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Substrate formulation and cultivation trials of *Laetiporus sulphureus* from tropical northern Thailand

Chukeatirote E[1*], Luangharn T[1], Hyde KD[1], Wang R[2] and Song C[2]

[1]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand.*
[2]*Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, Shanghai 201403, P. R. China.*

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Abstract

Laetiporus sulphureus is a globally distributed polypore fungus valued for its culinary, medicinal, and biotechnological applications. Despite its economic potential, this species remains notably under-domesticated, and almost no cultivation protocols exist for tropical regions. In northern Thailand, *L. sulphureus* is rare and seasonal, and is harvested exclusively from the wild, highlighting the need for reliable cultivation methods. This study provides the first systematic assessment of substrate suitability for Thai strains of *L. sulphureus* using both hardwood logs (mango, para rubber, and castanopsis) and para-rubber sawdust across 12 substrate formulations. Two strains (MFLUCC 12-0546 and MFLUCC 12-0547) were successfully propagated on sorghum grains, producing high-quality spawn. Log cultivation resulted in limited and transient colonization in *Castanopsis*, with no fruiting after six months. In contrast, sawdust-bag cultivation revealed clear substrate-dependent performance: formulas F11 and F12 supported rapid and complete colonization and induced primordia formation, whereas nutrient-poor formulas (F1–F5) failed to support growth. Although mature fruiting bodies were not obtained, this study identifies the first substrate formulations capable of initiating reproductive development, including primordia formation, in Thai *L. sulphureus* strains and it provides quantitative baseline data on colonization behavior under tropical conditions. These findings highlight the species' sensitivity to substrate composition, nutrient balance, and environmental triggers such as temperature and humidity. The study establishes foundational parameters for future optimization and represents a critical step toward domestication of *L. sulphureus* in Southeast Asia, with potential long-term benefits for local mushroom production and commercial diversification.

Keywords – fungal domestication – non-traditional cultivated fungi – polypore – Thailand

Introduction

Mushrooms are ecologically and economically important fungi that play key roles in nutrient cycling, plant symbiosis, and the decomposition of organic matter. Many species are highly valued for their nutritional, medicinal, and bioactive properties, leading to growing global interest in domesticating edible and medicinal wild fungi. Among these, *Laetiporus sulphureus* ("chicken of the woods") is a distinctive and widely distributed polypore characterized by its bright orange fruiting bodies and its ability to cause cubical heart rot in hardwood trees (Gilbertson & Ryvarden 1986, Song et al. 2014). This species has attracted scientific attention due to its unique chemical composition,

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Corresponding Author: Ekachai Chukeatirote – e-mail – ekachai@mfu.ac.th

antioxidant and antimicrobial activities, and edible potential when young (Turkoglu et al. 2007, Radic et al. 2009). Despite these promising attributes, *L. sulphureus* remains one of the least domesticated edible fungi. Only a limited number of studies have reported successful primordia induction or fruiting under controlled conditions (Olennikov et al. 2009, Pleszczyńska et al. 2013), and no standardized cultivation method currently exists. The challenges are likely related to species-complex diversity, substrate specificity, and physiological requirements that differ from those of more commonly cultivated saprobic mushrooms. As a result, commercial-scale production is virtually absent worldwide.

In Thailand and other tropical regions, *L. sulphureus* occurs sporadically and only seasonally, making it an uncommon but high-value wild edible mushroom. Although the species is widely recognized and appreciated as a choice edible fungus in Europe and North America, it has not achieved comparable popularity in Thailand. Survey literature does not indicate that it is commonly consumed or culturally prominent mushroom in Thai cuisine, suggesting limited local popularity compared to other wild edible species (Chandrasrikul et al. 1985). Consequently, the mushroom is rarely encountered in local markets and remains primarily a wild-harvested, niche food resource rather than a mainstream edible mushroom. Market prices for wild mushrooms in Thailand often exceed those of cultivated species, suggesting strong economic incentives for domestication (Sanmee et al. 2003, Srikram & Supapvanich 2016). However, tropical environmental conditions differ substantially from those in temperate climates where *L. sulphureus* is most commonly reported, and no systematic cultivation trials have been conducted for Southeast Asian strains. This represents a significant knowledge gap in both fungal biology and applied mycology. The present study aimed to address this gap by evaluating the cultivation potential of two Thai *L. sulphureus* strains using locally available hardwood logs and para-rubber sawdust, a readily available local by-product, with 12 substrate formulations. Fruiting body production was an intended outcome; however, given the absence of established cultivation protocols for Southeast Asian strains, the primary focus was to (i) assess substrate compatibility for mycelial colonization on both log and sawdust systems, (ii) identify substrate formulations capable of initiating reproductive development, particularly primordia formation, and (iii) establish baseline cultivation parameters under tropical conditions. By systematically comparing substrate performance under semi-natural log conditions and controlled bag cultivation, this study provides foundational data to support future optimization of fruiting and domestication efforts for this underexplored species in Thailand.

