

# Utilization of Agricultural Waste for the Cultivation of *Pleurotus* Mushrooms in Thailand

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## Abstract

Thailand is one of the major agricultural production countries in the world. Therefore, large amounts of agricultural waste are generated as by-products of the agroindustry. The wastes are usually discarded or burnt, resulting in environmental pollution. The main goal of this research was to evaluate the use of agricultural waste for mushroom cultivation. A total of 15 mushroom isolates were recovered from mother spawns and basidiocarps. They were screened for their cellulolytic enzyme activity on Czapek agar using carboxymethyl cellulose (CMC) as the sole carbon source. Two isolates of the oyster mushrooms, *Pleurotus pulmonarius* PP6 and *Pleurotus ostreatus* PO3, produced the best enzyme activities. To observe the mycelial growth on agricultural waste, the two oyster mushroom candidates were cultured in jars containing five different types of agricultural waste: corn husk, rice straw, coconut meal, coconut husk and sugarcane bagasse, and the jars were incubated at 25°C for six weeks. The results show that both isolates grew best on coconut meal, producing very densely packed mycelia. Meanwhile, corn husk and rice straw were also good sources for oyster mushroom cultivation. This study shows that these three substrates have the potential to be utilized in mushroom cultivation on a commercial scale.

**Keywords:** Agricultural Waste, Coconut, Corn, Oyster Mushroom, Sugarcane

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## INTRODUCTION

Edible mushrooms have been cultivated and used as a source of food for centuries because they can be grown on a wide range of substrates and are highly nutritious. *Pleurotus*, the members of which are commonly known as oyster mushrooms, is a genus of gilled mushrooms that includes numerous widely consumed mushrooms. Indeed, the genus includes some of the most common and often cultivated edible mushrooms.<sup>1</sup> *Pleurotus* mushrooms are white rot fungi so they can produce various lignocellulolytic enzymes. Therefore, by-products from agricultural crops containing cellulose, hemicellulose, and lignin can be commonly used as substrates.<sup>2</sup>

In Thailand, agriculture plays an important role and forms the backbone of the Thai economy. A wide range of crops are grown, and they are consumed domestically or exported internationally. Each year, huge amounts of fruits and vegetables are harvested, and subsequently large quantities of agricultural waste are generated.<sup>3</sup> After the crops are harvested, the waste is usually burnt causing soil depletion and critical air pollution. There is an urgent need to solve this problem.<sup>4,5</sup> Previous researchers have given various instances of the use of agricultural waste as substrate for mushroom cultivation.<sup>6</sup>

Sawdust, a by-product from logging and timber mills, is widely used for mushroom

cultivation because it is suitable as a growth medium for mushrooms and is reasonably cheap and cost effective. However, the use of sawdust has become difficult due to increasing concerns about deforestation.<sup>7</sup> Such concerns have driven demand for sawdust, which has in turn led to increasing production costs for mushroom growers. Therefore, there is the challenge to find other sustainable sources of substrates. In this study, five types of agricultural waste that are abundant in Thailand, namely corn husk, rice straw, coconut meal, coconut husk and sugarcane bagasse, were evaluated for their utility in the cultivation of oyster mushrooms. The main objectives of this study were (1) to isolate the oyster mushrooms and screen for their ability to produce cellulolytic enzymes, and (2) to evaluate agricultural waste for mushroom cultivation.

