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Animal Posters

A-2000

Efficient Serum-free Cryopreservation of Cell Lines at -20°C and -80°C. CYNTHIA L. GOODMAN[1], Xu Han[2,4], Yuping Huang[1], Claire Dubos[1], Joseph Ringbauer, Jr[1], Megan M. Augustin[3], and David Stanley[1]. [1]USDA/ARS Biological Control of Insects Research Laboratory, 1503 S. Providence Rd., Columbia MO 65203; [2]University of Missouri, School of Medicine, One Hospital Drive N403, Columbia MO, 65211; [3]Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132; and [4]CryoCrate LLC, 1601 S. Providence Rd., Columbia, MO 65211. Email: cindy.goodman@ars.usda.gov

Optimizing the cryopreservation of cell lines for short-term and long-term uses is vital for biomedical and agricultural research. Current storage techniques often involve serum in the freezing media and specialized liquid nitrogen facilities, limiting the feasibility of cryopreservation. In this study, we used a serum-free commercially available cell cryopreservation medium, C80EZ®, which is designed for storage in standard laboratory freezers (-20°C to -80°C). C80EZ prevents cell aggregation and ice recrystallization during storage at -20°C and below. We worked with commercially available cell lines, including an insect cell line (Sf9), canine kidney cell lines (MDCK and 293T) and human cancer cell lines (MDA-MB-468 and PANC-1). Cells were frozen in a 1:1 mixture of C80EZ and serum-free cell culture medium with 5% DMSO at either -20°C (directly) or -80°C (using standard freezing devices). After long-term storage at -80°C (6 mos), the post-thaw viability of the Sf9 cells was higher than control groups using 90% v/v FBS in the cell culture medium plus 5% DMSO for liquid nitrogen storage (76.4± 2.4% alive, C80EZ+ DMSO; 48.7±1.0% alive, FBS+DMSO). The production of a steroid alkaloid, verazine, by post-thaw cells (stored 1 month, -80°C) resulted in similar verazine production levels in the C80EZ cryopreserved cells and unfrozen cells (2.8±0.4 ng/ml vs. 2.3±0.3 ng/ml). Combining C80EZ+DMSO with an antioxidant, lactobionate (LA), enhanced cell viability when stored at -20°C for two weeks: 76.4+2.4% alive for C80EZ+ DMSO+LA vs 34.1+1.1% alive for FBS+DMSO+LA. Mammalian cell lines registered >90% post-thaw viability with C80EZ (8 mos, -80°C), with control groups having < 10% viability. We infer that C80EZ has strong potential as a reliable and efficient approach to long-term storage of cell lines without using FBS and liquid nitrogen facilities.

A-2001

Evaluating Potential Genotoxicity of Imidacloprid with Fish Cell Lines. BRENNA N. HAY and Lucy E. J. Lee. Department of Biology, University of the Fraser Valley, Abbotsford BC, CANADA. Email: b_hay@live.ca

Imidacloprid is currently the most widely used insecticide for agricultural crops worldwide. It is a systemic pesticide (taken up by plants and retained within plant tissues) that breaks down slowly in soil and tends to move into water, where it poses risks of concern to aquatic organisms including fish. Literature reports have indicated that imidacloprid is generally non-toxic to fish; however, recent studies indicate the possibility of genotoxic effects in some fish species, thus an in vitro evaluation for genotoxicity using various fish cell lines is being carried out. Preliminary experimentation using the micronucleus assay indicated that imidacloprid at the tested concentrations of 0.1 mg/L, 0.5 mg/L, 1.0 mg/L, and 2.0 mg/L all had statistically significant genotoxic effects on the killifish embryo cell line KFE-5, indicating that at these high concentrations, tested, double stranded DNA breakages may occur. At these concentrations, more binucleated cells had micronuclei, and the mean values of micronuclei were significantly higher than those of the negative control. Our preliminary results confirm other scientific research that has shown that imidacloprid has significant cytotoxic and genotoxic effects on non-target organisms such as fish. Additional research is in progress, which includes performing Comet assays and further micronucleus assays using lower doses and different types of fish cell lines. The effects on cells that may come into direct contact with pesticides are of specific interest, thus cell lines derived from gills, integument, and gastrointestinal cells are currently being evaluated.

