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Microbial Diversity in Composting: identification and analysis of fungal and bacterial communities for enhanced organic waste recycling

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INTRODUCTION & AIM

Composting is an eco-friendly method of managing waste by converting organic materials into nutrient-dense compost through the action of microbes [1]. This process unfolds in four distinct stages: initial mesophilic, thermophilic, secondary mesophilic, and maturation [1]. Several factors influence microbial activity and organic matter breakdown, including the carbon-to-nitrogen (C/N) ratio, moisture content, temperature, particle size, pH level, oxygen availability, and microbial composition [1,2]. Bacteria, fungi, and actinomycetes are key organisms in decomposing complex compounds like cellulose, hemicellulose, and lignin [1,2]. As composting advances, microbial dominance typically shifts from bacteria to fungi (Nemet et al., 2021). Introducing specific microbial strains can enhance the efficiency of the composting process [3]. The resulting compost serves as a sustainable soil amendment, improving soil structure, boosting the effectiveness of fertilizers, and supporting plant growth [3]. This practice supports circular economy principles by minimizing the environmental footprint of waste disposal [3]. The aim of this study was to identify fungal and bacterial species involved in composting and to optimise their contribution to organic matter decomposition. The compost samples were subjected to microbiological analysis, employing selective growth media, macroscopic and microscopic examinations, DNA extraction, PCR amplification and Sanger sequencing, with the objective of achieving precise fungal and bacterial identification.

METHODS

A 25 g compost sample, derived from one year of fruit and vegetable residue collection (Figure 1), was mixed with 225 mL of sterile peptone water and incubated at 25°C with agitation at 120 rpm for 30 minutes at room temperature (López-González et al., 2013). To analyse the microbial communities, the resulting mixture was used to inoculate various culture media.

Inoculation on different culture media:

- ↳▶ Nutrient Agar → Mesophilic bacteria (25°C, 48h)
- ↳▶ GYC → Acetic acid bacteria (25°C, 96h)
- ↳▶ MRS → Lactic acid bacteria (Anaerobic, 25°C, 48h)
- ↳▶ Aaronson Medium → Actinobacteria (45°C, 72–120h)
- ↳▶ Sabouraud with chloramphenicol → Filamentous fungi (25°C, 7 days)

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Phenotypic characterization:

- ↳▶ Bacteria: Gram staining and catalase test
- ↳▶ Fungi: Microscopic morphology observation

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Species identification:

- ↳▶ DNA extraction
- ↳▶ PCR → Bacteria: 16S rRNA amplification
- ↳▶ PCR → Fungi: ITS region amplification
- ↳▶ Sanger sequencing

Figure 1- Compost derived from one year of fruit and vegetable residue collection

RESULTS & DISCUSSION

Several isolates (e.g., B1–B10) were identified as *Bacillus* spp. (Table 2), showing expected Gram-positive and catalase-positive traits (Table 1), confirming consistency between sequencing and phenotypic tests. Isolates B13 (*Pseudomonas aeruginosa*), B14 (*P. fluorescens*), and B11 (*Serratia fonticola*) were Gram-negative and catalase-positive, as typical of their genera. Less common compost isolates like B7 (*Sphingobacterium kitahiroshimense*), B12 (*Chryseobacterium sp.*), and B2 (*Devosia sp.*) (Table 2) also matched their known Gram-negative and catalase-positive profiles (Table 1) .

Table 1- Bacteria Gram staining and catalase test

Sample	Gram stainig	Catalase test
B1	+	+
B2	-	+
B3	+	+
B4	+	+
B5	+	+
B6	+	+
B7	-	+
B8	+	+
B9	+	+
B10	+	+
B11	-	+
B12	-	+
B13	-	+
B14	-	+

