Rafidain Journal of Science
https://rsci.mosuljournals.com
Vol. 32, No.2, pp.10-19, 2023

Screening of Conditions for Manganese Peroxidase Production from White Rot Fungi (*Pleurotus djamor*)

Eze C. G.
Department of Science Laboratory Technology (Biochemistry)/ University of Nigeria/ Nsukka

Institute of Biological Environmental and Rural Science/ Aberystwth University/ Wales/ UK.
Eze Martha I.
Medical Center/ University of Nigeria/ Enugu Campus

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Article information

Received: 15/2/2023
Revised: 2/3/2023
Accepted: 9/3/2023

DOI: 10.33899/rjs.2023.178571

corresponding author:
Eze C. G.
eze.gabriel@unn.edu.ng

ABSTRACT

The need for alternative and environmentally friendly methods for waste clean-up has led to the use of enzymes in bioremediation. In this study, white rot fungi were isolated from decaying plant parts using standard microbiological and biochemical techniques. The isolated fungi were identified and screened using standard substrates (2, 6-DMP) to determine their capability to produce manganese peroxidase. Pure manganese peroxidase was achieved after four distinct purification phases. Crude extract of the homogenate proteins obtained through optimized submerged fermentation system were precipitated using ammonium sulfate. Ammonium sulfate used at 60% concentration at pH 4.5 precipitated proteins with highest Manganese peroxidase activity (322 U/mg). The precipitated proteins were desalted through dialysis for twelve hours with buffer exchange at interval of six hours and activity of 343.91 U/mg was recorded afterwards. DEAE-cellulose was used for the ion exchange purification of the dialyzed protein, NaCl gradients of 0.3-0.6 M was found to be best in washing off bound protein from the exchange resin and activity of 434.18 U/mg was recorded from the pooled fraction tubes. Sephadex G-100 was used for separation of the proteins into molecular sizes and weights. 2.8 purification folds of the enzyme were achieved after ion exchange (DEAE-cellulose) and gel filtration (sephadex G-100) with percentage yield of 2.20%. Specific activity of the enzyme increased to 602.00% after gel filtration. The partial purified enzyme was further characterized for determination of optimal pH (4.5), temperature (40 ^{O}C) and kinetic properties K_m of 3.4 mM and V_{max} of 250µmol/min.

Keywords: Manganese Peroxidase, White rot fungi, Ion exchange Chromatography, Gel filtration.

INTRODUCTION

Manganese peroxidase (MnP, EC 1.11.1.13) is an implicated housekeeping enzyme with an oxido-reduction catalytic pattern. The enzyme is characterized with four manganese ions in the allosteric sites and oxidizes its substrates usually reduced phenols or aromatic amines with the evolution of reduced oxygen in the form of water (Eze *et al*., 2010). There are three distinct activity phases of MnP which include: cleavage of covalent bond between the oxygen molecules of H_2O_2 by heme Fe^{+3} resulting in the evolution of the first compound, a ferry iron porphyrin cation radical (Fe^{+4}=O) (Martin, 2002). Compound I then convert to compound II (Fe^{+4}=O) inducing an oxidation of Mn^{+2} to Mn^{+3} by MnP. Compound II triggers the second reduction of $Mn^{+2}$with concomitant conversion of MnP to its original resting state (MnP-Fe^{+3}). pH prevailing in the catalytic initial step is inconsequential in the reaction medium whereas the progress of the reaction is highly dependent on the hydrogen or hydroxyl ion concentrations of the reactor. Time dependent inactivation of the enzyme is brought about high by substrate at pH 3.0-3.5 in which compound II is converted to compound III.

Manganese peroxidase are wide ubiquitous in bacteria, fungi, plants, animals and certain specialized tissues (Mishra *et al*., 2012; Xu *et al*., 2011). Among the microbes, lignocellulosic active basidomycete are active prolific producers of these house-keeping enzymes. In the fermentation system, manganese peroxidases are choicely produced through selected fermentation system of solid matrices; organismal producers of the proteins are said to be exo-secretory within the optimized culture broth (Xu *et al*., 2011).