A-2002

The Role of Gut Microbes in Regulating the Constitutive Androstane Receptor and Metabolism. ELESA POTERES, Iara Ibay, Lauren Alt, Kelly Keeler, Matt Pytynia, Allison Isabelli, and Kristina Martinez-Guryn. Department of Biomedical Sciences, Midwestern University, Downers Grove IL. Email: epoteres49@midwestern.edu

The constitutive androstane receptor (CAR) is a nuclear hormone receptor which plays a role in the regulation of xenobiotic, glucose, and lipid metabolism. Intriguingly germ-free mice, that are resistant to diet-induced obesity, display upregulated CAR and target gene expression in the liver compared to conventionally-raised mice, suggesting a microbial role in its regulation. Our preliminary results show that CAR knockout (KO) mice fed a high fat diet (HFD) exhibit increased adiposity and altered microbiota composition compared to wild-type mice. Thus, we hypothesized that CAR KO mice fed a HFD and given antibiotic treatment would have a lean phenotype similar to those seen in a germ-free model. Before conducting the proposed study, we sought to determine the most effective antibiotic treatment; one that significantly decreases bacterial load and has a minimal effect, if any, on CAR activation. Therefore, we conducted a study using 30 mice on a HFD or a low-fat diet (LFD) (n = 15, n = 15 respectively) that received: water control (n = 5), rifaximin, (non-absorbable broad-spectrum antibiotic, n = 5) or an antibiotic cocktail consisting of metronidazole, cefoperazone, vancomycin, and neomycin (n = 5) to determine which antibiotic group reduced bacterial load, weight gain, and body fat percentage, and increased cecum size. We also intend to measure mRNA levels of CAR and target genes involved in lipid metabolism. Thus far, we have determined that, regardless of diet, the antibiotic cocktail increased cecum size and decreased body fat percentage compared to the control and rifaximin groups. Therefore, the antibiotic cocktail is expected to be a useful model for determining host-microbe interactions involving CAR and host metabolism.

A-2003

Bioavailability of Artemisinin from *Artemisia annua:* Gender Differences in Absorption Distribution Metabolism and Excretion and Inhibition of Hepatic Cytochrome P450 Enzymes by *A. annua* Extracts and Phytochemicals. MATTHEW DESROSIERS[1], Alexis Mitteleman[2], and Pamela J. Weathers[1]. [1]Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester MA and [2]Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester MA. Email: mrdesrosiers@wpi.edu

The medicinal plant *Artemisia annua* is a promising therapeutic candidate for the treatment of malaria as it is the main producer of the antimalarial drug artemisinin (AN). Semisynthetic derivatives of AN make up the major component of artemisinin combination therapies, the frontline treatment for malaria in most of the world. However, these drugs are often unavailable or too expensive to those in need. Previously we showed that AN delivered as powdered dried leaves of *A. annua* (DLA) is about 40-fold more bioavailable in mice, about 4 times more soluble in intestinal fluid, 37% more permeable to the intestinal membrane, and distributes in higher quantities to several organs in male rats when compared to pure AN. Here, using female rats, absorption, distribution, metabolism, and excretion (ADME) studies were performed to compare with male rats. Groups of rats were orally dosed with pure AN or a slurry of DLA. After 1 hour, rats were euthanized, organs were collected, extracted, and analyzed for AN content. Compared to males, female rats had significantly more AN in several organs after 1 hour regardless of AN delivery method. It has been hypothesized that secondary phytochemicals produced by DLA may inhibit AN-metabolizing enzymes in the liver leading to increased bioavailability of AN when delivered as DLA. To investigate the effect of DLA on hepatic AN metabolism, an *in vitro* human liver microsome system was used to determine if DLA phytochemicals inhibit CYP2B6, the cytochrome P450 isoform responsible for AN metabolism in humans. Using a luciferin-based probe for CYP2B6, extracts of *A. annua* and pure phytochemicals from *A. annua* were tested for their inhibitory effect on CYP2B6. Methanolic extract of *A. annua* had an IC_{50} about 4-fold lower than pure artemisinin indicating other phytochemicals in *A. annua* inhibit CYP2B6. Testing of individual phytochemicals is ongoing to determine which phytochemicals are the strongest CYP2B6 inhibitors. Together these results help explain the greater bioavailability of artemisinin from *per os* consumption of dried *A. annua* leaves vs. pure artemisinin.

