I. INTRODUCTION

Streptococcus pyogenes (Group A β-hemolytic streptococcus, GAS) is one of the most common human pathogens and is responsible for many types of infections. Infections with GAS include pharyngitis, impetigo, scarlet fever, erysipelas, pneumonia, septic arthritis, toxic shock-like syndrome, and septicemia (Bisno, 1991). Some of these patients will develop acute glomerulonephritis or acute rheumatic fever if antibiotics are not appropriately administered (Fischetti, 1989).

Because the incidence of serious GAS infections steadily declined in developed countries after the 1940's (Bisno, 1991; Massel *et al.*, 1988), such infections and their sequelae were no longer considered a major public health problem. However, during the second half of the 1980s the incidence of rheumatic fever (RF) and invasive GAS infection increased (Veasy *et al.*, 1987; Steven *et al.*, 1989; Martin and Hoby, 1990). RF has been associated with overcrowding and poverty in developing countries (Land and Bisno, 1983; Markowitz, 1985; Gordis, 1985).

The important virulence factor of GAS is an M protein (Lancefield,1962; Fischetti,1989; Kehoe,1994). It is an antiphagocytic fibrillar layer on the bacterial cell surface and is a particularly relevant marker because type-specific antibody to this protein is thought to confer immunity to subsequent infection by the same strain (Lancefield, 1962). Thus, most epidemiological studies of GAS infections depend on serological typing of this protein. However, M-serological typing is associated with a higher failure rate than T-typing. In Thailand and Southeast Asia, more than 80% of GAS strains are non-typable (Pruksakorn *et al.*, 1990; Kaplan *et al.*, 1992; Jamal *et al.*, 1995). M protein genes were demonstrated but the strains were not M-typable (Pruksakorn, unpublished data, Tran *et al.*, 1993). The maintenance of a complete bank of

M-antisera is cumbersome since there are more than 100 recognized M serotypes (Musser *et al.*, 1995). Complete M-antisera banks are now limited to only a few laboratories in the world (Fox, 1974; Kaplan *et al.*, 1992). This has limited the usefulness of the M-serological typing method.

The T protein, a surface protein, has also been used in epidemiological studies of GAS (Swift *et al.*, 1943). T proteins from various strains of GAS are identified by one of several established serological assays utilizing the approximately 20 conventional anti-sera (Williams, 1958; Moody *et al.*, 1965; Rotta, 1978; Efstratiou, 1980). It is common for a single GAS strain to have more than one T-protein antigens, resulting in a pattern of T-proteins rather than a single type. This existence of T patterns has been a source of confusion, since incomplete patterns are commonly found (Johnson and Kaplan, 1993).

Characterization of GAS may also be accomplished by the detection of serum opacity factor (OF) production, which is consistently associated with specific M-protein containing streptococcal strains (Top and Wannamaker, 1968; Widdowson *et al.*, 1970), but it was less discriminative for epidemiological studies.

Other methods have been sought to replace the traditional serological M-typing. Restriction fragment length polymorphism (RFLP) has been shown to be a reproducible and discriminative method for GAS typing (Cleary *et al.*, 1988; Bingen *et al*, 1992b; Mylvaganam *et al.*, 1994; Seppälä *et al.*, 1994b). However, the major limitation of RFLP is that it is labor-intensive, and difficult to read the fragments. This limits our ability to evaluate larger number of strains. Ribotyping of GAS has not been shown to be superior to RFLP for strain differentiation (Bingen *et al.*, 1992b). Multilocus enzyme electrophoresis is an elegant tool for epidemiological mapping, although identical electromorphs might be sequentially heterogenous, particularly at the DNA level of genetic sequence, because of silent mutations (Selander *et al.*, 1986; Bert *et al.*, 1995). Random amplified polymorphic DNA (RAPD) analysis is faster, technically

easier and more economical than the older genomic typing method (Seppälä *et al.*, 1994a). However, identifying suitable primers and conditions that provide consistent, reproducible results may be difficult. The discriminatory power of the technique is also uncertain (Maslow *et al.*, 1993; Seppälä *et al.*, 1994a). The M-protein gene specific oligonucleotide probes, *emm*-specific probes, for hybridization of GAS chromosomal DNA has yielded high sensitivity and specificity for GAS typing (Kaufhold *et al.*, 1992). But a single *emm*-specific probe cannot identify all members of M type 1, as defined by conventional serotyping using polyclonal antisera (Penny *et al.*, 1995). *vir*-Typing was also investigated and found that it was useful for GAS typing (Gardiner *et al.*, 1995).

This study reports the amplification of *emm* genes of standard M-typable GAS and M-nontypable strains isolated from patients in Thailand by Polymerase Chain Reaction (PCR), and subsequent digestion of the PCR products with restriction enzymes to analyze the polymorphism of *emm* genes. The N-terminus sequences of the *emm* genes and the chromosomal RFLP between each strain were compared.