Media optimization of antimicrobial activity production and beta-glucan content of endophytic fungi *Xylaria* sp. BCC 1067

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**A B S T R A C T**

Fungi is a notable asset for drug discovery and production of pharmaceuticals; however, slow growth and poor product yields have hindered industrial utilization. Here, the mycelial biomass of *Xylaria* sp. BCC 1067 was examined in parallel with the assessment of antimicrobial properties by using media-type selection. To enhance both mycelial content and antifungal activity, the media replacement approach was successfully applied to stimulate fungal growth and successively switched to poorer malt-peptone extract media for metabolite production. This simple optimization reduced fungal cultivation time by 7 days and yielded 4-fold increased mycelial mass (32.59 g/L), with approximately 3-fold increased antifungal activity against the model yeast *Saccharomyces cerevisiae* strain. A high level of β-glucan (115.84 mg/g of cell dry weight) and additive antibacterial effect against *Propionibacterium acnes* were also reported. This simple strategy of culture media optimization allows for investigation of novel and rich source of health-promoting substances for effective microbial utilization.

1. Introduction

Microbes, especially fungi, have been perceived as a huge unexploited source of potentially incredible novel pharmaceutical products for the advancement of medication and nutraceuticals [1]. Their ability to produce a wide variety of organic compounds, notably penicillin, lovastatin, and other medicines, has drawn much attention from researchers and companies concerning bioprospecting for potential bioactive compounds in fungi [2]. Among others, beta-glucans are the most commonly found polysaccharides that have gained significant attention due to their therapeutic importance as an immunomodulator for the treatments of several diseases including cancers and health promotion in gastrointestinal diseases by lowering cholesterol and immune reactions [3].

Glucans are significant constituents, and thus a trademark, of fungal cell walls and are considered to be health-promoting compounds. In general, beta-glucans make up 50–60% of the fungal cell dry weight [4]; in fact, they are one of the key characteristics of fungi used in therapeutics, cosmetics, and the food industry [5]. Several studies have reported that beta-glucans also have various antitumor properties and may impact on the balance of macrophage activity [6].

In addition to beta-glucan discovery, endophytic fungal species are also well-known and promising sources for the development of novel drugs and the treatment of infectious and non-infectious diseases [7]. *Xylariales* is largely perceived as one of the most diversified and largest groups of Ascomycota, widely distributed in various regions of the world, with broad environmental diversity [8]. By the end of the year 2020, the Index Fungorum has recorded over 800 epithets of *Xylaria* [9]. *Xylaria* species are generally found in nature as endophytes or as saprophytes. They grow on a variety of substrates, including decaying and dead wood, as well as nests of termites and ants [11]. Fungal *Xylaria* creates a good platform to investigate the potential of bioactive metabolites for drug discovery, and are also an exceptionally productive source of polyketides, cytochalasins, terpenoids, succinic acid derivatives, etc. [12, 13]. A study conducted on the fruiting body fraction of *Xylaria curta* indicated that this species is capable of inhibiting the growth of human infected fungi and A-549 cancer cells [14]. Moreover, *Xylaria* sp. Acra L38 endophyte is found to produce Zifomarin, with strong antifungal activity against *Candida albicans* [15]. In addition, *Xylaria* sp. produced antimicrobial helvolic acid, which was active...
against the gram-positive microbes *Bacillus subtilis* and methicillin-safe *S. aureus* (MRSA), with MIC of 2 and 4 μg/mL, respectively [16].

Invasive fungal infections are a common cause of the increase in mortality rate. Indeed, even with the use of best-in-class symptomatic testing and antifungal treatments, mortality remains high due to persistent threats including drug resistance, and the emergence of new fungal pathogens. Among other species, *Xylaria* sp. BCC 1067 is the first to be identified as producing an antifungal agent [17] and was later shown to have antifungal activity against *S. cerevisiae*, with good synergy with the clinical antifungal agent azoles [18]. Induced intracellular ROS levels are shown following treatment with *Xylaria* extract, suggesting a fungicidal mechanism of a medicinal fungus [18]. The poly-ketide 19,20-epoxycholesalogen Q (ECQ) is then identified as one of the bioactive compounds present in *Xylaria* sp. BCC 1067 extract drives the disruption of actin depolymerization [19] and the induction of intracellular ROS, leading to cell death [20].

Despite its high potential as a platform to uncover more promising novel antifungals and bioactive agents, little is known regarding the bioactivity of *Xylaria* sp. BCC 1067 mycelial fraction. One of the key obstacles to fungal exploration for many years is the difficulty of finding a suitable cultivation process and the tedious purification. For these reasons, most urgent research such as antibiotics has been put off despite the global need for new and more effective antimicrobial and antifungal agents. In fact, like many fungi, *Xylaria* cultivation requires a relatively lengthy time, and a low yield of mycelial content and extract are often encountered, hindering its potential applications. Previously, the optimization of its culture condition has been investigated in various ways to increase mycelial biomass and metabolite production [, 22]. Some general-purpose media are commonly used in fungal culture, namely Sabouraud’s or potato dextrose media, which are considered nutrient-rich media. Several studies have revealed the importance of sucrose, which is the main carbon in yeast extract sucrose (YES), to maximize the growth yield better than the use of glucose in the cultivation medium [53]. Malt-peptone extract (MEB) has been used as a poorer nutrient source to grow the *Xylaria* sp. in various studies. For example, Ibrahim et al. discovered natural compounds from *Xylaria* sp., including new diplosporin and agistatine derivatives [24]. Others have also investigated the presence of antifungal and antifungal agents in the extract of *Xylaria* sp. BCC 1067 with MEB as the carbon source [18, 25]. Potato dextrose, malt extract, Czapek’s (CZA), and Sabouraud’s (SB) have been used previously to optimize the culture conditions of *Xylaria* sp. So11, with Sabouraud’s giving the highest metabolite production [22].

Apart from nutritional factors, environmental factors such as light condition, incubation status, pH of media, and temperature may also impact the growth and production of fungal metabolites [, 27]. When using a light pulse signal or growing under illumination, fungi initiate considerable changes in cell metabolism [54]. Different light conditions have affected the fungal growth and the biosynthesis of xylotetral B in *Xylaria* sp. 2508 [29]. The most common medium optimization strategy used in most studies is the one factor at a time (OFAT) strategy, which tracks down the ideal arrangement while keeping different variables at a consistent level [30]; however, this method is tedious and time-consuming. In comparison with the cultural fraction, fewer studies are reported on the optimization of the mycelial fraction of *Xylaria* sp. BCC 1067 and its biological activity. Thus, the effects of media type and light were evaluated here on the mycelial content as well as the bioactivity of the mycelial fraction. The model yeast *S. cerevisiae* mutants Δ*Δpdr5* and Δ*Δerg6* strains which lacks a key drug transporter and drug-targeted enzyme involved in the biosynthesis of plasma membrane component ergosterol, respectively, and *Propionibacterium acnes* were used for antimicrobial activity of *Xylaria* mycelial fraction. The glucan content was also examined to determine the broad-spectrum medicinal properties of *Xylaria* mycelial extract.

