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Fructose Mediated Catabolite Repression in *Sinorhizobium meliloti*

Clint Timmerman

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Submitted to the University of Redlands in partial fulfillment of the requirements for the degree
Bachelor of Science in the Department of Biology
Abstract

Plants are unable to utilize the N\textsubscript{2} form found in the soil environment. Bacteria known as rhizobia are capable of fixing this nitrogen into a form usable by the plant and initiate a symbiotic relationship in the legume root. One species of rhizobia, *Sinorhizobium meliloti*, has been thoroughly investigated in terms of its biochemical properties. Our research focuses on the concept of catabolite repression, a mechanism in which a preferred carbon source catabolized for growth represses the genes responsible for catabolizing a different carbon source such as lactose, as seen in *Escherichia coli*. *S. meliloti* displays succinate-mediated catabolite repression and glucose-mediated catabolite repression. Much research has focused on the mechanisms involved in succinate-mediated catabolite repression, while only a handful of these mechanisms are understood regarding glucose. Fructose may be an important entryway into the Entner-Doudoroff pathway with regards to glucose-mediated catabolite repression. This research seeks to answer the question of how far fructose must be metabolized in order to cause catabolite repression; is it in fact the process of phosphorylation or the formation of a metabolite such as 6-phosphogluconate that is necessary? In order to investigate this phenomenon, *S. meliloti* was subjected to *Tn5* mutagenesis and was screened in order to find a mutant that showed fructose-inhibited growth. This bacterium was also subjected to ampicillin enrichment, as another technique used to obtain a mutant that is unable to grow on fructose. These techniques led to a fructose growth mutant, SmCT2141. Catabolite repression was assessed based on the growth of the bacteria on media plates containing X-Gal, lactose and fructose, while quantitative analyses were performed via the measurement of β-Gal (the enzyme responsible for initial catabolization of lactose).
Introduction

In the soil environment, nitrogen exists mainly as atmospheric N\textsubscript{2}, which is triple bonded and unusable by plants, as they lack the enzyme needed to reduce this form of nitrogen into a form usable by the plant. Rhizobia are a family of soil bacteria that are able to fix nitrogen, converting it from its atmospheric N\textsubscript{2} form to ammonia, NH\textsubscript{3}. Although this may seem trivial, the role that rhizobia play is tremendously important.

Rhizobia infect legumes and take on a symbiotic role with the plant forming root nodules (Graham 2000). In this relationship the rhizobia provide nitrogen in the form of ammonia for the plant, while the plant provides carbon sources for the bacteria to use for growth. These plants require nitrogen to form amino acids and proteins. When these bacteria exist as saprophytes, or free living bacteria, they occupy an area known as the rhizosphere, which is a region surrounding the root of the plant about 1 mm wide (Lines-Kelly 2005). In the rhizosphere rhizobia sense flavonoids secreted by the plant, which are secondary metabolites created by the plant that act as chemical signals to the rhizobia. These chemical signals trigger the expression of nod factors, which are lipochitooligosaccharides responsible for signaling to the legume to begin root hair curling (Graham 2000). The infection thread (as seen in Figure 1 below) is an intracellular tube that is formed within the curled root hair. Once in the root cortex, the rhizobia continue to divide and grow, and they become bacteroids; which is a term that describes the rhizobia in their symbiotic state within the plant root cells (Timmers et. al 2000). As bacteria continue to divide, the plant cells do as well, creating vascular tissue that supplies the bacteria with other carbon sources and essential nutrients as well as carrying away waste much like veins and arteries in the human body.
After this has occurred, a full nodule forms as seen in the diagram below.

Figure 1. Cross section of a root showing the formation of a root nodule via root hairs

<http://imgkid.com/plant-vascular-tissue.shtml>

This nodule serves as a central location where the rhizobia bacteria are provided for nutritionally, and are able to provide nitrogen in a form that the legume can utilize. One such bacterium that participates in this symbiotic relationship is *Sinorhizobium meliloti*. This particular species of rhizobia has been studied for the past 40 years.
Research into this organism has looked at various aspects of its biochemical properties; ranging from nitrogen metabolism to usage of various carbon sources by *S. meliloti*. Carbon sources available to the rhizobium include glucose, succinate, fructose, mannose, gluconate, galactose and others (Geddes & Oresnik 2014). Rhizobia encounter several different environments on their way to becoming bacteroids within the plant nodule, the first of which is the bulk soil, next the rhizosphere, then the infection thread followed by the root cortex. By far the most nutrient rich, in terms of carbon sources, is the root nodule, which provides a mixture of the various carbon sources mentioned above. According to Geddes & Oresnik (2014), there is no prior research that pertains to the carbon compound make-up of the rhizosphere. Therefore, we can only assume that the sources mentioned above are present in varying concentrations.

