

Generating a Natural Product Library by Genetically Manipulating *mcrA* in marine *Penicillium rubens* to Discover Bioactive Compounds Against Alzheimer's Disease

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Bridge UnderGrad Science (BUGS) Summer Research Program

Abstract

Fungal secondary metabolites (SMs) are diverse organic compounds often associated with medicinal and industrial properties. These natural products are encoded by biosynthetic gene clusters (BGCs) in the fungal genome. Because most BGCs are silent under normal laboratory conditions, it is crucial to find new ways to activate them. Doing so will allow us to create a natural product library and discover compounds with bioactivity that can potentially treat conditions such as Alzheimer's Disease.

Our strain of focus, *Penicillium rubens* (YAP001), was collected from sea anemone off the coast of Florida. By using the CRISPR-Cas9 genome editing system, we knocked out the negative global regulator, *mcrA*, which suppresses many BGCs. We confirmed the deletion of *mcrA* by designing primers to perform diagnostic PCR and comparing the growth of wild type (WT) and *mcrA* knockout (*mcrA*Δ) strains under different culture conditions. SM production was analyzed with high-performance liquid chromatography (HPLC) procedures. Ultimately, we aim to use a seeding assay with tau protein to investigate whether the SM compounds are bioactive against Alzheimer's Disease.

Objectives

- Verification of *mcrA* knockout strain creation through diagnostic PCR.
- Cultivation of YAP001 in different conditions comparing WT and *mcrA* knockout.

Anti-SMASH

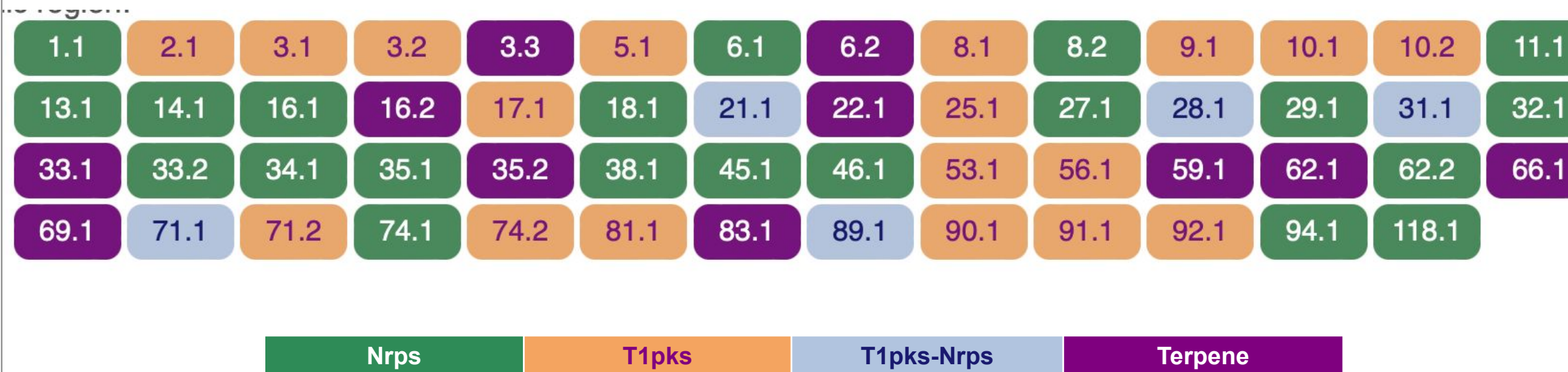


Figure 1. Anti-SMASH analysis demonstrates predicted biosynthetic gene clusters of YAP001.