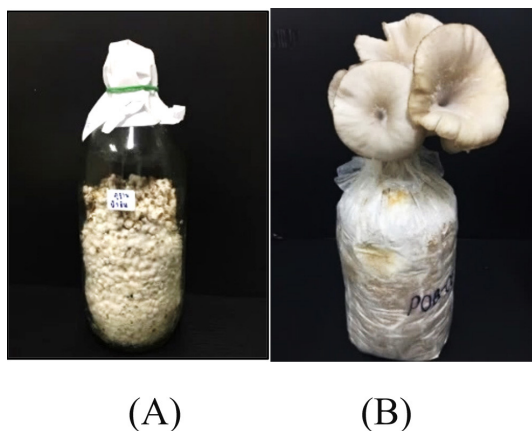
## MATERIALS AND METHODS

### Collection and isolation of *Oyster* mushrooms

Two types of oyster mushrooms, mother spawns and basidiocarps, were collected in this study (Figure 1). The samples were collected from commercial farms and local markets in the central parts of Thailand. The details of isolation codes, locations and numbers of samples are shown in Table 1. Isolation of a pure culture from a mother spawn was done by transferring a seed with fungal mycelium onto potato dextrose agar (PDA). For the fresh basidiocarp, a specimen was dissected using a flame sterilized blade, and pieces of mycelium (1x1 cm<sup>2</sup>) were cut from inside the cap and stalk of the basidiocarp. They were transferred onto PDA. The PDA plates were incubated at 25°C for seven days. After the pure cultures of oyster mushrooms were recovered, they were maintained on PDA slants and kept at 4°C.

### Study of cellulolytic enzymes

Fifteen isolates of *Pleurotus* mushrooms were cultured on PDA for 7 days at 25°C. The screening method was modified from a study by Sazci *et al.*<sup>8</sup> Agar discs were cut from the PDA using a Cork borer no. 1 (4 mm in diameter) and transferred to the center of CMC agar plates (Czapek agar supplemented with carboxymethyl cellulose as a carbon source). The plates of CMC agar were incubated at 25°C for 11 days.



**Figure 1.** Samples of oyster mushrooms collected from commercial farms in Thailand. (A) A mother spawn growing on sorghum seeds, and (B) Basidiocarps growing out from a substrate

The negative control was CMC agar without inoculation. Afterwards, the plates were dyed by flooding with 20 ml of 1% Congo red solution (1 mg/ml) for 15 min. Then the plates were decolorized with 0.85% normal saline solution. The diameters of the mycelium circles on each plate were measured. There were five replicates (five plates) for each treatment. The data is presented as mean value  $\pm$  standard error.

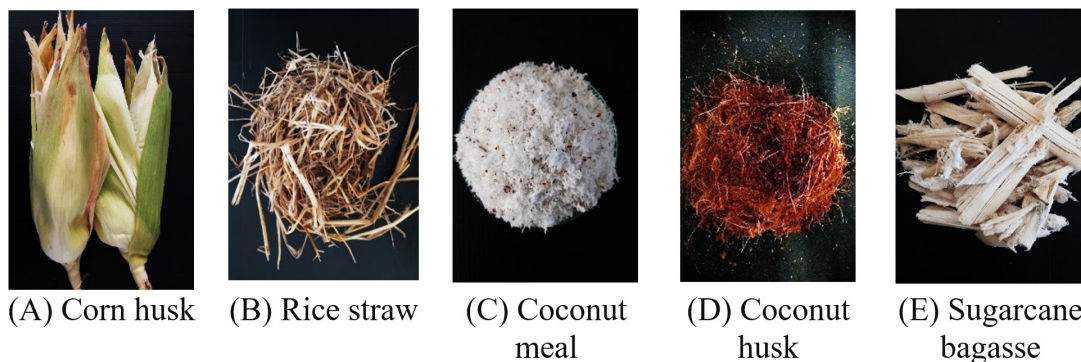
### Cultivation of mushrooms in jars

Five types of agricultural waste were investigated for use as carbon sources for the cultivation of oyster mushrooms (Figure 2). They were collected from local farms and markets free of charge because they were seen as by-products

and waste. After they had been allowed to dry in the sun during the day for 12 hours, they were chopped and ground into fiber and fine powder. Twenty grams of each substrate was added into 500 ml jars and 1.2 g of potato dextrose broth medium and 50 ml of distilled water were added into every jar. The jars were autoclaved at 121°C for 1 hour. After cooling down to room temperature, the oyster mushroom cultures were inoculated into the jars. This experiment was done in three replicates (three jars) for each substrate. The mushroom cultures were incubated at 25°C for six weeks. They were observed weekly, and the photographs of the jars were taken at the final week of incubation.

