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Production of Laccases by Fungi Isolated from Soil in Sokoto, Nigeria

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Abstract A research was carried out on production of laccases by fungi isolated from soil within Sokoto, Nigeria. Soil samples were obtained from saw mill and were immediately transported to mycology laboratory for analysis. Carboxyl methyl cellulose (CMC) was applied on the media at different concentrations of 0.5g and 1g. on the media The fungi fungal isolates that can produce laccase were identified as *Rhizopus oryzae, Fusarium sporotrichioides* and *Absidia corymbifera.* From the results obtained, *Rhizopus oryzae* had the highest percentage frequency of occurrence of 50% followed by *Fusarium sporotrichioides and Absidia corymbifera* had with 12.5% each. *Rhizopus oryzae* and *Fusarium sporotrichioides* had a diameter of zone of inhibition of 43mm vertical and horizontal using both 0.5g and 1g carboxyl methyl cellulose (CMC). *Absidia corymbifera had* 17mm horizontal, 14mm vertical using 0.5g CMC and 18mm horizontal, 16mm vertical using 1g CMC. On the basis of the results found, it can be concluded that laccases can be produced by fungi isolated from the soil in our environment.

Keywords Laccases, Fungi, Production, Soil

1. Introduction

Laccases are multicopper enzymes belonging to the group of blue oxidases that use molecular oxygen to oxidize various aromatic and non-aromatic compounds by a radical-catalyzed reaction mechanism (Morozova *et al.,* 2007). The enzymes are involved in the pathogenicity, immunity and morphogenesis of organisms and in the metabolic turnover of complex organic substances such as lignin or humic matter (Hattaka *et al.,* 2001). Owing to their high non specific oxidation capacity, laccases are useful biocatalysts for diverse biotechnological application. Phylogenetically, laccases are members of the multi-copper protein family including ascorbate oxidase, ceruloplasmin and bilirubin oxidase. Because it belongs to the oxidase enzyme family, it requires oxygen as a second substrate for the enzymatic action. Laccase was first studied by Gabriel Bertrand in 1894.

Laccases play a role in the formation of lignin by promoting the oxidative coupling of monolignols, a family of naturally occurring phenols. Others such as ones produced by the fungus *Pleurotus ostreatus* play a role in the degradation of lignin and can therefore be included in the broad category of ligninases (Condi, *et al.*, 2007). They are widely distributed in higher plants, bacteria, fungi and insects. In plants, laccases are found in cabbages, turnip, potatoes, pears, apples and other vegetables.They have been isolated from Ascomyceteous, Deuteromyceteous and Basidiomyceteous fungi to which more than 60 fungal strains belong (Gianfreda *et al.,* 1999).

The white-rot Basidiomycetes fungi efficiently degrade lignin in comparison to Ascomycetes and Deuteromycetes which oxidize phenolic compounds to give phenoxy radicals and quinine (Eggert *et al.,* 1996). In the recent years, these enzymes have gained application in the field of textile, food industry, paper and pulp industry, synthetic chemistry, cosmetics, biofuel cells, as a medical diagnostics tool, soil bioremediation and biodegradation of environmental phenolic pollutant and removal of endocrine disruptors (Couto *et al.,* 2006). These enzymes are used for pulp delignification, pesticide or insecticide degradation, organic synthesis (Faccelo *et al.,* 2008), waste detoxification, textile dye technological uses and biosensor and analytical application. In Nigeria, little or no effort has been made to improve the detoxification of environmental pollutants. This research work is therefore aimed at isolating and producing laccase enzyme from soil in our local environment to improve the detoxification of environmental pollutants.

2. Materials and Methods

2.1. Sample Collection

A total of eight (8) samples of saw dust were collected

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from four different areas designated as; A3i, A3iii, B3iii, C3iii, C3ii, D3i, D3ii and D3iii. The samples were collected in clean polyethene bags and were immediately transported to Mycology laboratory of Usmanu Danfodiyo University Sokoto for analysis.

2.2. Serial Dilution

One gram of each sample was diluted three (3) times in sterile physiological solution. Serial dilution was carried out in accordance with the procedure of Cheesbrough, (2000).

2.3. Isolation of Fungi

The Suspension obtained after vigorous mixing of the of soil samples were left to stand for 30 mins. 0.5ml of different aqueous dilutions of soil suspensions were inoculated on Sabouraud Dextrose Agar plates. to which Streptomycin was had been incorporated to reduce bacterial contamination. The inoculated plates were incubated upright at 25°C for 5 days. All observed colonies were subculture to obtain and pure cultures were obtained.

2.4. Subculture

The Colonies from the plates were observed for morphological difference. Each colony that differs morphologically from another was picked with a sterilized inoculating needle and inoculated on a freshly prepared Sabouraud Dextrose agar plates and incubated at 25°C for 3-5 days in order to obtain pure colonies of the isolates.

2.5. Identification of Fungi

The growth rate, colour, texture, colonial morphology and diffusible pigmentation of each sample were examined macroscopically. Tease mount using lactophenol cotton blue was adopted and microscopic features such as spore and hyphae morphology were observed and compared with the standard colored atlas as described by of Cheesbrough, (2000).

2.6. Production of Laccase

A total of five (5) fungal strains were inoculated onto the plates containing plain agar with the following composition ($g.l^{-1}$): peptone 3g, $K_2PO_{4\ 0.4g}$, $MgSO_4$ 0.5g, $ZnSO_4$ 0.001g, $FeSO_4$ 0.005g, $MnSO_4$ 0.5g and Carboxyl methyl cellulose (CMC) 0.5g and 1g respectively. The plates were incubated at 30°C for 5 days.

