Advanced and Sustainable Technologies
ASET
E-ISSN 2976-2294
Volume 4, No 2, December 2025 [429-435]

Identification of Microbes from Effective Microbes (EM) Formulations and Spent Mushroom Substrate (SMS) Using Morphological Analysis

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Received 5 September 2025, Revised 10 October 2025, Accepted 25 October 2025

ABSTRACT

Effective Microbes (EM) formulations and spent mushroom substrate (SMS) are valuable in sustainable agriculture due to their beneficial microbial content. This study aims to culture, isolate, and identify microbial populations in EM and SMS using morphological analysis techniques. The samples included in this study are: Sample 1 (control): distilled water, Sample 2: spent mushroom substrate (SMS), Sample 3: EM with SMS, and Sample 4: EM. pH extraction was performed for each sample to assess conditions, yielding the following values: Sample 1 – 7.20, Sample 2 – 6.50, Sample 3 – 3.67, and Sample 4 – 6.72, indicating varying acidity levels. Microorganisms were cultured on Potato Dextrose Agar (PDA) media, promoting growth, with tests conducted three times for each sample as the replication. Pure colonies were isolated via the four-quadrant streaking method. All samples exhibited successful growth on PDA media, with distinctive colony formations. EM primarily produced creamy, round bacterial colonies, while SMS and SMS + EM samples displayed fungal structures. Microscopic examination identified cocci-shaped bacteria in EM samples and yeast cells in SMS and SMS + EM samples. While this study focused on morphological identification, it does not provide precise species-level resolution and may overlook non-culturable organisms. Nonetheless, it presents a practical, low-cost approach for characterizing microbial communities in EM and SMS, offering foundational data for future research and applications in organic farming and soil enrichment.

Keywords: Effective microbes, Microbial identification, Morphological analysis, Potato dextrose agar, Spent mushroom substrate.

1. INTRODUCTION

1.1 Overview of Effective Microorganisms (EM)

Effective microorganisms (EM) are a diverse group of beneficial bacteria, yeasts, fungi, and actinomycetes that enhance soil fertility and plant health. Originally developed by Professor Teruo Higa in Japan during the 1970s, EM was created as an alternative to chemical fertilizers, which were causing long-term environmental damage and reducing soil health. EM typically contains lactic acid bacteria, photosynthetic bacteria, yeasts, and fermenting fungi. These microorganisms work synergistically to suppress harmful pathogens, promote nutrient cycling, and produce bioactive compounds beneficial to plants and soil ecosystems [1].

Lactic acid bacteria, a primary component of EM, help decompose organic matter, produce lactic acid, and enhance soil sterilization, making nutrients more accessible to plants. Photosynthetic bacteria are also critical, as they synthesize essential compounds such as amino acids and

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hormones from root secretions and organic waste, fostering an environment conducive to plant growth [2]. Yeasts in EM contribute bioactive substances that stimulate root growth and improve plant resistance to environmental stress. Additionally, actinomycetes are effective in breaking down complex organic materials, providing essential nutrients, and preventing pathogen growth, which collectively support healthier plants and improved soil structure [3].

1.2 Mushroom Waste as an Agricultural Medium

Mushroom cultivation generates substantial organic waste, often termed spent mushroom substrate (SMS), which is usually disposed of but has high potential as an agricultural medium. Mushroom waste is rich in organic matter, lignocellulosic compounds, nitrogen, and essential nutrients that can enhance soil fertility and support plant growth[4]. Studies show that SMS can improve soil texture, increase water-holding capacity, and serve as a natural fertilizer. However, the slow decomposition rate of mushroom waste and its high lignin content can limit its immediate usability in agriculture without proper treatment [5].

The primary composition of SMS includes undigested substrate materials (e.g., straw, sawdust), fungal mycelium, and a variety of macro- and micronutrients that contribute to soil fertility when properly decomposed. Despite these advantages, raw SMS can be challenging to manage due to its high bulk density and potential for phytotoxicity. The introduction of EM has been explored as a strategy to accelerate SMS decomposition, transforming it into a more bioavailable and nutrient-rich substrate suitable for plant growth [7].

1.3 Mushroom Waste treated with Effective Microorganism (EM)

The combination of EM and mushroom waste represents a promising approach to sustainable agriculture. EM facilitates the breakdown of organic material in mushroom waste, releasing nutrients that would otherwise remain locked in complex compounds. The microbes in EM produce enzymes that accelerate lignin and cellulose degradation, increasing the availability of nitrogen, phosphorus, and other essential nutrients [6].