**Table 1.** *Pleurotus* mushrooms collected in this study, their isolation code, locations, and types of samples

Mushroom	Code	Province	Farms' Name	Type
<i>P. pulmonarius</i>	PP1	Nonthaburi	Katom Hed	Mother spawn
<i>P. pulmonarius</i>	PP2	Nonthaburi	Pothong	Mother spawn
<i>P. pulmonarius</i>	PP3	Bangkok	Baan Hed	Basidiocarp
<i>P. pulmonarius</i>	PP4	Kanchanaburi	Aunty Chin	Mother spawn
<i>P. pulmonarius</i>	PP5	Nakornprathom	Kasetsart University	Basidiocarp
<i>P. pulmonarius</i>	PP6	Kanchanaburi	Takratum	Basidiocarp
<i>P. ostreatus</i>	PO1	Nonthaburi	Pothong	Mother spawn
<i>P. ostreatus</i>	PO2	Kanchanaburi	Aunty Chin	Mother spawn
<i>P. ostreatus</i>	PO3	Bangkok	Krisana	Basidiocarp
<i>P. ostreatus</i>	PO4	Kanchanaburi	Aunty Chin	Mother spawn
<i>P. ostreatus</i>	PO5	Kanchanaburi	Aunty Chin	Mother spawn
<i>P. ostreatus</i>	PO6	Nakornprathom	Kasetsart University	Basidiocarp
<i>P. cornucopiae</i>	PCN1	Nonthaburi	Pothong	Mother spawn
<i>P. djamor</i>	PD1	Nonthaburi	Pothong	Mother spawn
<i>P. cystidiosus</i>	PC1	Kanchanaburi	Aunty Chin	Mother spawn
Net (Isolates)	15			



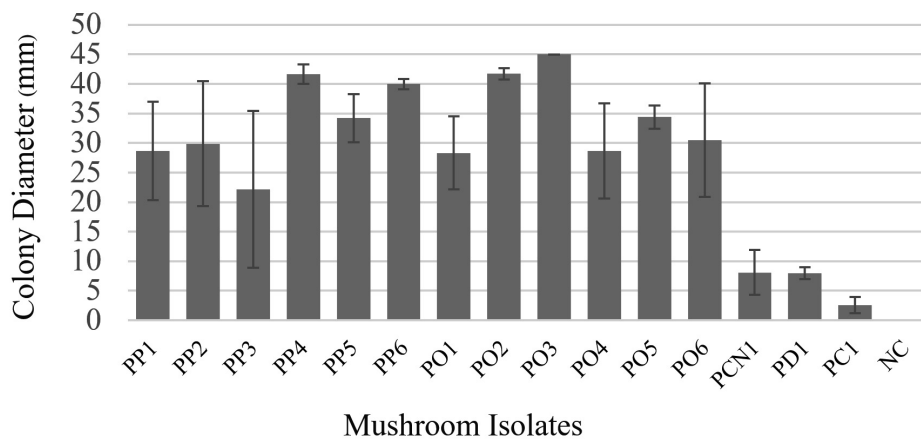
**Figure 2.** Five types of agricultural waste used for cultivation of oyster mushrooms