Honing in on the uptake/utilization of these carbon sources for growth leads to the research into catabolite repression in this bacterium. Catabolite repression is a mechanism in which one carbon source, such as glucose, represses the genes responsible for catabolism of another carbon source. Essentially, the bacterium prefers one carbon source over another and shuts down the machinery responsible for using the other, less preferred carbon source (Ninfa & Atkinson 2000). This mechanism is significant as it allows the bacteria to only spend its energy on a source of carbon that will grant the largest payoff. If one carbon source requires less energy to break down or gives more back in terms of products and energy it should be preferred and the mechanisms used to break down other carbon sources should be shut down so that the bacteria can use the optimum source. This can be seen when looking at a growth curve illustrating catabolite repression, where it exhibits a lag phase in the curve, the flat area, where no growth occurs as the primary carbon source has been used up and the bacterium must now activate other catabolic mechanisms (Figure 2). This stops catabolite repression and the lac operon is activated.
as seen by the sharp increase in β-Gal activity in the bottom curve. Note that before this lag phase β-galactosidase (β-gal) activity is delayed/repressed. This is known as diauxic growth as it involves growth on two different carbon sources involving a shift in usage. Figure 2 displays both the O.D. readings, which correspond to bacterial growth, and β-Gal activity in S. meliloti undergoing succinate-mediated catabolite repression.

Figure 2. Succinate mediated catabolite repression seen in S. meliloti β-Gal activity is seen as the bottom curve, while optical density is the top curve (Ucker and Signer 1978).

In the model organism Escherichia coli, catabolite repression is achieved via glucose repressing the uptake and utilization of lactose, thereby repressing the lac operon. The mechanism here involves the interaction of the IIB, IIC, and IIA proteins of the glucose transport system with a phosphate source. Glucose is imported via these enzymes then phosphorylated via the IIA enzyme, which when dephosphorylated no longer activates adenylyl cyclase and inhibits lactose
from entering the cell via lactose permease, encoded by the lacY gene located on the lac operon (Figure 3). This mechanism, known as inducer exclusion, inhibits lactose uptake (Figure 3) (Bringhurst & Gage 2002). In addition, the phosphotransferase system (PTS) (Figure 3) involves halting the production of cAMP. cAMP acts as a positive regulator of the lac operon by binding to the CAP protein, which allows easier binding and transcription of RNA polymerase. Thus the lac operon is forced to stay in its repressed form, as the lactose molecule is unavailable to bind to the repressor and free its binding from the lac operon (Figure 4). Note that this is the mechanism used in *E. coli*, but not in *S. meliloti*.

Figure 3. Phosphotransferase system (PTS) and its inducer exclusion effect on lactose in *E.coli*.

Inducer exclusion was seen in *S. meliloti* but via a different mechanism, as *S. meliloti* lacks a complete PTS system (Geddes & Oresnik 2014, Garcia et. al 2010, Bringham & Gage 2002). In addition, *S. meliloti* has a different lac operon when compared to *E. coli*, as demonstrated by Jelesko and Leigh (1994). There is no CAP protein which normally acts as an inducer for the lac operon in *E. coli*; this lac operon also contains a different set of genes, some of whose functions are unknown, (Figure 5).

Figure 4. Lac operon in *E. coli* in both repressed and unpressed form

<http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-23/23_03.jpg>
Figure 5. Lac operon of *S. meliloti*. Lac Z encoding β-Gal, Lac R a repressor–like protein (Jelesko & Leigh 1994).

