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Importance of cariogenic biofilm formation in *S. mutans* carbohydrate metabolism.Palina Vyhoukaya¹, Anna Skalniak², Jakub Piątkowski², Anna Jurczak³, Dorota Kościelniak³, Ryszard Drożdż¹ and Wirginia Krzyściak^{1*}¹Jagiellonian University, Poland²Genetics Laboratory, Department of Endocrinology, Jagiellonian University, Medical College, Krakow, Poland³Department of Pediatric Dentistry, Institute of Dentistry, Jagiellonian University, Medical College, Krakow, Poland

Background: *Streptococcus mutans* is known as one of the important etiologic agents of dental caries, a widespread disease in Polish children. Recognition of novel features determining the pathogenicity of this pathogen may contribute to understanding of the mechanisms of bacterial infections. Carbohydrate metabolism is one of the key metabolic pathways subject to changes during *Streptococcus mutans* cariogenic biofilm formation which could determine its pathogenicity. The aim of the study was to evaluate the activity of pyruvate kinase (PK) and to illuminate the role of the enzyme in *S. mutans* cariogenic biofilm formation. This enzyme catalyzes the reaction of phosphate transfer from a phosphoenolpyruvate (PEP) to ADP, and the decline is observed in physiological biofilm species. Therefore it could be a target for newly synthesized compounds of potential anticaries activity.

Material and methods: The study was performed on isolates derived from children with deciduous teeth (n = 80) aged 4±1.2 years: the experimental group – 40 children with caries, control group – 40 children without caries. Enzymatic typing of isolated bacterial strains was performed based on the enzymatic profiles obtained from the commercially available test, and evaluated by 16SrDNA gene sequencing. The enzymatic activity of PK was purified, precipitated and determined spectrophotometrically. Biofilm assay was evaluated using a close model based on a microtiter plate using ATCC and clinical strains. Biomass of biofilm was measured spectrophotometrically and fluorimetrically. In this study, we revealed at first the activity of PK in various biotypes and formation time of *S. mutans* cariogenic species biofilm.

Results: The activity of PK was examined spectrophotometrically after purification and precipitation during different model of biofilm formation. We observed a slight increase in the activity of the glycolytic enzyme during cariogenic biofilm formation compared to physiologic biofilm structures. In case of forming the *Streptococcus mutans* cariogenic biofilm, the activity of glycolytic enzymes also grew after 14 and 18 hours in the context of *S. mutans* biotypes I-II proposed in the study (clusterization method).

Conclusions: The increase in the activity of glycolytic enzymes during the biofilm formation (due to the effect of low pH) in categorized biotypes allow for better differentiation of *S. mutans* species and thus may contribute to recognition of transmission mechanisms of pathogenic bacteria and facilitate treatment. Bacterial cells adapt to new conditions better in mixed-clinical species biofilm than in the mono- and dual- biofilms – the increase of the glycolysis rate associated with increased activity of glycolytic enzymes reflects this phenomenon very well. Therefore, inhibition of glycolytic enzymes might be an essential step in the reduction of mixed-species cariogenic biofilm, what could be a useful tool in prevention of caries.

Biography

Palina Vyhoukaya has completed her MSc in Laboratory Medicine at Jagiellonian University Medical College in Krakow, Poland. She is currently working in the field of Laboratory Medicine at Jagiellonian University Medical College in Krakow Poland.

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