

Molecular Characterization of *Trichoderma asperellum* and Lignocellulolytic Activity on Barley Straw Treated with Silver Nanoparticles

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Silver nanoparticles (AgNPs) have been applied as an antifungal agent, which results in AgNPs contamination of agricultural waste that interferes with the lignocellulosic enzymes produced by fungi. Therefore, this study examined the production of carboxymethylcellulase (CMCase) and manganese-dependent lignin peroxidase (MnPase), using barley straw treated with AgNPs. *Trichoderma asperellum* growth was not inhibited at 25 ppm AgNPs, while negligible growth inhibition was observed at 50 ppm AgNPs, which was not observed with *Aspergillus terreus* and *Curvularia lunata*. *T. asperellum* was the highest producer of CMCase and MnPase using barley straw with or without 25 ppm AgNPs versus *A. terreus* or *C. lunata*. AgNPs addition to barley straw before *T. asperellum* inoculation played a role in repressing enzyme activities (CMCase 156.33 U/mL and MnPase 1.28 U/mL); however, addition of AgNPs (50 ppm) after 10 days of incubation showed the highest activity (CMCase 160.67 and 1.35 U/mL MnPase). Optimum temperature for enzyme production by *T. asperellum* using untreated and treated barley straw was 35 °C and 30 °C, respectively. Enzyme activities increased with increasing polyoxyethylenesorbitan monooleate surfactant concentrations up to 0.25 mL/g substrate without AgNPs, whereas the activity decreased with AgNPs (25 ppm). The exception to this observed trend was at low concentrations of the surfactant (0.10 mL/g substrate).

Keywords: *Trichoderma asperellum*; Lignocellulolytic activity; Barley straw; Silver nanoparticles; Tween 80

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INTRODUCTION

Agro-industrial wastes, as well as other lignocellulosic biomass sources, represent the largest renewable reservoir of fermentable carbohydrates on earth (Kamm and Kamm 2004; Mtui and Nakamura 2005). Agro wastes include pre- and post-harvest losses from crops, and wastes from the food processing industries. There has been considerable interest in utilizing this lignocellulosic waste due to its abundance and renewability for the production of value-added products (Foyle *et al.* 2007). Several agricultural wastes, due to their abundant availability, have been used for production of enzymes *via* fermentation processes. Wheat bran, sugarcane bagasse, rice straw, wheat straw, corn cobs, soy bran, rice husk, coffee husk, and barley are the most common agro-industrial wastes used as substrates (Sánchez 2009; Hanbyul *et al.* 2017).

Lignocellulases are key enzymes involved in the depolymerization of lignocellulosic biomass, and they have a vast array of industrial applications. These enzymes, particularly cellulases, are produced during the fermentation of lignocellulosic biomasses; cellulases are employed in numerous industrial applications, such as food, textile, pulp and paper, and detergent production (Graminha *et al.* 2008; Hebeish *et al.* 2009). Lignin peroxidases (LiPase), manganese-dependent peroxidases (MnPase), and laccase are the three major lignin-degrading enzymes; they have great potential for industrial applications (D'Souza *et al.* 2006). Hanbyul *et al.* (2017) mentioned that utilization of agricultural wastes for enzyme production will contribute to an increase in economical and environmentally sustainable production of valuable products.

Filamentous fungi, particularly basidiomycetes, are the main decomposers of lignocellulosic biomasses in nature. This is due to their development of fungal hyphae, which enables them to penetrate plant tissues, and their new enzymatic pathways that allow degradation of the structural compounds of plant tissues (Reese 1976; Rabinovich *et al.* 2004; Boer *et al.* 2005). Singhania *et al.* (2009) successfully demonstrated that several *Trichoderma* spp. can be cultivated onto various lignocellulosic materials. Studies conducted with *T. asperellum* have reported the potential of this fungus for producing a range of glycoside hydrolases (GHs), which can degrade the carbohydrates in the plant cell walls to release proteins, bioactive polymers, and simple sugars (da Silva Aires *et al.* 2012; Bech *et al.* 2015; Selvamani *et al.* 2015). *T. asperellum* will grow on substrates, such as cellulose (Raghuwanshi *et al.* 2014). Enzymes produced by *Trichoderma* spp. have been investigated for the decomposition of a range of substrates, such as wheat straw (Alvira *et al.* 2013), wet-exploded corn stover (Rana *et al.* 2014), and rice straw (Zhang *et al.* 2009; Kogo *et al.* 2017). *T. harzianum* produced high levels of filter-paperase (FPase) and cellobiohydrolase (CBH) from barley straw (Hanbyul *et al.* 2017).

