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A BIOTECHNOLOGICAL APPROACH ON SCREENING AND PRODUCTION OF MANGANESE PEROXIDASE BY *FUSARIUM SP* FROM ARECA NUT HUSK

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ABSTRACT

The family of extracellular ligninolytic enzymes typically includes lignin peroxidases laccases, manganese peroxidases, versatile peroxidases and other accessory enzymes. Out of these enzymes MnP is thought to play the most crucial role in lignin degradation. Manganese peroxidase, an ubiquitous enzyme among ligninolytic fungi, is an extracellular heme-containing enzyme known to catalyze the oxidation of Mn2+ to Mn3+ in a reaction requiring appropriate manganese chelator. This enzyme catalysis the oxidation of Mn2+ to Mn3+, which in turn can oxidize a variety of phenolic substrates, including lignin model compounds Six *Fusarium sp* were isolated from soil samples from different regions in Bangalore. Isolated fungal strains were screened for Manganese peroxidase production by plate assay. *Fusarium sp* NPSV 01 were selected as best Manganese peroxidase producer (9 mm). The *Fusarium sp* NPSV 01 were employed for further fermentation kinetics studies through solid state fermentation by using areca nut husk. The different pH (6), temperature (30^0C) and inoculum size (1.25 ml) were optimized and it showed 0.381U/ml of manganese peroxide activity.

KEYWORDS: Manganese peroxidase, solid state fermentation, Areca nut husk, *Fusarium* and Plate assay. phytoconstituents, isolation, characterization.

1. INTRODUCTION

Plant cell walls have lignin in their structure as the most abundant component (Patrica lopes de oliveira et al., 2009). Lignin is a heterogeneous, branched and complex polymer consisting of phenylalanine-derived aromatic Subunits2. Lignin is degraded by a narrow array of microbes4. Fungal attack on lignin is attributed to certain secreted nonspecific oxidoreductases, which produce low molecular weight mediators able to intrude recalcitrant biopolymers (Juho Jarvinen *et al*., 2012).

The family of extracellular ligninolytic enzymes typically includes lignin peroxidases (LiP, EC 1.11.1.14), laccases (EC 1.10.3.2), manganese peroxidases (MnP, EC 1.11.1.13), versatile peroxidases (VP, EC 1.11.1.16) and other accessory enzymes. Out of these enzymes MnP is thought to play the most crucial role in lignin degradation (Juho Jarvinen *et al*., 2012)

Manganese peroxidase (EC 1.11.1.13), an ubiquitous enzyme among ligninolytic fungi, is an extracellular heme-containing enzyme known to catalyze the oxidation of Mn2+ to Mn3+ in a reaction requiring appropriate manganese chelator. This enzyme catalysis the oxidation of Mn2+ to Mn3+, which in turn can oxidize a variety of phenolic substrates, including lignin model compounds (Raziye Ozturk Ureka et al., 2005). Ligninolytic enzymes have a potential in several industrial and biotechnological processes within a wide variety of organic and inorganic substrate specificities. Such applications include the detoxification of industrial effluent, mostly from, textile and petrochemical industries, bleaching and delignification processes in the paper and pulp industries, removing the phenolic compounds from the beer and wine in the food industry. In addition, their capacity to remove xenobiotic substances and produce polymeric products makes them a useful tool for bioremediation purposes,(Emre Erden et al.,2009). Ligninolytic cultures as well as their enzymes have been reported to degrade and decolorize various dyes. (Pradeep Verma and Datta Madamwar, 2002)More recently, the production of ligninolytic enzymes including MnP was demonstrated in *Phyllosticta*, *Aspergillus*, *Fusarium*, and *Penicillium*. (Pradeep Verma and Datta Madamwar, 2002) The present study deals with isolation screening and production of Manganese peroxidase by solid state fermentation using Areca nut husk from *Fusarium sps*.

2. MATERIALS AND METHODS

2.1 Isolation and screening of manganese peroxidase producing fungi

The fungal strains were isolated from different soil samples which were collected from different places in

and around of Bangalore city on Czapek dox agar medium containing (g/L of distilled water)Sucrose-30g; Potassium Hydrogen Phosphate (KH2PO4)-02g; Potassium Chloride (KCl)-0.52; Magnesium sulphate (MgSO4.7H2O)-0.52g; Iron sulphate (FeSO4.7H2O)-0.01gtrace and agar-20g. The fungal strains were periodically subcultured on czapek dox agar and preserved at 4^{o}c.

