Preparing Inoculants Using Diluted Cultures of Rhizobia and Presterilized Peat

The production capacity of small-scale inoculant production plants using presterilized peat can be increased by using diluted liquid cultures of rhizobia. In this exercise, fully grown cultures are diluted in water and other diluents of different formulations prior to incorporating of presterilized peat in packages or in polypropylene trays. The multiplication of rhizobia in the inoculants is studied.

KEY STEPS/OBJECTIVES

1. Culture Rhizobium sp. and Bradyrhizobium sp.
2. Make culture dilution flasks.
3. Prepare diluents in dilution flasks.
4. Prepare and package peat.
5. Sterilize peat in packages and polypropylene trays.
6. Prepare yeast-mannitol broth (YMB) + peat blanks and check for sterility.
7. Examine yeast-mannitol agar (YMA) congo red (CR) plates plated with YMB-peat blanks.
8. Perform viable counts on late-log-phase cultures.
9. Prepare diluted cultures.
10. Inject diluted cultures into peat.
11. Mix diluted cultures with autoclaved peat in trays and package.
12. Perform viable counts on inoculants at 2 weeks.
13. Perform viable counts on inoculants at 8 weeks.
14. Record and analyze the data.
a. Culturing Rhizobia in YMB (Key Step 1)

Prepare 500 ml of YMB in each of two 1-liter Erlenmeyer flasks. Inoculate one flask with Bradyrhizobium sp. (e.g., B. japonicum TAL 102) and the other with Rhizobium sp. (e.g., TAL 1145 from Leucaena leucocephala). Both rhizobia should have antisera available for strain recognition and confirming purity (by serology) to be done later in the experiment. Incubate the inoculated flasks at 25–30°C on a shaker. To obtain late-log-phase cultures, allow the fast- and slow-growing rhizobia to grow for 4–7 days, respectively. At the end of the specified growth period, check the purity of the culture by Gram stain and by serology (simple tube agglutination or by the fluorescent antibody (FA) technique as described in Section II).

b. Making a Culture Dilution Flask and Its Operation (Key Step 2)

The culture dilution flask is basically a 2-liter Erlenmeyer flask modified by a short glass-tubing outlet at the base of the flask as shown in Figure 28.1. Seek the assistance of a skilled glassblower for fitting the glass tubing to the base of the flask. Five culture dilution flasks are required per rhizobial strain (four for diluents and one for the undiluted culture as control, Table 28.1).

Attach a piece of surgical latex tubing of suitable size to the glass tubing outlet of each dilution vessel. Close the open end of the latex tubing with a plug made from a short piece of glass rod. Add appropriate diluent, close the flask, and sterilize the entire

![Diagram of a culture dilution flask](image-url)