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Surface Sterilization Soybean Nodules for Microbiological Studies

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We use this protocol and it's working

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Disclaimer

This protocol has been tested in a standard microbiology laboratory setting. Users must ensure all safety precautionsare followed, including biosafety measures, proper disposal of hazardous materials, and aseptic handling of microbial samples. Bleach solutions are corrosive, and glycerol stocks should be stored properly to prevent contamination. The authors are not responsible for misuse or procedural errors.

Abstract

This protocol describes the surface sterilization and storage of root nodules for microbiological research, ensuring the removal of external contaminants while maintaining viable internal microbial communities. The process includes a saline wash to remove debris, bleach sterilization, and glycerol stock preparation for storage. The sterilized nodules are plated on LBA media to verify sterility before downstream applications.

Guidelines

- Ensure all steps from bleach sterilization onwards are performed under aseptic conditions.
- Do not over-vortex the nodules in saline or bleach solutions, as this may damage them.
- Dispose of bleach solutions properly in a designated discard container.
- Use freshly autoclaved saline and DDI water to avoid contamination.
- Do not leave nodules longer in bleach solution than the recommended time, as this may damage them.

Materials

Glassware & Consumables

- Autoclaved filter paper No. 2 – For drying nodules
- 50 mL sterile Falcon tubes – For sample handling
- 1.5 mL sterile flip-cap tubes – For storage
- LBA plates – For sterility testing
- 1 mL pipette & tips – For precise liquid handling
- Large waste discard beaker – For bleach disposal
- Sterilized funnel – For transferring nodules (optional)
- Plastic wrap or parafilm – For sealing plates

Reagents & Solutions

- 0.9% autoclaved NaCl (saline solution) – For initial washing
- 1.8% NaClO (bleach solution, 20% high-strength bleach) – For sterilization
- Autoclaved DDI water – For rinsing residual bleach
- 80% glycerol stock solution – For preservation

Equipment

- Mesh sieve – For rinsing nodules
- Vortex mixer – For agitation during washing
- Forceps or measuring scooper – For handling nodules

Safety warnings

- Bleach is corrosive: Always wear appropriate PPE (gloves, lab coat, and eye protection) and handle bleach under the hood.
- Aseptic technique is critical: Ensure all steps after the saline wash are performed under sterile conditions to avoid recontamination.
- Waste disposal: Autoclave or neutralize bleach waste as per your institution’s guidelines.

Ethics statement

This protocol does not involve animal or human subjects; however, researchers should follow biosafety guidelineswhen handling microbial samples.

Before start

- Ensure that all reagents and solutions required for the bleach wash and subsequent rinsing are freshly prepared and autoclaved.
- Verify that non-sterile materials used only during the saline rinsing (e.g., initial saline aliquots) are clearly distinguished from those used in aseptic conditions.
- Set up the biosafety hood with all necessary equipment (forceps, scooper, pipettes, waste beaker, etc.) before beginning the bleach sterilization process.
- Prepare a workspace with LBA plates, filter paper, and sterile tools before starting the sterilization process.
- Ensure waste disposal beakers and bleach solutions are ready before handling nodules.
- Work inside a biosafety hood from Step 9 onward to maintain sterility.
- Wear appropriate PPE (lab coat, gloves, safety goggles) when handling chemicals and bacterial cultures.

Procedures

1 **Labeling and Initial Preparation**

1. In the biosafety hood, label all LBA plates and flip-cap tubes with the appropriate cultivar and treatment details.
2. Prepare three flip-cap tubes per sample (three replicates): reserve one tube specifically for glycerol stock preparation.

2 **Saline Washing of Nodules**

1. Combine nodules into their respective 50 mL Falcon tubes (this step may be performed outside the hood since the bleach wash has not started).
2. To avoid contaminating the original autoclaved saline solution, aliquot a small volume of 0.9% saline into a separate container for washing.
3. Fill the Falcon tube with nodules to approximately 20–25 mL with saline.
4. Vortex gently at very low speed for 30 seconds, ensuring the nodule surfaces are not damaged.
5. Take the tube to the sink and carefully decant as much liquid as possible without discarding the nodules.
6. Transfer the nodules onto a mesh sieve and rinse with DI water.
7. Rinse the empty tube with DI water and return the nodules using sterile forceps or a scooper.
8. Repeat steps 4–9 two more times (total of three saline washes) until the vortexed solution shows little to no visible dirt.

Note: All steps after saline washing should be performed under the biosafety hood to maintain aseptic conditions.

3 **Bleach Sterilization and Rinsing**

1. Prepare a fresh 1.8% bleach solution and place a discard beaker under the hood alongside containers of DDI water, LBA plates, and glycerol stock.
2. Pour the bleach solution into the washed Falcon tube until the nodules are just covered. Process one sample at a time to avoid confusion.
3. Vortex the tube for 30 seconds and let it sit for 1 minute.
4. Carefully pour the bleach solution into the waste beaker, ensuring that no nodules are lost.
5. Rinse the nodules by filling the tube with DDI water (20–25 mL), vortexing for 30 seconds, and discarding the water into the waste beaker.
6. Repeat the bleach wash once more and then perform four successive rinses with DDI water to remove all traces of bleach.

4 **Drying and Sterility Verification**

1. Transfer the rinsed nodules onto sterile filter paper to dry. Invert the tube and gently tap it against the filter paper at multiple spots to release all nodules.

2. Once dry, carefully transfer the nodules onto an LBA plate. Roll the nodules across the plate's surface and repeat on two additional plates (three plates total).
3. Incubate the plates upside down for 5 days. Check for any microbial growth to verify complete surface sterilization.

5 **Storage Preparation**

1. Under sterile conditions, transfer nodules into three labeled sterile 1.5 mL flip-cap tubes. Use any preferred method (sterile forceps, funnel, or folded sterile filter paper) to ensure complete transfer while maintaining sterility.
2. In the tube filled halfway with nodules, add 1 mL of 80% glycerol stock solution.
3. Store the tubes at -20°C in a labeled storage box.

6 **Expected Results**

- **Sterile nodules:** No microbial growth should be observed on LBA plates after incubation.
- **Successful storage:** Nodules preserved in glycerol stock are ready for downstream microbiological or genetic analyses.

Protocol references

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