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Screening of fungi isolated from damaged plant materials for the production of lignocellulolytic enzymes with decolorizing ability

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Abstract

Lignocellulolytic fungi are the major sources of enzymes, mainly cellulase, xylanase, and oxidative enzymes. In this study, three fungal species, identified as strains TV-1, PLS-21#7, and PSA-3, were isolated from damaged areas of infected plants. Based on morphological observation, the species were classified into *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, respectively. The fungi were grown on agar media containing carboxymethyl cellulose and Beechwood xylan, due to which clear zones surrounded the colonies on both agar media. The results indicate that strains *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 have cellulolytic and xylanolytic activities. The fungal isolates were grown on ground cassava pulp and durian peel at the temperature of 25 ℃ for ten days to determine their growth ability and enzyme production profile. Unlike durian peel, cassava pulp induced cellulase and xylanase synthesis for the three fungal strains. Although the crude enzyme of *Phanerochaete* sp. TV-1, grown on cassava pulp, showed higher cellulase and xylanase activities than those of *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, the decolorizing ability against Indigo carmine was not different among the three strains (p-value>0.05), showing around 40-60% decolorization within three days. Our study demonstrates that the new *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 strains can be used as hosts for enzyme production in low-cost media. These enzymes can be further applied in biomass saccharification or treatment of synthetic dye-containing wastewater.

Keywords: *Curvularia* sp., *Fusarium* sp., Decolorization, Food waste, Lignocelluloytic enzyme, *Phanerochaete* sp., Solid-state fermentation

1. Introduction

Lignocellulosic biomass includes forestry, agricultural, agro-industrial, and food by-products. This material is accumulated in large quantities and considered waste, thus requiring good management practice for waste removal. This waste is mainly composed of carbohydrates and lignin. Therefore, it can be used as an inexpensive renewable energy source, with immediate availability, and produce several value-added products, such as ethanol, food additives, organic acids, and enzymes [1,2].

Lignocellulolytic enzymes, including cellulase, xylanase, and oxidative enzymes, have gained considerable attention due to their immense applications in various industries. These enzymes play a crucial role in converting carbohydrates in lignocellulosic material into fermentation sugar for chemical and biofuel production. Cellulases and xylanases are used for clarification of brewery, wine, and fruit juice. In animal feed, cellulases and xylanases

are used as an additive to improve digestion and nutrition value. Moreover, xylanases and laccases are used in pulp and paper bleaching to increase paper brightness [3]. However, the industrial application of enzymes could lead to high production costs as enzymes are expensive. Therefore, microorganisms with hyperactive-enzyme production ability and the development of cost-effective, large-scale enzyme production are needed [1].

Various fungal species produce cellulases, xylanases, and ligninases to degrade cellulose, xylan, and lignin in the plant cell walls, respectively [2,4-6]. These enzymes are secreted across the cell membrane to the external environment, thus offering easy enzyme harvesting. In addition, fungi can grow on solid substrates because lignocellulosic wastes, such as sugarcane bagasse, rice straw, corn stover, cassava pulp, and durian peel, contain high amounts of proteins, carbohydrates, and minerals, presenting a suitable low-cost substrate for fungal growth.

Phanerochaete has been a topic of interest for several researchers. This basidiomycete secretes lignin-decomposing peroxidases, cellulases, and hemicellulases (xylanases) [7], making it a model white-rot fungus for deconstructing plant cell walls. However, the major problem with using this organism for enzyme production is its unstable productivity. Also, *P. chrysosporium* strains require aeration during fermentation, which is an expensive process. Thus, controllable conditions are needed for enzyme production by this species.

Curvularia is a group of filamentous fungal pathogens and saprobes associated with humans, plants, and animals [4]. Besides pathogenesis, *Curvularia* spp. are known to produce (ligno) cellulolytic enzymes, whereas lignocellulosic waste can induce the enzyme system. For example, *C. affinis* produced high exoglucanase (3.64 U/g) and endoglucanase (0.92 U/g) when it was cultivated on *Phaseolus vulgaris* bean waste for eight days [4]. The hydrolytic enzymes of *C. verruculosa*, including cellulase (0.5 U/mL), xylanase (2.5 U/mL), and protease (2.0 u/mL), were induced in a corn stalk-containing medium, while pectinase (0.8 U/mL) and lipase (0.7 U/ml) were constitutive in a glucose-containing medium [4,8].