Effective production of the enzyme in the fermentation system lies hugely on the existing constitution of the media, fermentation prevailing physiological conditions and the organisms' typed strains and adaptation in the fermentation process. Fermentation nutrients for effective production of proteins from organisms are constitutively of carbon, nitrogen and phosphate groups; these macro nutrients are said to catabolite inducers for operon switching during protein productions through various translational and post-translational modification processes (Valerro, 2010). However, most enzyme production are cost implicative, the use of cheap renewable agro-wastes for optimum manganese peroxidase production is a key element for sustainable green chemistry and millennium goal achievements in the field of biotechnology

A more recent interest in this enzyme centers on its stabilization effects on fungal consortium and their active participation in bioremediation of petroleum hydrocarbons in contaminated sites.

MATERIALS AND METHODS

Chemicals, standardized prepared solutions, equipment used in the present study are standardized, products of sigma-Aldrich, Bristol, Merck, Gallehmp wood companies.

The present study was carried out in three distinct stages including: microbial isolations and identifications, enzyme productions through optimizations processes and its assaying.

Decaying wood collection

Decaying plant branches was collected at a bush path located at Ibagwa aka, Nsukka, Enugu state. Collected samples were taken to the laboratory in percolated sample tubes as conducted by Ezeonu *et al*. (2013).

Microbial isolations and identification

Lignocellulosic fungi were isolated from the decaying wood from Ibagwa-aka south L.G.A of Enugu state. Isolation was carried out through microbial culturing on prepared media and identified through biochemical test on the cultured cells as described by Ezeonu *et al.* (2013).

Screening of the isolates for manganese peroxidase production

Identified strains of white rot fungi was analyzed for the production of manganese peroxidase using 2,6 Dimethoxyphenols (DMP) as standard substrate as described by Atalla *et al*. (2017).

Enzyme production and optimization of production parameters

Manganese peroxidase was produced from the lignocellulosic fungi under solid state fermentation system as described by Silva *et al*. (2012). Production manganese peroxidase under

different physiologic parameters such as incubation time, pH, carbon and nitrogen availability was optimized during the production.

Assay for Manganese Peroxidase Activity

Manganese peroxidase activity was determined using the assay protocol described by De Jong *et al*. (1992). 2,6 dimethoxyphenol (2,6 DMP) was used as the standard substrate.

Determination of Protein Concentration

Protein content was determined using the method described by Lowry *et al*. (1951) bovine serum albumen was used as the standard protein.

RESULTS

Microbial Isolation and Molecular Characterization

Pure colonies of *Pleurotus* sp. were obtained using potato dextrose agar culture medium (Plate 1).

Fungal microscopic features under the objectives of a light microscope (100 X) revealed clusters of filamentous fungal mycelium in a safranine stained dye (Plate 2).

Morphology features of *pleurotus* sp. isolates from crude oil contaminated soil (Table 1). Morphological characteristics of the fungi showed that the isolate are: filamentous, spore-forming and non-motile fungi with optimum growth at range 25-40^{o}C.

Electrophoresis of the amplified ITS region from genome of *Pleurotus* showed a typical band at 550 bp as shown in the Fig. (6). *Pleurotus djamor* showed the best evolutionary relatedness based on the phylogenic tree shown in Fig. (1).

Plate 1: Pure strains of *whiterot* fungi isolated from a decaying wood part.