This approach not only improves the efficiency of mushroom waste recycling but also enhances the quality of the soil substrate, promoting healthier root systems and improving plant resilience. Research indicates that EM-treated mushroom waste supports higher plant biomass, enhanced root development, and greater resistance to soil-borne pathogens compared to untreated organic materials [8].

1.4 Environmental and Economic Benefits of EM and Mushroom Waste Integration

The application of EM to mushroom waste presents environmental and economic benefits. First, the recycling of mushroom waste reduces the environmental impact associated with disposal and prevents the accumulation of waste in landfills. By converting waste into a productive input for agriculture, this approach aligns with principles of the circular economy and sustainability [9].

Economically, the use of EM-enriched mushroom waste provides farmers with a cost-effective, nutrient-rich growing medium, reducing reliance on expensive chemical fertilizers. Moreover, by improving soil health and plant productivity, this sustainable approach can enhance crop yields and provide mushroom farmers with additional revenue from repurposing their waste [10].

2. MATERIAL AND METHODS

2.1 Sample Collection

Four treatments were prepared for microbial analysis: (i) distilled water, used as a control to confirm sterility and minimize contamination bias; (ii) spent mushroom substrate (SMS) obtained from a commercial mushroom farm; (iii) a commercial effective microorganism (EM) formulation; and (iv) a mixture of SMS and EM, designed to evaluate microbial interactions and potential synergistic effects.

2.2 pH Analysis

The pH of each sample was determined using a calibrated digital pH meter. Measurements were conducted in triplicate to ensure reproducibility and accuracy. The pH data were subsequently analyzed to examine potential correlations between microbial growth patterns and environmental conditions.

2.3 Culturing and Isolation

Microorganisms were cultured on Potato Dextrose Agar (PDA) to facilitate the growth of fungi, yeasts, and selected bacteria. The four-quadrant streak plate technique was employed, and streaking was repeated three successive times per sample to obtain pure colonies from mixed populations. Culture plates were incubated at 28 ± 2 °C for 48–72 hours, with daily observations to monitor colony development.

2.4 Microscopic and Morphological Analysis

Pure isolates were examined under a compound light microscope for microscopic and morphological characterization. Observations included spore formation, hyphal structure, pigmentation, and cell shape. Preliminary identification of isolates was carried out by comparing observed morphological characteristics with standard microbiological references.

Table 1: pH analysis 4 samples used.

Sample	pH	Interpretation
Control (Distilled Water)	7.20	Slightly alkaline; unsuitable for microbial growth.
SMS	6.50	Near neutral; supports diverse microbial communities.
EM	3.67	Strongly acidic; favors lactic acid bacteria growth.
SMS + EM	6.72	Mildly acidic; suitable for both fungi and beneficial bacteria.

Figure 1: Microscopic image obtained from SMS sample isolation.

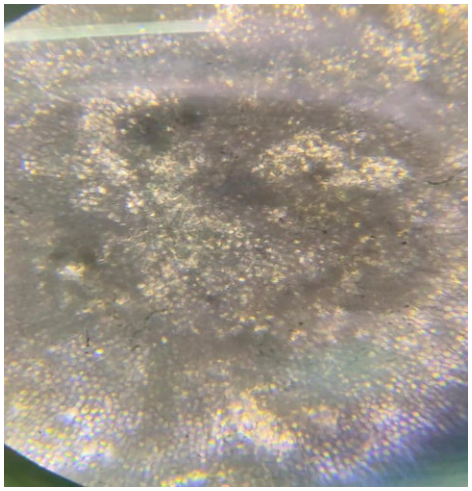

Figure 2: Microscopic image obtained from EM sample isolation.

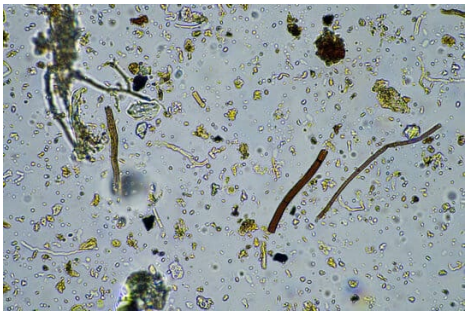

Figure 3: Microscopic image obtained from EM + SMS sample isolation.

