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Microbial Diversity Assessment in Soil from Dumping Sites: Isolation, DNA Extraction and Electrophoretic Analysis

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Abstract:- This study aims to investigate the microbial diversity present in soil samples collected from various dumping sites, which are known for their complex and heterogeneous waste environments. The primary objective is to isolate and characterize the microbial communities inhabiting these soils, providing insights into their composition and potential ecological roles.

Soil samples were collected by digging 5 cm deep at multiple dumping locations to ensure a representative sampling of the microbial populations. The collected soil samples underwent a serial dilution process, extending up to 10^-9 and 10^-10 dilutions, to facilitate the isolation of individual microbial colonies. These diluted samples were then inoculated onto Standard Potato Dextrose Agar (PDA) media, a nutrient-rich medium conducive to microbial growth. Following an incubation period, distinct bacterial colonies were observed and isolated for further analysis.

DNA was extracted from the isolated bacterial colonies using a standard DNA extraction protocol. The quality and purity of the extracted DNA were assessed through gel electrophoresis, a technique that separates DNA fragments based on their size. Remarkably, the electrophoretic analysis revealed clear and distinct DNA bands along with RNA bands, indicating successful extraction of high-quality microbial DNA. Notably, this was achieved without the use of proteinase treatment, which is typically employed to remove protein contaminants from DNA samples.

The results of this study underscore the robustness of the methodologies employed in isolating and characterizing microbial DNA from soil samples collected at dumping sites. The presence of distinct DNA and RNA bands highlights the effectiveness of the DNA extraction process, even in the absence of proteinase treatment. These findings provide valuable insights into the microbial diversity within dumping site soils, suggesting a rich and varied microbial community that may play significant roles in soil health and waste decomposition.

I. INTRODUCTION

➢ *Background*

Soil microbial diversity plays a crucial role in nutrient cycling, soil fertility, and the degradation of organic matter. Dumping sites, often rich in organic and inorganic waste, provide a unique environment for microbial communities. Understanding the microbial diversity in such sites can provide insights into the ecological impact of waste disposal and the potential for bioremediation.

➢ *Research Question/Hypothesis*

This study aims to assess the microbial diversity in soil from dumping sites by isolating bacteria and analyzing their DNA.

➢ *Objectives*

- To collect soil samples from various dumping sites.
- To perform serial dilution and inoculate samples on PDA media.
- To isolate bacterial colonies and extract their DNA.
- To analyze the extracted DNA using gel electrophoresis.

II. LITERATURE REVIEW

➢ *Existing Research*

Previous studies have demonstrated the presence of diverse microbial communities in soil samples from various environments, including agricultural fields, forests, and urban areas. However, less is known about the microbial diversity in dumping sites, which are characterized by a mixture of organic and inorganic waste materials.

Fierer, N., & Jackson, R. B. (2006). "The diversity and biogeography of soil bacterial communities." *Proceedings of the National Academy of Sciences*, 103(3), 626-631. DOI: 10.1073/pnas.0507535103

Upadhyay, A., & Roy, R. B. (2014). "Assessment of microbial diversity in soil of landfill sites in Pune, India." *International Journal of Current Microbiology and Applied Sciences*, 3(5), 1032-1042.

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Tian, Y., Zhang, Y., & Li, Y. (2017). "Method for isolation of soil microorganisms and its application." *Chinese Journal of Applied and Environmental Biology*, 23(1), 150-154. DOI: 10.3724/SP.J.1141.2017.01050

➢ *Theoretical Framework*

The study is grounded in the principles of microbial ecology and molecular biology, focusing on the identification and characterization of microbial communities using culture-dependent methods and molecular techniques.

III. MATERIALS AND METHODS

A. Soil Sample Collection

➢ *Materials*

- Sterile gloves
- Trowel
- Sterile sample containers
- Permanent marker
- Notepad

➢ *Method*

Soil samples were collected from different dumping sites by digging 5 cm deep. Approximately 50 grams of soil from each site were placed into sterile containers, labeled, and transported to the laboratory for analysis.

Fig 1 Dry Waste Dump(I)

Fig 2 Wet Waste Dump(II)

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B. Serial Dilution and Inoculation Materials

- Sterile water
- Test tubes
- Pipettes
- Standard Potato Dextrose Agar (PDA) plates
- Incubator

➢ *Method*

Soil samples were subjected to serial dilution up to 10^-9 and 10^-10 dilutions. Diluted samples were inoculated onto PDA media plates and incubated at 37°C for 48-72 hours. Bacterial colonies were observed and counted.

