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

L. A. Channa Bhathiya Jayasekara [a], Attaporn Poonsawad [a], Kwanrutai Watchaputi [a], Songsak Wattanachaisaereekul [b], Nitnipa Soontorngun [a,*]

[a] Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, 10150, Thailand
[b] School of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand

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ABSTRACT

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,]. 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

* Corresponding author.
E-mail address: nitnipa.soo@kmutt.ac.th (N. Soontorngun).

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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 antiplasmodial 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 polyketide 19,20-epoxycytochalasin 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 many 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 antiplasmodial 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 Sabouroud's (SB) have been used previously to optimize the culture conditions of *Xylaria* sp. Sof11, with Sabouroud'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 xyloketal 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 *Δpdr5* and *Δerg6* strains which lacks a key drug transporter and drug-targeted enzyme involved in the biosynthesis of plasma membrannne 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 BIOTEC Culture Collection (BCC culture 6200032292; 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. The *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 weighed 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 mycelium fraction was then treated with ethyl acetate in a 1:2 ratio (w/v) (EtOAc, QREC, New Zealand). Ethyl acetate was selected because of its chemical and biological properties, such as minimum 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 (MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0), *Δpdr5* (BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) *Δpdr5*::*kanMX4*), and *Δerg6* (BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) *Δerg6*::*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 OD_{600} at 0.1. Cell cultures were subjected to incubation for 5–6 h to obtain an OD_{600} of 0.6–0.8, and the cell cultures were then diluted to obtain an OD_{600} 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, Δ*pdr5* and Δ*erg6* strains were grown on YPD agar plates. After, the cell density was adjusted to 1 * 10^6 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 7×10^5 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 OD_{595} values, 100 μL of extract and 100 μL of cell culture were added to 96 well plates (Corning® Costar®, SigmaAldrich, China) and the OD_{595} values of the cultures were monitored with an automated microplate reader (M965+; 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 salicyclic 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 hypoxylone*, 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

Table 1
Total glucan consists of α-glucan, and β-glucan contents of the mycelium of *Xylaria* sp. BCC 1067.

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 xyloketal 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.

Table 2
Cell Dry weight of mycelium and crude production by *Xylaria* sp. BCC 1067. Cells were cultured using different growth media, under light or dark conditions, prior to the extraction of mycelium.

Growth media	Cell Dry weight of mycelium (g/l) Mycelial extract (g/g)			
	Dry mass (light)	Dry mass (dark)	Crude: mycelium (light)	Crude: mycelium (dark)
MEB	6.81 ± 0.16^{c}	8.14 ± 0.68^{c}	0.032 ± 0.002^{a}	0.034± 0.003^{a}
Sabouroud's	17.01 ± 0.18^{b}	18.27± 1.24^{b}	0.013 ± 0.001^{b}	0.014 ± 0.001^{b}
PDB	14.44 ± 0.17^{b}	17.09 ± 0.2^{b}	0.012 ± 0.001^{b}	0.012 ± 0.001^{b}
Czapek's	15.75 ± 0.45^{b}	14.23 ± 0.7^{b}	0.027 ± 0.005^{a}	0.032 ± 0.003^{a}
YES	57.16 ± 3.32^{a}	61.83 ± 1.59^{a}	0.005 ± 0.001^{c}	0.005 ± 0.001^{c}

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

The pleiotropic 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 MIC_{50} 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 MIC_{50} of 1160 mg/L, and then the Czapek's and PDB media showed MIC_{50} 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 MIC_{50} 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 MEB under light showed the highest antifungal activity, with a MIC_{50} 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 MIC_{50} of 1290 mg/L (Table 3 and Fig. 2A). The MIC_{50} 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 2.7×10^{7} CFU/mL of viable cells in the control. Treatment with at the highest concentration of *Xylaria* extract from MEB under dark condition showed lower cell count of 1×10^{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 MIC_{50} 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

