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MANGANESE PEROXIDASE (MnP) ACTIVITY OF SOME NIGERIAN EDIBLE MUSHROOMS

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ABSTRACT

Mushrooms are able to secrete manganese peroxidase (MnP), an important ligninolytic enzyme involved in degrading the highly abundant, complex and recalcitrant polymer, lignin. Ligninolytic enzymes produced by these mushrooms are essential for lignocellulosic waste degradation and detoxification in the environment. Few species especially from Nigeria have been screened for ligninolytic enzymes. Six species of the Nigerian edible mushroom were screened for manganese peroxidase (MnP) activity. Three different media, namely, Nutrient broth medium, minimal salt broth (MSB), medium supplemented with 10% crude oil and MSB medium supplemented with 1% glucose were used for this purpose. The specific activities of the enzymes were determined spectrophotometrically. The growth rate on Sabouraud dextrose agar (SDA), minimal salt agar (MSA) supplemented with starch and MSA supplemented with cellulose were studied. Sensitivity of the isolates to streptomycin and erythromycin was also evaluated. Enzymatic activities were observed in all the isolates, namely, *Clitocybe gibba*, *Gerronema chrysophyllum*, *Pleurotus porrigens*, *Clitocybe familia*, *Auricularia auricular* and *Lepiota procera*. The highest MnP activity was observed in nutrient broth with the best being from *Pleurotus porrigens* (25.81U/ml) and the least from *Lepiota procera* (16.55U/ml). While *P. porrigens* and *G. chrysophyllum* fully colonized SDA plates, MSA + starch, MSA + cellulose on the third, fourth and fifth day respectively, *L. procera* grew at a much slower rate. All isolates tested were resistant to the antibiotics. This screening should be extended to more species and enzymes of species presenting best activities characterized for their potential application in various biotechnological fields such as bioremediation. Research towards the development of grain spawns should be encouraged for commercialization purposes.

INTRODUCTION

Ligninolytic enzymes are highly versatile and ubiquitous in nature, known for their role in the degradation of various complexes and recalcitrant polymers (Chandra and Chowdhary, 2015). The most common ligninolytic enzymes are manganese peroxidase (MnP), lignin peroxidase (LiP),and versatile laccase (Lac). MnP and LiP are known to catalyze the initial depolymerization of lignin while Lac is believed to participate in lignin degradation through the oxidation of phenolics. Both MnP and LiP are extracellular haem protein peroxidases (Shin *et al.*, 2005). MnP is synthesized by almost all the lignin-degrading white rot fungi (WRF) and is the most crucial in lignin biodegradation. The MnPs are peroxide-dependent extracellular glycoproteins with one molecule of heme (Dashtban *et al*. 2010). In the presence of hydrogen peroxide, they oxidize Mn (II) to highly reactive Mn (III). Mn (III) further chelates with organic acids, which attack and oxidize lignin and other recalcitrant compounds (Van Aken and Agathos 2002).According to Singh *et al.,* (2011), MnP has the properties of both an oxidase and a peroxidase. It not only catalyzes the lignin and phenolic lignin model compounds, but also catalyse the non-phenolic ones in the presence of certain compounds to polycyclic aromatic hydrocarbons by oxidation of Mn^{2+} to Mn^{3+} with H_2O_2 as an oxidant (Shin *et al*., 2005). They are efficient degraders of many recalcitrant compounds including different synthetic dyes, herbicides, polycyclic aromatic hydrocarbons, mycotoxins, estrogens, explosives, and antifouling compounds (Wang *et al.,* 2018). The presence of MnP increases the degree of dye decolorization (Christian *et al*., 2003).

Mushrooms are widespread in nature and are the earliest form of fungi known to mankind (Okhuoya, 2010). They have been reported to have medicinal and bioremediation potentials (Fen *et al*., 2014). Most of these mushrooms belong to the group of fungi causing the white rot of wood and other lignocellulosic materials. This is because of their ability to degrade the recalcitrant lignin polymer that protects polysaccharides in vascular plants. Lignocellulosic wastes are the most plentiful biopolymer in nature and pose a great deal of degradation challenge, as a result of its lignin constituents. The quantity of these waste generated by several agro-based enterprises, such as paper and pulp industries, distilleries, crop residues, and the food industrial waste, demonstrates the reach of the issue and contributes significantly to global pollution.

