

鞭毛蛋白遺伝子塩基配列に基づくボレリア種の 系統分類とライム病ボレリアの Molecular Typing への応用

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Phylogenetic Analysis of *Borrelia* Species Based on Flagellin Gene Sequences and Its Application for Molecular Typing of Lyme Disease *Borreliae*

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ABSTRACT We determined almost complete flagellin gene sequences of various *Borrelia* species and aligned them together with previously published sequences. A neighbor-joining phylogenetic analysis showed that the genus *Borrelia* was divided into three major clusters: New World relapsing fever borreliae (*B. turicatae*, *B. parkeri*, and *B. hermsii*), Old World relapsing fever borreliae (*B. crocidurae*, *B. duttonii*, and *B. hispanica*), and Lyme disease borreliae (*B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*). Agents of animal spirochetosis (*B. coriaceae* and *B. anserina*) and species unknown in pathogenicity (*B. miyamotoi* and *B. lonestari*) were related to relapsing fever borreliae. Although the Lyme disease borreliae, their relative species (*B. japonica* and *B. andersonii*), and newly described genomic groups (PotiB2, VS116, DN127, Hk501, and Ya501) were closely related to each other, each taxon formed an independent branch on the phylogenetic tree. The data obtained in this study indicate that the flagellin genes are useful in *Borrelia* taxonomy. To distinguish the Lyme disease borreliae from related organisms easily, we designed an oligonucleotide primer set for the flagellin gene and performed a

PCR-restriction fragment length polymorphism (PCR-RFLP) analysis. The primer set amplified an approximately 580-bp DNA fragment that including species-specific restriction sites, and *Hap*II, *Hha* I, *Cel*II, *Hinc*II, or *Dde* I digestion of the product resulted distinctively different PCR-RFLP patterns. The PCR-RFLP typing method which we developed should facilitate rapid identification of Lyme disease borreliae and related organisms obtained from biological and clinical specimens.

抄録 鞭毛蛋白遺伝子の塩基配列比較に基づく系統解析により、ボレリア属細菌は新世界回帰熱ボレリア, 旧世界回帰熱ボレリア, ライム病ボレリアに大別され、その系統樹が既存の種の分類とよく一致することから、ボレリア属細菌内の分類には鞭毛蛋白遺伝子を用いた比較が有用であることが示唆された。そこで、ボレリア種同定の試みとして鞭毛蛋白遺伝子を標的とした PCR プライマーセットを設計し PCR-RFLP 解析を行った。解析の結果、ライム病ボレリアで種特異的な分離パターンが得られたことから、この鞭毛蛋白遺伝子による PCR-RFLP 解析が臨床材料などからのライム病ボレリア種の迅速同定へ応用可能であることが示された。

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