## RESULTS

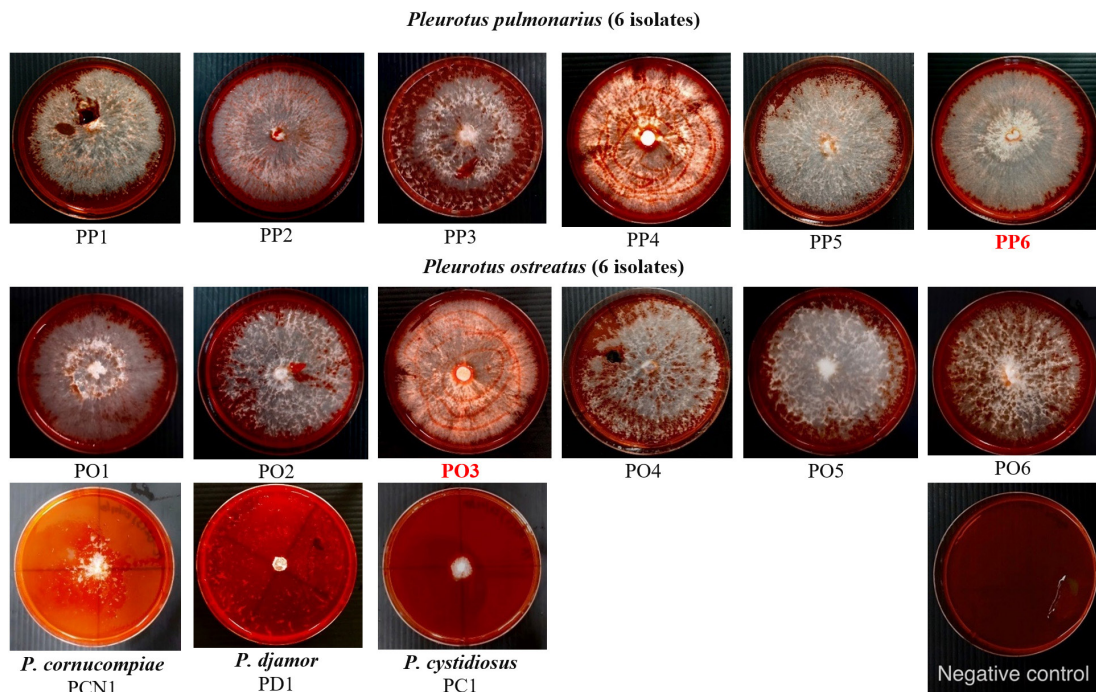
### Isolation of mushrooms

Five species of oyster mushrooms were collected, and they were *P. pulmonarius*, *P. ostreatus*, *P. cornucopiae*, *P. djamor* and *P. cystidiosus*. They were collected from four provinces in central parts of Thailand. There

were six isolates each for *P. pulmonarius* and *P. ostreatus*, while there was one isolate for each of the other three species. Isolation codes for each isolate are shown in Table 1. The pure cultures of oyster mushrooms were isolated from five and 10 samples of mother spawns and basidiocarps, respectively.



**Figure 3.** Colony diameters of *Pleurotus* mushrooms on CMC agar incubated at 25°C for 11 days. The colony diameters are expressed as means with standard deviation (mean±SD). Note: NC stands for negative control



**Figure 4.** A screening of cellulase activity by *Pleurotus* mushrooms on CMC agar incubated at 25°C for 11 days



**Screening of cellulase enzyme**

These 15 mushroom isolates were studied for their cellulolytic enzyme production activity. Most of them were able to utilize cellulose on CMC agar (Figures 3 and 4). They were categorized into three groups: (1) Isolates with low or without activity (2) Isolates with moderate activity, and (3) Isolates with high activity.

Group (1) There were three isolates (PCN1, PD1 and PC1), of which one isolate (PD1) did not grow on CMC agar, showing that it was unable to utilize cellulose as a substrate. The