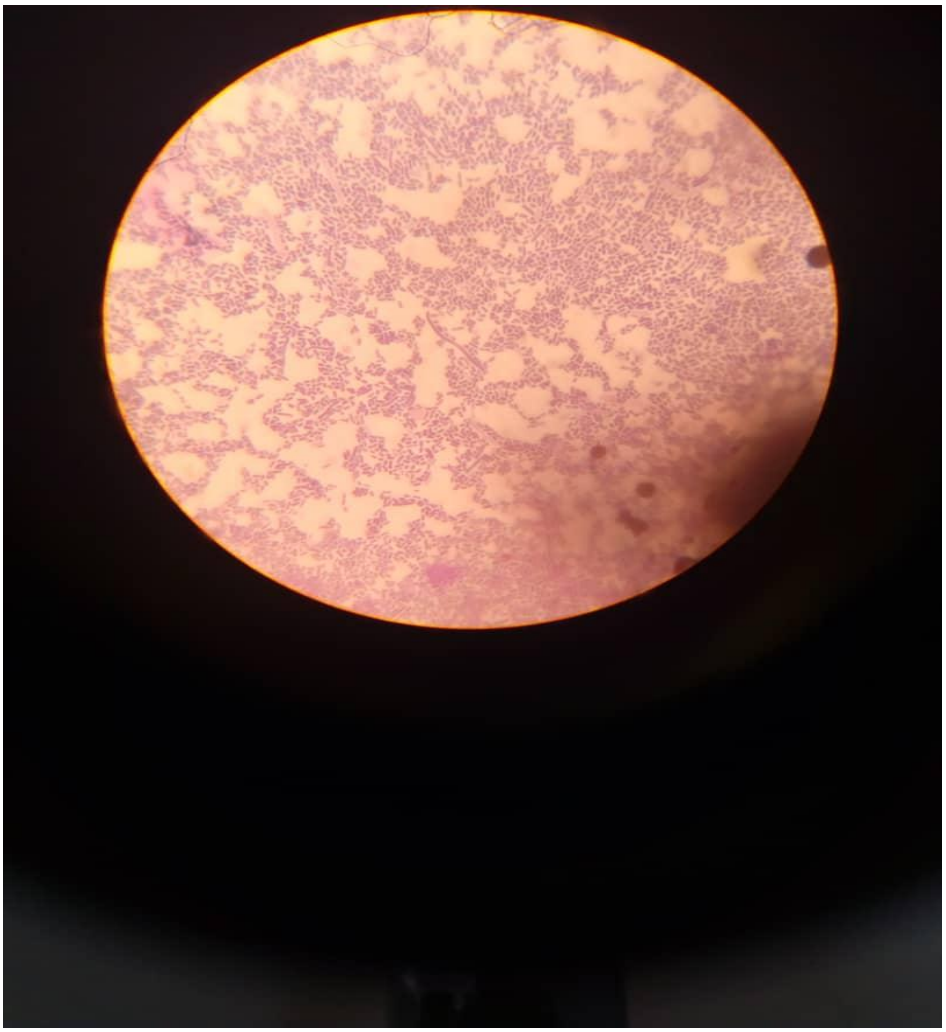

Plate 2: Micrograph of the isolated *whiterot* fungi (40X).

Table 1: Basic Morphology and Biochemical features of the *Whiterot* fungi

Morphology	**Biochemical Tests**
Round	
Sporulating	Starch hydrolysis (+)
Filamentous	
Whitish	
Non-motile	
	Temperature range (37-40 ^{o}C)
	Glucose fermentation (+)

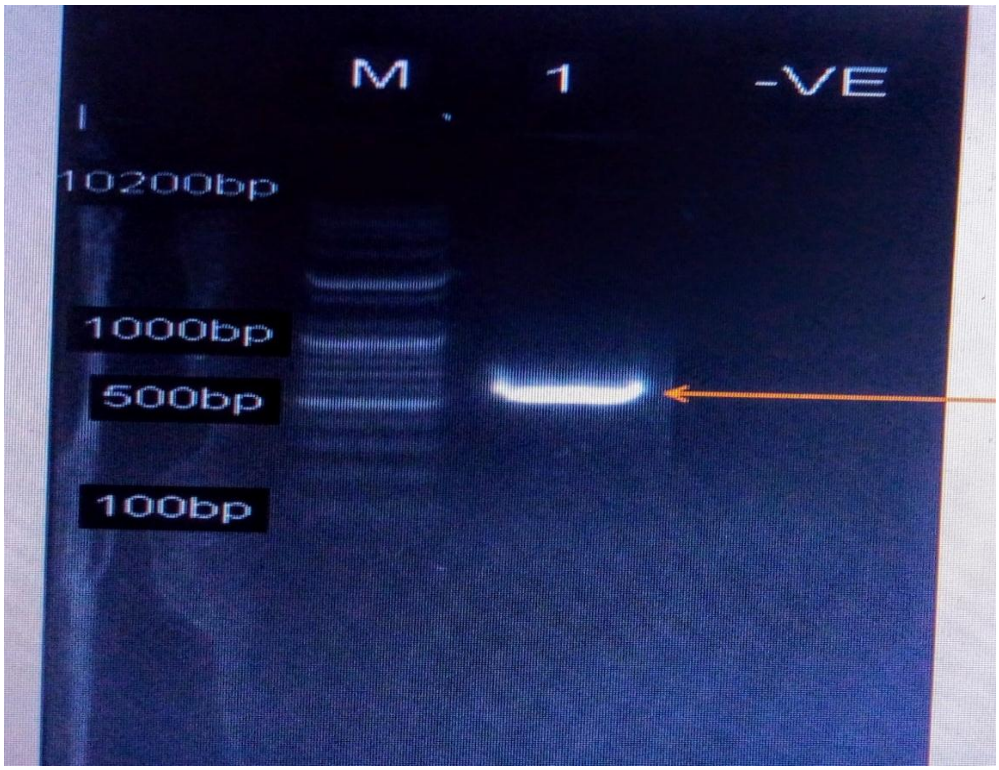

Fig. 1: Electrophoresis of the ITS region from *Pleurotus* sp. genome.