Screening for manganese peroxidase producing fungi was carried out by inoculating fungal strain onto czapek dox agar medium supplemented with 0.2% tannic acid and incubated at 30^{o}c for 5 days as described by Anuja Sharma *et al* (2017).

2.2 Identification of manganese peroxidase producing fungi

Manganese peroxidase producing fungi was identified microscopically using lacto phenol blue stain at 40x magnification as described by Geethanjali and Jayashankar.(2017)

2.3. Production of manganese peroxidase

The Manganese Peroxidase was produced by solid state fermentation medium containing 10g of sterilized and inoculated and medium was incubated for 5days, after the incubation period 200ml of distilled water was added to the fermentation medium and subjected to filtration using Whatman No.01 filter paper the filtrate was centrifuged at 5000rpm for 15 minutes to obtain crude enzyme extract and for further studies.

2.4 Inoculum preparation

The fungal spore suspension was prepared from a 4 day old culture grown on Czapek dox agar slants. 10ml of 20% tween -80 solution was added and scraped with loop to suspend spores. The spore suspension was stored in screw cap bottles for further use.

2.5 Optimization of moisture content for the production of mnp

The substrate areca nut husk were used for production of Manganese peroxidase by varying the moisture content of the fermentation medium from 35% - 65%, and the medium was incubated at 30^{o}C. Manganese peroxidase activity was checked every 24 hours upto 120 hours.

2.6 Optimization of ph for the production of mnp

Keeping optimized moisture content constant, the optimization of pH was studied by manganese peroxidase production for 5 days by varying the pH of the medium from 3.0 to 9.0.

2.7 Optimization of Temperature for the Production of MnP

With the optimized moisture content and pH, Manganese peroxidase production was studied by optimizing temperature of the fermentation medium at variable temperature conditions from 25^{o}C - 40^{o}C

2.8 Optimization of inoculum size for the production of mnp

The inoculum was prepared by 168h freshly prepared culture of *Fusarium* sp at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 mL and then inoculated and fermentation studies were carried out. As it was mentioned before, the media was extracted during every condition of pH or incubation temperature or inoculum size to prepare crude enzyme for each and was used to assay the Manganese peroxidase activity.

2.9 Manganese peroxidase assay

Manganese peroxidase assay was performed as described by Ayyappa et.al (2016) using guaiacol as a substrate. The reaction mixture consists of 0.5M sodium tartrate buffer 0.5ml, 0.5ml enzyme, 0.5ml 1mM guaiacol substrate, 1ml 1mM manganous sulphate, the reaction mixture was incubated for 5 minutes at room temperature after the incubation period 1Mm hydrogen peroxide was added to the reaction mixture and the absorbance was measured after 1minute at 465nm.

Unit: One unit of Manganese peroxidase activity is the activity of enzyme that catalyzes the conversion of 1µm of guaiacol per minute.

3. RESULT AND DISCUSSION

3.1 Isolation of fungi

In the present study, six potent fungal strains were designated, out of six isolates were isolated from soil samples collected at varying environmental stress conditions. They were isolated from in and around Bengaluru Karnataka, India. The eco-stressed soil was selected as a source for isolation of fungal Manganese peroxidase producers for current studies. In the present study soil samples were collected from in and around Bangalore, six fungal isolates were obtained from the soil, the isolates were designated as NPSV 01, NPSV 02, NPSV 03, NPSV 04, NPSV 05, and NPSV 06. The fungal strain *Fusarium sp* NPSV was identified microscopically as species of *Fusarium* by lacto phenol cotton blue staining.(Figure-1)

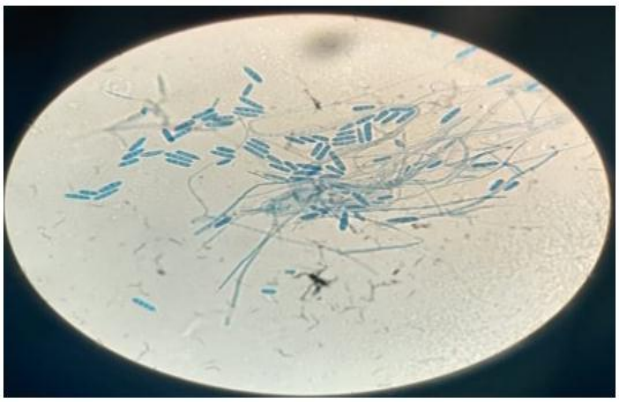

Plate-1:Microscopic view of NPSV01 strain.

