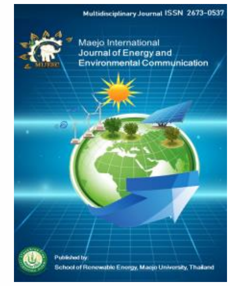
Maejo International Journal of Energy and Environmental Communication 3-3 (2021) 45-55

Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Effective microbes (EM) and their potential on mushroom commercialization in Malaysia

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ARTICLEINFO

Article history:
Received 18 October 2021
Received in revised form
10 November 2021
Accepted 21 November 2021

Keywords:
Mushrooms
Pleurotus ostreatus
Effective microorganisms
Biotechnological techniques

ABSTRACT

The mushroom cultivation industry in Malaysia is one of the fastest growing agricultural sectors due to its high return value and low production cost. With the Malaysian government forecasting an RM300 million worth of mushroom exporting by 2020, demand for high-quality mushrooms would rise. Commercially grown mushrooms in Malaysia include Pleurotus ostreatus, Pleurotus djamor, Pleurotus eryngii, and Schizophyllum commune. The mushroom cultivating technique is critical to achieving large yields. More mushrooms can be harvested in less time by employing Effective Microbes (EM) as a yield performance booster. However, EM is not yet commercially accessible, despite the fact that numerous potential EM exist, including dominant bacteria from mushroom production, bacteria bioinoculant, and anti-listerial agent. Furthermore, the EM activator found inside the substrate represents another yet-to-be-commercialized yield performance booster. Several possible EM activators, including as dairy wastewater, rice husk biochar, and tea compost, can be transformed from industrial waste with a steady increase in industrial waste. This paper reviewed the potential of EM in the mushroom cultivation industry. Additionally, the potential EM activator and how it enhances the mushroom yield performance is as well summarized.

1. Introduction

Sustainability is a direction every country strives for in order to safeguard the lives of future generations. Efforts have been made by producing sustainable food, which having several requirements to be met in order to be categorized as sustainable food. There are three requirements as stated by Pandey et al. (2018); (1) provide a healthy environment, (2) gain profit economically, and (3) ensure equity in social and economy.

In addition, the production of sustainable food must be safe to be consumed, contain high nutritional value, as well as affordable for consumer. However, the issue of making sustainable food profitable for both society and the economy poses a new challenge for researchers seeking to increase yield or exponential growth in food production. Besides, producing sustainable food that is environmentally friendly is essential in ensuring the health of the communities, which can be accomplished by reducing the usage of chemicals. As demand grows, the notion of sustainable food has

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gotten more challenging, yet most countries and organizations are moving in the right way (Li & Kallas, 2021).

Mushroom has increasingly gained attention as one of the sustainable foods nowadays. This is due to the number of nutritional benefits produced by mushroom, which includes antioxidants, polysaccharides, proteins, and terpenes. As a result of these compounds, mushrooms are a promising possibility for dietary items that can aid in the treatment of a variety of degenerative disorders (Ma et al., 2018). There are approximately 2000 species of edible mushrooms worldwide, where 35 species of them are commercially cultivated (Rathore et al., 2017). Mushroom cultivation has long been thought to be a straightforward profit generator; nevertheless, understanding of spawning and cultivation, as well as technical abilities, are essential to secure profitability (Bhuyan, 2020). Several intrinsic and extrinsic factors should be taken account in ensuring successful cultivation, such as nitrogen source, pH level, moisture, and mineral content for intrinsic factors; and temperature, humidity, luminosity, and air composition for extrinsic factors (Bellettini et al., 2019).

Farmers must have appropriate expertise before beginning commercial mushroom cultivation since, according to a survey, the majority of farmers do not know how to control disease, have poor harvesting management, and have low and poor spawn quality (Kant Raut, 2019). Besides lacking knowledge on cultivation, farmers are also lacking in government advice especially in establishing new mushroom farm and lacking in facilities and equipment in making good quality compost, casing, and mushroom spawn. In addition, knowledge on handling diseases and pest is much required in order to prevent cultivated mushroom from spoilage, which in return causes profit loss to the farmers. However, there is limited choice and supply of bio-pest control products in the local market that can be applied throughout the cultivation process (Rosmiza et al., 2016). Nowadays, with advancement of technology, a supplementary compound can be used in practice to enhance the yield performance of commercial mushrooms.

