Pulse-field-gel-electrophoresis development for *Salmonella* species using PaeR7 1 enzyme

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Abstract

Pulse field gel electrophoresis (PFGE) currently has been preformed on 22 *Salmonella* spp. isolates using a new method that distinguishes between different species of *Salmonella* and gives reproducible results using Pae R7 1 enzyme. This PFGE data, when using the Pae R7 1 enzyme, was compared to the Xba 1 restriction enzyme that is used by CDC (Center for Disease Control) for *Salmonella* spp. isolate comparisons. PFGE results were analyzed using cluster analysis and results were comparable between Pae R7 1 and Xba 1 enzymes for distinguishing differences.

Introduction

*Salmonellae* is a major cause of food-borne illness in the United States (1). Pulse-field gel electrophoresis (PFGE) is one of several approaches available for determining relationship between isolates (2). For our purpose, PFGE represents an opportunity to evaluate the relationship between *Salmonella* isolates associated with sample collected at the farm and at the packing plant. The pattern associated with PFGE is enzyme and isolate unique and should be useful in discriminating isolates. Several enzymes are available and the goal of this project was to evaluate the discrimination ability of Pae R7 1 compared to the Xba 1 restriction enzyme. The null hypothesis was that a cluster of isolates identified by Xba 1 would be identified as a cluster using Pae R7 1.

Materials and methods

*Salmonella* isolates were obtained from rectal swabs, lymph tissue, skin, gut and head meat swabs from swine slaughter at a commercial plant. Isolates identified as *Salmonella* were then grown overnight on blood agar plates then resuspended in cell suspension buffer (100 mM Tris-HCL, 100 mM EDTA, pH8.0) to obtain an absorbance reading of 1.2 to 1.4. 300 ul of suspension was added to 15 ul of proteinase K (20 mg/ml) and 300 ul of 1.2% SeaKem agarose (FMC Bioproducts), mixed, and then allowed to solidify in a 100-µl plug mold (Bio-Rad Laboratories). Plugs were incubated for 2 hours at 55°C in 5 ml of Cell lysis buffer (50 mM Tris-HCL [pH 8.0], 50 mM EDTA [pH 8.0], 1.0% SDS, and 25 ul proteinase K [20 mg/ml]), washed twice for 15 min with ddH2O followed by four 15 minute washes with TE buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA). A 2 mm slice of each plug was incubated in 200 µl of restriction buffer (Buffer 4, BSA and PaeR7 1) at 37°C for 2 to 4 h. The DNA fragments were separated on 1.0 % agarose (FMC Bioproducts). Electrophoresis was performed on CHEF Mapper XA system (Bio-Rad) for 32 h at 14°C at 6V/cm in 0.5xTBE buffer with varying pulse times (begin - 0.47s, end - 10.0s). MidRange1 PFG marker (NEB) was used as DNA size marker. The DNA restriction patterns were compared using BioNumerics software (Applied Math).

For Xba 1 enzyme the procedure was provided by Centers for Disease Control.

Results

22 isolates were available for comparison. The number of bands produced by the Pae R7 1 was greater than the Xba 1 enzyme. On average, 20 - 25 bands are identified with Pae R7 1 and 10 -12 with Xba 1. Results for seven similar isolates using Pae R7 1 enzyme showed 90% correlation by
BioNumerics software analysis whereas Xba 1 enzyme showed 88% correlation between these same isolates. Another group of 4 isolates showed 96% similarity when using the Pae R7 1 enzyme and 82% similarity between these isolates when using the Xba 1 enzyme. A third group of 3 isolates showed a 76% correlation between isolates for Pae R7 1 and a 73% correlation between isolates when using Xba 1. When individually comparing isolates to each other for each enzyme and taking into account that similar isolates had been ran on different gels then both enzymes were able to group the 22 isolates into the same 11 groupings. However, the relationships between isolates, of different banding patterns, are not always the same when comparing the 22 patterns generated by Pae R7 1 and Xba 1.

Discussion

In studies directed at looking for clusters of organisms the use of Pae R7 1 may be comparable to Xba 1. However, epidemiological studies examining association between isolates using Xba 1 or Pae R7 1 would likely reach different conclusions due to differences between cluster associations identified by the enzymes. A draw back of the Pae R7 1 enzyme is the increased processing time required. Pae R7 1 analysis of 11 isolates required 32 hours and used shorter pulse times (0.47 seconds to 10 seconds) compared to just 18 - 20 hours and longer pulse times (.47 seconds to 63 seconds) for Xba 1.

Conclusion

Initial use of the Pae R7 1 enzyme gives comparable results to Xba 1 enzyme. This procedure has been comparable at distinguishing Salmonella isolates for the purpose of strain relatedness. When comparing banding patterns of individual isolates to each other both enzymes identify the same 11 groups. When using the BioNumerics Software similar correlations between similar isolates are seen between the two enzymes. Initial results may show more variation between runs due to laboratory conditions and experience of technicians. However, continued comparisons of these two procedures are still taking place and final analysis of results is still to be determined.

References
