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Isolation and Morphological Characterization of Fungal Isolates from Leachate of Namo Bintang Landfill, North Sumatra

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Abstract: This study aims to isolate and characterize the morphological characteristics of fungal colonies obtained from leachate water at a landfill site. To obtain fungi from leachate water, the following steps were taken: Fungi were isolated using the pour plate method with Potato Dextrose Agar (PDA) medium; morphological characterization of fungi was performed macroscopically and microscopically. Macroscopically, by observing the shape, edges, texture, and color of the fungal colonies, and microscopically, by observing the shape of the hyphae and the presence of asexual spores. The results showed that seventeen fungal isolates were successfully isolated from the leachate of the Namo Bintang landfill with different morphologies. The macroscopic characteristics of the fungal colonies showed that 15 fungal isolates had round colonies, and the other two isolates (NKD 07 and NKT 14) had irregular colonies. The edges of the fungal colonies varied, ranging from flat, wavy, to serrated. The color of the fungal colonies was predominantly gray and green. The texture of the fungal colonies varied from powdery, hairy, smooth, to cotton-like. Microscopic characteristics of the fungi show that most fungal isolates have septate hyphae and varied conidia shapes, such as round, oval, crescent, and tubular. These findings indicate that the leachate from the Namo Bintang landfill contains diverse fungi with different colony morphological characteristics, making it possible to develop them as biological agents.

Keywords: Bioremediation; fungal diversity; isolation; landfill leachate; morphological characterization

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INTRODUCTION

The human environment is surrounded by various wastes generated from daily activities includes from households, industrial and commercial activities. This waste is collected and eventually disposed of in landfills. Landfills have become a global issue because they account for about 95% of the waste generated worldwide (Oziegbe et al., 2021). In Indonesia, the OECD (2019) reports that 30% of solid waste is not collected and managed due to limited landfill management systems, which will exacerbate the problem of leachate pollution that causes problems for the environment and living creatures. The leachate created by the leakage of rainwater falling into the landfills of the solid waste contains organic pollutants, inorganic salts, and heavy metals that cannot be biodegraded (Garcete et al., 2022; Yildirim et al., 2022). Leachate migration into the environment can contaminate soil, groundwater, surface water, air (through unpleasant odors), and food products grown around the landfill area, and harm the health of humans living around the landfill site and consuming contaminated products (Daniel et al., 2021).

Fungi are known as the primary decomposers of complex organic matter, involved in the decomposition process, and known to be capable of decomposing wood, including lignin and cellulose. The biodegradation capacity of fungi is almost universally associated with oxidative enzymatic reactions mediated by various types of oxidases and peroxidases (Muksy & Kolo, 2023; Vaksmaa et al., 2023). Fungal

enzymes have the potential to transform pollutants and detoxify and remediate polluted environments. Viswanath et al. (2014) reported that laccase acts on phenolic and nonphenolic compounds associated with lignin and highly resistant environmental pollutants, enabling it to degrade lignin, decolorize, detoxify industrial waste from paper and pulp, textiles, wastewater treatment, and xenobiotic degradation. Karigar & Shwetha (2011) reported that the main role of manganese peroxidase and lignin peroxidase is lignin degradation.

Fungi isolated from extreme environments exhibit diverse morphology and high adaptability. This is influenced by habitat characteristics such as the high organic matter/nutrient content of waste, as well as the presence of heavy metals and toxic substances. This diversity is likely due to rapid and continuous genetic drift, which consolidates adaptive alleles in small populations that reproduce asexually (Zegzouti et al., 2020). Bazzicalupo et al. (2020) found genetic variation, including single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), that enable resistance in contaminated environments. This variation, which affects genes responsible for metal excretion, storage, immobilization, and detoxification of reactive oxygen species (ROS), appears to be under selective pressure in isolates exposed to pollution.

Environmental factors also influence the geographical diversity of fungi, including pH, dissolved organic carbon (DOC), volatile solids (VS), and concentrations of ammonium (2NH₄⁺), nitrite (3NO₂⁻), and nitrate (4NO₃⁻) (Ye et al., 2020). The high toxicity or mutagenicity of many chemical compounds found in landfill leachate can affect metabolism and biodiversity. Gotvajn & Pavko stated that ammonia nitrogen toxicity to fungi affects fungal growth in landfill and also inhibits the biological treatment of leachate. The presence of heavy metals in leachate can select for microbes present in the leachate, causing the extinction of sensitive species, while species resistant to metal stress thrive, thus contributing to an overall increase in fungal diversity (Mohammad et al., 2015). However, information on the diversity and morphological characteristics of fungi originating from leachate in Indonesia is still very limited. This condition indicates the need for research focused on the exploration and characterization of fungi from local contaminated sources such as leachate from Namo Bintang lamdfill.