Fungal sensitivity to silver nanoparticles (AgNPs) has been recently investigated (Abdel-Ghany *et al.* 2017). There are reports that AgNPs are able to bind to yeast cell walls and cell membranes, which contaminates the intracellular components (Nasrollahi *et al.* 2011). Lee *et al.* (2010) stated that AgNPs inhibit the budding process and mycelia growth. AgNPs inhibits fungal growth and sporulation processes (Abdel-Ghany 2013; Abdel-Ghany *et al.* 2013; Pinto *et al.* 2013; Abdel-Ghany *et al.* 2017). Previous research conducted by Gutarowska *et al.* (2012) showed that AgNPs applied to different technical materials, such as paper, leather, wood, and textiles, were more effective at suppressing biological growth from fungi than from bacteria and yeasts. The AgNPs caused changes to the activities of the enzymes secreted by the fungi (Pietrzak *et al.* 2015). For *Penicillium chrysogenum*, the activities of the enzymes α - and β -glucosidase, acid phosphatase, and *N*-acetyl- β -glucosamidase were altered. The activities of alkaline and acid phosphatase, esterase (C4), β -galactosidase, α - and β -glucosidase, and naphthol-AS-BI-phosphohydrolase enzymes secreted by *Aspergillus niger* were affected by AgNPs, as reported by Pietrzak *et al.* (2015). Although antimicrobial activities, as well as microbial immobilization of secreted enzymes, by AgNPs have been reported, it has also been published that lower concentrations of AgNPs promote the activities of β -glucosidase in soil media (Durga *et al.* 2015). Soil fungi are known to be tolerant to the presence of heavy metals (Baldrian 2003; Tuomela *et al.* 2005); however, the sensitivity of fungi to heavy metals can vary among species and strains (Baldrian 2003; Fomina *et al.* 2007). Degradation of cotton fabrics has been evaluated by enzymatic hydrolysis with cellulase. Silver, and particularly TiO₂, nanoparticles suppressed the biodegradation of cotton fabrics

by such enzymes (Lazić *et al.* 2015). Recently, Milošević *et al.* (2017) suggested that AgNPs hinders the biodegradation of cotton and cotton/polyethylene terephthalate (PET) fabrics. The aim of this study was to assess the capability of *T. asperellum*, *Aspergillus terreus*, and *Curvularia lunata* to biosynthesize certain lignocellulolytic enzymes when grown on agricultural waste treated with different concentrations of AgNPs.

EXPERIMENTAL

Isolation of Fungi Resistance to Silver Nanoparticles with Lignocellulolytic Activity

Sterile Whatman filter papers were immersed in solutions containing 50 ppm of AgNPs (<100 nm; Sigma-Aldrich). The treated filter paper was placed into a glass beaker containing 5 cm of agricultural soil, which was then covered by an additional 5 cm of soil. The beaker was incubated at 30 °C for 4 weeks. Sterile Whatman filter papers without AgNPs were used as a control and were prepared in a similar manner. The appearances of fungal spots on the filter papers were isolated and identified according to established literature protocols (Ellis 1971; Raper and Fennell 1973; Samuels *et al.* 1999). The identification of fungal species that are resistant to AgNPs was confirmed by using molecular characterization, where the genomic DNA was extracted by freeze fracturing in liquid nitrogen in accordance to the procedure described by Sharma *et al.* (2007). Approximately 0.2 g of the mycelia was placed into liquid nitrogen in a test tube for 10 min and vigorously homogenized. Then, 500 µL of a DNA extraction buffer (200 mM tris-HCl buffer (pH 8.0), 240 mM NaCl, 25 mM EDTA, and 1% SDS (sodiumdodecyl sulfate) was pipetted into the test tube and mixed for 5 min; afterwards, the mixture was centrifuged for 5 min at 10,000 rpm. The separated supernatant was gently mixed with an equal volume of 1:1 (v/v) phenol-chloroform solution for 30 min, which was then centrifuged at 12,000 rpm. The lighter (top) phase was gently withdrawn and mixed with a 0.1 volume portion of 3 M CH₃COONa solution (pH 5.2) and a 2 volume portion of 96% ethanol for 60 min at -20°C. The collected DNA pellets from this treatment, after centrifugation, were washed with 70% ethanol, dried, and re-suspended in 100 µL of distilled water.

PCR Amplification

Trichoderma asperellum was identified based on Internal transcribed spacer (ITS) rDNA sequence (18S- 28S rRNA), according to White *et al.* (1990). The sequences of the ITS1 and ITS4 primers were 5'TCCGTAGGTGAACCT TGC GG3' and 5'TCCTCCGCTTATTGATAT GC3', respectively. The PCR analysis was carried out according to Sambrook *et al.* (2001). Molecular Evolutionary Genetic Analysis software (version 6; MEGA6) was used for the phylogenetic analyses (Tamura *et al.* 2013). The closest homologues to the sequences were selected, and multiple sequence alignments were carried out using the Clustal W program in the MEGA6 software. A phylogenetic tree was constructed using the neighbor joining method with 1,000 bootstrap replicates based on the ITS gene sequences in order to show the phylogenetic relationships between the fungal isolate and the closely-related strains retrieved from the National Center for Biotechnology Information (NCBI) Gen Bankdatabase.