A-2004

Fabricating Three-dimensional Plant-based Biomaterials to Support Mammalian Cell Growth and Tissue Formation. C. J. NIEMIEC and J. Z. Gasiorowski. Midwestern University, Biomedical Sciences, 555 31st St, Downers Grove, IL 60515. Email: cniemiec99@midwestern.edu

Tissue injury can cause a wide range of complications including loss of nerve function or sensation, or loss of blood flow. Biomaterials can help rebuild these tissues by providing a scaffold for cells to grow on before or after being implanted into the body. Unfortunately, fabricating scaffolds that have both macro-scale (>mm) geometries that can both support

physiologically functional tissues and micro-scale (< μm) topography to direct individual cell behaviors is often an expensive and significant technical challenge. A possible solution to this challenge is to decellularize and repurpose abundant plant-based extracellular matrix materials. Previous studies have demonstrated that simple leaves, plugs, and discs of plant-based biomaterials can maintain mammalian cellular growth. Our goal was to craft more complex, physiologically relevant 3-dimensional shapes with inexpensive plant materials and supplies. In particular, we have been able to reproducibly fabricate tube structures with varying external and internal luminal diameters using decellularized apples with a highly controllable range of inner to outer diameter ratios of 0.57 – 0.65. These diameters and ratios did not significantly change after the decellularization and wash process. Scanning electron microscopy revealed qualitatively consistent nano- and micro-scale surface topography after all processing methods. After processing, we were able to successfully seed mammalian fibroblasts directly onto and into the decellularized apple tubes, or onto collagen and fibronectin coatings applied to the luminal surface of the apple tubes. The tube structures supported cell growth under all of these conditions, but growth was enhanced two-fold with collagen coatings. As we further optimize our processing and seeding methods, the decellularized apple tubes can be used for in vitro tissue and toxicology studies, or for in vivo implantations for tissue regeneration strategies.

A-2005

Edging Closer to Developing Marine Invertebrate Cell Lines. PETRA W. C. LEE[1], Anthony U. Miyagi[1], Gilian C. Opolko[2], and Lucy E. J. Lee[1,2]. Departments of Biology, [1]University of the Fraser Valley, Abbotsford, BC, CANADA and [2]Wilfrid Laurier University, Waterloo, ON, CANADA. Email: lucy.lee@ufv.ca

Extensive research into establishing continuous cell lines from marine invertebrates have been unsuccessful, despite concerted efforts being made for over half a century. Among marine invertebrates, crustacean cell cultures have been most frequently attempted. This report provides an update on our decade long efforts to establishing marine crustacean cell lines. Shrimp and lobsters of various stages were cultured under varying conditions, with a series of substrates and chemical components. Microbial contamination has been the biggest obstacle, although these can be kept at bay using several antimicrobial compounds. Enzymatic dissociation as well as tissue explant methods yielded culturable cells, some lasting up to 8 months. Coating cell culture flasks with attachment factors such as fibronectin, laminin, collagen or chitin provided variable results. Maintaining appropriate osmolarity was key and changing media formulations and supplements were tested. Leibovitz-15 and Grace's insect medium, have been most commonly used with addition of various sugars, amino acids and other defined and undefined supplements to optimize growth media. Incubation temperatures varied and while lobster cells derived from *Homarus americanus* were kept as low as 4°C, shrimp cells from *Litopenaeus vannamei* grew best at 27°C. This study will help further investigations for establishing an immortal cell line from crustaceans.

A-2006

Effects of the Phytochemical, Naringenin, on Rainbow Trout Intestinal Epithelial Cells. N. C. BOLS[1], P. G. Pumputis[1], V. R. Dayeh[1], and L. E. J. Lee[2]. [1]University of Waterloo, Department of Biology, Waterloo, ON N2L 3G1, CANADA and [2]University of the Fraser Valley, Department of Biology, Abbotsford, V2S 7M8, CANADA. Email: ncbols@uwaterloo.ca

