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Optimization of Mycelial Biomass Production and Bioactivities of Four *Oudemansiella canarii* Isolates from Central Luzon Region, The Philippines

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Abstract

Mushrooms are recognized for their nutritional and therapeutic potential, yet studies on optimizing culture conditions and evaluating their bioactivities of the genus *Oudemansiella* remain limited. This study was carried out to investigate the mycelial biomass production of four *Oudemansiella canarii* isolates (OCNu, OCTa, OCBu and OCPa) in fruit-based liquid media and to assess the anti-inflammatory, anti-oxidant, and anti-diabetic activities of their mycelial extracts and exopolysaccharides (EPSs). Among the tested media, 50% Saba banana puree produced the highest biomass of OCNu (714 mg 100 mL^{-1}), OCBu (723 mg 100 mL^{-1}) and OCPa (739 mg 100 mL^{-1}), while OCTa achieved its maximum yield of 780 mg 100 mL^{-1} in 40% puree. Optimal growth conditions were pH 5–6, 30°C, and 150 rpm agitation, which significantly increased biomass compared to static cultures ($P \leq 0.05$). In bioactivity assays, the OCNu mycelial extract exhibited cyclooxygenase (COX) 1 and 2 inhibition of 73% and 71%, respectively, whereas EPSs of OCTa and OCNu showed the highest COX-1 (50%) and COX-2 (50%) inhibition. DPPH radical scavenging activities ranged from 59% to 63% for mycelial extracts and 60% to 74% for EPSs. In antidiabetic assays, mycelial extracts at 10 μg mL^{-1} displayed negligible α-glucosidase inhibition, while OCPa EPS showed modest inhibition of 3% to 5%. These results demonstrate that *O. canarii* is a promising source of bioactive compounds with anti-inflammatory and anti-oxidant properties and highlight the feasibility of using fruit-based substrates as cost-effective media for mycelial biomass production.

Keywords: α-glucosidase; Cyclooxygenase; DPPH radical scavenging; Exopolysaccharide; Mycelial biomass

Introduction

Mushrooms are recognized as promising sources of nutritional and pharmaceutical compounds, attracting significant attention for their potential in reducing various health risks. Despite this interest, many wild mushrooms remain under-utilized. Researchers attribute this gap to cultivation difficulties, limitations in nutrient optimization, and other external factors (Dulay and Damaso 2017; Aguilar *et al.* 2023a). Some reports suggest that controlling conditions such as pH, nutrient type, temperature, light, and agitation can markedly influence mycelial biomass (Agudelo-Escobar *et al.* 2017; Aguilar *et al.* 2023a; Asasira *et al.* 2025). Other studies highlight the role of substrate formulation and the time required for ramification and pinhead initiation in determining fruiting efficiency (Kalaw *et al.* 2022; Aguilar *et al.* 2024).

While numerous mushrooms are already commercialized, many are not yet fully studied. *Oudemansiella canarii* is one of those understudied species. This species has been reported from at least 14 countries, including China and the Philippines (Dulay 2023). Alberti *et al.* (2021) described it as a wood-decaying fungus that colonizes twigs and branches. Cultivation trials of this mushroom using different substrates show variability in growth. For example, Ruegger *et al.* (2001) noted that sugarcane bagasse produced denser and faster ramification of its mycelium compared to eucalyptus sawdust. In the Philippines, Dulay and Damaso (2017) reported the first successful cultivation, where malt extract agar promoted rapid mycelial growth and cracked corn supported thick colonization. A mixture of rice straw (80%) and sawdust (20%) also resulted in high biological efficiency for fruiting bodies. Despite these advances, knowledge about the species remains limited.

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Mushrooms are increasingly recognized for their bioactive compounds that exert significant health promoting effects. Their mycelia and fruiting bodies contain polysaccharides, phenolics, terpenoids and other secondary metabolites that contribute to their anti-inflammatory, anti-oxidant and anti-diabetic activities (Singh *et al.* 2025; Zade *et al.* 2025). Anti-inflammatory effects are primarily mediated through inhibition of pro-inflammatory enzymes and modulation of the immune response, as mushroom polysaccharides exhibit potent anti-inflammatory and immunomodulatory properties (Mizuno and Minato 2024). Anti-oxidant activity has also been widely observed; polysaccharides are frequently mentioned as strong radical scavengers with possible roles in diabetes prevention (Arunachalam 2022). Likewise, inhibition of α-glucosidase and α-amylase has been observed, mechanisms that may explain improved postprandial glucose control and enhanced insulin sensitivity (Stojkovic *et al.* 2019; Ferraro *et al.* 2024). Although results vary across species, the evidence points to mushrooms as promising functional foods that could help manage oxidative stress, inflammation, and metabolic disorders.