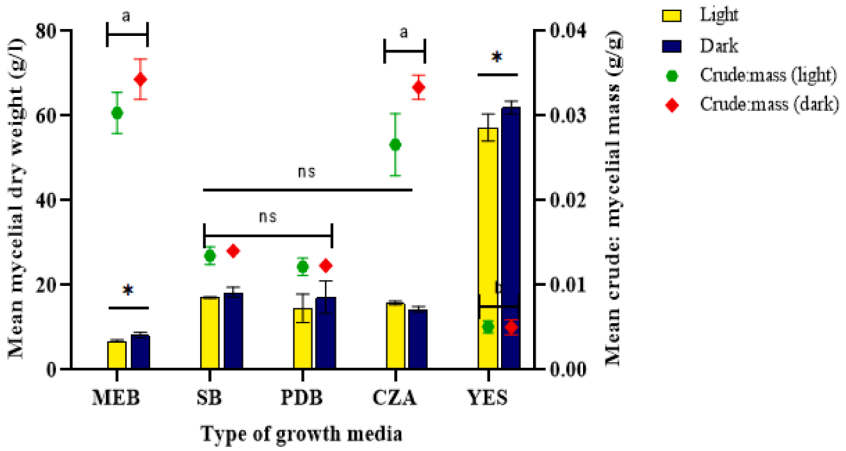

Fig. 1. Comparison of dry mycelial mass and crude to mycelial mass ratio of *Xylaria* sp. BCC 1067.
Dry mycelial mass of *Xylaria* sp. BCC 1067 and crude to mycelial mass ratio under different growth media. Data were represented by the means of two independents, with three replicates, and the small vertical 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).