Fusarium is a plant-pathogenic species that produce abundant and diverse lignocellulolytic enzymes, including cellulases, xylanases, and laccases [6,9]. The biochemical and functional properties of a number of xylanases under the GH10 and GH11 families from the wild-type fungus *Fusarium oxysporum* have been described [10], indicating that this fungus is a strong xylanase producer. Moreover, studies on laccase expression in *F. oxysporum* sp. lycopersici 4278 revealed at least six putative laccase-encoding genes in the genome. The genes were expressed during fungal growth, and the enzymatic activity after 12 days and six days of cultivation on rice straw and wood chips were 186 U/mL and 2.5 U/mL, respectively [9]. Moreover, some *Fusarium* species showed the ability to remove synthetic dyes [11,12].

The major limitations of fungal enzyme production in the industry are the yields of target enzymes, desired characteristics, workability under diverse conditions, and high production costs [13,14]. Thus, exploring new fungi with a complete set of lignocellulolytic enzymes and good protein secretion from diverse environments is one promising approach to obtain enzymes with desired properties [15-17]. The objectives of this study were to isolate and investigate the potential of plant-associated fungi collected from damaged plant materials. The fungi were screened for cellulolytic/xylanolytic activity using agar plate assays containing carboxymethyl cellulose and Beechwood xylan as substrates. The strains were screened for their growth ability on lignocellulosic waste, including cassava pulp and durian peel, and the crude enzyme extracts were measured for protein concentrations and the cellulase and xylanase activities. Moreover, the crude enzymes from different fungal isolates were tested to decolorize synthetic dye Indigo carmine.

2. Material and methods

2.1 Fungal isolation and morphological study

Fungi were isolated from the surface scraping of different plant materials, namely mango trees, and rice plants. These fungi were cultured in plates with potato-dextrose agar (PDA) medium, containing 100 mg/L of chloramphenicol (Sigma, Singapore) to inhibit bacterial growth. The plates were incubated at 25 ℃ for several days. The differential colonies were transferred to new plates with PDA until the pure colonies were obtained.

The isolated fungi were preliminarily identified based on their morphological characteristics (i.e., color, appearance, and diameter of colonies) after PDA growth under a light microscope. Smears of the isolated fungi were prepared in Lactophenol cotton blue and examined with a compound binocular microscope for microscopic appearance. Fungal identification was made based on morphological characteristics, mycelia, fruiting bodies, and conidia arrangements [18,19].

2.2 Plate assay for cellulase and xylanase activity

The fungal isolates were screened for their ability to degrade cellulose and xylan using Luria-Bertani agar (LB) (Difco™, France) containing 1% (w/v) carboxymethyl cellulose (CMC) (Megazyme, Ireland) as the substrate for cellulases and 1% (w/v) beechwood xylan (BWX) (Megazyme, Ireland) as the substrate for xylanase. A 0.5 cm fungal plug was inoculated onto the plates and incubated at 25℃ for five days. After that, the culture

plates were stained with 1% (w/v) Congo red dye and left for 30 min, then destained by washing twice with 1 M NaCl for 20 min with mild shaking. Transparent hydrolytic zones (clear zone), where cellulose and xylan were degraded into simple sugars, were observed. The enzymatic index (EI) was calculated by measuring the clearance zone and using the following expression [20]:

Enzymatic index (EI) = Diameter of hydrolysis zone (in cm)/ Diameter of the colony (in cm) (1)

Each experiment was performed in triplicate.

2.3 Crude enzyme production (solid-state fermentation)

A two-stage cultivation technique was employed. The fungi were grown on a PDA plate at 25℃ for four days in the first stage. In the second stage, solid-state fermentation was carried out in a 50-mL centrifuge tube containing 1.0 g of lignocellulosic material (cassava pulp or durian peel). Cassava pulp and durian peel were dried and milled into small pieces (3-5 mm). An inoculum of one mycelial disc (0.5 cm) was added to the tube and incubated under static conditions at 25℃ for ten days. After incubation, 20 mL of 0.1 M sodium acetate buffer (4° C) was added to each tube. The samples were then grown at 25°C with shaking at the speed of 180 rpm for 60 min. The crude extract obtained was centrifuged at 5,000 rpm, 4°C for 15 min, and filtered with a 0.45-µm filter disc to obtain the crude enzyme.

