JACKIE ALBERT Due 10/4/18

Research Plan/Project Summary Instructions

The Research Plan/Project Summary should include the following:

a. **RATIONALE:** Include a brief synopsis of the background that supports your research problem and explain why this research is important and if applicable, explain any societal impact of your research

Curcumin is a xenohormetic compound, and is the product of the stress response of the turmeric plant. This compound confers stress tolerance benefits in mammals in certain doses. It does this through the evocation of the mammalian stress response, which includes increased cell autophagy and phagocytosis. This increased stress tolerance causes longevity in the cases of caloric restriction and the ingestion of other xenohormetic compounds, and thus the effect of curcumin on longevity of *C. elegans* should be observed to see whether curcumin can lead to longer lifespan. The increased autophagy and phagocytosis also leads to a decrease in protein aggregates with some xenohormetic compounds, so this effect will also be explored with curcumin.

Aggregates of the protein α-synuclein are characteristic of Parkinson's disease, a neurodegenerative disease common in older people. These aggregates form Lewy bodies, a pathological hallmark in the onset of the disease.

b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:** How is this based on the rationale described above?

Will the evocation of the hormetic response by the xenohormetic compound curcumin fight α-synuclein aggregation in aging wild type *C. elegans*?

c. Describe the following in detail:

- **Procedures:** Detail all procedures and experimental design including methods for data collection. Describe only your project. Do not include work done by mentor or others. Include detail regarding chemical concentrations and drug dosages.
- *C. elegans strains and maintenance*
    - Obtain NL5901 (worms display YFP tagged alpha synuclein aggregates in body wall muscle cells) worms from Caenorhabditis Genetics Center in Minnesota.
    - Maintain all NL5901 strain *C. elegans* according to standard protocol on NGM agar with *E. coli* OP50 at 22°C.
- *Preparation of curcumin plates*
    - Dissolve 0.7276 g of curcumin powder in 85 ml DMSO (dimethyl sulfoxide) and 50 ml dH20 to create a stock solution with a molarity of 148.148 mM.
    - Add 10 ml of the prepared stock solution to 87 ml of DMSO to get a molarity of 20 μM.

- Add 26 mL of the 20 μM solution to 174 mL of molten NGM agar.
- Pour the curcumin agar into 60cm dishes for testing.

- *Nematode Growth Medium agar preparation*
  - Autoclave 1 ml of 1 M $CaCl_2$, 1 ml of 1 M $MgSO_4$, and 25 ml of 1 M KPO4 buffer separately each for 15 minutes.
  - Autoclave a solution of 3 g of NaCl, 17 g of agar, 2.5 g of peptone, and 975 ml of H_2O for 50 minutes.
  - Let the solution cool to 55°C, then mix in the previously autoclaved $CaCl_2$, $MgSO_4$, and KPO_4 buffer along with 1 ml of 5 mg/ml cholesterol dissolved in ethanol.
- *Preparation of bleaching solution*
  - Prepare bleaching solution immediately before performing the age synchronization procedure.
  - Mix 6.26 ml of 4 M NaOH with 2.5 ml of NaOCl and 50 ml of M9 buffer.
- *Adding OP50 lawn*
  - Fill petri dishes up to ⅔ of their volume with the NGM agar, and once dry, let the plates sit at room temperature for 2-3 days.
  - Prepare a streak of OP50 *E. coli* from a glycerol stock by selecting one colony to grow in LB overnight at 37 °C with agitation to ensure proper aeration.
  - Once excess liquid has evaporated from the plates, add OP50 *E. coli* to the center of the plate with a pipette.
  - Allow the OP50 *E. coli* lawn to grow overnight before adding worms to the plate.
- *Age synchronization*
  - Wash a plate of *C. elegans* with 1 ml of M9 buffer two times and place in a centrifuge tube.
  - Pellet the worms at 3,884 × g (rcf; 8,500 rpm) for 1 min and discard the supernatant.
  - Add 200 μl of Distilled Water (D.W.) and 800 μl of bleaching solution to the centrifuge tube.
  - When 90% of nematodes have lysed and several embryos are released, after about 20 minutes.
  - Centrifuge the tube at 3,884 × g (rcf; 8,500 rpm) for 1 min and discard the supernatant, avoiding pellet disruption.
  - Wash three times with M9 buffer (1 ml each): Spin down at 137 × g (rcf; 1,000 rpm) for 1 min and discard the supernatant.
  - Transfer embryos with 1 ml of M9 buffer to a conical tube containing 1 ml of M9 (total 2 ml).

- Incubate overnight at 20 °C with shaking at 30 rpm to allow L1 larvae to hatch

- *Curcumin screening*
  - Transfer age synchronized L1 NL5901 worms to the curcumin-free plates for one day, and then transfer the worms to the curcumin assay plates via subculturing.
  - Subculture the worms on to new dishes every three days.
- *Alpha synuclein aggregation*
  - Wash the worms with M9 buffer, and mount them on slides. To mount worms on slides, transfer them into a drop of M9 buffer and allow M9 to evaporate.
  - View mounted worms under the fluorescence microscope.
  - Take photos of the worms under the fluorescent microscope once every 4 days after L1 stage.
  - Aggregate intensity will quantified by determining YFP fluorescence intensity using ImageJ software.

- **Risk and Safety:** Identify any potential risks and safety precautions needed.
- Gloves and goggles will be necessary to avoid contamination of hands and eyes when dealing with curcumin and *C. elegans,* and care will be taken to ensure that no transgenic animals escape the laboratory setup. DMSO, hypochlorite bleach, and M9 buffer will also be handled with gloves, and with goggles on, to avoid irritation of hands and/or eyes.
- **Data Analysis:** Describe the procedures you will use to analyze the data/results.
  - ImageJ will be used to determine fluorescence intensity of worms from each condition.
    - Fluorescence will be measured for each individual worm using the integrated density measurement
  - A T-test will be performed to determine the statistical significance of the results

d. **BIBLIOGRAPHY:** List major references (e.g. science journal articles, books, internet sites) from your literature review. If you plan to use vertebrate animals, one of these references must be an animal care reference.

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Items 1–4 below are subject-specific guidelines for additional items to be included in your research plan/project summary as applicable.

3. **Potentially hazardous biological agents research:**
   a. Give source of the organism and describe BSL assessment process and BSL determination

The source of the organism is the Caenorhabditis Genetics Center. The *C. elegans* are BSL 1.

   b. Detail safety precautions and discuss methods of disposal

Gloves and goggles will be worn when dealing with *C elegans.* To dispose of cultures, especially those with live worms, bleach will be poured onto the plates and left for 20 minutes, and then plates will be drained and thrown in the garbage can.

4. **Hazardous chemicals, activities & devices:**

- Include detail regarding chemical concentrations and drug dosages
    - Curcumin, used in 200 micrometer dosages.
    - Hypochlorite bleach solution, prepared as described.
    - M9 buffer, prepared as described on WormBook.
- Describe the risk assessment process
    - Hazardous when in contact with eyes
    - May irritate skin upon skin contact
    - May cause drowsiness and dizziness when ingested
    - Flammable
- Supervision
    - Supervised by research teacher.
- Safety precautions
    - Goggles will be needed to protect the eyes.
    - Keep locked up and away from heat.
    - Wear a mask to prevent inhalation of chemicals.
- Methods of disposal
    - Bleach plates after assays and dispose
    - Autoclave all laboratory instruments.
    - All waste is placed into the regulated waste bins.