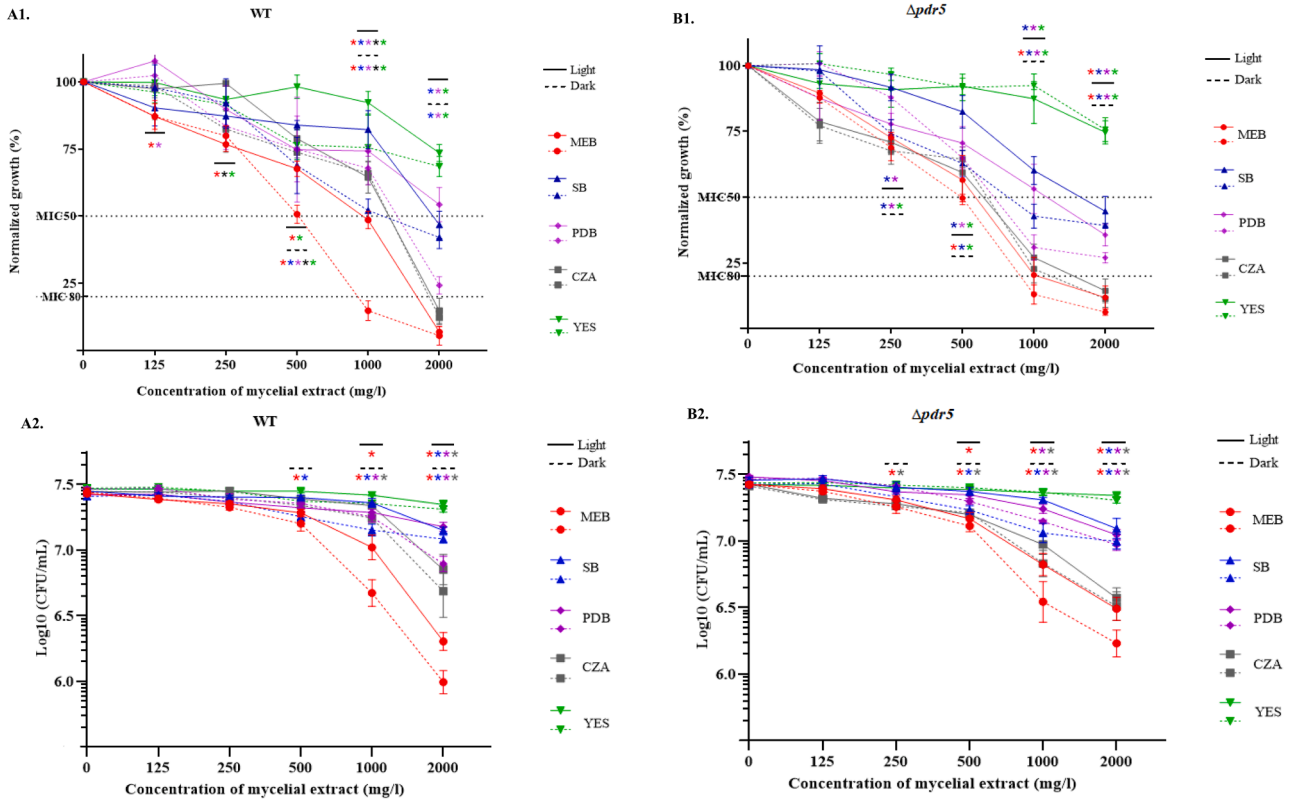

Fig. 2. Antifungal activity of *Xylaria* sp. BCC 1067 mycelial extracts
The antifungal activity under different media and light conditions against *S. cerevisiae* strains. (A) Antifungal potential of the mycelial extracts against *S. cerevisiae* WT. (B) Antifungal potential of the mycelial extracts against *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).

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 Sabouroud'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. 2B). 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. 2C). The mycelial extracts from all media types showed better antifungal activities against *Δerg6* strains than the *Δpdr5* strain, with the

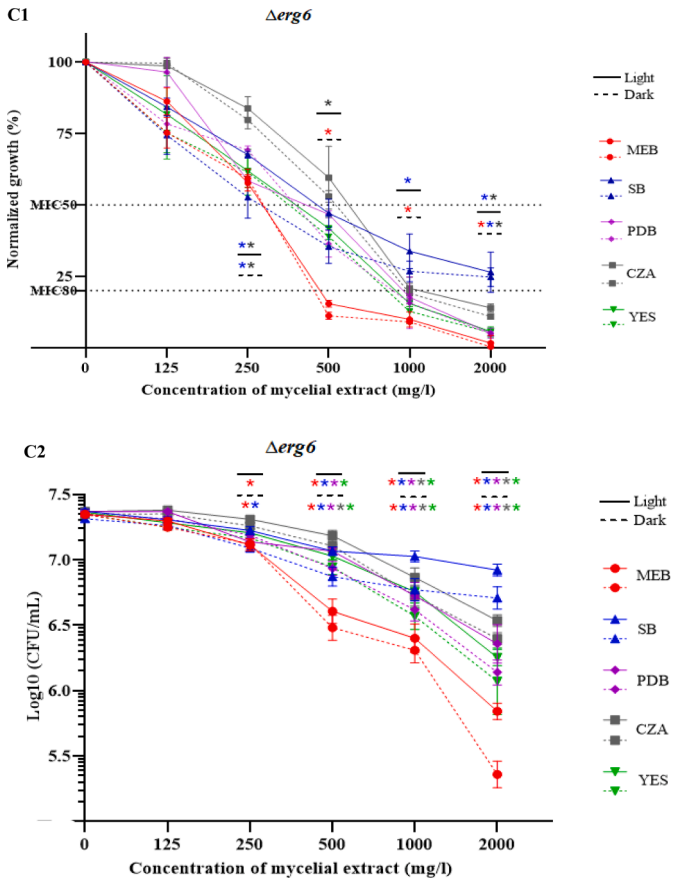

Fig. 2. (*continued*).

Table 3
Minimum inhibitory concentrations, MIC_{50}, and MIC_{80} values, of mycelial fraction against *S. cerevisiae* strains.
MICs were obtained from *Xylaria* sp. BCC 1067 mycelial fraction cultured using various media types under light or dark conditions as indicated.