### 2. Materials and methods

#### 2.1. Fungus, yeast, and bacteria strains

The fungal strain *Xylaria* sp. BCC 1067 was obtained from the BIO-TEC Culture Collection (BCC culture 620032292; National Science and Technology Department Agency, Bangkok, Thailand). *S. cerevisiae* BY4742 (WT, Δpdr5 & Δerg6 strains) was used as an indicator strain for antifungal assays (Open Biosystems). *P. acnes* was purchased from the Department of Medical Sciences, Ministry of Public Health, Thailand.

#### 2.2. Determination of glucan content in the mycelia of *Xylaria* sp. BCC 1067

Mycelium samples were maintained with MEB media; the flasks were incubated at 25°C for 28 days. The mycelia were harvested by drying at 42°C for 24 to 36 h. The total glucan content, including α- and β-glucan content, in the mycelium of *Xylaria* BCC 1067 samples were determined using a mushroom and yeast β-glucan kit (K-YBGL - Megazyme International, Wicklow, Ireland) [31]. The glucan analyzing kit consists of exo-1,3-β-glucanase, β-glucosidase, amyloglucosidase invertase, and glucose determination reagent (including 4-amino antipyrine and glucose oxidase peroxidase), and glucose standard solution. The control yeast β-glucan samples were also included. The glucan calculations were performed using megazyme Mega-Calc™, which is available at the megazyme website ‘www.megazyme.com’. All glucan contents were expressed as a percentage (w/w) of mycelial dry weight.

#### 2.3. Media selection for optimal growth and antifungal activity

The basal media for optimal growth and antifungal activity consisted of MEB containing malt extract (12.75 g/L), and peptone (2.25 g/L); potato dextrose broth (PDB) containing potato extract + dextrose (29.0 g/L); Sabouroud’s broth (SB) containing dextrose (20.0 g/L), and peptone (10.0 g/L), YES containing yeast extract (20.0 g/L), sucrose (150 g/L), and anhydrous magnesium sulfate (0.5 g/L); Czapek’s broth (CZA) containing sucrose (30.0 g/L), magnesium sulfate (0.5 g/L), sodium nitrate (2.0 g/L), dipotassium phosphate (1.0 g/L), and ferrous sulfate (0.1 g/L), which is modified to Czapek’s yeast extract broth by adding yeast extract (5.0 g/L). *Xylaria* sp. BCC 1067 was cultivated according to the technique by [25], with certain alterations to select the best growth medium. It was first grown on solid media with 2% (w/v) agar with each medium mentioned above, for 7 days, and then transferred aseptically to a 250 mL Erlenmeyer flask containing 50 mL of basal liquid medium. The flasks were incubated at 25°C for 28 days at a steady state, with shaking at 150 rpm. Light and dark conditions were selected as two variable factors for growth optimization.

#### 2.4. Growth optimization via the medium replacement approach

According to the preliminary media selection studies, higher biomass was obtained from YES, while the highest activity was observed using MEB under dark conditions. Given the interesting fungicidal property of mycelial extract from *Xylaria* sp. BCC 1067, a media replacement approach was applied to scale up the mycelium biomass and the production of the crude extract. Starter cultures were maintained in YES broth. Mycelia were cut into small pieces and transferred aseptically to 250 mL Erlenmeyer flasks containing 50 mL of basal liquid broth. Flasks were then incubated at 25°C for 7 days at a steady state under dark conditions. After 7 days, the YES broth was completely removed from the Erlenmeyer flasks and replaced with MEB medium to achieve high antifungal activity. *Xylaria* sp. BCC1067 cultures were incubated for 21 days. The same culture procedures were applied for data acquisition in terms of growth and antifungal activity. The growth of *Xylaria* sp. BCC 1067 mycelium was determined using two independent experiments, each performed in triplicate.
2.5. Determination of mycelial dry weight

The dry weight of mycelial biomass was taken into account to measure mycelial growth. Mycelia were harvested by filtration using a Buchner funnel, using Whatman™ no. 1 filter papers, and dried at 42 °C for 24 to 36 h, until the samples achieved constant weight. To determine the mycelial growth of the Xylaria species after 28 days, the Xylaria growth was determined in terms of the dry weight under light and dark conditions. In each step, three replicates were weighted to calculate the mean and SD.

2.6. Mycelial extraction

Prior to the mycelial extraction process, the dried mycelia were ground to a fine powder. The mycelial fraction was then treated with ethyl acetate in a 1:2 ratio (v/v) (EtOAc, QREC, New Zealand). Ethyl acetate was selected because of its chemical and biological properties, such as minimal cell toxicity, biphasic action, and polarity. Each collected ethyl acetate fraction was treated with anhydrous magnesium sulfate to remove water and was then filtered (Whatman™ no. 1 filter paper). The filtered fractions were subjected to evaporation using a rotary vacuum evaporator (BUCHI, Thailand Ltd.), adjusting the temperature to 40 °C and the pressure to 100 bar. The dry extracts were stored below 4 °C and dissolved in 5% (v/v) methanol prior to use, as this methanol concentration does not affect the growth of yeast cells.

2.7. Antifungal assay

The antifungal activity test was conducted against the model yeast S. cerevisiae strains WT (MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0, Δpdr5 (BY4742 (MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δpdr5:kanMX4)), and Δpdr6 (BY4742 (MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δpdr6:kanMX4)). The minimal inhibitory concentrations (MICs) of the Xylaria sp. BCC 1067 extract was determined according to the microdilution reference method from the National Committee for Clinical Laboratory Standards, with some modification [32]. Based on the reference method for measuring the MIC values, twofold mycelial extract dilution steps were carried out using a microdilution tray. A standard culture media (YPD) (HIMEDIA, India) for yeast strains was used for the determination of MIC values. The yeast strains were grown overnight in YPD at 30 °C and 150 rpm, and the cultures were adjusted to start the OD600 at 0.1. Cell cultures were subjected to incubation for 5–6 h to obtain an OD600 of 0.6–0.8, and the cell cultures were then diluted to obtain an OD600 of 0.001. Then, a two-fold dilution of the extract was prepared in flat bottomed 96 well plates, at a volume of 100 µL of the extract, and 100 µL of cell culture was then added (Corning® Costar®, SigmaAldrich, China). The highest concentration used was 2000 mg/L of Xylaria extract. The 96 well plates were incubated at 30 °C 150 rpm for 24 h, following the OD600 values were detected. For the determination of colony-forming units (CFU), 100 µL of cell culture of each well obtained from the same plates used in the MIC analysis plates was subjected to 10-fold dilution prior to spread on agar plates. The plates were incubated for 48 h at 30 °C for the determination of colony-forming units.

