

論 文 内 容 要 旨

Factors involved in transmission of *Streptococcus mutans*

(ミュータンスレンサ球菌の伝播因子に関する研究)

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Introduction

Mutans streptococci (MS) group harbors the oral cavity and its prevalence from previous study is about 33 – 75% in children, in which these bacteria possess the risk of dental caries. The predominant organisms of MS in the human oral cavity that related to the risk of dental caries are *S.mutans* and *S.sobrinus*. Dental caries is a transmissible infectious disease which could be occurred by means of intra-familial transmission between father, mother and child, or extra-familial transmission. Parents, particularly mother, are the major source of the transmission of MS alongside the fact that mother acted as the first contact and as the primary care giver for the children. In addition, another studies showed that father may also transmit MS to his children. Colonization of MS is associated to its factor in transmission, such as ability to produce bacteriocin which variant in MS is mutacin. Mutacins are antimicrobial peptides that inhibit the growth of the other bacterial which are genetically related or closely related species. The ability to produce broad spectrum of mutacins could promote the colonization of the *S.mutans* producers which in turn could promote dental biofilm. The other potential factor in transmission is adhering to host surface. The adherence of *S.mutans* and *S. sobrinus* is related to metabolism of insoluble glucans which would provide site for avid colonization by these microorganisms. Glucan formation is catalyzed by an enzyme group glucosyltransferase (GTF) which is encoded by *gtfB*, *gtfC* and *gtfD*. The aims of this study are, to identify *S.mutans* from current study place, to observe genotype transmission of *S. mutans* and to examine factors that involved in the transmission of *S.mutans*.

Material and methods

Thirty seven children (18 boys and 19 girls) aged from 1 to 6 years old whose at the nursery in Hiroshima University were examined. To detect *S.mutans*, dental plaque was collected by brushing all of the erupted teeth with sterile toothbrush then washed in dH₂O. The plaque samples were prepared for chromosomal DNA using standard mini prep method. Detection for *S.mutans* performed using primers as described by Igarashii et al (1996; 2000). Experiment followed by isolation of colony from the parent's plaque sample in child who harbors *S.mutans*. Then, restriction endonuclease analysis using *EcoRI* and *HindIII* were performed to observe transmitted and non-transmitted genotype in family. In this study, bacteriocin activity, biofilm formation and real time PCR analysis were performed for detection virulence transmitting factor from *S.mutans*. In bacteriocin activity assay using agar diffusion, overnight supernatant from transmitted and non-transmitted genotype were inoculated to agar using multi inoculators' pin , continued with overnight incubation. Then, inhibition zone which emerge was measured. Experiment was performed in quadruplicate. The biofilm formation analysis was performed in triplicate by quantifying the biofilm formation stained with crystal violet using spectrophotometer at 630 nm. The mRNA expression of *gtf* by real time PCR analysis was performed in triplicate. The expression of the encoded *gtf* gene was performed by using a reverse transcript real- time polymerase chain reaction. The primers used in this study for *gtfB* , *gtfC* , *gtfD* and 16S rRNA is according to Yoshida et al (2003; 2005)

Result

Detection of *S.mutans* revealed that there are one children who harbor *S.mutans* and seventeen children harbor *S.mutans* and *S.sobrinus*. Isolated colony from eleven children's and parent which already confirmed as *S.mutans* were digested. The result showed that 34 genotypes are positively *S.mutans* in 27

subjects. Moreover, our study also showed that 27 genotypes of *S. mutans* are positively detected in 8 families. From the study obtained 8 transmitted (in which 2 children showed similar genotype) and 18 non-transmitted genotype. Result shown that all of the children in this study harbor only one genotype and there are children who harbor similar pattern in the same family and also from different family. The examination of bacteriocin related to the virulence transmitting factor, showed no significant difference of the inhibition zone between transmitted and non-transmitted genotypes. In the other hand, biofilm formation analysis showed differences, though not statistically significant between transmitted and non-transmitted genotypes of *S. mutans*. The mRNA expression of *gtf* suggested there are statistical significant difference with student's *t*-test ($p < 0.05$) in *gtfC* expression, whereas *gtfB* and *gtfD* showed no significant difference.

Discussion and Conclusion

In this study there are 17 children who harbor *S. mutans* and *S. sobrinus* and 1 child who are detected for *S. mutans* from 37 children. This condition is multifactorial that may involve host, bacteria and environment. One of the possible reasons from results obtained in this study is because children received fluoride application every month. The colonization of *S. mutans* might be disrupted by fluoride application. From fingerprint *S. mutans* analysis show 34 genotypes in 27 subjects. There are 8 transmitted (2 children showed the similar genotype) and 18 non-transmitted *S. mutans* genotype. There are the similar genotype at the two children may be due to daily activities such as sharing food, drinks and utensils. Study suggests, bacteriocin facilitates the transmission of *S. mutans* with inhibition activity to other bacteria. Results obtained in this study showed no obvious difference in inhibition zone. This condition in accordance with another study, found that no association between the inhibitory spectrum of the infecting strain and caries experience or the level of MS infection of the host. Therefore, another analysis was performed to examine the factors involved in transmission by biofilm formation analysis. Biofilm formation analysis from transmitted and non-transmitted genotype showed differences, however statistically non-significant. The biofilm differences that occur in this study may be due to adhesion, which is modulated by genes. Genes that related to adherence are *gtfB*, *gtfC* and *gtfD*. Therefore, from the differences in this study, continued with the examination of mRNA *gtf* gene expression. From *gtf* genes expression analysis, showed that there is statistically different in *gtfC* expression. *gtfC* which is encoded GTF C enzyme, produce water insoluble glucan that create sticky environment, resulting initial adherence which induce colonization related to transmission of *S. mutans*. With high expression of genes *gtfC*, the water insoluble glucans produced also increased, thus enhanced *S. mutans* adherence. Next, the virulence of transmitted *S. mutans* genotypes elevated and transmission occurs. In conclusion, it was found *gtfC* gene might affect the virulence transmission genotype of *S. mutans*.