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Characterization of BPA Sensitive Strains in C. elegans

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Characterization of BPA Sensitive Strains in *C. elegans*

By

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Abstract

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Bisphenol A (BPA) has been present in our environment since 1891 when it was first synthesized by Aleksandr Dianin. BPA has since been a topic of interest due to the possibility of diseases associated in human consumption of the chemical. Interestingly, *Caenorhabditis elegans*, commonly known as C. *elegans* have shown to be able to tolerate the chemical. This organism is an advantageous model organism for research since they are simple organisms to study, cheap, produce a large brood size, and have a short life cycle. Previously a forward genetic screen was done to isolate 2000 healthy strains in order to determine which strains showed sensitivity to BPA. *bin-1*, a mutant strain, was isolated and was fully sequenced to determine the gene responsible for BPA sensitivity. It was found that *bin-1* was essential for BPA sensitivity because the BIN-1 protein caused the animals to be able to survive in the presence of BPA. The BIN-1 protein is predicted to function as a glycosylase, although this has yet to be tested. Research done by Nakajima has found that other animals such as freshwater algae and tobacco cell lines have been able to glycosylate BPA (Nakajima et al. 2004 and Nakajima et al. 2007). Therefore, my research is centered upon (1) characterizing BPA- sensitive strains found in a forward genetic screen and (2) prioritizing which strains are most sensitive to BPA. This was performed through daily sensitivity tests. I expected to find some strains to show sensitivity and some to not. With the results of my research, I will be able to determine which strains showed increased sensitivity and need to be further tested to see why they are showing sensitivity to BPA.

Background

1.1 BPA

Bisphenol A, commonly known as BPA, is a synthetic phenol that is readily found throughout the environment. In 2008, 52 million metric tons of BPA were made worldwide (Arnold 2013). BPA has been manufactured in many plastics and epoxy stains and impacts humans; a large volume of research has been conducted to characterize the impacts of BPA on humans. BPA was first synthesized in Russia in 1891 by Aleksandr Dianin by combining acetone and two similar phenols (Jalal et al. 2017). However, it was not used in manufacturing until around the 1950s. Thereafter, it was widely used in products such as bottles, plastic containers, and industrial products because of its flexibility, toughness, and water-resistance (Gao et al. 2014). However, because of its broad use and ability to leach into food and beverages that are in BPA-containing containers, it became a topic of interest. BPA has become a common environmental contaminant and has been linked to many human diseases and disorders, such as asthma, diabetes, obesity, cancer, and reproductive anomalies (Lang et al. 2008, Wang et al. 2008). Products that contain BPA polycarbonate products include, but are not limited to electronics, electrical goods, household products such as utensils, containers, and bottles, and products with epoxy resins that include car coatings and protective coverings in buildings (Arnold 2013). A significant amount of BPA appears to enter the environment during the production of BPA-containing products at industrial facilities. BPA containing waste that enters waste water treatment plants makes its way into adjacent aquatic environments. Median concentrations of BPA found in the water in North America and Europe were 0.081 μg/1 and 0.01 μg/1, respectively. To put these numbers into context, the 95th percentiles (i.e. that the

numbers are 95% higher than other continents) of the two continents are 0.47 μg/1 and 0.35 μg/1. Even though BPA had been around for a significant amount of time, in April 2008, the controversial topic hit national news because of the scientific, political, and economic important discussion based on the safety of the chemical. The public was informed of the possible health impacts associated with BPA exposure. In 2008, the Canadian government was the first to take action and categorize BPA as a toxin (Vogel 2009). Even though the European Food Safety Authority and the US Food and Drug Administration (FDA) announced that certain levels of exposure were deemed to be safe in 2006 ("Bisphenol A"). However, many did not want to take the chance, so stores began to stop selling plastic bottles that contained BPA. After, the United States Congress took a year to pass a bill saying that BPA could not be produced in baby bottles or containers for kids' food (Vogel 2009).

