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Isolation, Identification, and Antimicrobial Activity of Soil Bacteria in Sana'a City Against Some Pathogenic Bacteria with GC-MS Profiling of Active Extracts

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Article

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Abstract

Background

Soil serves as a vast reservoir of microbial diversity capable of producing biologically active secondary metabolites. The escalating global threat of antimicrobial resistance mandates the exploration of natural environments for novel bioactive agents.

Objectives

This study aimed to isolate and identify soil bacteria from various sites in Sana'a City, Yemen, evaluate their antibacterial activity against clinical pathogens, and profile the chemical composition of their crude extracts using Gas Chromatography-Mass Spectrometry (GC-MS).

Methods

Fifty soil samples were collected across ten locations. Bacterial isolates were identified via morphological characterization, Gram staining, and biochemical assays (oxidase, catalase, citrate utilization, and TSI). Antimicrobial efficiency was screened against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the agar well diffusion method. Crude secondary metabolites were extracted using chloroform and ethanol (1:1 v/v) and analyzed by GC-MS.

Results

Fifty bacterial isolates were recovered, with the highest yield obtained from Art College (22%) and Alhasabah Street (16%). Sixteen isolates demonstrated antibacterial activity, among which four representative species (*Pseudomonas fluorescens*, *Bacillus subtilis*, *Acinetobacter baylyi*, and *Azotobacter vinelandii*) exhibited potent broad-spectrum inhibition. Isolate B10 showed the highest inhibition against *S. aureus* (19 mm) and *P. aeruginosa* (15 mm). GC-MS analysis revealed complex chemical profiles rich in bioactive fatty acids (e.g., n-hexadecanoic acid, octadecanoic acid), phenolic compounds, amides (octadecanamide), esters, and terpenoids (squalene).

Conclusion

Soil bacteria from Sana'a City synthesize diverse secondary metabolites with marked antimicrobial efficacy. These findings highlight local soil microbiomes as potential sources for novel therapeutic applications.

1. Introduction

Soil is a highly complex, heterogeneous, and nutrient-limited ecosystem supporting an extraordinary diversity of microorganisms. Among microhabitats, the rhizosphere represents a dynamic hotspot of microbial abundance and metabolic exchanges driven by plant root exudates. Soil microbes have historically served as the primary natural source of clinically vital antibiotics.

Antibiotic resistance has emerged as a major global public health challenge, leading to increased morbidity, mortality, prolonged hospital stays, and escalating healthcare costs. Pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* present severe clinical complications due to resistance mechanisms including extended-spectrum β-lactamases (ESBLs), multidrug efflux pumps, and biofilm formation.

Soil bacteria synthesize diverse secondary metabolites, broadly categorized into volatile organic compounds (VOCs) and soluble compounds, which mediate ecological competition and pathogen inhibition. Specific antimicrobial metabolites are reported in approximately 50% of *Bacillus* species and over 88% of *Pseudomonas* species inhabiting rhizospheric environments.

To address the shortage of effective therapeutics, this study was conducted to isolate bacterial strains from soil environments in Sana'a City, Yemen, evaluate their antagonistic activity against clinical pathogens, and characterize the chemical constituents of their bioactive extracts via GC-MS profiling.

2. Materials and Methods

2.1. Sampling Sites and Sample Collection

Fifty soil samples were systematically collected from the rhizosphere (April–August 2024) across ten distinct sites in Sana'a City:

1. Science College (July 6, 2024)
2. Art College (July 6, 2024)
3. Al_Ahsan Garden (July 7, 2024)
4. New Sana'a University (July 8, 2024)
5. Altahrer Street (July 9, 2024)
6. Alhasbah Street (July 12, 2024)
7. Sawan Garden (July 13, 2024)
8. Home’s Soil (July 16, 2024)
9. Almadenah Street (July 18, 2024)
10. Motaher Taqi Street (July 19, 2024)

Soil samples (~ 5 g) were taken using sterile spatulas and collected in clean, dry, sterile plastic bags.

2.2. Isolation and Phenotypic Identification

One gram of each soil sample was dissolved in 10 mL of sterile distilled water to prepare soil suspensions. Suspensions were inoculated onto Nutrient Agar (NA, OXOID, England) plates by streaking and incubated at 37°C for 24 hours. Pure single colonies were picked and transferred into Nutrient Broth (NB). Isolates were identified based on colony morphology, Gram staining, and standard biochemical tests (Oxidase, Catalase, Citrate Utilization, and Triple Sugar Iron tests).