2.4 Protein determination and enzyme assays

The protein concentration in the crude enzyme was determined by Bradford assay (Bradford 1976) using bovine serum albumin as a standard. Cellulase (CMCase) assay was carried out at 55 °C in a reaction mixture containing 80 µL of the crude enzyme and 100 µL of 2% (w/v) CMC in 25 mM sodium acetate buffer (pH 5.5), with a final volume of 200 µL. The reaction sample was incubated for 18 h while shaking at 300 rpm. The xylanase activity was determined by incubating 80 µL of the crude enzyme with 100 µL of 2% (w/v) BWX in 25 mM sodium acetate buffer (pH 5.5), with a final reaction volume of 200 µL at 55 °C for 18 h with a shaking speed of 180 rpm. The reaction was stopped by adding 150 µL of 3,5-dinitrosalicylic acid (DNS) reagent and boiling it for 10 min [21]. Reducing sugar concentration was determined by measuring the absorbance at 540 nm using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (ThermoFisher Scientific, USA). The data obtained were expressed as mean ± standard deviation (SD).

2.5 Indigo carmine (IC) decolorization

Decolorization assay was performed in 25 mM sodium acetate buffer (pH 4.8) in a 96-well plate with a total volume of 200 µL, containing 0.1% (w/v) Indigo carmine and crude enzyme 40 µL. The mixture was incubated at 30 °C in the dark. After incubation for 0, 24, 48, and 72 h, the decolorization rate was measured at the initial dye absorbance maximum (608 nm). The absorbance value at 608 nm was used to evaluate the reduction of decolorization in terms of percentage. The decolorization percentage was calculated according to the following formula:

% Decolorization = (1 - (initial absorbance - final absorbance)/ (initial absorbance) x 100 (2)

Control samples run in parallel containing heat-inactivated crude enzyme.

2.6 Statistical analysis

Statistical analyses were performed using JMP®, Version 13 (SAS Institute Inc., Cary, NC, 1989-2019) software. The data were tested for normality, and the one way-ANOVA was applied. The Tukey test was applied to highlight significant differences between means.

3. Results and discussion

3.1 Sources and growth of fungi

A total of 15 fungal-infected and damaged plant samples were collected from different provinces of Thailand. The samples were immersed in distilled sterilized water and incubated at 25 °C shaken at 200 rpm for 1 h. After incubation, the suspension was taken, serially diluted, and plated on PDA. The plates were incubated at 25 °C for five days to observe fungal growth. PDA cultivation was first performed to give fungi any possibility to grow on

a basic medium and gain a high number of fungal species. Following this step, three fungal strains with fast growth were isolated, and their growth rates were determined based on the growth ratio, which is an increase in the hyphal diameter [20] (Table 1). It was found that strain TV-1, re-isolated in our laboratory from the contaminated culture, showed the highest growth ratio, increasing from 4.0 to 17.0 after 3-day cultivation (p-value < 0.05). Strains PLS-21#7, isolated from the fungal infected rice plants in Patumthanee province, grew from 3.4 to 8.0, and strain PSA-3, isolated from the damaged mango tree in Pitsanulok province, grew from 1.8 to 5.4 within three days. After five days of incubation, the growth ratio of strains continuously increased (14.0 and 8.4 mm, respectively). The growth of strain TV-1 could not be measured as the mycelia spread over the plate since day-4 of incubation.

Table 1 The hyphal diameters and growth ratio of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 grown for five days at 25°C on PDA.

Fungal strains	Day 1st		Day 3rd		Day 5th	
	Diameter (mm)	Ratio*	Diameter (mm)	Ratio*	Diameter (mm)	Ratio*
Phanerochaete sp. TV-1	20.0±1.4	4.0±0.3^{a}	85.0±1.4	17.0±0.3^{a}	>85.0±1.4**	>17.0±0.3^{a}
Curvularia sp. PLS21#7	17.0±0.7	3.4±0.1^{a}	40.0±0.7	8.0±0.1^{b}	70.0±0.7	14.0±0.1^{b}
Fusarium sp. PSA-3	9.0±0.7	1.8±0.1^{b}	27.0±0.7	5.4±0.1^{c}	42.0±1.4	8.4±0.3^{c}

* Ratio = Hypha diameter / Initial diameter of disc (5.0 mm)
** The mycelia reached the edge of the petri dish on day-4.
Different letters in superscripts indicate significant differences in growth rates among each group. The alpha used in ANOVA and Tukey's multiple comparison tests was 0.05.

