Karlsruhe Institute of Technology

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Recombinant synthesis of organic compounds in cyanobacteria

Introduction of a new organism into cyanobacterial biotechnology

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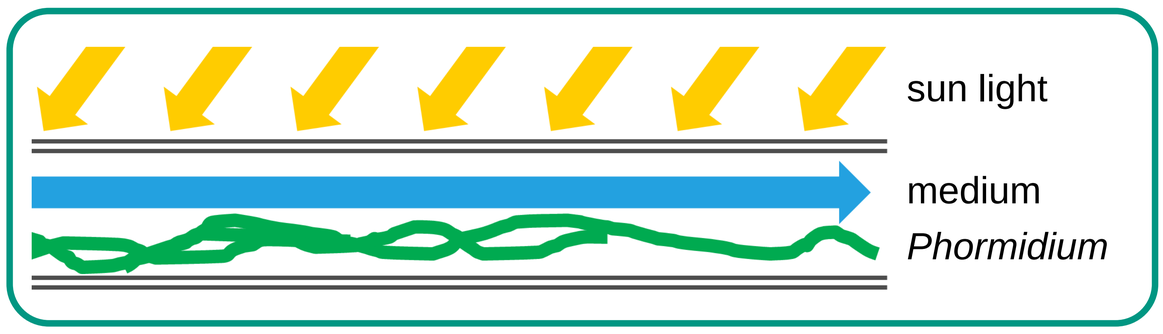
Abstract

For the world's energy supply, biofuels are getting an increasing impact. The third generation of biofuels consists of energy rich compounds isolated from eukaryotic microalgae or cyanobacteria. This includes metabolites that are originally produced by a wild type organism or whose synthesis is introduced via genetic engineering.
The main objective of this work is to establish and optimize the recombinant production of ethanol in different strains of the cyanobacterium *Phormidium*. With *Phormidium* we want to introduce a new, robust organism to cyanobacterial biotechnology that has promising growth rates and also facilitates new possibilities for cultivation due to its filamentous growth in suspension culture and on surfaces.
The first cornerstone is to establish stable transformation of *Phormidium* through homologous recombination. In the next step, different promoters are characterized in terms of gene expression via reporter gene assays and ethanol synthesis. In subsequent experiments, the cultivation of selected *Phormidium* clones in bioreactors will be investigated. Ethanol synthesis will be further optimized using metabolic engineering methods. The envisioned concept for optimized ethanol production is considered a suitable model for the synthesis of further compounds in *Phormidium* and closely related species.

