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Identification of *Pseudomonas* sp. using fatty acid methyl ester (FAME) analysis

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Protocol status: Working

We use this protocol and it's working



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Disclaimer

This protocol has been optimized for FAME analysis of *Pseudomonas* sp. Researchers must follow biosafety and chemical handling regulations when working with microbial cultures and hazardous reagents. The authors are not responsible for procedural errors or misuse.

Abstract

This protocol describes the extraction, methylation, and gas chromatography (GC) analysis of cellular fatty acids from a *Pseudomonas* sp. The Fatty Acid Methyl Ester (FAME) method enables bacterial identification based on characteristic fatty acid profiles. The procedure involves saponification, methylation, extraction, and sample cleanup to prepare FAMES for gas chromatography (GC) analysis, ensuring high accuracy and reproducibility.

Guidelines

- All reagent transfers must be done in a fume hood to prevent exposure to hazardous chemicals.
- Maintain aseptic conditions throughout to prevent contamination.
- Ensure proper labeling of all tubes and vials to avoid sample mix-ups.
- Monitor water bath temperatures to prevent overheating or tube breakage.
- Dispose of hazardous waste properly according to institutional safety guidelines.

Materials

Reagents & Solutions

Reagent 1 (Saponification Solution):

- 45g sodium hydroxide
- 150mL methanol
- 150mL distilled water

Reagent 2 (Methylation Solution):

- 325mL certified 6.0N hydrochloric acid
- 275mL methyl alcohol

Reagent 3 (Extraction Solution):

- 200mL hexane
- 200mL methyl tert-butyl ether

Reagent 4 (Sample Cleanup Solution):

- 10.8g sodium hydroxide dissolved in 900mL distilled water

Consumables & Equipment

Glassware & Tubes:

- Pyrex glass tubes (for reaction steps)
- Brown screw-cap vials (for GC sample storage)

Lab Equipment:

- Hot plate (for water bath heating)
- Water bath (98–100°C)
- Thermometer (for temperature monitoring)
- Vortex mixer (for sample mixing)
- Pipettes & Large Tips (for reagent transfers)
- Pebble box (80°C)
- Ice bath (for rapid cooling)
- Clinical rotator (optional) (for sample mixing)

Other Supplies:

- T-streak plates with bacterial strains
- Plastic inoculation loops
- Ethanol (for cleaning hands and tools)
- Parafilm (for plate wrapping)
- Permanent markers (for labeling tubes and vials)

Safety warnings



- Perform all reagent handling in a fume hood.
- Wear gloves, lab coats, and safety goggles when handling chemicals.
- Dispose of chemical waste according to institutional safety guidelines.

Ethics statement

This protocol involves the Fatty Acid Methyl Ester (FAME) analysis of bacterial strains using gas chromatography (GC). No animals or human subjects were involved in this study. However, researchers must adhere to institutional biosafety and chemical safety regulations when handling bacterial cultures and hazardous reagents such as sodium hydroxide, hydrochloric acid, and organic solvents (hexane, methyl tert-butyl ether).

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.

Proper biosafety, waste disposal, and ethical considerations must be followed throughout the experimental process to ensure safe and responsible research practices.

Before start

- Ensure all reagents are freshly prepared and properly stored.
- Label all tubes and vials before sample processing.
- Set up a clean, contamination-free workspace.
- Set up the water bath to reach 100°C using a hot plate. Monitor the water level carefully to ensure it does not overflow or submerge the Pyrex tubes when boiling. Too much water can cause the tubes to be fully submerged, leading to contamination and potential loss of samples. Too little water may result in uneven heating and inefficient saponification.
- Prepare fume hood for safe reagent handling.

1 Sample Preparation

1. Obtain one Pyrex glass tube for each bacterial strain and ensure it is clean and dry.
 - If the tube has been cleaned with soap, ensure no residual soap particles remain, as they may interfere with downstream analysis.
2. Label each tube with the corresponding bacterial strain name to avoid sample mix-ups.
3. Place the labeled tubes in a rack inside a Biological Safety Cabinet (BSC) to maintain sterility and prevent contamination.
4. Prepare the water bath by heating it to 100°C using a hot plate.
 - Use a thermometer to confirm the water reaches and maintains 100°C before proceeding.
 - Avoid sudden temperature fluctuations by adjusting the hot plate gradually.
5. Using a sterile inoculation loop, collect bacterial biomass from the third quadrant of a T-streak plate and lightly smear the bacteria at the bottom of the tube.
 - Ensure a sufficient bacterial mass is collected for reliable fatty acid analysis.
6. Wrap T-streak plates with parafilm after inoculation to maintain aseptic conditions and prevent desiccation.
7. When placing tubes in the water bath, ensure that the caps remain above the water level to prevent:
 - Contamination from external sources.
 - Unintentional dilution of the sample with water from the bath.