Effective microorganisms (EM) are an example of supplementary compound that can be used in mushroom agriculture. EM is defined as the mixed cultures of microorganisms that provides natural benefits when it is applied as inoculant to a soil ecosystem, meanwhile, in mushroom cultivation, it is the supplement to increase the microbial diversity in the substrate ecosystem (Joshi et al., 2019). These microbes discovered in EM are known as Plant Growth Promoting Rhizobacteria (PGPR), consisting of numerous species such as Bradyrhizobium, Bacillus and Pseudomonas (Singh, 2018b). This PGPR plays their respective roles in the substrate ecosystem, for example, Bacillus subtilis was found to be able to prevent green mold disease (Potocnik et al., 2018), while Bradyrhizobium elkanii able to increase biological efficiency of mushroom cultivation via nitrogen-fixing (Agrawal et al., 2020). On the other hand, lactic acid bacteria provide sterilizing properties, which will protect the mushroom substrate from disease spread (Joshi et al., 2019).

The purpose of this review is to provide an insight on mushroom industry in Malaysia, where the type of mushroom grown for commercialization will be discussed. This review also provides a summary on techniques involved in mushroom cultivation in ensuring profitable income. Finally, the potential of EM and their activator in enhancing the yield performance of the mushroom will be explained further. It was discovered that all the reviewed potential EM and its activator has benefits towards the microbial within the substrate that will enhance the mushroom yield production. Therefore, it is anticipated that this review will provide relevant and practical information to researchers investigating cultivation of mushroom towards large scale commercialization.

2. Mushroom industry in Malaysia

Mushroom cultivation is one of Malaysia's fastest growing and most promising agricultural sectors, owing to its low production costs and high return value in a brief period. Furthermore, the favorable climatic conditions for cultivation have contributed to the considerable mushroom market potential in this country (Ahmad Zakil et al., 2020). In fact, Malaysian government has set a target of RM300 million in mushroom exports by 2020 (Mat Amin et al., 2017). As the world's population and consumption per capita expanded, so did the demand for fresh mushrooms, causing their production in Malaysia to receive increasing importance and support from the agricultural industry (Tariqul Islam et al., 2017). In addition, The Ministry of Agriculture and Agro-Based Industry Malaysia estimates mushroom consumption in 2020 to be 2.4 kilos per person, propelling its demand to roughly 72,000 tonnes per year (Husain & Huda-Faujan, 2020).

Traditional Chinese medicine practitioners, chiropractors, acupuncturists, and herbalists use mushroom-based products comprehensively., which make it recognizable and listed under Malaysia’s National Agro-Food 2011-2020 Policy (Wong, 2018). The most popular mushroom cultivated and commercialized in Malaysia is oyster mushroom, and among the wide variety of mushroom cultivated, 90.89 % of all mushrooms farmed and marketed is Pleurotus ostreatus. This is due to their ability of decomposing a range of lignocellulosic materials that makes it the most marketed fungus in this country (Ali et al., 2018). Fresh, dried, and mushroom-based goods are all projected to see increased demand, especially fresh oyster mushroom as it is highly demanded by consumers (Mohd Zaffrie et al., 2014). Besides, imitation meat products made from mushrooms have a bright future in Malaysia, due to its meat-resembling taste and texture. This is in line with the government quest of finding Halal meat-resembled food.

2.1 Commercially cultivated mushroom in Malaysia

Malaysia, with its mixed population and multiracial culture, exploits mushrooms in its cuisine. Various edible mushroom species are harvested in the wild as well as cultivated with the advancement of technologies. Oyster mushroom and split gill mushroom are two well-known cultivated mushrooms among others in Malaysia. These edible mushrooms have been found to have nutritional and therapeutic benefits based on previous studies (Samsudin & Abdullah, 2019).