Much like *E. coli*, *S. meliloti* utilizes glucose-mediated catabolite repression (Jelesko and Leigh 1994), but is also known to undergo succinate mediated catabolite repression (Ucker & Signer 1978). Glucose mediated catabolite repression is interesting as the machinery within *S. meliloti* is not the same as *E. coli*. Our research raises the question: what has to happen to glucose metabolically to induce glucose mediated catabolite repression? This would most likely have to take place in the Entner Doudoroff pathway within *S. meliloti* as this is the primary pathway for glucose metabolism, taking glucose-6-phosphate and transforming it into 6-phosphogluconolactone, 6-phosphogluconate, and 2-keto-3-deoxy-6-phosphogluconate (Figure 6) (Geddes & Oresnik 2014).
Returning to succinate mediated catabolite repression, this occurs via succinate which is a tricarboxylic acid cycle intermediate that is used (by the citric acid cycle) to generate NADH for aerobic energy production, which is essential for *S. meliloti*. NADH plays a key role in the electron transport chain, which is part of aerobic respiration; *S. meliloti* cannot grow anaerobically. The mechanism for succinate-mediated catabolite repression (SMCR), remains to be conclusively determined; however various characteristics of regulation have been identified. HprK, an enzyme in the PTS system has been shown to regulate SMCR in *S. meliloti* (Pinedo and Gage (2009). Once succinate enters the cell via the DctA membrane import protein, it has
some effect on HPrK. In the presence of succinate, HPrK does not phosphorylate the serine residue on HPr, resulting in a building up of HPr with a phosphorylated histidine residue. This form of HPr results in SMCR, but is not the sole reason for SMCR activation; as explained by Pinedo and Gage (2009) there is another mechanism that induces SMCR. The incomplete proposed mechanism is shown in Figure 7. In this version of the PTS mechanism, the EI enzyme is present; however the EII enzyme is not. Inducer exclusion in *S. meliloti* is accomplished by inhibiting the lactose permease protein via succinate mediated catabolite repression, the specific proteins involved are unknown and hence the mechanism is incomplete.

![Proposed mechanism of succinate mediated catabolite repression in *S. meliloti*.](image)

**Figure 7.** Proposed mechanism of succinate mediated catabolite repression in *S. meliloti*.

(Pinedo and Gage 2009).
As explained earlier, phosphorylation is key for catabolite repression in *E. coli*, but it could be the generation of some later metabolite that causes catabolite repression in *S. meliloti*. The reasoning behind this logic is data found in the saprotrophic soil bacterium *Pseudomonas putida* that also exhibits catabolite repression. Similar to *S. meliloti*, *P. putida* prefers succinate as its primary carbon source. This bacterium uses 2-keto-3-deoxy-6-phosphogluconate (KDPG) as the signal molecule for catabolite repression of the aromatic phenylacetic acid (Kim et. al 2009). KDPG is a breakdown product of glucose that occurs in the Entner-Doudoroff pathway. Thus, the key component to catabolite repression in *S. meliloti* can simply be phosphorylation or it could rely on a breakdown product somewhere down the road of the Entner-Doudoroff pathway.

Fructose is known to cause catabolite repression as well, which is the focus of this research (Artmann et. al 2012). Fructose is a monosaccharide, frequently bound to glucose via glycosidic bond forming the molecule sucrose; much as glucose and galactose form lactose. *S. meliloti* imports fructose via an ATP-binding cassette (ABC) mechanism (Lambert et. al 2001). ABC transporters utilize ATP hydrolysis to drive active transport of the fructose molecule across the cell membrane. This begins when it is phosphorylated much like glucose but via a different enzyme, fructokinase, which converts fructose to fructose-6-phosphate (Gardiol et. al 1980). Fructose-6-phosphate is then converted to glucose-6-phosphate via phosphoglucoisomerase (Arias et. al 1979). This glucose-6-phosphate molecule is further broken down via the Entner-Doudoroff pathway. Alcohol sugars such as mannitol and sorbitol are oxidized to fructose and then follow the fructose metabolic pathway. Figure 8 below shows the catabolism pathways for *S. meliloti* (Gardiol et. al 1980).
FK (Fructokinase) and PGI (Phosphoglucoisomerase) are the main enzymes responsible for fructose breakdown (Arias et., al 1979).

In glucose mediated catabolite repression, either an Entner-Doudoroff pathway intermediate (as seen in *P. putida*) or a simple phosphorylation step such as the conversion of fructose to fructose-6-phosphate may be responsible for catabolite repression. These two reasons are the most likely mechanisms for catabolite repression in *S. meliloti* (Artmann et., al 2012).