2.8. Disk diffusion assay

The antifungal disk diffusion assay was conducted according to CLSI M44-A2 guidelines with modifications [33]. According to the results obtained from the broth microdilution antifungal assay, selected mycelial extracts including MEB, YES, and the media replacement approach were used for the disk diffusion assay. Wild-type, Δpdr5 and Δpdr6 strains were grown on YPD agar plates. After, the cell density was adjusted to 1 × 10⁶ cells/ml. 100 µL of cell culture was plated on each agar plate before placing the disks. Different concentrations of Xylaria mycelial extracts were applied to the disks. The plates were incubated for 24 h at 30 °C. The images of cells were captured for determination of the zone of inhibition.

2.9. Antibacterial assay

Indicator strain P. acnes were cultured in BHI broth for 72 h at 37 °C and used for the antibacterial activity test of the mycelial fraction of Xylaria. Prior to examining the bacterial susceptibility to the mycelial extract, the bacterial density was adjusted to 7 × 10⁵ CFU/mL. The broth dilution method was used to determine the minimum bacterial concentration (MBC) values, and 2000 mg/L of the mycelial extract was used as the maximum concentration. To determine the OD₉₅₀ values, 100 µL of extract and 100 µL of cell culture were added to 96 well plates (Corning® Costar®, SigmaAldrich, China) and the OD₉₅₀ values of the cultures were monitored with an automated microplate reader (M9651; Metertech, Taipei, Taiwan) after 72 h exposure to Xylaria sp. BCC 1067 extract.

The checkerboard method was used to investigate combination tests and to elucidate the interaction between the mycelial extract and antibiotic drugs, including citric acid and salicylic acid, against bacterial cells (P. acnes). The effects of the combination are evaluated by calculating the fractional inhibitory concentration index (FICI) of each combination, by determination of the MIC value at 50% inhibition. Synergy was defined as a FICI lower than 0.5, while a FICI between 0.5 and 4.0 indicated that there was no interaction or an additive effect, and a FICI of more than 4.0 indicated that there was antagonism between the two agents [34].

2.10. Data acquisition

At least two independent studies, each performed in triplicate, were carried out in each experiment. The statistical data manipulation was performed by two-way ANOVA followed by Tukey’s multiple comparisons test using the SPSS program, and all the graphs were generated using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA; www.graphpad.com).

3. RESULTS

3.1. Determination of the β-glucan content of the Xylaria mycelial fraction

Few studies related to Xylaria sp. have reported on the beta-glucan; for example, Xylaria hypoxylon, which is the most studied and the second closest species to Xylaria BCC 1067, has been identified as a glucan source [35]. This study aimed to measure the β-glucan present in the mycelial fraction of Xylaria sp. BCC 1067, using controlled acid hydrolysis to estimate the total glucan content, while α-glucan was estimated using an enzymatic technique (AOAC method 996.11) [36], and the β-glucan content was determined by the difference in total and α-glucan contents. Using MEB media, the extracted mycelial mass of Xylaria sp. BCC 1067 was found to contain a total of 122.58 ± 1.33 mg/g of dry mycelial mass, while the beta and alpha glucan contents were measured as 115.84 ± 2.64 mg/g and 6.75 ± 2.13 mg/g, respectively (Table 1). To test the accuracy of the glucan assay, the glucan content of the control samples obtained in the assay were compared to the exact

| Glucan     | Control (mg/g of dry mycelium) | Xylaria sp. BCC 1067 (mg/g of dry mycelium) |
|------------|-------------------------------|--------------------------------------------|
|            | Mean  | SD   | Mean  | SD   |
| Total Glucan | 511.73 | 0.1  | 122.58 | 1.33 |
| Beta Glucan  | 505.42 | 0.3  | 115.84* | 2.64 |
| Alpha Glucan | 6.34  | 0.37 | 6.75*  | 2.13 |
content of the glucan present in the control samples provided by the Megazyme kit [36].

3.2. Effect of media type on mycelial yield and its antifungal activity

Culture optimization is a common approach to increase the production of metabolites and biomass of fungi. Since the cultivation media is a key determinant factor in this process, five common growth media were selectively used to grow Xylaria sp. BCC 1067, including MEB, SB, PDB, CZA, and YES. The YES media was highlighted as the best media to increase fungal biomass, shown by the highest amount of cell dry weight, with mycelial yields of 57.16 ± 3.32 g/L and 61.83 ± 1.59 g/L under both light and dark conditions, respectively (Table 2). A significant difference in the growth of Xylaria sp. could be observed (Fig. 1), with Sabouroud’s, PDB, and Czapek’s showing a moderate impact on the mycelial growth, which was approximately one-fourth of that produced using the YES media under both light and dark conditions (Table 2 and Fig. 1). Notably, MEB showed the least mycelial growth under both conditions, with yields of 6.81 ± 0.16 g/L and 8.14 ± 0.68 g/L, respectively (Table 2 and Fig. 1). The media type accounted for 98.7% of the total variance and was therefore considered to have a significant effect on the mycelial growth of Xylaria BCC 1067. Thus, different types of growth media appear to play a key role in mycelial and metabolite production, which also depends on the metabolites produced and the fungal strains. Likewise, medium optimization studies on Xylaria sp. 2508 also showed sucrose to be the best carbon source to maximize growth, however, the induction of bioactive metabolites was low compared to the other carbon sources [21].

3.3. Effect of light and dark on mycelial biomass and antifungal activity

Light and dark conditions were examined as a variable factor for growth and metabolic production although no significant effect was observed on the mycelial content of each cultivation. However, the two factors of growth medium and light conditions showed significant interaction, and the effect of one variable depended on the other variable for the growth and development of the mycelial extract. A better antifungal potential has been observed from the mycelial fraction using the dark culture condition. Under various light conditions, different metabolic pathways are activated or inactivated, which prompts the metabolism shifts, as shown by the fungal growth and the biosynthesis of antifungal xylotekal B in Xylaria sp. 2508 [29]. Here, the amount of crude extract from mycelial biomass was calculated as the amount of mycelial extract per one gram of mycelium. The highest crude extract production per one gram of mycelium was calculated as 0.034 ± 0.003 (g/g) using MEB media grown under dark conditions, followed by Czapek’s media grown under dark conditions, with a value of 0.032 ± 0.003 (g/g) (Table 2); however, these results showed no significant difference. Sabouroud’s, PDB, and Czapek’s showed a moderate crude production under both conditions, which did not show any significant difference, while YES medium accounted for the lowest crude production per 1 g of mycelial cells, with a value of 0.005 (g/g) under both light and dark conditions (Fig. 1). Nevertheless, the statistical analysis excluded the null hypothesis, resulting in an overall interaction between the two factors for the production of mycelial extract. Thus, the effect of the growth media interacts with the light condition during the production of mycelial extract.