YAP001 WT and *mcrA*Δ Strains

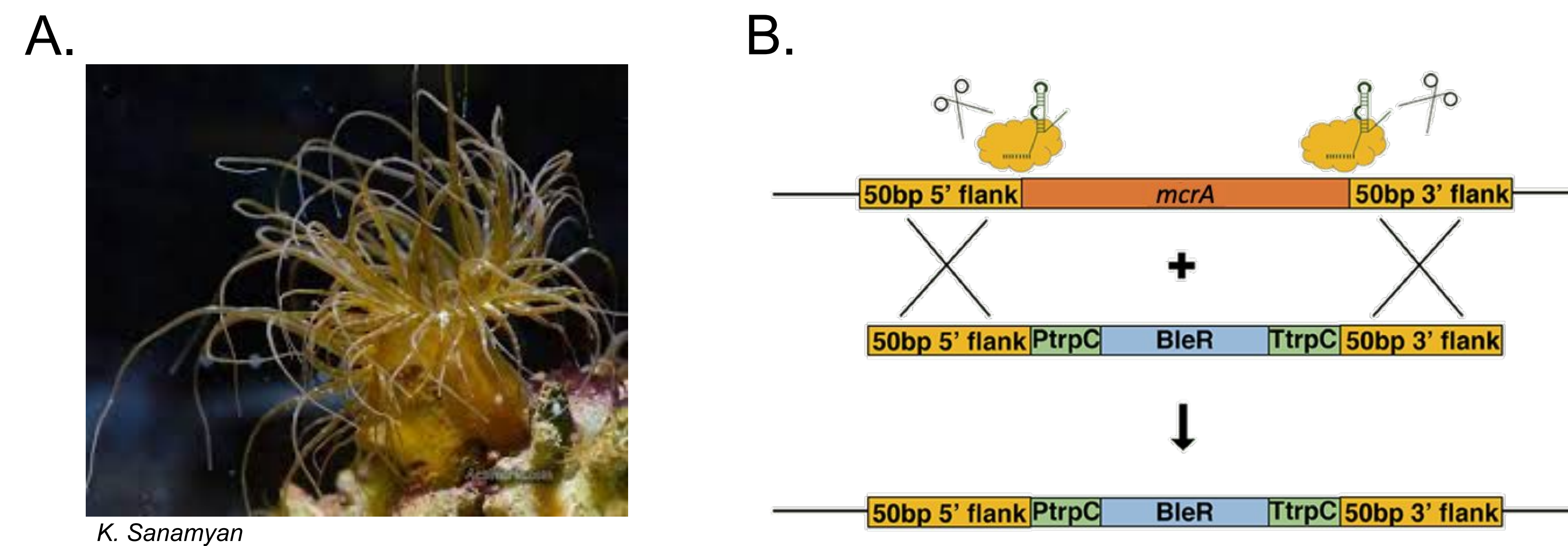


Figure 2. Origins of strains used in this project. (A) YAP001 was isolated from sea anemone *Exaiptasia diaphana* off the coast of Florida. (B) *mcrA*Δ strain generated using *in vitro* CRISPR-Cas9.