		Minimal Inhibitory concentration (mg/l)					
		Light			Dark		
Medium		WT	Δ*pdr5*	Δ*erg6*	WT	Δ*pdr5*	Δ*erg6*
MEB	MIC_{50}	965 ± 2.94	590 ± 5.56	295 ± 0.97	510 ± 3.35	500 ± 5.20	298 ± 0.52
	MIC_{80}	1700 ± 1.96	1050 ± 6.65	470 ± 0.97	930 ± 3.68	900 ± 5.56	450 ± 0.96
SB	MIC_{50}	1910 ± 2.35	1650 ± 5.26	460 ± 4.90	1160 ± 5.70	820 ± 4.94	290 ± 8.85
	MIC_{80}	≥2000	≥2000	≥2000	≥2000	≥2000	≥2000
PDB	MIC_{50}	≥2000	1170 ± 9.33	430 ± 5.68	1410 ± 6.14	720 ± 4.15	400 ± 1.44
	MIC_{80}	≥2000	≥2000	960 ± 1.18	≥2000	≥2000	900 ± 9.65
CZA	MIC_{50}	1290 ± 5.65	660 ± 8.41	620 ± 7.08	1300 ± 5.85	670 ± 3.13	550 ± 2.87
	MIC_{80}	1900 ± 5.64	1550 ± 5.01	1100 ± 1.26	1860 ± 2.53	1250 ± 6.38	980 ± 2.05
YES	MIC_{50}	≥2000	≥2000	390 ± 2.77	≥2000	≥2000	370± 3.28
	MIC_{80}	≥2000	≥2000	920 ± 1.08	≥2000	≥2000	860 ± 4.37

MIC_{50} 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 MIC_{50} 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

