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# Isolation and identification of fungi (mold and yeast) on BSF Maggot (*Hermetia illucens*) growing media using livestock waste

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## Abstract

If the issue of trash with high organic content is not handled correctly, it will affect both the environment and human health. BSF maggots can convert it through a bioconversion process to get over this issue. Fungi are one type of microorganism in the medium that can contribute to this process. This study attempts to separate and characterize fungi (yeast and mold) in BSF maggot media that come from organic kitchen waste, dairy cow dung, and milk waste sludge, both before and after the BSF maggots break them down. The data was descriptively examined using an exploratory approach with four treatments and five replications. 100% organic kitchen waste (R0), 50% organic kitchen waste and 50% dairy cow manure (R1), 50% organic kitchen waste and 50% dairy waste sludge (R2), and 33.3% organic kitchen waste, 33, 3% dairy cow manure, and 33.3% dairy waste sludge (R3) were the treatments given. T-test findings showed that there was no significant change in the number of prints. However, there was a significant difference ($P<0.05$) in the number of yeasts in treatments R2 and R3. *Aspergillus sp., Rhizopus sp., Mucor sp., Penicillium sp., and Trichoderma sp*. is a type of mold found. *Saccharomyces* and *Candida* are the types of yeast found.

**Keywords:** Dairy cow feces; Mold; Yeast; Waste; BSF maggot

## 1. Introduction

Both dairy farm waste from off-farm sources and livestock industry trash can be found on farms. Organic waste from the livestock business is one type of waste that can contaminate the environment. 1.8–2.4% nitrogen, 1.0–1.2% phosphorus, 0.6–0.8% potassium, and up to 75% organic matter are all present in dairy cow manure. The ideal moisture content range for fly laying is between 27% and 85% in cow dung. also promotes the BSF fly larvae's growth and development (Swapentha Buana et al., 2021). In addition, there is milk waste sludge, which according to Indonesian Government Regulation No. 101 of 2014 is classified as B3 waste (toxic and hazardous waste). Because of its high nutritional value, milk sludge can be used to develop BSF maggots. Additionally, BSF maggots can develop in the nutrients found in kitchen organic waste (SOD). 1–15% crude protein and 5–38% crude fiber are present in SOD made from fruit and vegetable waste (Jalaludin et al., 2016).

Black Soldier Fly (BSF) larvae can be used as a biodecomposition agent for waste management. It has been demonstrated that BSF larvae, often known as BSF maggots, may turn organic waste into useful products like compost and maggots for use as extra feed for fisheries and animals (Lalander & Vinnerås, 2014). BSF maggots have a high protein content of 61.41%, making them suitable for use as feed (Rachmawati et al., 2015). According to Augustin et al. (2023), BSF maggots can minimize organic waste by 56% when they use it as a development medium.

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Maggot waste is the term for media that has been broken down by maggots (frass). During the maggot-induced degradation process, it is imperative to consider the microorganisms present in organic waste. Microorganisms such as mold and yeast contribute to the breakdown of organic waste, minimize volume, and create nutrient-rich products (Widyastuti & Sardin, 2021). These microbes have the ability to convert complicated substances into simpler ones. To ascertain the kinds of molds and yeasts that contribute to the degrading process carried out by maggots, it is necessary to isolate and identify the molds and yeasts present in the maggot development conditions. This research aims to isolate and identify the types of mold and yeast found in BSF larval growth media which consists of a mixture of fermented dairy cow feces, dairy industry waste sludge, and kitchen organic waste.

## 2. Materials and methods

### 2.1. Material

Dairy cow dung, milk sludge, organic waste from the kitchen, and PDA which is used as a growing medium for yeast and mold are the principal ingredients.

Utilization: instruments for the cultivation of maggots (trays, wire ram, scales, 25 kg barrel, thermometer, pH meter, etc.); instruments for the separation and detection of molds and yeasts (micropipettes, test tubes, petri dishes, osse, object glass and cover glass, colony counter, microscope, and additional supporting instruments).