Poisoned Food Technique for Anti-Fungal Assay of AgNPs

Potato dextrose agar (PDA) media before autoclaving were supplemented with different concentrations of AgNPs (0, 25, 50, or 100 ppm) (Abdelghany *et al.* 2017). About 25 mL of the growth medium was poured into each Petri dish and allowed to solidify. A 5 mm disc of a 5-day old culture of the fungal isolates was placed at the center of the Petri dish, which was then incubated at 30 °C for 7 days; the fungal radial growth was then measured. PDA medium without the AgNPs served as control. The colony radius was measured after 6 days. Inhibition of the fungal growth in relation to the control treatment was calculated as,

$$I = \left(\frac{C-T}{C} \right) * 100\% \quad (1)$$

Where I is the fungal growth inhibition (%), C is the radial fungal growth with the control (cm), and T is the radial fungal growth with the AgNP treatment (cm).

Agriculture Waste Preparation Prior to Fungal Treatment

Fresh barley straw without any deterioration was harvested, washed with sterile water, and air-dried. The straw was cut into approximately three cm lengths, milled in an electric grinder, and sieved to obtain uniform particles (1 mm). The milled straw was used as an enzyme substrate once it had been treated with 5% NH_4OH for 72 h at 4 °C and neutralized with 2 N $(\text{NH}_4)_2\text{SO}_4$. The neutralized mill straw was swollen with water, which was then used as the substrate for enzyme assays.

Enzymes Production at Different AgNPs Concentrations

Each five grams of the above prepared barley straw were soaked separately in 20 mL solutions that contained 25, 50, or 100 ppm of AgNPs in 250 mL Erlenmeyer flasks. The substrate was wetted with 20 mL of a moistening agent (distilled water) as a control sample and then autoclaved. A 5 mm disc of a 5-day old active fungal culture was used to inoculate each flask. The flasks were incubated at 30 °C for 15 days. After the incubation period, 50 ml of distilled water were added to each flask and shaken for 60 min with a shaker set to 200 rpm. All of the contents of the flask were filtered through a muslin cloth on a glass funnel into a clean and dried flask. The cooled filtrates were centrifuged at 80,000 rpm for 10 min. The clear supernatants obtained after centrifugation were used as a crude source of cultured fungal enzymes.

Effect of AgNPs on Enzymes Production after 10 days of Inoculation

Five grams of the prepared barley straw were soaked in solutions that contained 25, 50, or 100 ppm of AgNPs in 250 mL Erlenmeyer flasks. At the same time, five grams of prepared barley straw were soaked in sterile distilled water without AgNPs, which were then filtered. The substrate was wetted with 20 mL of a moistening agent and then autoclaved. A 5 mm disc of a 5-day old active fungal culture was used to inoculate each flask, which were then incubated at 30 °C for 15 days. Different concentrations of AgNPs were added to untreated (*i.e.*, without AgNPs) and inoculated straw after 10 days of incubation under aseptic conditions. After the completion the incubation period to 15 days, the enzymes were prepared as described in the above section.

Effect of Temperature on Enzyme Production at Different Concentrations of AgNPs

Five grams of the prepared barley straw were soaked in solutions that contained 25, 50, or 100 ppm of AgNPs in 250 mL Erlenmeyer flasks. The substrate was wetted with 20 ml of a moistening agent and then autoclaved. A 5 mm disc of a 5-day old active fungal culture was used to inoculate each flask. The flasks were incubated at 15, 20, 25, 30, 35 or 40 °C for 15 days. After the incubation period, the enzymes were prepared as described earlier.

Effect of Surfactant on Enzyme Production at Different Concentrations of AgNPs

The polysorbate 80 (Tween 80) surfactant was added at concentrations 0.10, 0.15, 0.20 or 0.25 mL/g substrate to prepared barley straw treated with different concentrations of AgNPs. The same conditions of enzyme production (*i.e.*, incubation period of 15 days at 30 °C) were used as described in the previous experimental procedures.

Carboxymethylcellulose (CMCase) Assay

Carboxymethylcellulase activities were measured as described by Wang *et al.* (2008). A reaction mixture containing 1.0 g of carboxymethylcellulose (CMC) in 100 mL sodium acetate buffer (pH 5.0) was prepared. One mL of the supernatant was mixed with 1 mL of the 1% CMCase in acetate buffer in a test tube. The test tube was incubated at 63°C for 30 min; the amount of reducing sugars liberated was measured by the dinitrosalicylic acid (DNS) method as described by Miller (1959). The absorbance of the test tube solution was measured at 540 nm using an UvikonXs/60/99-90289 /110-240V/50-60Hz spectrophotometer made in France by Schott. A blank solution containing 1 mL of distilled water was used in place of 1 mL of supernatant (enzyme). The concentration of the reducing sugars were determined using a glucose standardization curve. One activity unit (1 U) for CMCase was defined as the quantity of enzyme that was needed to release 1 μmol of glucose per mL of culture filtrate per minute at standard assay conditions.

