Localization of the Genus *Rothia* in the Oral Cavity

Satoshi Uchibori¹, Osamu Tsuzukibashi², Taira Kobayashi³, and Masahiro Aida³

¹Nihon University Graduate School of Dentistry at Matsudo, Crown Bridge Prosthodontics, Matsudo, Chiba 271-8587, Japan
²Department of Laboratory Medicine for Dentistry, and ³Crown Bridge Prosthodontics, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

**Abstract**

Among the genus *Rothia*, *R. dentocariosa* and *R. mucilaginosa* have been isolated from the human oral cavity. Currently, we reported that new selective media were developed for the isolation of each species. The clinical efficacy of the genus *Rothia* was evaluated from samples of oral cavities, such as pit and fissure plaque, buccal surface plaque, the buccal membrane surface, the dorsum of tongue, gingival crevicular fluid (GCF), and the mucosal surface of denture base using these selective media. *R. dentocariosa* and *R. mucilaginosa* were detected in all sites from pit and fissure plaque, buccal surface plaque, the buccal membrane surface and the dorsum of tongue. Therefore, both *Rothia* species are common members of oral cavity. *R. mucilaginosa* was predominant at the dorsum of tongue with 28.15% to total streptococci. Results show that the dorsum of tongue is the main habitation area of *R. mucilaginosa*. *R. dentocariosa* and *R. mucilaginosa* were also detected in GCF. These bacteria were detected to a certain extent on the mucosal surface of denture base. Therefore, it seems that these bacteria are members of denture plaque.

**Introduction**

The genus *Rothia* isolated from the oral cavity of humans are *Rothia dentocariosa* and *Rothia mucilaginosa* (1). *R. dentocariosa* was isolated in several laboratories from a variety of human sources, not only from the oral cavity, carious teeth, and periodontal pocket crevice of periodontitis, but also from blood, respiratory secretions, abscesses, wounds, and the eye (2–6). Furthermore, *R. dentocariosa* has been reported to be a cause of endocarditis and lung cancer (7–9). *R. mucilaginosa* was also isolated in several laboratories, not only from the oral cavity and upper respiratory tract, but also from the throat, lungs, blood, and dental plaques (10–13). Recently, Yamane et al. (14) reported isolation and identification of *Rothia mucilaginosa* from persistent apical periodontitis lesions. Furthermore, this organism is a responsible pathogen for a variety of opportunistic infections (11, 15–18). Immunocompromised patients are very often infected with this pathogen, leading to endocarditis (11, 12, 17, 19), sepsis (20, 21), peritonitis (17), and infections associated with tissue and organ transplantations and implants. Complications are also caused in patients with tumors, diabetes, and other diseases (22).

It is important to know the distribution of these organisms in oral cavities as they may cause opportunistic infections. Recently, we developed new selective media RDSM (23) and RMSM (24) for the isolation of *R. dentocariosa* and *R. mucilaginosa*, respectively. The aim of study is to investigate the localization of the genus *Rothia* in human oral cavities using each selective medium.

**Materials and Methods**

**Subjects**

Clinical specimens were collected from anonymous Japanese volunteers with no antibiotic use in the past three months who agreed to participate in the study after signed and informed consent (Table 1). This study was approved by the Ethics Committee of Nihon University School of Dentistry at Matsudo, Japan (EC 11–020).
Clinical sample sites

All samples were obtained after saliva was removed by a blast of air. Intact dental surfaces around lower and upper molars were selected for pit and fissure plaque and buccal surface plaque sampling, respectively. Plaque samples were collected using an explorer (YDM, Co., Tokyo, Japan) and spoon excavator (YDM). Gingival crevicular fluid (GCF) samples were collected from periodontal sites of healthy volunteers (pocket depth \( \leq 2 \) mm) using endodontic paper points. Samples were placed in a sterile microcentrifuge tube containing 0.5 mL of 0.05 M Tris-HCl buffer (pH 7.2) or reduced transport fluid in the case of paper points (25).

Mucosal swabs were collected from the buccal membrane surface and the dorsum of tongue using a sterile cotton swab (Kawamoto, Osaka, Japan). The mucosal surface of denture base of worn dentures was also obtained using a sterile cotton swab. Each swab was placed in a sterile microcentrifuge tube containing 1 mL of 0.05 M Tris-HCl buffer (pH 7.2).