1.2 Health Impact

BPA negatively impacts human health. This is concerning due to being found in many objects because of its affordability. BPAt is found in numerous common products including the lining of canned foods, and dental sealants, which leads to the high incidence of human exposure. It has been found that 6.6 μg of BPA per person is consumed each day as a result of eating canned food. As BPA enters the bloodstream, it can interfere with the endocrine system and has been connected to many human diseases and disorders, such as asthma, diabetes, obesity, cancer, and reproductive anomalies (Lang et al. 2008, Wang et al. 2008). Specifically, it was found to impact the number of eggs that mature and can be fertilized (Ehrlich et al. (2012). Hunt et al. (2003) also noted that when mice are exposed to oral dosing each day, the chemical causes meiotic aneuploidy (i.e., an abnormal number of chromosomes). Furthermore, fetus postnatal

development along with sexual maturity were also negatively impacted in pregnant mice exposed to BPA (Sugiura-Ogasawara et al. 2005). Hunt et al. (2003) also found that when mice are exposed to BPA, there is a distinct increase in mitotic chromosome abnormalities and nondisjunction. Hunt et al. (2003) studied mice meiosis to determine how BPA was affecting the biological process; chromosomes do not line up correctly. These findings are important because such problems with alignment are directly linked to the incidence of aneuploidy in women.

Rodents are often used as a model for humans in drug studies. Even though rodents and humans metabolize waste differently, the movement of drugs throughout the body is quite similar. Therefore, the impact of BPA on rodent reproduction justifies the investigation into the effects of exposure to this chemical in humans. Therefore, it is not surprising that Seguira-Ogaswara et al. (2005) found a correlation between high concentrations of BPA and women who experience recurrent miscarriages, which can be a result of the levels of BPA found in a woman's body (Sugiura-Ogasawara et al. 2005). This could be linked because 40-70% of reported miscarriages are due to issues associated with aneuploidy, and aneuploidy was also observed rodents exposed to BPA (Hunt et al. 2003). In addition to miscarriage, chromosome abnormalities are associated with congenital defect and mental retardation (Hunt et al. 2003). Sugiura-Ogaswara et al. (2005), factors such as age, similar body mass index, occupation, and living environment were either kept constant or individuals were put into specific groups. They also found that women who were having 3-11 miscarriages had a higher concentration of BPA in their bodies. In addition to Suigiera- Ogasawara et al. (2005) research, Hunt (2003) found some similar things in mice.

Moreover, BPA is not only impacting female reproduction, it also has shown to impact male sperm count and function, which alters the ability to reproduce. Meeker et al. (2010)

performed a study on 190 men for four years. The authors found that when an increased amount of BPA was found in men's urine, those men had decreased sperm counts, decreased sperm motility, and sperm DNA was shown to be damaged (Meeker et al. 2010). Even though many studies have shown a correlation with increased BPA exposure and reproductive issues, there are still many areas that have not been tested.

1.3 *C. elegans*: Model Organism

Caenorhabditis elegans are a free-living, transparent nematode that are about 1mm in length, and thrives in temperate soil environments. Additionally, the animals can either be male or hermaphroditic. Hermaphrodite is animal that is female who produce sperm, and can self-fertilize. Before adulthood, these animals develop during four larval stages (Corsil et al. 2015). These organisms are an especially effective model used in a variety of biological disciplines, including genetics, cell biology, and developmental biology. Some of the advantages of working with *C. elegans* are that it is simple organism to study, cost inexpensive, reproduces quickly, and has a short life cycle. Since there is high homology, 38% of genes are similar, between humans and worms we can apply what we learn about *C. elegans* to a much broader context (Shaye and Greenwald 2011).

C. elegans has been shown to be surprisingly tolerant to BPA exposure (Allard and Colaiácovo 2010). Therefore, we want to understand how *C. elegans* can tolerate BPA exposure, and identify potential pathways used to excrete or detoxify BPA in the animal. A forward genetic screen was previously done by Allen and Kroetz to identify mutant strains of *C. elegans* that have increased sensitivity to BPA. My research focus is to better characterize these previously identified strains that have shown increased sensitivity to BPA.

1.4 BPA Impact on *C. elegans*

BPA has shown to impact mice, humans, and the *C. elegans* germline. When an organism is exposed to BPA, the toxin can cause sterility, the inability to have progeny, and affect survivorship. With BPA being found in many plastics and epoxy resins around us, it is hard to escape this chemical. Extensive research has been performed by Allard and Colaiácovo (2010) regarding how BPA impacts worms and have found that the process of meiosis is a primary target. Meiosis is the cellular process during which haploid eggs and sperm are made, and this process is vital for sexual reproduction and genetic variability.