Manganese Peroxidase (MnPase) Assay

MnPase activities were measured by UV/Vis absorbance using one cm path length quartz cuvette and an UvikonXs/60/99-90289/110-240V/50-60Hz spectrophotometer made in France by SCHOTT. The reaction mixture of enzyme assay consists of 1 mL of 2 mM of 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonate (ABTS) and 1 mM MnSO₄ in a McIlvaine buffer (pH 5.0, then 100 μL of supernatants containing enzyme were added to this mixture. The peroxidase activity was then initiated by the addition of 0.4 mM H₂O₂ (Field *et al.* 1996; Garzillo *et al.* 2001). The enzymatic activities were estimated by monitoring the absorbance change at 420 nm (ABTS) using an ϵ of 36 mM⁻¹cm⁻¹ at 30 °C.

RESULTS AND DISCUSSION

During an extensive soil fungi cultural screen for AgNPs resistance, it was discovered that *T. asperellum* out-performed *A. terreus* and *C. lunata* with regard to fungal growth with different AgNPs concentration levels. The identification of *T. asperellum* fungus was confirmed using molecular characterization, which is based on ITS rDNA (Fig. 1). This method of molecular identification of fungi to the species level is primarily based

on the variable nature of the DNA's ITS regions (Romanelli *et al.* 2010; Delgado-Serrano *et al.* 2016).

The 18S rRNA sequence of the *T. asperellum* isolate was searched on a database (Basic Local Alignment Search Tool (BLAST)) using multiple sequence alignment (Fig. 1b) with the MEGA6 software. From the alignment profile results, it was concluded that the *T. asperellum* strain 29R(1) 18SrRNA amplicon closely matched other *T. asperellum* isolates (at > 99%). The constructed phylogenetic relatedness (Fig. 1) of the whole sequence of *T. asperellum* strain 29R(1) 18S rRNA was compared to the closely related strains from the database (blast.ncbi.nlm.nih.gov/Blast.cgi). This procedure revealed the molecular identity of the isolated fungus strain of this study.

T. asperellum growth was not inhibited at 25 ppm AgNPs, and it was very slightly inhibited at 50 ppm. This differed from the observations of *A. terreus* and *C. lunata*, where the growth of these fungi decreased with increasing AgNPs concentration (Table 1 and Fig. 2). A 100 ppm AgNPs dosage strongly inhibited the growth of the three isolated fungi. Pulit *et al.* (2013) found that 50 ppm of AgNPs inhibited the growth of *Cladosporium cladosporoides* and *Aspergillus niger* by 90% and 70%, respectively. From the results of this study, *T. asperellum* was considered resistant to AgNPs, particularly at 25 and 50 ppm levels, when compared to the other two isolated fungi. Fungal resistance to AgNPs has been attributed to the fungi's ability to produce secondary metabolites (Pulit *et al.* 2013), such as polyketides, ribosomal and nonribosomal peptides, and terpenoids (Andersen *et al.* 2013). This implied that the presence of enzymes capable of reducing silver cations, which consequently causes some resistance to these metal nanoparticles (Alghuthaymi *et al.* 2015). AgNPs are used as antifungal agents against phytopathogens; hence, there are some agricultural wastes that may contain trace levels of AgNPs. The observed results suggested that *T. asperellum* produced lignocellulosic enzymes that are required to degrade agricultural wastes treated with AgNPs. *T. asperellum* had the ability to produce lignocellulolytic enzymes (CMCase and MnPase) in the presence of AgNPs when compared with other fungal isolates using a barley straw substrate (Table 2). Lignocellulolytic enzymes were not detected when using *A. terreus* and *C. lunata* that were treated with 100 ppm of AgNPs. On the other hand, *T. asperellum* expressed the highest activities of lignocellulolytic enzymes using straw that was untreated or had 25 ppm of AgNPs. These observations were in agreement with observations reported by the Marx *et al.* (2013) study, which stated that *T. asperellum* had considerable potential for the production of lignocellulolytic enzymes. In our study the production of lignocellulolytic enzymes by *A. terreus* and *C. lunata* was low, although in another studies the production of cellulases on agricultural wastes under submerged fermentation was documented by *A. terreus* (Padmavathi *et al.* 2012; Olanbiwoninu and Odunfa 2016) and *C. lunata* (Nitharwal *et al.* 1991). In the present study, lignocellulolytic enzyme production by *T. asperellum* was induced at 25 ppm of AgNPs; this may be due to growth and activity of *T. asperellum* not being suppressed at 25 ppm of AgNPs. An earlier study reported that starch hydrolysis using amylase was enhanced after the use of AgNPs as catalysts (Ernest *et al.* 2012). Also, numerous studies have stated that AgNPs can be used as effective nanocatalysts in the degradation of starch by amylase (Rangnekar *et al.* 2007) and the degradation of cellulose by cellulase (Salunke *et al.* 2015). AgNPs increased the carbohydrate degradation reaction rates, although the exact binding mechanisms are unknown. Baldrian (2003) demonstrated that some heavy metals at low concentrations are essential for fungi to degrade lignocellulosic materials, although they may be toxic when present in higher concentrations.

