Biofilm forming ability of *Actinomyces naeslundii* strains obtained from children with aggressive periodontitis

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- Objectives

We have encountered cases of aggressive periodontitis with premature loss of deciduous teeth due to rapid alveolar bone destruction. In our previous study, a significantly higher percentage of such patients were found to possess *Actinomyces naeslundii*, a pathogenic bacterium associated with biofilm formation. The present study was conducted to investigate the biofilm formation capability of *A. naeslundii* strains isolated from those patients.

- Materials and Methods

Four *A. naeslundii* strains isolated from four patients as well as *A. naeslundii* JCM8350 were used. Each was grown overnight at 37°C, then inoculated into Trypticase soy broth. Growth curves were determined by measuring changes in optical density at 550 nm at one-hour intervals at 37°C under an anaerobic condition. Next, biofilms formed by each strain were stained with 1% crystal violet and absorbance at 570 nm was measured using a microplate reader. Furthermore, biofilm formed by the bacterial cells was labeled with 10 mM hexidium iodide and examined, with observations were performed using a confocal scanning laser microscope.

- Results

The growth rate of an *A. naeslundii* strains isolated form a patient with severe alveolar bone resorption was significantly lower as compared to that of the strains isolated from the other patients as well as JCM8350, while the quantity of biofilm it formed was also tended to be lower. Additionally, the biofilm associated with that strain showed a non-uniform three-dimensional structure with interlinked bacteria, while biofilms formed by the other strains were found to be comprised of the two parts, rigid and loose.

- Conclusion

These results suggest that *A. naeslundii* strains have varied biofilm forming abilities related to the pathogenicity of aggressive periodontitis.

Antimicrobial resistance of *Streptococcus mutans* organisms isolated from children undergoing hematopoietic stem cell transplantation

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Objectives

For pediatric patients undergoing hematopoietic stem cell transplantation (HSCT), high-dose chemotherapy and radiation therapy are given as a conditioning regimen, along with continuous antimicrobial agent administration for the resulting immunocompromised state. Following HSCT, oral conditions and flora generally worsen, leading to dental caries development. Using samples isolated from children undergoing HSCT, the present study investigated the antimicrobial resistance of *Streptococcus mutans*, a cariogenic bacterium, in those isolates.

Materials and Methods

The Okayama University Ethics Review Committee approved this study (Lab 1508-016). Saliva samples were collected from pediatric patients before, and one and three months after undergoing HSCT, then plated in Mitis Salivarius agar including bacitracin to isolate *S. mutans.* Minimum inhibitory concentration (MIC) values were determined using an antibacterial susceptibility test. RNA sequencing analysis was also performed to confirm the expression status of all antimicrobial resistant *S. mutans* genes. Furthermore, using fluorescent probes, the plasma membrane fluidity of antimicrobial-resistant *S. mutans* organisms was determined and compared to that of *S. mutans* MT8148.

Results

In samples obtained one month after HSCT, all *S. mutans* organisms showed resistance to various antimicrobial agents. The MIC of the organisms for showing resistance to β-lactams, penicillin, cephems, and carbapenems at one month was higher as compared to before as well as three months after treatment. Furthermore, 94 genes demonstrated increased expression in *S. mutans* organisms with as compared to without antimicrobial resistance, including genes related to ABC transporter and permease drugs. All antimicrobial-resistant *S. mutans* had lower levels of plasma membrane fluidity than that noted for MT8148.

Conclusion

S. mutans was found to acquire antimicrobial resistance following HSCT, thus reducing the effects of extracellular antimicrobials.

Analysis of ABC-transporter related genes in *Streptococcus mutans* associated with biofilm formation

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Objectives: *Streptococcus mutans* has been implicated as a primary causative agent of dental caries in humans. One of the important virulence properties of *S. mutans* organisms is their ability to form biofilm, resulting in dental plaque on tooth surfaces. In general, biofilm formation is initiated by interactions between planktonic bacteria and the surface in response to environmental stress factors, with the mechanism likely related to ATP-binding cassette (ABC) transporters. However, details related to of this mechanism remain unclear. The present study examined gene expressions in biofilm-forming bacteria and extracted *SMU922*, an ABC transporter-related gene, then, biological functions were investigated using *SMU922*-deficient mutant strains.

Materials and Methods: The DNA fragment encoding *SMU922* was amplified by PCR using specific primer sets and ligated into a pGEM-T Easy Vector. Next, the resultant plasmid was digested with EcoRI and inserted into an erythromycin resistance cassette, linearized with SalI, and used to transform *S. mutans* MT8148, which was termed *SMU922* deletion mutant strain (Δ*922*). To examine bacterial growth, MT8148 and Δ*922* were grown for 18 hours at 37°C, then inoculated into Todd Hewitt (TH) broth at 37°C. Cell density was determined by measuring he optical density (OD) at 600 nm. In addition, MT8148 and Δ*922* were inoculated into 100 μl of TH broth containing 0.5% or 1% sucrose in 96-well polystyrene microtiter plates. Following incubation for 48 hours, crystal violet staining was performed and biofilm formation quantified by measuring OD570.

Results: The growth rate of Δ*922* was similar to that of MT8148. On the other hand, the quantities of biofilm formed by Δ*922* were significantly reduced as compared to those formed by MT8148 in broth containing 0.5% or 1% sucrose.

Conclusion: These results suggest that *SMU922* is associated with biofilm formation.