3.2 Morphological study

The morphological study of individual strains was observed under a light microscope. Strain TV-1 developed white, cottony, and aerial colonies on a PDA medium. It contained blastoconidia [22], which indicates that this strain is lodged into *Phanerochaete* species.

Strain PLS-21#7 showed flattened greyish colonies on PDA medium and black pigmentation on the backside of the plate. The conidia were fusiform in shape, curved to slightly curve at the second cell with three septate. Both end cells of conidia are usually subhyaline [23]. These morphological properties suggest that strain PLS-21#7 belongs to the genus *Curvularia*.

Strain PSA-3 is lodged into the genus Fusarium, characterized by its white-to-cream mycelium and orange pigments on PDA medium [24]. It showed typical curved macroconidia widest in the middle of their length. The microconidia were oval to elongated oval and septated into 3-7. Therefore, based on the morphological classification, strains TV-1, PLS-21#7, and PSA-3 were designated as *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, respectively.

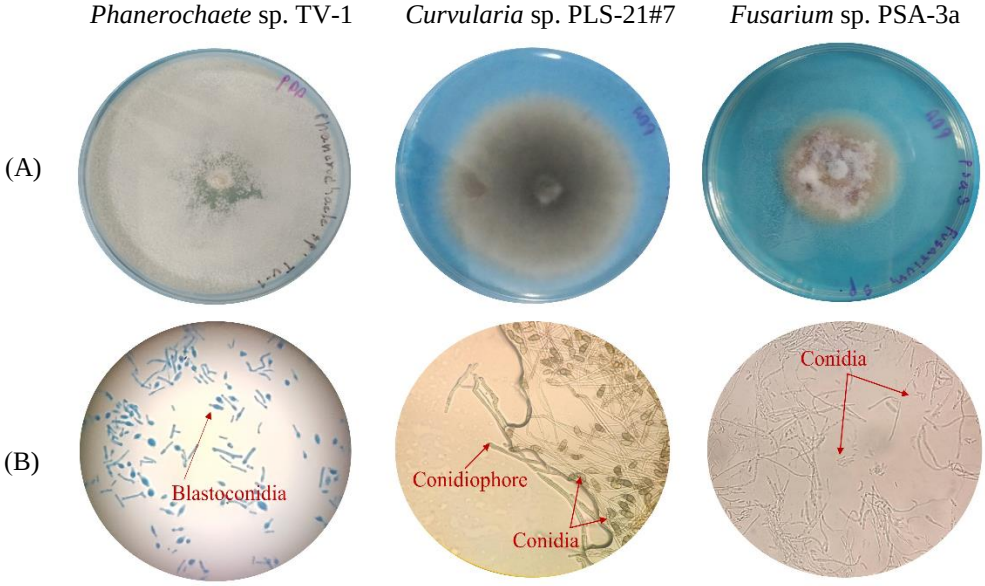

Figure 1 Morphological characteristics of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, (A) Colonial morphology of fungal isolates after 4-day incubation at 25°C on PDA, (B) Fungal morphology under a light microscope (40X Magnification).

3.3 Screening of cellulase and xylanase producing fungi

A plug of the 3-day grown fungal isolates was transferred by a sterile 5-mm diameter cork borer to PDA containing 1% (w/v) CMC and 1% (w/v) BWX to screen the cellulolytic and xylanolytic activities, respectively. The fungal isolates were incubated at 25°C for five days. After that, the agar medium was stained with Congo red, a dye that interacts with β-1,4 glycosidic bonds of polysaccharides, to visualize clear zones around fungal colonies [25].

The clear zones, indicating degradation of polysaccharides, were observed for all fungal isolates grown on CMC and BWX agar media (Figure 2), which suggests that *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 are cellulolytic and xylanolytic fungi that produce cellulases (CMCase) and xylanases to degrade CMC and BWX substrates. The highest cellulase activity was detected in *Phanerochaete* sp. TV-1 (EI=2.1±0.2), followed by *Curvularia* sp. PLS21#7 (EI = 1.5±0.1), and *Fusarium* sp. PSA-3 (EI=1.1±0.0) (p-value<0.05). Also, the xylanase activities were not significantly different among the three fungal isolates (p-value>0.05) (Table 2).