3.4. Antifungal activity of the Xylaria mycelium fraction against S. cerevisiae

The pleotropic drug resistance or PDR is a conserved phenomenon found in both prokaryotes and eukaryotes. It leads to the failure of treatment and normally occurs via many mechanisms, including the overexpression of drug efflux pumps [37]. The contribution of the PDR system in conferring resistance to the mycelial extract was evaluated using a yeast strain lacking a key multidrug transporter gene PDR5 and the drug target enzyme ERG6. The antifungal activity of mycelial extracts of Xylaria sp. BCC 1067 grown with different media types, namely MEB, Sabouroud’s, PDB, Czapek’s, and YES, were examined and compared using the microdilution reference method [32].

To examine the antifungal potential of different extracts from each media type, the susceptibility of the mutant S. cerevisiae yeast strains Δpdr5 and Δerg6 were examined and compared to the parental BY4742 strain (wild-type, WT) since, in most cases, they are the targets of the antifungal drugs. The growth of mutant strains Δpdr5 and Δerg6 was normalized compared to the WT strain, at increasing concentrations of the mycelial extracts, ranging from 0 to 2000 mg/L (Fig. 2A). The highest antifungal activity against the WT S. cerevisiae strain was shown using MEB as a culture media under the dark condition, with a MIC50 of 510 mg/L (Table 3 and Fig. 2A). Mycelial extracts from Sabouroud’s medium under dark conditions showed the second-highest antifungal activity against S. cerevisiae WT, with a MIC50 of 1160 mg/L, and then the Czapek’s and PDB media showed MIC50 of 1300 mg/L and 1410 mg/L, respectively, under dark conditions (Table 3). However, the YES medium did not show much antifungal activity against S. cerevisiae WT, with a MIC50 of 2000 mg/L (Table 3 and Fig. 2A). The varying sensitivity of the mycelial extracts suggested that the media type had an effect on antifungal production. Considering the effect of light, Xylaria sp. grown in MEW under light showed the highest antifungal activity, with a MIC50 of 965 mg/L against S. cerevisiae WT, whereas the extract of Czapek’s media was observed to inhibit S. cerevisiae WT by 50% at a MIC50 of 1290 mg/L (Table 3 and Fig. 2A). The MIC50 of Sabouroud’s medium was found to be 1910 mg/L, while PDB and YES media showed little antifungal activity against the S. cerevisiae WT strain (Table 3 and Fig. 2A). Additionally, screening of colony-forming units showed the viable cells treated with different Xylaria mycelial extracts at different concentrations. The least viable cell count of S. cerevisiae WT strain was found using the MEB culture under dark conditions followed by MEB culture under light condition (Fig. 3). At higher concentrations of extract, noticeable decrease in viable cell counts were observed with a total of $2.7 \times 10^7$ CFU/mL of viable cells in the control. Treatment with the highest concentration of Xylaria extract from MEB under dark condition showed lower cell count of $1 \times 10^6$ CFU/mL of viable cells.

Interestingly, the highest activity of the mycelial extract was found using MEB as a media and incubated under dark conditions. MICs of 500 and 900 mg/L of Xylaria sp. extract were found to inhibit the growth of S. cerevisiae Δpdr5 strains by 50% and 80%, respectively (Fig. 2B). The mycelial extracts from Xylaria BCC 1067 grown under dark conditions in Czapek’s medium, PDB medium, and Sabouroud’s medium showed antifungal activity with MIC50 values of 670 mg/L, 720 mg/L, and 820 mg/L, respectively, against S. cerevisiae Δpdr5 strains. Extracts from YES medium did not show any significant antifungal activity against S. cerevisiae Δpdr5 strains (Fig. 2B), while both MEB and Czapek’s media

Table 2

| Growth media | Cell Dry weight of mycelium (g/l) | Mycelial extract (g/g) |
|--------------|---------------------------------|-----------------------|
|               | Dry mass (light) | Dry mass (dark) | Crude: mycelium (dark) | Crude: mycelium (light) |
| MEB          | 6.81 ± 0.16       | 8.14 ± 0.68       | 0.032 ± 0.003           | 0.034 ± 0.003           |
| Sabouroud’s  | 17.01 ± 0.18      | 18.27 ± 1.24      | 0.013 ± 0.014           | 0.014 ± 0.001           |
| PDB          | 14.44 ± 0.17      | 17.09 ± 0.23      | 0.012 ± 0.012           | 0.012 ± 0.001           |
| Czapek’s     | 15.75 ± 0.45      | 14.23 ± 0.39      | 0.002 ± 0.002           | 0.023 ± 0.003           |
| YES          | 57.16 ± 3.32      | 61.83 ± 1.59      | 0.005 ± 0.005           | 0.005 ± 0.001           |

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showed high activity against the Δpdr5 strain compared to other media in light conditions, with MIC$_{50}$ values of 590 mg/L and 660 mg/L, respectively. Mycelial extracts from Sabouraud’s and PDB media under light conditions showed moderate antifungal activity, while YES media showed far less activity compared to all other media types (Fig. 2 B). A similar pattern of viable cell count was observed against S. cerevisiae Δpdr5 strain while the least amount of viable cell count was observed in the culture treated with Xylaria extracts from MEB under dark conditions with an approximate value of 1.4 × 10^6 CFU/mL (Fig. 2).

Previous studies investigating the antifungal activity of cultural fractions of Xylaria sp. BCC 1067 and the bioactive compound ECQ present in the cultural fraction showed strong antifungal activity against S. cerevisiae Δerg6 strains with a MIC$_{50}$ value of 295 mg/L [19]. Regarding to the mycelial fraction of Xylaria sp. BCC 1067, the highest activity against S. cerevisiae Δerg6 was shown using the MEB cultural media, which was much higher when compared with the other media types, with the MIC$_{50}$ of 295 and 298 mg/L and MIC$_{80}$ of 470 and 450 mg/L for Xylaria sp. extract under light and dark conditions, respectively (Fig. 2 C). The mycelial extracts from all media types showed better antifungal activities against Δerg6 strains than the Δpdr5 strain, with the

Fig. 1. Comparison of dry mycelial mass and crude to mycelial mass ratio of Xylaria sp. BCC 1067.
Dry mycelial mass of Xylaria sp. BCC 1067 and crude to mycelial mass ratio under different growth media. Data were represented by the means of two independents, with three replicates, and the small vertical bars show the standard errors. The symbols with lines indicated the significant differences of the growth medium for mycelial growth. The alphabet letters with capped lines indicated the significance of the medium, as the light and dark conditions do not show any significant difference for crude production. (P-value < 0.05 according to the Tukey HSD test).

