The Salmonellae are ubiquitous, gram-negative, enteric bacteria whose pathogenicity is seen in food-borne salmonellosis and in typhoid and paratyphoid fevers. The genus Salmonella includes more than 2,300 serotypes, of which only about 15-20 are clinically relevant, making this one of the more difficult microbes to identify. The traditional typing method for Salmonella, serotyping, offers a precise method for differentiating isolated strains. However, this method is time consuming and expensive, often requiring the use of over 150 specific antisera. Therefore, serotyping is not available to all laboratories which creates the demand for a more universally available subtyping and subsertype typing method. Molecular methods, such as repetitive sequence-based PCR (rep-PCR), have been shown to provide a viable alternative method to this and other expensive traditional methods. A semi-automated method utilizing a microfluidics platform has recently been developed for rep-PCR applications, reducing the turn-around time and labor costs while increasing throughput. This experiment demonstrates the concordance of the semi-automated method versus the manual method of rep-PCR using samples previously characterized by serotyping.

All 24 samples were successfully fingerprinted by the manual gel-based protocol (Figure 2a) and the semi-automated microfluidics protocol (Figure 3a). Cluster analysis with UPGMA of the rep-PCR fingerprints with both the BioNumerics basic package (Figure 2b) and DiversiLab System software (Figure 3b) separated each serotype into well-defined clusters. The monolatradic groups found with both the gel and microfluidics methods showed 100% concordance with those found by serotyping. Furthermore, the DiversiLab System reduced the time to answer to less than 4 hours.

Comparison of the manual gel-based rep-PCR and the automated DiversiLab System to differentiate Salmonella serotypes

K. Reece, R. Webb, J. Huong, T. Bittner, J. Manry, J. Bassett, and M. Healy

Bacterial BarCodes, Inc., 8000 North Stadium Drive, Suite 1200, Houston, TX 77054, USA, (866-473-7727), www.bacterialbarcodes.com

BACKGROUND

The Salmonellae are ubiquitous, gram-negative, enteric bacteria whose pathogenicity is seen in food-borne salmonellosis and in typhoid and paratyphoid fevers. The genus Salmonella includes more than 2,300 serotypes, of which only about 15-20 are clinically relevant, making this one of the more difficult microbes to identify. The traditional typing method for Salmonella, serotyping, offers a precise method for differentiating isolated strains. However, this method is time consuming and expensive, often requiring the use of over 150 specific antisera. Therefore, serotyping is not available to all laboratories which creates the demand for a more universally available subtyping and subsertype typing method. Molecular methods, such as repetitive sequence-based PCR (rep-PCR), have been shown to provide a viable alternative method to this and other expensive traditional methods. A semi-automated method utilizing a microfluidics platform has recently been developed for rep-PCR applications, reducing the turn-around time and labor costs while increasing throughput. This experiment demonstrates the concordance of the semi-automated method versus the manual method of rep-PCR using samples previously characterized by serotyping.

METHOD

Twenty-four isolates were obtained from a well-characterized collection that included 6 different serotypes. The samples were cultured and the DNA was extracted using the UltraClean® Microbial DNA Kit (Mo Bio Laboratories, Inc., CA). Amplification was performed using the DiversiLab System Bacterial Fingerprinting Kit (Bacterial BarCodes, Inc., TX). Amplicons were separated both by gel electrophoresis and by the Caliper 1000 Analyzer (Caliper Technologies, CA). Gel electrophoresis was performed with a 1.5% agarose gel containing 3 µg/ml ethidium bromide in TAE buffer containing 1 mg/ml ethidium bromide at 30V for 3-4 hours. The Caliper 1000 utilizes a DNA microfluidics chip as seen in Figure 1 and takes approximately 45 minutes for setup and sample separation. Data analysis was performed using BioNumerics software (Applied Maths, Belgium) for gel data and DiversiLab System software (Bacterial BarCodes, Inc.) for microfluidics data.

RESULTS

This data, and other published data, demonstrate that rep-PCR is an accurate and reproducible method for Salmonella serotyping. Rep-PCR fingerprinting can be successfully performed via a semi-automated, high-throughput system, the DiversiLab System. The DiversiLab System significantly reduces the time to answer when compared to gel-based fingerprinting and traditional serotyping methods. Because the DiversiLab System is easy to use, accurate, and reproducible, it allows for high-throughput capability, which is an invaluable necessity in today’s microbiology laboratories. The DiversiLab System can be a powerful tool for discriminating and identifying Salmonella serotypes.

REFERENCES