Research has also extended beyond fruiting bodies. Mycelia are attractive because they can be harvested more quickly. Submerged fermentation is commonly used to accelerate their production, yet the liquid left behind is often discarded. This is notable since recent reports show it can be used to recover exopolysaccharides (EPS), thereby maximizing yields (Agudelo-Escobar *et al.* 2017; Guo *et al.* 2023). During fungal growth, EPS are secreted as carbohydrate polymers that enable microorganisms to tolerate stress and avoid phagocytosis (Angelin and Kavitha 2020). Their biological effects are wide-ranging. For instance, Dulay *et al.* (2021) described anticancer activity in mycelial extracts, while Aguilar *et al.* (2023a, b) reported antibacterial properties. Antioxidant activity has also been noted (Alcazar *et al.* 2021). EPS fractions, on the other hand, are often associated with anti-oxidant (Kheni and Vyas 2017), anti-bacterial (Dutta *et al.* 2024), anti-inflammatory (Li *et al.* 2020) and anti-diabetic effects (Sonawane *et al.* 2020). Together, these outcomes indicate that both mycelia and EPS value further study.

In a similar context, tropical fruits are gaining importance in biotechnological applications. In the Philippines, mango, banana, and coconut serve as key agricultural products and are extensively utilized for the production of juices, jams, and preserved goods. However, yields have been affected by climate change (García-Mahecha *et al.* 2023). Processing also produces a large volume of waste. In the case of mango, 35–60% of the fruit biomass is discarded, which raises both environmental and economic concerns. Banana waste is even greater; nearly 60% of the biomass is roughly 114 metric tons worldwide that is lost each year and contributes significantly to greenhouse gas emissions (Alzate-Acevedo *et al.* 2021). The OAG (2022) report also highlighted that 50 million tons of banana waste are generated annually due to browning reactions. In the case of coconut, mature coconut water, often discarded in local markets, has been successfully utilized as a culture medium for fungi and in plant micropropagation studies (Dulay *et al.* 2021; Bautista and Valentino 2023). Given this context, the present study was carried out to evaluate select fruits as substrates for submerged fermentation to produce mycelial biomass and EPS of four *O. canarii* isolates from Central Luzon, Philippines, and to determine their anti-inflammatory, anti-oxidant, and anti-diabetic properties, which contributes to sustainable and cost-effective production for pharmaceutical, nutraceutical and industrial applications.

Materials and Methods

Collection of wild fruiting bodies

Wild *O. canarii* fruiting bodies were obtained from the four provinces of Central Luzon Region, Philippines, namely Nueva Ecija, Tarlac, Bulacan and Pampanga (Fig. 1). Collection of mushrooms was done from July 2024 to October 2024. Using Global Positioning System (GPS), the place of origin, coordinates, elevation, date of collection and substrate were recorded (Table 1). Wild fruiting bodies of *O. canarii* were collected and individually placed in properly labeled brown paper bag and brought to the laboratory for tissue culture to rescue their cell lines (Fig. 2).

Mushroom tissue culture

The collected fruiting bodies were cleaned using a tissue paper and prepared for tissue culture. In a disinfected laminar flow hood, a healthy fruiting body was cut into half to expose the inner portion. A small mushroom tissue was sliced using a scalpel and obtained using a sterile inoculating needle. The tissue was aseptically inoculated onto plates containing potato dextrose agar (PDA). Inoculated plates were incubated at 28°C and allowed to grow for 7 days. Successful cultures were used to prepare mycelial discs inoculant for the optimization study.

Culture media and initial pH evaluation

Fruits such as Saba banana (*Musa acuminata* × *balbisiana*), Indian mango (*Mangifera indica*) and mature coconut (*Cocos nucifera*) were evaluated as culture media in submerged production of mycelium biomass. Fruit puree and coconut water were prepared at varying concentrations (10%, 20%, 30%, 40% and 50%) and adjusted to pH 6.5. Each medium (100 mL) was dispensed into a flask plugged with cotton, and sterilized for 30 min at 121°C and 103 kPa pressure. Mycelial discs (10 mm-diameter) were inoculated and incubated at 28°C for 10 days. Mycelia were harvested, washed, and oven-dried at 40°C for 3–5 days. The mycelial dry weight was recorded to determine the most suitable culture medium type and concentration. Using the best medium, the effect of varying pH levels from pH 4.0 to 8.0 on the mycelial biomass yield was also determined.

Table 1: Geographic location, coordinates, elevation, collection date and substrate of the collected fruiting bodies of the four isolates of *O. canarii* in Central Luzon, Philippines

Code	Place of Origin	Coordinates	Elevation	Date	Substrate
OCNu	Agupalo Este, Lupao, Nueva Ecija	15°50'43.8"N 120°53'30.2"E	339 ft. ASL	12-07-24	Mango log
OCTa	San Andres, Victoria, Tarlac	15°34'41.5"N 120°39'47.8"E	84 ft. ASL	13-09-24	Mango log
OCBu	Sta Ines, San Miguel, Bulacan	15°09'50.4"N 121°01'15.2"E	130 ft. ASL	17-10-24	Mango log
OCPa	San Agustin, Magalang, Pampanga	15°12'58.9"N 120°41'31.5"E	104 ft. ASL	18-10-24	Mango log

