Extraction of Semi-Rough or Smooth LPS (Hot Phenol-Water Extraction)

MATERIALS

- Bacterial cells (Gram negative)
- Phenol redistilled (Sigma, 328111)
- Dialysis tubing (1,000 MWCO, regenerated cellulose)
- Oil bath
- Heating and stirring plate
- Chemical Hood
- Cold centrifuge

Preparation of 90% phenol solution:

- Weigh out 18 g of crystalline phenol and dissolve it in 2.2 mL of water to give 20 mL of a 90% Phenol solution.

Preparation of the bacterial sample:

- Gram negative bacterial cells having smooth, semi-rough or rough LPS are grown to their late log phase.
- Harvest the bacterial cells by centrifugation and wash the harvested cells gently once with water to remove the adhere media.

PROCEDURE (Extraction Procedure Should Be Done in a Chemical Hood):

1. Pre heat the oil bath to 68 °C and make sure the oil bath maintains the temp ± 2°C.

2. Re-suspend 3-4 g of washed bacterial pellet in 20 mL of water and preheat the suspension in the oil bath with continuous stirring for 10 min.

3. Preheat the phenol solution to 68°C for 10 min.

4. Add the heated phenol solution slowly using a glass pipette while stirring the cell suspension. A milky white appearance will appear upon the addition of phenol.

5. After the complete addition of phenol, stir the suspension vigorously for 30 min.

6. Immediately cool the reaction mixture on an ice-water bath to <10 °C. Continuous stirring helps bring down the temperature quickly.

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7. Centrifuge the extract at 3500 rpm at 10°C for 45 min. This will separate out the mixture in three distinct layers.

8. Carefully transfer the upper layer to into a 50 mL polypropylene Falcon tube. This is the phenol saturated water layer, smooth and semi-rough LPS extracts are in this layer.

9. Preheat water at 68 °C. Extract the residual phenol phase (bottom layer) and interphase (insoluble cellular components) with additions of 20 mL of the preheated water at 68 °C for 30min.

10. Cool down and centrifuge the cellular extract as mentioned from Step 7.

11. Pool the upper layer from both extraction and dialyze against 4L of water using 1,000 MWCO dialysis tubing. Continue the dialysis for at least 4 days with two changes of water per day.

12. Dry the dialysate in a lyophilyzer.

13. The lyophilized material contains LPS, soluble nucleic acid and proteins. The major portion of soluble nucleic acid and proteins can be removed by Ultracentrifugation at 105,000g for 4 hrs.

14. Remove and discard the supernatant. Re-suspend the LPS precipitate in water and lyophilize.

    NOTE: If required, further purification can be performed by treating the LPS with DNase, RNase and ProteinaseK followed by dialysis and ultracentrifugation.

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