Gene editing in *A. niger* using RNP-mediated CRISPR-Cas9

Paving the Way for Smarter Fermentation

Bothof, T; Raats, M.; Verschuren, T.
Research Group: Smart Fermentation
Contact information: tijnbothof@gmail.com, mwj.raats@avans.nl, t.verschuren3@avans.nl
Date: 12-06-25

Introduction

A. niger is a fungus that is heavily used in industrial purposes for the production of high-value compounds (Ritika, et al., 2024). Gene editing can increase production of these compounds or even produce entirely different ones!

CRISPR-Cas9 is a cutting-edge tool to delete and insert genes, using proteins derived from the bacterial defense system to induce double-strand breaks at the target site (Gasiunas, et al., 2012). This project uses the novel RNP-based method, which reduces off-target mutations.

The goal?

Validating a protocol for the deletion of the PyrG gene in *A. niger*.

Method

The transformation of *A. niger* is a complicated protocol, requiring numerous different steps:

1. *A. niger* is cultivated in liquid and on solid medium. The spores are used for future cultivation, whilst the mycelium is used for **DNA-extraction**.

2. PCR is used to generate a **repair template** for the transformation. Regions flaking the gene-of-interest are amplified and fused.

3. Mycelium is treated with enzymes to create **protoplasts**. These are mixed with the **RNP-complexes** and repair template to create mutants without the PyrG gene.

4. The transformants are grown on selective plates and sequenced using **Nanopore sequencing**. The library was prepared from 10 samples from 5 transformants in duplicate. The library was sequenced with the ONT **PromethION**.

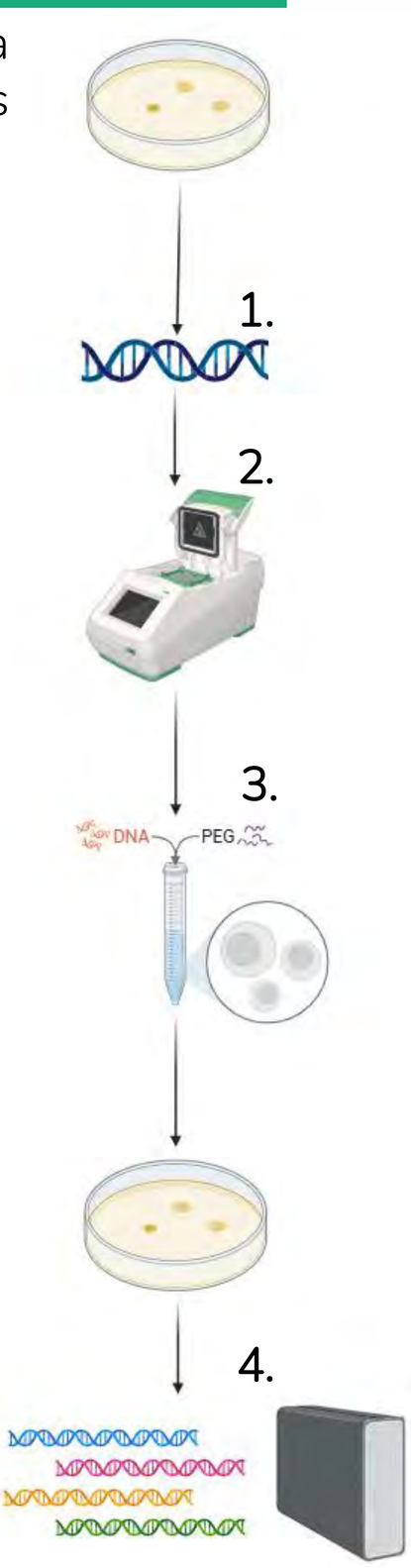

Figure 2. A simplified overview of the RNP-mediated CRISPR-Cas9 transformation process in. *A. niger*. Made using Biorender.

Results

Multiple colonies showed clear growth on the selective plates. This means the transformation was a success.

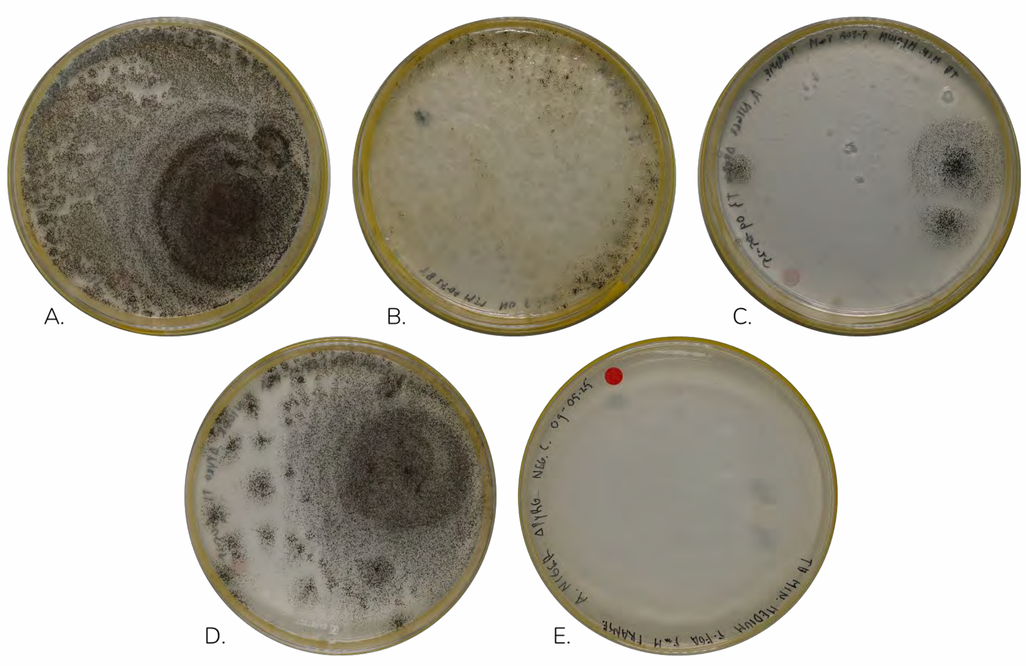

Figure 1. The five transformation plates after 11 days, showing growth on all plates except the control without repair template.

Sequencing shows a deletion in one of the mutants, but phenotypically all transformant colonies are 5-FOA resistant. This shows sequencing did not successfully detect deletions in the other four transformants.

Conclusion/Recommendations

This improved method can reliably generate successful PyrG gene deletion-transformants. The selective plates are sufficient in inhibiting growth of non-transformed cells. The bioinformatics tools used here may not detect small mutations and SNPs created during these transformations.

Increasing the amount of repair template added during the transformation may increase homologous repair rates.

References

1. Ritika, et al. (2024, November 1). CBS 146.36. *Fuel*. doi:10.1016/j.fuel.2024.132593
2. Gasiunas, G., et al. (2012, September 25). *Proceedings of the National Academy*. doi:10.1073/pnas.1208507109

Acknowledgements

I would like to express my sincerest gratitude to my supervisors Mirthe Raats and Tim Verschuren.
This project is financially supported by the European Just Transition Fund via project FermiChem (JTFZ-00022)