The effects of naringenin (N) on the rainbow trout intestinal epithelial cell line, RTgutGC, was investigated in order to establish a framework for screening phytochemicals for their potential to boost fish intestinal health. The flavanone N was chosen because of its ability to enhance intestinal barrier integrity *in vitro* with the human intestinal cell line, Caco-2, and *in vivo* with mice. RTgutGC cells showed no decline in viability at concentrations below 100 mM N, while at 30 mM N cells appeared more flattened and had more prominent cellular borders. RTgutGC monolayers were examined by confocal microscopy for F-actin with FITC-phalloidin and for the tight junction proteins, ZO-1 and claudin-3, by immunofluorescence staining. N decreased F-actin staining in cytoplasmic stress fibers but increased it in the cellular circumference. N caused no change in the staining for ZO-1 and claudin-3. Intestinal barrier function was evaluated by transepithelial electrical resistance (TEER) and Lucifer Yellow (LY) transport. Increasing concentrations of N increased TEER and reduced LY permeability. In a wound healing assay, RTgutGC migration was inhibited by 75 mM N. These results suggest that at some concentrations N might promote fish intestinal health and RTgutGC might be useful in identifying phytochemicals beneficial to fish.

A-2007

Analyzing the Potential Effects of Environmental Changes on Fish Cell Lines. ARIANNE M. QANBERY, Taylor Boyd, Tessa Webb, and Lucy Lee. Department of Biology, University of the Fraser Valley, Abbotsford, BC, CANADA. Email: Arianneqanbery@gmail.com

Climate change is causing dramatic effects on poikilothermic animals such as fish. In this study we evaluated the effects of higher or lower temperatures from the normal growth temperatures on fish cell lines derived from warm- and cold-water fish species. Preliminary observations indicated changes in cell morphology as well as mitochondrial structure. Using phase contrast and fluorescence microscopy with specific fluorescent markers such as ActinGreen, NucBlue and rhodamine 123, as well as with immunofluorescence for specific proteins, we noticed morphological changes with changing temperatures in the cytoplasm, nucleus and mitochondria of all fish cell lines tested. Most cells became enlarged and mitochondria became elongated with lower temperatures, while warmer temperatures caused shortening of mitochondria and irregular cytoplasmic changes. This occurred for all fish cell lines tested including cold-water derived cell lines such as salmonid cell lines, and warm-water fish derived cell lines such as EelB, GFSk-S1 and KFE-5 from eel, goldfish and killifish respectively. Further research is being conducted to examine the significance of these changes.

A-2008

Bioproduction and Purification of Prenylated Stilbenoids from Hairy Root Cultures of Peanut and Assessment of Their Cytotoxicity and Induction of Apoptosis in Triple Negative Breast Cancer Cells (MDA-MB-231). SEPIDEH MOHAMMADHOSSEINPOUR[1,2], Lingling Fang[2], and Fabricio Medina-Bolivar[2,3]. [1]Molecular Biosciences Graduate Program, [2]Arkansas Biosciences Institute, and [3]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: sepideh.mohammad@smail.astate.edu, fmedinabolivar@astate.edu

Prenylated stilbenoids are phenolic compounds produced as a defense mechanism against biotic and abiotic stresses in peanut. In addition to their role in plant defense, they have potential applications in human health due to their anticancer and antiviral properties. Previous studies have described the anticancer activity of selected prenylated stilbenoids in HeLa and leukemia HL60 cells, however their effects on triple negative breast cancer (TNBC) cells have not been studied. In order to develop new treatments for TNBC, we studied the effect of prenylated stilbenoids on cytotoxicity and caspase-mediated apoptosis in the TNBC cell line MDA-MB-231. A previously established hairy root line of peanut was used as a stilbenoid bioproduction system. The hairy root cultures were co-treated with methyl jasmonate, cyclodextrin, hydrogen peroxide and magnesium chloride and then the stilbenoids were extracted from the culture medium after 198 hours of treatment. High performance liquid chromatography (HPLC) analyses of the medium extract showed the presence of the non-prenylated stilbenoids resveratrol and piceatannol and the prenylated stilbenoids arachidin-1, arachidin-2, arachidin-3, and arachidin-5. The most abundant stilbenoids were arachidin-1 and arachidin-3 with yields higher than 300 mg/L. To purify the prenylated stilbenoids, the extracts were separated by semi-preparative HPLC. MDA-MB-231 breast cancer cells were treated with the purified prenylated stilbenoids for 24, 48 and 72 hours. Higher levels of cytotoxicity were obtained at 72 hours. The most cytotoxic compound was arachidin-3 with an IC_{50} of at least 10-fold lower than its non prenylated analog resveratrol. The increased cytotoxicity of arachidin-3 in the TNBC cells correlated with the activation of the apoptosis markers caspase-3 and caspase-7. In conclusion, prenylation increased the anticancer potency of the stilbenoids in TNBC cells. Further studies will investigate the *in vivo* anticancer effects of these natural products from peanut.

