I. INTRODUCTION

Chlamydia trachomatis is an obligate intracellular bacteria. Its infection is unique in that distinct clinical manifestations are caused by different serotypes, two of which have been major world health problems. Urogenital infection, caused principally by *C. trachomatis* serotypes D - K (D, Da, E, F, G, H, I, Ia, J and k), has been probably the world' s second most commonly encountered sexually transmitted disease and possibly the leading cause of both infertility and opthalmia neonatorum. Trachoma, associated with *C. trachomatis* serotypes A, B, Ba, and C, has been a leading cause worldwide of preventable blindness, whereas serotypes L1, L2, L2a and L3 have given rise to the relatively rare condition of lymphogranuloma venereum.

Chronic or asymptomatic *C. trachomatis* infection in both men and women caused an endemic of this organism. Therefore, a highly sensitive and specific screening method for *C. trachomatis* was necessary.The gold standard method for *C. trachomatis* detection was the cell culture technique. After cultivation, *C. trachomatis* was detected by staining with iodine or fluorescein conjugated with a monoclonal antibody to demonstrate the intracytoplasmic inclusions. The technique for detecting *C. trachomatis* antigens was a direct fluorescent antibody (DFA) method and enzyme immunoassay (EIA), with a used monoclonal antibody to detect *C. trachomatis* antigens on the major outer membrane protein (MOMP). Direct detection of *C. trachomatis* nucleic acid, used as a specific probe, was also developed. All these methods could detect organisms directly from specimens. However, the detection of *C. trachomatis* still had some limitations if samples contained only a few chlamydial organisms. Moreover, the culture technique was time consuming and labour intensive. It took 3-5 days to complete the test and required access to specialized facilities.

The recently developed polymerase chain reaction (PCR) technology allowed the detection of even the smallest amounts of specific nucleic acids from clinical samples, by using the repeated amplification steps of a respective target sequence. The PCR was shown in general to be highly sensitive for the detection of *C. trachomatis* and other pathogens in clinical samples.

Differentiation of *C. trachomatis* serotypes was necessary in epidemiologic studies to establish the regional prevalence of the organism. Serotype classification was based originally on immunological reaction with monoclonal antibodies that recognized antigenic determinants located on the MOMP. The microimmunofluoresent (MICRO-IF) test was the reference method. However, the serotyping of these organisms was limited, due in particular to the commercial unavailability of the panel of antisera for typing and their poor growth in the culture system.

Developed serotyping methods for *C. trachomatis* were based on the analysis of the nucleotide variation of the MOMP gene, which was responsible for the antigenic variation of the protein. The MOMP consisted of five constant domains separated by four variable ones and carried antigenic determinants for each serotype. These methods were known as restriction fragment length polymorphism (RFLP) and nucleotide sequencing.

The PCR-RFLP was based upon the differential restriction endonuclease digestion of PCR amplified MOMP sequence and proved to correlate well with the serotypes performed by the immunological assay. However, a frequent problem was an amplified large fragment of the nucleotide sequence that usually resulted in a complex banding pattern of the digested fragments. Furthermore, the amplification of a small fragment of DNA, which encompassed only the VD4 (the largest variable domain of MOMP gene), was performed by PCR and continually classified for serotyping by using four restriction endonuclease. At least 10

characteristic patterns of 15 serotypes of *C. trachomatis* were differentiated successfully , in which the predominated serotypes : E, F and D were included. These were to be the reference RFLP patterns in this study. However, the RFLP method did not cover the nucleotide sequence polymorphism of the VD4-MOMP gene, since only nucleotide changes, in which the recognition sequence was detectable as an altered RFLP pattern, could cause the number of variants to be underestimated.

The nucleotide sequencing of the MOMP gene of *C. trachomatis* was applied to molecular epidemiology. In at least 18 serotypes, the nucleotide sequence homology of all 4 VDs was separated into 3 serotype groups: B-complex group B, Ba, D, Da, E, L1, L2 and L2a; intermediate group G and F; and C-complex group A, C, H, I, Ia, J, K and L3. Moreover, the detailed nucleotide sequence polymorphism studies of the MOMP gene of *C. trachomatis* showed the amount of MOMP sequence variation in clinical isolates and characterized possible new *C. trachomatis* variants. The sequence variation was found mostly in VD1, VD2 and VD4, and they produced a new variant that could escape immune neutralization and establish infection or persist in the patient more readily . The genetic mechanism for MOMP diversification could not only represent the point mutation, but also the genetic recombination.

The point mutation was clustered in the nucleotide substitution for closely related serotypes, and for deletion or insertion of those distantly related. The genetic recombination exhibited the nucleotide sequence of some VDs that were mostly close to one serotype, while other VDs resembled other serotypes. It depended on the co-infection of the host with multiple *C. trachomatis* serotypes, presumably in response to immune selection.

In this study, the amplification of the VD4-MOMP gene of *C. trachomatis* from clinical samples in Chiang Mai was performed by PCR and the amplified 350 bp. Fragment continued genotyping by using RFLP with 4 restriction endonuclease: *Alu*I, *Hind*III, *Dde*I and *EcoR*II. The RFLP pattern was analyzed and

compared with its own reference. Furthermore, nucleotide sequencing of the VD4-MOMP gene of *C. trachomatis* from position 928 to 1017 or 1020 was obtained from 50 clinical samples. This genomic region was amplified directly by PCR from extracted DNA and sequenced by the dye-terminator method of ABI (Perkin-Elmer, Applied Biosystems, USA). The nucleotide sequence was resolved by a 310 automated DNA sequencer (Perkin-Elmer, Applied Biosystems, USA). The sequence, combined with the VD4-MOMP gene of reference *C. trachomatis* generated from the GenBank database, was phylogenetically analyzed by using computer programme DNASIS version 2.1. *C. trachomatis* was classified into specific genotypes and the nucleotide sequence polymorphism of the VD4-MOMP gene characterized in order that it could be useful for prospective molecular epidemiologic studies to determine the antigenic variation of MOMP for rational vaccine development.

Aims of the study

1. To study the nucleotide sequence polymorphism of the VD4-MOMP gene *of C. trachomatis* detected in Chiang Mai.

2. To compared the genotypes of amplified products in the VD4-MOMP gene by using RFLP and nucleotide sequencing method.

3. To study the molecular epidemiology of *C. trachomatis* infection.