2.3. Test Pathogens and Antibacterial Screening

Test pathogenic strains (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) were obtained from Aulaqi Laboratory.

Antibacterial screening was performed using the agar well diffusion method on Mueller-Hinton Agar (MHA, OXOID). Standardized bacterial suspensions were spread to form uniform lawns on 150 mm Petri dishes. Wells of 6 mm diameter were cut using a sterile cork borer, and 10 μL of bacterial extract was added per well. Plates were incubated at 37°C, and clear inhibition zone diameters (mm) were recorded.

2.4. Extraction of Secondary Metabolites

Bacterial isolates were inoculated in NB Erlenmeyer flasks and incubated on a rotary shaker at 110 rpm at room temperature for 7 days. The culture broth was filtered, and secondary metabolites were extracted using chloroform and ethanol separately in a 1:1 (v/v) ratio. Chloroform-treated broths were separated using a separating funnel to collect the solvent layer.

2.5. GC-MS Analysis

GC-MS profiling was conducted at the Yemen Standardization, Metrology and Quality Control Organization (YSMO), Sana'a, using a Shimadzu GCMS QP2020 NX system (Japan) and Perkin–Elmer GC Clarus 500 equipped with an Elite-5MS capillary column (30 m × 0.25 µm ID × 0.25 µm df). Carrier Gas: Helium (99.999%) at 1.0 mL/min. Mass Spectra: Electron ionization at 70 eV; scan range 45–450 Da. Spectra were matched against the National Institute of Standards and Technology (NIST) reference library.

3. Results

3.1. Soil Isolates Recovery

A total of 50 bacterial isolates were recovered from 50 soil samples across 10 locations in Sana'a City. The highest distribution was observed in Art College (n=11, 22%), Alhasbah Street (n=8, 16%), and Science College (n=7, 14%). See table 1

Table 1: Distribution and percentage of soil bacterial isolates across sampling sites in Sana'a City.

Sample No.	Sampling Site	Number of Isolates	Percentage (%)
1	Science College	7	14%
2	Art College	11	22%
3	Al_Ahsan Garden	3	6%
4	New Sana'a University	2	4%
5	Altahrer Street	3	6%
6	Alhasbah Street	8	16%
7	Sawan Garden	4	8%
8	Home’s Soil (from plant)	2	4%
9	Almadenah Street	4	8%
10	Motaher Taqi Street	6	12%
Total		**50**	**100%**

3.2. Antimicrobial Screening against Pathogens

Out of 50 isolates, 16 demonstrated measurable antagonistic activity, and 4 isolates exhibited potent broad-spectrum activity. See table 2

Table 2: Antimicrobial activity (inhibition zone diameter, mm) of soil bacterial isolates against pathogenic bacteria.

Isolate Code	S. aureus	K. pneumoniae	E. coli	P. aeruginosa
A/4	10	9	8	-
A/5	6	-	6	12
B/7	**18**	**-**	**13**	**10**
B/8	**16**	**8**	**-**	**13**
B/10	**19**	**-**	**13**	**15**
G/2	**16**	**16**	**-**	**13**
G/4	10	8	10	-
H/1	10	13	10	13
H/2	12	14	13	10
W/1	8	11	12	-
W/2	9	10	10	-
W/3	12	12	12	10
W/4	9	10	15	-
P/3	**15**	**10**	**15**	**12**
P/4	12	13	10	-
P/6	12	7	7	-

Note: (-) indicates no zone of inhibition.

3.3. Biochemical Identification of Key Potent Isolates

Four representative isolates showing major broad-spectrum activity were identified to the species level. See table 3.1

- **Table 3.1: Biochemical profile of *Pseudomonas fluorescens***
    - Gram stain: Gram-negative rods
    - Oxidase: Positive
    - Catalase: Positive
    - Citrate Utilization: Positive
    - TSI Test: Alkaline slant / Alkaline butt (K/K), No H_2S, No Gas. See table 3.2
- **Table 3.2: Biochemical profile of *Bacillus subtilis***

- Gram stain: Gram-positive rods (spore-forming)
- Oxidase: Positive
- Catalase: Positive
- Citrate Utilization: Positive
- TSI Test: Acid slant / Acid butt (A/A), Gas positive, No H_2S. See table 3.3