Therefore, this study isolates and describes fungi from landfill leachate as baseline data for bioremediation research. This study aims to isolate and characterize the morphological characteristics of fungal colonies obtained from leachate water at a landfill site.

METHOD

This research was conducted in the Microbiology Laboratory of the Universitas Sumatera Utara from Augst 2024 to July 2025. The tools used in this study were petri dishes, erlenmeyer flasks, cork borers, bunsen flasks, duran bottles, cool boxes, while the materials used were leachate, distilled water, chloramphenicol, and PDA (Potato Dextrose Agar) media.

Experimental Procedure

1. Leachate Collection

Leachate was collected from Namo Bintang landfill. Leachate with black color and pungent odor was collected from water pooling and placed in 250 ml sterile duran bottles, then stored in a cool box. Leachate was collected from 3 points. Point 1 (3°27'59.028"N 98°39'6.642"E 284° W), point 2 (3°27'59.0216'N 98°39'6.072"E 302°

NW), point 3 (3°27'59.214"N 98°39'5.802"E 320° NW). The map of leachate sampling locations can be seen in Figure 1.



Figure 1. Leachate sampling locations at the Namo Bintang Landfill at three points:

A) Point 1, B) Point 2, C) Point 3

2. Isolation of Fungi

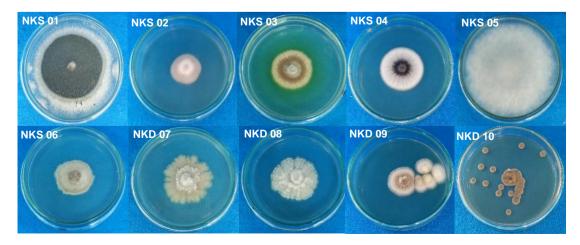
Fungi were isolated from leachate. Isolation of fungi was performing using the pour plate method. A total of 1 ml of leachate was added to a sterile petri dish and mix with with sterile liquid PDA medium supplemented with chloramphenicol, then homoginized. Plate was incubated at room temperature for 7 days. The growing colony were subculture in new PDA media.

3. Morphological Characterization of Fungi

Morphological characterization of fungi is performed macroscopically and microscopically. Macroscopically, by observing the shape, edges, texture, and color of the fungal colony. Microscopically, by observing the shape of the hyphae and the presence of asexual spores. Lactophenol cotton blue stain is used for microscopic observation of fungi. Fungi are characterized based on their microscopic morphology.

RESULT AND DISCUSSION

Seventeen fungal isolates were successfully isolated from leachate of Namo Bintang landfill on Potato Dextrose Agar (PDA) media with different morphology. Six isolates were obtained from point 1 (coded NKS), seven from point 2 (codede NKD) and four isolates from point 3 (coded NKT). Figure 2 below shows fungi growing on PDA media.



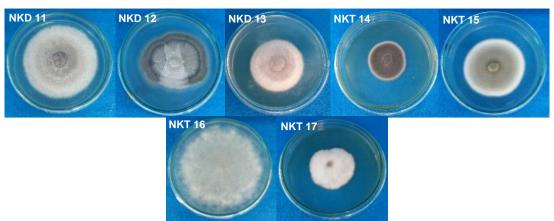


Figure 2. Fungal colonies isolated from Namo Bintang landfill leachate on Potato Dextrose Agar (PDA) media

Based on the results of fungal isolation in Figure 2, the most fungi were obtained from point 2, with 7 fungal isolates (NKD 07, NKD 08, NKD 09, NKD 10, NKD 11, NKD 12, NKD 13) and the least were obtained from point 3, with 4 fungal isolates (NKT 14, NKT 15, NKT 16, NKT 17). The difference in the number of fungal isolates obtained is likely due to the environmental conditions at each location, such as the presence of heavy metals in the leachate. The Cd level in the point 2 leachate, analyzed by AAS, was 0.045 ppm, while it was higher at point 3, measuring 0.056 ppm. The Zn level analyzed was 0.068 ppm, which consequently influenced the presence of fungi in the leachate. Xu et al. (2019) reported that heavy metal toxicity results in a decrease in fungal population and leads to a reduction in fungal diversity (Ashraf & Ali, 2007). Garcete et al. (2022) successfully isolated 15 fungal isolates from leachate, and Titilawo et al. (2023) successfully isolated 17 fungal isolates belonging to the genera Aspergillus, Alternaria, Chrysosporium, and Penicillium from a landfill site.