Allard and Colaiácovo (2010) have found that exposure to BPA results in several errors inmeitotic functionality in *C. elegans*. These include difficulty with chromosomal pairing during meiosis, changes in how DNA double strand breaks are repaired, which impairs the following activation of DNA damage checkpoint kinases, and increased germ cell apoptosis. Although BPA has shown to be problematic in other animals, it was not clear if they would influence reproduction as a whole in *C. elegans*. Worms were exposed to varying amounts of BPA including 100μM, 500μM, and 1 mM concentrations. They also used an ethanol, cholesterol, and a culture method to analyze if reproduction of *C. elegans* was being impacted. Using ethanol, they were able to strongly see the phenotype due to how quickly BPA is able to dissolve in ethanol. Cholesterol has been found to cover up the endocrine- disrupting chemicals in *C. elegans*, so cholesterol was taken out of the medium. They found that the best "recipe" for the experiment was to expose the animals to BPA that was completely dissolved in ethanol with no presence of cholesterol, and 1 mM of BPA for four days. As a result of increasing amount of exposure to BPA, the number of progeny continued to decrease as in embryonic mortality increased (Allard and Colaiácovo 2010).

1.5 Previous Work in the Kroetz Laboratory

Significant previous work was conducted by Allen and Kroetz to look at the effects of BPA on reproduction in *Caenorhabditis elegans.* Allen began his thesis work by conducting a genetic screen to determine which gene(s) confer tolerance to BPA. He used ethyl methanesulfonate (EMS) mutagenesis at a concentration that causes the genome of each cell to have ~20 point mutations (Allen)

Allen followed an EMS protocol developed for *C. elegans*. First, Allen exposed the wild-type strain of *C.elegans* to EMS to mutate the animals of interest (Figure 2). After the exposure to EMS, the animals were washed repeatedly to remove EMS and the mutagenized animals were placed on a 10 cmNematode Growth Media (NGM) plate and allowed to recover at 15° C. The next day, Allen picked 60 young adult hermaphrodite mutagenized animals and placed 2 animals on an NGM plate, which resulted in 2 animals on 30 plates. Those animals will be called the parental (PO) generation for the rest of the discussion. These animals should have on average 20 different mutation in each of their germ cells. Four days later, after the parental generation was allowed to reproduce, the F1 generation was selected. These animals were heterozygous for the mutation, so the animals would have shown a normal phenotype if the mutation was recessive. Four L4s (the oldest larval stage before adulthood) hermaphrodites of the F1 generation were moved onto 30 new NGM plates resulting in 125 plates for a total of 500 animals. Picking only L4s would ensure that mating did not occur prior to being transferred to new plates. Four days after being in the 20° C incubator, a single L4 hermaphrodite was transferred onto a NGM plate totalling 2,000 F2 animals on 2,000 plates. These animals are the F2 generation, which would now, on average, have both recessive alleles for ¼ of the induced mutations or approximately 5 mutations per genome. To ensure good health, the number of progeny and the amount of food

source left on the NGM plate was evaluated. Healthy animals were the ones which (1) exhausted the food source as quickly as N2 (a distinct wild-type used in laboratories) wild-type animals and (2) showed a wild-type phenotype. The animals classified as unhealthy were disposed of. Next, the 1,080 animals labeled as healthy were then tested for BPA sensitivity. Allen transferred animals on NGM plates containing 1mM of BPA in ethanol and an ethanol control plate with a Q-tip. After adequate time for the animals to self-fertilize, the animals were observed for BPA sensitivity by (1) the amount of progeny and (2) the amount of food source exhausted. He then selected the 98 animals that were significantly healthier on the control than on the BPA containing media.

It was important to rule out as many strains of animals that are sensitive to BPA for reasons that are not specific to an interaction with BPA. Therefore, we wanted to determine which animals were sensitive to BPA but not a second, unrelated toxicant. These animals that are sensitive to toxicants in general would not inform us how BPA is mechanistically impacting in the worm. The second screen performed with boric acid was used to assure that the animals showing BPA sensitivity were actually sensitive to BPA and did not have other underlying conditions. An important factor dictating how *C. elegans* will react when exposed to BPA, is how their cuticle layer functions. The worm cuticle is the protective layer that surrounds a worm. It is extremely important to make sure that the cuticle is working properly as a barrier to chemicals. If not, this can skew results. If the animal showed sensitivity to BPA and boric acid, Allen could conclude that the animals are sensitive to chemicals and toxins in general rather than to BPA in particular, and these mutant strains could then be eliminated.