In nature, different organisms degrade lignin; however, fungi, in particular WRF, are the most potent sources of the enzymes (Placido *et al*., 2015). Macrofungi just like other fungi are heterotrophic and obtain the nutrients necessary for their growth through three different ways: saprophytism, parasitism (pathosystic symbiosis) and mutualistic symbiosis (Anastasi*et al*., 2009). Saprobe mushrooms

(wood decomposing and litter decomposing fungi) secret these enzymes when growing in their natural habitat to degrade the lignin in the substrate on which they grow (Petre*et al.,* 2014). Also, the Termitomyces mushrooms species which live in a mutualistic association with termites provide the ligninolytic enzymes needed by their termite's partners to breakdown lignin in wood while the termites in turn provide a suitable environment for the mushroom growth (Tibukha, 2012).

Since the discovery of MnP in *P. chrysosporium* (Glenn and Gold, 1985), MnPs have been found in other basidiomycetes, such as *Bjerkandera sp*. strain BOS55 (Mester and Field, 1997), *Bjerkandera sp.* (Palma *et al.,* 2000), *Agaricusbisporus* (Lankinen *et al.,* 2001), *Lenzitesbetulinus* (Hoshino *et al.,* 2002), *Phanerochaeteflavido-alba* (de la Rubia *et al*., 2002), *Phanerochaetesordida* (Harazono *et al*., 2003), *Panustigrinus* (Lisov *et al*., 2003), *Ganoderma lucidium* (Novotný*et al*., 2004), *Dichomitussqualens* (Eichlerová *et al*., 2005), *Lentinula edodes* (Boer *et al*., 2004), *Nematolomafrowardii* b19 (Hildén *et al.,* 2008), *Pleurotuspulmonarius* (dos Santos Bazanella *et al*.. 2013), *Trametes versicolor*, *Pleurotusostreatus*, *Irpexlacteus* (Zhao *et al*., 2015), and *Trameteshirsuta* (Vasina *et al.,* 2017).

Nigerian mushrooms have not yet been extensively documented and the potentials of known species are still poorly studied.

Considering the wide application of MnPs, the current study was conducted to screen some Nigerian edible mushrooms for manganese peroxidase activity.

MATERIALS AND METHODS

Collection of mushrooms

Local mushrooms confirmed to be edible by villagers in Uburu, Oha-Ozara Local Government Area of Ebonyi State, Nigeria were collected (both from the soil and wood). They were transported in polythene bags to Microbiology Laboratory unit of Nnamdi Azikiwe University, Awka, Anambra State. Fully mature and healthy mushrooms were separated from the rest based on their basidiocarps and the presence of visible symptoms.

Identification of the mushrooms

A Botanist, Mr. P. O. Ugwuozor from the Department of Botany from the same institution assisted in the identification of the mushrooms

Isolation of the mushrooms into pure cultures

The sorted mushrooms were washed in sterile water and allowed to stand for five minutes in water to rehydrate the basiodiocarps. The rehydrated basiodiocarps were then washed thoroughly in sterile water containing 5% sodium hypochlorite. A sterile knife was used to cut the mushrooms and a sterile forceps used to collect a small piece of the tissue according to Onyango *et al.* (2011). The small piece of the mushroom tissue was carefully inoculated onto the center of Petri dishes containing sterile solidified Sabouraud Dextrose Agar (SDA) with chloramphenicol to prevent bacterial contamination. This was done in triplicates

for each mushroom. The inoculated plates were incubated at room temperature for 5 - 8 days to enable mycelia establishment. Colonies growing on the triplicate plates were compared against each other to determine their uniformity. To obtain pure cultures for storage, sub-culturing was done on fresh SDA plates and incubated for five days at room temperature. They were then transferred to SDA slants, incubated for five days at room temperature and then stored in refrigerator at 4ºC (Adekunle and Adebambo, 2004).

Screening of isolates for Manganese peroxidase (MnP) activity

The isolates were screened for manganese peroxidase (MnP) production in three different media, namely: Nutrient broth medium, minimal salt broth (MSB) medium supplemented with 10% crude oil, and minimal salt broth (MSB) supplemented with 1% glucose

Eight-millimeter agar plugs of the test mushrooms (72 hours old) were used as inocula in 10ml of the different media. All the test tubes were incubated at room temperature for seven days. At the end of the stipulated incubation periods, the samples were centrifuged and the MnP activity of supernatants was determined by the methods described by Patricia *et al*., (2009). The reaction mixture for this assay contained 0.1ml of 0.25M sodium lactate, 0.05ml of 2mM MnSO4, 0.2ml of 5% serum albumin bovine, 0.1ml of 0.1%phenol red, 0.5ml crude enzyme and 0.05ml of 2mM H_2O_2 in 0.2m sodium phosphate buffer (pH 8.0). The mixture was left at room temperature for 5 minutes and the reaction was ended with 0.04ml NaOH 2N. The absorption at 610nm was measured against a blank. Three isolates (two from wood and one from soil) with high MnP activity were selected for further study.

