introduction

There are more than 2000 different types of *Salmonella enterica*. The two major serological varieties (serovars) that are most common among humans are *Salmonella* Typhimurium and *Salmonella* Enteritidis. For this research project, we used clones of *Salmonella enterica* isolated from store-bought chicken. In order to identify which serovar of *Salmonella enterica* has colonized these commercially grown chickens, the following specific aims were carried out: 1.) 19 clones periodically isolated from store-bought chicken from 2017-2019 will be cultured and DNA will be extracted. 2.) PCR of three genes was performed to determine which serovar of *Salmonella enterica* had been isolated. The three genes that we worked with are: *InvA* for *Salmonella enterica, FimA for Salmonella* Typhimurium, and *SefA for Salmonella* Enteriditis.

Methods and Materials

Cultures and Maintenance:19 clones isolated from 2017-2019 from regional grocers in the greater Kansas City region are maintained at -80 degrees Celsius. These isolated clones will be cultured and maintained on TSA plates at room temperature. Thus far, only one culture has been tested. **DNA:**Cells were cultured in TSB and the total DNA was extracted using the Promega Wizard Genomic Isolation kit.**PCR:**PCR was then performed on extracted DNA from the 1 wildtype strain of *S*. Enteriditis, using the 3 primer sets.

Results

Analysis: PCR results help facilitate serovar identification without costly serotyping kits.

- Figures 1, 2, and 3 represent results of three cycles using primers for the marker genes *InvA, FimA*, and *SefA*
- Each of these were done using a stock culture of *Salmonella* Enteriditis in an attempt to gain a positive control for future assays
- The ladder that was used : 100 bp DNA ladder
- *Negative controls represent extracted DNA without primers.*
- Additional lanes consisted of extracted template DNA with no additives, other than sample dye.

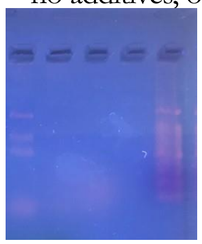
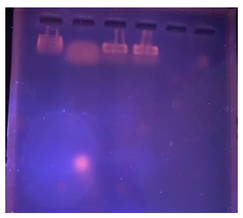


Figure 1: shows a gel with the PCR run with *InvA* primers. this picture goes from right to left, Well 1 is the ladder, well 2: Negative Control, Well 3 Template DNA, Well 4: DNA INVA , Well 5: ladder

Figure 2: shows a gel with the PCR run with *SefA* primers. Well 1 is the ladder (blurred), well 2: Negative Control, Well 3: Template DNA, Well 4: DNA SefA , Well 5: template DNA



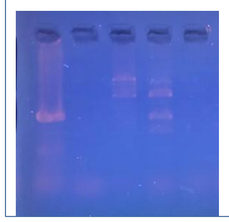


Figure 3: Shows a gel with PCR run with FimA Primers. (right to left) . Well 1 is Negative control, well 2: Ladder, Well 3: Ladder), Well 4: DNA FimA , Well 5: Ladder

Conclusions and Future Directions

- Primers for *FimA and SefA* appear to amplify (or not in the case of *FimA*) their genes in extracted *Salmonella* Enteriditis DNA.
- *InvA* did not demonstrate that the clone was *Salmonella.*
- *InvA* will be re-run using both *Salmonella* Enteriditis and *Salmonella* Typhimurium because it should have remained positive
- Error on the part of the experimenter led to 100 bp ladder being loaded multiple times

In the spring of 2020, I plan on running more PCR analysis on the 19 clones that were isolated from 2017-2019, using each of the primers above and *Salmonella* Enteriditis as a positive control for my PCR.

Selected References

A. A. K. Jawad, A. H. Al-Hamadani And H. A. M. Al-Karawy. 2010. "Detection of FimC Gene as Fingerprinting for *Salmonella* Typhimurium Isolates by Using Polymerase Chain Reaction." *Al- Qadisiyah Journal of Veterinary Medicine Sciences*, vol. 9, no. 3, p. 97.,

Groisman, Eduardo A., and Howard Ochman. 1997 "How *Salmonella* Became a Pathogen." *Trends in Microbiology*, vol. 5, no. 9, Sept., pp. 343–349.,

Parsek, Soumet, C., et al. 1999 "Identification by a Multiplex PCR-Based Assay of *Salmonella* Typhimurium and Salmonella Enteritidis Strains from Environmental Swabs of Poultry Houses." *Letters in Applied Microbiology*, vol. 29, no. 1,, pp. 1–6

Sunar, N. M. et al.2010."Molecular techniques to characterize the invA genes of *Salmonell*a spp. for pathogen inactivation study in composting." *University of Tun Hussein Onn Engineering Department.*