Fig. 2. Antifungal activity of Xylaria sp. BCC 1067 mycelial extracts
The antifungal activity under different media and light conditions against S. cerevisiae strains. (A) Antifungal potential of the mycelial extracts against S. cerevisiae WT. (B) Antifungal potential of the mycelial extracts against S. cerevisiae Δpdr5 strains. (C) Antifungal potential of the mycelial extracts against S. cerevisiae Δerg6. The different colored symbols indicate the significant differences between extracts from each medium in each concentration level. (P-value < 0.05 according to the Tukey HSD test).
MIC50 of 300 mg/L and 550 mg/L obtained under dark conditions. However, the extracts obtained from mycelium grown in light conditions also showed good activity against the Δerg6 strain, with MIC50 values ranging between 295 mg/L and 620 mg/L (Fig. 2C). The depletion of ergosterol content in the Δerg6 strain renders the cell sensitive to antifungal extract which may result in a higher accumulation of antifungal compounds inside the yeast cells. Nevertheless, the mycelial extract obtained using the MEB showed the best activity against S. cerevisiae as compared to the mycelial extracts obtained from other culture media types. Thus, better antifungal activity was observed against the Δerg6 strain with the least amount of viable cell count when compared to the WT and Δpdr5 strains. The least viable cell count was observed from the culture treated with MEB under dark condition with a total of 2.3 × 10^5; thereby inhibiting more than 95% of cells as compared to the untreated condition (Fig. 2).

### 3.5. Optimization of the mycelial yield via the media replacement approach

Next, we attempted to increase the mycelial biomass of the mycelial extract of Xylaria sp. BCC 1067, along with the antifungal activity. It is assumed that the bioactive substances, including antifungals, present in the mycelial fraction may be increased with its biomass content; therefore, the media replacement approach was employed. Briefly, the starter Xylaria culture used YES, which was selected based on its high fungal growth promotion (Table 2 and Fig. 1). Then, on day 7, the YES medium was removed and replaced with MEB culture medium, which produces a mycelial extract with the best antifungal activities (Table 3 and Fig. 3B). The mycelial biomass and antifungal activities of the S. cerevisiae strains were monitored until day 28. Based on the findings, during the first 7 days, mycelium growth increased rapidly using the YES medium.
The antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts on growth of *S. cerevisiae* strains. (A) Growth curve of *Xylaria* BCC 1067, cell dry weight analyses of media replacement approach, along with single media on the left y-axis. The symbols indicated the simple effects within columns or incubation period for the growth of mycelial biomass. \( P < 0.05 \) according to the Tukey HSD test. The symbols indicated the main effects within the media for mycelial crude production. \( P \)-value < 0.05 according to the Tukey HSD test). The right y-axis showed the comparison of the crude to mycelial mass ratio between medium replacement and single media as indicated by signs with black frame at day 28. (B) Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts from the medium replacement approach against *S. cerevisiae* wild-type. (C) Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts from the medium replacement approach against *S. cerevisiae* Δ*pdr5*. Data were represented by the means of two independents, with three replicates, and the small vertical bars show the standard errors. The symbols indicated the simple effects at each concentration compared to the incubation period. \( P \)-value < 0.05 according to the Tukey HSD test).

**Fig. 3.** Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts on growth of *S. cerevisiae* strains. (A) Growth curve of *Xylaria* BCC 1067, cell dry weight analyses of media replacement approach, along with single media on the left y-axis. The symbols indicated the simple effects within columns or incubation period for the growth of mycelial biomass. \( P < 0.05 \) according to the Tukey HSD test. The symbols indicated the main effects within the media for mycelial crude production. \( P \)-value < 0.05 according to the Tukey HSD test). The right y-axis showed the comparison of the crude to mycelial mass ratio between medium replacement and single media as indicated by signs with black frame at day 28. (B) Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts from the medium replacement approach against *S. cerevisiae* wild-type. (C) Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts from the medium replacement approach against *S. cerevisiae* Δ*pdr5*. Data were represented by the means of two independents, with three replicates, and the small vertical bars show the standard errors. The symbols indicated the simple effects at each concentration compared to the incubation period. \( P \)-value < 0.05 according to the Tukey HSD test).

The crude to mycelial mass ratio at day 28 was also plotted along with the mycelial growth (Fig. 3A). The crude to the mycelial mass ratio was best when using MEB, as shown by the high amount of crude extract per one gram of dry mycelial mass, and showed a significant difference between the YES and MEB growth media compared to the media replacement approach (Fig. 3A). In contrast, the crude extract yield was very poor when using YES, despite a much higher mycelial biomass. When comparing the media replacement approach, we could obtain a higher crude production than with the YES media, but lower than that of the MEB (Fig. 3A). The amount of mycelial extract and the antifungal of the mycelial extract produced during different time intervals was also determined in the replacement approach. However, no significant difference was found between the amount of mycelial extract produced per one gram of mycelial mass in each case (Fig. 3A). Therefore, the higher fungal cell content was directly proportional to the amount of mycelial extract from *Xylaria* sp. BCC 1067.

### 3.6 Enhanced antifungal potential of *Xylaria* mycelium via the media replacement approach

The antifungal assay conducted based on the medium replacement approach showed that the mycelial extract obtained from day 28 had better activity against the *S. cerevisiae* WT and Δ*pdr5* strains compared to the extracts obtained on days 14 and 21 (Fig. 3B). The results suggested that the effect of concentration depends on the incubation time, as the interaction of the two factors was extremely significant as shown by statistical analysis. A higher antifungal potential was observed against the Δ*erg6* strain, with a MIC\(_{50}\) of 110 mg/L at day 21 of cultivation (Table 4 and Fig. 3B). No significant difference was seen in the antifungal activity of the mycelial extract obtained on day 21 or day 28, suggesting an optimal cultivation time. The interaction between various factors including culture condition and the incubation period was found to have an extremely significant effect on the antifungal potential. With the medium replacement approach, we were able to maximize the growth yield compared to the original growth medium (MEB); however, the antifungal activity of the mycelial fraction from the medium replacement approach was less than when using the MEB medium alone. The main reason behind this is that the medium replacement approach stimulates the growth but might not produce as much of the relevant antifungals or might produce different types of compounds. The further investigation shall be carried out to search for the relevant antifungal compounds produced by the mycelial fraction of *Xylaria* sp. BCC 1067 although the polysaccharide glucan content with reported antifungal activity is found [38]. In fact, several studies conducted on different *Xylaria* sp. have found different types of bioactive compounds in the mycelial fractions, including polyphenols, triterpenoids, adenosine, and intracellular polysaccharides [39]. Additionally, flavonoid contents were also found in the mycelial extracts of *Xylaria* sp. [40] while ergosterol and linoleic acid methyl esters have been found in the fruiting body fraction of *Xylaria polymorpha* [41].

