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Identification of Bacteria using BIOLOG GEN III Assay

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Md Sahadat Ali[1], Fatima Tuz Zohora Mony[1], Jonathan D. Eisenback[1]

[1]Virginia Tech

Md Sahadat Ali
Virginia Tech

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We use this protocol and it's working

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Disclaimer

This protocol is optimized for BIOLOG GEN III analysis of *Pseudomonas* sp. Researchers must follow institutional biosafety regulations when handling bacterial cultures and using spectrophotometers and microplate readers. The authors are not responsible for procedural errors or misuse.

Abstract

This protocol describes the BIOLOG GEN III Assay used to analyze the metabolic and chemical sensitivity profiles of *Pseudomonas* sp. The assay assesses carbon source utilization (columns 1–9) and chemical sensitivity (columns 10–12) using BIOLOG GEN III microplates. The procedure includes bacterial culture preparation, inoculum standardization, microplate inoculation, incubation, and data collection using a Synergy plate reader.

Guidelines

- Use freshly grown bacterial strains to ensure maximum viability and metabolic vigor.
- Pre-warm BIOLOG GEN III microplates and Inoculating Fluid (IF A) before starting.
- Maintain aseptic techniques throughout the procedure.
- Ensure consistent pipetting and proper well-filling to avoid cross-contamination.
- Record OD (turbidity) correctly to maintain standard inoculum concentration.

Materials

Reagents & Supplies

- BIOLOG GEN III Microplates – For metabolic and chemical sensitivity analysis
- BUG+B Medium – For optimal bacterial growth
- Inoculating Fluid A (IF A) – For preparing bacterial suspension
- Sterile Disposable Transfer Pipettes – For accurate liquid handling
- Multichannel Pipettes (Electric Pipettes Used) – For uniform inoculation
- Reservoirs – For handling inoculum

Equipment

- BIOLOG Inoculator & Streaker – For transferring bacterial culture
- Spectrophotometer – For turbidity measurement
- Synergy Plate Reader – For kinetic data collection
- Incubator (28°C) – For controlled bacterial growth

Safety warnings

- Handle bacterial cultures aseptically to avoid contamination.
- Follow institutional biosafety protocols when using spectrophotometers and microplate readers.
- Properly dispose of used microplates and inoculating fluids according to lab guidelines.

Ethics statement

This protocol involves the BIOLOG GEN III assay for bacterial metabolic and chemical sensitivity analysis. No animal or human subjects were used in this study. However, researchers must follow institutional biosafety regulationswhen handling bacterial cultures, inoculating fluids, and microplates.
If this protocol is adapted for experiments involving animal or human-derived samples, prior approval must be obtained from an Institutional Animal Care and Use Committee (IACUC) or an equivalent ethics committee. Researchers must comply with internationally accepted ethical standards, and any relevant permit numbers and committee approvals should be documented accordingly.
All laboratory personnel must adhere to proper aseptic techniques, biosafety precautions, and waste disposal guidelines to ensure safe and responsible research practices.

Before start

- Review the protocol and precautions carefully.
- Label all tubes, microplates, and reservoirs appropriately.
- Ensure all reagents and microplates are at room temperature before starting.
- Set up the Synergy plate reader with the correct kinetic settings.

Procedure

1 **Culture bacteria on BUG+B Medium**

1. Grow *Pseudomonas* sp. on LB agar plates to obtain isolated colonies.
2. Subculture the strain on BUG+B medium for maximum metabolic activity.
3. Incubate the culture at 28°C for 20 hours to prevent entry into the stationary phase.

Note: Fresh bacterial cells are required to maintain optimal metabolic activity.

2 **Prepare Inoculum**

1. Adjust bacterial suspension to the desired turbidity to ensure standard inoculation.
2. Determine the target cell density using spectrophotometry (95%T at 600 nm).

- **Equation:**
- Absorbance = 2 - log (%T)
- Example: 95%T → Absorbance = 2 - log(98) = 0.009

3. Standardize the inoculum concentration:

- Measured OD = 0.041 (for example)
- Calculation:
- Given volume (V1) = 10 mL
- Target OD (S1) = 0.009
- V1 × S1 = V2 × S2
- 10 × 0.009 = V2 × 0.041
- V2 = 2.2 mL
- Required Inoculating Fluid (IF) to dilute sample to 0.009 OD = 7.8 mL

4. Use a streaker to pick up bacterial colonies from the agar plate.
5. Transfer bacterial cells into IF A and mix well to obtain a uniform suspension.
6. Pour 1 mL of bacterial suspension into a cuvette and measure OD at 600 nm.
7. Adjust the OD to 0.009 by adding more IF A if necessary.

Note: A precise OD adjustment is critical for assay reproducibility.

3 **Inoculation of BIOLOG GEN III Microplate**

1. Transfer the bacterial suspension into a multichannel pipette reservoir.
2. Attach 8 sterile pipette tips onto the 8-Channel Repeating Pipettor.
3. Fill pipette tips with bacterial suspension.
4. Dispense 100 µL into each well of the BIOLOG GEN III microplate.

- Avoid cross-contamination by preventing splashes.
- Ensure equal filling of all wells for consistent results.

5. Cover the microplate with its lid to prevent contamination.

Note: Carefully ensure no carryover or uneven pipetting

4 **Incubate the Microplate**

1. Place the microplate in an incubator at 28°C for 48 hours.
2. Collect data at multiple time points (12, 16, 24, and 48 hours).

Note: Longer incubation times allow for more distinct metabolic reactions.

5 **Data Collection Using Synergy Plate ReaderPlate Reader Settings**

- Temperature Setpoint: 28°C
- Kinetic Run: Start (0:03:00), Interval (0:00:50)
- Shaking Mode: Orbital (Continuous)
- Read Mode: Absorbance at 600 nm
- End Kinetic: Plate Out

6 **Expected Results**

1. **Carbon Source Utilization (Columns 1–9):**

- Compare wells to A-1 (Negative Control Well).
- Negative (-): No color change (same as A-1).
- Positive (+): Purple color development (stronger than A-1).
- Borderline (\ ): Faint purple or weak reaction.

2. **Chemical Sensitivity Assays (Columns 10–12):**

- Compare wells to A-10 (Positive Control Well).
- Negative (-): Less than 50% color intensity of A-10 (bacterial growth inhibition).
- Positive (+): Normal or near-normal purple color (resistant to chemical).
- Borderline (\ ): Unclear results.

Note: Some genera may exhibit faint reactions, which should be interpreted carefully.