Growth rate of isolates on SDA

Sterile plates of solidified SDA were inoculated with 4mm agar plugs from test isolates and incubated at room temperature. Colony diameter in each plate was measured along two axes perpendicular to one another every day, and the average of the two measurements taken and final diameter given in mm/day (Kim *et al*., 2005).

Growth rate of isolates on different carbon sources

The ability of the isolates to utilize different carbon sources was studied. MSB supplemented with 1% starch, or 1% cellulose were inoculated with 4mm agar plugs from 72 hours old cultures of selected isolates and incubation was at room temperature. Colony diameter in each plate was measured according to Kim *et al.*, (2005).

Sensitivity of isolates to antibiotic

Well in agar method was used to test the sensitivity of isolates to some antibiotics produced by soil organisms. The inoculum was prepared by dispensing a 4mm agar plug of isolate in 1ml of sterile saline. Using an inoculating wire loop, the test organisms were evenly spread on the surface of sterile SDA plates. Streptomycin (10 microgram) and erythromycin (10 microgram) were dispensed into wells made in the agar. The plates were left for a while to allow the antibiotic to absorb into the agar and then

incubated at room temperature for 3 – 5 days (Magaldi*et al.*, 2004). Sensitivity was determined by zones of clearing around the antibiotic wells.

RESULTS

Identification of edible mushrooms

Collected mushrooms were identified as *Clitocybe gibba, Gerronema chrysophyllum, Pleurotus porrigens, Clitocybe familia, Auricularia auricular,* and *Lepiota procera.* Plate 1 shows the pictures and sources of the different mushrooms used.

Manganese peroxidase (MnP) activity of isolates

All the isolates showed their highest MnP activity in nutrient broth and the least in minimal salt broth (MSB) supplemented with crude oil. *P. porrigens* showed the highest MnP activity (20.89U/ml) in MSB medium supplemented with crude oil. While *C. familia* had the least MnP activity (5.77U/ml) in same medium, as shown in Figure 1

Growth rate of isolates on Sabouraud Dextrose Agar (SDA)

*P. porrigens*and *G. chrysophyllum* fully colonized the SDA plates on the third day at 82mm as shown in Figure 2. *L. procera* grew at a slower rate reaching only 27mm on the third day and 68mm on the eighth day.

Growth rate of isolates on minimal salt agar (MSA) + starch

All the isolates were able to utilize starch as sole carbon source. However, as shown in Figure 3, *P. porrigens*and *G. chrysophyllum*rapidly colonized the plates on the fourth day at 82mm. while *L. procera* grew more slowly showing its suboptimal utilization of this substrate.

Growth rate of isolates on minimal salt agar (MSA) + cellulose

Full colonization of the plates was observed on the fifth day by *P. porrigens, G. chrysophyllum* and *G. molybdities* at 82mm, while the growth of *L. procera* was at 19mm as represented in Figure 4. Thus, all the isolates were able to utilize cellulose as sole carbon source, although at different rates.

Sensitivity of isolates to antibiotics

The well in agar method was used to test the sensitivity of the isolates to streptomycin and erythromycin. Isolates were resistant to tested antibiotics. The results are shown in Table 1