Table 2 Growth and enzyme activities of isolated fungal strains

Fungal strains	Cellulase activity			Xylanase activity		
	Hydrolysis zone diameter (cm)	Colony diameter (cm)	Enzyme index (EI)	Hydrolysis zone diameter (cm)	Colony diameter (cm)	Enzyme index (EI)
Phanerocheate sp. TV-1	2.0±0.0	0.9±0.1	2.1±0.2^{a}	2.7±0.2	1.9±0.5	1.3±0.1^{a}
Curvularia sp. PLS21#7	5.0±0.7	3.5±0.7	1.5±0.1^{b}	6.2±0.7	5.0±0.7	1.2±0.1^{a}
Fusarium sp. PSA-3	5.3±0.4	4.8±0.4	1.1±0.0^{b}	5.5±0.0	4.6±0.2	1.2±0.0^{a}

Different superscript letters indicate significant differences in growth rates among each group. The alpha used in ANOVA and Tukey's multiple comparison tests was 0.05.

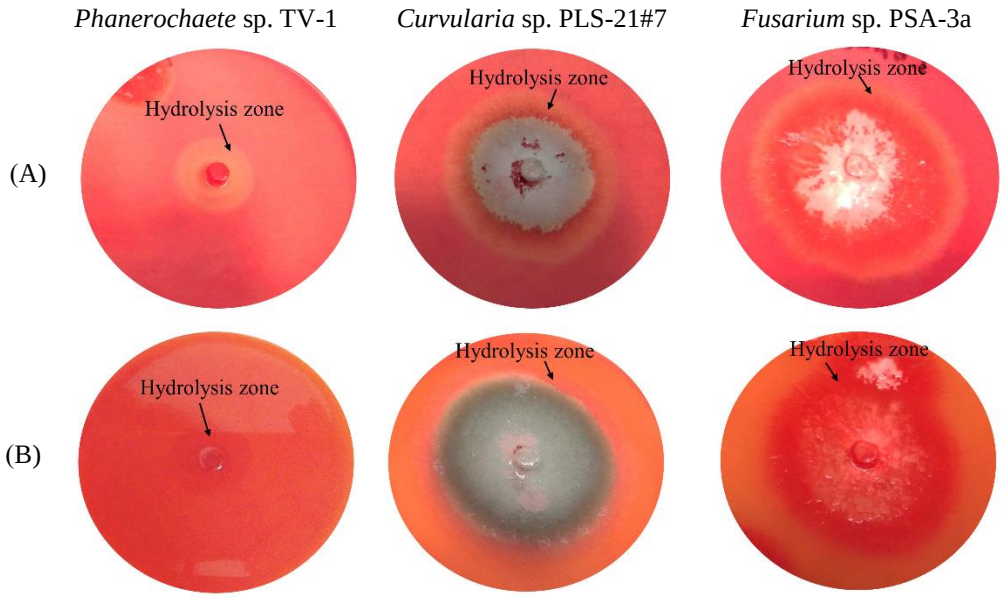

Figure 2 Detection of enzymatic activity from the isolated fungal strain on agar plates. Plates showing the zone of hydrolysis by *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, (A) Cellulolytic activity (plate with CMC), (B) Xylanolytic activity (plate with BWX)**.**

3.4 Growth of fungal isolates on cassava pulp and durian peel

Carbon is an essential factor for fungal growth, enzyme production, and production cost. Using agro-industrial wastes as the sole carbon and energy source could reduce enzyme production costs and, in turn, make the process more cost-effective. In this study, cassava pulp and durian peels were selected and used as a growth substrate for fungal isolates, as they are by-products of the food industry and contain a high carbohydrate content. Based on the chemical composition analysis, cassava pulp contains 75.0% starch, 4.11% cellulose, 4.2% hemicellulose, and

1.15% lignin [26], and durian peel contains 25.7% cellulose, 18.5% hemicellulose, and 15.9% lignin [27]. The sum of polysaccharides of the individual materials can be used as a carbon source.

Here, *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 were grown on ground cassava pulp and durian peels (3-5 mm) with 50% moisture content at 25℃ for ten days. After incubation, the samples were extracted using a cold buffer, and the supernatants were taken to determine protein concentration. It was found that all fungal isolates could grow well on cassava pulp, suggesting their capacity to utilize the polysaccharides. However, the fungal isolates were poorly grown on durian peels. This is likely due to the structural polysaccharides in durian peels resist enzymatic degradation owing to their complicated network, or durian peels might contain some compounds that inhibit fungal growth (Figure 3).