ASL = Above sea level

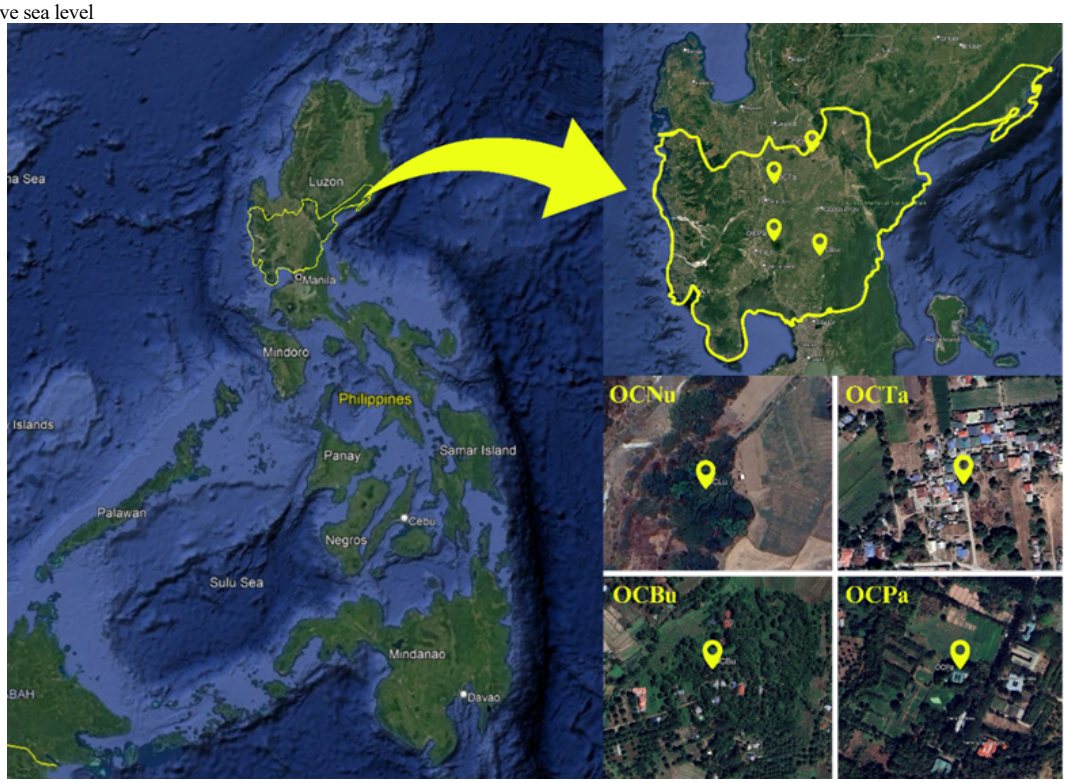

Fig. 1: The geographic locations of the four isolates of *O. canarii* in the Philippines. OCNu = Nueva Ecija isolate; OCTa = Tarlac isolate; OCBu = Bulacan isolate and OCPa = Pampanga isolate

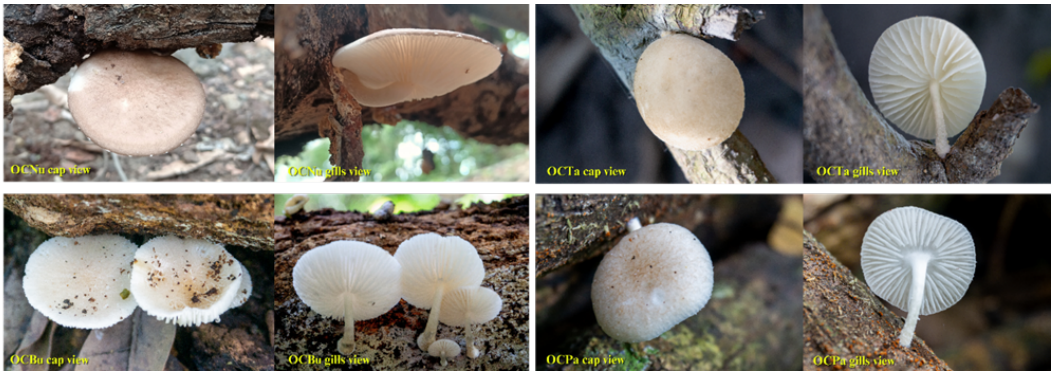

Fig. 2: The top and bottom view of the fruiting bodies of the four isolates of *O. canarii* growing in their natural habitat

Temperature and agitation evaluation

Mycelium biomass yield as affected by temperature and agitation were also determined. For the temperature, mycelial discs were inoculated in flask containing the most suitable medium and optimal pH and incubated at three temperature conditions for 10 days. Each condition was tested in triplicate. Mycelia were harvested, washed and oven-dried at 40°C for 3–5 days. For agitation, cultures were incubated in static, shaking at 100 rpm and 150 rpm at the previously identified favorable temperature. Harvesting and determining the yield of mycelia were the same in the preceding section.

Mass production and extraction of EPS and mycelia

Following the established requirements for submerged culture, 50 culture bottles were prepared for each *O. canarii* isolate to allow large-scale production of mycelia and EPS. After a 10-day incubation period, the mycelial mats were collected, air-dried and set aside for subsequent extraction. The residual broth from the culture was also recovered and used as the source of EPS. The protocol of EPS isolation was followed after El-Mahdy *et al.* (2023) and Diaz *et al.* (2025). The ethanolic extraction procedure of mycelia was followed after Dulay *et al.* (2014). The bioactivities of EPS and extract were assessed.

