Abstract

Hydrocarbons are organic molecules composed of carbon and hydrogen. Specifically, linear hydrocarbons are predominantly found on the cuticle of plants, providing protection from the sun, insects, and water loss. These linear hydrocarbons are desirable molecules for the production of bio-renewable fuels. Several proteins have been identified that directly impact hydrocarbon synthesis in discrete organs of plants, including GLOSSY1 from maize and CER1 from Arabidopsis. These proteins were previously expressed and purified in the Nikolau laboratory and used to raise monoclonal antibodies in mice at Iowa State University’s Hybridoma Facility. In this project, we used an ELISA (Enzyme-linked immunosorbent assay) to screen 184 different fusion cell lines for antibodies against GLOSSY1 and CER1. The cells that screened positive with ELISA will be re-screened using western blots to identify the antibodies with the highest affinity for their respective proteins. The final monoclonal antibodies will be used for immuno-pull down experiments, to identify binding partners and help elucidate the function of these proteins. Also, these antibodies will be sent to collaborators to confirm heterologous expression of GLOSSY1 and CER1 in Debaryomyces hansenii (yeast). Through these efforts we hope to better understand the function of these proteins and how they act in the biosynthesis of hydrocarbons.

Research Objective

Mice serum is being screened to identify monoclonal antibodies with the best affinities to the maize GLOSSY1 and Arabidopsis CER1 proteins, which both function in the biosynthesis of hydrocarbons.

Introduction

The cuticle of plants is composed of small crystal-like cuticular waxes that uniformly coat the aerial tissue of a plant. The cuticle plays a major role in protecting the plant from UV damage, dust, pollen, and insects, and also prevents water loss. Major constituents that comprise the cuticle are linear hydrocarbons, which have potential applications to the development of advanced biofuels. While hydrocarbons are prevalent in the cuticle of many plants, the biosynthetic pathway by which these hydrocarbons are produced is not fully understood.

Two proteins of particular interest to our research group are GLOSSY1 from maize and CER1 from Arabidopsis. In the absence of either GLOSSY1 or CER1 (i.e. gene knock-outs), there is a dramatic reduction in the hydrocarbon fraction of the cuticular waxes. (Kooimeef, et al 1989 and Hansen et al 1997).

Methods

Protein Purification

We purified the His-tagged GLOSSY1 protein using a nickel affinity column. Unwanted proteins were removed by washing the column with increasing concentrations of imidazole. Our protein was then eluted from the column with 200 mM of imidazole and concentrated to 0.8 mg/mL.

Results/ Discussion

Purification of GLOSSY1

15% SDS-PAGE Stained with Coomassie Blue

Western Blot

We were successful in purifying GLOSSY1!!

ELISA

CER1

This is a representative of one of several ELISA plates. The wells that have a greenish-blue color are examples where the primary antibody bound tightly to the protein.

From a total of 194 antibodies screened by ELISA, we identified 16 antibodies against CER1 and 23 against GLOSSY1 that showed some level of interaction. In future work, the 41 candidates will be screened via western blot analysis to identify the antibodies that have the strongest affinities to the target proteins. Western blots have higher sensitivity than ELISA because we first separate the proteins, which allows for assessment of the affinity of each antibody for our protein of interest.

Future Work

The final monoclonal antibodies will be available for collaborators to confirm heterologous expression of GLOSSY1 and CER1 in Debaryomyces hansenii (yeast), as well as immuno-pull down experiments that will help us identify binding partners.

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