

論文要旨等報告書

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学位論文内容の要旨

Introduction

Oral bacteria represent one of the most complex bacterial communities that inhabit the human body. In healthy subjects, oral bacteria are normally present without causing any pathological condition. They are noted to be stable over given period of time, and specific in structure for each individual. However, normal oral bacterial flora can be influenced by the use of mouthwashes or administration of antibiotics. In medically compromised patients, due to immunological diseases or administration of immunosuppressive drugs, oral bacteria can be pathogenic, causing intraoral and systemic lesions and infections. For long, oral bacteria were screened and detected using conventional culture methods. Thus many uncultivable species remained undetected and uncharacterized. Recent approaches based in molecular analysis of bacterial 16S rRNA genes found that oral bacteria is highly diverse than previously thought, with more than 500 species isolated from oral cavity.

The aim of this study is to reveal followings; 1) the effects of long-term use of commercially available mouthwashes on oral bacteria of healthy subjects, 2) the transition of oral bacterial flora in patients receiving bone marrow transplantation (BMT) as an example of immunocompromised subjects. This study utilized both culture methods and molecular analysis, namely terminal restriction fragment length polymorphism (T-RFLP) and clone library analysis (CLA).

Materials and Methods

Nine healthy subjects and 7 patients were involved in this study. Healthy subjects were instructed to use Listerine[®], GUM[®] and saline solution as oral rinse following tooth brushing for a period of 3 months. Saliva samples were collected on 0, 1, 2 and 3 month. Oral bacteria were cultured onto Luria-Bertani agar plates. Numerical analysis of cultured colonies was performed using naked eye. Swab samples from sites of developing mucositis were collected from patients on -7, 7 and 14 days of BMT. Identification of species by culture analysis was performed on all samples by plating the samples onto brain heart infusion agar plate.

T-RFLP analysis was performed on all samples. Labeled PCR products were prepared using labeled universal primers targeting 16S-rDNA, later digested by endonuclease *MspI*. Lengths and sizes of digested Terminal-Restriction-Fragments (TRFs)

were determined using ABI-Prism-310 Genetic Analyzer and ABI-Peak-Scanner software respectively. Cluster analysis to detect similarity between samples of each group based on data of T-RFLP was performed using MicrobiotaProfiler software. Clone library analysis was performed on three samples from one patient (n = 90 clones/sample) using unlabelled universal primers. Nearest possible candidates were detected using online DNA Data Bank of Japan.

Results

A) Healthy subjects

1. Numerical analysis of cultured colonies showed limited effect of oral rinses on oral bacteria. In Listerine group, a sharp drop in number of colonies was noted one month from baseline, while the number of colonies returned back to baseline level after two months.
2. T-RFLP analysis showed similar patterns between samples of individual subjects over the experimental time. Major species were shown by equal size peaks, indicating stable oral bacterial flora.
3. Dendrogram showed tendency of cluster formation between samples of each group. This was more noted in the control group than Listerine® or GUM® groups.

B) BMT patients

1. Culture analysis detected 5 species in all patients' samples. α -hemolytic *Streptococcus* species were detected in samples of all patients. Five patients tested positive to *Neisseria* species in at least one sample. Less frequently detected species were *Stomatococcus*, γ -hemolytic *Streptococcus*, and coagulase-resistant *Staphylococcus*
2. T-RFLP analysis showed drastic changes in patterns of samples from individual patients. Peaks representing major bacterial species were diminished in samples collected 14 days after BMT, indicating simple oral bacteria.
3. Dendrogram lacked the appearance of clusters between samples of individual patients, indicating changes in composition of oral bacteria over the study period.
4. CLA could isolate more than 20 species from 3 samples of one patient. Major species included *Streptococcus* species, *Capnocytophaga* species, *Abiotrophia defective* and *Prevotella* species. Most of isolated species were opportunistic. *Streptococcus pneumoniae* was detected in high levels in the first sample. Less number of species was isolated from the third sample, indicating more simple oral bacteria. The results confirmed findings by T-RFLP.

Discussion and Conclusion

Our results of analysis of samples from healthy subjects show relatively limited effect of mouthwashes on healthy oral bacterial flora. Analysis of samples from patients receiving BTM showed drastic changes in the types and amount of oral bacteria over the study period. This demonstrated the influence of altered immune response and the effect of antibiotic therapy. Culture method could only identify few number of species compared to CLA. Opportunistic and pathogenic bacteria were frequently isolated by CLA. High population of *Streptococcus pneumoniae* points out the possibility of development of systemic infection from oral bacteria. Our findings stress on the importance of oral care for immunocompromised patients and close examination of oral bacteria in critical cases.

論文審査結果の要旨

Background:

Oral bacterial flora is normally present without causing any pathological condition. They are noted to be stable over given period of time and specific in structure for each individual. However, normal oral bacterial flora can be influenced by the use of mouthwashes or administration of antibiotics. In medically compromised patients, due to immunological diseases or administration of immunosuppressive drugs, oral bacteria can be pathogenic, causing intraoral and systemic lesions and infection. Although oral bacteria have been screened and detected using conventional culture methods, many uncultivable species remain undetected and uncharacterized. Recent approaches based in molecular analysis of bacterial 16S rRNA genes found that oral bacterial flora is highly diverse than previously thought, with more than 500 species isolated from oral cavity. The aim of this study is to reveal followings; 1) the effects of long-term use of commercially available mouthwashes on oral bacteria of healthy subjects, 2) the transition of oral bacterial flora in patients receiving bone marrow transplantation (BMT) as an example of medically compromised subjects. This study utilized both culture methods and molecular analysis, namely terminal restriction fragment length polymorphism (T-RFLP) and clone library analysis (CLA).

Research Findings:

Analysis of samples from healthy subjects showed relatively limited effect of mouthwashes on oral bacterial flora. Analysis of samples from patients receiving BMT showed drastic changes in the types and amount of oral bacteria over the study period. This finding demonstrated the influence of altered immune response and the effect of antibiotic therapy. Culture method could only identify small number of species compared to CLA. Opportunistic and pathogenic bacteria were frequently isolated by CLA.

Importance of findings:

This study showed the usefulness of applying molecular analysis tools for detection of oral bacteria and monitoring of oral flora. The findings also stress on the importance of oral care and monitoring of oral bacteria for immunocompromised patients.

The reviewers committee is satisfied that the thesis meets the requirements for award of Doctor of Philosophy in Dental Science.