Identification of Major Cultivable Aerobic Bacteria in the Oral Cavity of Malaysian Subjects

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Abstract: Culture dependent and culture independent methods have shown that about 600 species of bacteria inhabit the human oral cavity. While some oral microorganisms have a direct link to dental caries, periodontal disease and halitosis, opportunistic pathogens may be responsible for systemic diseases such as bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight, coronary heart disease and cerebral infarction (or stroke). This study employs bacterial 16S rDNA sequences to rapidly identify the major cultivable aerobic bacteria in the oral cavity of Malaysian subjects. The data obtained shows that the oral cavity of healthy volunteers contains a number of potentially pathogenic organisms including Streptococcus pneumoniae and Staphylococcus aureus. The need to profile and characterize these microorganisms using rapid detection methods can go a long way in developing future management strategies in clinical setting to enhance oral health in the Malaysian population.

Key Words: 16S rDNA sequences, oral cavity, pathogenic

INTRODUCTION

Culture-dependent and culture-independent methods have estimated that about 600 species of bacteria inhabit the human oral cavity. The oral microbiotas play critical roles in oral health and are directly linked to diseases such as dental caries, periodontal disease and halitosis. Streptococcal species (S. mutans, S. sobrinus), Lactobacilli, Actinomyces and occasionally Candida yeasts have been implicated in dental caries[8]. Porphyromonas, Bacteroides, Prevotella species have been associated with periodontal disease and halitosis[9,10].

The oral cavity is also inhabited by many types of lactic acid bacteria that are able to inhibit oral pathogens by producing hydrogen peroxide, bacteriocins and organic acids. Such bacteria include Lactobacillus rhamnosus GG, Lactobacillus casei, Bifidobacterium, Streptococcus oligofermentas, Streptococcus mutans, Weissella cibaria and Streptococcus salivarius[1,2,4,12].

The oral cavity may serve as a reservoir for many pathogenic bacteria. Opportunistic oral bacteria have been documented in causing systemic diseases such as bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight, coronary heart disease and cerebral infarction or stroke[11].

The development of the oral community involves competition as well as synergy among these bacteria. The bacterial populations in the human oral cavity are in a dynamic state of change. The need to profile and characterize these microorganisms using an appropriate rapid identification method can go a long way in enhancing oral health management in the Malaysian population. The aim of this study was to identify the major cultivable aerobic microbial population of the oral cavity by using 16S rRNA gene sequencing analysis.

MATERIALS AND METHODS

Sampling sites for bacteria were the dorsum of the tongue, teeth surface and gingival crevice of healthy subjects. Bacteria were collected using either the Gracey curette (teeth surface and gingival crevice) or cotton swab (tongue). Swabbed samples were suspended in Reduced Transport Fluid (RTF) and
Table 1 shows the highly abundant bacteria from each sampling site. In this study, S. mitis, S. pneumoniae, S. oralis, S. australis, S. infantis, S. sanguinis, S. pseudopneumoniae, A. naeslundii, A. viscosus, N. subflava, N. mucosaa, Capnocytophaga granulosa, Pseudomonas aeruginosa, Staphylococcus aureus, Rothia mucilaginosa, Kingella oralis, Lautropia sp. have been detected as the major species in the oral cavity using 16S rDNA gene sequencing analysis. Typical 16S sequences obtained are shown in Fig. 1a-c for C. granulose, N. mucosa and S. australis respectively.

Table 1: Major Bacterial Species Identified

| Gingival crevice | Teeth surface | Tongue surface |
|------------------|---------------|----------------|
| Streptococcus    | Streptococcus | Streptococcus  |
| Pneumoniae       | Pneumoniae    | Pneumoniae     |
| Streptococcus    | Streptococcus | Streptococcus  |
| Mitis            | sanguinis     | mitis          |
| Streptococcus    | Actinomyces   | Actinomyces    |
| Oralis           | viscosus      | viscosus       |
| Actinomyces      | Actinomyces   | Actinomyces    |
| Neisseriadii     | naeslundii    | pseudopneumonia|
| Subflava         | Lautropia sp. | Lautropia sp.  |
| Capnocytophaga   | Kingella oralis | Neisseria subflava |
| granulosa        | Neisseria mucosa | Lautropia sp.  |
| Pseudomonas      | Neisseria mucosa | Lautropia sp.  |
| Aeruginosa       | Neisseria subflava | Neisseria subflava |
| Staphylococcus   | Neisseria subflava | Neisseria subflava |
| Aureus           | Rothia mucilaginosa | Rothia mucilaginosa |