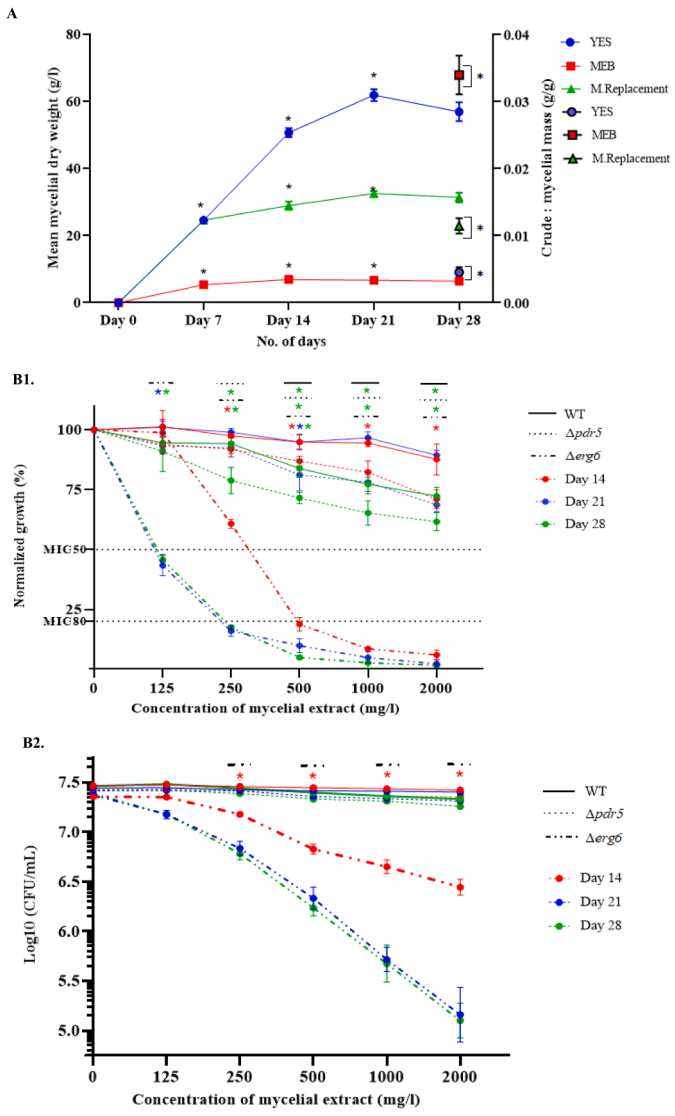

Fig. 3. Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067
The antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts on growth of *S. cerevisiae* strains. (A) Growth curve of *Xylaria* BCC 1067, cell dry weight analyses of media replacement approach, along 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 Δpdr5*. D. Antifungal activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts from the medium replacement approach against *S. cerevisiae Δerg6*. 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).

media as the starter culture broth and continued to rise until day 14, reaching a stationary phase at day 21 (Fig. 3A). When comparing the dry mass achieved from the media replacement approach, by the end of day 28, when the mycelial extract was collected, the mycelial yield was approximately half of that obtained using the YES media but was four-fold higher yield than that of the MEB media (Fig. 3A).

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 *Δpdr5* 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 *Δerg6* 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 *Xykaria* 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.cervisiae Δerg6* 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 *Δerg6* 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 *Δpdr5* strains at 9.67 ± 1.13

Table 4
MIC values of mycelial fraction from medium replacement approach against *S. cerevisiae* strains.

Media	Day	MIC 50 (mg/l)			MIC 80 (mg/l)		
		WT	*Δpdr5*	*Δerg6*	WT	*Δpdr5*	*Δerg6*
Replacement	Day 14	≥2000	≥2000	298 ± 1.89	≥2000	≥2000	485 ± 2.81
Replacement	Day 21	≥2000	≥2000	110 ± 4.24	≥2000	≥2000	225 ± 2.31
Replacement	Day 28	≥2000	≥2000	112 ± 2.19	≥2000	≥2000	235 ± 2.27
YES	Day 28	≥2000	≥2000	370 ± 3.28	≥2000	≥2000	860 ± 4.37
MEB	Day 28	510 ± 3.35	500 ± 5.20	298 ± 0.52	930 ± 3.68	900 ± 5.56	450 ± 0.96

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

Citric acid and salicyclic 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,]. Citric acid has been used as a cleaning agent in cosmetic products, whereas salicyclic 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_{50} 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 combinational assay between *Xylaria* sp. extract and some antimicrobial agents (citric 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 salicyclic 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 fungi'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 $NaNO_3$ 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 $NaNO_3$, 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 $NaNO_3$ 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