The main question of this research is: Is fructose mediated catabolite repression in *S. meliloti* caused at the beginning via simple phosphorylation or does fructose have to be converted to a glucose metabolite to cause catabolite repression?
Objectives

The research that I have been conducting focuses on fructose mediated catabolite repression in *S. meliloti*. I am currently trying to determine how far fructose has to be metabolized to cause repression of the genes responsible of utilizing other carbon sources such as lactose. This involves focusing on two main enzymes: fructokinase (FK) and phosphoglucoseisomerase (PGI). The first objective was to obtain a mutant strain that lacks or is deficient in either of these enzymes, as it is imperative to my work in order to determine what affect these enzymes have on catabolite repression compared to normal wild-type strains. The second objective was to characterize all mutants I obtained, meaning they would be classified according to what specific mutation they have. Lastly, these mutants were to be analyzed in terms of catabolite repression; growing them on a combination of fructose and lactose as well as a variety of other sugars to determine if catabolite repression is occurring, under which conditions it is occurring, and at what steps in catabolism.

Methods

Media.

The two growth media used in this project were LB and M9. A 1 liter volume of M9 required 42 mM Na$_2$HPO$_4$·7H$_2$O, 22 mM KH$_2$PO$_4$, 8 mM NH$_4$Cl, and 9 mM NaCl (Miller 1972). To conserve reagents, M9 was made at a 2X concentration. This mixture was autoclaved to ensure sterility as well as making sure the salts dissolve into solution. Agar was also autoclaved separately at the same time in deionized water so that the two could be combined at a hot temperature to prevent the agar from cooling down and solidifying too quickly (for a final concentration of Agar 1.5%). M9 also includes 1 mM MgSO$_4$, 0.25 mM CaCl$_2$, 5 ng/mL CoCl$_2$. 
1 ug/mL biotin, .0025% tryptone, along with .4% of a carbon source that was filter sterilized using a 0.2 micron filter (Miller 1972, Glazenbrook & Walker 1991). Because these solutions were already sterile, they were combined into a small test tube in the sterile hood and added to the M9 solution after it was autoclaved. Making 1 L of LB media for plates required 15 g agar, 5 g yeast extract, 10 g NaCl, and 10 g tryptone. This mixture was added to 1 liter of deionized water in a large 2 liter flask, then autoclaved. This process results in roughly 40 petri dishes of media. When making selection plates 400 ug/mL streptomycin and 200 ug/mL neomycin were added to the mixture. Liquid media was similar, only lacking agar.

**Tn5 Mutagenesis.**

*Tn5* mutagenesis required taking the *Tn5* plasmid from bacteria, such as the *E. coli* strain MM294/PRK602, and conjugating with *S. meliloti*. The *Tn5* confers resistance to neomycin while inserting randomly into the chromosomal DNA of bacteria that it has conjugated with. This was achieved by growing both strains of bacteria in 16 mm test tubes containing 4 mL of LB overnight in a 30 °C water shaker bath, neomycin was added to the *E. coli* tube to ensure the plasmid was retained. 500 uL of *S. meliloti* 1021 (wild type) or *E. coli* were pipeted into microfuge tubes and centrifuged at 12000 RPM for 1 minute. The supernatant was then poured off and the pellet was resuspended in 500 uL of LB. The tube was centrifuged once again followed by pouring off the supernatant, and the pellet was resuspended in leftover supernatant (approximately 100 uL). One 1021 tube and one MM294/PRK602 tube were combined for conjugation and allowed to interact for 5-10 minutes. Then 17 uL of MM294/PRK602, 1021, and the combination of both were spread onto filters that lie on LB plates. Two filters/plates were used for the 1021, MM294/PRK602 mixture to maximize the chance of obtaining conjugated
bacteria. These were allowed to grow overnight at 30 °C. Each filter was vortexed in a test tube in 1 mL of DI water and 1 mL of M9 salts (Miller 1972).

After this step the two main techniques, use of selections plates and ampicillin enrichment for obtaining mutants were used. The first involved spreading a dilution onto LB supplemented with fructose, streptomycin and neomycin at a concentration of .4%. PGI mutants should grow slower on these plates, appearing as small colonies known as “pukies”. These pukies were picked and patched via a sterile toothpick to LB plates and allowed to grow for a couple of days. These colonies were then replica plated to both M9 glucose and M9 fructose plates to determine if fructose minus mutants were obtained. The second technique required spreading the cells onto M9 succinate plates, later changed to M9 glucose due to PGI issues. These were then scraped and followed up with the ampicillin enrichment described in the next paragraph. Volumes pipeted onto plates were 50 uL for 1021 and MM294/PRK602, and 10 uL for each of the conjugation mixtures. Two plates served as controls, while 28 others were used for the conjugation strains.