PCR – *mcrA* Knockout Confirmation

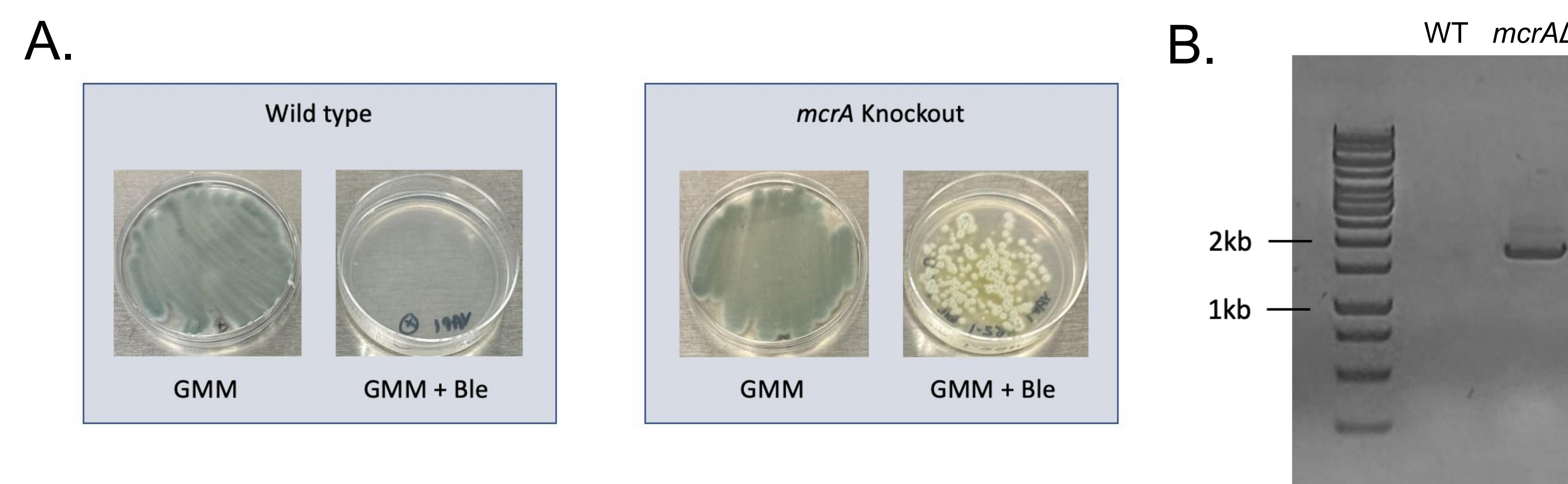


Figure 3. Using CRISPR-Cas9, we deleted *mcrA* and replaced it with a phleomycin resistance gene to select for transformants following transformation. (A) WT strain is susceptible to phleomycin (Ble), whereas *mcrA*Δ is resistant (B) Results of diagnostic PCR amplification of *mcrA* coding regions in WT and *mcrA*Δ strains.

Screening Different Conditions

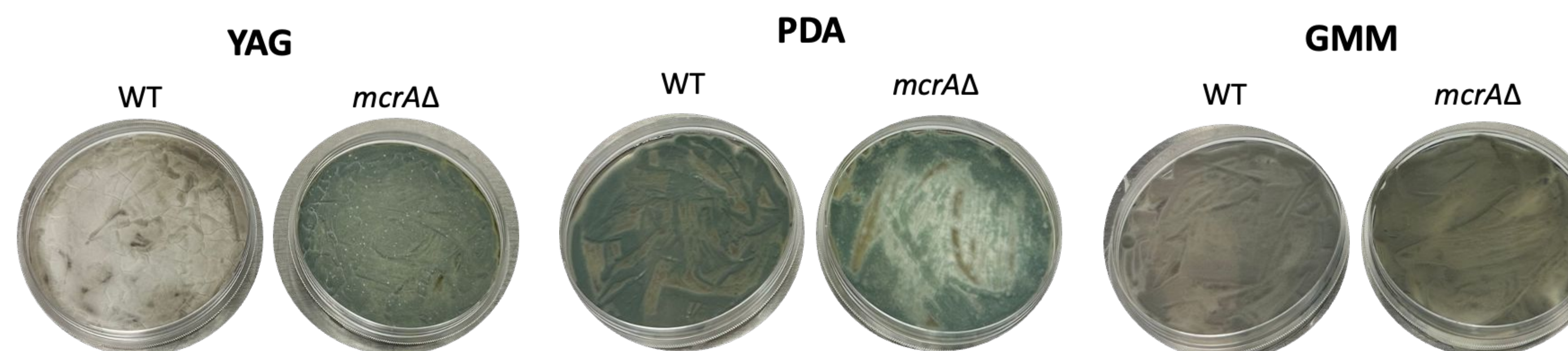


Figure 4. Growth of WT strain and *mcrA*Δ strain on different media conditions: Yeast extract agar medium (YAG), Potato Dextrose Agar (PDA), and Glucose Minimal Media (GMM). Fourteen different media conditions were tested. Above represents the three most promising conditions that we have chosen for further analysis.

