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*, *N. naeslundii*, *N. viscosus*, *N. subflava*, *N. mucosa*, Capnocytophaga granulosa, *Pseudomonas aeruginosa*, Staphylococcus aureus, Rothia mucilaginosa, *K. oralis*, *L. 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

<table>
<thead>
<tr>
<th>Gingival crevice</th>
<th>Teeth surface</th>
<th>Tongue surface</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus</em></td>
<td><em>Streptococcus</em></td>
<td><em>Streptococcus</em></td>
</tr>
<tr>
<td><em>Pneumoniae</em></td>
<td><em>oralis</em></td>
<td><em>pneumoniae</em></td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td><em>sanguinis</em></td>
<td><em>mitis</em></td>
</tr>
<tr>
<td><em>Mitis</em></td>
<td><em>Actinomycyes</em></td>
<td><em>Streptococcus</em></td>
</tr>
<tr>
<td><em>Oralis</em></td>
<td><em>viscosus</em></td>
<td></td>
</tr>
<tr>
<td><em>Actinomycyes</em></td>
<td><em>Streptococcus</em></td>
<td></td>
</tr>
<tr>
<td><em>N. naeslundii</em></td>
<td><em>naeslundii</em></td>
<td><em>pseudopneumoniae</em></td>
</tr>
<tr>
<td><em>Neisseria</em></td>
<td><em>Lautropia sp.</em></td>
<td><em>infantis</em></td>
</tr>
<tr>
<td><em>Subflava</em></td>
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</tr>
<tr>
<td><em>Capnocytophaga</em></td>
<td><em>Kingella oralis</em></td>
<td><em>Neisseria subflava</em></td>
</tr>
<tr>
<td><em>granulosa</em></td>
<td></td>
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</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td><em>Neisseria mucosa</em></td>
<td><em>Lautropia sp.</em></td>
</tr>
<tr>
<td><em>Aeruginosa</em></td>
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</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td><em>Neisseria subflava</em></td>
<td></td>
</tr>
<tr>
<td><em>Aureus</em></td>
<td><em>Rothia mucilaginosa</em></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Major Bacterial Species Identified

RESULTS

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 rRNA gene sequencing is a rapid and reliable method that could profile bacteria of the oral cavity including potential pathogens. The authors wish to thank University of Malaya and the Malaysian Ministry of Science, Technology & Innovation for facilities and the award of research grant E-science 12-02-03-2076 (8422076) to carry out this project.

REFERENCES