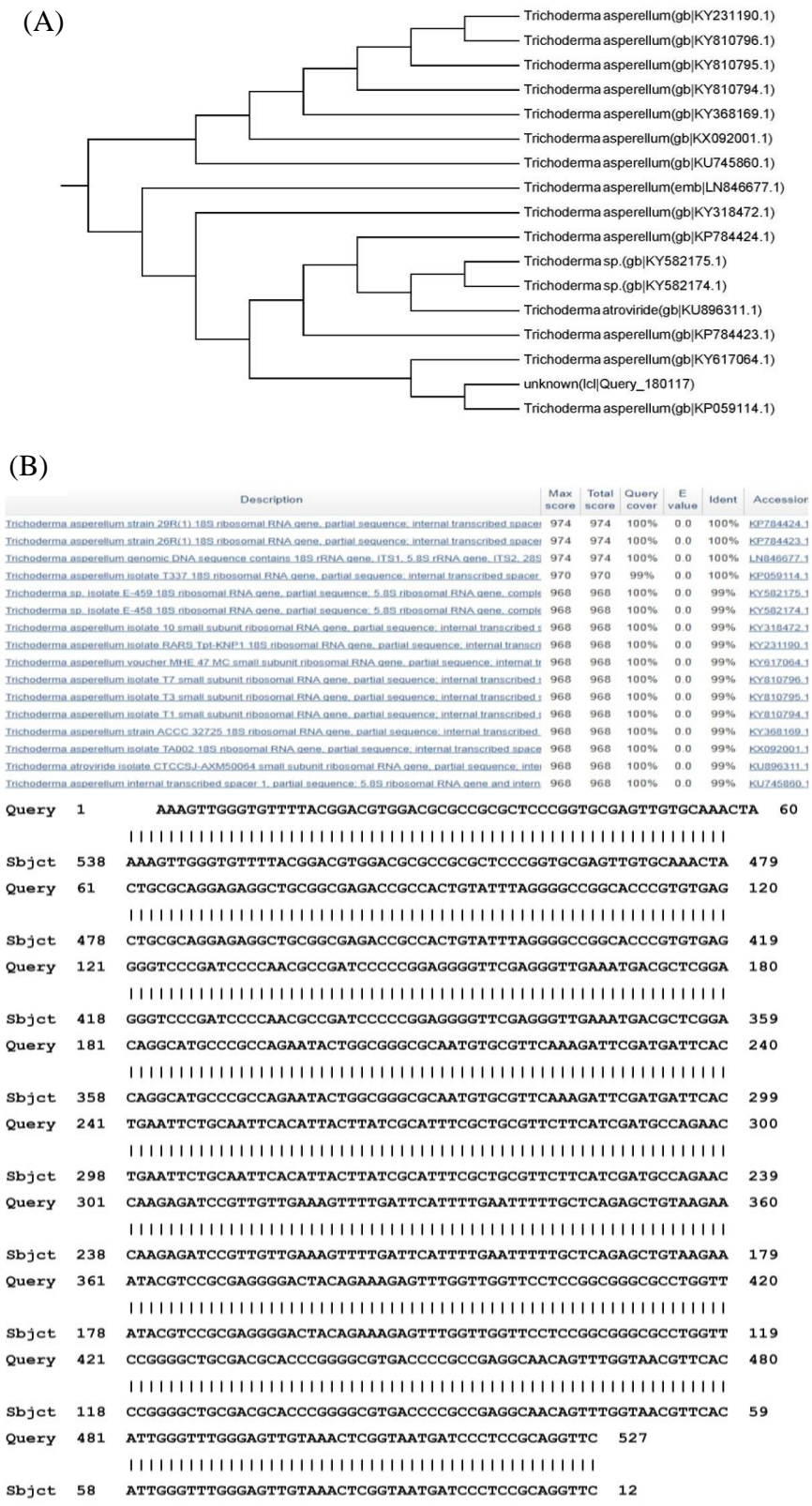
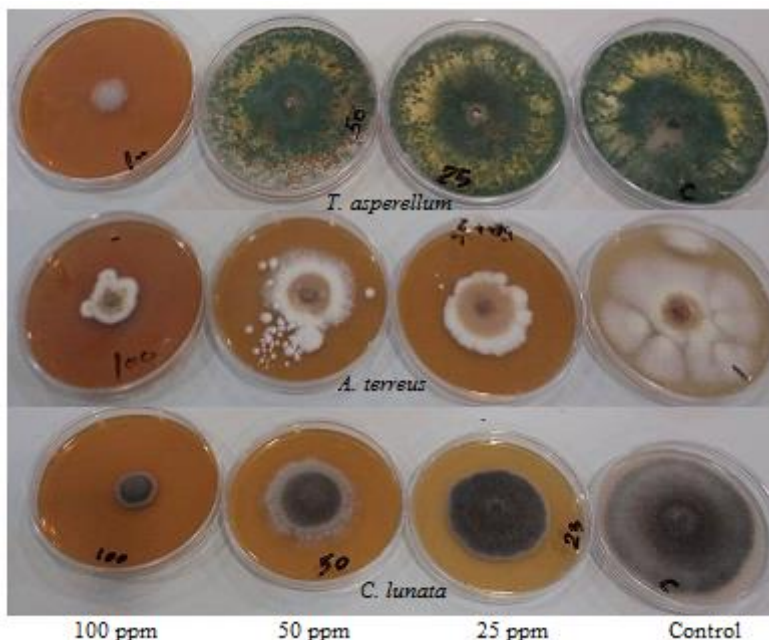