Anti-inflammatory assay

A stock solution (10,000 μg mL^{-1}) was prepared in DMSO and diluted to obtain a working concentration of 200 μg mL^{-1}. Using sonicator, centrifuge, and vortex mixer, the samples were homogenized. For assay preparation, a clean scintillation vial received 5184 μL of 100 m*M* Tris buffer (pH 8), followed by the addition of 96 μL of enzyme solution (250 U mL^{-1}). The enzyme-cofactor mixture was prepared by combining COX enzyme (human recombinant COX-2 and sheep COX-1) with 480 μL of 20 μ*M* hematin. To each test well preloaded with 50 μL buffer, 120 μL of the enzyme-cofactor solution was dispensed. Subsequently, 10 μL of sample solution or positive control (160 m*M* indomethacin) or negative control (DMSO at 5%) was added. Each treatment was tested in duplicate with two replicates (n = 2, t = 2). The mixture was incubated at 25°C for 15 min, after which 10 μL of Amplex Red (200 μ*M*) and 10 μL of arachidonic acid (2000 μ*M*) were introduced. The reaction was mixed, purged with nitrogen and monitored for 2 min using a CLARIOstar plate reader. Fluorescence was recorded at an excitation wavelength of 535 nm and emission wavelength of 590 nm with measurements taken every 12 sec.

Anti-oxidant assay

Following the protocol of Kolak *et al.* (2006), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined with modifications. One mL of each sample and ascorbic acid at 1000 μg mL^{-1} was prepared and separately mixed with 4 mL of 0.1 m*M* DPPH solution and incubated at 37°C for 30 min in the dark condition. After incubation, the colorimetric absorbance at 517 nm was read using UV-VIS spectrophotometer. The radical scavenging activity percentage was computed.

Anti-diabetic assay

The anti-diabetic activity of the mycelial extracts was evaluated using α-glucosidase inhibition assay which depends on the enzymatic hydrolysis of the substrate to release p-nitrophenol, corresponds with sample's activity in comparison to ethanol as negative control. The p-nitrophenyl-α-D-glucopyranoside adjust at 1.86 m*M* concentration as stock substrate. The enzyme glucosidase adjusted at 120 mU mL^{-1} concentration were prepared as stock enzyme solution. Samples at 100 μg mL^{-1} final concentration were also prepared. The samples were homogenized in dimethyl sulfoxide (DMSO) using vortex mixer, followed by centrifugation. In separate wells of a 96-well microtiter plate, 190 μL of 50 m*M* sodium phosphate buffer solution (pH 6.8) containing NaCl (0.1 *M*) was mixed with 10 μL sample to make a sample concentration of 15 μg mL^{-1}. This was followed by the addition of 50 μL of the enzyme solution to the mixture, which was incubated for 10 min at 37°C. After which, 50 μL of the substrate was added, making a total volume of 300 μL in each well. Absorbance of the liberated p-nitrophenol was measured every 30 sec for 30 min at 405 nm using UV-VIS Spectrophotometer. The inhibitory activity of the samples and the positive control was recorded in percentage.

Statistical analysis

The optimization experiment followed a one-factor-at-a-time approach. Treatments were arranged in completely randomized design and analyzed using ANOVA in GraphPad Prism. Mean values were compared using Tukey's HSD at 5% significance level.

Results

Effect of fruit-based liquid culture media

The effects of varying concentrations of three fruit-based liquid media including puree of Saba banana and Indian mango, and coconut water on the yield of mycelial biomass of four *O. canarii* isolates namely, OCNu, OCTa, OCBu, and OCPa, were evaluated in this study. Mycelial biomass yields of the four *O. canarii* isolates grown in the different liquid media for 10 days is presented in Fig. 3. Among the tested fruit-based media, 50% of Saba banana puree produced the highest mycelial biomass yield for all the isolates, except for the OCTa, which yielded the highest mycelial biomass in 40% of Saba banana puree. Although 40% of Saba banana puree yielded the second highest biomass of three isolates, this was not significantly different from those of 50% of Saba banana puree ($P > 0.05$) in OCNu and OCPa, but found significantly different in OCBu ($P \leq 0.05$). In Indian mango puree, 40% and 50% did not show significant difference ($P > 0.05$) in both OCNu and OCTa. However, 40% mango puree resulted in a higher biomass yield for OCBu, whereas 50% was optimal for OCPa ($P \leq 0.05$). Coconut water-based media consistently resulted in the lowest mycelial biomass production across all *O. canarii* isolates. Among all concentrations tested, 10% fruit-based media formulations produced the lowest biomass yields, regardless of the fruit type or isolate.