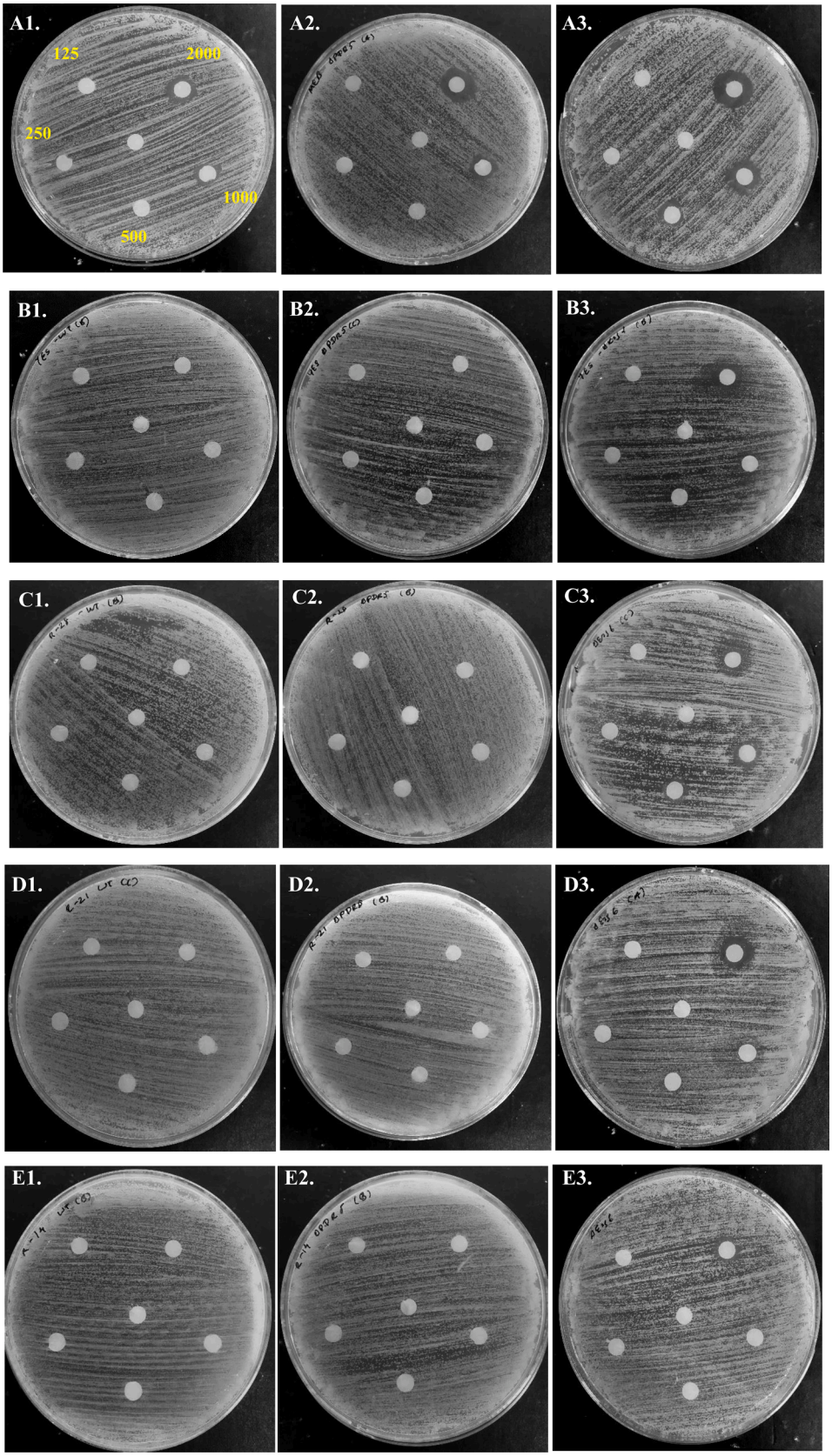

Fig. 4. Antifungal activity of selected *Xylaria* mycelial extracts against *S.cerevisiae* using the disk diffusion assay. Cell culture was treated with *Xylaria* extracts against *S. cerevisiae* wild-type, *Δpdr5*. and *Δerg6* from (A) MEB under dark condition. (B) YES medium (C) media replacement approach at day 28 culture (D) media replacement approach at day 21 culture (E) media replacement approach at day 14 culture.

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

poor nutrient content. However, the carbon and nitrogen sources utilized in the growth media also rely upon the strain cultivation process [53]. Thus and from an industrial point of view, the major optimization strategies using selected components including peptone have been laid as the foundation for customized media for *Xylaria* metabolite production.

In fact, apart from the nutritional composition, light has played a significant role in mycelial production. When comes to life, light is a significant ecological factor practically in all the environments as an energy source. Adaptation of the organisms to environmental changes is affected by the circadian timing mechanisms which undergo daily changes for diverse organisms. Filamentous fungi are composed of several photoreceptors which permits them to utilize light via signal transduction pathways as a sign to prompt versatile reactions including sporulation and virulence [, 55]. Here, the highest mycelial biomass and antifungal activity are observed under dark condition (Figs. 1–2). 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 achievements of clinical treatment [62]. Additionally, the *pdr5* mutant can be used to study the functions and the site of action of the new compounds due to its prominent role in expelling toxic matter out of cells. The highest activity against the wild-type strain is shown using MEB, and in dark conditions compared to the other conditions. The wild-type *S. cerevisiae* strain shows the most resistance to the mycelial extract, while the mutant strain displays increased sensitivity to the mycelial extract (Fig. 2).