HPLC Analysis of WT and *mcrA*Δ

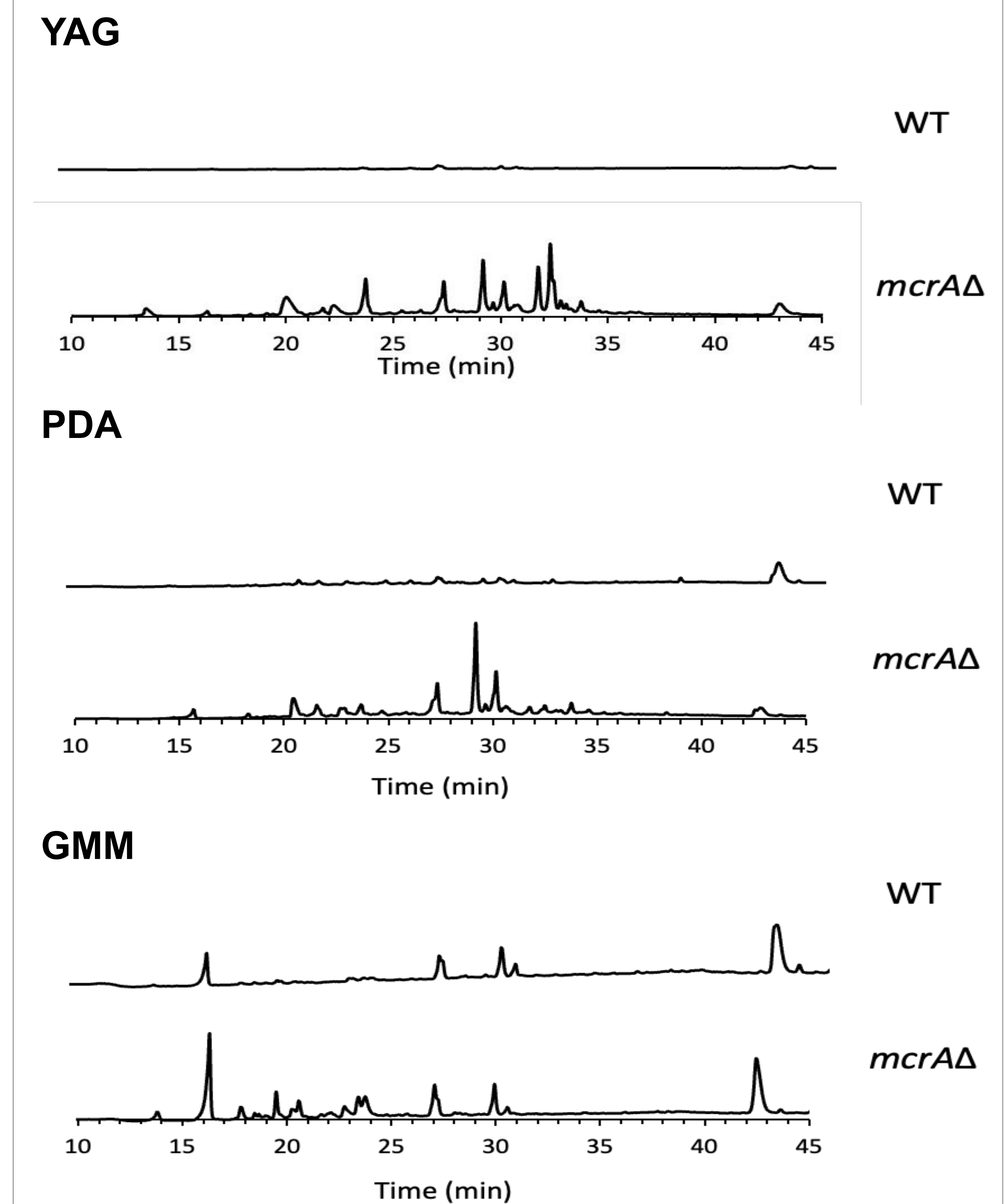


Figure 5. Results from HPLC for both strains grown on different media. This analysis allows us to check for the upregulation of secondary metabolites after deleting *mcrA*.

Summary

Our diagnostic PCR test results affirm that we successfully modified the genome sequence. We created a natural product library with a range of secondary metabolites that are potentially beneficial to treating Alzheimer's disease. We were also able to identify YAG, PDA, and GMM as potential culture conditions to scale up, as the compounds of the *mcrA*Δ strain was more intensely upregulated. A key next step is implementing the bioactivity seeding assay with tau protein to determine the anti-Alzheimer's properties of the secondary metabolites found.

References

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- photo of K. Sanamyan: specimen from reef tank