Microbiological methods

All samples were dispersed by sonication for 30 s in an ice bath (50 W, 20 kHz, Astrason® System model XL 2020, NY., USA). Portions (100 \( \mu \)L) of appropriate dilutions of these samples were placed on Difico™ Mitis Saliivarius Agar (MS agar, Becton, Dickinson and Co., Sparks, MD, USA) for total streptococci, Bact™ Brain Heart Infusion (BHI, Becton, Dickinson and Co.) supplemented with 1% Bact™ Yeast Extract (Y, Becton, Dickinson and Co.) agar (BHI–Y agar), or BBL™ Brucella agar (Becton, Dickinson and Co.) supplemented with 5% blood, hemin 5 \( \mu \)g/mL, and menadione 1 \( \mu \)g/mL (Brucella–blood agar) for total bacteria, and selective medium plates RDSM and RMSM for the genus Rothia. Plates for total streptococci and bacteria were cultured as shown in Table 1. Selective medium plates were cultured at 37°C for 48 h in aerobic conditions, and the number of colony–forming units (CFU)/ml was calculated.

Results

Clinical examination

To investigate the microflora of hard tissues in oral cavities, the recovery of R. dentocariosa and R. mucilaginosa taken from samples of each oral site on RDSM and RMSM relative to MS agar for total streptococci is shown in Fig. 1. The detection ratios of R. dentocariosa and R. mucilaginosa were 4.12% (range: 0.52–11.97%) and 0.30% (range: 0.06–4.15%) from pit and fissure plaque, and 1.70% (range: 0.02–4.78%) and 0.15% (range: 0.01–0.75%) from buccal surface plaque, respectively. Furthermore, we investigated the microflora of soft tissues in oral cavities. The detection ratios of R. dentocariosa and R. mucilaginosa agar were 0.77% (range: 0.19–2.46%) and 2.30% (range: 0.87-
from the buccal membrane surface, and 1.14% (range: 0.08-3.53%) and 28.15% (range: 6.36-48.24%) from the dorsum of tongue, respectively.

For samples of GCF, the recovery of bacteria taken from subjects on RDSM and RMSM relative to Brucella agar with supplemented blood, hemin, and menadione for total bacteria is shown in Table 2. *R. dentocariosa* and *R. mucilaginosa* were also detected at 0.94% (range: 0.03-3.21%) and 0.12% (range: 0.02-0.29%) from GCF, respectively.

For samples of the mucosal surface of denture base, the recovery of bacteria taken from subjects on RDSM and RMSM relative to BHI-Y agar for total bacteria is shown in Table 3. On the mucosal surface of denture base, the detection ratios of *R. dentocariosa* and *R. mucilaginosa* were 1.27% (range: 0.05-3.62%) and 1.16% (range: 0.02-3.79%), respectively.

### Discussion

Currently, we reported that *R. dentocariosa* and *R. mucilaginosa* were detected at 2.62% (range: 1.0-4.6%) and 3.41% (range: 0.6-7.1%) of the total bacteria in paraffin-stimulated whole saliva samples, respectively (23, 24). Therefore, *R. dentocariosa* and *R. mucilaginosa* are members of oral microflora. In this study, these bacteria were ordinarily detectable in pit and fissure plaque and buccal surface plaque. In fact, since the detection ratios of these bacteria were almost the same as that of stimulated saliva, it may suggest that they are part of the common bacteria in oral microflora and compose the biofilm of oral cavities. About the related point of view of prosthodontics, these bacteria were detected to a certain extent on the mucosal surface of denture base. Therefore, it seems that these bacteria are also members of denture plaque and there is a possibility of kinds of opportunistic infection bacteria such as the genus *Candida* which is detected from denture plaque in the same way as the genus *Rothia*. Gordon (10) reported that *R. mucilaginosa* was detected from the surface of the human tongue. However, the detection ratios of this bacterium were not described. Results of the detection ratios in the dorsum of tongue were achieved with this research, and showed that the dorsum of tongue may be a main habitat for *R. mucilaginosa*. Gibbons et al (26) reported that *Streptococcus salivarius* adhered to the dorsum of tongue’s surface of humans in high proportions. Indigenous organisms colonized the bases of papillae on the anterior tip and lateral edges of the tongue as discrete microcolonies. It is thought that *R. mucilaginosa* may adhere to the tongue’s surface in a similar manner to that of *S. salivarius*. *R. dentocariosa* and *R. mucilaginosa* were also detected in GCF. However, they are numerical inferiorities in healthy periodontal sites.

It appears that this study is the first to investigate the distribution and localization of the genus *Rothia* in oral cavities using selection media. For future research, distributions and detection ratios of the genus *Rothia* in the oral region or focus such as carious teeth, periodontal disease and apical periodontitis will be investigated from the standpoint of its potential role in causing opportunistic infections.
Acknowledgments
The authors are grateful to Professors M. Hirasawa and K. Takada for their direction and helpful advice. We thank the members of the Department of Oral Microbiology for their continuous and kind support.

References