Fig 3 Test Tubes used for Serial Dilution Process

Fig 4 Incubation & Media Preparation

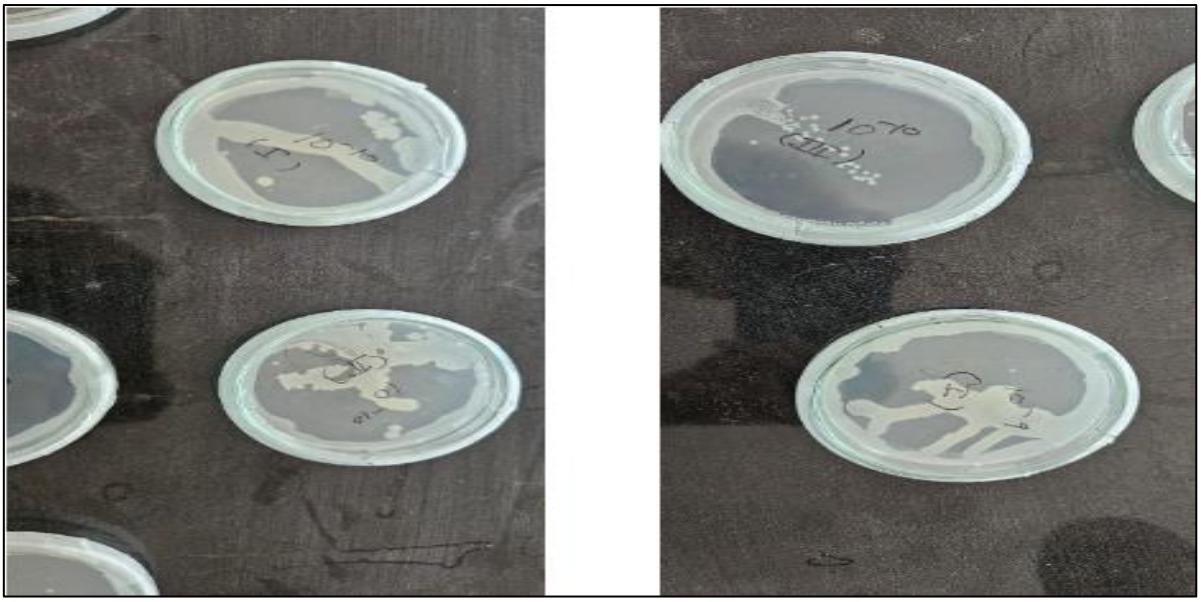

Fig 5 Growth of Bacterial Colonies

C. DNA Isolation

➢ *Materials*

- Bacterial colonies
- DNA extraction kit
- Microcentrifuge tubes
- Centrifuge
- Pipettes

➢ *Method*

Bacterial colonies were picked and used for DNA extraction following the protocol of the DNA extraction. The extracted DNA was quantified and assessed for purity.