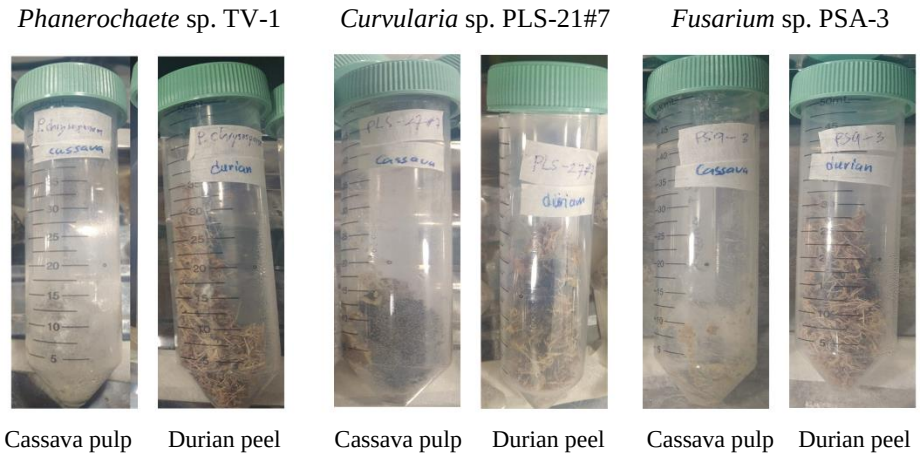

Figure 3 The growths of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 on cassava pulp and durian peel after ten days of cultivation at 25°C.

For soluble protein production, the protein concentrations were 36.7±1.1, 99.0±4.1, and 179.2±11.9 µg/mL for *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, grown on cassava pulp, respectively, while the protein concentrations were around 1-10 µg/mL for the three fungi grown on durian peel. The culture of three fungal isolates grown on cassava pulp yielded relatively higher protein concentrations than that for durian peels. This result suggests that cassava pulp might contain more digestible carbohydrates, with easy accessibility, for fungal assimilation, or some components of cassava pulp might trigger protein (enzyme) expression, resulting in high protein yields. Among the three fungal strains, *Fusarium* sp. PSA-3 exhibited the highest protein content when grown on cassava pulp and durian peel (Figure 4).

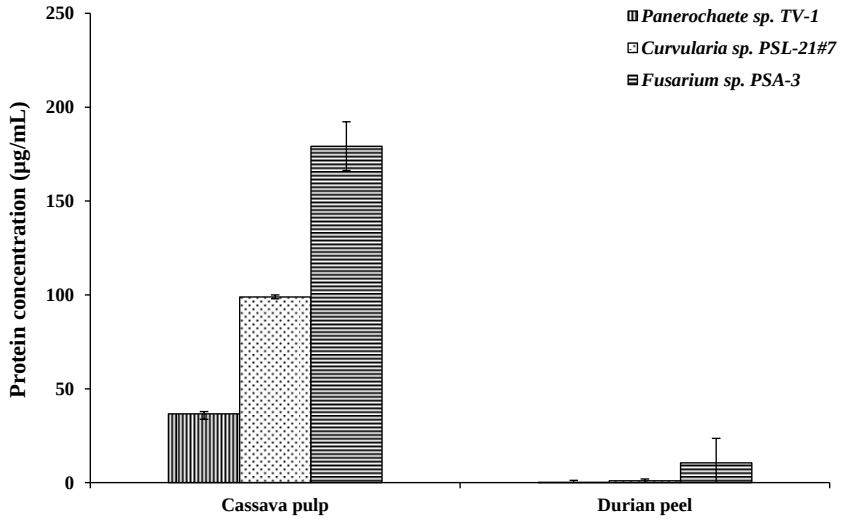

Figure 4 Crude enzyme production of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3. The soluble protein concentration of crude enzyme preparations determined by the Bradford method.

