BIO PROCESS AND SYSTEMS ENGINEERING TUHH

BioInSys: Development of Biofilm Inhibitors Using a Systems Biology Approach

WP7 - Proteomic and metabolic analyses

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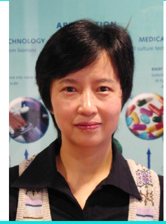

Goals and approaches:

Carrying out **proteomics** and **metabolomics** to study, in parallel to gene expression profiling (WP5), the inhibitory effect of Carolacton on the biofilm formation of *S. mutans* and identify potential targets of Carolacton. Proteomic, peptidomic and metabolic data generated will be used for integrated metabolic and regulatory network analysis (WP3) and dynamic modelling (WP4).
To this end, two-dimensional gel electrophoresis combined with mass spectrometry (2DE/MS) as well as liquid chromatograpgy mass spectrometry (LC/MSMS) will be applied for **differential proteomic analysis**. In addition, a global characterization of **phosphoproteome** is planned, aiming to understand Carolacton-related phosphorylation-mediated cellular signal sensing and transduction processes. Furthermore, a method for the enrichment and LC/MSMS analysis of secreted peptides (**peptidomics**) as signalling molecules will be developed by starting with the competence-stimulating peptide (CSP). Metabolomic analysis will be performed to characterize the physiological states of *S. mutans* cells in response to Calolacton treatment. The analysis will be focused on intracellular and extracellular metabolites involved in sugar metabolism, which is directly related to biofilm formation.

Achievements and Outlooks:

A systematical characterization of Carolacton effect on the biofilm formation of *S. mutans* wild type UA159 under different conditions pointed to a cell growth-related inhibitory effect of Carolacton. 2DE/MS based proteomics revealed that Carolacton damages the *S. mutans* cell membrane integrity. Analyzing membrane protein fraction by LC/MSMS led to the identification of membrane proteins, whose expression levels changed upon treatment by Carolacton, including proteins involved in peptidoglycan biosynthesis and cell division. This is in agreement with the results of transcriptomic analysis (WP5) and the observation that growing cells treated by carolacton were strongly elongated. Further quantitative membrane proteomics is ongoing. Stilll under development is a 2DE-based phosphoproteomics using ProQ-Diamond staining.

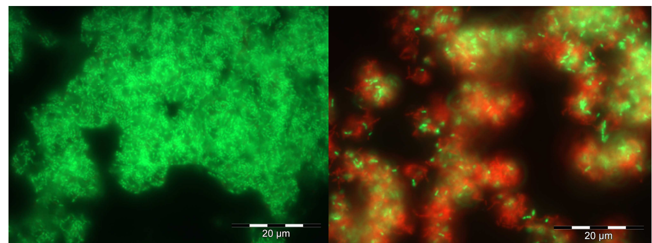

Fig. 1: Cell viability of *S. mutans* without (upper) and with (lower) Carolacton treatment (Fluorescent staining, green: live cells, red: dead cells

A comparative proteome analysis on *S. mutans* UA159 and its non-biofilm forming *vicK* knockout mutant discovered significant differences in the global protein expression patterns, which should help us to better understand the working mechanism of Carolacton, since *vicK* was also found to be one of the earliest changed genes at transcriptional level after carolacton treatment (WP5).
Cultivation of *S. mutans* UA159 in a minimal medium to facilitate the analysis of secreted peptides by LC/MSMS was realized. A method for multistep peptide enrichment has been developed using synthetic CSP and will be applied for monitoring the dynamic formation and degradation of CSP as well as for the identification of other secreted peptides by LC/MSMS.

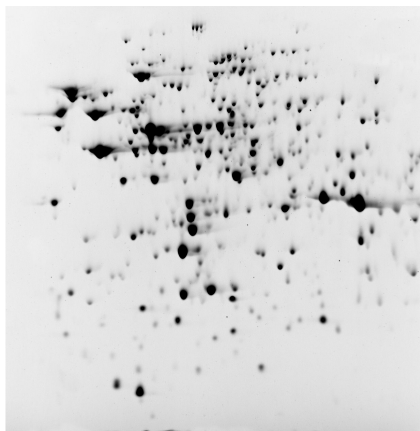

Fig. 2: Protein expression pattern of S. mutans UA159 by 2D gel electrophoresis

Intracellular sugar phosphates of the central carbon metabolism and extracellular acid formation will be analyzed by HPLC and HPIC.

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