### 3.7 Disk diffusion assay

The Disk diffusion assay was carried out to examine the antifungal activity using the *Xylaria* extracts obtained from the MEB media under dark condition, based on the observed highest antifungal activity, from YES media and the replacement media under dark condition were also selected as controls. On YPD plates, the zone of inhibition was observed clearly after 24 h incubation at 30 °C. The best antifungal activity was observed against *S. cerevisiae* Δ*erg6* strain with a zone of inhibition of 13.33 ± 0.57 mm at the *Xylaria* mycelial extract concentration of 2000 mg/L and a 9.00 ± 1 mm inhibition zone at 1000 mg/L (Fig. 4A). The extracts from YES medium was effective only against Δ*erg6* strain with an inhibition zone of 12.00 ± 1.2 mm at 2000 mg/L and a 6.33 ± 0.57 mm zone was observed at 1000 mg/L (Fig. 4B). However, smaller zones of inhibition were observed against WT and Δ*pdr5* strains at 9.67 ± 1.13 mm.
mm and 10.66 ± 0.57 mm, respectively, at the highest concentration of Xylaria extracts of MEB media.

All three extracts obtained from the medium replacement approach showed antifungal activity against the Δerg6 strain. The best effect was found using the extract at the end of day 28 culture with 13.23 ± 1.02 mm at 2000 mg/L and 7.66 ± 0.57 mm at 1000 mg/L (Fig. 4). The extracts obtained by the end of day 21 showed smaller zones of inhibition at 11.67 ± 0.57 mm at 2000 mg/L and 7.0 ± 1.00 at 1000 mg/L of mycelial extract concentration (Fig 4D). Xylaria extracts obtained from day 14 culture was only effective when using the highest concentration with a zone of inhibition at 7.33 ± 0.66 mm (Fig 4E). Thus, these results obtained using disk diffusion indicated a similar pattern of antifungal activity as compared to those of the broth microdilution assay (Table 5).

### 3.8. Antibacterial activity of the Xylaria mycelium fraction against P. acnes

Citril acid and salicylic acid are key components of many common mycotic drugs used in the current treatment of acne, and they have been a pillar of skin inflammation treatments in many instances [42, 43]. Citric acid has been used as a cleaning agent in cosmetic products, whereas salicylic acid is used in various lotions and creams at a concentration of 10% or 0.5–2%, respectively [44]. In this study, antibacterial and survival assays of the mycelial extract of Xylaria sp. BCC 1067 against P. acnes strains were also conducted using MEB as the culture media. The mycelial extracts showed antibacterial activity against P. acnes, with a MIC of 1000 mg/L and a MBC of 2000 mg/L (Fig. 5). The complete inhibition of bacterial growth was observed, with no colony formation on the agar plates at the higher concentrations of the mycelial extract. The antibacterial potential of cultural and mycelial extracts of Xylaria sp. strain R005 has previously been shown against multidrug-resistant S. aureus and Pseudomonas aeruginosa, demonstrating that the mycelial extract exhibits better antibacterial activity than the cultural fraction [45].

Next, due to high concentration of required mycelial extract of Xylaria, the drug combination assay between Xylaria sp. extract and some antimicrobial agents (citril or salicylic acids) against P. acnes was performed using 0–50 mM of chemical agents and 0–2000 mg/L of the mycelial extract on 96 well plates via the checkerboard method. Synergy was defined as a FICI lower than 0.5, whereas a FICI greater than 4.0 was considered as antagonism between the two agents, and FICI values between 0.5 and 4.0 indicated that there is an additive effect. The combination of Xylaria mycelial extract and citric acid or salicylic acid exhibited an additive effect, with the lowest FICI values being around 0.73 and 0.515 with the concentrations of the mycelial Xylaria extract at 500 mg/L and 30 mg/L and the citric and salicylic acid at 12 and 6 mM, respectively (Table 6).

### 4. Discussion

The nutritional necessities of fungi are significant for the fruitful development of research and for industrial fermentation processes. In general, most fungal growth media consist of carbon and nitrogen sources, with a trace of fungal-specific elements [46]. The most common media used for fungal growth are malt extract and potato extract, which provide all the basic nutrients for growth and sporulation [22]. The selection of better fermentation media will improve the fungal yield or the production of primary and secondary metabolites. Here, the vegetative mycelium of Xylaria sp. BCC 1067 is evaluated using various media types, with the high growth being observed using YES media compared to other poorer media types, while MEB produced the lowest biomass (Fig. 1). It is quite evident that the supplementation of higher amounts of carbon, such as in YES (150 g/L sucrose) and Czapek’s (30 g/L sucrose) affected the higher biomass yield (Fig. 1). This is likely due to the nutrient content of each media, including the carbon and nitrogen sources. In addition, different sugars also affect the growth of fungi, as well as their saccharide levels [47]. A fungus’s use of saccharides for energy and as a carbon source actually depends on the availability of sugar, the cultural conditions, and the adaptation of the strain on the substrate. During glycolysis, phosphorylation of glucose takes place as the first step to glucose-6-phosphate, which is then converted to fructose-6-phosphate via an isomerization reaction. Indeed, the supply of fructose, instead of glucose, converts this sugar to fructose-6-phosphate in a straightforward manner. Moreover, sucrose seems to be a commercially inexpensive source for mass biotechnological production and is viewed as the ideal carbon source to maximize the growth of fungi, including Xylaria species. Indeed, several studies have revealed the importance of sucrose rather than glucose or other carbon sources in the cultivation medium to maximize the growth yield [53].