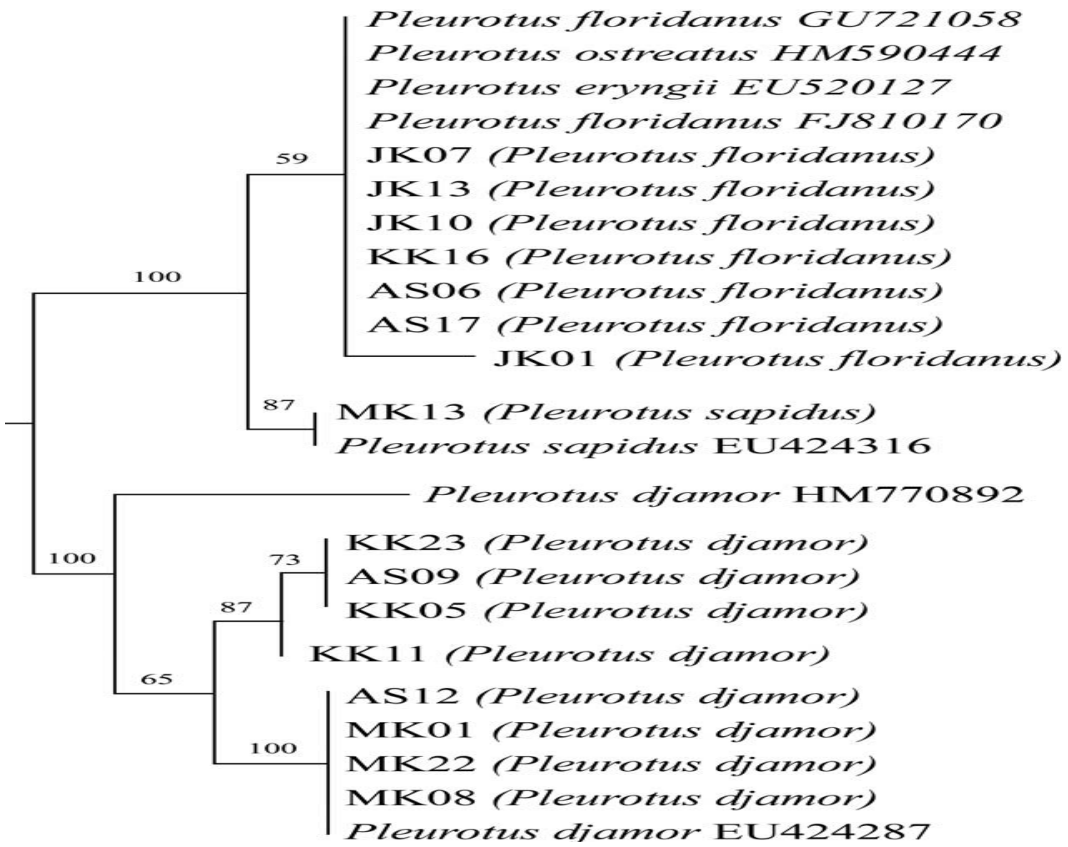

Fig. 2: Phylogenetic evolutionary relatedness of *Pleurotus djamor*.

Screening of the Isolates for Manganese Peroxidase Production

Studies on screening of the fungal isolates for manganese peroxidase production showed that 2,6 DMP infused in the medium colourized to yellow after three days of incubation (plate 3).

Plate 3: Chromogenic screening of the isolates for manganese peroxidase production.

Optimization of Physiological Conditions for Production of Manganese peroxidase

Effect of Incubation Time on Manganese Peroxidase Production

Manganese peroxidase activity from *Pleurotus djamor* activity peaked on day 12 (112.56µmol/min) of the study Fig. (3).

Effect of pH on the Production of Manganese Peroxidase

Fig. (4) shows the impact of pH on the production of manganese peroxidase from *Pleurotus djamor;* pH 6.0 was favorable for optimum MnP production.