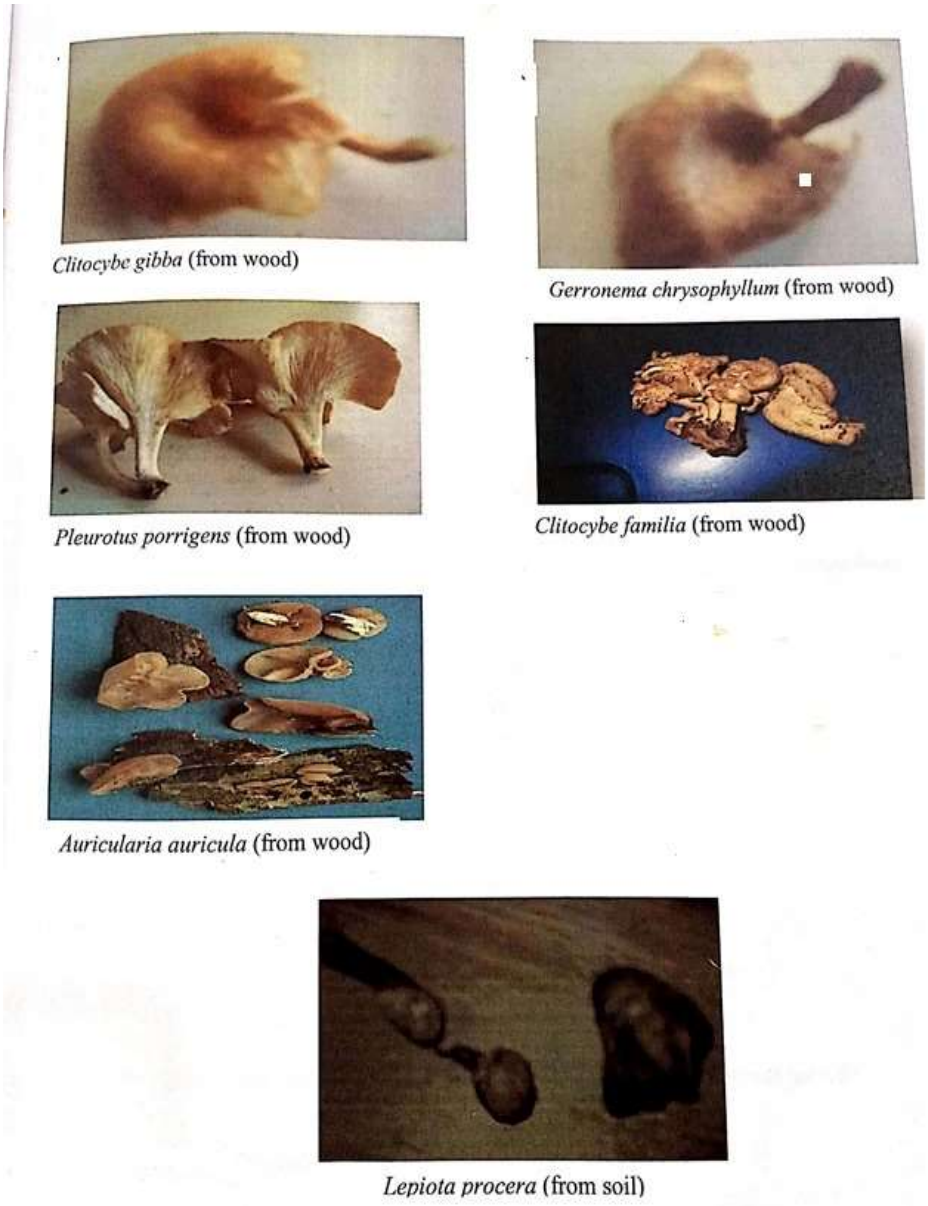


Plate 1: Source, name, and pictures of collected mushrooms