### 2.2. Method

*2.2.1. Exploratory and experimental methodologies are used in this study.*

Total Plate Counting, or TPC, is the procedure used in sample cultivation. R1 = 100% kitchen organic waste, R2 = 50% kitchen organic waste: 50% dairy cow dung, R3 = 50% kitchen organic waste: 50% milk waste sludge, and R3 = 33.3% kitchen organic waste: 33.3% dairy cow manure: 33.3% milk waste sludge were the four treatments in the experimental design, which employed the T-test. The number of mold and yeast colonies both before and after BSF maggot cultivation were the criteria that were noted. Prior to and following the cultivation of BSF maggots, the varieties of mold and yeast were observed using the exploration approach.

### 2.3. Observed Variables

The variables observed in this research are

- Number of molds before and after BSF maggot cultivation
- Number of yeast before and after cultivating BSF maggots
- Types of mold before and after BSF maggot cultivation
- Types of yeast before and after cultivating BSF maggots

### 2.4. Research Procedure

*2.4.1. Number of Molds and Yeasts*

- Samples were isolated using PDA (Potato Dextrose Agar) media.
- Take 1 ml of the sample resulting from a 10-3 dilution into a petri dish, then put the PDA media containing the antibiotic mixture at a temperature of 45 °C into the petri dish containing the sample.
- The petri dish is shaken in a figure eight shape until it is homogeneous, then wait until it freezes.
- Petri dishes are stored at room temperature for 5-7 days.
- Count and record the number of mold and yeast colonies by looking directly at the colony counter on each sample.

*2.4.2. Mold observation*

- Mold samples that have been isolated and counted are then identified using slide culture.
- Take a drop of liquid PDA and place it on a glass slide until it solidifies.
- Select several mold colonies and collect them using an osse needle aseptically, pricking them in the center of the frozen PDA. Then cover using a cover glass.
- Store it in a place where there is water at the bottom to create a humid atmosphere.
- Store at room temperature for 4-5 days.

- Observe the preparation under a 10x40 microscope.
- Note the characteristics and identify the type.
- Compare with textbook pictures.

*2.4.3. Yeast Observations*

- Mold samples that have been isolated and counted are then identified using wet preparation.
- Take a sample of the suspension using a pipette, place it on a glass slide.
- Add a drop of methyline blue, then cover with a cover glass.
- Observe the preparation under a 10x100 microscope.
- Note the characteristics and identify the type.
- Compare with textbook pictures.

## 3. Results and Discussion

### 3.1. Effect of Treatment on Molds Amount

Research results the number of molds before being degraded by BSF maggots is shown in Table 1.

**Table 1** T-test for the number of molds before being degraded by BSF maggots

| Treatment | Number of molds | | Sig ≤ 0.05 (2-tailed) |
|---|---|---|---|
| | Before degradation (....x$10^3$ CFU/g) | After degradation (....x$10^3$ CFU/g) | |
| R0 | 1.14 ± 0.51 | 0.84 ± 0.37 | 0.4350 |
| R1 | 1.92 ± 0.86 | 1.82 ± 0.81 | 0.6336 |
| R2 | 3.00 ± 1.34 | 1.67 ± 0.75 | 0.6174 |
| R3 | 3.44 ± 1.54 | 1.41 ± 0.63 | 0.1747 |

Description: R0 (100% organic kitchen waste); R1 (50% organic kitchen waste, 50% dairy cow feces); R2 (50% organic kitchen waste, 50% dairy waste sludge); R3 (33.3% organic waste, 33.3% dairy cow feces, 33.3% milk waste sludge)

Table 1's T-test analysis results indicate that each treatment's fungal population declined, but not considerably (P > 0.05). The R3 treatment (33.3% kitchen organic waste, 33.3% dairy cow excrement, and 33.3% milk lime water sludge) had the highest concentration of mold. Generally speaking, the substrate, water content, pH, and chemical compounds included in these materials all have an impact on mold formation. Materials containing carbohydrate, pectin, protein, and lipids are susceptible to mold growth (Nasution & Periadnadi, 2017). If the waste has low pH, low water content, low nitrogen, and some nutrients are missing, mold will grow rapidly. Generally, mold thrives best at pH 4-5 (Dewi et al., 2014).