Allen continued to do further study on the 41 animals that showed BPA sensitivity and no sensitivity to boric acid. Later, Allen found inconsistencies in whether or not the mutant strains

continued to show sensitivity to BPA. He attempted a battery of tests to find one that had reproducible results. He found that the results were the most reproducible when he controlled for (1) the age of the animal tested and (2) a similar environment of the animal tested. Allen tested L1 stage worms, which is the first larval stage allowing for the most developmental exposure to BPA. To have similar environmental conditions, he first transferred a single L4 hermaphrodite onto a NGM plate in order to lay eggs for two days to a week, and tested L1 progeny for BPA sensitivity from this plate every day from the second day when enough L1s were present to test until the seventh day when there was no bacterial food source left and therefore the environmental conditions would no longer change daily. The environmental conditions that change each day would include the birth order of the animals, the amount of food present, and the presence of ascarosides, which are small molecules that act as pheromones, that are produced by *C. elegans* and regulate behavior (Edison 2009). Any of these environmental conditions, or a combination of them could influence the BPA sensitivity of the strains. Two to three days after the L1 were placed on a BPA- sensitive plate and a control plate he checked the survival rate of the L1 to adulthood. A strain was marked sensitive if 30% or less of the 20 animals survived on the BPA containing plate but more than 75% of the animals survived on the control plate. There was some conditional effect that certain strains were sensitive on some days, but not others. Though the idea of BPA sensitivity tests seemed crazy, the outcome was advantageous for Allen's project.

Out of the 41 strains he found to be sensitive, he further characterized one mutant strain which he named *bin-1.* In order to find which gene allows the worm to detoxify BPA, Allen performed eight consecutive back crosses between *bin-1* and the wild-type animal. Backcrossing is a process to "clean" or get rid of background mutations. In Allen's project, this was important

because during the EMS mutagenesis, the animals acquired many mutations and in order to proceed with his project, he wanted to obtain animals with the desired mutation. Each time a backcross is done, the genome of the mutant becomes increasingly more identical to the wild type, so the DNA is more like the wildtype. After the crossing was complete, he isolated the genomic DNA of the wild-type and *bin-1* strain and searched for those mutations that were in the protein coding areas that were present the *bin-1* strain and not the wild-type animal. Kroetz found that there were three mutations in protein coding genes in the *bin-1* strain, one of which was a nonsense mutation in F59C6.8. A nonsense mutation is characterized by a change in DNA that causes the protein to terminate translation before expected. This means that there is a stop codon in an unprecedented location, which results in shortened or non-effective proteins being expressed. To verify that the mutated F59C6.8 gene was causing the BPA sensitivity, Kroetz removed the entire open reading frame of the F59C6.8 gene in an otherwise wild-type animal using CRISPR, and the animals showed increased sensitivity to BPA. Therefore, the results showed that *bin-1* is essential for detoxifying BPA in *C. elegans.*

My project aims to find more strains that are reproducibly sensitive to BPA. This entails going through and characterizing the remaining 41 strains that displayed BPA sensitivity. Through the BPA sensitivity tests,i t is possible to be able to find more BPA intolerant nematodes or bin-2, bin-2, bin-3, etc.

Project Overview

We are interested in how *C. elegans* have shown a tolerance for BPA, so we want to identify the potential pathways that are used to help excrete or detoxify BPA in *C. elegans.* Previously, a genetic screen was performed in order to find which genes are allowing these animals to be tolerant to BPA. Furthermore, I am continuing to characterize the remaining strains of interest to determine their BPA sensitivity by employing daily BPA- sensitivity tests. Daily BPA sensitivity tests are employed to find out in which part of the worm's life cycle a strain is more sensitive to BPA. These tests were performed seven days a week. The test provided vital information which told us if the strain showed increased sensitivity to BPA or not.

The objectives of this study were to:

(1) characterize the two BPA- sensitive *C. elegans* strains A.4.2.1 and A.4.2.3 identified by Allen and Kroetz in a previous forward genetic screen.