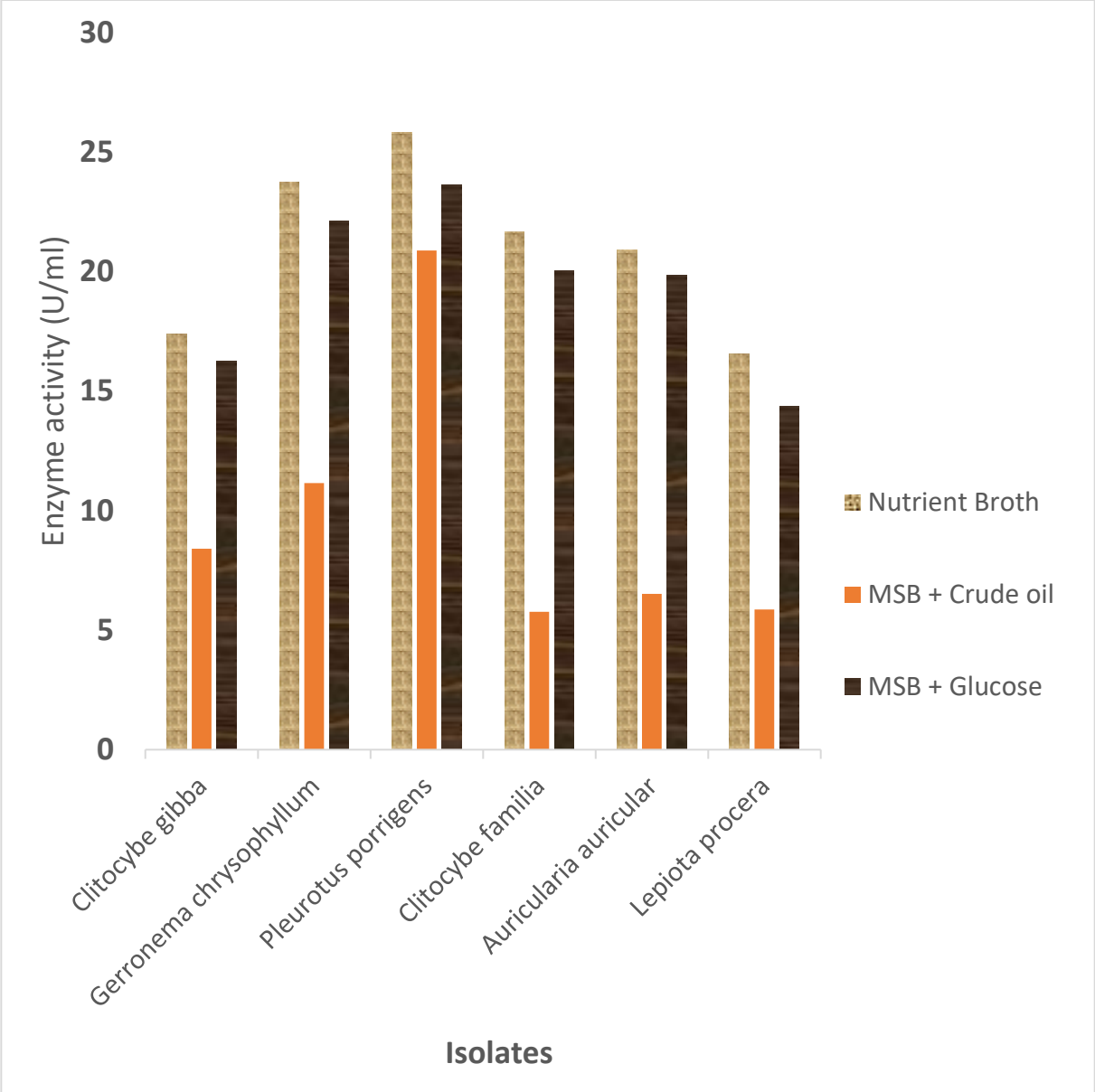


Figure 1: Manganese peroxidase activity of isolates after growth in different media

