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Antimicrobial potential of fungal laccase isolated from lignocellulolytic waste soil

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ABSTRACT

The present study aims to investigate the antimicrobial properties of lignocellulytic fungal laccase. Antimicrobial enzymes target the different bacterial biofilms and their cellular components which can be used for bacterial control in healthcare, food manufacturing and environmental protection. Lignocellulytic fungi were isolated from lignocellulolytic waste soil sample (coconut waste soil, paddy straw waste soil, banana waste soil, citrus peel waste soil and flower waste soil) collected from Bhilai-Durg region of Chhattisgarh India. A total of 85 fungal strains were isolated by soil dilution technique on the fungal isolation medium *i.e.,* Potato Dextrose Agar medium and Czapek Dox Agar medium. Primary screening was done by guaiacol plate assay method where guaiacol was used as indicator substrate. Only 17 fungal culture showed positive result for laccase production. Further all the laccase positive culture used for antimicrobial potential using the Kirby Baur well diffusion method against four Gram positive bacteria (*Staphylococcus aureus*, *Myccrococcus leutis*, *Listeria monocytogens* and *Bacillus cereus*) and six Gram negative bacteria (*Salmonella enterica*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeurogenosa*, *Aeromonas hydrophilia*, and *Enterococcus faecalis*) were it showed better results against Gram positive bacteria than the Gram negative bacteria.

Key word: *Antimicrobial, Laccase, Enzyme, Lignocellulolytic, Primary screening*

Introduction

Laccase (EC 1.10.3.2) are copper containing lignolytic enzyme which helps in substrate catalysis by oxidation reaction (Kiiskinen *et al*., 2004). They belongs to protein family including ascorbate oxidase, bilirubin oxidase and ceruloplasmin (Baldrian, 2006). During the past few years enzyme used in various fields of application is of the higher importance. Laccase are extracellular enzyme that have been broadly studied in fungi, yeast, bacteria, plants and insect and estimated for removal of toxic phenolic compounds (Manusamy *et al*., 2008). They are applicable in various industries such as removal of toxic phenolic compounds from industries and degradation of agricultural waste water, intractable xenobiotics (Maysa *et al*., 2012), pulp delignification, soil bioremediation, effluent and waste detoxification by catalyzing a wide range of substrate like diphenols, polyphenol, amine using of molecular hydrogen and produce water molecules (Gupta *et al*., 2014). Their extensive applications are included ethanol production, paper and pulp processing, dye bleaching, food industries, pharmaceutical industries and textile industries (Adinarayana *et al*., 2007).

Antimicrobial resistance properties of any microorganism such as bacteria, fungi, virus and parasites can be described as when microorganism change over the time and no longer response to any medicine (WHO, 2020). Many laccase enzyme producing fungi isolated from different environment and

screened for the antimicrobial compound. They serve as a source of bioactive compound with anticancer, antiviral, antimicrobial, antifouling and anti-inflammatory compounds (Barbosa *et al*., 2020).

Materials and Methods

Sample collection

Lignocellulolytic soil samples were randomly collected from the different places of Bhilai-Durg region of Chhattisgarh state.

Test Bacterial culture

Test Bacterial culture were purchased from MTCC Chandigarh, India.

Isolation of fungi

Fungal culture were isolated by the serial dilution method on PDA (Waskman, 1922).

Primary screening for laccase producing fungi

The pure fungal isolates were screened for production of laccase enzyme by guaiacol plate assay technique on PDA (Adiveppa and Basappa, 2015).

Morphological identification of positive fungi

The fungal isolates which produced laccase enzyme were identified through morphological characterization at Agharkar Research Institute, Pune, India.

Antimicrobial activity

Antimicrobial activity were performed by Kirby Baur technique (Nair *et al*., 2021).

Results and Discussion

A total of 85 fungal culture were isolated from lignocellulolytic waste soil sample in fungal isolation medium. Primary screening were performed by guaiacol plate assay method, only 17 fungal culture showed reddish brown colour halo in the medium which indicated the positive laccase production showed in Fig. 1. Positive culture were morphologically identified from Agharkar Research Institute, Pune, India, showed in Table 1. All the laccase positive culture used for antimicrobial potential using the Kirby Baur well diffusion method showed good results against Gram positive bacteria (*Staphylococcus aureus*, *Myccrococcus leutis* and *Listeria monocytogens*) and Gram negative bacteria (*Salmo*8. All the laccase positive culture used for antimicrobial potential against four Gram positive and six Gram negative bacteria were it showed better results with Gram positive bacteria than the Gram negative bacteria. Similar results found by the other Authors, Nair *et al*. (2021) isolated *Neurospora crassa* AJAS1 marine laccase producing fungi after testing their ability to antimicrobial activity against gram positive (*Proteus mirabilia* and *Staphylococcus aureus*) and negative (*Enterococcus sp*. and *Klebsiella sp*.) bacteria. Chin *et al*. (2021) studied the antimicrobial activity of marine laccase producing fungi against three Gram positive bacteria *i.e*., *Bacillus cereus*

Table 1. Identification of fungal isolates on the basis of their morphological characters.