Fig. 1. (A) Phylogenetic relationships between the *T. asperellum* strain 29R(1) and the ITS sequences of closely related fungal strains retrieved from the NCBIGen Bank database; (B) cluster analysis of *T. asperellum*

Table 1. Fungal Growth at Different AgNPs Concentration Levels

Silver Nanoparticles Concentration (ppm)	<i>T. asperellum</i>		<i>A. terreus</i>		<i>C. lunata</i>	
	Colony radius (cm)	Inhibition %	Colony radius (cm)	Inhibition %	Colony radius (cm)	Inhibition %
Control(0)	8.5 ± 0.2	0.00	7.0 ± 0.5	0.00	7.5 ± 0.1	0.00
25	8.5 ± 0.5	0.00	4.0 ± 0.4	42.9	5.0 ± 0.2	33.3
50	8.3 ± 0.2	2.40	3.5 ± 0.5	50.0	4.0 ± 0.2	46.6
100	1.0 ± 0.3	88.2	2.0 ± 0.2	71.4	1.5 ± 0.1	80.0

**Fig. 2.** Fungi growth at different AgNPs concentrations levels**Table 2.** Lignocellulolytic Enzymes Activities of Fungal Isolates Incubated at 30 °C for 14 Days

Silver nanoparticles concentration (ppm)	<i>T. asperellum</i>		<i>A. terreus</i>		<i>C. lunata</i>	
	CMCase (U/mL)	MnPase (U/mL)	CMCase (U/mL)	MnPase (U/mL)	CMCase (U/mL)	MnPase (U/mL)
Control	174.33 ± 0.58	1.66 ± 0.02	89.33 ± 0.58	1.03 ± 0.02	66.67 ± 1.53	0.65 ± 0.01
25	189.33 ± 0.58	1.69 ± 0.02	63.67 ± 1.53	0.71 ± 0.01	59.33 ± 1.53	0.51 ± 0.01
50	156.33 ± 1.53	1.28 ± 0.02	32.67 ± 1.15	0.27 ± 0.02	47.00 ± 0.10	0.0 ± 0.00
100	13.67 ± 1.53	0.30 ± 0.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

± SD, standard deviation (three replicates)

AgNPs addition to the barley straw substrate prior to *T. asperellum* inoculation played an important role in the activity repression of lignocellulolytic enzymes, but the addition of AgNPs to barley straw after 10 days of inoculation showed highest activity of lignocellulolytic enzyme. For example, CMCase and MnPase activities were 156.33 and 1.28 U/mL, respectively when 50 ppm of AgNPs was added at the 1st day of *T. asperellum* inoculation but the activities of CMCase and MnPase were 160.67 and 1.35 U/mL respectively when 50 ppm of AgNPs was added to barley straw at 10 days of *T. asperellum* inoculation (Table 3). This may explained that the highest quantity of enzymes produced

by *T. asperellum* before the addition of AgNPs that may affect *T. asperellum* growth and the production of their enzymes. Dhawale *et al.* (1992) stated that the inhibition of ligninolytic enzymes is often associated with the presence of toxic pollutants in the growth medium. In an earlier study, Ibrahim *et al.* (2013) noted that *T. asperellum* that was isolated from rotten oil palm fruit bunches produced the highest amount of cellulase after 7 days.