Materials & Methods

Mushroom strains and culture maintenance

Two strains of *Laetiporus sulphureus* (MFLUCC 12-0546 and MFLUCC 12-0547) were used in this study (Fig. 1). These strains were the only isolates collected and available from wild fruiting bodies in northern Thailand. Both strains were previously well characterized and taxonomically verified by Luangharn et al. (2014), making them reliable representatives of the species for experimental purposes. The strains were maintained on potato dextrose agar (PDA) at 25 °C and sub-cultured monthly to ensure viability. Actively growing margins of 7-day-old cultures were used for all experiments.

Spawn preparation

Sorghum grains were selected as the spawn substrate due to their favorable nutrient content and proven suitability for basidiomycete propagation (Klomklung et al. 2012). Fifty grams of thoroughly washed sorghum grains were dispensed into 100-mL glass bottles and hydrated to 55–60% moisture content. Bottles were sterilized at 121 °C for 15 min and cooled to room temperature. Each bottle was inoculated with one 1-cm mycelial plug from the PDA cultures under aseptic conditions. Bottles were incubated at 30 °C in darkness for 14 days. Mycelial growth was monitored every two days, and the colonization percentage was determined at the end of the incubation period by visually assessing the proportion of the substrate surface covered by mycelium. Colonization was

expressed as a percentage of the total substrate area that was fully colonized by fungal mycelium. Spawn was considered ready for use when the grains were fully colonized and formed a dense, cohesive mycelial network.

Fig. 1 – Wild basidiomata of *Laetiporus sulphureus* collected in northern Thailand: a strain MFLUCC 12-0546. b strain MFLUCC 12-0547.

Log cultivation

Log cultivation was conducted under monitored outdoor (semi-natural) conditions rather than in a climate-controlled facility. Three hardwood species commonly available in northern Thailand were tested: mango (*Mangifera indica*), para rubber (*Hevea brasiliensis*), and castanopsis (*Castanopsis* sp.). Logs were cut into 80–100 cm lengths, seasoned outdoors for 4–6 weeks to reduce sap content, and stored under shade to prevent cracking. Holes (1.2 cm diameter; 3 cm depth) were drilled along each log in a staggered pattern at 7.5 cm intervals. Approximately 5 g of sorghum spawn were packed into each hole, which was then sealed with sterile paraffin wax. Five replicate logs were prepared per wood species. Inoculated logs were arranged horizontally on elevated racks in a shaded, well-ventilated outdoor laying yard. Temperature (26–32 °C) and humidity (65–85%) were monitored daily. Logs were misted twice daily to maintain surface moisture. Mycelial colonization around inoculation points was recorded weekly for six months.

Bag cultivation

Rubber tree sawdust was used as the primary substrate. Twelve formulations (F1–F12) were prepared by combining sawdust with different proportions of rice bran, calcium carbonate, and additional organic or inorganic supplements (Table 1). Substrate moisture was adjusted to 65–70%. For each formulation, 1 kg of substrate was packed into polypropylene bags (6.5 × 12.5 inches). A 20 g layer of rice husk was added on top of the substrate prior to sealing. The husk layer was applied to reduce surface compaction after sterilization and to facilitate air exchange near the bag opening; it was not intended as a nutritional supplement. A 5-cm central opening was fitted with a polypropylene neck ring, plugged with sterile cotton, and covered with clean kraft paper. Bags were sterilized at 121 °C and 15 psi for 6 hours, then allowed to cool overnight and inspected for contamination or bag deformation. Under a laminar flow hood, bags were inoculated with 25 g of sorghum grain spawn from either strain. Bags were incubated in darkness at 25 ± 1 °C. Mycelial colonization was assessed every three days, and complete colonization was recorded when the entire substrate mass appeared uniformly white and compact. Five replicate bags were used for each substrate formulation (n = 5 per formula).