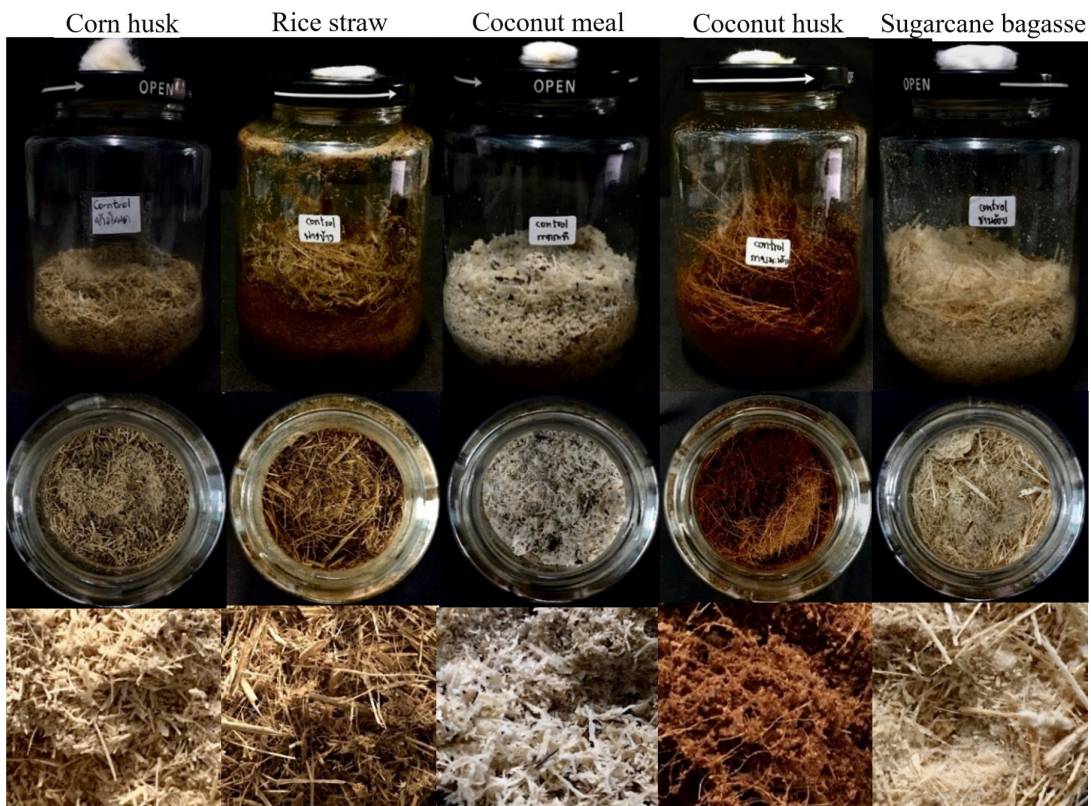
other two isolates (PCN1 and PC1) produced small colonies that ranged from 2.58 – 8.10 mm in diameter.

Group (2) There were eight isolates (PP1, PP2, PP3, PP5, PO1, PO4, PO5, PO6) that grew well on CMC agar. They produced colonies ranging from 22.17-34.40 mm in diameter. However, they produced loosely flat mycelia on the medium.

Group (3) There were four isolates (PP4, PP6, PO2 and PO3) which grew very well on CMC agar. They produced large colonies of >40.00 mm

**Table 2.** The development of fungal mycelia of *Pleurotus* mushrooms on five types of agricultural waste

Treatment	Substrates				
	Corn husk	Rice straw	Coconut meal	Coconut husk	Sugarcane bagasse
Control	–	–	–	–	–
<i>P. palmonarius</i> PP6	++	+++	++++	+++	+
<i>P. ostreatus</i> PO3	+++	+++	++++	+++	+++



**Figure 5.** Control treatments of five types of agricultural waste. The jars containing these substrates were incubated at 25°C for 6 weeks

in diameter. Two isolates, *P. pulmonarius* PP6 and *P. ostreatus* PO3, were selected for further study because they produced clearly visible white circles with dense mycelia as shown in Figure 4).

#### Growth of *Pleurotus* mushrooms on agricultural waste

Fungal mycelial development was categorized into 4 levels (Table 2). These were: (–) = no mycelial growth; (+) = mycelial growth on the surface; (++) = mycelial growth on the surface and inside substrate; (+++) = mycelial growth on the surface and inside substrate with a loose mass of mycelia; and (+++++) = mycelial growth on and inside substrate with a dense mass of mycelia. Figures 5-7 show mycelial development in the jar experiment. The top, middle and bottom rows show the side view, top view, and close-up top view of the jars, respectively, for each substrate.

In the control treatments, in which no mushroom inoculation took place, there was no physical or appearance changes of substrates. All of them were still intact after they were incubated at 25°C for 6 weeks (Figure 5). They were used