Besides sugar and carbon sources, effective nitrogen sources such as yeast extract, peptone, and NaNO₃ could contribute to the mycelial formation of Xylaria sp. BCC 1067. Malt-peptone extract and Sabouroud’s media include peptone as a nitrogen source, while yeast extract is included as the nitrogen source in YES media. Meanwhile, Czapek’s medium contains both yeast extract and NaNO₃, while PDB media has potato extract as a nitrogen source. These media types affect the growth of Xylaria to a varying extent (Fig. 1). Furthermore, yeast extract, peptone, and NaNO₃ are also important for the production of metabolites in fungi [48]. A complex hydrolysate of yeast extract provides a wide range of nutrients and growth factors that are vital for the growth of the organism, including carbon, sulfur, trace nutrients, and vitamin B complex, among others. Peptones are known to be excellent natural sources of amino acids, peptides, and proteins. Natural products such as animal tissues (meat peptone, fish peptone, etc.), milk, plants, or microbial cultures are subjected to enzymatic digestion or acid hydrolysis results in the formation of peptone. Such rich media promote the growth of Xylaria and allow for the collection of precious biomolecules, including antifungal agents and beta-glucans.

During the media replacement approach, YES and MEB medium are used as a strategy to maximize the growth of Xylaria sp. and produce better antifungal activity of Xylaria extract in a shorter period of cultivation. YES contains yeast extract as the nitrogen source, while the malt extract supplies the fungal cells with peptone. However, the impact of peptone supplementation on the metabolic behavior of cells is not properly known [51]. Davami et al. [52] has suggested that the cell growth and productivity-enhancing effect of peptones is reliant upon the basal medium nutrient composition. Moreover, peptone supplementation may result in low-nutrient in contrast to other sources with higher nutrient content [52]. This could potentially explain the better antifungal activity of Xylarial extract obtained using the MEB media with...
Fig. 4. Antifungal activity of selected *Xylaria* mycelial extracts against *S. cerevisiae* using the disk diffusion assay. Cell culture was treated with *Xylaria* extracts against *S. cerevisiae* wild-type, Δpdr5, and Δerg6 from (A) MEB under dark condition. (B) YES medium (C) media replacement approach at day 28 culture (D) media replacement approach at day 21 culture (E) media replacement approach at day 14 culture.
sporulation and virulence [55]. Here, the highest mycelial biomass and affected by the circadian timing mechanisms which undergo daily energy source. Adaptation of the organisms to environmental changes is significant ecological factor practically in all the environments as an adapted to low or dark light environments [59].

Low-lit woodland floors thereby they naturally and metabolically inhibited by green and blue lights as fungi are sensitive to light in the hindering the fruiting of mushrooms. In fact, mycelium growth is antifungal activity are observed under dark condition (Figs. 1 – 2). Exposure of mycelium to white light affects the growing pattern possibly affected the growth of the mycelium, better in the dark conditions [57]. Several studies have also shown that light condition has significantly affected the growth of the mycelium, better in the dark conditions [57]. Exposure of mycelium to white light affects the growing pattern possibly hindering the fruiting of mushrooms. In fact, mycelium growth is inhibited by green and blue lights as fungi are sensitive to light in the blue to near UV range while being unaffected by red light [58]. Besides, as being decomposers, the main reason behind the higher production of mycelium under dark conditions is their natural habitats are often low-lit woodland floors thereby they naturally and metabolically adapted to low or dark light environments [59].

To summarize on antifungal activity production, the antifungal potential of mycelial extracts from Xylaria sp. BCC 1067 is determined in parallel with the media optimization of growth culture. Several studies conducted on fungal pathogenesis have suggested that the overexpression or expanded movement of membrane transporters in fungal cells is caused by multidrug resistance [61]. The PDR5 gene encodes a key multidrug efflux pump, which is responsible for the elimination of toxic substances [37]. The overexpression of carriers in fungi, especially pdr5-like proteins, is perceived as a significant danger to the deliver of the mycelial extract and 0–50 mM of indicated chemical agent. The heat plots of the percent additive effect were defined by the normalized growth between the mycelial extract and chemical agents including citric acid and salicylic acid, at concentrations of 0–2000 mg/L of the mycelial Xylaria extract and 0–50 mM of indicated chemical agent.

Table 5
Antifungal susceptibility, the zone of inhibition from the disk diffusion assay.

| Medium | Strain          | Concentration (mg/L) | Zone of Inhibition (mm) |
|--------|-----------------|----------------------|-------------------------|
| MEB    | WT              | 2000                 | 9.67 ± 1.13             |
|        | Δpdr5           | 2000                 | 10.66 ± 0.57            |
|        | Δerg6           | 2000                 | 13.33 ± 0.57            |
|        | 1000            |                      | 9.00 ± 1                |
| YES    | Δerg6           | 2000                 | 12.00 ± 1.2             |
|        | 1000            |                      | 6.33 ± 0.57             |
| Replacment day 28 | Δerg6 | 2000                 | 13.23 ± 1.02            |
|        | 1000            |                      | 7.66 ± 0.57             |
| Replacment day 21 | Δerg6 | 2000                 | 11.67 ± 0.57            |
|        | 1000            |                      | 7.0 ± 1.00              |
| Replacment day 14 | Δerg6 | 2000                 | 7.33 ± 0.66             |

Fig. 5. Antibacterial activity of the mycelial fraction of Xylaria sp. BCC 1067 extracts against P. acnes. (A) Percentage normalized growth relative to untreated cells. Data are represented by the means of two independents, with three replicates, and the small vertical bars show the standard errors. Drug interaction between the mycelial extract of Xylaria sp. and (B) citric acid or (C) salicylic acid against P. acnes. The heat plots of the percent additive effect were defined by the normalized growth between the mycelial extract and chemical agents including citric acid and salicylic acid, at concentrations of 0–2000 mg/L of the mycelial Xylaria extract and 0–50 mM of indicated chemical agent.

To search for antifungal agents, the mutants of the ergosterol biosynthesis pathway are commonly tested, such as genes in the ergosterol biosynthesis and ergosterol function that are vital to fungal cell survival. Various antifungal drugs, including azoles and polyenes, targets ergosterol biosynthesis: ergosterol defection results in cell sensitivity and death [63]. Although the ERG6 gene is considered a non-essential gene, it involves catalyzation steps in the ergosterol biosynthesis pathway. Moreover, it causes an alternative pathway in ergosterol biosynthesis [64]. Thus, the absence of the ERG6 gene results in increased permeability and fluidity to drugs and other compounds [65]. Recently, the Δerg6 strain has been found to increase susceptibility to cytochalasin treatment and display defective actin depolymerization.
Table 6
Fractional Inhibitory Concentration (FIC) and FICI of the combination of the *Xylaria* sp. BCC 1067 mycelial extract with citric or salicylic acid.