After complete colonization, bags were transferred to a controlled-environment fruiting chamber. Fruiting initiation followed the cold-shock method adapted from Pleszczyńska et al. (2013), in which 300 mL of sterile water at 10 °C was injected into each bag, which was then incubated at 4 °C for 24 h. After cold treatment, the bag surface was cut open to expose the substrate. Bags were

then maintained at 25 ± 1 °C, 70–85% relative humidity, and diffuse light (200–300 lux). Mist watering was performed twice daily under aseptic conditions. The appearance of primordia and fruiting bodies was photographed and recorded in a tabular format.

Data recording and analysis

The quantitative data recorded included mycelial colonization rate (%), time required to achieve full colonization (days), proportion of bags that formed primordia, and log colonization success for each hardwood species tested. Environmental parameters (e.g., temperature and humidity fluctuations) were monitored throughout the cultivation period. Owing to the exploratory nature of this study and the limited number of replicates, data were summarized solely through descriptive analysis. Formal statistical analyses were not performed, as the primary objective was to establish baseline observations and identify general trends to inform future, more rigorous experimental designs.

Table 1 Composition of the twelve substrate formulations evaluated for *Laetiporus sulphureus* growth and primordia induction.

Component (per 100 kg substrate)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Rubber tree sawdust (kg)	100	100	100	100	100	100	100	100	100	100	100	100
Rice bran (kg)	4	5	7	8	10	12	13	14	15	15	15	15
Pumice sulfate (g)	40	50	80	100	150	300	500	700	800	900	1000	1000
Calcium carbonate (kg)	0.5	0.5	1	1	1.5	1.5	2	2.5	3	4	3.5	3
Brewer's yeast (g)	–	–	200	150	200	300	400	500	500	2000	1500	1000
Magnesium sulfate (kg)	–	–	–	–	1	1.5	2	2.5	3	4	3.5	3
Rice husk (kg)	–	–	–	–	–	–	1	2	2.5	3	3.5	4
Sorghum powder (kg)	–	–	–	–	–	–	–	1	2	3	2	2
Corn cobs powder (kg)	–	–	–	–	–	–	–	0.5	1	2	1	1
Sucrose (kg)	–	–	–	–	–	1	1.3	1.5	1.5	1.5	1.5	1.5
EM solution (ml)	–	–	–	–	–	100	200	300	400	500	700	1000
Molasses solution (ml)	–	–	–	–	–	100	200	300	400	500	700	1000
Salt solution (ml)	–	–	–	–	–	500	750	800	800	900	800	800

Note: All amounts are given per 100 kg dry substrate. EM = Effective Microorganisms.

Results

Mushroom spawn production

Both *Laetiporus sulphureus* strains (MFLUCC 12-0546 and MFLUCC 12-0547) colonized sorghum grains efficiently under the incubation conditions used. Complete colonization was achieved within 14 days at 30 °C, with 100% colonization in all bottles (n = 10). The resulting mycelium was dense, white, and uniform across the grain surface, indicating strong substrate compatibility (Fig. 2A). No contamination was observed during spawn production. The consistency of colonization across replicates confirmed sorghum's suitability as a substrate for high-quality spawn preparation.

Log cultivation

Mycelial colonization on hardwood logs differed among the three species tested. Only *Castanopsis* logs exhibited visible surface colonization around the inoculation points (Fig. 2B). A thin mycelial layer appeared within 3–5 days after inoculation, but this growth collapsed within 48 hours. A second episode of surface colonization was observed approximately four weeks later, although it remained weak and localized. After six months of incubation, no primordia or fruiting bodies were observed on any log (Fig. 2C). Mango and para-rubber logs showed no visible external mycelial growth throughout the incubation period. Based on surface observations (n = 15), visible colonization was limited (6.7%), and no reproductive development was recorded (Table 2).

Colonization was evaluated by visual inspection of growth around the inoculation points, and logs were not destructively sampled to assess internal mycelial spread. Therefore, limited internal colonization within the wood cannot be excluded.