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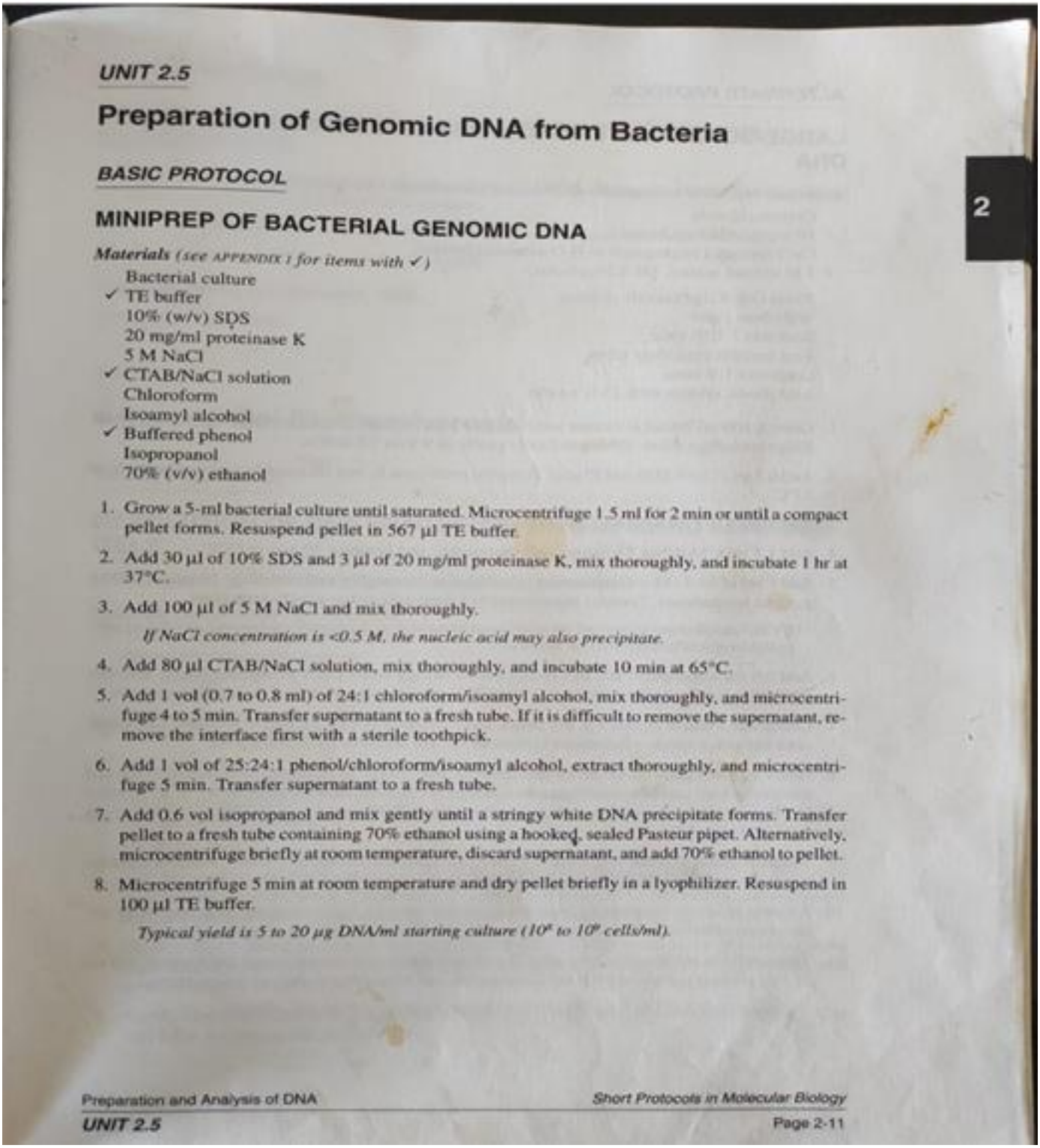

UNIT 2.5

Preparation of Genomic DNA from Bacteria

BASIC PROTOCOL

2

MINIPREP OF BACTERIAL GENOMIC DNA

Materials (see APPENDIX 1 for items with ✓)

Bacterial culture
✓ TE buffer
10% (w/v) SDS
20 mg/ml proteinase K
5 M NaCl
✓ CTAB/NaCl solution
Chloroform
Isoamyl alcohol
✓ Buffered phenol
Isopropanol
70% (v/v) ethanol

1. Grow a 5-ml bacterial culture until saturated. Microcentrifuge 1.5 ml for 2 min or until a compact pellet forms. Resuspend pellet in 567 µl TE buffer.
2. Add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K, mix thoroughly, and incubate 1 hr at 37°C.
3. Add 100 µl of 5 M NaCl and mix thoroughly.

   If NaCl concentration is <0.5 M, the nucleic acid may also precipitate.
4. Add 80 µl CTAB/NaCl solution, mix thoroughly, and incubate 10 min at 65°C.
5. Add 1 vol (0.7 to 0.8 ml) of 24:1 chloroform/isoamyl alcohol, mix thoroughly, and microcentrifuge 4 to 5 min. Transfer supernatant to a fresh tube. If it is difficult to remove the supernatant, remove the interface first with a sterile toothpick.
6. Add 1 vol of 25:24:1 phenol/chloroform/isoamyl alcohol, extract thoroughly, and microcentrifuge 5 min. Transfer supernatant to a fresh tube.
7. Add 0.6 vol isopropanol and mix gently until a stringy white DNA precipitate forms. Transfer pellet to a fresh tube containing 70% ethanol using a hooked, sealed Pasteur pipet. Alternatively, microcentrifuge briefly at room temperature, discard supernatant, and add 70% ethanol to pellet.
8. Microcentrifuge 5 min at room temperature and dry pellet briefly in a lyophilizer. Resuspend in 100 µl TE buffer.

