International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 4, 2019, pp.-1550-1556.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

AN INSIGHT INTO THE POTENTIAL MACROFUNGAL LACCASE PRODUCERS OF NORTH EAST, INDIA

BORGOHAIN T., HAZARIKA D.J., GAUTOM T., PARVEEN A., KAKOTI M., DULLAH S., BAROOAH M. AND BORO R.C.*
Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, 785013, India
*Corresponding Author: Email - robinboro@gmail.com

Received: April 04, 2019; Revised: April 26, 2019; Accepted: April 27, 2019; Published: April 30, 2019

Abstract- Macrofungal laccases are versatile group of metalloenzymes that oxidize both phenolic and non-phenolic compounds with simultaneous four-electron reduction of molecular oxygen to water. The practical applications of laccases have led to a search for sources of the enzyme from macrofungi of North Eastern (NE) region, India. In this study, thirty previously collected macrofungal strains were screened for laccase activity, of which only nine strains tested laccase positive. Detectable amount of laccase production was observed in all the nine macrofungal strains from 2nd day till 10th day of inoculation. Among the nine strains, it was observed that *Trametes versicolor* (1), *Ganoderma lucidum* and *Trametes coccinea* produced maximum enzyme on 6th day; *Trametes versicolor* (2), *Pleurotus pulmonarius, Leiotrametes lactinea* and *Panus lecomtei* on 8th day whereas *Trametes cubensis* and *Trametes sanguinea* showed maximal production on 10th day after inoculation. The partially purified macrofungal laccases exhibited pH optima in acidic pH range (pH 2.5 - 4.5) and were able to resist high temperature ranging from 50 ºC to 70ºC. Zymogram analysis showed significant differences typifying the diversity of macrofungal laccases. All the nine macrofungal strains were capable of oxidizing different ortho- substituted aromatic substrates to varying degrees. Among the nine strains tested, a remarkably high laccase activity was observed by *Panus lecomtei* (7511 U/L) when ABTS was used as asubstrate.

Keywords- *ABTS, Ganoderma lucidum, Guaiacol, Laccase, Macrofungi, Panus lecomtei, Trametes versicolor, Zymogram*

Citation: Borgohain T., *et al.*, (2019) An Insight into the Potential Macro Fungal Laccase Producers of North East, India. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 4, pp.-1550-1556.

Introduction

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are versatile group of copper metalloenzymes that catalyse the concomitant oxidation of phenolic compounds whilst simultaneously undergoing the four-electron reduction of molecular $O_2 \rightarrow H_2O$. These enzymes have the ability to catalyse the oxidation of phenolic compounds similar to that of the subunits present in lignin. The mineralization of lignin in nature, which is a unique phenylpropanoic biopolymer, is thought to be aided by laccase [24]. Laccases are remarkably nonspecific as they have a wide range of substrate specificities and the oxidation of substrate varies from one laccase to another. Substrate oxidation by laccase is a single electron reaction generating a free radical [28]. To name a few, some phenolic-based substrates that are utilized by laccases include polycyclic aromatic hydrocarbons (PAH) and polychlorinated bi phenols (PCP's), both of which are widespread in soil and water as contaminants [13]. An important feature of laccase is the presence of a covalently-linked carbohydrate moiety (10–45% of total molecular mass) that may give high stability to the enzyme [15]. The catalytic site of laccase consists of four copper atoms per molecule. The type 1/T1 copper atom performs one electron oxidation of both phenolic and non-phenolic groups and the type 2/T2 and type 3/T3 copper atoms forms a trinuclear copper cluster and are responsible for reduction of molecular oxygen to water. For instance, the artificial laccase substrate 2,2´ azino –bis (3- ethybenzothiazoline -6 sulphonic acid) or ABTS has the capacity to act as a mediator enabling the oxidation of non-phenolic compounds. Despite their common role in degrading phenolic compounds 'laccase' in fact presents the possibility to oxidize activated metoxiphenols like syringaldazine, hydroquinone, pyrogallol [18]. Laccases are ubiquitously produced by fungi, plant and bacteria. In nature, there exist certain macrofungal species that can efficiently degrade lignin in wood and are the only organisms capable of completely mineralizing lignocelluloses, the most abundant recalcitrant material available in nature.

Macrofungi are regarded as those fungi that form large fructifications which are visible without the aid of a microscope. Among which, ascomycetes, deuteromycetes and most white-rot basidiomycetes are the most well-known and have long been investigated as one of the most abundant producers of extracellular laccase [1,19]. Laccase activity has been demonstrated in many macrofungi belonging to the basidiomycetes and ascomycetes species and the enzyme has already been purified from many species [30]. Macrofungi act as stable and reliable source of extracellular laccases. The possibility of the production of large quantities of macrofungal laccase along with its ease of extraction makes it a quintessential candidate in enzyme- based industrial applications. However, to be viable for biotechnological applications, huge amount of laccases at low production cost and with good properties is required. However, the production of laccase is reported to be strongly affected by the nature and amounts of nutrients, especially nitrogen and trace elements in the growth medium [1],[30]. The North Eastern (NE) region of India is a spectacular biodiversity hotspot and a habitat to diverse macrofungal species [23]. Therefore, the present research was designed to explore the potential macrofungal laccase producers of North eastern region of India by analysing and understanding the intriguing properties in a more cost effective way in order to make it a perfect candidate for many industrial processes.

Materials and Methods

Macrofungal strains

Thirty previously isolated macrofungi from different regions of NE India, were obtained from the Microbial Biotechnology Lab, Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat. All the isolates were grown on potato dextrose agar (PDA) medium, stored at 4ºC and sub cultured periodically to maintain their viability.