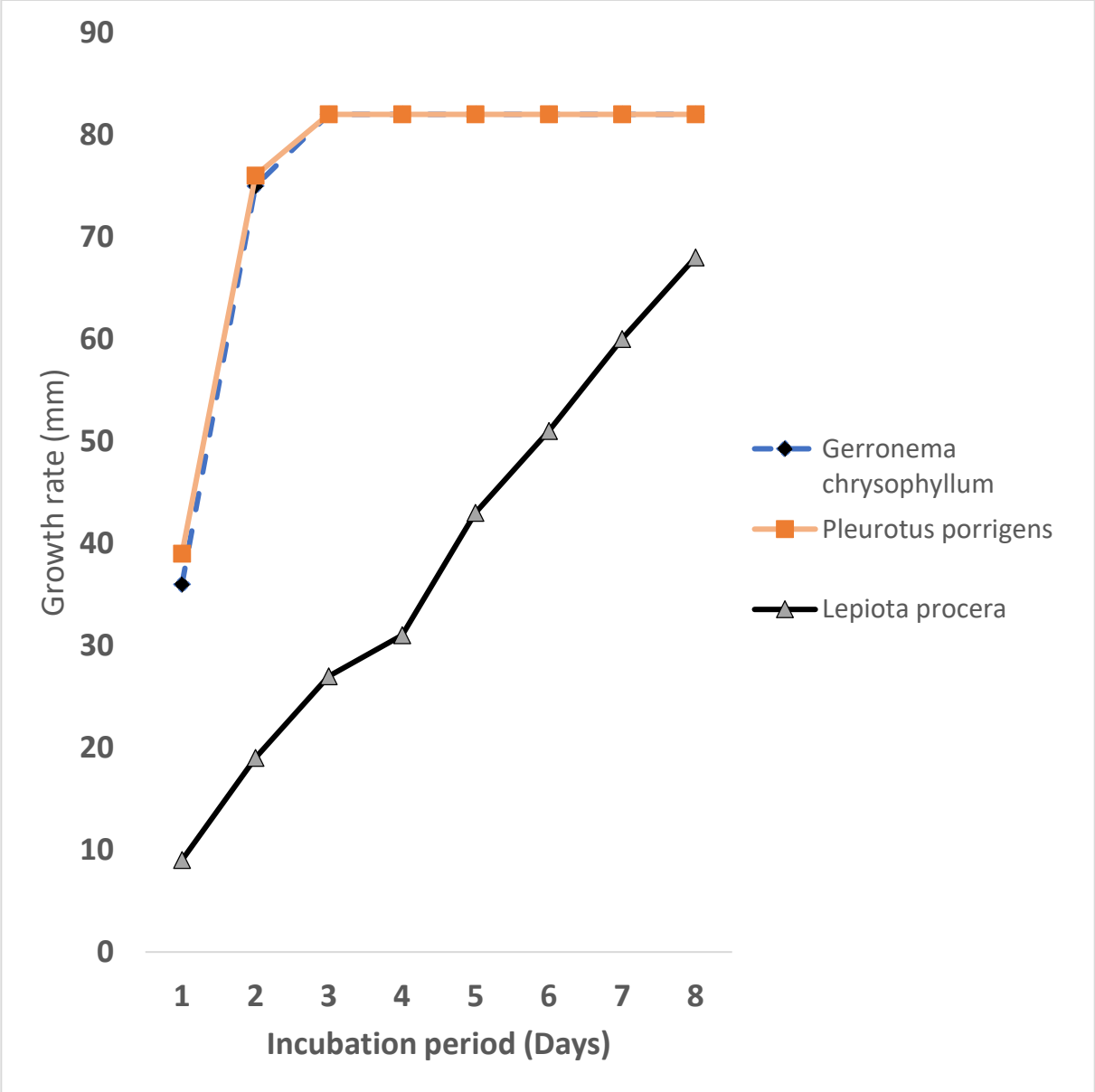


Figure 2: Colony growth rate of isolates on SDA

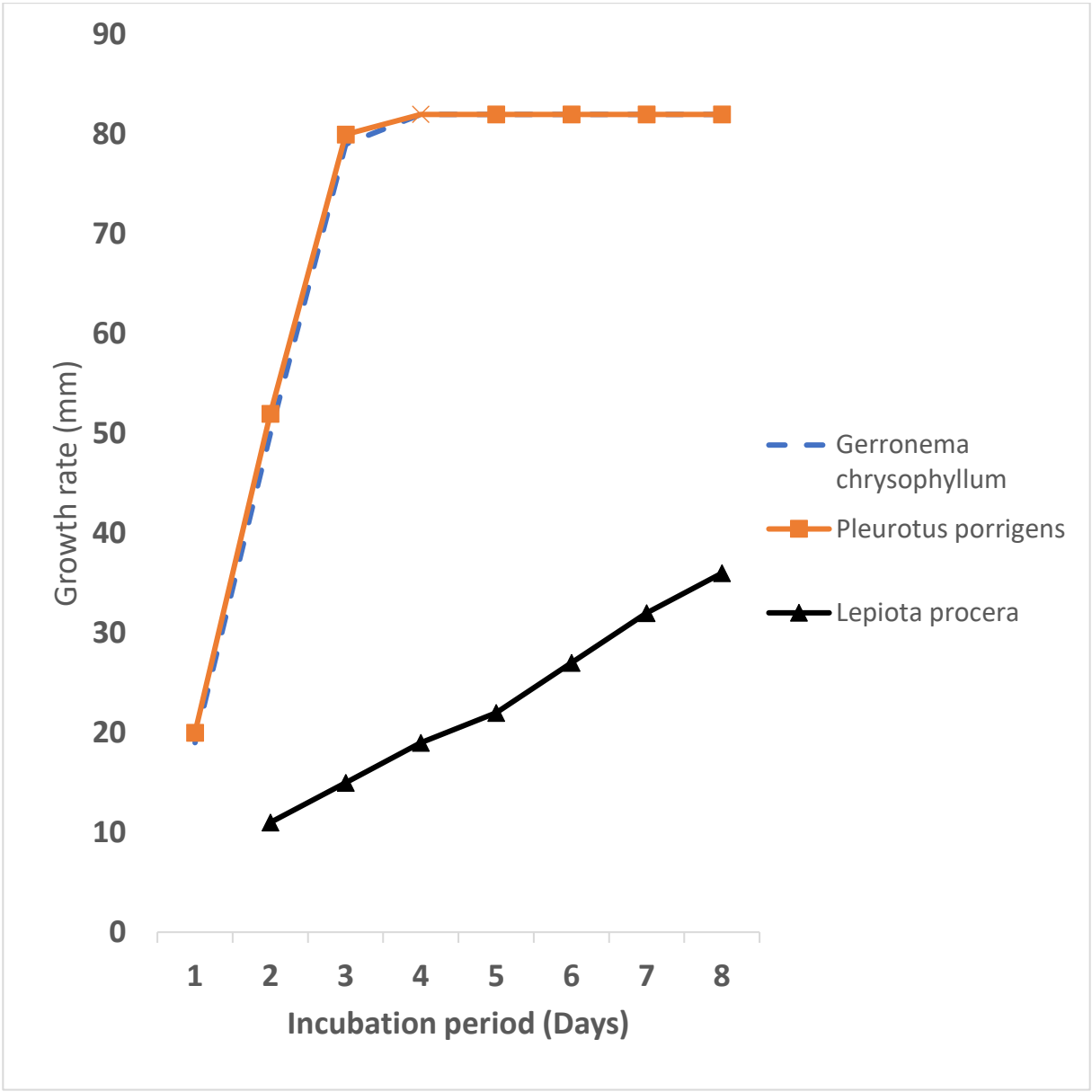


Figure 3: Colony growth rate of isolates on MSA + Starch

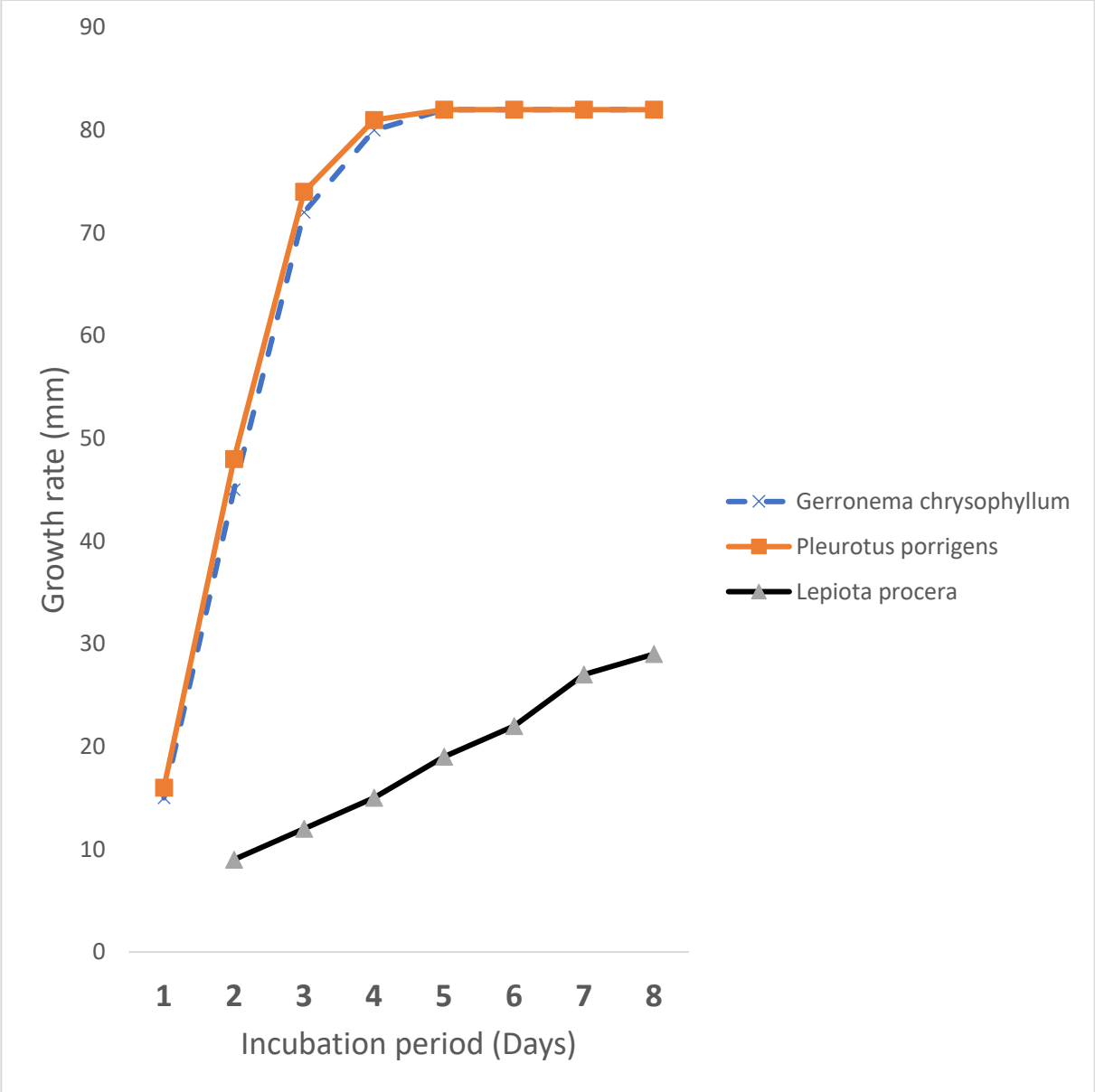


Figure 4: Colony growth rate of isolates on MSA + Cellulose

Table 1: Sensitivity of isolates to antibiotic

Isolates	Streptomycin	Erythromycin
G. chrysophyllum	-	-
P. porrigens	-	-
L.procera	-	-

- **No zone of inhibition**

DISCUSSION

The screening of Nigerian edible mushroom for MnP activity was investigated. The isolates obtained include: *Clitocybe gibba*, *Gerronema chrysophylum*, *Pleurotus porrigens*, *Clitocybe familia*, *Auricularia auricular* and *Lepiota procera*. All the six isolates showed MnP activity in Nutrient broth medium and MSB supplemented with either crude oil or glucose. MnP activity was highest in NB medium and least in MSB supplemented with crude oil (Figure 1). *P. porrigens* had the highest MnP activity in all the medium tested while *L. procera* had the least. Based on high MnP activity and source, three isolates, namely, *P. porrigens* (from wood), *G. chrysophyllum* (from wood)and *L. procera* (from soil)were selected for further studies.