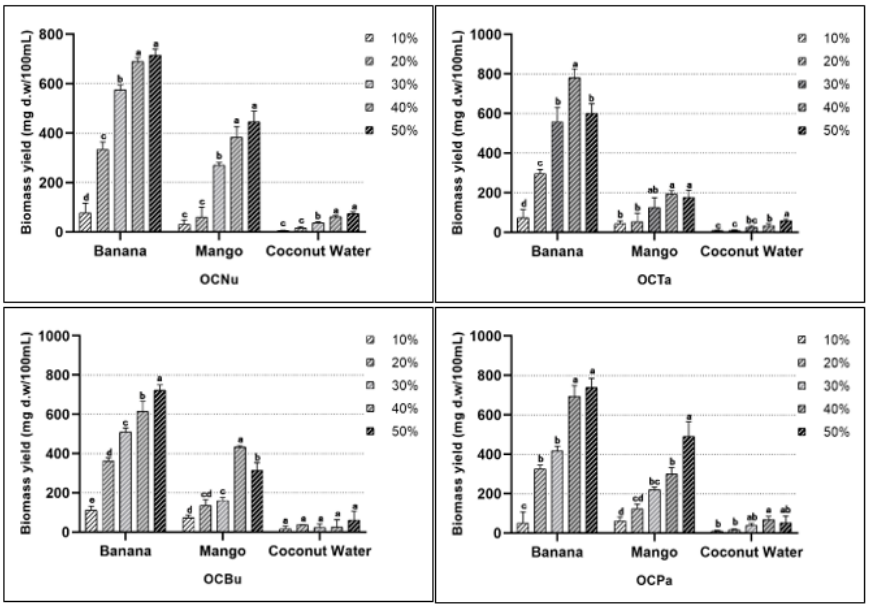

Fig. 3: Mycelial biomass yield of the four isolates of *O. canarii* as affected by the varying concentrations of fruit-based liquid culture media after 10 days of incubation at 28°C. Values within a column that share the same superscript letter do not differ significantly based on Tukey's HSD test at the 5% level

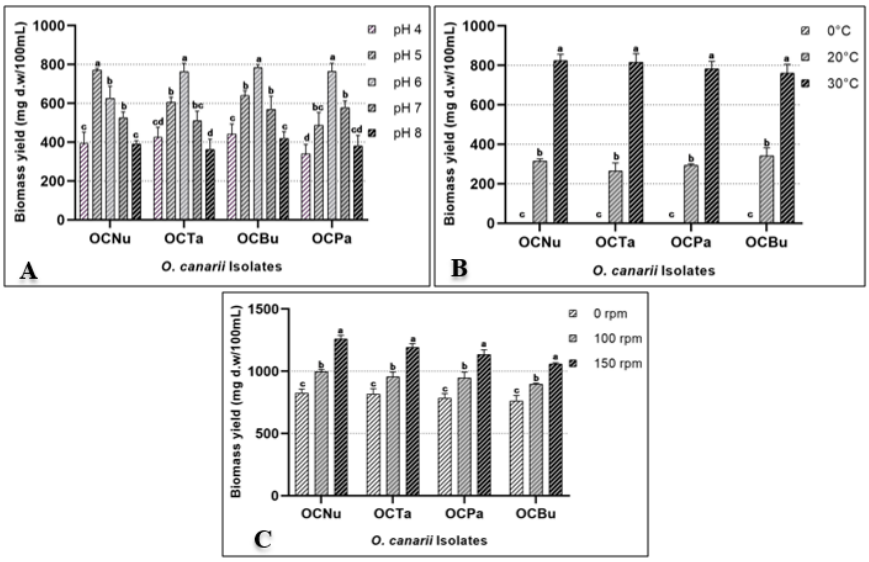

Fig. 4: Mycelial biomass yield of the four isolates of *O. canarii* as affected by the (**A**) varying pH levels of the best medium, (**B**) temperature and (**C**) agitation conditions after 10 days of incubation. Values within a column that share the same superscript letter do not differ significantly based on Tukey's HSD test at the 5% level

Effect of pH, temperature and agitation

In this study, 50% (for OCNu, OCBu and OCPa) and 40% (for OCTa) of Saba banana puree were adjusted to varying levels of pH. The effects of pH on the biomass yields of the four mushroom isolates in 10 days of incubation are shown in Fig. 4A. Saba banana puree with an initial pH of 6 recorded the significantly highest biomass yields for all evaluated *O. canarii* isolates, except for the OCNu, which registered the significantly highest yield in Saba banana puree with initial pH of 5 ($P \leq 0.05$). In contrast, lowest yield of mycelial biomass was observed at pH 4 and 8 for all evaluated isolates.

To determine the effect of temperature, culture bottles containing Saba banana puree at its previously determined optimal concentration and pH were prepared, inoculated with mycelial discs of mushroom isolates, and incubated under three temperature conditions. The resulting biomass yields of the four *O. canarii* isolates are presented in Fig. 4B. All tested isolates exhibited the significantly highest yield of mycelial

Table 2: COX-1 and COX-2 inhibition activity of mycelial extract and EPS of four isolates of *O. canarii*

Mushroom Isolate	Cyclooxygenase-1 Inhibition (%)		Cyclooxygenase-2 Inhibition (%)	
	Mycelial Extract	Exopolysaccharide	Mycelial Extract	Exopolysaccharide
OCNu	72.70 ± 1.33^{b}	39.69 ± 6.67^{c}	70.72 ± 4.56^{b}	49.96 ± 5.02^{b}
OCTa	22.51 ± 7.25^{d}	49.67 ± 2.83^{b}	22.69 ± 8.13^{d}	43.93 ± 5.96^{b}
OCBu	41.59 ± 7.43^{c}	30.80 ± 12.60^{c}	39.94 ± 2.91^{c}	29.51 ± 11.26^{c}
OCPa	26.31 ± 6.62^{d}	30.01 ± 15.05^{c}	25.54 ± 7.26^{d}	22.95 ± 9.21^{c}
Indomethacin	89.94 ± 4.35^{a}		90.55 ± 1.77^{a}	