3.2 Screening for manganese peroxidase producing fungi

All the six isolates NPSV 01 to NPSV 06 were inoculated on to czapek dox agar medium supplemented with 0.2% Tannic acid, out of six isolates NPSV 01 was potential towards the production of manganese peroxidase by the formation of clear zone around the colony with 9mm diameter. Figure 2 represents the result of NPSV 01 strain towards screening of manganese peroxidase enzyme. The remaining strains of *Fusarium sp* NPSV02-NPSV 06 were also showed manganese peroxidase activity in moderate range.

Figure-2: Plate assay.

3.3 Optimization of moisture content for the production of manganese production

The results obtained in the present study on the effect of moisture content in solid state fermentation on Manganese peroxidase production by *Fusarium* NPSV 01 is represented in Figure-3. It reveals that the Manganese peroxidase production with the increasing moisture 40% to 60% and then further increase in moisture content caused the declining of Manganese peroxidase. These increasing peaks were observed up to 96 hours of fermentation period and thereafter the decreased yield as fermentation period increased. Maximum manganese peroxidase activity of 0.35 U/ml was obtained at 60% moisture using NPSV 01 fungal strain under solid state fermentation using areca nut husk as substrate. Muhammed Irshad et al (2011). carried out solid state fermentation using banana stalks from *schizophyllum commune* IBL-06 found optimum moisture content is 60% for the production MnP, which similarly correlates to our work.

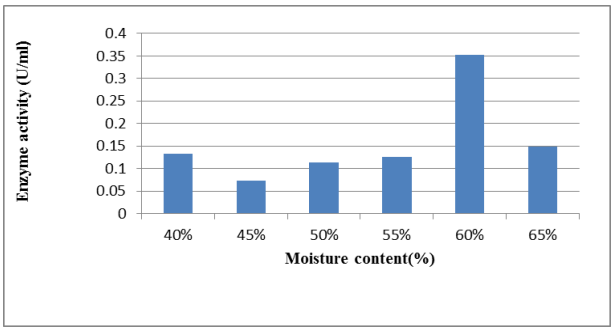

Figure-3: Effect of moisture content on manganese peroxidase production.

3.4 Optimization of pH for the Production of Manganese production

The results obtained in the present study on the effect of initial pH in solid state fermentation fermentation of Manganese peroxidase production by *Fusarium* NPSV 01 is represented in Figure-4. It reveals that the Manganese peroxidase production with the increasing of pH of the medium from pH 3.0, up to pH 6.0 and then further increase in initial pH caused the declining of Manganese peroxidase yield. These increasing peaks were observed up to 96 hours of fermentation period and thereafter the decreased yield as fermentation period increased. The optimum production of manganese peroxidase under solid state fermentation using areca nut husk as substrate with the activity 0.380U/ml was obtained at pH 6.0 for 96 hours of fermentation period. The least manganese peroxidase activity was obtained at pH 9.0. Rajan *et. al* (2010), and Geethanjali and Jayashankar (2017), were reported on optimum pH is 6.0 for the production of manganese peroxidase using areca nut husk as a substrate from *Phanerochaete chrysosporium* and *Fusarium Oxysporium* respectively.

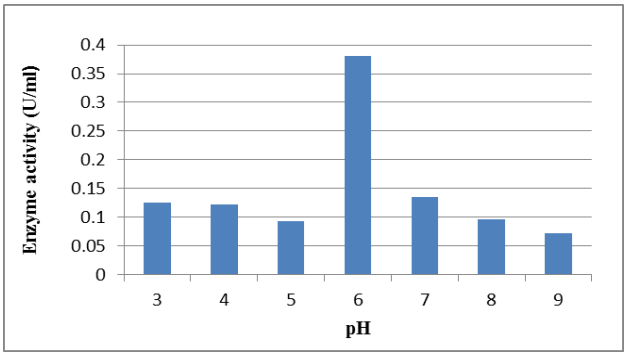

Figure-4: Effect of pH on Manganese peroxidase production.

