Crude Dauer Pheromone Preparation

Adapted from Bargmann lab protocol by Erik Andersen on March 17, 2011

1. Inoculate two 5 ml cultures of LB with HB101 and grow overnight at 37ºC.
2. Add each culture to 1 L of Superbroth in a 4 L Erlenmeyer flask and grow overnight at 37ºC.
3. Centrifuge the cultures to pellet and wash three times with S basal.
4. Resuspend washed pellets in S medium and bring the final volume up to 50 mL.
5. Make 1 L of S medium in a 2.8 L Fernbach flask.
6. Wash two nearly starved out 10 cm plates of N2 worms to the flask using M9.
7. Add 25 mL of the 50 mL of HB101 in S medium to the culture. Leave the remaining HB101 at 4ºC.
8. Grow the worms at 20ºC shaking at 200 rpm for seven days.
9. After the culture has clarified (around seven days), add the remaining 25 mL of HB101 from the stock at 4ºC.
10. After four more days, centrifuge the culture and keep the supernatant.
11. Filter the supernatant through a Buchner filter funnel (medium frit) under vacuum.
12. Filter the remaining supernatant through several Nalgene Vacuum Filter Units with 0.2 μm PES membranes to remove any remaining worms and bacteria. Multiple filter units will have to be used as the bacteria clog the filters quickly.
13. Concentrate the supernatant using a rotary evaporator at room temperature for 4 hours.
14. Lyophilize the supernatant after reducing to 50 mL volume on the rotary evaporator.
15. Add 100 mL of ethanol, mix, and crush the solids.
16. Let the solids settle and remove ethanol to rotovap flask.
17. Repeat for three extractions.
18. Concentrate with the rotary evaporator until dryness.
19. Resuspend in 5 mL of sterile water.
20. Store at -20ºC in 1 mL aliquots.
21. Test dauer formation on plates and in liquid.