Values are expressed as mean ± SD. Values within a column that share the same superscript letter do not differ significantly based on Tukey's HSD test at the 5% level. Ethanolic extracts and EPSs at 10 μg mL^{-1} and indomethacin (positive control) at 2,862 μg mL^{-1} were assayed in two independent experiments and two replicate tests

Table 3: DPPH radical scavenging activity of mycelial extract and EPS of four isolates of *O. canarii*

Mushroom Isolate	DPPH Radical Scavenging (%)	
	Mycelial Extract	Exopolysaccharide
OCNu	60.87 ± 2.46^{b}	59.95 ± 3.18^{d}
OCTa	63.31 ± 3.69^{b}	74.09 ± 3.46^{bc}
OCBu	59.02 ± 5.89^{b}	70.69 ± 5.54^{c}
OCPa	60.47 ± 7.05^{b}	62.51 ± 7.81^{d}
Ascorbic acid	92.17 ± 0.35^{a}	

Values are expressed as mean ± SD. Values within a column that share the same superscript letter do not differ significantly based on Tukey's HSD test at the 5% level. Ethanolic extracts and EPSs at 1000 μg mL^{-1} and ascorbic acid (positive control) at 1000 μg mL^{-1} were assayed in two independent experiments and triplicate tests

Table 4: Inhibition of α-glucosidase by mycelial extract and EPS of four *O. canarii* isolates

Mushroom Isolate	α-glucosidase Inhibition (%)	
	Mycelial Extract	Exopolysaccharide
OCNu	2.21 ± 4.33^{b}	2.57 ± 3.17^{c}
OCTa	1.82 ± 2.13^{b}	4.15 ± 1.98^{bc}
OCBu	0.00 ± 0.00^{b}	4.43 ± 4.65^{bc}
OCPa	0.00 ± 0.00^{b}	5.28 ± 1.92^{b}
Acarbose	89.82 ± 0.89^{a}	

Values are expressed as mean ± SD. Values within a column that share the same superscript letter do not differ significantly based on Tukey's HSD test at the 5% level. Ethanolic extracts and EPSs at 10 µg mL^{-1} and acarbose (positivie control) at 1000 μg mL^{-1} were assayed in two independent experiments and two replicate tests

biomass at 30°C compared to the other temperature treatments ($P \leq 0.05$), indicating this as the most favorable temperature for growth. In contrast, no mycelial growth was observed at 0°C, confirming that such low temperature completely inhibits the growth of *O. canarii* mycelia.

The influence of static and agitation (speed of 100 and 150 rpm) on the mycelial production of four *O. canarii* isolates was also investigated. As shown in Fig. 4C, all evaluated mushroom isolates yielded the significantly highest mycelial biomass when cultured in 150 rpm agitated condition ($P \leq 0.05$). This was followed by those grown in 100 rpm agitated condition. Contrastingly, the lowest yield was significantly recorded in static cultures.

Anti-inflammatory activity

The anti-inflammatory potentials of the *O. canarii* extracts and EPSs were evaluated through cyclooxygenase inhibition assay and the percentage inhibitory activities are summarized in Table 2. Among the mycelial extracts, OCNu exhibited the highest inhibitory activity ($P \leq 0.05$) and was the only extract to exceed 50% inhibition against both COX-1 and COX-2. Conversely, OCTa mycelial extract displayed the lowest activity, which was statistically comparable to that of OCPa in both assays. With respect to EPS, OCTa showed the highest inhibitory activity ($P \leq 0.05$) against COX-1, whereas OCNu recorded the highest inhibition of COX-2, although not significantly different from OCTa. The lowest inhibitory activity of EPS against COX-1 and COX-2 was recorded in OCPa, followed by OCBu, which did not differ significantly.

Anti-oxidant activity

The abilities of the mycelial ethanolic extracts and EPSs of *O. canarii* isolates to scavenge DPPH radicals were assessed in this study. The percentage DPPH radical scavenging activities of the tested samples are shown in Table 3. All mycelial extracts exhibited DPPH scavenging activities ranging from 59.02% to 63.31% ($P > 0.05$). For EPS, OCTa demonstrated the highest scavenging activity, although statistically comparable to OCBu ($P > 0.05$). The lowest scavenging activity was recorded in OCNu, which did not differ significantly from OCPa.

Anti-diabetic activity

The α-glucosidase inhibition abilities of the *O. canarii* extracts and EPSs were assessed to determine their anti-diabetic potential (Table 4). All mycelial extracts tested at 10 μg mL^{-1} did not show significant inhibitory activity against α-glucosidase. However, among the EPSs, OCPa demonstrated the highest α-glucosidase inhibitory activity, which did not differ significantly from OCTa and OCBu (*P*

> *0.05*). Nevertheless, the inhibitory activities of all EPS samples were significantly far lower than that of acarbose.