- **Table 3.3: Biochemical profile of *Acinetobacter baylyi***

  - Gram stain: Gram-negative coccobacilli/rods
  - Oxidase: Negative
  - Catalase: Positive
  - Citrate Utilization: Positive
  - TSI Test: Alkaline slant / No change butt (K/NC), No H_2S, No Gas .See table 3.4

- **Table 3.4: Biochemical profile of *Azotobacter vinelandii***

- Gram stain: Gram-negative oval/large rods
- Oxidase: Positive
- Catalase: Positive
- Citrate Utilization: Positive
- TSI Test: Slant/Butt metabolic reactions consistent with non-fermentative nitrogen-fixer .

3.4. GC-MS Profile of Secondary Metabolites

1. *Pseudomonas fluorescens* Extract

Major bioactive components detected:

- Fatty acids: n-Hexadecanoic acid, Octadecanoic acid
- Phenolic compounds & Ester derivatives
- Cyclic siloxanes & Terpenoids (e.g., Squalene)

2. *Bacillus subtilis* Extract

Major bioactive components detected:

- Tyramine
- Hexadecanoic acid & Octadecanoic acid
- Octadecanamide
- Fatty acid esters and phenolic derivatives

3. *Acinetobacter baylyi* and *Azotobacter vinelandii* Extracts

Major bioactive components detected:

- Saturated/unsaturated fatty acids
- Esterified compounds and hydrocarbon derivatives

4. Discussion

Soil environments in Sana'a City represent competitive microbial habitats where bacteria synthesize secondary metabolites for survival and niche colonization. The recovery of 50 isolates and the subsequent identification of 16 antagonistic strains affirm that Yemeni soil microbiomes possess significant biological potential.

S. aureus exhibited high susceptibility to the extracts, with isolate B10 producing an inhibition zone of 19 mm. Gram-negative pathogens (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) were also inhibited by selects isolates (e.g., B10, G2, P3). Inhibition of *P. aeruginosa* (up to 15 mm by isolate B10) is notable given its intrinsic resistance and outer membrane impermeability.

The antibacterial mechanisms are supported by the GC-MS chemical profiling:

1. **Fatty Acids (n-Hexadecanoic acid & Octadecanoic acid)**: Known to disrupt bacterial cell membranes, alter membrane fluidity, and induce cell lysis.
2. **Tyramine & Amides (Octadecanamide)**: Interference with essential bacterial cell wall synthesis and metabolic pathways.
3. **Phenolics & Terpenoids (Squalene)**: Exert antioxidant and membrane-permeabilizing activity.

The broad-spectrum inhibitory action observed in the crude extracts is likely driven by synergistic interactions among multiple constituent compounds rather than a single isolated metabolite.

5. Conclusion and Recommendations

5.1. Conclusion

This study confirms that soil bacteria isolated from Sana'a City, particularly *Pseudomonas fluorescens*, *Bacillus subtilis*, *Acinetobacter baylyi*, and *Azotobacter vinelandii*, synthesize potent antimicrobial secondary metabolites. Integrating classical microbiological assays with GC-MS chemical characterization validated the biological efficacy and identified key chemical classes responsible for pathogen inhibition.

5.2. Recommendations

1. Purification and isolation of individual bioactive compounds from crude extracts using column chromatography and HPLC.
2. Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) for isolated pure compounds.
3. Conducting *in vivo* toxicity and biocompatibility assays to evaluate pharmaceutical safety.
4. Exploring biotechnological and agricultural applications of these isolates as biocontrol agents against plant and human pathogens.

Declarations

Ethics Approval and Consent to Participate:

Ethics declaration: Not applicable. This study involved the isolation and characterization of environmental soil bacteria and did not involve any human participants, human biological samples, or live animal subjects. No institutional ethics committee approval was required for environmental soil sampling

Consent to Participate declaration:

Not applicable.

Consent for Publication:

Not applicable.

Availability of Data and Materials:

All data generated or analyzed during this study are included in this published article.

Competing Interests:

The authors declare that they have no competing interests.

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Authors' Contributions:

Miadah Mojahed Mohammed Al-Odle performed sample collection, laboratory experiments, and manuscript drafting. Abdulrhman Abdullah Hasan Humaid designed and supervised the study, interpreted the data, and revised the manuscript. Both authors read and approved the final manuscript.

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