学位論文内容の要旨

Abstract of the dissertation

博士の専攻分野の名称：博士（獣医学）　　氏名：SIRIPORN　KONGSOI

Name

学位論文題名

The title of the doctoral dissertation

Studies on *Salmonella* Typhimurium DNA gyrase and the impact of *gyrA* mutations to quinolone susceptibility

（*Salmonella* Typhimurium が有する DNA ジャイレースの性状と A サブユニット遺伝子上の変異のキノロン剤感受性への影響）

Quinolones exhibit good antibacterial activity against *Salmonella* isolates and are often the choice of treatment for life-threatening salmonellosis due to multi-drug resistant strains. To assess the properties of quinolones, we performed an *in vitro* assay to study the antibacterial activities of quinolones against *S.* Typhimurium recombinant DNA gyrase. We expressed the *S.* Typhimurium GyrA and GyrB subunits in *E. coli.* GyrA and GyrB were obtained at high purity (>95%) by Ni-NTA agarose resin column chromatography as His-tagged 97-kDa and 89-kDa proteins, respectively. Both subunits were shown to reconstitute an ATP-dependent DNA supercoiling activity. IC_{50}s or CC_{25}s demonstrated that quinolones highly active against *S.* Typhimurium DNA gyrase share a fluorine atom at C-6. The relationships between MICs, IC_{50}s and CC_{25}s were assessed by estimating a linear regression between two components. MICs measured against *S.* Typhimurium NBRC 13245 correlated better with IC_{50}s (R = 0.999) than CC_{25}s (R = 0.969). These findings suggest that the quinolone-inhibited DNA supercoiling assay may be a useful screening test to identify quinolones with promising activity against *S.* Typhimurium. Furthermore, to clarify the significance of amino acid substitutions in *S.* Typhimurium GyrA to quinolone resistance, we expressed recombinant WT and five mutant gyrases in *E. coli* and characterised them *in vitro*. WT and mutant gyrases were reconstituted *in vitro* by mixing recombinant GyrA and GyrB. Correlation between the amino acid substitutions and resistance to quinolones, CIP, LVX, NAL and SIT, was assessed by the quinolone-inhibited DNA supercoiling assay. All mutant DNA gyrases showed reduced susceptibility to all quinolones when compared to WT gyrases. Double mutations exhibited the lowest quinolone susceptibility amongst all mutant gyrases. In addition, low IC_{50} revealed the effectiveness of sitafloxacin on mutant gyrases even for DNA gyrase with two simultaneous amino acid substitutions. Current results suggest that SIT having halogen atoms at R1 and R8 might be a good choice for the treatment of salmonellosis caused by CIP-resistant *S.* Typhimurium.