2.1.1. Oyster mushroom (Pleurotus sp.)

The oyster mushroom was discovered growing naturally on rotting wood before becoming one of the most widely cultivated mushrooms in the world. Oyster mushroom is classified in Basidiomycetes under family of Agaricaceae that can grow in temperate and tropical climates parasitizing dead and degrading wood materials (Agrawal et al., 2020). Among all, white oyster mushroom, or scientifically known as Pleurotus ostreatus, are the most cultivated for the numerous health benefits provided. High concentration of protein, carbohydrates, minerals, fibers, and vitamins is discovered in this species of mushroom with several amino acids that plays an important role in anti-tumor, antioxidant, and anti-hypercholesterolemia (Khan et al., 2019; Tupamahu & Budiarso, 2017). Moreover, *P. ostreatus* has been the subject of several studies in revealing its nutritional and therapeutic properties, which increasing its market worth (Raman et al., 2021). Besides, other species of mushroom such as button mushroom (*Agaricus bisporus*) and shiitake mushroom (*Lentinula edodes*) are dominating the global market of mushroom as well.

Pink oyster mushroom (*Pleurotus djamor*) is another species of oyster mushroom that is commonly cultivated in Malaysia for commercialization. It is known for its unique, pink-coloured fruiting bodies, and commonly referred to as roseus mushroom. It can be grown at temperature between 26-35°C with relative humidity of 80 % or higher (Lakshmanan & Sabaratnam, 2018). It feeds on decaying wood components as saprophyte and develops naturally in rotting wood during the wet season. Pink oyster mushroom is well known for having various phytochemicals within its fruiting bodies, such as tannins, flavonoids, and terpenoids (Silva et al., 2018). These compounds serve potential health benefits to the consumer. Furthermore, it was discovered that P. djamor exhibits hypolipidemic effect and contain antidiabetic properties (Acharya et al., 2017; Dulay et al., 2017).

In addition to P. ostreatus and P. djamor, Pleurotus eryngii or commonly known as king oyster mushroom is also commercially cultivated in Malaysia. It is a high value product due to its superior quality, texture, and flavour. King oyster mushroom is widely cultivated for commercialization in Europe, North America, Middle East, and several regions in Asia (Kikuchi et al., 2017). However, it is only native to North Africa and Asia. This mushroom can be distinguished from other mushroom through its thick and meaty stipes with small caps (Park et al., 2021). Similar to the others, king oyster mushroom also possessed high nutritional value as well as various biological activities, such as antitumor, modulating human gut microbiome, antioxidant, therapeutic properties, and cholesterol lowering activities (Juab, 2019; Romruen & Bangyeekhun, 2017; Yang et al., 2020). In addition, evidence showed that *P. eryngii* can regulate the production of inflammation mediators, and its anti-inflammatory activity has been proven although it is still rarely being explored in detail (Hu et al., 2018).

2.1.2. Oys Split gill muchroom (Schizophyllum commune)

Split gill mushroom or known as Schizophyllum commune, is one of the widely cultivated, edible, and medicinal mushrooms, and is consumed in various countries in Southeast Asia. It is cultivated in Malaysia and Thailand (Wanna & Sudhadham, 2018). The fruiting body of split gill mushroom has a width ranging from 1 to 5 centimetre. It has a unique shape of a fan and consists of water-soluble polysaccharide known as schizophyllan (Kumar et al., 2017). *S. commune* has been widely used as a molecular tool for studying hyphal fusion and development, heterologous expression of genes, gene deletions after it has been successfully genetically modified (Tovar-Herrera et al., 2018). Results from studies of lectin in split gill mushrooms have shown capability in triggering nitrite production, boosting production of macrophage-activating factors, and activating the lymphocytes (Zhao et al., 2020).

3. Techniques in mushroom cultivations

Mushrooms are cultivated since they are recognised to be a sustainable ad profitable agribusiness that generates revenue for local producers. China is the main producers for edible mushroom cultivation with over 30 billion kg produced in the year 2013 alone, which covers 87 % of total production worldwide (Royse et al., 2017) as shown in Figure 1. Meanwhile, Asia, Europe, America, and other countries involved in mushroom cultivation are producing a total of 4.3 billion kg altogether in 2013. Cultivating mushrooms in microbe-rich substrates with a diversity of interactions between beneficial bacteria and fungus can increase the yield performance of the mushroom, which in turn generates more profit. The cultivation of edible mushroom commonly done using conventional methods. There are two conventional methods that still in practice to this day, which is cylindrical bag log cultivation and wood tray cultivation.