3. Results

A total number of five (5) species of fungi were isolated. Table 1indicated the species of fungi isolated from the soil sampled. Table 2 indicated the results of fungal isolates producing laccase. The species include *Aspergillus niger, Absidia corymbifera, Aspergillus flavus, Fusarium sporotrichioides* and *Rhizopus oryzae*. 3 were laccase positive and 2 laccase negative. Table 3 showed the percentage frequency of occurrence of fungal isolates. From the results, it was observed that *Rhizopus oryzae* had the highest frequency of occurrence (50%) and *Fusarium sporotrichioides, Aspergillus niger*, *Aspergillus flavus* and *Absidia corymbifera* had 12.5% each.

Table 4 showed the measurement of diameter of zone of inhibition of the organism. Carboxyl methyl cellulose (CMC) was used at different concentrations of 0.5g and 1g. From the result, *Rhizopus oryzae* and *Fusarium sporotrichioides* had 43mm vertical and horizontal using both 0.5g and 1g CMC. *Absidia corymbifera had* 17mm horizontal, 14mm vertical using 0.5g CMC and 18mm horizontal, 16mm vertical using 1g CMC.

Table 1. Identification of Fungal Isolates from Soil

Isolates	Colony Description	Microscopy	Identified Organism
A_{3i}	It is white and colony with aerial growth	It has pigmented rhizoids and sporangiophore and numerous stolons	*Rhizopus oryzae*
A_{3iii}	It is white and colony with aerial growth	It has pigmented rhizoids and sporangiophores and numerous stolons	*Rhizopus oryzae*
B_{3iii}	It is white and colony with aerial growth	It has pigmented rhizoids and sporangiophores and numerous stolons	*Rhizopus oryzae*
C_{3iii}	It is black in colour and powdery, the reverse is yellow	The conidiophores terminate is vessels and the conidia are in chains	*Aspergillus niger*
C_{3ii}	It is green in colour and powdery	They have aerial hyphae bearing conidiophores	*Aspergillus flavus*
D_{3i}	Colony whitish and Aerial mycelium floccose or cottony	Conidiophores branched, formed in aerial mycelium bearing cylindrical phialides which often proliferate sympodially	*Fusarium sporotrichioides*
D_{3ii}	It is white and colony with aerial growth	It has pigmented rhizoids and sporangiophores and numerous stolons	*Rhizopus oryzae*
D_{3iii}	Colony floccose, light greyish and it covered the whole petri-dish	It has occasional septum often terminating in a large sporangium. Rhizoids borne on a swollen area of the stolon	*Absidia corymbifera*

Table 2. Fungal isolates producing laccase

Fungal isolates	Production of laccase
Rhizopus oryzae	+
Aspergillus niger	-
Aspergillus flavus	-
Fusarium sporotichioides	+
Absidia corymbifera	+

Key + = laccase positive
- = laccase negative

Table 3. Percentage frequency of occurrence of fungal isolates (%)

Identified Organism	Number of Organisms	Frequency (%)
Rhizopus oryzae	4	50
Aspergillus niger	1	12.5
Aspergillus flavus	1	12.5
Fusarium sporotichioides	1	12.5
Absidia corymbifera	1	12.5

Table 4. Diameter of zone of inhibition of the organism (mm)

Organism	CMC (g)	Diameter of zone of Inhibition (mm)
Rhizopus oryzae	0.5	43 vertical & horizontal
Rhizopus oryzae	1	43 vertical & horizontal
Fusarium sporotrichioides	0.5	43 vertical & horizontal
Fusarium sporotrichioides	1	43 vertical & horizontal
Absidia corymbifera	0.5	17 horizontal, 14 vertical
Absidia corymbifera	1	18 horizontal, 16 vertical

Key: CMC=Carboxyl methyl cellulose

4. Discussion

A total number of five (5) species of fungi were isolated. Three (3) of the organisms were laccase positive. The laccase producing fungi include *Fusarium sporotrichioides*, *Absidia corymbifera and Rhizopus oryzae*. *Aspergillus niger* and *Aspergillus flavus* were laccase negative. The presence of this organism is not surprising as their source could be from soil or sawdust in our environment.

From the research conducted it was found out that Rhizopus oryzae has the highest frequency of 50%, Fusarium sporotrichioides, Aspergillus niger, Aspergillus flavus and Absidia corymbifera had 12.5% each. Rhizopus oryzae and Fusarium sporotrichioides had a diameter of zone of inhibition of 43mm vertical and horizontal using both 0.5g and 1g carboxyl methyl cellulose (CMC). Absidia corymbifera had 17mm horizontal, 14mm vertical using 0.5gCMC and 18mm horizontal, 16mm vertical using 1g CMC. The findings of the present study agree with the works of Telke et al (2009) and Cullen and kersten (1996) who isolated similar groups of fungi from soil sample. From these findings, Penicillium rubrum, Stearum ostrea and Theliophora terristus were laccase positive while Aspergillus niger and Aspergillus flavus were not able to produce laccase enzyme. Laccase production by fungi has previously been shown to depend markedly on the composition of the cultivation medium for example carbon source, nitrogen content and phenolic inducer compounds have been reported to have significant effect on laccase production (Niku-paavola et al., 1990, Rogalski et al., 1991and Schlosser et al., 1997).

5. Conclusions

Laccases are widespread in nature, being produced by a wide variety of plants, fungi and bacteria. The functions of the enzyme differ from organism to organism and typify the diversity of laccase in nature. Laccases catalyse the oxidation of phenolic compounds whilst simultaneously reducing molecular oxygen to water. The present study showed that laccase producing fungi were obtained from the soil in our environment. Base on the results obtained, it can be concluded that laccases can be produced by fungi isolated from the soil in our environment.

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