CHARACTERIZATION OF CATALYTIC *α*-1,3-GLUCANASE ISOZYMES FROM *PAENIBACILLUS GLYCANILYTICUS* FH11 BY USING *BREVIBACILLUS* SYSTEM; ESSENTIAL FOR SUPPRESSION OF *STREPTOCOCCUS MUTANS* BIOFILMS

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S. mutans has been implicated in the etiology of dental caries by facilizing the colonization of tooth surfaces and playing a key role in the development of the virulent dental plaque. *α*-1,3-Glucan, which is a key structural constituent of the biofilm matrix (dental plaque), synthesized by glucosyltransferase type B (gtfB) in the presence of ingested sucrose. *α*-1,3-Glucanases also called mutanases, which hydrolyze *α*-1,3-glucan, are classified into two families of glycoside hydrolases, fungal (type 71) and bacterial (type 87). Because of being considered to degrade *α*-1,3-glucan, *α*-1,3-glucanases have been purified and characterized from various microbial sources. However, there are few reports on *S. mutans* biofilm study. For the host cell expression, *Brevibacillus* system is an effective bacterial expression system for secretory proteins. *B. choshinensis* is a gram-positive bacterium and easy to handle non-sporulating bacterium, lacking extracellular protease, that has been already shown to provide a high level of recombinant protein expression. Recently, many proteins are produced from this expression system and use for medical treatment, research study (1). Therefore, in this study we attempted to use *Brevibacillus* expression system to express, purify, and characterize of *α*-1,3-glucanase. In addition, we aimed to investigate the effect of recombinant enzyme on *α*-1,3-glucan biofilm produced by *S. mutans* from the viewpoints of formation and the effect of toothpaste agent on enzyme activity. Two novel catalytic domains of *α*-1,3-glucanase isozyme genes were cloned from *P. glycanilyticus* strain FH11 and heterologously expressed in *Brevibacillus* system. The recombinant isozymes, in termed CatAgl-FH1 and CatAgl-FH2, were purified to homogeneity with specific activity 0.70 U/mg and 0.77 U/mg respectively. The molecular mass of catalytic domain was estimated 62 kDa by SDS-PAGE. Both recombinant enzymes exhibited the different properties. The optimal pH of CatAgl-FH1 and CatAgl-FH2 were 5.5 and 6.0, respectively. The pH stability of CatAgl-FH1 and CatAgl-FH2 were in a range of pH 4.0-11.0 and 4.5-9.0, respectively. The optimal temperature of CatAgl-FH1 and CatAgl-FH2 were 60°C and 55°C, respectively and they were stable until 60°C. Thin Layer chromatography revealed their mode of hydrolysis towards *α*-1,3-glucan was endo-cleavage pattern. The major products of CatAgl-FH1 were di- and trisaccharide but mainly trisaccharide was for CatAgl-FH2. Both enzymes showed high tolerance against high concentration of sodium fluoride. However, each enzyme activity on surfactants were stepped down when sodium dodecyl sulfate and benzethonium concentration were increased.

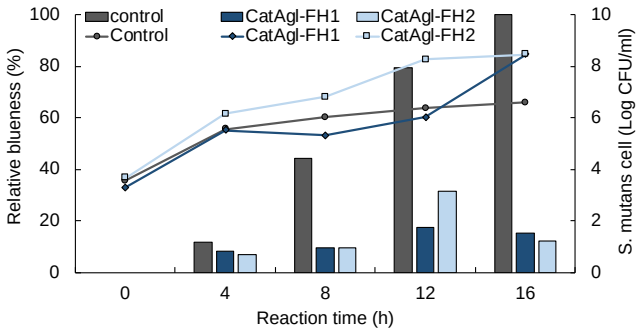

Figure 1 – The formation of S. mutans biofilm adhered to glass plate.

For *S. mutans* biofilms studies, artificial models were developed to verify the possibility of suppressing *α*-1,3-glucan biofilm that adhered to glass plate. Both of recombinant enzymes inhibited biofilm formation via hydrolyzing *α*-1,3-glucan more than 80% compared to untreated biofilm at 16 h reaction time without affecting bacterial growth. Therefore, the recombinant enzymes could be applied in oral hygiene products such as toothpaste, mouthwash and chewing gums for support mechanical cleaning of tooth surfaces.

Reference

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