A-2009

The Role of *Clostridium ramosum* in Promoting the Development of Obesity and Increasing Lipid Absorption. IARA CASSANDRA V. IBAY, Lauren Alt, Matt Pytynia, Kelly Keeler, and Kristina Martinez-Guryn. Midwestern University, Department of Biomedical Science, Downers Grove, IL Email: iibay66@midwestern.edu

Several studies have demonstrated that gut microbiota composition is significantly altered by diet and metabolic disorders. However, it is still unclear whether these shifts or specific strains of bacteria have a causative role in the development of obesity and associated disorders. In particular, *Clostridium ramosum* has been correlated with obesity especially under high-fat diet conditions and may involve upregulation of the long chain fatty acid transporter *Cd36* in the small intestine. However, a direct impact of *C. ramosum* on lipid absorption has not been definitively established and the exact mechanisms for this (including or beyond *Cd36* regulation) need to be further explored. We sought to determine the functional impact of *C. ramosum* on obesity and lipid absorption and the mechanisms behind this process. To gather preliminary results, 6 mice fed a high fat diet (HFD) were supplemented with or without *C. ramosum*. Body weight was measured bi-weekly for 3 weeks and glucose tolerance test conducted after 3 weeks of *C. ramosum* supplementation. There was no significant difference in body weight or glucose tolerance between the two groups. Further analyses will examine potential differences in adiposity and lipid absorption upon continued supplementation with *C. ramosum*.

A-2010

The Development of an Antisense Oligonucleotide-based Ointment for the Treatment of Squamous Cell Carcinoma of Human Skin: Preparatory Stage Research Using an Insect Model. K. V. LAIKOVA[1], E. Y. Bessalova[1], T. P. Makalish[1], R. Z. Useinov[2], A. M. Krasnodubets[2], Z. Z. Temirova[1], N. V. Gal'chinsky[2], I. A. Novikov[2], and V. V. Oberemok[2]. [1]V.I. Vernadsky Crimean Federal University, Medical Academy named after S.I. Georgievsky. Simferopol, UKRAINE and [2]V.I. Vernadsky Crimean Federal University, Taurida Academy. Simferopol, UKRAINE. Email: botan_icus@mail.ru

Squamous cell carcinoma is the most malignant epithelial tumor of the skin in humans. It belongs to the group of non-melanoma malignant neoplasms of the skin characterized by an aggressive course due to the high probability of metastasis. The purpose of this work was to search for new non-surgical ways to treat patients with squamous cell carcinoma of the skin. We decided to develop an ointment based on an antisense oligonucleotide with an active substance that blocks the division of cancer cells. Topical application of this ointment will allow convenient, targeted use of the medicine on the affected skin. To achieve this goal, we decided to use RNase H-recruiting antisense oligonucleotides. Preparatory experiments were carried out on a model insect, Lymantria dispar, the gypsy moth. At the larval stage L. dispar cells, including epithelium cells, divide very actively (over a period of 2 months, the insect mass increases on average 1000 fold) with a level of proliferation comparable to that of cancer cells. As a target, we chose 5.8S rRNA, which plays a key role in protein biosynthesis. Based on this, we developed an antisense fragment 11 nucleotides long (TGCGTTCG AAA, oligoRIBO-11) and applied this sequence topically to 2nd instar L. dispar larvae (6 pmol/caterpillar). On the 6th day after treatment, the greatest changes were observed in the nuclei, where the amount of heterochromatin increased (condensed in large clumps along the karyolemma) and the number and size of the nucleoli decreased. The nuclear cytoplasmic ratio increased significantly by 1.5 fold. These manifestations, characteristic of the decreased synthetic activity of cells, demonstrate their reduced functional activity and maturity, which prevents them from actively dividing. The next stage of the work will be devoted to the application of antisense oligonucleotides to 5.8S rRNA on mammalian skin squamous cell carcinoma lines. (This work was partially supported by V.I. Vernadsky Crimean Federal University Development Program for 2015-2024).