Effect of Nitrogen Sources on Production of Manganese Peroxidase

Studies on the effect of nitrogen sources on the production of manganese peroxidase from *Pleurotus djamor* showed that beef extract gave the optimum manganese peroxidase production while combination of ammonium sulfate and beef extract was the least source of nitrogen for the production of manganese peroxidase Fig. (5).

Effect of Carbon Sources on the Production of Manganese Peroxidase

Equi concentration of glucose and wheat bran (0.5% each) gave the best source of carbon for the production of manganese peroxidase while rice bran was antagonistic to the enzyme production.

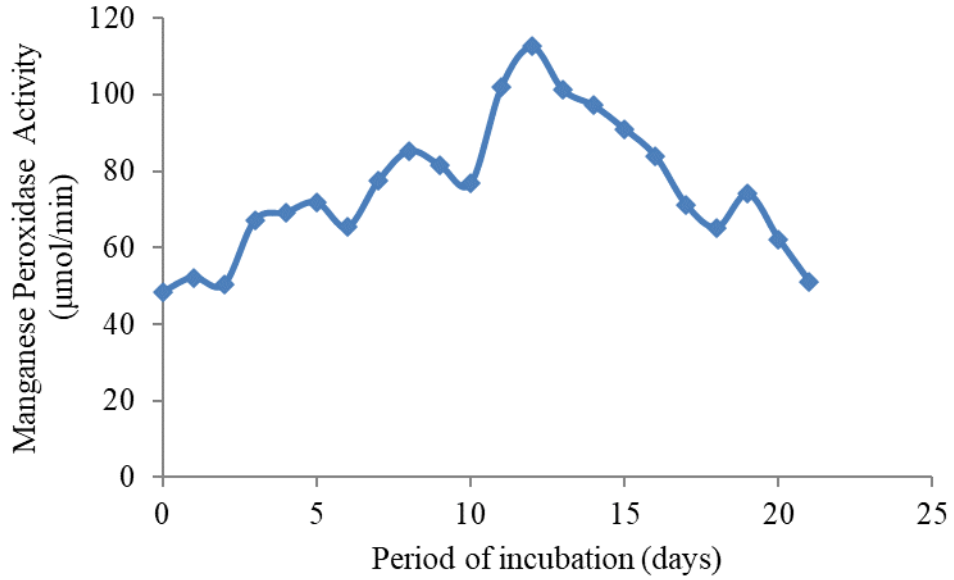

Fig. 3: Effect of nitrogen sources on the production of manganese peroxidase from *Pleurotus djamor*

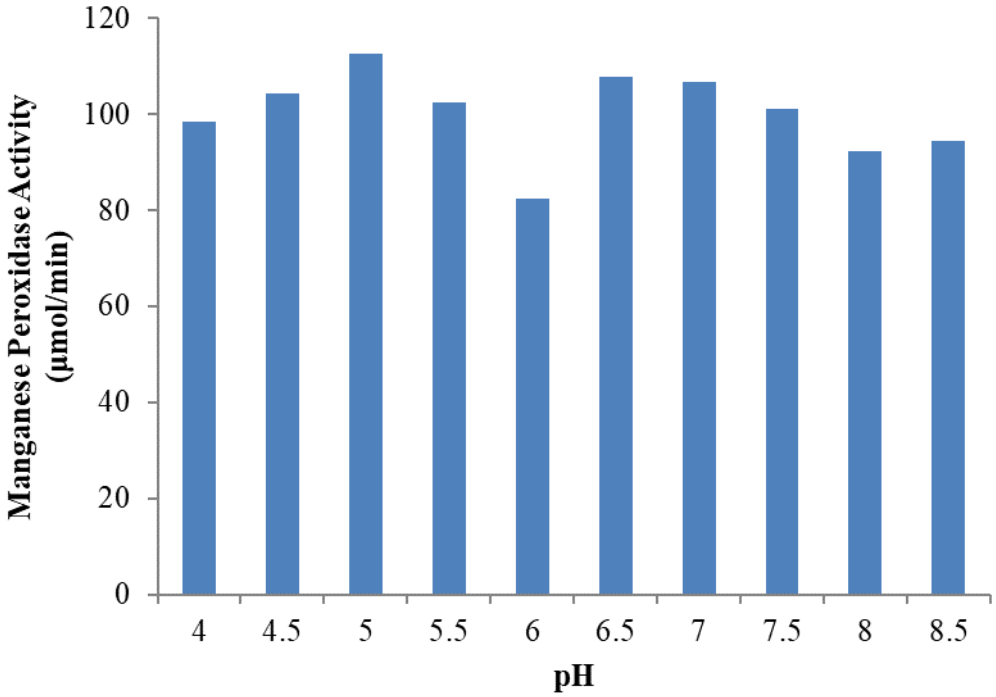

Fig. 4: Effect of pH on the production of manganese peroxidase from *Pleurotus djamor;* manganese peroxidase