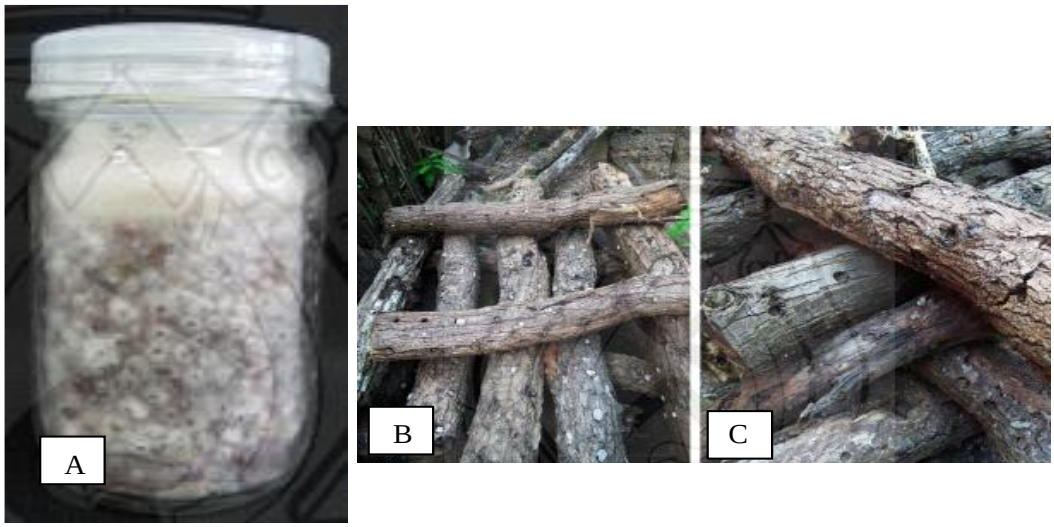

Fig. 2 – A Spawn production of *Laetiporus sulphureus* on sorghum grain. B Visible surface colonization around inoculation points on *Castanopsis* logs. No fruiting bodies were observed after 6 months of incubation. C Internal colonization was not assessed.

Bag cultivation

Substrate formulation had a strong effect on colonization. No mycelial growth occurred in formulations F1–F5, all of which consisted solely of rubber sawdust without nutrient supplementation. After 45 days, colonization remained at 0% in all replicates (n = 25). Formulations F6–F10 supported partial colonization, with colonization rates ranging from 20–40%. Mycelial spread was slow (mean 35–42 days to reach half-bag colonization), and growth was often patchy or inconsistent. None of these formulations produced primordia (n = 25). The best performance was achieved by formulations F11 and F12, both of which contained balanced organic (rice bran) and inorganic ($CaCO_3$) supplements. All bags in these treatments (100%, n = 10) reached full colonization within 21–22 days, forming a dense, compact mycelial matrix (Table 2; Fig. 3). These two formulations were the only ones capable of supporting reproductive initiation.

Table 2 Growth characteristics of *Laetiporus sulphureus* on different substrate formulas.

Treatment Formula	Mycelial Density	Complete Colonization (%)	Mean Colonization Time (days)	Primordia Formed
F1	–	0	–	No
F2	–	0	–	No
F3	–	0	–	No
F4	–	0	–	No
F5	–	0	–	No
F6	1+	20	35	No
F7	1+	20	35	No
F8	1+	40	42	No
F9	2+	40	42	No
F10	2+	40	42	No
F11	3+	100	22	Yes
F12	3+	100	21	Yes

Notes:

- Mycelial density scale: – (no growth), 1+ (sparse), 2+ (moderate), 3+ (dense).

- Full colonization % and mean colonization time are approximate ranges based on grouped formulas.
- Primordia formation indicates visible fruiting initiation.

Fig. 3 – Mycelial development of *Laetiporus sulphureus*. a–b No visible mycelial growth after 3 months of incubation. c moderate mycelial growth localized near the inoculation site after 3 months. d compact mycelial growth after 75 days. e–f primordia formation observed after 4 months of incubation.

