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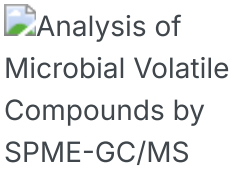
Analysis of Microbial Volatile Compounds by SPME-GC/MS



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We use this protocol and it's working

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Disclaimer

This protocol is intended solely for research and educational purposes. While the procedures have been validated in the authors' laboratory, results may vary depending on strain, media composition, and experimental conditions. Users are responsible for ensuring compliance with institutional biosafety and chemical safety regulations. The authors and Virginia Tech disclaim liability for misuse or misapplication of the protocol.

Abstract

This protocol describes the use of headspace solid-phase microextraction (SPME) and GC-MS to identify volatile organic compounds (VOCs) produced by '*Candidatus* Pseudomonas auctus' JDE115 under different culture conditions. VOCs are adsorbed on a 50/30 µm DVB/CAR/PDMS StableFlex® fiber, thermally desorbed in a GC injector, and analyzed on a DB-5 column with an Agilent 7890 GC–5975C MSD system. Compounds are identified by comparison to Wiley and NIST spectral libraries. The assay confirms bacterial production of 1-undecene and dimethyl disulfide (DMDS), both implicated in antifungal activity

Attachments

DOCX

SPME-GCMS Analysis o...

3.7MB

Image Attribution

All images used in this protocol, including photographs of agar plates and assay setups, were generated by the authors at Virginia Tech. Images are original and may be used for academic and research purposes with proper citation of this protocol.

Guidelines

- Use freshly prepared PDA medium in airtight vials.
- Pre-condition SPME fibers according to manufacturer's instructions.
- Run all samples, blanks, and controls under identical instrument conditions for reproducibility.
- Include both bacterial and fungal controls, plus medium-only blanks.

Materials

- '*Candidatus* P. auctus' JDE115 culture (LB agar, 48 h, 28 °C)
- *Agroathelia rolfsii* culture (optional co-culture condition)
- Potato dextrose agar (PDA) medium
- 20 mL glass vials with Teflon septa (airtight)
- 50/30 µm DVB/CAR/PDMS StableFlex® SPME fiber (Supelco, USA)
- Deionized water
- Helium (carrier gas, GC-grade)

Equipment

- Incubator (28 °C)
- Magnetic stirrer and stir bars
- Agilent® 7890 GC coupled to a 5975C mass selective detector (MSD)
- DB-5 capillary column (30 m × 250 µm I.D., 0.25 µm film thickness)
- Vortex mixer
- Analytical balance
- Pipettes and sterile tips

Safety warnings

- Handle all cultures in a biosafety cabinet when possible.
- Volatile metabolites may include hazardous compounds; avoid direct inhalation.
- GC-MS solvents and carrier gases must be handled in accordance with institutional chemical safety rules.
- Follow manufacturer instructions for safe handling of SPME fibers.

Ethics statement

This protocol involves only microbial cultures and chemical analysis, requiring no human or animal ethics approval. All procedures must be conducted in accordance with institutional biosafety and chemical safety guidelines.

Before start

- Pre-clean and bake glass vials to remove contaminants.
- Verify incubator temperature at 28 °C.
- Prepare labeled 20 mL airtight vials with Teflon septa.
- Confirm GC-MS system calibration and mass library availability.

Treatment Setup

1 **Culture conditions:**

- JDE115 (bacterial control)
- *A. rolfsii* (fungal control)
- JDE115 + *A. rolfsii* (co-culture)
- Medium-only (blank control)

Inoculate 10 mL PDA into 20 mL airtight vials, incubate at 28 °C.

Sample preparation

2

- Prepare airtight 20 mL vials with 10 mL PDA.
- Inoculate according to the four experimental conditions.
- Incubate at 28 °C until VOC production stage.
- Equilibrate samples at room temperature for 30 min.
- Insert pre-conditioned SPME fiber into vial headspace for 10 min exposure.
- Retract fiber and immediately inject into GC injector port.

GC-MS analysis

3

- Injector port: 200 °C
- Split ratio: 1:10
- Purge flow: 3 mL/min
- Carrier gas: Helium, 1.0 mL/min constant flow
- Oven program:

Initial 35 °C, hold 3 mins
Ramp 15 °C/min to 200 °C
Hold 200 °C for 5 min

- Transfer line: 220 °C
- Mass spectra: Electron ionization (EI) mode
- Library: Wiley and NIST

Data acquisition

4

- Collect chromatograms and spectra for each sample.
- Identify peaks by comparison to library spectra.
- Quantify target compounds by peak integration (e.g., DMDS, 1-undecene).

Protocol references

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