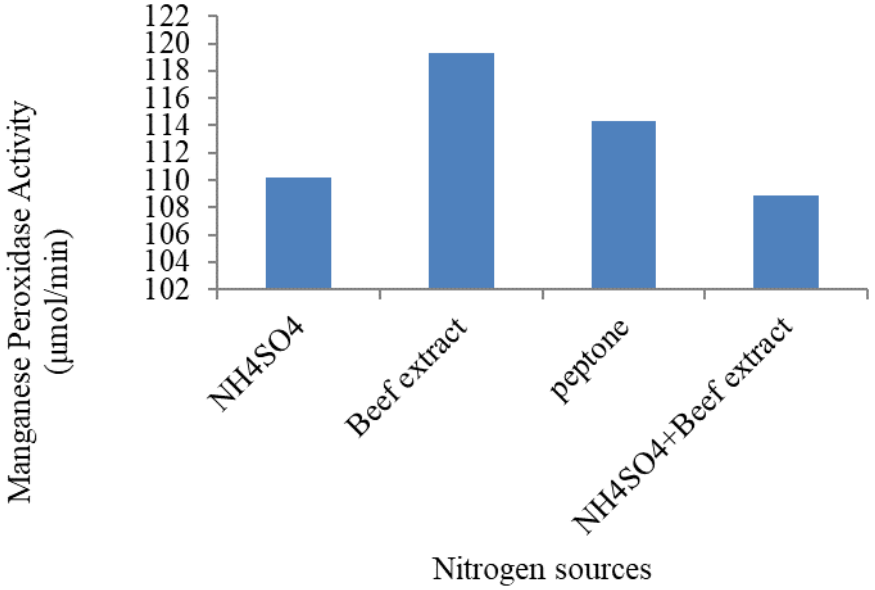

Fig. 5: Effect of nitrogen sources on the production of manganese peroxidase from *Pleurotus djamor*

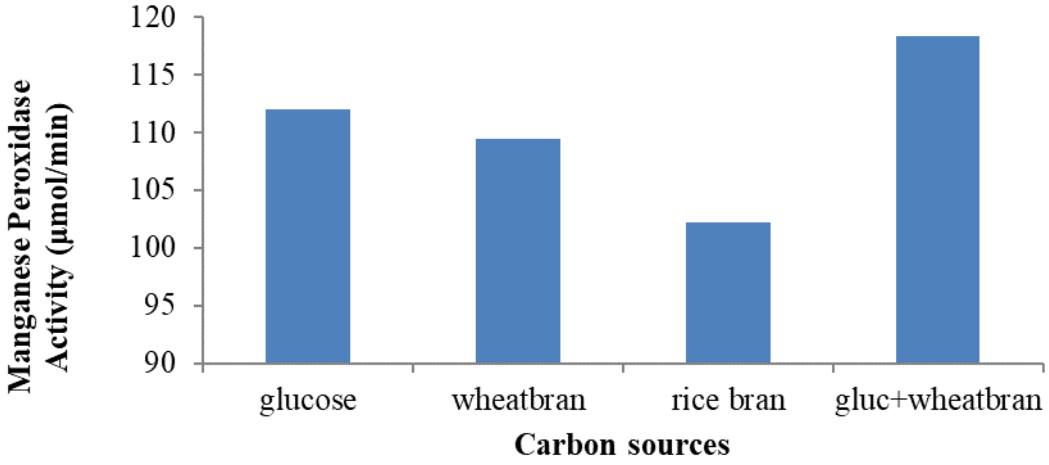

Fig. 6: Effect of carbon sources on the production of manganese peroxidase from *Pleurotus djamor*

DISCUSSION

It is factual to note that all living organism contain protein (enzyme) which without these proteins, their efficiency is drastically undermined. In this present study, *Pleurotus djamor* used for production of manganese peroxidase was isolated from the bark of a decaying wood at Ibagwa-Aka, Igbo-Eze South L.G.A of Enugu State.