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

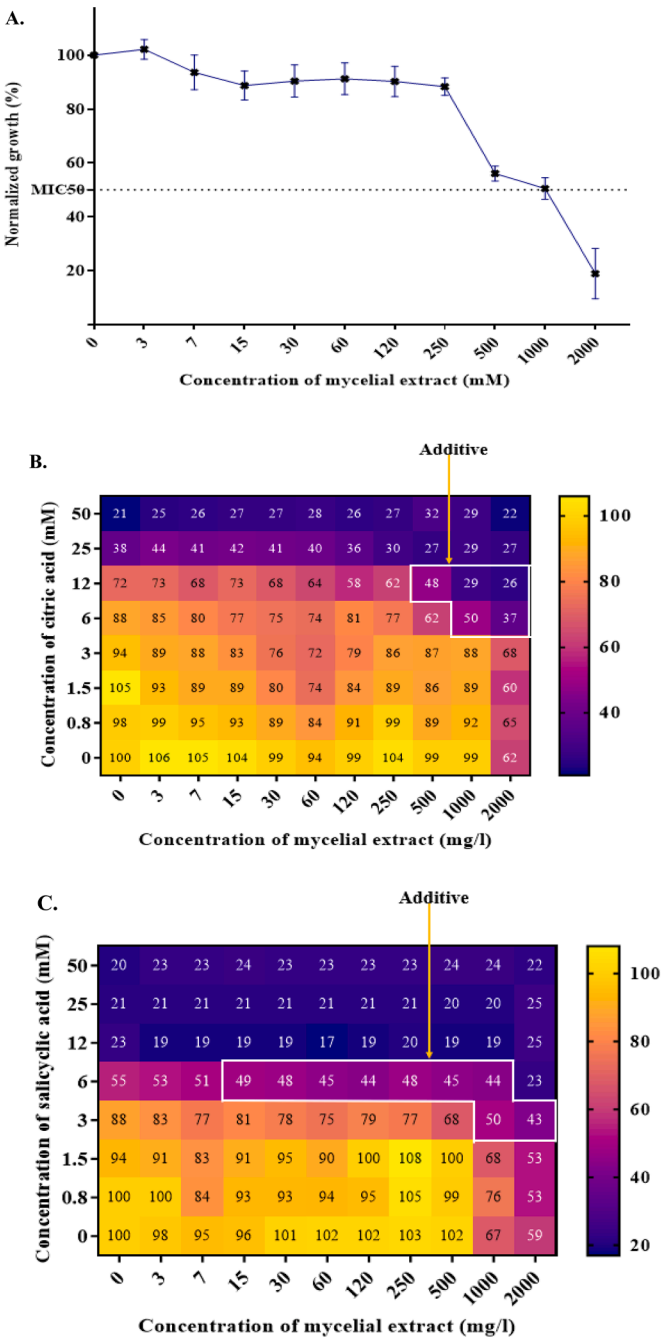

Fig. 5. Antibacterial activity of the mycelial fraction of *Xylaria* sp. BCC 1067 extracts against *P. acnes*. (A) Percentage normalized growth relative to untreated cells. Data are represented by the means of two independents, with three replicates, and the small vertical bars show the standard errors. 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.

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.

MIC_{50} (mM)Acid		MIC_{50} (mg/l)*Xylaria* Mycelial extract		FIC		FICI	Definition
Alone	Combination	Alone	Combination	Acid	Mycelial extract		
Citric acid							
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 acid							
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 mechanistically 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 combinational 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 salicyclic 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 polymorphia* [76]. Based on the above evidence, around three-folds of beta-glucans were extracted from *Xylaria* sp. BCC 1067 compared to *Xylaria polymorphia* (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 approache 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

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

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