Sustainable Agriculture: Using Synthetic Biology to Engineer Higher Yields with Rhizobacteria

Author: Jessica Roper

Major: Biology

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

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**Abstract:** Due to increasing agricultural demand, scientists are exploring the use of synthetic biology to create a more efficient biological 3-O-MSI pathway and nitrogen fixation. Using rhizobacteria, scientists have created the first transkingdom synthetic pathway for rhizopines. These rhizopines are hypothesized to be able to be controlled by the host plant instead of the rhizobacteria. In the future the synthetic biology can be used to create a biofertilizer to replace artificial fertilizers, increase crop yields by increasing nutrient uptake to make a more sustainable agriculture.

**Introduction**

It is estimated that by 2050, the average agricultural yield will need to increase by 70% in order to maintain the current population incline (Ke et. al). Due to the lack of new places suitable for agriculture, higher crop yields are needed with limited negative environmental and ecological effects (Ke et. al). Such effects are already common practice when producing a higher yield plant, this is due to the increase in artificial fertilizers and depletion of soil nutrients (Ke et. al). Another main problem with increasing plant yields is the need for additional nitrogen. This nitrogen can only be used by plants after nitrogen fixation. Nitrogen fixation is commonly found in root microbiota, specifically in rhizobia (Geddes et. al). This bacteria type is believed to increase agricultural productivity by increasing nitrogen fixation, solubility of soil compounds, and overall root growth (Geddes et. al).

Through studying rhizobia and plant relationships, other studies have determined a compound called rhizopines to be evident in the nodules of a legume plant (Murphy et. al). These rhizopines are different from other compounds because they are controlled by the soil bacteria while many other natural occurring compounds come from the plant itself (Murphy et. al). While these rhizopines are a relatively new discovery, scientists have determined 2 compounds that are commonly found within, 3-O-MSI and SIA 1 (Murphy et. al). In past studies, these compounds have been studied and attempted to be applied using traditional methods (Ke et. al). Many past studies have failed to successfully recreate the biological pathway of these compounds (Ke et. al).

To attempt to recreate the compounds pathways, a new technique called synthetic biology is being used. Synthetic biology is the creation of a system of genetically modified organisms to solve human based needs (Gerd et. al). This technique uses genetic engineering to either produce a new biological function or reduce or enhance a previously existing biological system (Gerd et. al). It is hypothesized that this new technique will be able to successfully recreate the biological pathway of 3-O-MSI and SIA. In this study Barney Geddes, Ponraj Paramasivan, and other scientists attempted to use synthetic biology to create an alternative biosynthetic pathway using the compound 3-O-MSI and SIA in *Hordeum vulgare* and *Medicago truncatula* using bioluminescence to indicate transkingdom signaling between the plants and rhizobia.

**Recent Progress**

This study was broken into several main parts; emission of rhizopines in rhizobial symbiosis, explaining the 3-O-MSI biosynthetic pathway, creating the biosynthetic pathway, and indicating that transkingdom signaling between the plant and bacteria occurred. For the study to begin, the scientists had to determine the presence of rhizopines in rhizobial symbiosis. This was done by using a mocB promoter on the genetic strand to encode protein binding in the rhizopine compound. A mocR was used to regulate the ATP transporter in the rhizopine locus of the mocB gene (Geddes et. al). The sensor responded to the presence of SIA 1, an enantiomer thought to be found in rhizopines from recent studies (Geddes et. al). Other studies have also indicated that rhizopines are enantiomers themselves (Geddes et. al). This indicates that the biosensor mocB reacted to the presence of rhizopine exudation.  All plants used in this study except for *Arabidopsis thaliana* indicated the presence of rhizopines (Geddes et. al).

Next, the 3-O-MSI pathway was explored using *Sinorhizobium meliloti* L5-30. In other studies, this bacterium was shown to indicate an early presence of rhizopines and it was believed to be a part of the beginning of the biosynthetic pathway (Geddes et. al). Other studies have suggested that the rest of the pathway is composed of the mosDEF genes (Geddes et. al). This was also consistent with this study when the Gas chromatography mass spectrometry indicated mosBCDEF genes in the rhizobia that encoded for the 3-O-MSI pathway. These results indicated that the mosDEF oxidizes hydroxyl groups to make the 3-O-methyl-scyllo-incose 1D-6 compound that the biosynthetic pathway is named after. The study also suggested that the presence of a membrane and/or an electron transport chain is needed for the oxidation process to be completed (Geddes et. al).

The biosynthetic pathway was then synthesized using a similar approach to the biological pathway. The synthetic pathway uses bacterial inositol dehydrogenase to oxidize the myo-inositol 3. It also uses mosB gene to catalyze glutamate to generate SIA 1. The synthesized pathway was tested in *N. benthamiana* and was able to produce SIA 1. It is believed that this pathway will be able to be synthesized into plants and to work similarly to the natural pathway between rhizobacteria and plants. This pathway was the first synthetic pathway to be successful in transferring the process of rhizopine synthesis (Geddes et. al).

To determine if this process of synthetic biology would occur transkingdomly, scientists used agrobacterium to put SIA 1 into the soil with a bioluminescent as a tracker. When tested, the luminescence was no longer present in the soil near the roots of the *Medicago truncatula*. In *Hordeum vulgare*, the luminescence was found in the nodules of the roots. This indicates that the rhizopines that were originally created in the bacterial kingdom were successfully taken in by a plant kingdom (Geddes et. al). This is the first study to successfully transfer the rhizopine process transkingdomly (Geddes et. al.

**Discussion**

This study was the first to successfully have a transkingdom rhizopine synthetic pathway transfer (Geddes et. al). Scientists believe that using this newly created pathway they can maintain and engineer the rhizobacteria using the mocR. They believe that by engineering the bacteria and the plant to work together, they can create a higher agricultural yield, lower disease, and create a more efficient nitrogen fixation due to the increased presence of rhizopines.

Ideally the synthetic pathway could be used to create a biofertilizer. One way this is possible is through increasing the signal between plants and rhizobacteria to increase the overall amount of nitrogen taken or to switch the nitrogen dependency into carbon. There has also been a synthesized enzyme, Nif, which allows for nitrogen fixation to occur at a faster rate (Ke et. al). While the use of biofertilizers is often unsuccessful due to bacterial resistance, it would remove the need for artificial fertilizers and their negative environmental impacts (Ke et. al).

Another possible study in the future is using this synthetic pathway and agrobacterium to gain control of the genome by inducing the plant to create signals for the bacteria. These signals can indicate a need for more nutrients or to control the gene expression of the rhizobacteria. Scientists believe that by placing the control of the nutrients given by rhizobacteria in a host plants control then it will increase the yield. They also postulate the overgrowth penalties will be lower when in the plants control (Geddes et. al).

These biofertilizers and other synthetic biological discoveries have been limited due to the restrictions around synthetic biology being used in an open environment. In order for synthetic biology to be used, it must have a way for the organism or pathway to be eliminated. These are commonly called containment mechanisms and are placed in the genome. Many kill switches deprive an essential enzyme in order to eliminate the synthetic biology. Once killed these organisms can still cause harm to the surroundings by having their rDNA be picked up by other bacteria. One way to limit this is to create a gene flow barrier. While the use of synthetic biology is highly regulated, it is being controlled in specific areas and is not in the general environment (Gerd et. al).

Using these recent discoveries, the biosynthetic pathway can be used in the future with the right precautions to help control the agricultural demand needed to support the growing human population. The use of new synthetic biology can be used in numerous ways to limit the negative environmental affects typically caused by supporting a larger population.

**References**

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