3.5 Measurement of enzymatic activity of the crude enzymes

The crude enzymes from the cultures of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, grown on cassava pulp and durian peel, were concentrated by ultrafiltration column, dialyzed against buffer to remove residual sugar from the growth culture, and measured for cellulase and xylanase activities. In this preliminary study, the cellulase and xylanase activities were considered based on the reducing sugar released from CMC and BWX substrates at a specific incubation time. It was revealed that the crude enzymes derived from cassava pulp-grown cultures of the three fungal strains showed better hydrolyzing performance against CMC and BWX than those from durian peel-grown cultures. The higher cellulase and xylanase activities in the cassava pulp-derived crude enzymes are possible due to two reasons: some chemical components in cassava pulp could trigger the cellulolytic and xylanolytic enzyme systems in the given fungi or due to the fast growth rates of the fungi on cassava pulp which yield higher protein (enzyme) concentrations, as cellulase and xylanase production has been associated with growth [10,28,29] (Figure 5).

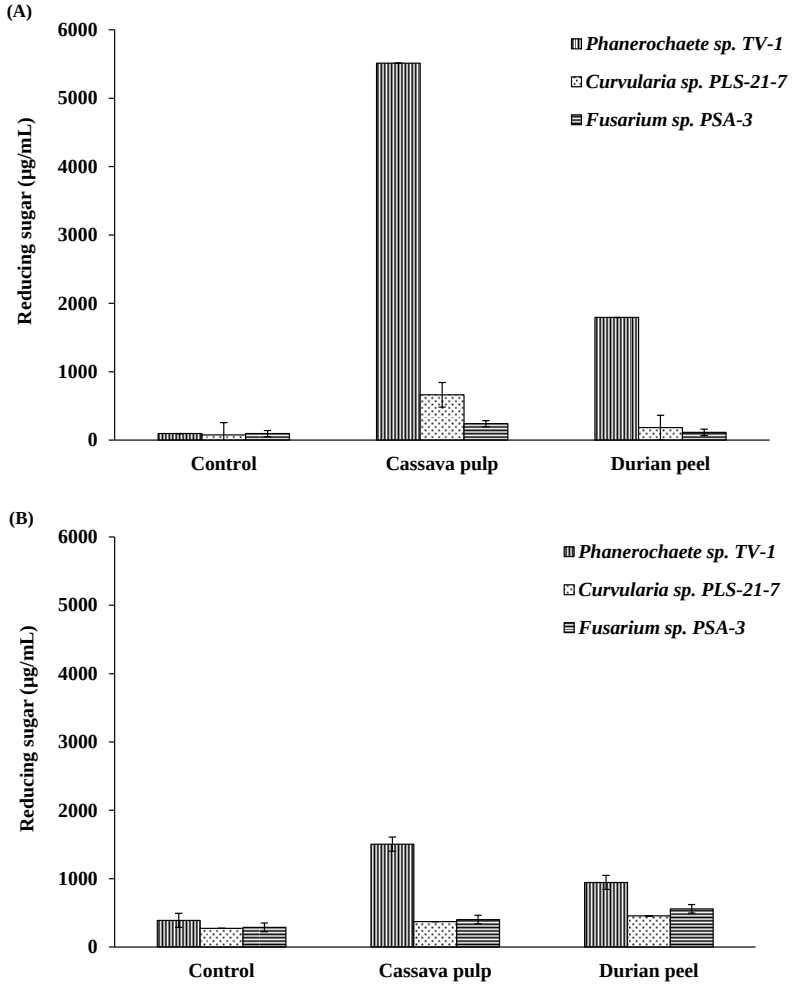

Figure 5 Crude enzyme activities of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, (A) Reducing sugar released from the hydrolysis of CMC and (B) BWX by the crude enzymes from different isolates.

It was observed that the cassava pulp-derived crude enzyme of *Phanerochaete* sp. TV-1 was highly active against CMC and BWX, yielding reducing sugar with concentrations of around 5,000 and 1,600 µg/mL from CMC and BWX hydrolysis, respectively. The hydrolysis of CMC and BWX by *Curvularia* sp. PLS21#7 crude enzyme yielded reducing sugar with concentrations of around 500 µg/mL and 320 µg/mL, whereas those by *Fusarium* sp. PSA-3 yielded reducing sugar with concentrations of around 180 µg/mL and 520 µg/mL, respectively (Figure 5A and B). On the contrary, the crude enzymes derived from durian peel cultures of different fungal strains showed similar hydrolyzing ability towards CMC and BWX to those from cassava pulp: *Phanerochaete* sp. TV-1>*Curvularia* sp. PLS21#7~*Fusarium* sp. PSA-3. However, the reducing sugar concentrations released from the hydrolysis of CMC and BWX by the durian peel grown cultures of individual

