Antimicrobial susceptibility phenotypic patterns and genotypic analysis of anaerobes isolated from healty people

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学位論文全文に代わる要約
Extended Summary in Lieu of the Full Text of a Doctoral Thesis

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学位論文題目：　健康人から分離された嫌気性菌の抗菌薬感受性パターンと遺伝子型解析
Thesis Title　Antimicrobial susceptibility phenotypic patterns and genotypic analysis of anaerobes isolated from healthy people

学位論文要約：
Summary of Thesis

Introduction: Anaerobic bacteria, including the most frequently isolated *Bacteroides fragilis* group (BFG) strains, are the normal resident gastrointestinal microbiota members. BFG is human opportunistic pathogens that cause severe intra-abdominal infections, postoperative wounds, skin, and soft tissue infections, together with aerobic bacteria. The number of antimicrobials effective against BFG bacteria is relatively limited. Increasing antimicrobial agent resistance in BFG strains has been reported in recent years and observed worldwide, including resistance to β-lactams, tetracyclines, macrolides, clindamycin, and fluoroquinolones. In literature, BFG is in the spotlight as an important clinical pathogenic anaerobic, but not so much available research on BFG from healthy people in different geographical areas.

Objectives: This dissertation conducted a study on BFG isolated from healthy individuals in Vietnam and Japan with four specific objectives as follows:

1. To investigate the major species of *Bacteroides* spp. in the microbiota of healthy individuals in Vietnam and Japan
2. To investigate the phenotypic and genotypic patterns of antimicrobial-resistance in *Bacteroides fragilis* group isolated from healthy individuals in Vietnam and Japan
3. To establish and validate a protocol from sample collection to BFG complete genome sequencing, control and assure genome sequences quality, and register to public databases.
4. To make whole-genome sequencing of meropenem–resistant *Bacteroides* for further analysis of carbapenem-resistant mechanisms

Methods: BFG was isolated from fecal samples of healthy individuals in Vietnam and Japan. Isolated strains were identified using MALDI-TOF MS, and the minimum inhibitory concentration (MIC) of 18 antibiotics was determined using the agar dilution method. Additionally, 20 antimicrobial resistance genes were detected using standard PCR. Whole-genome sequences of chosen strains were obtained by using a hybrid approach, combining Nanopore long-read sequencing and DNBseq short-read sequencing on the same qualified high molecular weight DNA. Genome sequences were obtained deposited at the DNA Data Bank of Japan (DDBJ) according to DDBJ, annotated by using the DDBJ Fast Annotation and Submission Tool (DFAST). Resistance genes were determined by ResFinder v4.0 and AMRFinderPlus.

Results:

1. A total of 139 BFG strains belonging to 11 BFG species were isolated from the two countries. *B. fragilis* was not the predominant species in either country, but the difference in

the prevalence of *B. fragilis* between Vietnam and Japan was significant. The first, second, and third most common species were *Parabacteroides distasonis* (35.5%), *Bacteroides thetaiotaomicron* (19.7%), and *Phocaeicola* (*former Bacteroides*) *vulgatus* (17.1%) in Vietnam, and *Bacteroides ovatus* (23.8%), *P. vulgatus* (22.2%), and *P. distasonis* (15.9%) in Japan.

2. Isolations from Vietnam and Japan showed some similarities in terms of MIC_{50} values, MIC_{90} values, and percentages of resistant strains. However, isolations from Vietnam showed significantly higher resistance to piperacillin, cefmetazole, clindamycin, tetracycline, and minocycline. *ErmB*, *tet36*, *tetM*, *nim*, *catA*, and *qnrA* were not found in either country. *CepA* was more common in *B. fragilis* than in non-fragilis *Bacteroides*. In contrast, *cfiA*, *ermG*, *mefA*, *msrSA*, *tetX*, *tetX1*, *bexA*, *qnrB*, and *qnrS* were found only in non-fragilis *Bacteroides*. *ermG*, *mefA,* and *msrSA* were statistically higher prevalence in Japan compared to Vietnam. In contrast, *linA* and *qnrS* were higher prevalence in Vietnam compared to Japan.
3. A protocol for whole-genome sequencing of BFG genome from sample collection to complete genome sequences, control and assure genome sequences quality and register to public databases was established and validated. The protocol was applied to analyzing genomes of three *P. vulgatus* isolated from a healthy individual in Japan in the scope of antimicrobial resistance. The whole-genome sequencing approach showed multiple advantages, not only in determining resistance genes but also locating them for further analysis on resistance mechanism and horizontal gene transfer. This work also contributed to the public genome database complete genomes of three *P. vulgatus*, that are not yet well-established.
4. Two meropenem-resistant BFG from Vietnam and Japan were sequenced and analyzed by the protocol mentioned above this protocol. Whole-genome analysis revealed multiple determinants for the multidrug-resistant phenotype of those strains that could not be fulfilled by other investigation methods such as standard PCR. Questions still remain about the meropenem-resistance mechanism since there were not any known carbapenem-resistance genes found.

Conclusion: This study is the first report on the antimicrobial susceptibility patterns in the BFG isolated from healthy individuals in Vietnam and Japan. BFG isolated from healthy people in Vietnam and Japan carried various types of antimicrobial-resistant genes and showed diversities in phenotypic resistant patterns. The distribution of various antimicrobial agent resistance genes also differed between the two countries. The whole-genome approach has many advantages and is beneficial for multi-drug resistant BFG for genome analysis. The development of recent technology in bioinformatic supports to have a better understanding of BFG genomes but still faces challenges due to the plasticity of BFG genomes.

Considerations and suggestions for future research: Antimicrobial resistance is a big concern in BFG isolated from healthy people in both Vietnam and Japan. With that high prevalence of resistance genes, BFG is a pool that can harm the host in many ways, as discussed. The horizontal gene transfer in BFG was presented and discussed in many publications, that very interesting to investigate in our samples.