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研究題目：Contribution of Ammonium Transport Proteins in *Streptococcus mutans* to biofilm formation

Objective

Nitrogen is an essential nutrient for Gram-positive bacteria, whereas ammonium as one of the alternative nitrogen sources can also be utilized. *Streptococcus mutans* biological properties such as acidurity, biofilm formation and natural competence, are thought to be associated with induction of dental caries. Nitrogen is an excellent ammonium source for many bacteria including *S. mutans*. However, the ammonium transporter in *S. mutans* remains to be elucidated. *S. mutanspossesses* several kinds of membrane transporters that function as import or export molecules, which is crucial for its survive. In gram-positive bacteria, NrgA protein encoded by the *nrgA* gene, controls the ammonium transporter, and is required for transport and utilization of ammonium at a low concentration. In this study, we analyzed the function of the *nrgA* operon in *S. mutans* in relationship to biofilm formation.

Materials and methods

Analysis of the complete genome of S. *mutans*UA159 in the Oralgen database（http://oralgen.lanl.gov/oralgen-tng/）indicated the *nrgA* gene as apossible *S. mutans* ammonium transporter. *S. mutans* MT8148（serotype *c*）which is a homologue to the ammonium transporter gene in *Bacillus subtilis* was used in this study. The DNA fragment encoding *nrgA* was amplified by PCR and then ligated into a pGEM-T Easy Vector. The resultant plasmid was digested with *Eco*RI and BamHI and ligated with a streptococcal-*E. coli* shuttle vector（pSF152）to generate pCA01, which was then introduced into *S. mutans* MT8148 by transformation to create an *nrgA*-deficient mutant（NRGD）. First we determine the growth rates of MT8148 and NRGD. Bacterial organisms were grown overnight at 37°C, then inoculated into Todd-Hewitt Broth（THB）and 20 and 40 mM ammonium and absorbance at 550nm was determined at hourly intervals using spectropohotometry. Next, the ability of both MT8148 and NRGD to form biofilms was assesses by growing the bacteria using 96-well polystyrene microtiter plates.Formed biofilms were stained with 1% crystal violet and absorbance was determined at 570 nm, while their structures were analyzed using LIVE/DEAD backlight viability staining, for which biofilm cells were stained and kept in the dark for 15 minutes, then examined under a fluorescence microscope.

Results

Both MT8148 and NRGD were capable of growth in medium with and without the presence of ammonium, but ammonium affects the bacterial growth, whereas a significant difference of NRGD growth was seen in the presence of ammonium. In addition, the quantity of biofilm formation by NRGD was considerably lower, while the morphology of biofilms generated was rough as compared to those by MT8148.

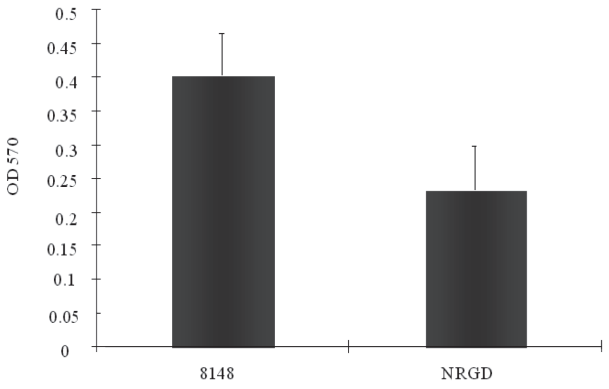


Fig.1　Assay of biofilm fromation in MT8148 and NRGD

Discussion

Ammonium transport is structurally related to the membrane transport protein involved in the movements of ions, molecules, and macromolecules across a biological membrane. The present results demonstrated the present NRGD mutant developed at a lower rateand showed structure alterations. Therefore, our findings show that the ammonium transporter in NrgA protein is important for bacterial growth and biofilm formation. Clearly, additional investigations of the structure and mechanisms of NrgA are required to elucidate the molecular basis of its properties.

Research achievement

2011 年 10 月　日本小児歯科学会近畿地方会（ポスター発表）

2012 年 5 月　第 50 回日本小児歯科学会（ポスター発表予定）

2012 May　Pediatric Dentistry Association of Asia in Bali（oral presentation）