S. No.	Culture code	Identification	Family
1.	BWB	*Dichotomopilus funicola*	Chaetomiaceae
2.	BWJ	*Curvularia pallescens*	Pleosporaceae
3.	BWM	*Curvularia pallescens*	Pleosporaceae
4.	BWW	*Alternaria padwickii*	Pleosporaceae
5.	BWY	*Penicillium sp.*	Trichocomaceae
6.	FWD	*Scytalidium lignicola*	Chaetomiaceae
7.	FWJ	*Humicola fuscoatra*	Chaetomiaceae
8.	FWN	*Fusarium sp.*	Nectriaceae
9.	Peel B	*Paecilomyces sp.*	Trichocomaceae
10.	Peel E	*Curvularia sp.*	Pleosporaceae
11.	Peel J	*Fusarium sp.*	Nectriaceae
12.	Peel S	*Aspergillus niger*	Trichocomaceae
13.	CWD	*Aspergillus fumigatus*	Trichocomaceae
14.	CWG	*Curvularia brachyspora*	Pleosporaceae
15.	PSA	*Chaetomium sp.*	Chaetomiaceae
16.	PSO	*Penicillium sp.*	Trichocomaceae
17.	PSP	*Histoplasma capsulatum*	Ajellomycetaeae

Table 2. Antimicrobial activity of laccase producing fungi on test bacteria.

S. No.	Potent Fungal Isolates	Gram +ve Bacteria Zone of Inhibition (diameter in mm)				Gram -ve Bacteria Zone of Inhibition (diameter in mm)					
		SA	ML	LM	BC	SE	EC	KP	PA	AH	EF
1.	*Dichotomopilus funicola*	-	-	12	-	-	-	-	-	-	-
2.	*Curvularia pallescens*	-	-	*10*	-	-	-	-	-	-	-
3.	*Curvularia pallescens*	-	-	-	-	-	-	-	-	-	-
4.	*Alternaria padwickii*	-	-	-	-	-	-	-	-	-	-
5.	*Penicillium sp.*	-	-	20	-	-	-	-	-	-	-
6.	*Scytalidium lignicola*	-	-	-	-	-	-	-	-	-	-
7.	*Humicola fuscoatra*	-	15	-	-	-	-	-	-	-	-
8.	*Fusarium sp.*	-	-	-	-	-	-	-	-	-	-
9.	*Paecilomyces sp.*	12	26	-	-	16	-	-	-	-	18
10.	*Curvularia sp.*	-	-	-	-	-	-	-	-	-	-
11.	*Fusarium sp.*	-	18	-	-	-	-	-	-	-	-
12.	*Aspergillus niger*	18	15	-	-	12	-	14	-	-	16
13.	*Aspergillus fumigatus*	16	25	-	-	21	-	-	-	-	17
14.	*Curvularia brachyspora*	-	16	-	-	-	-	-	-	-	-
15.	*Chaetomium sp.*	-	-	-	-	-	-	-	-	-	-
16.	*Penicillium sp.*	-	-	16	-	-	-	18	20	-	-
17.	*Histoplasma capsulatum*	-	18	-	-	-	-	-	-	-	12

Note:- SA- *Staphylococcus aureus*, ML- *Myccrococcus leutis*, LM- *Listeria monocytogens*, BC- *Bacillus cereus*, SE- *Salmonella enterica*, EC- *Escherichia coli*, KP- *Klebsiella pneumoniae*, PA- *Pseudomonas aeurogenosa*, AH- *Aeromonas hydrophilia*, EF- *Enterococcus faecalis*.

(ATCC 10876), *Enterococcus faecalis* (ATCC29212) and *Staphylococcus aureus* and three Gram negative bacteria *i.e.*, *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028).

Conclusion

The present study illustrates that 17 fungal culture has the capability to produce reddish brown halos on guaiacol containing Potato Dextrose Agar plate. Morphological characterization of fungal isolates belonged to 5 genera of fungi. The laccase producing fungi showed promising antimicrobial properties. These can be useful for the searching of antimicrobial compounds against resistant bacteria.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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