### 3.2. Effect of Treatment on Yeast Amount

The results of the study of yeast numbers before and after BSF maggot degradation can be seen in Table 2.

**Table 2** T-test results for the number of yeasts before and after the BSF maggot degradation process

| Treatment | Number of yeast | | Sig ≤ 0.05 (2-tailed) |
|---|---|---|---|
| | Before degradation (....x$10^3$ CFU/g) | After degradation (....x$10^3$ CFU/g) | |
| R0 | 55.90±25.00 | 22.61 ± 10.11 | 0.2462 |
| R1 | 50.24 ± 22.47 | 21.43 ± 9.58 | 0.1974 |
| R2 | 32.14 ± 14.37 | 19.47 ± 8.71 | 0.0098* |
| R3 | 47.67 ± 21.32 | 16.85 ± 7.53 | 0.0638* |

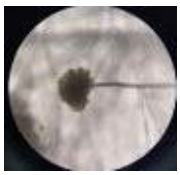
Note: the sign (*) means there is a real difference.

Table 2's T-test analysis results indicate that R2 and R3 had a substantial decline ($P<0.05$), although R0 and R1 did not alter significantly ($P>0.05$). Of course, a number of variables, including nutrients, oxygen, pH, and humidity, have a significant impact on yeast growth (Yurliasni & Zakaria, 2013). The media has a high water content (72%–82%), which results in a high humidity level. The number of colonies is also impacted by microbial contamination from air, water, and medium processing equipment (Wulandari et al., 2012; Abil Said et al., 2023). Environmental factors and the nutrients found in the substrate—simple sugars, carbohydrates, nitrogen, and oxygen—have an impact on yeast reproduction (Ahmad RZ, 2005; Dewi et al., 2014).

### 3.3. Identify mold types

The results of identifying mold types before and after degradation by BsF maggots can be seen in Table 3.

**Table 3** Identification of mold types before and after degradation by BSF maggots

| Treatment | Before Degradation | After Degradation | Characteristic | | | |
|---|---|---|---|---|---|---|
| | | | Hyphae | Spore Head | Asexual Spores | Clan |
| All maintenance |  |  | Clear | Round or oval | Conidiophores | *Aspergillus* |
| R1 |  | - | Do not accept | Round | Sporangiophore | *Rhizopus* |
| R1 and R2 |  |  | Clear | Round like a broom | Conidiophores | *Penicillium* |
| R2 |  |  | Do not accept | Round, oval, ovoid | Sporangiophore | *Mucor* |
| R3 |  |  | Clear | Cells are round or oval in shape and are single attached to each other | Conidiophores | *Trichoderma* |

Source : Nurholipah & Qurrota Ayun Prodi Biologi, 2021; Saif et al., 2020

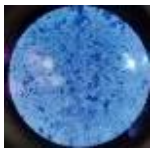
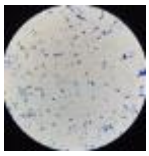
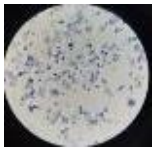
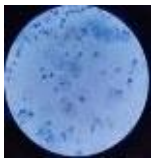
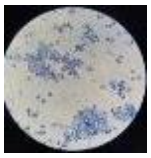
*Aspergillus sp., Mucor sp., Penicillium sp.,* and *Trichoderma sp*. grew both before and after the degrading process, according to Table 3's results of mold identification. Organic kitchen waste is most likely the source of *Aspergillus* fungus development. The claim that *Aspergillus* mold typically grows on a variety of substrates, such as soil, spices, food ingredients, seeds, leaf surfaces, and air, lends credence to this (Lee et al., 2016). Mold is more efficient than bacteria at decomposing trash (Purwadaria et al., 2003; Nasution & Periadnadi, 2017). *Aspergillus sp*. is a filamentous mold that belongs to the *Aspergillaceae* family and the Ascomycota division. It is mostly composed of chain cells that produce hyphae structures. *Aspergillus niger*, on the other hand, possesses the traits of black colonies and has skeptic hyphae, which include the Trichoromaceae family and the division of Ascomycota. identifying the type of mold Typically, *Rhizopus sp*. is present in culinary products. According to Sriherwanto C et al. (2017), this mold is distinguished by its

