Identification of Swine *Salmonella* serotypes Using Pulsed-field Gel Electrophoresis of Conserved *Xba1* Fragments

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Abstract

Swine *Salmonella* isolates (n=674) from various locations throughout the United States and Canada were analyzed via pulsed-field gel electrophoresis (PFGE) with *Xba1*. PFGE subtypes were analyzed by cluster analysis and compared to conventional serotyping results. The analysis showed a correlation of serotype to PFGE subtype. In addition, conserved fragments were identified within the restriction patterns that were unique to each serotype. PFGE using *Xba1* restriction provides a screening method for identifying swine *Salmonella* serotypes.

Introduction

Identifying the serotype of a *Salmonella* isolate in combination with molecular subtyping by pulsed-field gel electrophoresis (PFGE) has proven helpful in determining the relatedness of individual cases, indicating an outbreak and its possible source. PFGE has been used to identify and characterize strains within serotypes and has become an important tool in epidemiology. The Center for Disease Control and Prevention (CDC) has developed standardized methods and a reporting system called PulseNet for several major food borne pathogens. The result, of having standard methods, has been uniform results among different laboratories and a large database of PFGE results. Liebana, et al. found that five different serotypes of *Salmonella* could be separated with PFGE. This study was developed to determine if conserved DNA fragments of *Salmonella* were able to aid in determining the serotype of an unknown isolate.

Materials and Methods

DNA, from each isolate, was extracted, embedded into an agarose plug, and cut with the restriction enzyme, *Xba1*. The fragments were separated on a 1% agarose gel in 0.5X TBE buffer on a CHEF 3 system (BioRad). The settings were 6V/cm, included angle 1200 initial switch time 2.2 sec, final switch time 64 sec, and 21 hours run time.

The gel was stained with ethidium bromide and recorded with a Gel Doc system. The gel was recorded into BioNumerics and PFGE subtypes were determined. The subtypes were analyzed by cluster analysis and compared to conventional serotyping results.

Results and Discussion

The 674 isolates, representing 12 *Salmonella* serotypes, were separated into 66 different *Xba1* PFGE subtypes. When the 66 different subtypes were analyzed by cluster analysis, the subtypes were separated into groups of identical serotypes based on their PFGE bands. The groups of individual serotypes were separated from other serotypes at a 70% similarity level (Figure 1), with 56% similarity over all isolates. The similarity within each serotype grouping was associated with fragments conserved within the *Xba1* patterns associated with each serotype.

Examples of these conserved fragments are illustrated in Figure 2 using the serotype Typhimurium. These results indicated that when unable to serotype by conventional methods, PFGE would be a possible alternative in serotype determination. Our results indicated that PFGE characterization would be useful in determining the serotype of an isolate of *Salmonella* based on bands conserved within the serotypes' *Xba1* PFGE subtypes when analyzed by BioNumerics software and compared to a large database.

Such PFGE characterization might be especially useful when an isolate cannot be serotyped by conventional methods, or when a laboratory does not have access to standard serotyping.

PFGE using *Xba1* restriction provides a screening method for assisting in the determination of swine *Salmonella* serotypes.

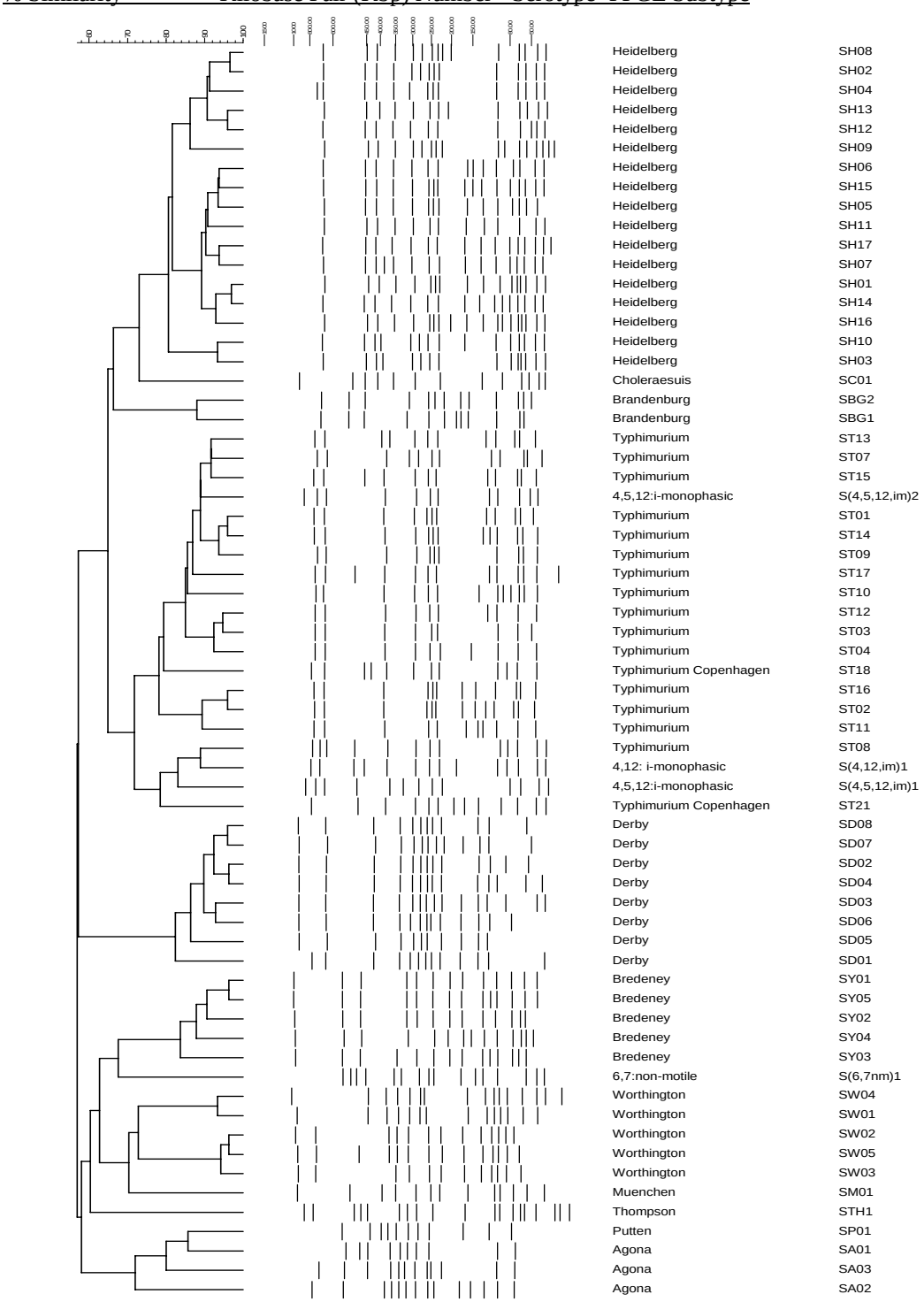

Figure 1. Cluster analysis of the pulsed-field gel electrophoresis *Xba1* fragment pattern of *Salmonella* serotypes, showing clustering by serotype (Dice Similarity Coefficient, band tolerance 0.8%).