Genetically Modifying *Penicillium rubens* by Manipulating *mcrA* to Enhance Production of Secondary Metabolites for Anti-Cancer Therapeutics

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Abstract

Filamentous fungi produce natural products that have been proven to have medicinal and industrial potential. These compounds, also known as secondary metabolites (SMs), are produced by biosynthetic gene clusters (BGCs), most of which are silent. Due to the potential discovery of new SMs and bioactivities, it is essential to activate some of these silent pathways.

Our strain, *Penicillium rubens* (IMV00188), can produce compounds similar to the antibiotic penicillin, which has the potential to be used in medicine. To investigate additional potential useful SMs, we genetically manipulated this strain using *in vitro* CRISPR-Cas9. We targeted *mcrA*, which is a negative global regulator that suppresses silent BGCs. Knockout of this gene allows for the production of new compounds. Once we knocked out *mcrA*, the secondary metabolites produced by the wild type (WT) and mutant strains were grown in different conditions, extracted, and analyzed by high-performance liquid chromatography (HPLC). Ultimately, our goal is to create a natural product library and screen the bioactivity of these SMs for potential hits as cancer therapeutics.

Objectives

- Verification of *mcrA* knockout strain creation through diagnostic PCR.
- Cultivation of IMV0188 in different conditions comparing WT and *mcrA* knockout.

Anti-SMASH

1.1	2.1	3.1	3.2	3.3	5.1	6.1	6.2	8.1	8.2	9.1	10.1	10.2	11.1
13.1	14.1	16.1	16.2	17.1	18.1	21.1	22.1	25.1	27.1	28.1	29.1	31.1	32.1
33.1	33.2	34.1	35.1	35.2	38.1	45.1	46.1	53.1	56.1	59.1	62.1	62.2	66.1
69.1	71.1	71.2	74.1	74.2	81.1	83.1	89.1	90.1	91.1	92.1	94.1	118.1	

Nrps | T1pks | T1pks-Nrps | Terpene

Figure 1. Anti-SMASH analysis demonstrates potential biosynthetic gene clusters of IMV00188.

IMV00188 Wild type and *mcrA*Δ Strains

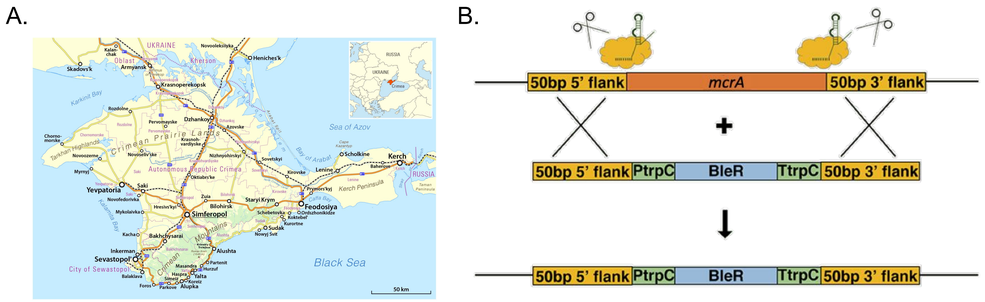

Figure 2. Origins of strains used in this project. (A) IMV00188 was isolated from soil on a mountain slope located in Crimea. (B) *mcrA*Δ strain generated using *in vitro* CRISPR-Cas9.