Table 3. Lignocellulolytic Enzymes Activities on Barley Straw Treated with Silver Nanoparticles before and after 10 Days of *T. asperellum* Inoculation

Silver Nanoparticles Concentration (ppm)	Before Fungal Growth		After 10 Days of Fungal Growth	
	CMCase(U/mL)	MnPase(U/mL)	CMCase(U/mL)	MnPase(U/mL)
Control	174.33 ± 0.58	1.66 ± 0.02	174.33 ± 0.58	1.66 ± 0.02
25	189.33 ± 0.58	1.69 ± 0.02	195.67 ± 1.15	1.88 ± 0.01
50	156.33 ± 1.53	1.28 ± 0.02	160.67 ± 1.15	1.35 ± 0.01
100	13.67 ± 1.53	0.30 ± 0.04	99.33 ± 0.58	0.92 ± 0.01

± SD, standard deviation (three replicates)

In the current study, the optimum temperature for *T. asperellum* growth may not necessarily have been the optimum temperature for the production of lignocellulolytic enzymes (Table 4). The optimum temperature for the production of lignocellulolytic enzymes using untreated barley straw was 35 °C (*i.e.*, CMCase and MnPase activities were 188.33 and 1.68 U/L, respectively), whereas the optimum temperature for barley straw treated with AgNPs was 30 °C. The latter temperature is considered to be the optimum temperature for *T. asperellum* growth. At 25, 50, and 100 ppm of AgNPs, the CMCase activities at 30 °C were 189.33, 156.33, and 13.67 U/mL, respectively, as compared to their activities at 35 °C which were 180, 140.17, and 13.33 U/mL, respectively. Kheng and Omar (2005) stated that the incubation temperature is a critical factor in enzyme production. Ibrahim *et al.* (2013) found that maximum productivity of cellulases (0.8 U/mL), CMCase (24.7 U/mL), and β -glucosidase (5.0 U/mL) by *T. asperellum* occurred at 35 °C when using oil palm empty fruit bunch as the lignocellulosic substrate. The combined effect of low (20 °C) or high (40 °C) temperature with high concentrations of AgNPs (50 or 100 ppm) was found to strongly inhibit the production of lignocellulolytic enzymes. This may explain why the fungus, while under two different stress factors, failed to produce these enzymes.

The present study examined in detail the effects of polyoxyethylene sorbitan monooleate (Tween 80), a surfactant, on the lignocellulolytic activities of *T. asperellum* in the presence of AgNPs (Table 5). Generally, lignocellulolytic enzyme activities increased with increasing surfactant concentration up to 0.25 ml/g substrate in the absence of AgNPs. The specific mechanism why surfactants enhance the extracellular enzyme production with filamentous fungi has not been elucidated (Wang *et al.* 2008). Zheng and Obbard (2001) have stated, however, that surfactants, especially polyoxyethylene sorbitan monooleate, can increase the bioavailability of less soluble substrates for the fungus, and can stimulate the growth of the fungal spores. Moreover, polyoxyethylene sorbitan monooleate has the capability to affect the cell membrane structure and to promote the excretion of ligninolytic enzymes from fungal cells into the growth medium (Rodrigues *et al.* 2008). In recent studies, the production of cellulase and laccase was increased two-fold with addition of polyoxyethylene sorbitan monooleate as compared to fermentation without this surfactant (Domingues *et al.* 2000, Asgher *et al.* 2011).

Table 4. Effect of Different Temperatures on Lignocellulolytic Enzymes Activities of *T. asperellum* with Different AgNPs Concentrations

Temp.. (°C)	CMCase activity (U/mL) at AgNPs (ppm)				MnPase activity (U/mL) at AgNPs (ppm)			
	0	25	50	100	0	25	50	100
15	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
20	129.33 ± 0.58	133.83 ± 0.76	0.0 ± 0.00	0.0 ± 0.00	1.33 ± 0.03	1.36 ± 0.01	0.0 ± 0.00	0.0 ± 0.00
25	170.67 ± 1.15	185.17 ± 0.29	132.33 ± 0.29	11.67 ± 0.58	1.63 ± 0.03	1.66 ± 0.01	1.22 ± 0.02	0.25 ± 0.02
30	174.33 ± 0.58	189.33 ± 0.58	156.33 ± 1.53	13.67 ± 1.53	1.66 ± 0.02	1.71 ± 0.06	1.32 ± 0.01	0.23 ± 0.03
35	188.33 ± 1.15	180.00 ± 1.0	140.17 ± 0.29	13.33 ± 0.58	1.68 ± 0.02	1.69 ± 0.02	1.28 ± 0.02	0.30 ± 0.04
40	162.50 ± 0.50	128.83 ± 0.76	0.0 ± 0.00	0.0 ± 0.00	1.44 ± 0.01	1.55 ± 0.13	0.0 ± 0.00	0.0 ± 0.00