Pleurotus species has been reported to produce significant amount of MnP (Lang, 1998; dos Santos Bazanella*et al.* 2013). Also. In our study likewise, they produced the highest amount of MnP in the three different media studied.

A species of Gerronema, *G. nemorale* hasbeen reported to produce MnP (Antonín*et al*., 2008). According to them, *G. nemorale* produced more of laccase than MnP. In this study, focus was on MnP activity and *G. chyrsophyllum* was the second best in terms of MnP activity.

L. procera isolated from the soil had the least MnP activity. However, some authors have reported Lepoitasp belonging to the Agaricales as one the basiodiomycetes capable of efficient ligninolytic enzyme production (Jayasekara *et al*., 2019). This was not the case in our study and could be as a result of geographical influence.

The growth rate of organisms has a role to play on how rapid they can colonize/penetrate any substrate. Asamudo *et al.,* (2005) reported that the faster the growth rate/mycelia thickness, the higher the rate of mechanical penetration and breaking down of substrate. This leads to higher bioremediation capabilities. In this study, *P. porrigens*and *G. chyrsophyllum* had the highest growth rates in all media studied and *L. procera*, the least (Figures 2, 3 and 4). It was also observed that isolates with high MnP activity also had higher growth rate and vice versa. This suggests that there is a close relationship between peroxidase activity and growth rate. Hence,species with high MnP activities grew faster. This concurs with the report of Xu *et al.*, (2013).

Some soil microorganisms produce antibiotics which affect the functions/activities of other sensitive organisms. All the isolates showed resistance to streptomycin and erythromycin (Table 1). Thus, they can effectively grow and compete with soil microorganisms producing these antibiotics.

CONCLUSION

The result from this study show that Nigerian edible mushrooms have the ligninolytic enzyme system for breaking down recalcitrant molecules found in agrowastes and environmental pollutants, with each species having varying levels of manganese peroxidase activity and growth rate. It would be interesting to extend the screening of these enzymatic activities to other species of the area, characterize the enzymes especially those that presented the best activities like *P. porrigens*, optimize their production, develop their spawns, and apply them in various biotechnological fields. Therefore, they could be potential sources of commercial enzymes for industrial use in bio-bleaching of pulp, decolourization of textile dyes and bioremediation of polluted environment among other uses.

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