Discussion

The culture media serves as the primary source of different essential nutrients that are required for the growth and development of mushrooms. In this study, we evaluated three fruit-based liquid culture media prepared at varying concentrations in order to determine the most suitable medium in maximizing the yield of the biomass of the four different isolates of *Oudemansiella canarii* under submerged fermentation conditions. The Saba banana puree at 40% and 50% concentrations produced the highest mycelial biomass among all the tested media in all of the four *O. canarii* isolates. Similarly, the 30% concentration of Saba banana puree was reportedly to yield the highest mycelial biomass of *Agrocybe cylindracea* and *Pleurotus djamor* (Diaz *et al*. 2025). Moreover, Silva *et al.* (2018) reported that the saba sucrose gulaman (SSG) supported the highest mycelial growth of *P. djamor* compared to other three tested varieties of banana. Aside from cultivation of mushroom, the banana puree has also been demonstrated to be an affective medium for lactic acid fermentation, supporting the development of probiotic products using *Lactobacillus paracasei* CBA L74 as the starter culture (Gallo *et al.* 2021).

The suitability of the banana as a culture medium is attributable to its essential nutrients that it contains as it promotes the growth and development of mycelia. The USDA Food Data Central (2020) reported that for 100 g of ripe bananas, it contains carbohydrates (21.2 g), sugars (15.8 g), dietary fiber (1.7 g), protein (0.74 g), ash (0.7 g), fat (0.29 g), nitrogen (0.12 g), organic acids, minerals and vitamins. The nutrient compositions of banana are essential in the physiology, morphogenesis, growth, and development of mushrooms. Nitrogen is important in enhancing and accelerating growth of mycelia and also in the subsequent differentiation of mycelium into fruiting bodies (Shelonik 2024). Organic acids are key metabolites of the tricarboxylic acid (TCA) cycle and contribute to the growth and development of mycelium through their metabolic and regulatory roles (Patyshakuliyeva *et al.* 2013). Potassium is the most abundant mineral composition of banana. Potassium regulates osmotic balance, maintains cellular hydration, and activates enzymes involved in protein and carbohydrate metabolism, thereby promoting vigorous mycelial expansion (Kalač 2010; Sun *et al.* 2022). Vitamin C is the predominant vitamin found in banana, acts as an antioxidant, protecting fungal cells from oxidative stress during rapid metabolic activity and supporting the synthesis of certain biomolecules essential for cell growth (Soh *et al.* 2021). Overall, these nutrients are naturally present in the banana for the growth medium is indeed necessary for supporting or supplementing the growth and development of mycelia of the four isolates of *O. canarii*.

Another growth factor for mycelia and its physiology is the pH, as it influences the medium ionic balance, modulates the hyphal structure and morphology, regulates the important physiological activities of fungal cells, and impacts both the assimilation of nutrients and its production of secondary metabolites (Deacon 2006). In our study, three isolates were observed to have the best performance at pH 6 and one at pH 5. Likewise, in our previous work, the culture medium that has pH 5 and 6 showed the highest biomass production among the 14 different *Lentinus* isolates that belongs to the six species (Dulay *et al.* 2021). Additionally, this preference of mycelia in slightly acidic medium are also observed in other mushrooms such as *Ganoderma lucidum*, *Volvariella volvacea*, and *Pleurotus flabellatus* (Dulay *et al.* 2015; Mohamad *et al.* 2015; Agudelo-Escobar *et al.* 2017).

The four *O. canarii* isolates grew most actively at 30°C, which suggests that they behave as tropical mushrooms. Similar trends were observed by Dulay *et al.* (2015, 2016) and Kupradit *et al.* (2020) in other Philippine mushrooms such as *P. ostreatus*, *G. lucidum*, *C. cinerea* and *V. volvacea* wherein 30°C seems to be connected to their adaptation to the tropical climate where these fungi are commonly found. Temperature is also a regulator of secondary metabolism. For instance, the study of Jiang *et al.* (2018) demonstrated a controlled temperature shifting during the incubation of *Phellinus baumii*, wherein the incubation temperature was gradually reduced continuously from 28°C to 24°C for 43-90 h of submerged fermentation, and the results shows that it significantly enhanced the flavonoid biosynthesis in *P. baumii*. These exhibit the importance of temperature in mushroom physiology, where not just affect the biomass accumulation but also the synthesis of bioactive compounds.

In submerged cultivation, agitation enhances the availability of dissolved oxygen, promotes uniform nutrient distribution, and sustains the concentration gradient between the intracellular and extracellular environments (Elisashvili 2012; Dulay *et al.* 2015). In this study, we demonstrated that the highest biomass yield was obtained in shake-flask culture at 150 rpm, suggesting that agitation is an important factor in the mycelial biomass production of the four isolates of *O. canarii*. Similarly, shake-flask at 70 rpm, 100 rpm, 124 rpm and 180 rpm cultures yielded the highest mycelial biomass production of *C. cinerea*, *G. lucidum*, *L. squarrosulus* LSQBot, *P. ostreatus* and *P. albidus*, respectively (Wahyudi *et al.* 2015; Dulay *et al.* 2015, 2016, 2021; Kirsch *et al.* 2016). Contrastingly, mycelial cultures of *L. swartzii* BIL4618, *L. sajor-caju* C005, *L. sajor-caju* LSCBot, *L. squarrosulus* LSQOs, *L. tigrinus* BP32 and *L. strigosus* BIL1324 significantly produced higher biomass yields in static-flask culture (Dulay *et al.* 2021). Using the optimal conditions, mycelia and EPS were mass-produced, extracted, and assayed.