(2) prioritize *C. elegans* strains that are most sensitive to BPA.

Experimental Methods/ Project Design

Naming Convention

To track the percentage of each strain of *C. elegans* that were mutagenized via EMS, we used a naming system. For strain A 4.2.1, the "A" indicated that this is the first screen performed in the Kroetz lab, "4" comes from the 4th plate of mutagenized animals isolated, "2" indicated the F1 generation, and the final number "1" comes from the F2 generation. Therefore A 4.2.1 and A 4.2.16 are derived from the same plate of F1 animals and perhaps the same animals, whereas A 4.3.16 and A 4.2.16 are derived from the same plate of mutagenized animals.

Determining BPA- Sensitivity

To determine BPA sensitivity of isolated strains of *C. elegans,* daily BPA sensitivity tests were performed. During these daily tests, one strain was tested at a time (Table 1). On day zero, we transferred one individual L4 (larval stage 4) animal of the desired strain onto a fresh NGM plate. On day two, 20 of her progeny in the first larval stage after hatching (L1) were transferred to a plate with BPA- containing medium; 20 L1 animals were also transferred onto an ethanol control plate. We included a control plate to ensure that if the animal showed sensitivity, we knew it was because of the mutation and not the particular strain. We continued to transfer twenty L1 animals onto BPA and ethanol control plates each day, until progeny exhausted the food source from the original day zero plate. The food source typically ran out on day seven after placing an L4 on an NGM plate. Two days after transferring the L1 animals onto the BPA and ethanol plates, we counted the animals that survived. Worm survival counts were used to determine sensitivity. A strain was deemed sensitive on an individual day if six or fewer animals (<33%) survived on the BPA plate and fifteen or more animals (>75%) survived on the ethanol

control plate. Although sensitivity should be consistent through time, some sources of observed variability in worm survival include: 1. worms can and often do crawl off of plates, 2. errors can occur in picking worms from plates, and 3. there can be unexplained variability in worm survival numbers.

Sanger Sequencing

Two strains that were identified to be BPA- sensitive were derived from the same F1 plate. These strains are A4.2.1, and A4.2.16. Previously the genetic mutation leading to BPA-sensitivity for A4.2.16 was determined to be a nonsense mutation in F59C6.8. We wanted to determine if A4.2.1 and A4.2.3 had the same nonsense mutation and so Sanger sequencing was used.

Results

BPA Sensitivity Tests

These additional experiments determined that some strains were in fact BPA- sensitive where other strains were no longer found to be BPA- sensitive in the more rigorous daily tests (Table 1). Some animals were completely insensitive, MBK33 showed sensitivity on everyday, but day two, while A10.2.13 showed increased sensitivity on day two of the study (Table 1). Variability is possible due to worms crawling off of plates, picking the wrong larval stage animal, or there can be some outside factor that is occurring that we just are not aware of.

Sanger Sequencing

Sequencing indicated that *bin-1* has a nonsense mutation (red box), but A.4.2.1 and A.4.2.3 surprisingly did not carry this mutation, suggesting that different mutation elsewhere in the genome was making the two strains sensitive to BPA (Figure 1).

Discussion and Future Direction

Classification of 41 BPA- sensitive strains

We began with a list of 41 strains that we wanted to test BPA sensitivity. Of the 41 strains, *bin-1* (A.4.2.16) was classified by Allen and Kroetz. Some of the remaining strains were not frozen and lost, and some were lost when the freezer lost power, and some were tested before my project began. A.4.2.1 and A.4.2.3 were two strains of interest that had been previously tested in which we believed would have the same mutation because both strains came from the same parent plate as *bin-1*, however through sequencing we found out that they did not have the same mutation. By the end of my work, we tested 10 strains including the N2 wild- type strain.

In the future, the strains that have show increase sensitivity through the daily BPA sensitivity test, will be followed up on. It is imperative to follow up on them because there can be something unique that is making them sensitive to BPA, which would deem interesting to study in more detail. A.4.2.1 and A.4.2.3 will be followed up on to confirm both strains are sensitive. Secondly, we would find what chromosome the mutation is on to determine the possible genes that can be making the animals tolerant to BPA. To narrow which chromosome the mutation is on, we would perform chromosomal location mapping. In addition, the two strains that we found to have sensitivity to BPA, A 4.2.1 and A 10.2.13, through the daily BPA sensitivity tests, will be followed up on by retesting to make sure sensitivity was not an error of some sort and then we would test what chromosome the mutation is on to further condense the possible genes that may be making the animal tolerant to BPA.