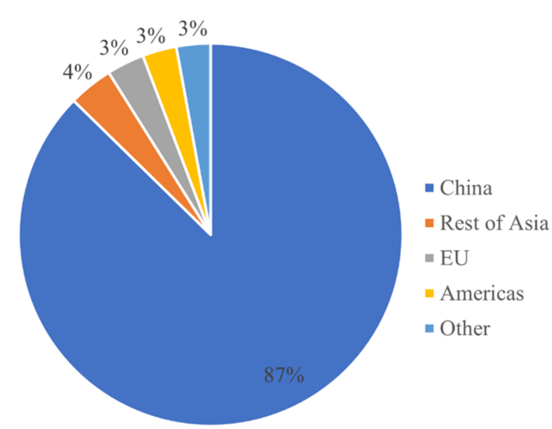

Figure 1 Percentage of mushrooms cultivated worldwide in 2013 from the total production of 34.8 billion kg.

3.1 Cylindrical bag log cultivation

The cylindrical bag log cultivation is the most popular and efficient method for edible mushroom. It is commonly made of plastic bags with a variety of material, such as polypropylene (PP), polyvinyl chloride (PVC), and polyethylene (PE) with the dimension of 15 cm x 30 cm (Khusnul et al., 2021). A hole is made at one end of the bag log, generally with a ring neck, to allow the mushroom to protrude, allowing inoculation and harvesting. There is various substrate utilized in mushroom cultivation, but sawdust and rice bran are chosen as the major components because of their high lignocellulosic content, which aids in mushroom development by delivering nutrients (Nam et al., 2018). Besides, calcium carbonate and water are also included in the substrate to maintain the pH of the substrate and increase moisture content, respectively, which aids in the movement of nutrients from the mycelium to the mushroom's fruiting bodies (Bellettini et al., 2019).

The cylindrical bag log with approximately 1 kg weight is capped and autoclaved using a steamer to kill competing microorganisms, any presence pests, and to create a sterile environment in the bag log. Following sterilisation, the bag log is set aside to cool to ambient temperature. Mushroom spawn is injected and inoculated into the bag log once it has cooled (Shrestha et al., 2021). The bag log should be kept in a well-ventilated area since oxygen is essential for the fructification of the mushrooms. The temperature in the room should be kept between 23°C and 18°C throughout the running and growth of the mycelium and between 18 and 25°C during the mushroom fructification process (Abdurrahman et al., 2019). Furthermore, the room should be completely dark, and the humidity of the bag log should be maintained by spraying it with water twice a day. The mycelia will take around 30 to 35 days to proliferate across the entire substrate, after which fructification will occur (Milenkovic, 2017).

3.2 Wood tray cultivation

The wood tray cultivation generally done in rectangular wood tray with standard size of 100 cm x 50 cm x 15 cm. Mushroom spawn is combined with substrate and placed in the wood tray ahead of time. This is the spawning phase, during which the room temperature is kept at 25°C and the humidity is kept at a constant level by spraying water on the walls and floor. Upon spawning duration ended, a sheet of paper, commonly newspaper, is used to cover the wood tray top, where water is sprayed on (Wan Mahari et al., 2020). During the incubation period, the room is sealed for approximately 12-15 days to avoid fresh air from penetrating in, in order to maintain ambient temperature within the room. Once the mycelium has grown and spread across the substrate bed, the ventilation in the room is improved to ensure that air is circulated throughout the space, and the humidity of the room is maintained (Bellettini et al., 2019).

3.3 Advantages of conventional cultivation techniques

Both conventional cultivation approach has its benefits and drawbacks. The wooden tray approach takes less time for spawn running than the bag log culture method because the wooden trays have a bigger surface area that can speed up mycelial development. Nonetheless, bag log mushroom cultivation is still the best for commercial reasons, according to Julian et al. (2019), since it provides better mushroom output owing to the prevention of evaporation and maintains a constant carbon dioxide level in the bag log for spawn running and fructification. In addition, bag log culture technique for mushroom would be the finest solution for commercial applications to earn better income (Atila, 2017).