Cyclooxygenase-1 and 2 are proteins strongly linked with inflammation, pain, and tumor progression (Hossen *et al.* 2021). In present study, we found that OCNu isolate showed the strongest inhibitory response and was the only

sample to exceed 50% inhibition for both COX-1 and COX-2. This suggests the activity may be due to fungal metabolites that naturally inhibit COX enzymes. Compounds such as ergosterol and ergosterol peroxide are abundant in higher fungi and have been associated with the downregulation of COX-2 activity (Rangsinth *et al.* 2023). For instance, ergosterol from *Scleroderma polyrhizum* was shown to reduce LPS-induced lung injury in mice by lowering COX-2 expression (Zhang *et al.* 2015). A fraction rich in ergosterol from *Cordyceps militaris* also displayed neuroprotective effects, as it decreased nitric oxide production in LPS-stimulated BV2 microglial cells (Nallathamby *et al.* 2015). Other than ergosterol, several classes of metabolites have also been recognized as natural COX-2 inhibitors. These include phenols, terpenoids, flavonoids, alkaloids, stilbenes, and quinones (Cui and Jia 2021). We also found that the EPS derived from the OCTa isolate exhibited the strongest inhibition of COX-1, whereas the EPS from the OCNu isolate showed the highest inhibition of COX-2. Fungal EPSs are naturally secreted biopolymers with diverse structural and functional characteristics, making them promising candidates for industrial applications and medical innovation. Several mushroom β-glucans, such as lentinan, grifolan, scleroglucan and schizophyllan have also been reported to inhibit colon cancer by inducing anti-inflammatory cytokines, activating leukocytes, and suppressing metastasis through modulation of tumor growth (Moniruzzaman *et al.* 2025).

Mushrooms are well recognized as rich sources of antioxidants, which play a crucial role in protecting human cells from oxidative damage linked to chronic diseases such as cancer and cardiovascular disorders by scavenging free radicals (Du *et al.* 2018). The mycelial extract and EPS of the four *O. canarii* isolates both showed antioxidant activity, with DPPH scavenging greater than 50%. Such effects appear connected to secondary metabolites. Compounds including polysaccharides, phenols, tocopherols, flavonoids, glycosides and organic acids have often been described as contributors to antioxidant capacity (Zade *et al.* 2025). Acharya *et al.* (2019) demonstrated that *O. canarii* basidiocarps reported an EC_{50} of 0.912 μg in the DPPH assay, a total antioxidant capacity of 15.33 μg ascorbic acid equivalent per mg extract, and an ABTS value of 12.91 μM Trolox equivalent per mg extract. Our results, together with the previous works, suggest that mushrooms are valuable candidates for natural antioxidants.

Numerous mushrooms have been reported for their anti-diabetic activity (Shamim *et al.* 2023). At the tested concentration, we found out that none of the mycelial extracts exhibited significant inhibitory activity whereas all EPSs showed very low inhibitory activities against α-glucosidase. The relatively low activity of *O. canarii* extracts and EPSs may be attributed to their structural characteristics and compositional profiles. The α-glucosidase inhibitory effect of mushroom-derived polysaccharides has been reported to depend on factors such as monosaccharide composition, molecular weight, higher structure, and type and location of glycosidic bond in order to bind effectively with the active site of α-glucosidase (Ji *et al.* 2023). Another possible explanation is that the compounds responsible for α-glucosidase inhibition may not be predominantly extracted in ethanol. Additionally, the concentration tested in our study may not have been sufficient to elicit strong α-glucosidase inhibition. Further fractionation using various solvents for mycelia, structural characterization of EPSs and evaluation of varying concentration would be essential to elucidate the α-glucosidase inhibition activity of *O. canarii*.

Conclusion

This study demonstrated that fruit-based liquid media, particularly 50% Saba banana puree, at pH 5–6, 30°C and agitation at 150 rpm significantly enhanced the mycelial biomass production of *O. canarii* isolates. Beyond biomass production, the mycelial extract and EPS of *O. canarii* isolates exhibited promising bioactivities, including anti-inflammatory effects through COX enzyme inhibition, antioxidant activity *via* DPPH radical scavenging, and very low α-glucosidase inhibitory activity, suggesting potential applications in managing oxidative stress and inflammation. These findings highlight the potential of *O. canarii* as a valuable source of functional food ingredients or nutraceuticals while also presenting fruit-based substrates as cost-effective media for large-scale cultivation. To fully realize its applications, further studies should focus on isolating and characterizing the active compounds, validating bioactivities through *in-vivo* models, optimizing large-scale fermentation systems and exploring additional therapeutic properties such as anticancer and immunomodulatory activities.

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Author Contributions

JARN conducted the experiments, performed data collection and initial analysis. RMRD supervised the study, assisted in data interpretation, and contributed substantially to manuscript writing and revision. SPK and AMDL provided guidance during experimental design and valuable comments during manuscript preparation. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Ethical clearance for this study was granted by the CLSU Ethics Review Committee.

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