These fungal isolated from leachate of Namo Bintang landfill showed different colony morphological characteristics. This is likely due to the physiological and genetic adaptability of fungi to environmental heterogeneity and stress factors such as heavy metals, toxic compounds, organic materials, and leachate pH. It should be noted that there is a certain variability in the influence of the environment, for example, the presence of heavy metals on microbial diversity, which is basically caused by differences in the tolerance of various taxa in the microbial community to heavy metal stress (Campillo-Cora et al., 2025). Fomina et al. (2024) state that the growth conditions on the surface trigger various morphological reactions in filamentous fungi, which specifically demonstrate the remarkable ability of the mycelium system to adapt to environments with high metal and mineral content. Poputnikova & Terekhova (2010) have shown that the influence of landfills as a source of environmental pollutants in the surrounding area stimulates the development of soil microbiota, the growth of fungal populations, and biomass and biological diversity (as indicated by the biomass of certain species).

Macroscopic Characterization of Fungal Colonies Isolated from Leachate

Macroscopic observations were conducted to identify the morphological characteristics of fungal colonies (shape, margin, color, and surface texture). The data from observations of fungal isolates from leachate at the Namo Bintang landfill are shown in Table 1.

Table 1. Macroscopic characterization of fungal colonies isolated from leachate

| Isolate | Colony shape | Margin | Color | Texture |
|---------|--------------|----------|-------------------------|----------|
| NKS 01 | Circular | Entire | Green | Powdery |
| NKS 02 | Circular | Undulate | White | Floccose |
| NKS 03 | Circular | Entire | Yellowish brown | Floccose |
| NKS 04 | Circular | Undulate | White with black center | Cottony |
| NKS 05 | Circular | Entire | White | Cottony |
| NKS 06 | Circular | Entire | Green | Smooth |
| NKD 07 | Irregular | Serrate | Green | Floccose |
| NKD 08 | Circular | Serrate | White | Cottony |
| NKD 09 | Circular | Entire | White with brown center | Floccose |
| NKD 10 | Circular | Entire | Green | Powdery |
| NKD 11 | Circular | Entire | Gray | Floccose |
| NKD 12 | Irregular | Undulate | Gray | Floccose |
| NKD 13 | Circular | Entire | Gray | Floccose |
| NKT 14 | Circular | Entire | Gray | Powdery |
| NKT 15 | Circular | Entire | Army green | Floccose |
| NKT 16 | Circular | Entire | White | Cottony |
| NKT 17 | Circular | Entire | White | Cottony |

Based on the morphological characteristics of fungal colonies in Table 1, fungal isolates obtained from leachate water showed varying morphological characteristics. Most isolates had round colonies, and two isolates (NKD 07 and NKT 14) had irregular colonies. The margin of the fungal colonies varied, ranging from flat, wavy, and serrated, with the color of the colonies dominated by gray and green. The texture of the fungal colonies varied, ranging from powdery, hairy, smooth, and cotton-like. The morphological differences between fungal colonies are not only determined by the genetics of the fungus species, but may also be greatly influenced by environmental conditions and the interaction between the two.

Research conducted by Shivayogimath & Watawati (2013) states that the presence of waste in landfills results in the formation of heavy metal ions such as Cu, Ni, Zn, Pb, Fe, Mn, Cr, Cd, and other toxic compounds. Soil and water contamination by heavy metals can cause the extinction of species that are sensitive to the stress they cause, and the growth of resistant species, thereby increasing microbial diversity (Mohammad et al., 2015). This condition becomes a form of environmental stress that affects metabolic activity and gene expression that play a role in the formation of fungal colony structures. Ploidy, mating type, and genotype-environment interactions also play a role in controlling colony morphology (Granek & Magwene, 2010). Gadd et al. (2001) explains that nutritional factors and the presence of toxic substances such as heavy metals can affect the growth and spread of fungal biomass, resulting in an uneven distribution of biomass within the colony, with some allocated to the edges. When nutrients are scarce, hyphae grow more slowly and their branching pattern changes, which can disrupt secondary metabolism, resulting in variations in the morphology of the fungal colony. In addition to morphological changes, heavy metals also cause the formation of colored or colorless mycelium due to the fungal response to heavy metal stress. Ban et al. (2012) stated that these changes occur as a response to melanin, a heavy metal detoxification pigment in fungi, due to heavy metal exposure. The formation of pigments by fungal cells is accompanied by the precipitation of metal ions on the fungal cell walls (Yazdani et al., 2010).

As a result, each isolate exhibits different macroscopic characteristics, such as round or irregular colonies, smooth to serrated edges, and various colors (green, gray, white). These differences reflect the fungi's ability to adapt and adjust their growth to environmental changes and indicate possible physiological differences between isolates.

Microscopic Characterization of Fungal Colonies Isolated from Leachate

Microscopic observations were conducted to identify the morphological characteristics of fungal colonies, including hyphae and conidia. The data from observations of fungal isolates from leachate at the Namo Bintang landfill can be seen in Table 2.