4. Discovery of effective microbes (EM) and its application in the agricultural sector

The effective microbes (EM) concentrates include 80 species of valuable microorganisms with the majority of which being lactic acid bacteria, photosynthetic bacteria, and yeast. EM was initially introduced in Japan in the 1990s in a combination of aerobic and anaerobic microorganisms. This combination is commercially available worldwide in various forms, including free cells, liquids, and mudballs (Sharip et al., 2020). EM was originally formulated for the application in the farming sector, especially for soil and crop enhancement, which later then discovered its application in the water sector (Mandalaywala et al., 2017).

Since then, the application of EM has been increasing in Asia, and study has shown EM is used in the agricultural sector for waste decomposition, wastewater treatment, as well as revival of degrading or contaminated soil (Ngilangil & Vilar, 2020). "Bokashi" is an example of EM used in these applications. "Bokashi" is a supplemented clay ball of EM that are thrown into water bodies to restore the water quality, preventing nearby plantations from using polluted water (Sitarek et al., 2017). Besides, "bokashi" is also applied as microbial growth medium by providing suitable growing environment in the soil, which will contribute to the environmental protection as the reliance on chemical pesticides and fertilizers is reduced (Li et al., 2020).

4.1 Potential of EM in mushroom industry

The application of EM in the agricultural industry is no longer novel. However, it is still relatively new in the mushroom farming applications. Mapanao et al. (2016) has stated in their study that Effective Microorganisms-I, a commercial agricultural microbial inoculant and performance enhancer, has a potential to be applied to the mushroom substrate, despite it has yet to be researched. The Effective Microorganisms-I contains lactic acid bacteria (LAB), yeast, photosynthetic bacteria, propionic bacteria, and actinomycetes, with LAB being the main component of the product. However, the finding of a study was unsatisfactory when the microbial inoculant was applied to pre-treated sterilized substrates. Nevertheless, it can be suggested an experimentation on treated sterilized substrates or the use of various mixes of effective microbes as a future potential.

4.1.1 Dominant bacteria isolated from the mushroom cultivation process as a compost quality stabilizer

The microbial community within mushroom substrate changes as the stages of mushroom cultivation changes. The interaction with the microbial community increased upon completion of substrate colonization by the spawn, which indicates the mushroom interacts with various microorganisms at various stages of development as the substrate matures. During the substrate composting stage, the largest microbial community is present as the raw materials are being converted into nutrient for the mushroom utilization in forming fruiting bodies (Familoni et al., 2018). With the advance of technology nowadays, the microbial community within the mushroom substrates can be identified. Metaproteomic research and Sanger sequencing are among the techniques applied for the identification of each of the microorganisms at a specific time of cultivation within the community.

Sanger sequencing performed on isolates collected from a given stage of the mushroom, while metaproteomic research focused on functions, identity, and abundance of proteins, which will then be applied to study each of the individual microorganisms. Previous studies have applied these techniques in analysing the changes impact in the microbial activity towards the mushroom growth (Robinson et al., 2021; Wang et al., 2021). As previously indicated, diverse microbial communities are present at various phases of mushroom development and inside the substrate. These microbial communities include dominant bacteria that contribute in various mechanisms to support the substrate in a specific growth stage. Previous study conducted by Familoni et al. (2018) support the presence of Pseudomonas during substrate preparation stage, which further classified the bacteria into mushroom growth-promoting bacteria (MGPB). These bacteria dominate especially in the growth of P. ostreatus and having the capability to trigger primordial development and enhance the mushroom yield.

On the other hand, the presence of Thermobispora spp. and Thermobacillus in substrate during substrate partial composting and maturing suggested that they are the example of thermophilic bacteria that can survive through pasteurization during the process of substrate preparation. The substrate colonization by thermophilic bacteria serves its own purpose through preparing the compost to stimulate and develop growth that will indirectly enhance the mushroom yield (Carrasco et al., 2018). This indicates that the listed bacteria have a purpose to be dominant, allowing us to manage the compost's quality. Table 1 summarises the dominant bacteria identified via sequencing techniques that involved at different phases of substrate processing for mushroom growth as well as their benefits.