dark-colored rhizoids and stolons, slightly rounded columella and umbrella-like apophyses, big, black sporangia at the apex of the sporangiospores, and non-conceptile hyphae. The hyphae of the *Mucor sp* mold type are seen to be non-separable, and the sporangia on top of the sporangium are spherical, solitary, or branching. Then, the Penicillium sp mold possesses an axis from which branches grow and a well-structured conidiophore. This mold is a member of the Aspergillaceae family and the Ascomycota division. According to Prayekti & Sumarsono's investigation, tofu fruit flesh had Penicillium sp fungus in 2019. This is consistent with studies that found a tiny quantity of tofu dregs in the medium, which had previously been employed as a medium for the hatching of BSF maggots.

### 3.4. Identify yeast types

The results of identifying yeast types before and after degradation with BSF maggots can be seen in Table 4.

**Table 4** Identification of yeast before and after BSF maggot degradation

| Treatment | Before Degradation | | | | After Degradation | | | |
|---|---|---|---|---|---|---|---|---|
| | **Results** | **Cell Shape** | **Asexual Cells** | **Type** | **Results** | **Cell Shape** | **Asexual Cells** | **Type** |
| R0 |  | Round | Forms ascospores | *Saccharomyces* |  | Oval | Does not form aspores | *Candida* |
| R1 |  | Round | Forms ascospores | *Saccharomyces* |  | Oval | Does not form aspores | *Candida* |
| R2 |  | Oval | Does not form ascospores | *Candida* |  | Round, oval | Forms ascospores | *Saccharomyces* |
| R3 |  | Round | Forms ascospores | *Saccharomyces* |  | Round, elliptical | Does not form aspores | *Candida* |

Source : (Ahmad R Z, 2005)

Table 4's presents the findings of the yeast identification. It indicates that *Saccharomyces* dominated the yeast prior to degradation, whereas *Candida* dominated the yeast following degradation. Round, oval, or cylindrical cells that can develop pseudohyphae but not real hyphae are the defining characteristics of the genus *Saccharomyces*. Polyhedral buds are used for asexual reproduction, while ascospores one to four or more per ascus are used for sexual reproduction. While yeast can digest carbohydrates like lactose and sucrose, it is unable to create the urease enzyme (Pratiwi & Akhdiya, 2020). A variety of shapes, including round, oval, cylindrical, elongated, seldom pointed, oval, triangular, or bottle-shaped, with or without pseudohyphae, are seen in the *Candida* genus. Pleiotropic budding is an asexual method of reproduction. Ascospores, arthrospores, melanoblastospores, and spores are not created by *Candida* types; nevertheless, certain types produce chlamydospores that are white to cream in color and devoid of carotenoid colors. It is not always the case that all types of *Candida* may ferment (Pratiwi & Akhdiya, 2020). According to *C. stellimalicola* and *Candida guilliermondii* da Cunha et al. (2018) and Pratiwi & Akhdiya (2020), *Candida* tropicalis, a member of the genus *Candida*, has the capacity to stimulate the growth of cowpea plants. It also has the ability to inhibit fruit rot caused by phytopathogenic fungi.

## Conclusion

The amount of mold on the frass was the same when cattle manure was treated with a mixed medium to encourage maggot growth. Molds were less prevalent in the R2 and R3 treatments. The mold types that were effectively isolated and recognized were *Rhizopus, Aspergillus, Penicillium, Mucor,* and *Trichoderma*. Meanwhile, *Saccharomyces* and *Candida* were the mold types that were effectively isolated and identified.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

There are no conflicts of interest to disclose.

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