Additional Rationale for Testing

The main purpose of this work was to isolate strains that showed increased sensitivity to daily BPA tests. BPA tests were implemented because it was found to be the most reproducible test by Allen and Kroetz. This method allowed us to work in time increments each day. Secondly, L1 were chosen to be the most appropriate stage to be tested. This was done because L1s are the youngest larval stage to give the longest exposure to BPA. The embryo would not be an appropriate stage to test because they do not move, so we would not be able to tell if they are alive. In addition, we studied L1 progeny of a single mutant hermaphrodite was tested. In addition, future work could be done to test whether it is the birth order of the animals, the amount food present on a given plate, and/ or presence of ascarosides (i.e. small molecules with pheromone like action that could be impacting the BPA tolerance).

Tables and Figures

Table 1. Daily BPA Sensitivity test data. As you can see from the chart, some strains are reproducibly sensitive to BPA on specific days and not sensitive to BPA on other days. For example, bin-1 and MBK33 are sensitive on days 5 and 6, but not sensitive on day 3. It is important to determine a reproducible test of BPA sensitivity to test the rest of the strains.

				Days L1's were put on plates				
Date Started	Strain	Condition	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
8/31/20	N2	1 mM BPA	20	19	20	20	19	20
8/31/20	N2	EtOH control	20	20	20	20	19	20
9/7/20	A3.4.5	1 mM BPA	18	13	17	17	18	17
9/7/20	A3.4.5	EtOH control	19	18	20	18	20	19
9/21/20	A9.1.16	1 mM BPA	10	12	10	12	15	12
9/21/20	A9.1.16	EtOH control	20	19	19	15	19	20
9/31/2020	A10.2.2	1 mM BPA	18	19	15	10	17	16
9/31/2020	A10.2.2	EtOH control	19	18	20	20	20	18
10/8/20	A6.1.5	1 mM BPA	16	20	19	20	18	14
10/8/20	A6.1.5	EtOH control	20	18	20	20	20	20
10/8/20	A8.2.6	1 mM BPA	15	10	7	11	12	14
10/8/20	A8.2.6	EtOH control	20	20	20	19	19	20
10/19/20	A10.2.13	1 mM BPA	2	15	20	9	19	16
10/19/20	A10.2.13	EtOH control	19	19	19	20	19	19
10/22/20	MBK33	1 mM BPA	12	3	2	0	0	0
10/22/20	MBK33	EtOH control	18	18	17	20	19	20
10/25/20	A12.4.12	1 mM BPA	14	20	16	15	15	13
10/25/20	A12.4.12	EtOH control	19	20	18	20	20	19
10/25/20	A13.3.2	1 mM BPA	18	6	11	15	13	15
10/25/20	A13.3.2	EtOH control	20	19	18	20	20	19