Table 2. Microscopic characterization of fungal colonies isolated from leachate

| Isolate | appearance of hyphae and spores | Description | Isolate | appearance of hyphae and spores | Description |
|---------|---------------------------------------|--|---------|---------------------------------------|---|
| NKS 01 | | Spherical conidia forming a fan-like arrangement | NKS 02 | | Septate hyphae, spherical conidia |
| NKS 03 | | Septate hyphae, conidia arranged in chains | NKS 04 | | Spherical conidia |
| NKS 05 | | Septate hyphae, spherical conidia | NKS 06 | | Septate hyphae, elongated tubular conidia |
| NKD 07 | | Septate hyphae, oval conidia | NKD 08 | | Aseptate hyphae, oval conidia |
| NKD 09 | | Spherical conidia forming a fan- like structure | NKD 10 | | Septate hyphae, spherical conidia |
| NKD 11 | | Septate hyphae, spherical conidia | NKD 12 | | Septate hyphae, spherical conidia |

| Isolate | appearance of hyphae and spores | Description | Isolate | appearance of hyphae and spores | Description |
|---------|---------------------------------------|--|---------|---------------------------------------|--|
| NKD 13 | | Septate hyphae, spherical conidia | NKT 14 | | Septate hyphae, spherical conidia |
| NKT 15 | | Aseptate hyphae, scattered spherical conidia | NKT 16 | | Septat hyphae, spherical conidia |
| NKT 17 | | Septate hyphae, crescent- shaped conidia | | | |

Based on Table 2 above, microscopic observations show that most fungal isolates have septate hyphae and varied conidia shapes, such as round, oval, crescent, and tube-like. The variation in the shape of hyphae and conidia of fungi isolated from landfill leachate may be due to each fungal species having unique genetic characteristics that result in different hyphae and conidia structures. In addition, the diversity in the shape of hyphae and conidia in fungal isolates from landfill leachate may reflect the fungi's ability to adapt to their environmental conditions.

Leachate contains dissolved and suspended materials, including carbon dioxide and methane, as well as hazardous contaminants resulting from the disposal of household or residential and industrial products and various legally disposed contaminants (Abdel-Shafy et al., 2024). Hyphae develop through polarized apical growth, and individual cells are separated from elongated hyphae by developing cross walls (septa). The branched hyphal network is adapted to efficiently explore the available space in the substrate (Nagy et al., 2017). The spherical shape of the spores minimizes the surface area to volume ratio and therefore maximizes the survival of the spores under harsh conditions (Calhim et al., 2018). Research by Colin et al. (2013) on Aspergillus niger fungi shows that environmental factors and growth media have a significant impact on hyphal morphology and conidia adhesion properties. Changes in factors such as pH, nutrient availability, and cell wall enzyme activity can affect the number of hyphal branches and the macroscopic growth type of fungi. The function of the β-N-acetyl-D-glucosaminidase (Mb-NAGase) enzyme has a negative relationship with hyphal growth unit length (IHGU), indicating that increased enzyme activity results in shorter and more branched hyphal structures. The adhesive ability of conidia also determines the growth pattern of colonies; conidia with high adhesive power tend to form dense aggregates (pellets), while conidia with low adhesive power will produce more widespread mycelium growth.

The differences in the morphology of hyphae and conidia in fungi isolated from landfill leachate are caused by a combination of genetic and environmental factors, pH, nutrient availability, and organic and inorganic compounds, triggering morphological adaptation. Cell wall enzyme activity and conidia adhesion ability also determine the shape and growth pattern of fungi. Therefore, variations in hyphae and

conidia morphology are the result of fungal adaptation to extreme environmental pressures such as landfill leachate.

CONCLUSION

Based on the research findings, it can be concluded that a total of seventeen fungal isolates were successfully obtained from the leachate of the Namo Bintang landfill in North Sumatra. These fungal isolates exhibited varying morphological characteristics of their colonies. Most of the fungal isolates had septate hyphae with conidia of various shapes, including spherical, oval, crescent, and tubular. This morphological diversity reflects the fungi's adaptation to extreme and polluted environments rich in organic matter and heavy metals. Therefore, landfill leachate is a potential source of fungal species with strong tolerance and adaptive abilities that can be explored and developed as agents for the bioremediation of polluted environments.

RECOMMENDATION

Further research is recommended to identify the type of fungus biomolecularly using a DNA-based approach to confirm the identification of its colony morphology characteristics. The potential of these fungal isolates can be explored in the bioremediation of contaminated environments, such as the presence of heavy metals, and how fungi tolerate and absorb heavy metals. These isolates can be developed as environmentally friendly bioremediation agents in managing leachate and other contaminated environments.

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