Primordia were observed only in F11 and F12. In F11, 60% of bags (3 out of 5) formed primordia 10–14 days after fruiting induction. In F12, 80% of bags (4 out of 5) produced primordia within a similar time frame. Primordia appeared as small, bright-yellow nodules, typical of early *L. sulphureus* development. However, none of the primordia progressed to mature fruiting bodies. Across all 12 substrate formulations (n = 60), the overall primordia formation rate was 11.7%, observed exclusively in the two nutrient-enriched treatments. No mature fruiting bodies were produced in any treatment. Following cold-shock induction and transfer to fruiting conditions, primordia desiccated or stalled after 4–7 days. Bags maintained high humidity (70–85%) and stable temperatures (25 ± 1 °C), suggesting that additional environmental triggers were required for full fruiting.

Discussion

This study represents the first comparative assessment of substrate suitability for Thai strains of *Laetiporus sulphureus* under tropical cultivation conditions. Although mature fruiting bodies were not obtained, the results provide important biological insights into substrate compatibility, environmental constraints, and early reproductive behavior of this species. These findings help explain why *L. sulphureus* remains difficult to domesticate and establish a framework for future optimization efforts. Because cultivation of Southeast Asian strains has not previously been reported, this study was designed as an initial domestication assessment rather than a fully optimized fruiting

trial. Log cultivation was conducted under monitored outdoor conditions in a shaded laying yard, whereas sawdust-bag incubation and fruiting induction were performed under controlled environmental conditions. This dual approach allowed evaluation of substrate compatibility and early reproductive responses while acknowledging that precise environmental optimization for mature basidiocarp development remains to be determined.

Substrate composition strongly influences colonization and primordia initiation

The twelve sawdust formulations tested demonstrated clear differences in mycelial performance, confirming that *Laetiporus sulphureus* requires more than a lignocellulosic base for reliable colonization. Nutrient-poor substrates (F1–F5) did not support mycelial growth, suggesting that the substrate alone does not provide sufficient nitrogen, carbon, or micronutrients to sustain metabolic activity. The absence of visible mycelial growth after 3 months of incubation is likely due to substrate- and species-specific factors rather than incubation conditions alone. Incubation was conducted at 25 °C in the dark, with a relative humidity of 60–75%. Under these conditions, *L. sulphureus* exhibited slow or inhibited mycelial colonization depending on substrate composition and nutrient availability (Luangharn et al. 2014). In particular, dense substrates, low available carbohydrates, improper moisture levels, or limited oxygen diffusion within the bags can significantly delay or prevent mycelial establishment. Additionally, *L. sulphureus* is a wood-decaying fungus that may require specific lignocellulosic characteristics or substrate pretreatment to initiate growth, which could explain the prolonged incubation period without visible colonization. Similar nutrient limitations have been reported in other cultivated wood-decaying fungi, including *Ganoderma lucidum* and *Lentinula edodes*, which require balanced carbon-to-nitrogen (C/N) ratios for effective vegetative growth (Shimomura & Hasebe 2004, Klomklung et al. 2012). In contrast, formulations F11 and F12, which were supplemented with rice bran and $CaCO_3$, achieved complete substrate colonization and primordia formation. Rice bran is a rich source of readily accessible carbohydrates, proteins, lipids, and B vitamins, all of which have been shown to accelerate mycelial growth and improve substrate utilization in tropical mushroom species (Kwon & Thatithatgoon 2004, Kumla et al. 2013). In addition to nutrient supplementation, $CaCO_3$ likely played a critical role by buffering substrate pH. During incubation, lignocellulosic substrates can become acidic due to microbial activity and the accumulation of organic acids, which may inhibit enzyme activity and mycelial development. The inclusion of $CaCO_3$ helps stabilize pH within a range favorable for lignocellulolytic enzyme function, nutrient uptake, and overall metabolic efficiency in basidiomycetes. The successful colonization observed in $CaCO_3$-supplemented formulations aligns with previous findings that enriched and pH-stabilized substrates support early developmental stages of *L. sulphureus*, including primordia initiation (Pleszczyńska et al. 2013). Collectively, these results suggest that *L. sulphureus* has relatively specific nutritional (e.g., C/N) and pH requirements compared to many commonly cultivated basidiomycetes, underscoring the importance of substrate fortification and pH regulation for successful mycelial establishment and potential domestication. In addition, a thin layer of rice husk was placed on the surface of each substrate bag to reduce compaction after sterilization and to maintain aeration near the opening. This approach follows common local bag-preparation practices but was not specifically optimized for *L. sulphureus*. Because the husk was not mixed into the main substrate and contains little available nutrients, its role was likely physical rather than nutritional. However, its effect on mycelial spread and primordia formation was not examined directly. Future studies should compare treatments with and without a rice husk layer to determine whether this step influences colonization dynamics or subsequent reproductive development.

