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Growing bacteria in Superbroth (“thick food”) V.2

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol shows you how to prepare "thick food" to grow *C. elegans* at higher densities.

Materials

MATERIALS

Potassium Dihydrogen Orthophosphate Certified AR for Analysis Fisher Chemical **Fisher Scientific Catalog #** P/4800/53

Superbroth for bacterial culture

Safety warnings

This protocol requires a Bunsen burner. Do not wear gloves around a Bunsen burner as they might catch fire and hurt you.

Before start

Make sure you have superbroth medium and potassium orthophosphates. If you work at the Gurdon Institute, you can order these solutions from the media kitchen and a week notice is ideal.

Prepare Super Broth

1 thickFood.docx

2 Add 1 bottle (100 ml) K-orthophosphates to each bottle of Super Broth (900 ml)

Prepare starter culture

3 Add 30 ml of Super Broth (plus K-orthophosphates) to a 100 ml flask. Inoculate *Escherichia coli* HB101 (also works with *E. coli* OP50, *Acinetobacter schindleri*, *Bacillus pumilus* and *Pseudomonas fragi* although not all these bacteria grow at 37 C) from plate in cold room. Use sterile flask and work under sterile conditions.
Incubate for several (8 hours) hours at 37 °C, shaking (200 rpm). This culture can also be left to grow overnight.

Prepare overnight cultures

4 Add 1 L Super Broth (plus K-orthophosphates) to a 2.5 L sterile flask.
Add ca. 5 ml starter culture (check that bacteria have grown!) to each 1L medium.
Incubate over night at 37 °C, shaking (180-200 rpm). You may need to book an incubator to accommodate several flaks.

Prepare the thick food

5 Transfer cultures to 1000 ml plastic bottles for centrifugation. Use the large SorvallRC 3B Plus centrifuge, rotor H-6000.

6 Spin cultures at 4000 rpm, 20 minutes, 4 °C.

7 Discard supernatant and re-suspend pellet in 25-30 ml of H_2O (sterile). Or b-broth.Work under sterile conditions to avoid contamination of bacteria.

8 Seed culture using electronic pipette on low speed by dropping one droplet at atime, 2 ml in total on each of 14mm plate. The rationale behind this is thatworms like the edges of a bacterial lawn and seeding droplets increases thetotal length of edges.

9 A 14mmplate can grow up to 80,000 animals.

10 The bacteria can be frozen at -20 C for later seeding. A postdoc in the lab says to freeze resuspended bacterial while another postdoc says to freeze pellet. I personally froze the resuspended bacteria so that I can seed them immediately after defrosting.