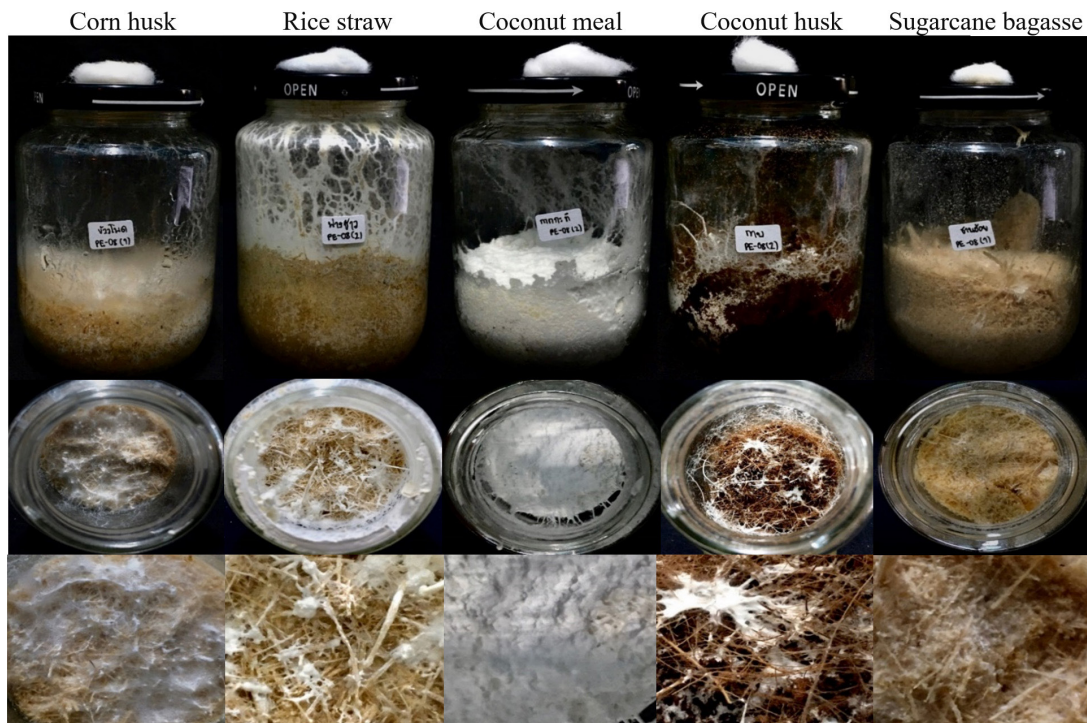
to compare with those that were inoculated with the two mushrooms to assess the mycelial development on the surface and in the interior layers of the substrates.

#### *Pleurotus pulmonarius* PP6

Isolate PP6 grew best on coconut meal, producing white mycelia both on the surface of and inside the substrate. When it was cultivated on rice straw, it produced dense white mycelia on the surface of the substrate as well as on the walls of the jar, and it produced a moderate amount of mycelia inside the rice straw. Growing on corn husk and coconut husk, Isolate PP6 grew moderately on the surface of these two substrates. However, it hardly grew on the surface of sugarcane bagasse, and only a tiny amount of mycelia was observed on the substrate.