RESULTS

Table 1: Major Bacterial Species Identified

| Gingival crevice | Teeth surface | Tongue surface |
|------------------|---------------|----------------|
| Streptococcus    | Streptococcus | Streptococcus  |
| Pneumoniae       | Pneumoniae    | Pneumoniae     |
| Streptococcus    | Streptococcus | Streptococcus  |
| Mitis            | sanguinis     | mitis          |
| Streptococcus    | Actinomyces   | Actinomyces    |
| Oralis           | viscosus      | viscosus       |
| Actinomyces      | Actinomyces   | Actinomyces    |
| Neisseriadii     | naeslundii    | pseudopneumonia|
| Subflava         | Lautropia sp. | Lautropia sp.  |
| Capnocytophaga   | Kingella oralis | Neisseria subflava |
| granulosa        | Neisseria mucosa | Lautropia sp.  |
| Pseudomonas      | Neisseria mucosa | Lautropia sp.  |
| Aeruginosa       | Neisseria subflava | Neisseria subflava |
| Staphylococcus   | Neisseria subflava | Neisseria subflava |
| Aureus           | Rothia mucilaginosa | Rothia mucilaginosa |

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diluted 10, 10² and 10³ times. Diluted samples were plated in duplicate on Columbia blood agar plates and incubated aerobically at 37°C for 3-5 days. Distinct colonies (2-3 colonies of apparently same morphology) were selected and streaked separately on agar plates. Single colonies obtained were characterized by gross (colour and shape) and microscopic morphology as well as by gram staining. The genomic DNA of pure cultures was prepared using i-genomic DNA extraction mini kit (iNtRON Biotechnology, Seongnam). Extracted DNA was evaluated for both quality and quantity by agarose gel (1%) electrophoresis. Universal primers for 16S rDNA (F: 5'-AGA GTT TCA TGG TCA TGG CTC AG and R: 5'-TAC GGC TAC CTT GGG TAC ACT GT) were used for PCR amplification. PCR conditions were: initial denaturation at 94°C for 5 min., denaturation at 94°C for 1 min., annealing at 52°C for 1 min., extension at 72°C for 1.5 min and final extension at 72°C for 10 min. PCR reaction mixtures (20µL total) contained 2µL of 10x PCR buffer, 2µL of dNTP mix (2.5mM each), 1 µL of each primer (10pmoles), 50ng of DNA template, 0.5µL of i-TaqTM DNA polymerase 5U/µL, iNtRON Biotechnology). The resulting PCR product was examined by electrophoresis on a 1.5% agarose gel and purified by PCR quickspin kit (iNtRON Biotechnology). DNA sequencing of the PCR product was done by Macrogen, Seoul. Sequences were blasted to NCBI databases using BlastN and bacterial species were identified by alignments with database 16S rDNA sequences.

Fig. 1a: 16S rRNA gene sequence of Capnocytophaga granulosa isolated from gingival crevice of subject

Fig. 1b: 16S rRNA gene sequence of Neisseria mucosa isolated from tooth surface of subject
Traditionally, oral bacteria have been studied by culture-dependent methods. Identification of the bacteria from culturing method relies on the characteristics observed in biochemical and physical properties of the known and reference strains under optimum growth condition. However, phenotypic characteristics can change under some circumstances like stress, even successful culturing does not necessarily give the correct identification. In comparison, 16S rDNA gene sequencing is a rapid and reliable method that could profile bacteria of the oral cavity including potential pathogens.

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S. pneumoniae is a major cause of infections of the respiratory tract, central nervous system and bloodstream, it also is a nosocomial pathogen. Furthermore, antibiotic resistant strains of this pathogen are common[3]. In recent years it has emerged as an important pathogen in HIV-infected patients and immunocompromised subjects[7].

CONCLUSION

The identification of oral bacteria by 16S rDNA gene sequencing is a rapid and reliable method that could profile bacteria of the oral cavity including potential pathogens.

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Fig 1c: 16S rRNA gene sequence of Streptococcus australis isolated from tongue surface of subject

Discussion

Traditionally, oral bacteria have been studied by culture-dependent methods. Identification of the bacteria from culturing method relies on the characteristics observed in biochemical and physical properties of the known and reference strains under optimum growth condition. However, phenotypic characteristics can change under some circumstances like stress, even successful culturing does not necessarily give the correct identification. In comparison, 16S rDNA gene sequencing is a rapid and reliable method that could profile bacteria of the oral cavity including potential pathogens.

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