Fruiting limitations suggest physiological constraints specific to tropical environments

Although primordia formation occurred, no bag produced mature fruiting bodies. Several factors likely contributed to this developmental arrest, reflecting both ecological and physiological constraints.

1. Temperature sensitivity

Laetiporus sulphureus occurs most frequently in temperate climates (Gilbertson & Ryvarden 1986, Song & Cui 2017), and fruiting often follows cool or fluctuating temperatures. Studies by Ota et al. (2009) and Olennikov et al. (2009) also indicate that low temperatures play a critical role in reproductive induction. In this study, colonization proceeded well at 25 °C, but fruiting may require lower temperatures, more prolonged cold exposure, or larger diurnal fluctuations than those provided.

2. Moisture and aeration dynamics

Although high humidity was maintained, bag cultivation systems provide relatively stable moisture. Many polypores require alternating wet–dry cycles or exposure to fresh air to trigger fruiting (Thawthong et al. 2014). Insufficient aeration or minimal moisture fluctuation may have prevented primordia from maturing.

3. Light and CO_2 regulation

Light plays an important role in basidiomycete morphogenesis, and wavelength and intensity can influence primordia differentiation (Anusiya et al. 2021). In the present study, fruiting was conducted under diffuse light (200–300 lux) in a controlled chamber. While this level is commonly used in mushroom cultivation, the specific light requirements of *L. sulphureus* remain poorly defined. Although the fruiting chamber was ventilated, the CO_2 concentration was not directly measured. Elevated CO_2 levels can inhibit pileus expansion and normal fruiting in some wood-decaying fungi (Shimomura & Hasebe 2004). Therefore, the influence of gas exchange cannot be fully excluded. Further trials incorporating direct monitoring of CO_2 levels and controlled airflow would help clarify the environmental triggers required for full fruiting development.

4. Species-complex variability

Laetiporus sulphureus is recognized as a species complex with considerable genetic diversity (Song et al. 2014, Song & Cui 2017). Isolates from tropical regions may differ physiologically from temperate populations, particularly in their responses to temperature, substrate composition, and environmental signals. Such genetic variation may contribute to inconsistent fruiting behavior reported from different geographic regions. Comparable patterns have been documented in other cultivated basidiomycetes. In *Lentinula edodes*, geographically distinct strains differ in temperature requirements and fruiting performance (Guo et al. 2023). Similarly, strain-level variation in growth rate, substrate utilization, and biological efficiency has been reported in *Pleurotus ostreatus* (Krupodorova et al. 2024). These observations indicate that intraspecific genetic diversity can substantially influence cultivation outcomes and support the interpretation that Thai isolates of *L. sulphureus* may respond differently from temperate strains.

Limited colonization on hardwood logs reflects ecological specificity

Only *Castanopsis* logs showed minimal colonization, whereas mango and para rubber logs showed none. Substrate specificity is well known in white rot polypores, which often depend on a particular wood chemistry for successful establishment (Gilbertson & Ryvarden 1986). *Castanopsis* may share lignocellulosic features similar to those of natural hosts reported for *L. sulphureus* in temperate regions. However, colonization was weak and short-lived, suggesting that the tropical hardwoods tested—generally denser and richer in extractives—may not be well suited to this species. Seasonal variation in wood moisture and the use of seasoned logs may also have reduced substrate suitability. Similar difficulties have been reported in early domestication attempts of *Lentinula edodes* and *Fomes fomentarius* (Thawthong et al. 2014). Spawn type may also have influenced colonization. Sorghum grain spawn was used to maintain uniform inoculum preparation. In commercial log cultivation of wood-decaying fungi, sawdust or wood chip spawn is more commonly used because it is better suited to woody substrates. Grain spawn may dry more rapidly and remain concentrated near the inoculation hole, potentially limiting mycelial spread into dense hardwood tissue. Comparative trials using alternative spawn types would help clarify this effect. Colonization

in this study was assessed based on visible growth around the inoculation points, and logs were not split to examine internal mycelial development. In log cultivation systems, mycelium is often more apparent beneath the bark or on freshly cut surfaces than on the outer bark. The absence of sustained surface growth, therefore, does not necessarily indicate a complete lack of internal colonization. Future studies should include periodic destructive sampling to confirm internal spread and to better relate vegetative growth to fruiting potential.