Table 1 Summary of the bacteria dominating different stages of substrate processing and their benefits.

Mushroom cultivation phase	Dominant bacteria	Benefits	References
Substrate preparation	Proteobacteria (*Pseudomonas* and *Sphingomonas* sp.)	*Pseudomonas* triggers primordial development and enhance mushroom yield	Banfie et al. (2021), Viera & Pecchia (2018), Familoni et al. (2018)
Substrate partial composting	Firmicutes (*Bacillus*, *Geobacillus*, and *Ureibacillus* spp.), *Pseudoxanthomonas*, and *Thermobispora* spp.	*Thermobispora* spp. prepares compost to stimulate and develop growth	Banfie et al. (2021), Carrasco et al. (2018)
Mature substrate	Actinobacteria, *Thermus* spp., and Firmicutes (*Bacillus*, *Geobacillus*, *Thermobacillus*, and *Ureibacillus* spp.)	*Thermobacillus* prepares compost to stimulate and develop growth	Banfie et al. (2021), Viera & Pecchia (2018), Carrasco et al. (2018)

4.1.2. Bacteria bio-inoculant as nutrient booster

Plant Growth-Promoting Rhizobacteria (PGPR) is a group of bacteria that has become one of the most popular study subjects among scientists, as it acts as bioinoculant that will naturally enter the roots and promote the host plant's growth via a variety of ways (Aasfar et al., 2021). PGPR can enhance the plant growth directly and indirectly. Directly, PGPR will stimulate the bioactive compounds production as well as boosting the nutrients availability at the rhizosphere, while indirectly, PGPR will enhance the plants' effect of pathogenic resistance against diseases (Scagliola et al., 2021). One of the bacteria that has been further studied for their role as PGPR is Azotobacter.

The Azotobacter is free-living, nitrogen-fixing, aerobic bacteria that can use atmospheric nitrogen for cellular protein synthesis resulting in nitrogen being added to the rhizosphere as a supplemental mineral (Sumbul et al., 2020). The versatility of Azotobacter as nitrogen-fixing bacteria is complemented by its capacity to produce plant growth-regulating compounds for the host (Pathak & Kumar, 2016). The Azotobacter, in combination with other bacterial bioinoculants, creates a potential bio-fertilizer that might be used on an industrial scale to augment commercial mushroom growth. A previous study conducted by Shukla et al. (2020) has applied the combination of Azotobacter with Phosphate solubilizing bacteria (PSB) on Pleurotus sp. and the results showed the yield has increased along with the fruit size and nutritional value (increase carbohydrate, protein, and fibre content, low fat content).

In addition to Azotobacter, other bacteria such as Bacillus, Paenibacillus, and Pseudomonas has exhibit potential as nutritional supplements in the form of bioinoculants by boosting mycelial development in grown mushrooms and showing antagonistic action against competing moulds in the mushroom casing. Various commercial biofertilizers focusing on fungal and bacterial plant development are available on the market, according to reports. However, Carasso et al. (2018) reported that there is still yet commercially available biofertilizer manufactured from mushroom growth-promoting microorganisms as of 2018.

4.1.3. Lactic acid bacteria as anti-listerial agent

A foodborne pathogen, Listeria monocytogenes, has recently been discovered in edible mushroom products in various countries, which may cause listeriosis in humans, as well as meningitis in elderly, and abortion in the pregnant women, in those who are most vulnerable. A study showed relatively 31.5 % of mushroom samples collected from South China, one of the largest mushroom producers in the world, was tested positive for *L. monocytogenes* (Chen et al., 2018). In addition, 30 patience and 4 deaths have been reported in a three-year period in United States, which associated with listeriosis cases for consumption of *L. monocytogenes* infected mushrooms. The revelation of the disease in edible mushrooms in various countries has presented a threat for mushroom producers (Yoon et al., 2020).