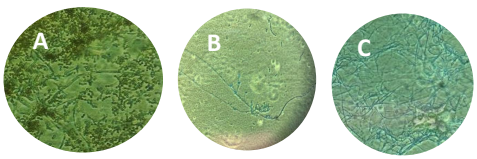
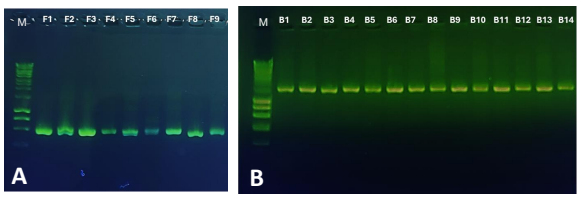


Figure 2-Microscopic visualization of sample F3 *Aspergillus heyangensis* (A), sample F2 *Cladosporium asperulatum* (B) and Sample F3 *Penicillium brevicompactum* (objective amplification of 40×).

The microbiological analysis of compost revealed a diverse community of both bacterial and fungal species (Figure 2, 3 and table 2,3) . Among the bacterial isolates, *Bacillus* species were the most dominant (Table 3), particularly *Bacillus subtilis*, which is well-known for its role in the decomposition of organic matter and production of extracellular enzymes like cellulase and protease . Other identified bacteria such as *Devosia sp.*, *Serratia fonticola*, and *Pseudomonas fluorescens* also contribute to nitrogen cycling and plant growth promotion [4].

In the fungal community (Table 2), *Penicillium brevicompactum* appeared in multiple samples, indicating its strong adaptability and role in organic matter degradation. *Aspergillus heyangensis* and *Aspergillus creber* were also identified, both known for producing enzymes capable of breaking down complex plant materials [5]. The presence of *Cladosporium asperulatum* and *Pestalotiopsis lespedezae* further highlights the diversity of filamentous fungi involved in composting, although some sequences showed lower identity percentages (Table 2), indicating either novel strains or incomplete matches in current databases.

Figure 3- Analysis of PCR products by 1.5% agarose gel electrophoresis.
(A)- 600-800 bp DNA fragments from Fungi.
(B)- 1400-1500-bp DNA fragments from bacteria, M-Marker: 10000 bp DNA marker



The successful amplification of the 16S rRNA and ITS regions (Figure 3), followed by Sanger sequencing (Table 2 and 3), allowed for precise microbial identification. The combination of culture-based techniques with molecular methods provided a comprehensive overview of the microbial dynamics during the composting process.

Table 2-Sanger sequencing results of fungal isolates

Code	Fungal Species	Percent identity (%)
F1	*Aspergillus heyangensis*	98.69
F2	*Cladosporium asperulatum*	81.85
F3	*Penicillium brevicompactum*	99.45
F4	*Penicillium brevicompactum*	99.43
F5	*Penicillium brevicompactum*	90.45
F6	*Penicillium brevicompactum*	93.73
F7	*Pestalotiopsis lespedezae*	78.64
F8	*Aspergillus creber*	99.25
F9	*Penicillium brevicompactum*	94.77

Table 3-Sanger sequencing results of bacterial isolates

Code	Bacterial Species	Percent identity (%)
B1	*Bacillus halotolerans*	96.3
B2	*Devosia sp.*	96.79
B3	*Bacillus subtilis*	97.08
B4	*Bacillus subtilis*	84.65
B5	*Bacillus amyloliquefaciens*	96.8
B6	*Bacillus mojavensis*	96.28
B7	*Sphingobacterium kitahiroshimense*	76.73
B8	*Bacillus subtilis*	96.52
B9	*Bacillus subtilis*	87.88
B10	*Bacillus subtilis*	97.35
B11	*Serratia fonticola*	98.15
B12	*Chryseobacterium sp.*	75.47
B13	*Pseudomonas aeruginosa*	77.21
B14	*Pseudomonas fluorescens*	97.84

CONCLUSION

This study confirmed the presence of a diverse and functionally relevant microbial community in compost derived from fruit and vegetable residues. The dominance of *Bacillus* and *Penicillium* species emphasizes their essential role in composting. Accurate identification of microbes through molecular tools supports targeted microbial management strategies to enhance compost quality and accelerate decomposition, contributing to sustainable organic waste recycling.

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