Chapter 1: INTRODUCTION

Pathogenic bacteria, such as *Salmonella* species often cause serious food poisoning outbreaks as well as sporadic diseases. In *Salmonella* spp., *S.* Typhi, *S.* Typhimurium and *S.* Enteritidis are three of the major serotypes that cause human infection. The latter two serovars are also common species that infect animals (Boonmar *et al.,* 1998 and Tsen *et al.,* 2002). *Salmonella* strains were isolated from patients of food poisoning cases, and animals and their products (Hau *et al.,* 2002). The ability to distinguish isolates of *Salmonella* is very important to trace the contamination source when a food poisoning outbreak occurs.

The methods that have been used for deciphering the relatedness among the isolates are divided into phenotypic and genotypic approaches. Phenotypic methods that have been used to subtype *Salmonella* species include biotyping (Maslow *et al.,* 1993), phage typing (Anderson *et al.,* 1977 and Ward *et al.,* 1987), serotyping (Kuffman, 1972), antibiogram typing (Horrevorts *et al.,* 1995) and bacteriocin typing (Towner and Cockayne, 1993).

Genotypic methods include plasmid profiling (Tenovar, 1985), Ribotyping (Grimont *et al.,* 1986), restriction fragment length polymorphism (RFLP) (Sambrook *et al.,* 1989), pulsed field gel electrophoresis (PFGE) (Schwartz *et al.,* 1984) and polymerase chain reaction (PCR) based methods. Among these, PCR-based typing methods are popular because of the ease of operation. PCR-based methods include repetitive extragenic palindromic (Rep-PCR) (Versalvoic *et al.,* 1991), arbitrarily primed polymerase chain reaction (AP-PCR) (Welsh and McClelland, 1990; Williams *et al*., 1990), insertion

sequence (IS200) (Millemann, 1995 and Baguar, 1993) and enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) (Hulton *et al.,* 1991; Sharples and Lloyd, 1990).

Due to the importance of *S.* Typhimurium in salmonellosis and food poisoning cases, subtyping of this *Salmonella* serovar has been carried out in many laboratories. For the subtyping of *Salmonella* Typhimurium phenotypic and genotypic methods using such as, phage typing, plasmid profiling (Wilshaw,1980; Holmberg, 1984; Nakamura, 1986 and Wray, 1998), ribotyping (Millemann, 1995 and Guerra, 1997), IS200 typing (Millemann, 1995 and Baguar, 1993), PCR-ribotyping (Nastasi, 1995), RAPD (Hilton, 1998) and PFGE (Schwarz, 1994 and Liu, 1995) have been reported.

In this study, a new PCR-based method known as resAP-PCR was performed for 49 *S.* Typhimurium isolates collected from sporadic cases during the years 1969 to 2006 in Malaysia.

AP-PCR is a variation of the PCR technique employing as single short primer that is not targeted to amplify any specific bacterial DNA sequence. Rather, at low annealing temperatures, the primer will hybridize at multiple random chromosomal locations and initiate DNA synthesis. If one copy of the primer binds to one strand of DNA, and another copy of the primer binds on the opposite strand of DNA but in proximity of the first primer, a DNA fragment will be synthesized and amplification of that fragment will occur. The resulting PCR products will represent a variety of different-sized DNA fragments that are visualized by agarose gel electrophoresis (Welsh and McClelland, 1990).

AP-PCR has been applied by many researches to investigate the evolutionary and *epidemiological relationships of several Salmonella serotypes* (Fadl *et al.* 1995).

In spite of its attractive efficiency, AP-PCR typing suffers from some problems such as less discriminatory power (Burr *et al*. 1997), less reproducibility and lack of consensus rules for interpretation of pattern differences (Struelens, 1998).

In hence, in this study a modified AP-PCR method was used. In this method, the genomic DNA was restricted with selected restriction enzyme, followed PCR to amplify restricted DNA fragments. This modified method is known as restricted AP-PCR (resAP-PCR).

The aim of this study was to determine the genotypic diversity of *Salmonella* Typhimurium strains by using the restricted AP-PCR approach and to evaluate its usefulness as a subtyping tool.