Biological significance of partial domestication

Despite the absence of fruiting bodies, this study reached a key domestication milestone: the formation of primordia. Primordia are a critical developmental stage marking the transition from vegetative to reproductive growth. Previous studies have noted that even inducing primordia in *L. sulphureus* can be difficult and strain-dependent (Olennikov et al. 2009, Pleszczyńska et al. 2013). Therefore, identifying formulations (F11 and F12) that support this stage provides meaningful insight into the nutritional and environmental conditions required by this species. The quantitative observations generated — colonization percentages, colonization times, and primordia frequency — represent the first baseline data for Thai *L. sulphureus* strains and contribute to understanding why tropical cultivation has been unsuccessful to date.

Another factor that may have influenced cultivation performance is strain maintenance during laboratory storage. In this study, cultures were maintained by periodic subculturing on PDA to ensure viability. While this method supports continued growth, repeated subculturing in basidiomycetes has been reported to lead to strain degeneration, reduced metabolic activity, and decreased fruiting ability over time (Danner et al. 2023). Such changes can occur even when mycelia appear healthy, potentially affecting colonization rate and reproductive development. The absence of a formal mother culture system in the present study may therefore have influenced strain vigor during cultivation trials. Future work should consider implementing a structured preservation strategy, such as cryopreservation, mineral oil storage, or regular renewal from original stock cultures, to maintain physiological stability and performance during long-term domestication studies.

Implications for future research and tropical mushroom cultivation

These findings identify several priority areas for continued research:

1. Temperature optimization: testing lower temperatures or fluctuating day–night cycles during fruiting (Ota et al. 2009).
2. Improved aeration and CO_2 control: using forced-air exchange or modified bag openings during fruiting.
3. Light studies: evaluating specific wavelengths known to stimulate basidiocarp formation (Anusiya et al. 2021).
4. Substrate refinement: adjusting C/N ratios, adding lignocellulosic enhancers, or forming compressed blocks that better mimic natural wood.
5. Fresh log trials: testing freshly cut logs obtained during the dormant season of deciduous hosts (after leaf fall or before bud emergence), when nutrient reserves are highest and wood is more suitable for colonization.
6. Strain comparison: screening additional Thai and regional isolates to identify more fruiting-responsive genotypes.

By identifying substrate formulations that reliably support colonization and primordia formation, this work lays the foundation for developing a tropical cultivation protocol for *L. sulphureus*. Although full fruiting was not achieved, the study contributes essential biological, ecological, and methodological insights that advance the domestication of this valuable but underexplored polypore.

Conclusions

This study provides the first comparative assessment of substrate suitability and early cultivation requirements for Thai strains of *Laetiporus sulphureus* under tropical conditions. Although mature fruiting bodies were not obtained, complete substrate colonization and primordia formation were achieved in sawdust formulations enriched with rice bran and $CaCO_3$ (F11 and F12). These results indicate that appropriate nutrient supplementation and pH buffering are essential for successful vegetative growth and early reproductive development in this species. In bag cultivation, where environmental conditions were controlled, substrate composition played a decisive role in colonization and primordia initiation. In contrast, limited colonization on hardwood logs under semi-natural outdoor conditions suggests ecological or substrate constraints associated with the tested wood species and environmental variability. The absence of fruiting underscores the need to refine fruiting-stage parameters, including temperature regime, moisture management, and strain selection. Overall, this study establishes baseline information on substrate formulation and early developmental responses of *L. sulphureus* in Thailand. Future research should focus on optimizing fruiting triggers, refining C/N ratios, testing freshly harvested hardwoods, and evaluating additional strains to identify genotypes with improved fruiting performance. Continued work in these areas will be necessary to advance the domestication and potential commercial cultivation of this species under tropical conditions.

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Accessibility of data

Not applicable.

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