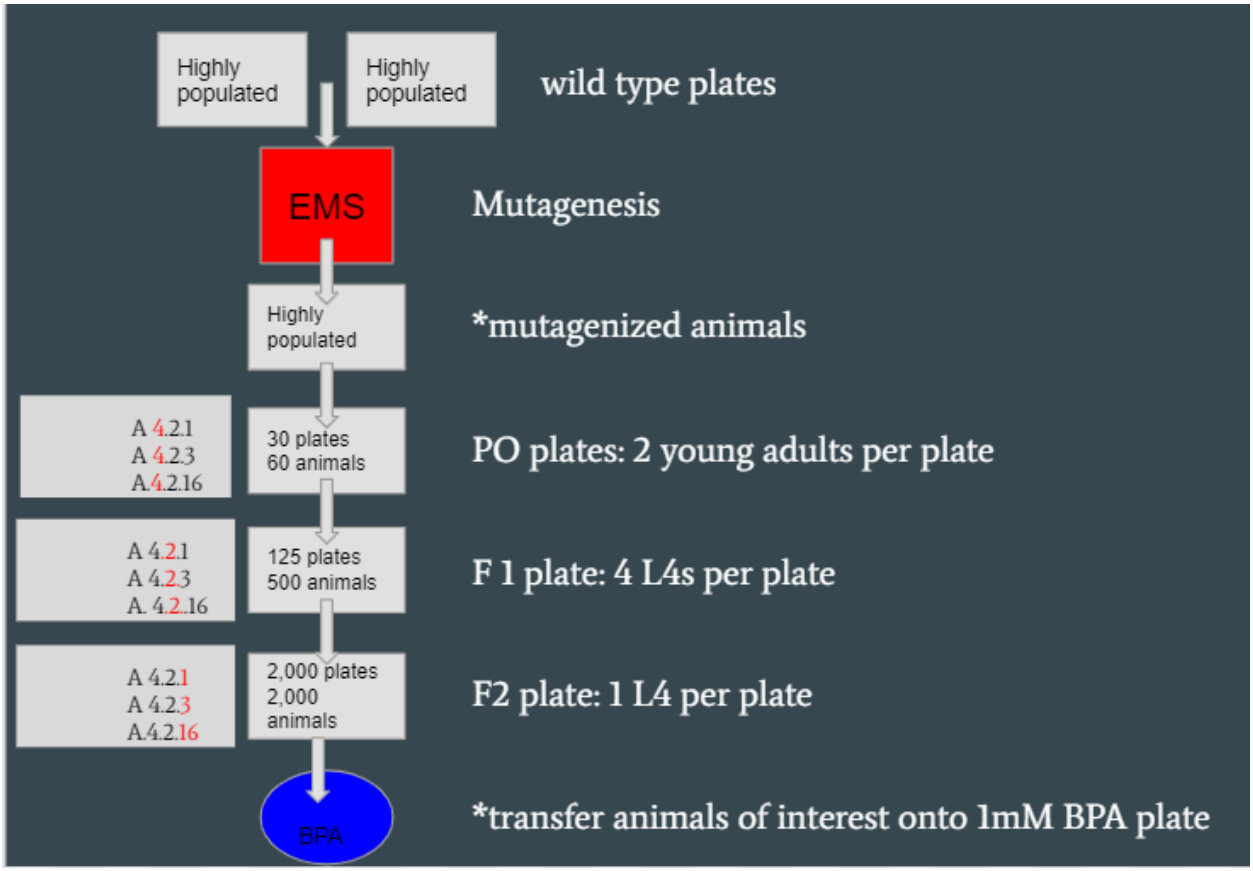

Figure 2. A diagram illustrating the ethyl methanesulfonate mutagenesis procedure. Squares represent NGM plate(s), while the REd box that is labeled “EMS” refers to the EMS that the worms were exposed to following the EMS protocol.

Figure 1. Sanger Sequencing Results found. The blue bos is highlighting the different nucleotides that each strain has. If you take a closer look *bin-1* has a stop codon mutation (red box), but A4.2.1 and A4.2.3 have different mutations. F59C6.8 is the wild type allele, so we sequenced it to show the wild- type DNA.

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Biographical Sketch

Giulianna I. Escobar was born in Marrero, Louisiana on June 22, 1999. She graduated from St. Mary's Dominican High School in 2017 and proceeded to obtain a Bachelor of Science degree in Biology with a minor in Spanish from the University of South Alabama in Mobile, Alabama in May 2021. During her time at South Alabama, she was in the Honors College, Active member of Mortar Board Honor Society, The Pre- PA Society, Alpha Epsilon Delta, Phi Mu Fraternity, and Eta Epsilon Delta Honor Society. She obtained the honor of being on the President's List for seven semesters and the Dean's list for one semester. During her time at the University, she served as the President of the Pre- PA Society (fall 2020- Spring 2021), Membership Director of Phi Mu Fraternity (Spring 2020-fall 2020), and Treasurer of the Pre- PA Society (fall 2019- Spring 2020). She also volunteered through her sorority's philanthropy, Children Miracle Network Hospitals, Alpha Epsilon Delta, and at Victory Health Partners, which is a non- profit healthcare clinic in Mobile. Her research focused on determining BPA sensitivity in *C. elegans* alongside Dr. Mary B. Kroetz. In May 2021, Giulianna earned a Bachelor of Science degree in Biology.