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論　文　内　容　要　旨

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学位論文題目	Molecular characteristics of an adhesion molecule containing cholesterol-dependent cytolysin-motif produced by Mitis group streptococci（ミチス群レンサ球菌が産生するコレステロール依存性細胞溶解毒素様の細胞接着分子の特性）		

内容要旨

Streptococcus pseudopneumoniae (SPpn) is a relatively new species belonging to the Mitis group of the genus *Streptococcus* (MGS) proposed in 2004. According to comparative genomic analysis, SPpn is closely related to *S. pneumoniae* (SPn) and *S. mitis* (SM). SPn is a well-known pathogen of humans responsible for disorders such as otitis media, meningitis, pneumonia, and sepsis. Although the species of MGS except for SPn have been considered as opportunistic pathogens with low pathogenicity against humans, the clinical significance of the strains of MGS about the causative bacteria for various infections such as endocarditis and fulminant infection is emerging. Regarding the pathogenicity of SPpn, it has been reported that SPpn showed potential pathogenicity from the investigation from other research group using experimental mouse peritonitis/sepsis model, suggesting that the relevance of SPpn to the history or exacerbation of chronic obstructive pulmonary disease (COPD) and in human fatal septicemia. Moreover, with regard to virulence factors, it has also been reported that the genes encoding various pneumococcal virulence factors are present on the SPpn genome. According to these information about SPpn, the strains belonging to MGS have the nature for potential pathogenicity against humans.

One of the pneumococcal virulence factors secreted from the SPpn is a homolog of pneumolysin (PLY) which is a typical cholesterol-dependent cytolysin (CDC) produced from the strains of *S. pneumoniae*. This PLY homolog from SPpn is named for pseudopneumolysin (PPLY). In addition to PPLY, another gene encoding the homolog of lectinolysin (LLY), an atypical CDC composed of 5 domains with an N-terminal additional domain deduced to have lectin activity. Furthermore, it was recently reported that a novel open reading frame (ORF) suggested to encode a CDC-like molecule with a molecular weight of ~100 kDa was identified on the genome of SPpn strain IS7493 possessing the genes encoding PPLY and LLY-homolog. Based on its nucleotide sequence information, it was suggested that the product of this novel gene might have multiple functions because of the presence of a lipase domain (lipase_3), tandem-arranged F5_F8_type_C domains, and a receptor-recognition domain of the CDC in this novel molecule. Therefore, this predicted CDC-related molecule is of interest as a novel potential virulence factor of SPpn and the related strains possessing the gene encoding this molecule. However, the function of this predicted molecule with potentially some of functional domains as well as its actual production from the bacteria has not been investigated so far.

In the present study, we investigated the molecular functions of this CDC-related molecule with

potential multiple functions *in vitro* using various recombinant proteins as well as the distribution and the production of this molecule in other oral streptococcal strains. Consequently, the gene encoding this CDC-related molecule was found not only in SPpn strains but also in the strains of other species belonging to MGS, such as SM and SPn. Moreover, the product of this gene was produced from the gene-possessing strains both as a secreted form and as a cell-bound form. The recombinant protein of this molecule prepared by *E. coli* expression system showed the functions for lipase activity and for the lectin domain(s)-dependent human cell-binding activity, although no detectable hemolytic activity. According to the information about the functions of this CDC-related molecule described above, and also based on the distribution of the gene encoding this molecule within the MGS, this novel CDC-related molecule was named mitilectin (MLC) and its contribution to the potential pathogenicity of the MLC-producing strains was further investigated. From the results of investigation about the function of MLC for an adhesion molecule, it was revealed that the treatment with anti-MLC antibody to human culture cell lines and the *mlc* gene-knockout in SM strain Nm-65 significantly reduced the target cell-binding activity of MLC-producing strains of SPpn and SM. Interestingly, it has also been confirmed that the strains possessing the *mlc* gene also carry the gene(s) encoding CDC(s) based on the genomic analysis *in silico*. Judging from these results, it is suggested that the MLC-dependent adhesion of the strains of MGS would enhance the cytotoxicity by the production and the action of these CDC(s), and may contribute to the potential pathogenicity of MLC-producing strains in MGS. Therefore, this novel multi-functional MLC was suggested to be important molecule with the function of cell-adhesion for considering the potential pathogenicity of the MLC-producing strains belonging to MGS, such as SPpn and SM.