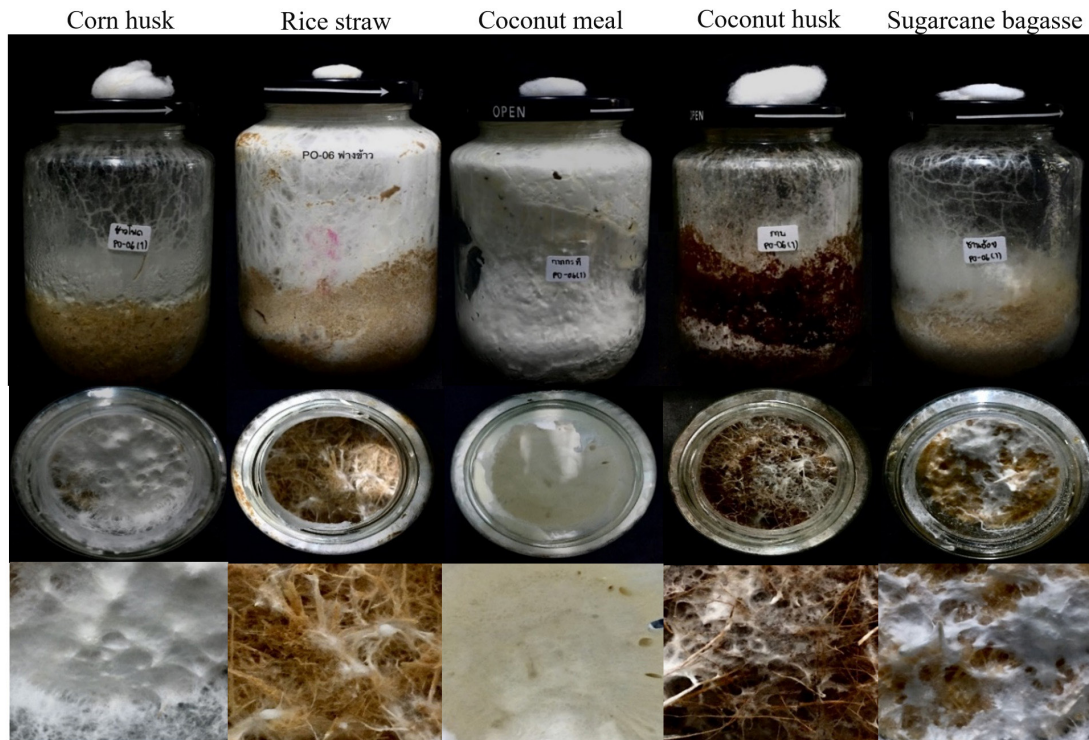
#### *Pleurotus ostreatus* PO3

Isolate PO3 also grew rapidly on coconut meal, producing white and densely compact and sticky mycelia on and inside this substrate. The mycelia also grew and reached the top of the jars.



**Figure 6.** Mycelial development of *P. pulmonarius* PP6 on five types of agricultural waste. The jars containing these substrates were incubated at 25°C for 6 weeks





**Figure 7.** Mycelial development of *P. ostreatus* PO3 on five types of agricultural waste. The jars containing these substrates were incubated at 25°C for 6 weeks

Similarly, it followed the same pattern by growing well on the surface of rice straw and produced dense mycelia on the walls of the jars. When this mushroom was cultivated on the other three substrates (corn husk, coconut husk and sugarcane bagasse), it also produced dense mycelia on each substrate as well as on the walls of the jars.

## DISCUSSION

The screening of cellulase-producing microbes using CMC as a carbon source and Congo red as dye indicator is widely used because the results generated by this plate method are clearly observable and easily interpreted.<sup>9</sup> The technique is frequently used as an initial screening method for detection of cellulases due to it being cost-efficient, simple, and convenient.<sup>10</sup> In this study, cellulases were chosen as key enzymes for the primary screening because they were essential enzymes for breaking down cellulose (a polysaccharide consisting of a linear chain of thousands of glucose units) into monosaccharides and oligosaccharides.

This breakdown is considered as a crucial step from an industrial and economic perspective because it makes a major constituent of agricultural waste available for further application. Previous studies by Velazquea-Cedeno *et al.*<sup>11</sup> and da Luz *et al.*<sup>12</sup> demonstrated that *Pleurotus ostreatus* and *Pleurotus pulmonarius* were good sources of numerous enzymes including cellulases. In this study, our results also indicated their high biotechnological applicability.

Production of fungal mycelial biomass is one the most important criteria for mushroom cultivation because it can be used as an indicator of how rapidly mushrooms can penetrate and colonize substrates. Therefore, the oyster mushrooms were cultivated in jars to observe both aerial and vegetative mycelia. Girmay *et al.*<sup>13</sup> reported that when *P. ostreatus* was cultivated on four substrates (cotton seed, paper waste, wheat straw and sawdust), it took from 14-19 days to colonize the substrates completely. If oyster mushrooms can colonize into cultivation materials within a short period of time, this is likely to indicate a shorter

spawn running step. This means the fruiting bodies of the mushrooms can be formed and harvested sooner. It also shortens the production cycle and enables more profit for farmers.