| Acid     | MIC50 (mM) | MIC50 (mg/L) | Mycelial extract | FIC  | Mycelial extract | FICI | Definition |
|----------|------------|--------------|------------------|------|------------------|------|------------|
|          | Alone      | Combination  | Alone            | Combination |      |       |      |
| Citric   |            |              |                  |        |      |      |      |
| 25       | 12         | 2000         | 1000             | 0.48  | 0.5  | 0.98 | additive |
| 25       | 12         | 2000         | 500              | 0.48  | 0.25 | 0.73 | additive |
| 25       | 12         | 2000         | 2000             | 0.48  | 1    | 1.48 | additive |
| 25       | 6          | 2000         | 2000             | 0.24  | 1    | 1.24 | additive |
| 25       | 6          | 2000         | 1000             | 0.24  | 0.5  | 0.74 | additive |
| Salicylic|            |              |                  |        |      |      |      |
| 12       | 6          | 2000         | 1000             | 0.5   | 0.5  | 1    | additive |
| 12       | 6          | 2000         | 500              | 0.5   | 0.25 | 0.75 | additive |
| 12       | 6          | 2000         | 250              | 0.5   | 0.125| 0.625| additive |
| 12       | 3          | 2000         | 2000             | 0.25  | 1    | 1.25 | additive |
| 12       | 3          | 2000         | 1000             | 0.25  | 0.5  | 0.75 | additive |

and aberrant accumulation of sterol intermediates [19]. However, the Δerg6 strain shows resistance to commercial drugs including Amphotericin B (AmB) and nystatin, and higher susceptibility to azoles, lovastatin, and fenpropimorph [63, 66]. Guan et al. [67] also indicated that the Δpdr5 strain is also resistant to polyene drugs such as Amphotericin B. It is one of the principal antifungal drugs commercially available. It is assumed to bind to the ergosterol in the fungal cell membrane, causing pores that lead to the loss of ions and cause fungal cell death. The loss of ergosterol content in the plasma membrane causes a reduction of available ergosterol to bind with AmB, leading to AmB resistance [68].

In this study, the mycelial extract from *Xylaria* sp. BCC 1067 increased cell susceptibility in the *S. cerevisiae* Δerg6 strain using MEB and other media types, suggesting the presence of antifungals that may target ergosterol (Fig. 2C). The antifungal effect of mycelial extract from the medium replacement approach has shown potential against the Δerg6 strain (Fig. 3). In contrast, mycelial extract from MEB medium has shown its potential against all three strains, while the potential of the YES medium mycelial fraction is very low (Fig. 2). Based on the above evidence, and on the highest sensitivity or lowest MIC values observed in the erg6 mutant as compared to the wild-type or the pdr5 mutant, it is hypothesized that the antifungal mycelial extract may better penetrate the Δerg6 strain with increased membrane permeability, and mechanically target cellular components, causing cell death. The absence of the ERG6 gene would make the cell progressively defenseless to antifungal compounds [68]. Although a clear mechanism of the extract is not well identified, the lower ergosterol composition of the cell membrane of the erg6 mutant may cause fungal cells to be prone to more mycelial extracts to enter the cell. This transformation has brought about significant fitness costs for the cell itself, related to the high inhibition rate as shown [70].

Furthermore, the strong and extraordinary health-enhancing properties of medicinal fungi has been shown by numerous pharmaceutical applications. We also report on the broad spectrum of antimicrobial activity exhibited by the mycelial fraction of *Xylaria* sp. BCC 1067 against the bacterial species *P. acnes*, which is known to cause acne and serious diseases [71]. These gram-positive bacteria are predominantly present in the pilosebaceous glands of human skin [72]. The complete inhibition of *P. acnes* is observed at higher mycelial extract concentrations, which indicates the medicinal value of this fungal extract. Despite the fact that monotherapy treatments are effective, the use of combination therapies may give a better advantage and reduce both the side effects and future drug resistance. The drug combination is vital, as the use of various classes of drugs may be able to overcome the limitation of monotherapies due to the single drug possibly not being able to cover all specific areas [73]. The results of this study show that the mycelial extract of *Xylaria* sp. BCC 1067 possesses measurable antibacterial activities against *P. acnes* in combination with citric and salicylic acids, with an additive effect (Fig. 5 and Table 5) that may enhance therapeutic outcomes. Additionally, several other *Xylaria* sp. have been reported with similar antibacterial potential against various pathogenic bacteria, including *Xylaria curta* [74] and *Xylaria* sp. strain R005 [45].

Lastly, over the last few years, the novel potential applications credited to polysaccharides have given a significant impetus that has increased scientific consideration. Several *Xylaria* sp. have been reported with the presence of beta-glucans, with beta-glucans in *Xylaria nigripes* being the major polysaccharide found [75], while 37.33 mg/g of beta-glucans have been reported from *Xylaria polymorpha* [76]. Based on the above evidence, around three-folds of beta-glucans were extracted from *Xylaria* sp. BCC 1067 compared to *Xylaria polymorpha* (Table 1). Their immunomodulatory and antitumor impact, thickening qualities, and stabilizer impact are among the most encouraging perspectives. Notably, understanding the molecular structure of these polysaccharides will have a significant impact on the application of these compounds for the well-being of mankind.

To conclude, *Xylaria* is a potential resource for natural product discovery. The mycelial extract of *Xylaria* sp. BCC 1067 contains antimicrobials and beta-glucan. High mycelial biomass and antifungal activity is achieved using media-type selection and, the media replacement approach reduces the cultivation time and enhances the bioactivity of mycelial extract. Thus, *Xylaria* sp. BCC 1067 is a promising beginning and cell factory for future bioactive product development. The knowledge gained here on the optimization process will prompt a new approach to maximize utilization of biotechnological products of fungi to cope with the rising of drug resistance problem in healthcare or agriculture as endophytic fungi as well as other biotechnological applications.

5. Credit authorship contribution statement

Conceptualization: L.A.C.B.J., & N.S., Data curation: L.A.C.B.J., Formal analysis: L.A.C.B.J., K.W., & N.S., Funding acquisition: L.A.C.B.J., & N.S., Investigation: L.A.C.B.J., & N.S., Methodology: L.A.C.B.J., K.W., & N.S., Project administration & Resources: N.S., Software: L.A.C.B.J., Supervision: S.W., & N.S., Validation: L.A.C.B.J., & N.S., Visualization: L.A.C.B.J., & N.S., Writing - original draft: L.A.C.B.J., Writing - review & editing: S.W., & N.S.

Declaration of Competing Interest

Nitipha Soontorngun reports financial support was provided by Thailand Science Research and Innovation. Nitipha Soontorngun reports a relationship with King Mongkut’s University of Technology Thonburi that includes: employment.

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