   Typical yield is 5 to 20 µg DNA/ml starting culture (10^8 to 10^9 cells/ml).

Preparation and Analysis of DNA — Short Protocols in Molecular Biology
UNIT 2.5 — Page 2-11

Fig 6 Protocol for DNA Isolation

D. Gel Electrophoresis

➢ *Materials*

- Agarose gel
- Gel electrophoresis apparatus
- TAE buffer
- DNA ladder
- Ethidium bromide
- UV transilluminator

➢ *Method*

The extracted DNA was subjected to gel electrophoresis using a 1% agarose gel in TAE buffer. The gel was stained with ethidium bromide and visualized under a UV transilluminator to assess the presence of DNA and RNA bands.

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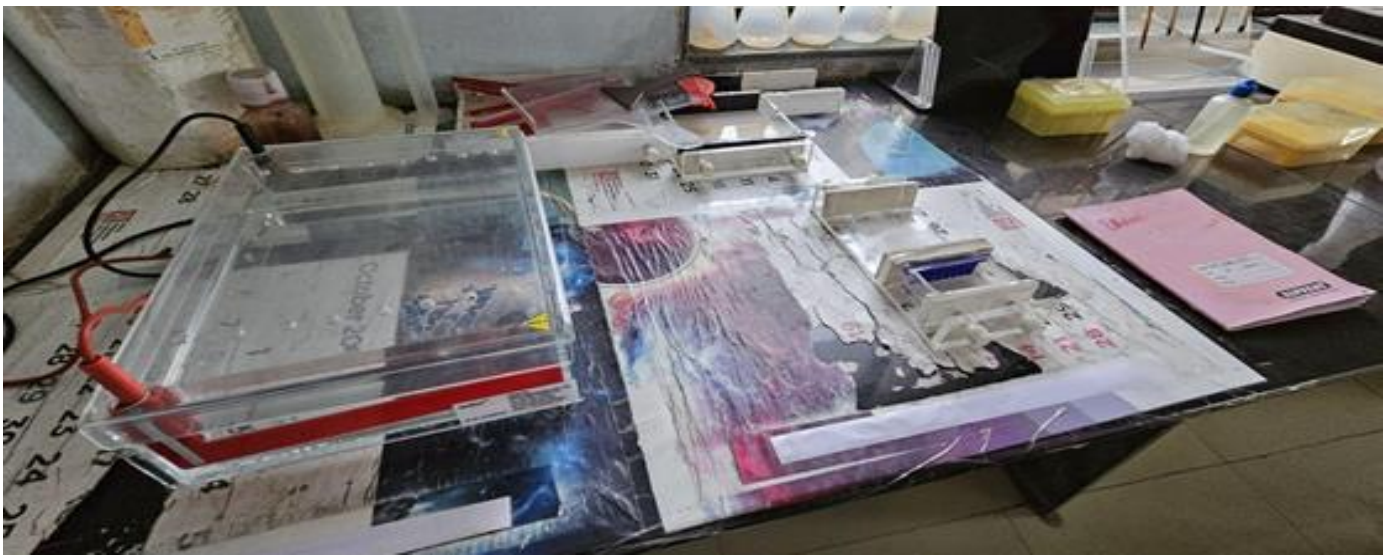

Fig 7 Gel Electrophoresis Assembly used for Electrophoresis

IV. RESULTS

➢ *Data Presentation*

Bacterial colonies were successfully isolated from the soil samples. The DNA extracted from these colonies showed distinct bands on the agarose gel. Both DNA and RNA bands were visible, indicating successful isolation of genetic material without protein contamination.

➢ *Figures and Tables*

Table 1 Number of Bacterial Colonies Isolated from Each Soil Sample.