The resistant of L. monocytogenes towards food processing technique has made it an indicator in ensuring safe consumption of food products (Pilevar et al., 2020). Therefore, to ensure edible mushrooms are not contaminated by L. monocytogenes, lactic acid bacteria (LAB) are applied as a biological agent, since their metabolites are able to inhibits the growth of pathogenic microorganisms. The LAB consists of Lactococcus, Lactobacillus, Streptococcus, Enterococcus, Pediococcus, Leuconostoc, Weissella, and Oenococcus (Bangar et al., 2021). These LAB contain various compounds such as antimicrobial (bacteriocin), antifungal (3-hydroxy fatty acids, phenyl-lactate, hydroxyphenyl-lactate, propionate), as well as low molecular weight metabolites (diacetyl, reuterin, fatty acids, reutericyclin), which makes it useful as biological agent (Castellano et al., 2017).

In a study conducted by Dygico et al (2019) in identifying isolates with anti-listerial activity, Lactococcus lactis subsp. lactis IL1403 was found to have closest homology to the genetic sequence obtained and classified under LAB group. This species exhibited inhibition against L. monocytogenes within the mushroom growing environment. On the other hand, L. lactis has shown inhibitory activity against *Escherichia coli*, which proves LAB can inhibit the pathogen growth in food products (Sharma et al., 2021). This occurrence might be due to the presence of high temperature-stabled bacteriocin called nisin produced by *L. lactis*, which acts by binding to the pathogens' cell wall precursor lipid II (Yoon et al., 2021). In addition, apart of having antimicrobial activity, nisin also exhibits bacteriostatic activity against bacterial spore, therefore, regarded as safe for consumption with GRAS status. A maximum safe dose of nisin for food consumption is 250 ppm (Zhao et al., 2017).

L. lactis, as a result, has the potential to be employed as an anti-listerial agent and as a bioinoculant in mushroom cultivars, allowing the microorganisms to produce nisin and display its anti-listerial action. This technology will aid mushroom growers in averting an increase in listeriosis cases caused by the eating of L. monocytogenes-contaminated mushrooms.

4.1 Industrial waste as EM activator in mushroom cultivation

Industrial waste that is not adequately processed before disposal can have negative repercussions for the environment, especially if it is deposited in one location and becomes a pathogenic microorganism pool (Freitas et al., 2021). Although agricultural waste such as pulps, leaves, seeds, peels, and pits are a source of secondary metabolites such as antibacterial, anticancer, and antioxidant, the expense of extracting the bioactive chemical from the waste is high (Ballesteros-Vivas et al., 2019; Peanparkdee & Iwamoto, 2019). Thus, some agricultural waste was utilized as mushroom cultivation substrate instead of extracting bioactive compounds, such as rice straw, sugarcane, cotton seed, and wood sawdust. These wastes contain EM within that can help breakdown substrates and provide nutrient for mushroom mycelium to absorb (Sardar et al., 2017), which provides high content of vitamins, proteins, calcium, iron, and potassium for mushroom fruiting bodies (Oliveira do Carmo et al., 2021). Hence the reason for Pleurotus ostreatus to be considered as a functional and nutritional food (González et al., 2021; Krakowska et al., 2020).

4.2.1. Treated dairy wastewater (DWW)

Dairy wastewater (DWW) includes vast levels of nutrients as a result of high wastewater consumption and several processing units, making effluent treatment crucial in the dairy products business. However, according to Al-Wasify et al. (2017), the existing treatment procedure for dairy wastewater is too expensive for businesses to afford since it requires expensive chemical agents and the processing of enormous volumes of solids and effluents. Organic nutrients such as fats, carbohydrates, and proteins will cause a decline in dissolved oxygen (DO) levels in water bodies like as rivers, if the nutrients are released to the environment untreated. Due to numerous nutrients that might boost the growth of undesired organisms and illnesses such as dengue fever, chicken pox, and malaria, this will become a disease-producing hotspot (Lakra et al., 2021; Reddy et al., 2021). Valorization of keratin waste biomass and its potential applications. Journal of Water Process Engineering, 40, 101707.).