3. RESULTS AND DISCUSSION

The pH values of the four treatments demonstrated distinct differences, reflecting their microbial composition and metabolic activity. The control (distilled water) remained neutral at pH 7.20, validating its role as a sterile baseline. The SMS sample recorded a slightly acidic pH of 6.50, a typical outcome of organic matter degradation and microbial metabolism in lignocellulosic residues [11]. The EM formulation exhibited a pH of 6.72, consistent with previously reported ranges for commercial EM products, which maintain mildly acidic conditions favorable for microbial stability [12]. Notably, the SMS + EM treatment showed a markedly lower pH of 3.67, suggesting enhanced fermentation activity. Such acidification is commonly associated with lactic acid bacteria and yeasts, which contribute to pathogen suppression and improved soil amendment properties [13].

All samples supported microbial growth on PDA, though with clear morphological differences. EM primarily produced creamy, round bacterial colonies, in line with the dominance of lactic acid bacteria commonly present in EM formulations [14]. SMS and SMS + EM samples, however, yielded fungal colonies with filamentous growth, reflecting the presence of fungi associated with the degradation of cellulose, hemicellulose, and lignin in mushroom substrates [15]. These findings align with earlier observations that SMS is a rich reservoir of microbial diversity, particularly saprophytic fungi, which can enhance soil fertility when applied as organic amendments [16].

Microscopic analysis further confirmed differences in microbial populations. Cocci-shaped bacteria were observed in EM samples, consistent with lactic acid bacteria frequently reported in EM formulations [17]. In SMS and SMS + EM samples, yeast cells and fungal hyphae were

identified, supporting the hypothesis that these substrates promote mixed microbial consortia. Previous studies have also isolated novel bacterial species, including Acinetobacter spp., from mushroom cultivation substrates, highlighting the ecological and biotechnological potential of such environments [18]. The coexistence of yeasts, fungi, and bacteria in the SMS + EM mixture suggests potential synergistic interactions that may accelerate organic matter decomposition and nutrient cycling, enhancing the value of these materials in sustainable agriculture [19].

The findings of this study corroborate the established roles of EM and SMS as valuable bioresources in agriculture. EM formulations are known to suppress plant pathogens, enhance nutrient availability, and improve soil quality through the activity of beneficial microbial consortia [20]. Similarly, SMS has been widely recognized as a cost-effective soil conditioner and biofertilizer due to its rich microbial and organic content [17]. The substantial reduction in pH in the SMS + EM treatment indicates enhanced fermentation processes, which may contribute to the inhibition of undesirable microorganisms while enriching soil microbial diversity [3].

Nevertheless, this study relied on culture-dependent and morphological methods, which may underestimate microbial diversity by excluding non-culturable organisms [5]. While these methods provide a practical, low-cost approach suitable for preliminary characterization, molecular techniques such as 16S rRNA and ITS sequencing would offer higher taxonomic resolution and capture unculturable taxa [19]. Despite these limitations, the present study demonstrates a simple and effective framework for characterizing microbial populations in EM and SMS, providing foundational data for their expanded application in sustainable and organic farming systems.

4. CONCLUSION

These findings highlight the complementary microbial communities present in EM and SMS, suggesting synergistic potential when combined for soil enrichment and crop production. Although culture-based and morphological methods provide valuable preliminary insights, they have inherent limitations in resolving species-level diversity and capturing non-culturable microorganisms. Future work employing molecular approaches such as 16S rRNA and ITS sequencing is therefore recommended to establish a more comprehensive understanding of the microbial ecology of EM and SMS. Overall, the study supports the integration of EM and SMS as low-cost, biologically active resources that can enhance soil fertility, suppress pathogens, and contribute to the development of environmentally friendly agricultural practices. By providing baseline data on their microbial communities, this work lays the groundwork for further research and practical applications in organic and sustainable farming systems.

ACKNOWLEDGEMENTS

We acknowledge the Biotechnology Laboratory, Department of Agrotechnology, Faculty of Agrosciences, University College of Agroscience Malaysia, for providing great facilities and equipment for the success of this study.

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Conflict of interest statement: The authors declare no conflict of interest.

Author contributions statement: Conceptualization, M.F.A.H Lim & A.Z.C Ali; Methodology, M.S.M Hamdan & O.E Jakily; Formal Analysis, M.S.M Hamdan & O.E Jakily; Investigation, M.S.M Hamdan & O.E Jakily; Writing & Editing, M.S.M Hamdan & O.E Jakily.