A set of optimized *P. taiwanensis* VLB 120 balancer strains with enhanced c-di-GMP levels

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PROMICOM

Harnessing the power of nature through PROductive MIcrobial CONsortia in biotechnology - measure, model, master

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Introduction

WP 3 aims at the optimization of minimal functional metabolic modules taking part in a target bioprocess and its further reassembly in the context of a synthetic microbial consortium. Following this scheme, bacterial farmers, producers and stabilizers are selected and / or optimized. In our project we aim at the photo-fermentative production of Hydrogen (H2) utilizing a defined microbial consortia composed of a photo-heterotrophic non-sulphur purple bacterium, a chemo-heterotrophic Pseudomonas and a photoautotrophic cyanobacterium.

In this consortium, the non-sulphur purple bacterium, namely *Rhodopseudomonas palustris*, is the producer strain, while the cyanobacterium serves as farmer and the Pseudomonas as balancer strain. In this specific example, the organisms grow attached to a surface, where they form a three dimensional structure termed biofilm promoted by the presence of the Pseudomonas. Biofilm formation is (amongst other factors) governed by cyclic di-GMP (c-diGMP), which is a global regulator in numerous organisms regulating various functions.

A stable biofilm is the basis for the realization of our process concept, which aims at the continuous photo-fermentative production of H_2 utilizing a capillary biofilm reactor (CBR). Thus engineering c-di-GMP formation in Pseudomonas resulting in a c-di-GMP overproducer could have a positive impact on biofilm formation of the consortia**.**

Construction of c-di-GMP overproducer

The balancer strain *Pseudomonas taiwanensis* VLB120 was transformed with the plasmid pS6311::DGC-244 containing a diguanylate cyclase resulting in *P. taiwanensis* VLB120/pS6311::DGC-244 (hereafter *P. taiwanensis* VLB120_DGC). A mutant variant of DGC from *Caulobacter crescentus*, termed DGC-244, was used to construct respective plasmid for inducible biofilm formation and was a kind gift from Prof. Urs Jenal (Biozentrum, University of Basel). This mutant lacks feedback inhibition by cyclic diguanylate. Diguanylate cyclase (DGC) catalyzes the reaction of two guanosine-5′-triphosphate (GTP) molecules to form c-di-GMP and thereby directly influenced c-di-GMP concentration in the organism.

Performance

Single species cultivation of the engineered balancer strain *P. taiwanensis* VLB120_DGC resulted in a thick and inhomogeneous distribution of the biofilm on the available growth surface (Figure 1). While *P. taiwanensis* VLB120 formed around 145 mg_{BDW} (BDW = Biofilm Dry Weight), 208 mg_{BDW} was determined for the engineered variant.

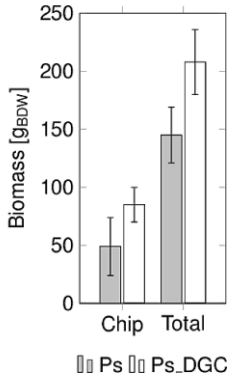

Figure 1: Comparison of total biomass formation by P. taiwanensis *VLB120 and* P. taiwanensis *VLB120_DGC on a chip based cultivation system after 12 days. The enhanced c-di-GMP levels in* P. taiwanensis *VLB120_DGC lead to significantly higher biomass yields.*

Beside the enhanced biofilm formation also biofilm formation was faster in the recombinant strain as compared to the wildtype.

Future perspective

In a next step we will quantify the intracellular c-di-GMP levels in the mutant. For this a Cyclic-di-GMP Assay Kit from Biovision has been ordered, but unfortunately takes much longer than anticipated to be delivered. As soon as it arrived, these quantification will be made. Furthermore, we will evaluate, how this enhanced biofilm formation impacts the overall consortia and H_2 Production.