LPS SILVER STAIN #1

REFRENCE: Hitchcock and Brown . (1983) *Journal of Bacteriology*, 154:269-277.

OBJECTIVE: To stain whole cells LPS gels.

METHOD:

1. After running the gel, fix overnight in 25% (v/v) isopropanol in 7% acetic acid. You do not need to use the rotary shaker.

2. The next morning, oxidize the gel for 5 minutes with 2.1g periodic acid and 8ml of isopropanol in 300ml of distilled water. Make this solution fresh before using.

3. Wash the gell 8 x 30 minutes in distilled water at room temperature on the rotary shaker.

4. Incubate for 10 minutes with silver stain on the rotary shaker.

   SILVER STAIN: 56ml 0.1N naOH
   230ml distilled water
   2ml 29.4% ammonium hydroxide

   Add 9.8ml of 20% silver nitrate solution slowly with shaking. Make this solution fresh right before using. It is written that you can dissolve 10 ml of the silver solution but I have found that in fact you can dissolve only 9.8. It is very important to keep the ammonium hydroxide tightly covered as its strength decreases rapidly if left open even for a short time.

5. 4 X 10 minute washes with distilled water on the shaker.

6. 10 to 20 minutes in the developer.

   DEVELOPER: 50 mg citric acid
   0.5 ml 37% formaldehyde
   1 litre distilled water

   Make fresh before use. Also raise the temperature of this solution to above 26°C to help preferentially stain the LPS.

7. Incubate the gel for 1 hour in a stop bath consisting of 20 ml of 7% acetic acid in 400 ml of distilled water.