Danai Pinyoowong 2009: Molecular Characterization of *Ehrlichia* and Related Genera in Dogs and Cats from Bangkok, Thailand. Doctor of Philosophy (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Associate Professor Arinthip Thamchaipenet, Ph.D. 121 pages.

Ehrlichiosis caused by *Ehrlichia* is of veterinary importance worldwide. In Thailand, there has been little information of *Ehrlichia* available on species, drug resistance and pathogenicity at molecular level. Genus-specific primers for *Ehrlichia* and *Anaplasma* were designed and used to amplify the *16S rRNA* gene from naturally infected canine blood samples. Both homology and secondary structure analysis of *16S rRNA* sequences indicated that they were novel *E. canis* and *A. platys* strains. Phylogenetic analysis revealed that the Thai *E. canis* strain was closely related and formed a single cluster with *E. canis* from different countries. *A. platys* found in this study showed close relationship with earlier report of *A. platys* from Thailand. These specific primers were further used to amplify *16S rRNA* genes from stray cat blood samples. However, the PCR products sequencing were failed to identify *Ehrlichia* and *Anaplasma* but surprisingly revealed similarity to other groups of alphaproteobacteria including *Bartonella clarridgeiae*, *Ochrobactrum intermedium* and *Sphingomonas paucimobilis*. Phylogenetic analysis showed that these species closely clustered but different from those of *Ehrlichia* and *Anaplasma*.

In order to understand drug resistance, a gene involved in bicyclomycin resistance, *bcr*, was isolated from *E. canis*-Bangkok by PCR. The hypothetical Bcr protein was analyzed and revealed close relationship with those of drug resistance transporter Bcr/CflA subfamily from bacteria in order Rickettsiales. Topology prediction using hidden Markov model algorithms indicated that the *E. canis*-Bangkok Bcr protein was an 11-transmembrane segment protein with N-terminal out of cell and C-terminal in the cytoplasm. It contained special motifs conserved in drug transporters belonging to major facilitator superfamily (MFS). Phylogenetic analysis showed that the hypothetical Bcr proteins of *E. canis*-Bangkok was closely related to those of *Rickettsia* and were segregated from other 12-TMS proteins. Therefore, they are likely to represent a new member of MFS.

In order to understand pathogenicity, patatin (*pat*) gene from *E. canis*-Bangkok was identified by PCR. Its protein sequence revealed characteristics of the conserved domains of bacterial patatins including those of phathogenic ones. The *pat* gene was then expressed in *E. coli* BL21 (DE3) and the crude enzyme was characterized for phospholipase A activities. The results showed that *E. canis*-Bangkok patatin could hydrolyze phospholipids that shorter than 10 carbon atoms because it could hydrolyze tributylglycerol but not trioleoylglycerol. The enzyme showed high activity at pH 8.0 and at temperature 40 °C which are the condition similar to environment in infected animals. It is purposed that *E. canis*-Bangkok patatin may be involved in hydrolysis of phospholipid membrane of mononuclear cells and enhance pathogenicity by spreading *Ehrlichia* to other cells.

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