± SD, standard deviation (three replicates)

Table 5. Effect of Different Polyoxyethylene Sorbitan Monooleate Concentrations on Lignocellulolytic Enzymes Activities of *T. asperellum* at Different AgNPs Concentrations

Tween 80 (mL/g)	CMCase activity(U/mL)at AgNPs (ppm)				MnPase activity(U/mL)at AgNPs (ppm)			
	0	25	50	100	0	25	50	100
Control	174.33 ± 0.58	189.33 ± 0.58	156.33 ± 1.53	13.67 ± 1.53	1.66 ± 0.02	1.69 ± 0.02	1.28 ± 0.02	0.30 ± 0.04
0.10	182.50 ± 0.50	192.33 ± 0.58	157.0 ± 1.15	0.0 ± 0.00	1.67 ± 0.01	1.67 ± 0.01	1.26 ± 0.02	0.0 ± 0.00
0.15	188.33 ± 0.29	184.33 ± 0.58	145.67 ± 0.58	0.0 ± 0.00	1.86 ± 0.01	1.30 ± 0.05	1.23 ± 0.01	0.0 ± 0.00
0.20	220.33 ± 0.58	198.00 ± 1.00	138.67 ± 0.58	0.0 ± 0.00	1.94 ± 0.03	1.28 ± 0.02	0.66 ± 0.04	0.0 ± 0.00
0.25	221.00 ± 1.00	219.00 ± 1.00	101.00 ± 0.87	0.0 ± 0.00	1.94 ± 0.02	0.88 ± 0.01	0.0 ± 0.00	0.0 ± 0.00

± SD, standard deviation (three replicates)

Recently, Lee *et al.* (2017) indicated that *T. harzianum* KUC1716 was morphologically optimized to increase the production of enzymes after adding polyoxyethylene sorbitan monooleate. In another study, the lipase activity from *Rhizopus delemar* was 63.68 U/L, which was approximately 13.5 times greater when polyoxyethylene sorbitan monooleate was used than the control without the surfactant (Açikel *et al.* 2011). The surfactant can also be used as the sole carbon source in the culture medium, as it is water miscible and does not inhibit fungal growth (Açikel *et al.* 2011). Previous studies noted that surfactants could affect the results of enzyme assays by reducing the irreversible binding of the enzymes to the assay substrates, which can result in higher measured values for enzyme activities (Reese and Maguire 1969; Qing *et al.* 2011).

It is possible that polyoxyethylene sorbitan monooleate increased the available surface area to *T. asperellum* by dispersing its mycelia, as well as its spores, which facilitated the fungus' ability to access nutrients in order for it to produce more enzymes. The lignocellulolytic enzyme activities were expected to increase with the addition of the surfactant in the presence of AgNPs; however, the results obtained from the current study did not support this hypothesis. It was observed that the presence the surfactant, when used at 0 to 25 mL/g, in combination with 100 ppm of AgNPs suppressed all lignocellulolytic activities of the fungus. The presence of polyoxyethylene sorbitan monooleate in conjunction with 50 ppm AgNPs decreased the activities of enzymes. On the other hand, it was observed that there was a slight increase in the activities of the enzymes when the surfactant was applied at 0.10 mL/g substrate in the presence of 25 ppm AgNPs (Table 5). It is hypothesized that polyoxyethylene sorbitan monooleate increased the antifungal activity of AgNPs against *T. asperellum*. Previous studies have also confirmed that the addition of sodium dodecyl sulfate, a surfactant, increased the fungicidal activities of AgNPs (Panáček *et al.* 2009). Chen *et al.* (2016) disclosed that polyoxyethylene sorbitan monooleate was the most effective surfactant for improving the antifungal activities of AgNPs.

CONCLUSIONS

1. This study assessed the capability of *T. asperellum* to biosynthesize lignocellulolytic enzymes when grown on agricultural waste treated with different concentrations of AgNPs. The result indicated that *T. asperellum* has the ability to produce lignocellulolytic enzymes in the absence or presence of AgNPs up to 50 ppm.
2. The optimum temperature for *T. asperellum* growth may not necessarily be the optimum temperature for the production of lignocellulolytic enzymes with using barley straw.
3. Lignocellulolytic enzyme activities increased with increasing polyoxyethylene sorbitan monooleate concentration up to 0.25 mL/g substrate of barley straw in the absence of AgNPs, but slight increase in the activities of the enzymes when the surfactant was applied at 0.10 mL/g substrate in the presence of 25 ppm AgNPs.
4. Overall, *T. asperellum* can be applied to barley straw wastes to effectively bio-convert it into enzymes, which can be further processed into value-added products. Fungal strains belonging to the genus *Trichoderma* may play an important role in the bio-

conversion of lignocellulosic substrates in the absence of AgNPs treatment, as also confirmed by the current study.

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