Confirmation of *mcrA* Knockout

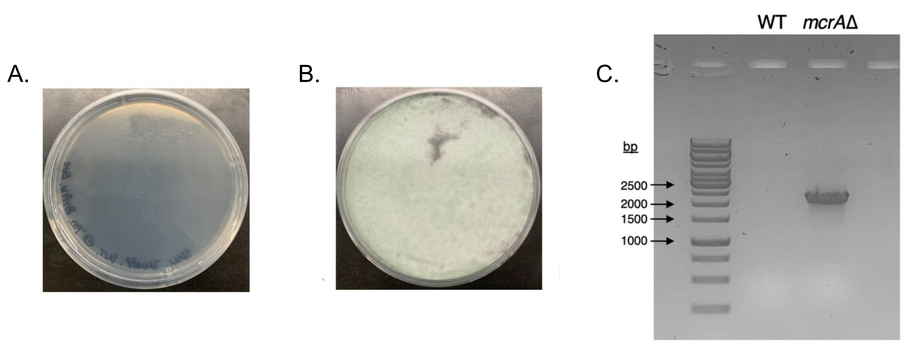

Figure 3. *mcrA* was deleted and replaced with a phleomycin resistance gene to select for transformants following transformation. (A) WT strain is susceptible to phleomycin. (B) *mcrA*Δ is resistant to phleomycin. (C) Results of diagnostic PCR amplification of *mcrA* coding regions in wild type and *mcrA* knockout (*mcrA*Δ) strains.

Screening Different Conditions

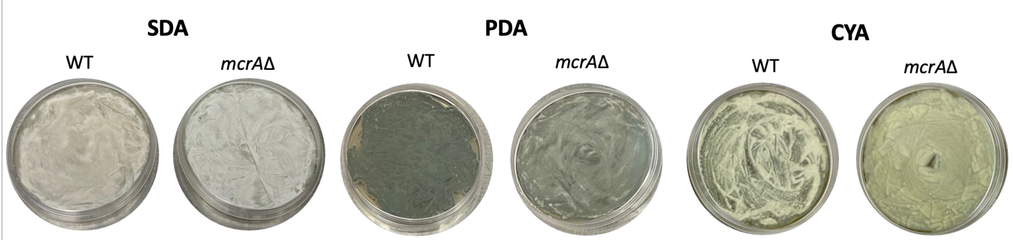

Figure 4. Cultivation of IMV00188 WT and *mcrA*Δ on different media. Fourteen different conditions were tested. Shown are the three most promising conditions that were chosen for further analysis: Sucrose Dextrose Agar (SDA), Potato Dextrose Agar (PDA), and Czapek Yeast Autolysate Agar (CYA)

HPLC Analysis of WT and *mcrA*Δ

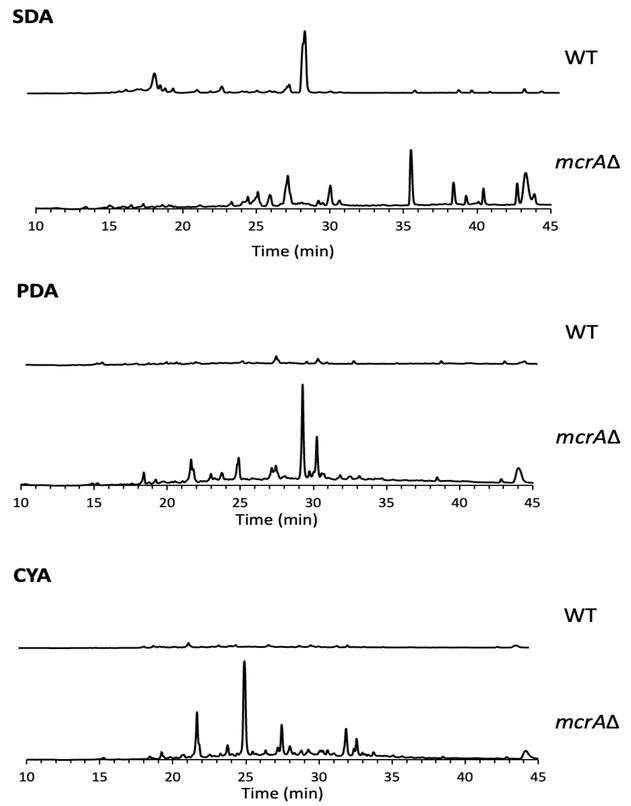

Figure 5. HPLC analysis of WT and *mcrA*Δ strains grown on different media. Variations in peaks show differences in SM production.

Summary

Our experiments showed how knocking out *mcrA* is effective in upregulating SM production in IMV00188 thereby fulfilling our goal of creating a natural product libaray. As a result, our next steps would be to scale up, isolate and identify the upregulated compounds in the *mcrA*Δ strains, and send them to our collaborators in order to test their bioactivity or ability to work as cancer therapeutics.

References

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- Maximilian Dörrbecker (Chumwa) - Own work, using OpenStreetMap data this file for the orientation map inset, CC BY-SA 2.0