DWW, on the other hand, has the potential to be a beneficial material for sectors such as food, medicines, and fuel health if appropriately processed using biotechnological processes. Biotechnological treatment solutions can help to minimise waste pollution in the environment, however further research on the optimal approach and cost effectiveness is still needed (Ahmad et al., 2019; Bhuyar et al., 2019; Gogoi et al., 2021a; Shen et al., 2022). According to Gogoi et al. (2021b), biofertilizer derived from treated DWW has demonstrated the capacity to increase yield for a variety of crops including maize, aloe vera, mung bean, sorghum Sudan grass, and lemongrass. Furthermore, spraying the button mushroom (*Agaricus bisporus*) substrate with DWW at a concentration of 75 % resulted in an increase in yield performance and a reduction in spawn running time to 15 days. The study's statistical data demonstrates that the substrate's nutritional content and physiochemical have rapidly grown (Kumar et al., 2021). As a result, the modified DWW may function as an EM activator within the substrate.

4.2.2. Rice husk biochar

The rice husk is a by-product of the processed rice, which contributed up to 150 million tonnes around the globe. Various techniques and methods have been examined to handle the waste materials in order to create a sustainable agriculture (Ebe et al., 2019). One of the ways applied to overcome the problem of excessive waste of rice husk is making it as a substrate for oyster mushroom cultivation. This is due to the high lignocellulosic content within rice husk that is required for the mushroom to grow (Taskirawati et al., 2020). However, the rice husk has to be used in combination with other lignocellulosic such as grass straw or rice bran in order to be used as substrate for better mycelium coverage (Hendrika et al., 2021).

The other ways of overcoming rice husk waste excess are by converting them into biochar. The biochar can be produced via pyrolysis process, where the rice husk will be thermally break down at temperature up to 700-800 ℃. The application of rice husk biochar is wide in agriculture industry. The biochar has been introduced into the soil of agriculture plantation to enhance the nutrients and structure of the soil (Bushra & Remya, 2020). Besides, the biochar has been proven to boost the amplification of microbial biomass when use in combination with inoculation of beneficial microbes (Singh et al., 2018a). Moreover, rice husk biochar was suggested as beneficial microbes' activator, especially from genus Bacillus sp. as it benefits soil the most (Ebe et al., 2020). Therefore, the application of rice husk biochar as mushroom substrate has a high potential to augment the yield performance of the mushroom.

4.2.3. Tea leaves compost

Apart of paddy straw, soybean stalk, and sugarcane bagasse, used tea leaves were also employed as substrate for mushroom production (Abid et al., 2020; Fufa et al., 2021). Tea is one of the widely consumed beverages in many nations around the globe, yet the waste produced still contains nutrients within (Perera et al., 2021). The compost of tea leaves contains beneficial components like antifungal properties, which is possible through the presence of caffeine compound (Rakatama et al., 2018). This indirectly can protect the mushroom cultivation process from the attack of fungal diseases, which if not contains, can bring damages and losses to the mushroom producers.

Atila et al. (2019) reviewed 40-60 % tea leaves content within mushroom substrates is the best for cultivation of Pleurotus ostreatus with high biological efficiency and shorter fructification time. In addition, the tea compost substrate aided microorganisms such as bacteria and actinomycetes in exhibiting more positive features for the host plant, such as disease prevention (Villecco et al., 2020). A microbiological investigation of the tea compost by González-Hernández et al. (2021) revealed that the substrate has a significant concentration of nitrogen-fixing bacteria and actinobacteria. Therefore, this evidence could be used in making argument on the potential of tea compost as substrate alternative in mushroom industry as it contains beneficial microorganisms as well as provide support as fungicide.

5. Conclusion

To summarise, the prospective EM that has been investigated is a strong contender for improving yield performance in mushroom production, particularly among commercial mushrooms in Malaysia. With biotechnological techniques for studying dominant bacteria, growth-promoting microorganisms, and converting industrial waste into beneficial material, the potential EM can be tested and further applied in other mushroom species, giving it a better chance of being marketed and benefiting more mushroom producers, boosting the Malaysian mushroom industry. In addition, potential EM activators like as rice husk biochar, tea leaves compost, and DWW are first industrial wastes that are then converted into usable products for other sectors, including the mushroom business. Due to the prevalence of paddy plantations, dairy products manufacturing, and tea farms in Malaysia, waste from these businesses may be collected and used to help the mushroom farming industry.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was funded by Universiti Malaysia Pahang (RDU212804 and RDU222801).

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