Ampicillin Enrichment.

The second technique, Ampicillin enrichment, uses the antibiotic ampicillin, which is similar to penicillin in that it inhibits cell wall production by the bacteria, leading to cell lysis of the cells that are actively replicating. Following the Tn5 mutagenesis procedure, scraped colonies were placed into 4 mL of M9 fructose liquid media. This was grown for 2-3 hours in a 30 °C water shaker bath, which is the around half the doubling time of S. meliloti. After this time had transpired, ampicillin was added to this growth tube at a concentration of 100 ug/mL. This concentration was established by various growth and kill tests performed at the beginning of this
investigation. Before ampicillin was added, the concentrated cell solution was diluted at a factor of at least 1:1000 due to this concentration of ampicillin being less effective when cell concentrations are very high. Ampicillin kills cells that are replicating, while leaving dormant bacterium intact. Thus, the only bacteria that survive this treatment should theoretically be mutants unable to grow in fructose (Miller 1972). These bacteria were then diluted and spread onto M9 glucose plates.

SmCT2141 was re-streaked several times onto LB to obtain single colony isolation. To confirm its inability to grow on fructose, it was streaked onto both M9 Glucose and M9 Fructose. Aside from plate data, growth data was also obtained. This was done by taking SmCT2141 (fructose mutant), and growing it in 4mL of LB, LB+glucose, and LB+fructose and taking absorbance readings at 600nm with a Spectrophotometer every two to three hours. Starting various growths at different times was done to ensure one set of data could be obtained and used to generate a growth curve.

In order to assess how catabolite repression is affected in these strains versus the wild type, the rhizobium was grown on a combination of carbon sources. A quick preliminary experiment performed to observe catabolite repression with the mutant involved growing SmCT241 and 1021 side by side on four different growth plates. Each contained LB, lactose at a concentration of 0.02%, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) at a concentration of 40 mg/mL. Each plate also contained either fructose, glucose, succinate, or no carbon source other than lactose at a concentration of 0.4%. These plates would give a rough sketch of what was going on in terms of catabolite repression. Growth on liquid media was also measured and will continue to provide growth rate data, concentrations of carbon sources were 0.4% for the corresponding carbon source. In addition other carbon sources will be used such as
gluconate, succinate, and galactose to assess their effects on the lac operon, as well as provide control replicates for the experiment. In order to determine if the lac operon is being repressed, β-galactosidase assays will be performed. This measurement determines the activity of the enzyme responsible for breaking down lactose into glucose and galactose which are usable carbon sources for the bacteria. β-Gal assays were performed, cells were suspended in Z buffer along with 1 drop of toluene to permeabilize the cells. The cells were then placed in a water shaker bath for 1 hour at 30 °C to remove any toluene and then at 28 °C without shaking for the assay. 0.2 mL of 4 mg/mL Ortho-Nitrophenyl-β-galactoside (ONPG) was added to the solution. The time of the reaction was recorded, and the reaction was stopped via the addition of .5 mL 1M Na₂CO₃ after the solution turned yellow (Miller 1972). This yellow color occurs due to β-gal cleaving ONPG to galactose and ortho-nitrophenol; the ortho-nitrophenol transmits yellow light. This activity was measured by the spectrophotometer at a wavelength of 420, 550 and 600 nanometers. Combining this data with the growth curve and plate data allowed me to determine what is occurring in terms of catabolite repression in both the mutant and the wild type versions of S. meliloti.

Results

To assess catabolite repression in S. meliloti, a mutant strain of fructose had to be obtained. This was done through the use of Tn5 mutagenesis. After growth on the LB+Fructose plates which also contained streptomycin and neomycin, pukies were picked and patched, roughly 10 per plate. Figure 9 shows pukies obtained while figure 10 shows successful picking and patching.
Figure 9. *S. meliloti* Colonies on LB plate, smaller colonies are deemed pukies (red circle) and removed from the plate to be tested for fructose growth capability
Figure 10. Possible *S. meliloti* Fructose mutant on M9 fructose plate (red circle).