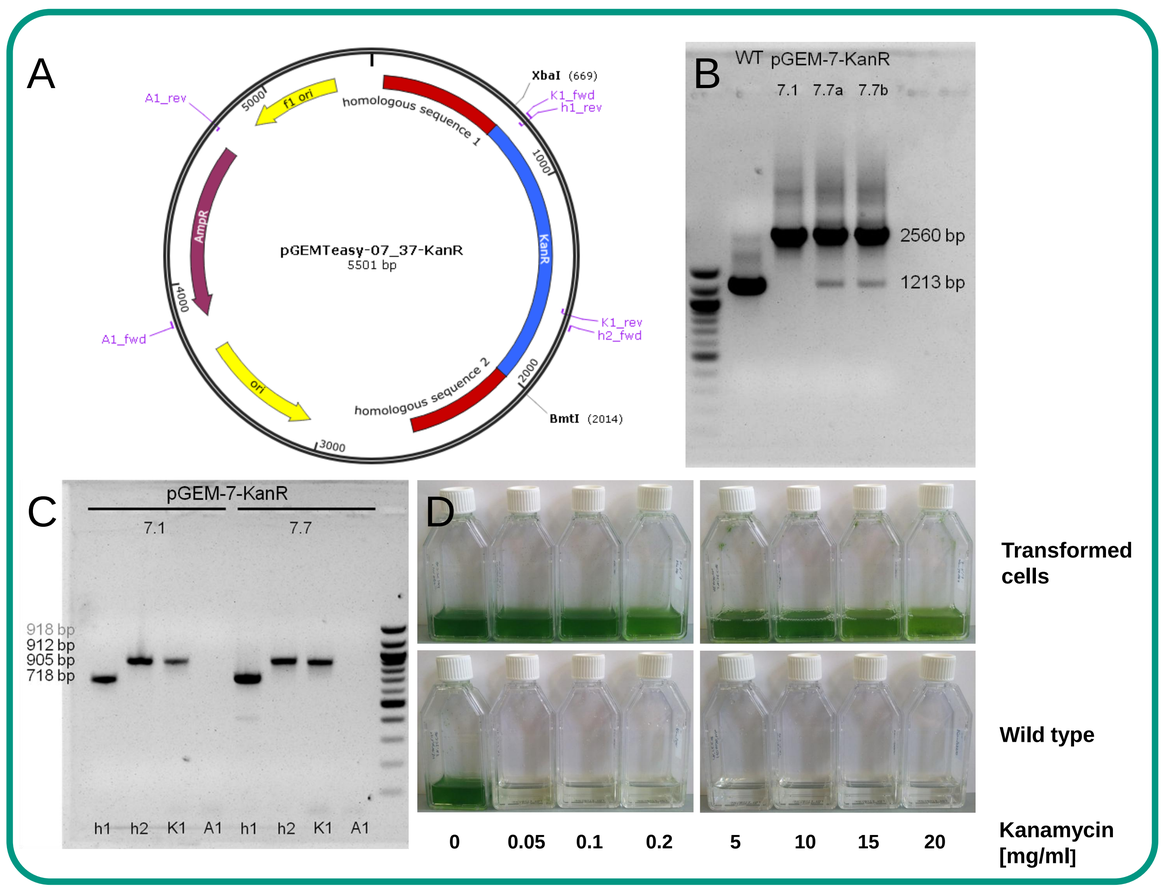
Introduction

The use of cyanobacteria in biotechnology research focuses mainly on the model organisms *Synechocystis* and *Synechococcus*. These are unicellular cyanobacteria with established cultivation and transformation methods. We want to introduce the filamentous and fast growing *Phormidium* to investigate whether a different lifestyle could bring new perspectives in cyanobacterial biotechnology. The adherent growth of *Phormidium* is a possible advantage concerning media exchange, prolonged cultivation and product purification.



Results

For the transformation of *Phormidium* the natural mechanism of homologous recombination is used and therefore a gene of interest has to be introduced into a sequence homologous to the *Phormidium* genome. In initial experiments, the gene for resistance against the antibiotic kanamycin was used to select transformed cells. For one homologous sequence a transformation protocol was established and it was tested successfully with three *Phormidium* strains. The success of transformation was confirmed via PCR and by high resistance of the transformed cells towards kanamycin.

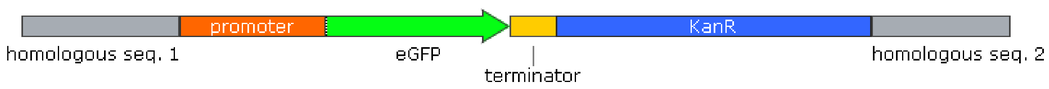


Conclusion

Until now, there was only one report of transformation in *Oscillatoria* (same genus as *Phormidium*) using a plasmid but lacking chromosomal integration. With the establishment of the transformation method in this work we have the necessary tool to perform stable transformations in several *Phormidium* strains using the natural mechanism of homologous recombination. Therefore it is possible in subsequent experiments to accomplish ethanol production in Phormidium and, in general, to generate mutants for basic biological questions.

Outlook

Since there is no pusblished experience with heterologous gene expression in *Phormidium* the next step of this work is to test different promoters and terminators. The basic design contains a promoter, an eGFP gene, a terminator, and a kanamycin resistance gene flanked by homologous sequences.



Promoter and terminator sequences with promising results from *Synechocystis* as well as selected ones originating from *Phormidium*, which are considered to be highly expressed, will be tested.

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