

PORK SAFETY

Title: Sequencing of 10 potential molecular epidemiological targets from a collection of food safety relevant *Salmonella* spp. - NPB #05-063

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Abstract:

Salmonella research is a top priority for the industry, especially related to pre-harvest reduction of pathogens with potential public health significance. The pre-harvest food safety research for *Salmonella* described here fell under the category of control strategy and responded to the directive from the NPB “Development and evaluation of evolving molecular, and other, diagnostic tools and monitoring techniques that can be used in epidemiological investigations”. The development and testing of molecular epidemiological tools for pre-harvest food safety applications continues to be an important and rapidly evolving issue. It is becoming vital to track contamination to its source during production, transport, and lairage. The ability to reliably distinguish between various isolates of the same serovar of *Salmonella* spp. will provide a valuable tool to the pork industry that would aid in tracking and identifying original sources of contamination. However, the development of reliable epidemiological methods for source tracking of *Salmonella* spp. represents a significant and real problem. One species of *Salmonella* (*Salmonella enterica*) has literally hundreds of very closely related subspecies or serotypes that must be distinguishable by any molecular method used. Many of the current attempts at the development of molecular epidemiological tools for *Salmonella* spp. are based upon methods that are applicable to other microorganisms. Because of the close genetic similarity of *Salmonella* spp. serotypes and isolates, most of these methods ultimately fail to provide reproducible results when used for *Salmonella* spp.

The current project will make available to food safety scientists a powerful comparative database that can be used to develop epidemiological methods that are specific to *Salmonella* spp. rather than attempting to adapt methods that have been used with other organisms. *Salmonella* spp. because of how closely related the serovars tend to be presents a significant challenge to the development and applicability of molecular epidemiological methods. Using the genome of *Salmonella* Typhimurium LT2 we have successfully aligned the entire genomes of 10 *Salmonella* spp. strains/serotypes. This data has been made available on the internet <http://liru.ars.usda.gov/salmonella2>. This research represented the first whole genome multiple alignment system available over the internet as well as one of the first base by base comparison of multiple bacterial genomes. During this National Pork Board funded research project we identified many regions of these genomes that show potential to serve as molecular epidemiology targets. It is this type of target region that

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taken individually or in groups tend to have the strongest potential for use as powerful and sensitive molecular epidemiology source tracking tools. Based upon those comparative genome analyses we have identified 10 regions that putatively could allow for molecular typing but also have potential to be useful for very isolate specific source tracking. In order to evaluate their usefulness as epidemiology markers we sequenced these 10 hypervariable regions (molecular epidemiological target regions METR) of the *Salmonella* genome from a collection of 50 different *Salmonella* serovars. Following the collection of the genetic information we established prototype databases which allowed for comparative analysis and query of unknown sequences. Finally, we obtained 6 “test isolates” of known serotype and sequenced the METR of these to determine if we could correctly identify their serotypes using the prototype databases. Although a statistical model and additional database tools must be developed to allow for automated analyses of results, in each case the 6 test serotypes were correctly identified by comparison to the METR databases. These results indicated the potential for the successful establishment of a molecular typing method which can be used for *Salmonella* spp. In its current form this typing method may not have the ability to distinguish between individual strains of a particular serotype, but the results indicate that further elucidation and extension of the METR regions and expansion of the collection of serotypes and strains contained within the database do have the potential for highly sensitive differentiation of serotype strains. This pilot project has established a METR prototype sequence databases for putative molecular typing of *Salmonella* spp at the serovar level.