Pure colonies of *Pleurotus* sp. were obtained using potato dextrose agar culture medium from the contaminated soil after three days of incubation. Microscopic features of the organism under a light microscope showed clusters of filamentous mycelia however, basic morphological features of the fungi showed that the isolate were: filamentous, spore-forming and non-motile fungi with optimum growth at 35-40^{o}C. These findings corroborate with that of basic manual for organism's isolations and identifications indicated by Ezeonu *et al*. (2013). Results from the electrophoretic clarification of the amplicons showed a typica band at 550 bp. *Pleurotus djamor* was identified after

genomic sequencing of the ITS region and given NCBI accession number of EU24287. Kumar *et al.* (2016) reported that band sizes of 500-700 bp are typical for fungi.

Microorganisms are active cells with clusters of catalytic proteins responsible for their actions (Okpokwasili and Amanchukwu, 1988). 2,6 DMP infused in the fermentation broth showed colour change to yellow after three days of incubation; the changes in the colour shows the activity of the enzyme from the organism to the substrate. 2,6 DMP however served as the better substrate for the enzyme. Juho *et al.* (2012) reported a similar observation, while screening for manganese peroxidase using 2,6 DMP as their standard screening substrate.

Effect of incubation period on the production of manganese peroxidase from *Pleurotus djamor* in a solid fermentation system showed that the manganese peroxidase activity peaked at day 12(112.56µmol/min) of the pilot study. Strains of *Pleurotus djamor* are said to be filamentous in morphology and fastidious in growth (Ezeonu *et al.*, 2013). The afore enlisted properties of the organism culminate in their relative time off front in metabolite productions, evidently seen in the lengthiness involved in manganese peroxidase production. Also, the presence of manganese in the catalytic architecture of the organisms increases the time of the organismal operon switching on and off. Basic biphasic curves were obtained during the production process. pH of 6.0 was optimum for the enzyme production while beef extract and combination of glucose/ wheat bran gave the best nitrogen and carbon sources for the enzyme production respectively. Optimum production of the enzyme at slight acidic range showed moderate tolerant at low pH which also reflected on its catalytic path as a house keeping enzyme in dismutation of oxides, peroxides and superoxides. As reported by Vasiljevic and Jelen, (2001), non-synthetic nitrogen is better in preference for fermenting organisms.

CONCLUSION

This research has demonstrated that *Pleorotus djamor* a basidomycete isolated from decaying wood can be a potential and sustainable source of manganese peroxidase which stands to be an integral part of evolving eco-toxicological sensors and biotechnological industries.

Funding Information

Eze Chukwuebuka G. of Department of Science laboratory technology (Biochemistry), UNN wholly funded the financial and otherwise expenses of the present work.

Conflict of Interest

There are no conflicting issues from the authors as regards to this article.

Consent to Participate

This is not applicable to the study.

Consent to Publish

This is not applicable to the article.

Author's Contributions

Eze C.G: Conceived and designed the experiments, performed the experiment and processed the data, analyzed the data and wrote the manuscript.

Eze M.I.: Guided the experimental design, performed the experiment and processed the data.

Oparaji, E.H: Analyzed the research design and methodology, interpreted the data and revised the manuscript.

Ethics

Authors declared no ethical issues that may arise after the publication of this manuscript.

Availability of Data and Materials

This is not applicable to this article.

REFERENCES

Atalla, M.; Zeinab, H.; Eman, R.; Amani, A.; Beer, A. (2010). Screening of some marine derived fungal isolates for lignin degrading enzymes production. *Agricult. Biol. J. North America*, **1**, 591-599.

Chilaka, F.; Nwachukwu, A.; Uvere, P. (2002). Thermal stability studies of β –galactosidase from germinating seeds of the brown beans, *Vigna unguiculata*. *Nigerian J. Biochem. Molec. Biol.*, **17**(1), 51-56.

Chikere C.; Okpokwasili, G.; Chikere, B. (2006). Bacterial diversity in typical crude oil polluted soil undergoing bioremediation. *African J. Biotechnol.,* **8**, 2535-2540.

Eze, S.O.O.; Chilaka, F.C.; Nwanguma, B.C. (2010). Studies on thermodynamics and kinetics of thermo-inactivation of some quality-related enzymes in White Yam (*Dioscorea rotundata*). *J. Thermodyn. Catalys.*, **1**, 104.