Figure 11. LB plate containing possible *S.meliloti* fructose mutants placed on sterile velvet before replica-plating
After picking and patching, replica plating was used to transfer large numbers of colonies quickly and efficiently, (as seen in figure 11). After this method was followed one colony exhibited no growth on M9 fructose, shown in Figure 12. This colony was traced back to the LB plate it was replica-plated from and picked and patched to an LB plate and later streaked on M9 glucose where it grew, as well as M9 fructose where it was unable to grow. This mutant strain was named SmCT2141 Figure 13 shows the streaking of SmCT2141 onto both M9 glucose and M9 fructose

Figure 12. M9 fructose plate containing Sm1021 colonies, red circle corresponds to fructose growth mutant SmCT2141
Figure 13. SmCT2141 grown on M9 Glucose (left) and M9 Fructose (right)

The mutant strain is able to grow on glucose (white streaks), while unable to grow on fructose, confirming SmCT2141 as a fructose minus mutant.

In developing the ampicillin enrichment technique, 100 ug/mL was the concentration deemed to be sufficient at killing dividing bacteria based on preliminary trials as stated above. This was determined by the data obtained in figure 15.
Figure 15. *Sm1021* grown with varying concentrations of ampicillin. Over a period of 6 hours, taking absorbance readings every 3 hours.

When subjected to ampicillin, *Sm1021* was unable to grow in LB; 100 ug/mL was chosen as the appropriate concentration as it was able to definitively kill all growing bacteria while also being at a high enough concentration to kill an unexpectedly large concentration of bacteria. The next highest concentration, 200 ug/mL, seemed to be overkill and a waste of ampicillin.

However, the technique requires dilution as learned in the second ampicillin experiment. If a dilution was not used before adding ampicillin, the bacteria concentration in the tube was too high, and the compound could not kill all of the growing cells. Another experiment was performed to ensure the ampicillin process was working correctly, this involved combining SmCT2141 and 1021 and subjecting them to the enrichment, this process yielded a SmCT2141 colony as expected from the dilution calculations, therefore the ampicillin killing is working...
correctly. Thus far the ampicillin enrichment procedure has not yielded any fructose mutants but this technique will continue to be developed in the hopes of obtaining the desired result.

The first round of ampicillin enrichment yielded no fructose minus mutants, so an additional ampicillin enrichment was performed that did not involve a dilution before adding ampicillin in the hopes that more mutants would be obtained from the process. However, this was not the case as colonies that were replica plated from the M9 glucose plates to M9 fructose plates showed growth as seen in Figure 14.

![Figure 14](image_url)

Figure 14. *S. meliloti* growth after being subjecting to ampicillin enrichment, spread onto M9 Glucose (right) and M9 Fructose (left)
Growth data was also obtained for the mutant as described in the methods section. Figure 16 below shows the growth data for SmCT2141 in LB, LB+ glucose and LB+ fructose over a 21 hour period.

![Absorbance vs. Time of Various SmCT2141](image)

Figure 16. Growth curve data for SmCT2141 grown in LB, LB+Fructose (LBF), and LB+Glucose (LBG) for 21 hours.

These data cannot be relied upon for its growth measurements involving LB and glucose as the starting absorbance was much higher than both LB and LBF. In this growth period, SmCT2141 does not show a large increase in growth rate when fructose is added to LB. When comparing it to that of LB alone, the plate data confirms that this mutant is indeed fructose minus.

In order to observe if any catabolite repression occurs in mutant SmCT2141, it was streaked onto LB+XGal plates described in the methods section. This lead to Figure 17, which is a qualitative measurement of B-Gal activity.
Figure 17. SmCT2141 and Sm1021 streaked onto LB+Xgal plates containing Fructose (Top), Glucose (Bottom left) and Lactose (Bottom right). Sm1021 labeled as WT.

The final experiment performed was a quantitative β-Galactosidase assay. This test was performed according to the Miller procedure (1972). This resulted in the results below; each sample contained .2% of each carbon source.
<table>
<thead>
<tr>
<th>Sample</th>
<th>OD600</th>
<th>β-Gal Activity</th>
<th>Standard Deviation</th>
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<tr>
<td>1021 Lactose</td>
<td>.384</td>
<td>108.1</td>
<td>7.16</td>
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<tr>
<td>1021 Glucose+Lactose</td>
<td>.418</td>
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<td>1021 Fructose+Lactose</td>
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<td>1021 Lactose</td>
<td>.478</td>
<td>118.6</td>
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</table>

Table 1. β-Galactosidase activity of 1021 and 2141 in various carbon sources. B-Gal activity is an average of 2-3 readings, OD600 is absorbance reading at time of harvest.