A-2011

Search for the Sequence of the Colorado Potato Beetle 5.8S Ribosomal RNA as a Prospective Target for DNA Insecticides. N. V. GAL'CHINSKY[1], I. M. Kenyo[2], K. V. Laikova[3], and V. V. Oberemok[1]. [1]V.I. Vernadsky Crimean Federal University, Taurida Academy. Simferopol, UKRAINE; [2]V.I. Vernadsky Crimean Federal University, Academy of Bioresources and Environmental Management. Simferopol, UKRAINE; and [3]V.I. Vernadsky Crimean Federal University, Medical Academy named after S.I. Georgievsky. Simferopol, UKRAINE. Email: pcr.product@gmail.com

Over the last decade, our group has mainly created DNA-based insecticides (RNase H-recruiting unmodified antisense oligonucleotides) to help address the damage caused to forests by the gypsy moth (*Lymantria dispar*). Our area of interest includes 5.8S ribosomal RNA (rRNA) as a prospective target for DNA insecticide development. 28S and 5.8S rRNAs constitute about 85-90% of total cellular RNA. 5.8S rRNA, which plays an important role in eukaryotic ribosome function and protein synthesis, is an excellent prospective candidate for this purpose. Our recent results demonstrate the potential use of antisense oligonucleotides (10-11 nucleotides long) complementary to the gypsy moth 5.8S rRNA in the development of insecticides. We have widened our investigations to include one more serious insect pest – the Colorado potato beetle (*Leptinotarsa decemlineata*, fam. Chrysomelidae). 5.8S rRNA for the Colorado potato beetle was not present in GenBank when we started our experiments. We decided to sequence the 5.8S rRNA for the Colorado potato beetle using cDNA and 2 primers: 5′-TGGATCACTTGGCT CG-3′ (forward) and 5′-CAGACAGGAGTGGTCC-3′ (reverse). We obtained a partial sequence (92 nucleotides long) of the Colorado potato beetle 5.8S rRNA: 5′-GTCACTTGTGAACTGCAGGACACATGAACATC GACATTTCGAACGCACATTGCGGTCCTCGG ATACTGTTCCTGGACCACTCCTGTCTGAGA-3′. The partial sequence for the Colorado potato beetle 5.8S ribosomal RNA obtained contains the universally conserved GAAC sequence region (letters 41-44) common to all 5.8S RNAs. The region surrounding it is often species specific. In GenBank we detected several species from the genera *Timarcha, Bruchus, Callosobruchus,* and *Calligrapha*, all belonging to the fam. Chrysomelidae, whose 5.8S rRNA partial sequences have 95-98% query cover with the target fragment. The partial sequence obtained will be used to create DNA insecticides to help control damage caused by the Colorado potato beetle.

A-2012

The Effects of β-hydroxybutyrate on *C. elegans.* A. LIN, D. Bhaumik, and G. J. Lithgow. The Buck Institute for Research on Aging, 8001 Redwood Blvd, Novato, CA 94945. Email: my.donc@gmail.com

Caenorhabditis elegans was used to examine the role of beta-amyloid (Aβ) in Alzheimer's disease. One strain of *C. elegans* expresses Aβ in the muscles, causing paralysis. β-hydroxybutyrate (BHB) has neuroprotective properties. We hypothesized that BHB can protect the nematodes from paralysis. All nematodes eggs are moved to new plates every two days. Paralysis assays and a Western blot of Aβ were done. Egg lay for GMC101 (expresses Aβ in muscles) and CL2122 (control) was synchronized. 46 hours after the egg lay, the nematodes were moved to BHB plates. The number of worms paralyzed was assessed. Extracts were placed on gel electrophoresis plates, for Western blot of Aβ, when 95% of control worms were paralyzed. Proteins were transferred to a membrane, which was probed with Aβ antibody. Blots were developed for analysis. Scoring percentages of worms not paralyzed showed that treatment of GMC101 with BHB reduced paralysis. The percentage of worms not paralyzed increased with increasing concentrations of BHB. The GMC101 worms treated with 100 mM showed the highest percentage of worms not paralyzed (96.18%), closely matching the percentage of CL2122 strain worms not paralyzed (100%). A Western blot analysis of Aβ peptides in GMC101 worms treated with 80 mM BHB was conducted. A quantitative analysis of the blot showed that the levels of Aβ oligomers were reduced in worms treated with 80 mM BHB. With respect to non-treated controls, the worms treated with BHB experienced 25% decrease in levels of Aβ oligomers. These results support the hypothesis that BHB affects the impact of Aß in *C. elegans*.