Note: Proper sample collection and handling are critical for accurate downstream fatty acid methyl ester (FAME) analysis

2 Saponification

1. In a fume hood, prepare an aliquot of Reagent 1 (Saponification Solution) instead of using it directly from the stock to minimize contamination risk.
2. Transfer 1 mL of Reagent 1 into each sample tube using a pipette:
 - Get the reagent aliquot as close to the sample tube as possible to prevent reagent loss from the pipette tip before reaching the tube.
 - Pipette up and down a few times before transferring to prevent reagent loss and ensure proper dispensing.
 - Change pipette tips between different samples to avoid cross-contamination.
3. Vortex each tube for 5 seconds at the highest setting to ensure proper mixing.
4. Place tubes in a 98°C water bath for 5 minutes, ensuring:
 - The water does not reach the tube caps to prevent contamination.
 - The solution level in all tubes is equal—if any tubes show a higher or lower level, it may indicate:
 - Water entered the tube (higher level) due to a loose cap or broken tube.
 - Evaporation occurred (lower level) due to improper sealing or heat exposure.
5. Cool tubes briefly (~ 2-3 minutes) in a rack before transferring them to an ice bath, otherwise sudden cooling may cause tube breakage.

6. Return tubes to the boiling water bath for an additional 25 minutes to complete saponification.
 7. After heating, cool tubes for ~ 2-3 minutes in a rack before transferring tubes to an ice bath to cool for the next step.
- Note:** Saponification breaks down lipid components to release free fatty acids for further processing in FAME analysis

3 **Methylation**

1. Label brown screw-cap vials to match Pyrex tubes.
2. In the fume hood, add 2 mL of Reagent 2 from an aliquot (Methylation Solution) to each tube.
3. Vortex briefly to mix.
4. Place tubes in an 80°C pebble box for 10 minutes.

Note: In this step, take care of time and temperature because this step is very time and temperature sensitive.

5. After the time has passed, take the tubes out and let them sit for a few minutes. Then return them to an ice bath for a few minutes.

Note: Methylation converts fatty acids into Fatty Acid Methyl Esters (FAMES) for GC analysis.

4 **Extraction**

1. In the fume hood, add 1.5 mL of Reagent 3 (Extraction Solution) to each tube, remembering to practice doing up and downs with the pipette in the reagent before transferring it into the tubes.
2. Recap tubes and mix by tumbling by hand or using a clinical rotator for 10 minutes. If experimenting with two people, hand-tumble half of the tubes for 5 minutes, switch with a partner, and hand-tumble the other half for 5 minutes (if no clinical rotator is available).
3. Carefully remove the bottom aqueous layer using a pipette and discard into a waste beaker. This step is tricky, you should keep proper observation of the level that separates the top part from the bottom.

Note: This step ensures separation of FAMES into the organic phase.

5 **Sample Cleanup**

1. Add 3 mL of Reagent 4 (Sample Cleanup Solution) to each tube.
2. Recap tubes and mix by hand tumbling for 5 minutes.
3. Once phase separation is clear, transfer 2/3 of the top organic phase into pre-labeled brown screw-cap vials using a pipette.
4. Avoid transferring bubbles or aqueous phase contaminants.

Note: Proper cleanup prevents contamination of the GC injection port and column.

6 **Storage and Analysis**

1. Store vials at -20°C for at least one hour to ensure complete phase separation.
2. If phase separation is incomplete, leave the vials overnight.
3. Transfer the clear organic phase into new vials if necessary.



4. Prepare samples for gas chromatography (GC) analysis.

Note: Ensure samples are completely separated before GC injection.

7 Expected Results

- Successful FAME extraction results in clear organic samples ready for GC analysis.
- GC chromatograms will display characteristic fatty acid peaks.
- Comparison with reference libraries will confirm the bacterial identity.

Protocol references

Kunitsky C, Osterhout G, Sasser M. Identification of microorganisms using fatty acid methyl ester (FAME) analysis and the MIDI Sherlock® microbial identification system. Newark (DE): MIDI, Inc.; 2006.