Discussion

The ampicillin enrichment process was attempted with little success, however after reviewing the procedure followed it was noted that ammonium chloride was not added to the M9 glucose plates or liquid medium used in the process. Thus, it can be assumed that this lack of nitrogen for the bacteria prevented their growth on such media leading to the lack of mutants.
from the enrichment technique. Using mutant SmCT2141 mixed with Sm1021 one colony was obtained after retrying ampicillin enrichment with the correct amount of ammonium chloride. This data suggests that this procedure may in fact be effective for obtaining growth mutants.

After using Tn5 mutagenesis, a fructose minus mutant was obtained and named SmCT2141. The purpose of obtaining this mutant was to characterize its mutation and observe its level, if any, of fructose mediated catabolite repression. Thus far the mutant has not been definitively characterized, but based on the growth data it seems it is not a PGI mutant, however this remains to be confirmed. PGI mutants show an inhibition of growth in the presence of fructose (Arias et. al 1979), and looking at the growth data this does not seem to be the case, but this experiment was done in LB instead of M9 glucose so this fact remains to be proven. The quick qualitative B-gal assay involving growth on LB+XGal suggests that the mutant does not show catabolite repression. It should be noted that the picture taken corresponds to a time period in which most of the fructose was already used, hence the blue coloring in the wild type streak. However, to what level or extent remains to be determined from the B-gal assays that will be performed. The final experiment that measured the β-Gal activity on a quantitative level gave interesting results. In both strains, the activity units were similar when grown solely in lactose. However, when grown in fructose and lactose, SmCT2141 shows a higher level of β-Gal activity than the 1021 strain, 30.3 and 2.92 units respectively. Although this was expected, the level of activity is not as high as the lactose only growth which suggests that catabolite repression is still occurring. The reason for this may be that the bacterium utilizes two mechanisms for fructose mediated catabolite repression, phosphorylation as well as some intermediate in the Entner-Doudoroff pathway causing catabolite repression. It also seems the fructose mutation in SmCT2141 has altered the glucose effect on catabolite repression as the glucose+lactose sample
shows less than half the activity compared to the same conditions in the 1021 strain. It must be mentioned that this experiment was only performed once; therefore the data obtained cannot be relied upon as definitive. Replicating this experiment will be necessary to confirm hypotheses.

Research will continue on this project, using techniques such as β-Gal assays and growth on plates containing X-gal, lactose, and carbon sources to answer the main question of where in the fructose pathway catabolite repression is activated. In addition to this, the ampicillin enrichment technique will be modified in the hopes of obtaining fructose mutants so that in the future it can be used as a much more efficient, faster way of obtaining mutant bacteria unable to grown on certain media. More growth data will be obtained using wild type controls to better affirm SmCT2141’s fructose minus phenotype. Lastly, in order to combat the springtime mold spores, a sterile box will be used when streaking or spreading bacteria onto growth plates to avoid contamination.

*Sinorhizobium meliloti* is a rhizobium bacterium capable of fixating nitrogen, allowing for a symbiotic relationship with legumes. However, this vastly understates the bacterium’s significance. Understanding the symbiotic relationship between rhizobia and their plant host is not only interesting but also important. Many legumes rely on this relationship in order to survive and thus need this family of bacteria to grow. The reason for this is that many plants do not receive nitrogen in the form they need, ammonia, and do not receive fertilizer or it is only given in small quantities at certain times of the year. The ability to manipulate these bacteria to nodulate more effectively allows for more productive plants such as alfalfa. In addition, controlling which rhizobia can invade and nodulate via catabolite repression is also a key tool that could be used. For example, if one strain is found that is better at nitrogen fixation than another, the rhizosphere or soil composition can be manipulated so that a carbon source that is
repressed by the original bacteria but preferred by the new bacteria is available. Thus, the newer more effective strain would be able to out-compete the other strain of rhizobia and effectively nodulate and provide for the legume host. The obvious effects here are that fertilizer will not be needed in great quantity, runoff of harmful chemicals into the water supply can be avoided, and a smaller ecological footprint made. Understanding the pathways and mechanisms involved in catabolite repression by *S. meliloti* could possibly allow one to further investigate other untested pathways and mechanisms involving other carbon sources. Using this knowledge may help in understanding catabolite repression by multiple sources, as well as the fact that further research may allow *S. meliloti* to act as a model organism for study much like *E. coli* has become, but as a soil bacterium instead.
References