fungal strains were much lower than those by the cassava pulp grown cultures (Figure 5A and B). The low cellulase and xylanase activities are possible due to the poor growth rates of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, when they were grown on durian peel, resulting in low enzyme concentrations (Figure 3). It should be noted that *Fusarium* sp. PSA-3, grown on cassava pulp, produces high soluble proteins but possesses low enzymatic (cellulase and xylanase) activity, compared to *Phanerochaete* sp. TV-1 and *Curvularia* sp. PLS21#7. The crude protein might contain other enzymes (such as amylase and pectinase) that are not determined in this study [6,30].

Taken together, cassava pulp is a suitable growth substrate for *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and Fusarium sp. PSA-3. It is a good inducer of extracellular cellulases and xylanases synthesis. Among the fungal isolates, *Phanerochaete* sp. TV-1 is a potent producer of cellulolytic and xylanolytic enzymes due to its high hydrolyzing ability towards CMC and BWX substrates.

3.6 Decolorization test

Several fungal genera are known to have the ability to decolorize synthetic dyes based on the presence of lignin-degrading enzymes, including laccases, lignin peroxidases (LiPs), and manganese dependent peroxidases (MnPs), in the genomes [9,11,12,31,32]. Some fungal species in genera, including *Phanerochaete*, *Curvularia*, and *Fusarium*, have been shown to contain lignin-degrading enzymes and degrade aromatic compounds [9,32,33]. In this study, the crude enzymes of *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3, grown on cassava pulp and durian peel, were tested for their ability to decolorize Indigo carmine.

It is observed that the crude enzymes derived from *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 cultures were able to remove Indigo carmine (Table 3). The crude enzymes obtained from either cassava pulp or durian peel cultures of individual strains showed similar decolorizing ability towards Indigo carmine, with 40-60% removal of the synthetic dye within 72 h. The previous study showed that *P. chrysosporium* produced LiPs and MnPs to degrade Indigo carmine [33]. Moreover, Balan and Monteiro [31] reported that *P. chrysosporium* showed 75% decolorization of Indigo blue within four days. On the contrary, *F. oxysporum* HUIB02 showed complete removal of Indigo carmine within 15 days [9], whereas *C. lunata* URM 6179 strain demonstrated a low decolorization rate, reaching 93% of decolorization in 10 days [32]. Although the crude enzymes from *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 strains could decolorize indigo carmine, the decolorization rate was slow. Our future work is focusing on optimizing enzymatic conditions and applying meditators to improve decolorization efficiency.

Table 3 Preliminary decolorization test of 0.01% indigo carmine by crude enzyme grown on cassava pulp (CVP) and durian peel (DP) *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and Fusarium sp. PSA-3.

Isolate	% Decolorization							
	Cassava pulp				Durian peel			
	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
Phanerochaete sp. TV-1	100±0.0	84.9±2.2	82.9±1.9	63.5±3.4	99.0±2.5	81.2±1.8	71.8±1.4	71.8±1.7
Curvularia sp. PLS-21#7	100±0.0	85.7±0.0	73.8±2.4	59.5±2.4	97.7±2.3	88.4±2.1	76.8±4.1	67.5±3.9
Fusarium sp. PSA-3	97.6±2.4	83.0±2.0	68.3±1.7	58.6±1.4	100±0.0	83.3±2.4	71.4±0.0	59.5±2.4

4. Conclusion

In this study, three fungal species, namely *Phanerochaete* sp. TV-1, *Curvularia* sp. PLS21#7, and *Fusarium* sp. PSA-3 were isolated from damaged parts of infected plants and classified into genus based on morphological observation. The three fungal isolates could grow on cassava pulp and durian peel, among which cassava pulp was a good carbon source for inducing cellulase and xylanase enzyme production. Among the three isolates, *Phanerochaete* sp. TV-1 exhibited the highest cellulase and xylanase activities; however, the decolorizing ability among the three isolates was not much different. Further research should focus on optimizing conditions using agricultural residues for fungal growth and the enzyme production of individual fungal isolates, identifying the fungal species using the molecular technique, and purifying the enzymes, particularly laccases, LiPs, and MnPs, for treating dyes contaminating wastewater.

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