Dede, A. P. O.; Akpaja, E. O.; Galillee, J. E. (2011). Effect of pH on the growth of the white root rot pathogen, *Rigidoporus lignosus* (Klotzsch) Imazeki, on selected para rubber sustaining soils in Nigeria. *African Scient.*, **12**, 3.

De Jong, E.; Field, J.; de Bont, J. (1992). Evidence for a new cellular peroxidase manganese inhibited peroxidase from the white rot fungus *Bjerkandera* sp. Bos 55. *FEBS letters*., **299**, 107-110.

Ezeonu, M.; Okafor, J.; Ogbonna, J. (2013). "Laboratory Exercises in Microbiology". 1st ed. Ephrata Publishing and Printing Company, Nsukka. Pp. 100-117.

Haluk, E.; Khawar, S.S.; Julia, M.; Tim, C.; Ricardo, C. (2012). Kinetic and thermodynamic characterization of the functional properties of a hybrid versatile peroxidase using isothermal titration calorimetry: Insight into manganese peroxidase activation and lignin peroxidase inhibition. *Biochim.,* **94**, 1221-1231.

Hiner, A.; Rodrguez-Lopez, J.; Garcia-Canovas, F.; Brisset, N.; Smith, A.; Arna, M.; Acosta, M. (2002). Reactions of the class II peroxidases, lignin peroxidase and *Arthromyces ramosus* peroxidase with hydrogen peroxide. *J. Biolog. Chem.*, **277**, 26879–26885.

Jiménez-Cedillo, M.; Olguín, M.; Fall, C.; Colin-Cruz, A. (2013). As (III) and As (V) sorption on iron-modified non-pyrolyzed and pyrolyzedbiomass from *Petroselinum crispum* (parsley). *J. Environm. Manag.*,**117**, 242–252.

Juho, J.; Sanna, T.; Ritva, I.; Heikki, O. (2012). Screening of white-rot fungi manganese peroxidases: a comparison between the specific activities of the enzyme from different native producers. *AMB express*. **2**, 62.

Lowry, O.; Roseburg, N.; Farr, A.; Randall, R. (1951). Protein measurement with Folin- Phenol reagents. *J. Biolog. Chem.,* **93**, 265-275.

Marin-Rangel, V.; Cortes-Martines, R.; Villanueva, R.; Garnica - Romo, M.; Martinez-Flores, H. (2012). As (V) biosorption in an aqueous solution using chemically treated lemon (Citrus aurantifolia swingle) residues. *J. Food Sci.*,**71**, 10–14.

Martin, H. (2002). Review: lignin conversion by manganese peroxidase (MnP). Enzyme and *Microbial. Technol.,* **30**, 454 – 466.

Mishra, V.; Balomajumder, C.; Agarwal, V. (2012). Kinetics, mechanistic and thermodynamics of Zn (II) ion sorption: a modeling approach. *Clean – Soil Air Water*., **40**, 718–727.

Matheiekal, J.; Yu, Q. (1999). Biosorption of lead (II) and copper (II) from aqueous solution pretreated biomass of Australian Marine algae. *Bioresource Technol.,* **69**, 223-229.

Silver, T.; Attilis Angelis, D.; Carvalho, A.; Silva, R.; Boscolo, M.; Gomes, E. (2005). Production of saccharogenic and dextrinogenic analyses by *Rhizomucor pussillus*. *J. Microbiol.*, **43**(6), 561-568.

Singh, R.; Kumar, V.; Kapoor, V. (2014). Partial purification and characterization of a heat stable α-amylase of thermophilic *Actinobacter*, *Streptomyces* sp. MSC 702. *Enzyme Research*: 1-8.

Song, J.; Xu, J.; Zhao, P.; L, Lu; Bao, J. (2011). A hydrogen peroxide biosensor based on direct electron transfer from hemoglobin toan electrode modified with Nafion and activated nanocarbon. *Microchim. Acta*, **172**(1), 117–123.

Volesky, B. (2010). Advances in biosorption of metals-selection of biomass types. *FEMS Microbial. Review,* **14**, 291-302.

Xu, X.; Zhang, T.; Wan; Zhang, C. (2011). A third-generation hydrogen peroxide biosensor based on horseradish peroxidasecross-linked to multi-wall carbon nanotubes. *Microchim. Acta*, **172**(1), 199–205.