Compound treatment assay for *C. elegans*

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NuRD Mediates Mitochondrial Stress-induced Longevity via Chromatin Remodeling in Response to Acetyl-CoA level

Detailed protocol：

1. *C. elegans* synchronization.
   a) Harvest gravid worms (adults full of eggs) with M9 buffer and transfer them into a 15 mL conical tube.
   b) Wash worms with M9 buffer and spin the tubes in a table-top centrifuge for 30 seconds at 2,000 rpm to pellet the worms.
   c) Add 1 mL of bleaching solution to the worm pellet and immediately shake vigorously for 5 mins to ensure the worms will be sufficiently dissolved.
   d) At the end of the bleach buffer incubation, immediately add 10 mL of M9 buffer to stop the bleaching. Spin the tubes at 2,500 rpm for 1 minute.
   e) Discard the supernatant, leaving behind the pellet of the eggs.
   f) Wash eggs by adding 10 mL of M9 buffer and resuspend the egg pellet. Spin and discard the supernatant.
   g) Repeat the wash step three times.
   h) Resuspend the egg pellet in 0.5 mL M9 buffer.
   i) Pipet the egg pellet onto NGM plates seeded with *Escherichia coli* OP50 or HT115 RNAi bacteria and grow them at 20 □ for one day.
   j) Collect synchronized L2 worms (~24 hrs after hatching) with M9 buffer and transfer worms to a 15 mL conical tube.
   k) Spin the tubes at 2,000 rpm for 30 seconds to pellet the worms. Repeat the wash step twice and discard the supernatant.
2. Prepare bacteria for worm liquid culture.
   a) Grow a starter culture of OP50/HT115 in a 15 mL tube with 3 mL LB medium at 37□ for 8 hrs on the day of worm synchronization.
   b) Transfer 1 mL starter culture into a 2 L conical flask with 1 L LB medium. Shake at 37□ overnight (10-16 hrs).
   c) Transfer the culture into two 500 mL centrifuge bottles and spin the cultures in a Beckman high-speed centrifuge at 4000 rpm for 10 mins.
   d) Discard the supernatant and resuspend all the bacteria with 20 mL S-medium (stored at 4□).
3. Transfer 5mL S-medium/bacteria mixture obtained from **step 2** into a 50 mL tube.
4. Add the tested compound into the 5 mL S-medium/ bacteria mixture, and mix well.
5. Transfer the L2-stage synchronized worms (~500 worms) obtained from **step 1** into the 5 mL S-medium/bacteria mixture containing the tested compound from **step 4**.
6. Incubate the cultures on an orbital shaker at 200 rpm at 20 °C for two days.
7. Continuously monitor until worms grow to the desired stage for testing.
8. Harvest and wash worms with M9 buffer before analyses.

Recipes:

1. **Bleaching solution** [6.25 mL 6% Sodium Hypochlorite, 3.25 mL 5M KOH, 15.5 mL sterile Milli Q H_2O.]
2. **M9 Buffer** [3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 mL 1 M $MgSO_4$, H_2O to 1 liter. Sterilize by autoclaving.]
3. **Nematode Growth Medium (NGM) plates**
   a) Add the following to a 2 L conical flask:
      3 g NaCl
      2.5 g Bacto Peptone
      17 g Bacto Agar
      Double distilled water to 1 liter.
   b) Stir bar
   c) Autoclave for 20 min at 121 ℃
   d) Wait until cooled at around 55 ℃
   e) Add the following
      1 mL of 1 M $CaCl_2$ sterile
      1 mL of 1 M $MgSO_4$ sterile
      25 mL of 1 M KH_2PO_4 pH 6.0 sterile
      1 mL of 5 mg/mL cholesterol (prepared in 95% ethanol, and stored at 4 ℃)
   f) Pour onto 60 mm sterile plates.
   g) Let dry for one night
   h) Seed with appropriate bacteria.
4. **LB medium** [10 g Bacto-Tryptone, 5 g Yeast extract, 10 g NaCl, H_2O to 1 liter. Sterilize by autoclaving.]
5. **S-Medium** [1 liter S Basal, 10 mL 1 M potassium citrate pH 6, 10 mL trace metals solution, 3 mL 1 M $CaCl_2$, 3 mL 1 M $MgSO_4$. Add components using sterile technique; do not autoclave.]
   a) S Basal [5.85 g NaCl, 1 g K_2HPO_4, 6 g KH_2PO_4, 1 mL cholesterol (5 mg/mL in ethanol), H_2O to 1 liter. Sterilize by autoclaving.]
   b) 1 M Potassium citrate pH 6.0 [20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate, H_2O to 1 liter. Sterilize by autoclaving.]
   c) Trace metals solution [1.86 g disodium EDTA, 0.69 g $FeSO_4$ •7 H_2O, 0.2 g $MnCl_2$•4 H_2O, 0.29 g ZnSO4 •7 H_2O, 0.025 g $CuSO_4$ •5 H_2O, H_2O to 1 liter. Sterilize by autoclaving. Store in the dark.]
   d) 1 M $CaCl_2$ [55.5 g $CaCl_2$ in 1-liter H_2O. Sterilize by autoclaving.]

Tested compound：

Reagent	Concentration	Source	Identifier
Sodium acetate	10 mM	Sigma	Cat#791741
Sodium pyruvate	10 mM	Sigma	Cat#V900232
D-glucose	10 mM	Sigma	Cat#G7021
Sodium butyrate	50 mM	MCE	Cat#HY-B0350A

This protocol is documented by Di Zhu. 2020/10/29