3.5 Optimization of Temperature for the Production of Manganese production

The results obtained in the present study on the effect of temperature in solid state fermentation of Manganese peroxidase production by *Fusarium* NPSV 01 is represented in Figure-5. It reveals that the Manganese peroxidase production was increased along with the increase of temperature of the medium from 25^{0}C, up to temperature 30^{0}C with optimized constant pH of 6.0. These increasing peaks were observed up to 96 h of fermentation period and thereafter the decreased yield as temperature levels and fermentation period increased.

The maximum production of managanese peroxidase under solid state fermentation, 30 ^{0}C was proven to be optimum with 0.364U/ml activity. The least Manganese peroxidase activity was obtained at temperature 40^{0}C. Akhila et al, (2010) the optimum temperature required for the production of manganese peroxidase from areca nut husk using *Phanerochaete chrysosporium* is 30 ^{0}C and according to Geethanjali and Jayashankar (2017), optimum temperature is 30^{o}c for manganese peroxidase production from areca husk using *Fusaium Oxysporium.*

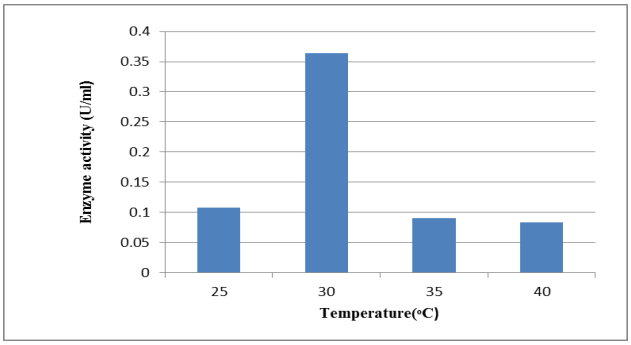

Figure-5: Effect of temperature on Manganese peroxidase production.

3.6 Optimization of inoculum size for the production of manganese peroxidase

The importance of inoculum size on microbial fermentation process is widely accepted. The results obtained in the present study on the effect of inoculum size in submerged fermentation of manganese peroxidase production by *Fusarium* NPSV 01 is represented in Figure-6. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 mL), 1.25 mL inoculum was found to be the most suitable for high production of manganese peroxidase by *Fusarium* NPSV 01 in solid state fermentation at 72 h of fermentation and it showed 0.381U/ml. It is clear that the manganese peroxidase production steadily increased with the increasing in the size of the inoculum until it reaches to the magnitude when enzyme productivity became maximum, thereafter no appreciable change in production of manganese peroxidase with high inoculum size could be observed. Sudha Hariharan and Padma Nambishan (2017) were carried out manganese peroxidase production using pineapple leaf from *Ganoderma lucidum* with 4ml of inoculum volume.

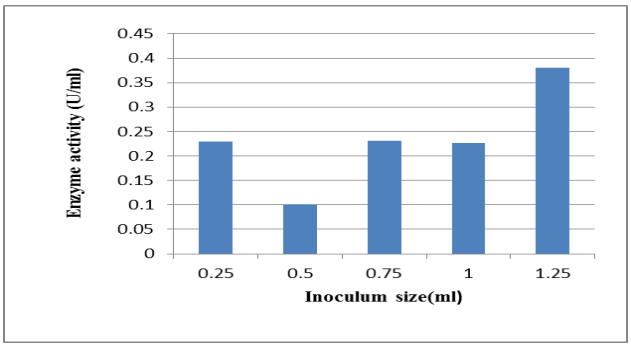

Figure-6: Effect of inoculum size on manganese peroxidase production.

4. CONCLUSION

From the present study, it was understood that solid state fermentation of agricultural husk (Areca nut husk) used as substrate will result in good yield of manganese peroxidase enzyme. The cost-effective technologies are needed for the production of enzyme and solid state fermentation is a suitable technology for economical production of manganese peroxidase using agrohusk residues as substrate. Areca nut husk fibre used as substrate for the production of manganese peroxidase using *Fusarium species* was found to be effective. Major parameters affecting the fermentation process for enzyme, production were studied and optimal levels were identified. Moisture content 60% , pH 6.0 ,temperature 30^{o} C and inoculum size 1.25ml was found to be suitable for the fungal organism *Fusarium* to produce manganese peroxidase at high rate. It is concluded from the findings that the strategy to produce manganese peroxidase from areca nut husk was successful.

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