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Characterization of *Salmonella* Serovars by PCR–Single-Strand Conformation Polymorphism Analysis

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PCR-restriction fragment length polymorphism (PCR-RFLP) and PCR–single-strand conformation polymorphism (PCR-SSCP) analyses were carried out on the 1.6-kb *groEL* gene from 41 strains of 10 different *Salmonella* serovars. Three *Hae*III RFLP profiles were recognized, but no discrimination between the serovars could be achieved by this technique. However, PCR-SSCP analysis of the *groEL* genes of various *Salmonella* serovars produced 14 SSCP profiles, indicating the potential of this technique to differentiate different *Salmonella* serovars (interserovar differentiation). Moreover, PCR-SSCP could differentiate strains within a subset of serovars (intraserovar discrimination), as three SSCP profiles were produced for the 11 *Salmonella enterica* serovar Enteritidis strains, and two SSCP profiles were generated for the 7 *S. enterica* serovar Infantis and five *S. enterica* serovar Newport strains. PCR-SSCP has the potential to complement classical typing methods such as serotyping and phage typing for the typing of *Salmonella* serovars due to its rapidity, simplicity, and typeability.

Salmonellae are the etiologic agents of different diseases collectively referred to as salmonellosis. Human salmonellosis can be divided into four syndromes: enteric fever (typhoid-like disease), gastroenteritis (food poisoning), bacteremia with or without gastroenteritis, and the asymptomatic carrier state.

On a global scale, the incidence of typhoid fever is decreasing, while that of nontyphoidal salmonellosis is increasing, although both remain major health problems. The World Health Organization has estimated that annually there are close to 17 million cases of typhoid fever, with nearly 600,000 deaths, and 1.3 billion cases of acute gastroenteritis or diarrhea due to nontyphoidal salmonellosis, with 3 million deaths (8, 23, 26).

To curb both typhoidal and nontyphoidal salmonellae, laboratory-based surveillance of human and animal infections is a necessary first step of any prevention strategy. Phenotypic methods play an important role in the identification to the genus level. Serotyping is used for primary typing of strains, while phage typing and antibiogram are used for subdivision of serotypes (33). However, serotyping of *Salmonella* is laborious due to the large number of recognized serotypes, i.e., over 2,400 (1, 27).

In addition, a number of molecular typing methods have also been used to try to improve the identification of salmonellae and also to differentiate strains below the level of serotypes. These DNA-related techniques include ribotyping (3), pulsed-field gel electrophoresis (PFGE) (18, 20, 32), IS*200* fingerprinting (4), PCR-ribotyping (12), ribosomal DNA intergenic spacer amplification and heteroduplex analysis (9), amplified fragment length polymorphism (1, 21), automated 5′ nuclease PCR assay (7), and random amplified polymorphic DNA analysis (30).

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In recent times, various molecular techniques that detect base sequence changes in bacteria have been used as tools in epidemiological typing. One of the most widely used techniques for the identification of point mutations, due to its simplicity, sensitivity, and rapidity, is PCR–single-strand conformation polymorphism (PCR-SSCP) (6, 22). SSCP was first designed to detect mutations in oncogenes and allelic variations in the human genome (22). Since then, this technique has played a role in bacterial typing (35) and in *Salmonella* studies (11, 34). Briefly, amplified double-stranded DNA is denatured to single-stranded DNA and subjected to nondenaturing polyacrylamide gel electrophoresis. The mobility of the single-stranded DNA in the gel is dependent not only on its length but also on its secondary structure, as determined by nucleotide sequence (6).

Here, we investigate the possibility of using PCR-SSCP to differentiate *Salmonella* strains both at the serovar level (interserovar) and at the intraserovar level, using nucleotide variation in the *groEL* gene, which encodes a heat shock protein (GroEL) that is a member of the stress response protein (HSP60) family (36).

MATERIALS AND METHODS

Bacterial strains. Forty-one epidemiologically unrelated strains from 10 different *Salmonella* serovars were studied (Table 1). These strains were kindly provided by Andre Burnens from the Swiss National Reference Laboratory for Foodborne Diseases, University of Berne, Berne, Switzerland. Five of the serovars (*Salmonella enterica* serovar Typhimurium, *S. enterica* serovar Newport, *S. enterica* serovar Hadar, *S. enterica* serovar Infantis, and *S. enterica* serovar Virchow) were the most common serovars isolated in Switzerland at the time of the study. The other five serovars (*S. enterica* serovar Enteritidis, *S. enterica* serovar Typhi, *S. enterica* serovar Arizona, *S. enterica* serovar Paratyphi A, and *S. enterica* serovar Paratyphi B) consisted of strains that belonged to the reference collection. All of the strains had been identified, biochemically tested, and serotyped at the institution from which they were obtained.

The bacteria were maintained on Luria-Bertani (LB) agar plates. Repeated subculturing of isolates was avoided, and for long term maintenance, all the isolates were kept in LB broth with 20% glycerol at −70°C (2).