Most of the research on mushroom production has been focused on coconut coir (sometimes called coconut fiber or coconut husk) because it is cheap and abundant.<sup>14</sup> In this present study, coconut meal, which is a by-product generated from coconut milk production, was focused on. We wanted to expand upon the body of knowledge to do with waste from coconut production. In our study, among the five kinds of substrates, coconut meal was considered the best candidate substrate for mycelium production of *Pleurotus* mushrooms. Carbon: Nitrogen ratio (C/N ratio) plays an important role for mushroom cultivation. The C/N ratio for coconut meal is 25.51.<sup>15</sup> While the C/N ratios for other 4 substrates including sugarcane bagasse, rice straw, corn husk and coconut coir are 65.88, 43.94, 15 and 10.77, respectively.<sup>15,16</sup> The optimal C/N ratio for *Pleurotus eryngii* is between 20-25.<sup>17</sup> Coconut meal also contains various soluble sugars, such as glucose, mannose, galactose and arabinose,<sup>18</sup> dozens of amino acids, and an abundance of minerals and vitamins.<sup>19</sup> These nutrients are a good substrate for mushrooms because they can act as precursors for fungal metabolism. This suggested that coconut meal can be used as a supplementary material to sawdust; therefore, it can enhance the mycelial growth of oyster mushrooms.

Corn husk is also an interesting alternative for mushroom cultivation. Adjapong *et al.*<sup>7</sup> evaluated three parts of corn residue (husk, cob and stalk) as growth media for the *P. ostreatus*. When the three of them were compared with sawdust, a common substrate used for commercial mushroom production, corn husk gave higher yield of weight of fruiting bodies per crop. Similarly, Mohd Rashid *et al.*<sup>20</sup>, who studied white oyster mushrooms (*Pleurotus florida*), also reported that corn husk could be used as a substitute for sawdust because when *P. florida* was cultivated with two substrates, corn husk gave the higher yield of all major indicators (the total number of fruiting bodies, fresh weight, and dry weight). Rice straw and coconut husk are also promising substitutes for sawdust because they are widely

studied and considered as low-cost substrates for oyster mushroom cultivation.<sup>5,21,22</sup>

Various studies suggested that sugarcane bagasse is a good source for the cultivation of *P. ostreatus*.<sup>23,24</sup> Their studies showed that biomass from sugarcane either used alone, or in combination with other substrates, such as rubber tree sawdust and wheat straw, can increase the yield of oyster mushrooms. In this study, *P. ostreatus* PO3 also grew well on sugarcane bagasse; however, *P. pulmonarius* PP6 hardly grew on the same substrate. Although they are members of the same genus *Pleurotus*, they probably differ at species level in their ability to utilize different substrates. In our study, we found that *P. ostreatus* grew well on sugarcane bagasse, but *P. pulmonarius* did not follow the same pattern. Thus, it cannot be assumed that mushrooms in the same genus can utilize the same substrate. This emphasizes the importance of evaluating substrates using different species of mushrooms.

Cultivation of oyster mushroom (*P. ostreatus*) by using combinations of two substrates offer a good alternative for better yield and cost effectiveness. De *et al.*<sup>25</sup> showed that combining rice straw and sugarcane bagasse improved yield of oyster mushroom. The mushroom grew faster and produced more weight when it was cultivated on a combined substrate compared to when it was cultivated on rice straw alone. Likewise, Elkanah *et al.*<sup>26</sup> mixed palm oil waste (shaft and bunch) with sawdust to cultivate oyster mushroom, the combined substrates gave higher yield of mushroom production. Our results show the coconut meal is the best candidate for oyster mushroom cultivation in a lab condition. Further studies on combining coconut meal with other substrates and optimal cultivation conditions are needed.

For the successful cultivation of mushrooms, three factors must be carefully considered: (1) the reliability of the mushroom strain, (2) the suitability of the substrate, and (3) the optimality of conditions. In this study, we provided useful knowledge related to the first two factors. We identified two promising isolates of oyster mushrooms, and we identified a suitable substrate, coconut meal.



## CONCLUSION

Among fifteen isolates of oyster mushrooms, the two candidates (*P. pulmonarius* PP6 and *P. ostreatus* PO3) that had the potential to use lignocellulosic materials, were chosen. When they were cultivated on different substrates, both produce dense and thick mycelia on coconut meal. However, *P. pulmonarius* PP6 grew rapidly on most substrates except for sugarcane bagasse. Of the five substrates, coconut meal proved to be the most promising source for the cultivation of oyster mushrooms.

## ACKNOWLEDGMENTS

None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

Not applicable.

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