Sample	Concentration	Type	Isolated Colonies
Site (I)	10^{-10}	Dry Waste	5
Site (II)	10^{-10}	Wet Waste	18
Site(I)	10^{-9}	Dry Waste	4
Site(II)	10^{-9}	Wet Waste	37

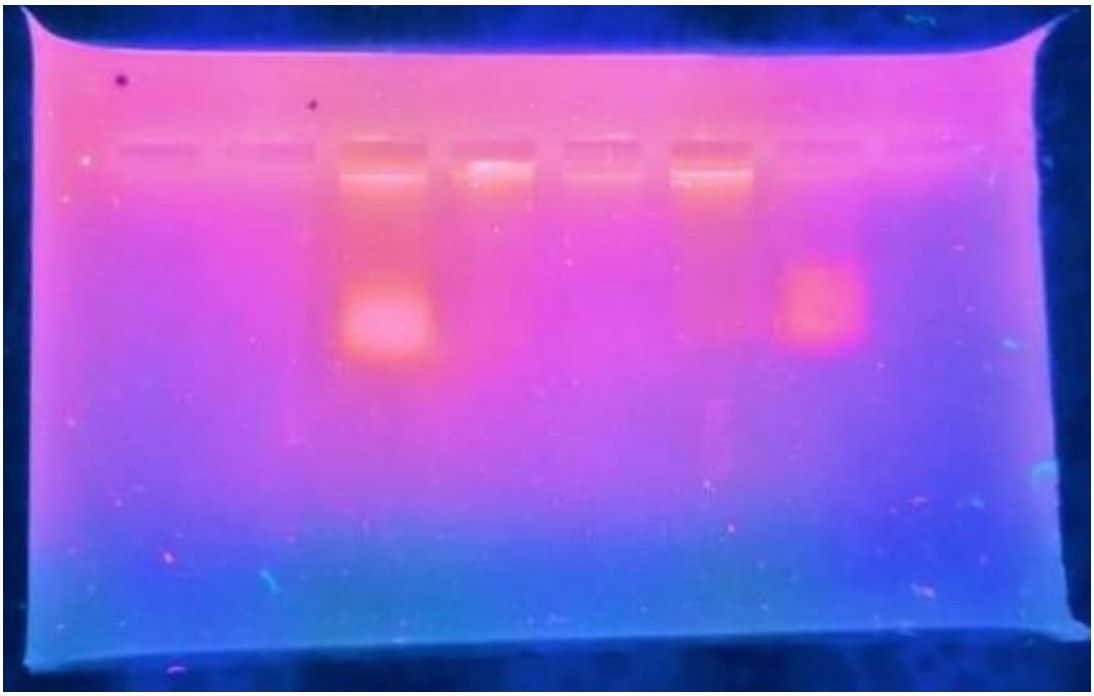

Fig 8 Agarose Gel Electrophoresis Results Showing DNA and RNA Bands.

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➢ *Key Findings*

- *Distinct Nucleic Acid Bands:*
  Successful extraction of DNA and RNA with clear, distinct bands observed.

- *High-Quality DNA:*
  Well-defined bands with minimal smearing, indicating good quality and integrity of extracted DNA.
- *Effective RNA Extraction:*
  Presence of RNA bands, demonstrating effective isolation of both DNA and RNA without proteinase treatment.

- *No Protein Contamination:*
  Absence of smeared bands, suggesting clean nucleic acid extraction without protein contamination.

- *Methodology Validation:*
  Effective use of serial dilution, PDA media inoculation, and DNA extraction techniques.

- *Reproducibility:*
  Consistent band patterns across samples, indicating reliable and reproducible experimental procedures.

V. CONCLUSION

➢ *Summary of Findings*

- Successfully isolated bacterial colonies from soil samples collected at various dumping sites.
- Extracted DNA from these colonies.
- Analyzed the extracted DNA using gel electrophoresis.
- Gel electrophoresis results revealed distinct and well-defined DNA bands.
- Presence of diverse microbial communities in the soil samples.
- DNA extraction methods were effective.
- High-quality DNA with minimal contamination was obtained.
- Methodologies for microbial isolation and genetic analysis were robust.

➢ *Future Directions*

- Further research is required to identify the specific bacterial species present and elucidate their roles in biodegradation and soil health.
- Expanding the scope of the study to encompass additional dumping sites and various seasonal conditions could yield a more comprehensive understanding of microbial diversity in waste-affected soils.
- This broader approach would enhance our knowledge of the ecological impacts and potential bioremediation applications of these microbial communities.

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