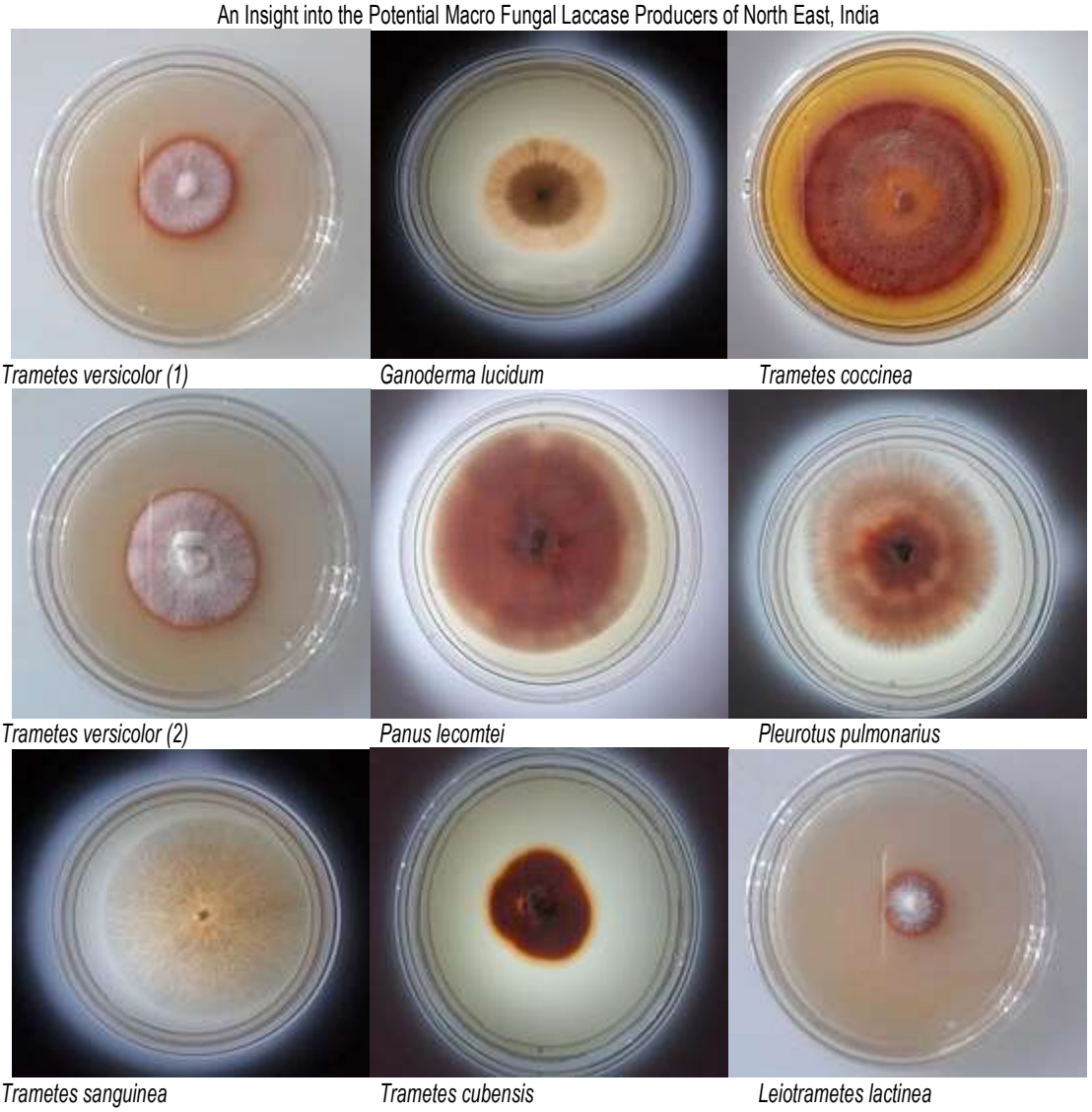

Fig-1 Photographs of macrofungal cultures showing positive results for laccase activity in guaiacol containing PDA medium

Preliminary plate screening

For preliminary screening, macrofungal strains were sub cultured on potato dextrose media (PDA) supplemented with 0.01% guaiacol and incubated at 25ºC - 27ºC for three to five days.

Quantitative estimation of laccase activity

For the analytical determination of laccases, one agar plug (diameter, 7 mm) each, from all actively growing macrofungus on PDA was transferred to separate flask containing 100 ml minimal medium as reported by Fang *et al* (2015), with slight modification [Composition : Yeast extract powder (1 g/l), D-glucose (10 g/l), KH_2PO_4 - (1 g/l), $MgSO_4.7H_2O$ - (5 g/l), NaCl - (5 g/l), lignin(0.1 g/l)] and incubated in a shaker incubator at 25ºC - 27ºC for 14 days [10]. To determine the optimal time of laccase production by the cultures, aliquots of 1 ml was collected every 48 h for monitoring laccase enzymatic activity by oxidation of ABTS. Macrofungal laccases are known to oxidize ABTS (green-colored molecule) to the cation radical ABTS + (dark green- colored molecule). The reaction was carried out in a cuvette, containing 2.2 ml of H_20, 0.6 ml of sodium acetate buffer (pH -4.5),0.1 ml of 15 mM ABTS, and 0.1 ml of enzyme extract and was incubated at 30 ºC for 10 min. Oxidation of ABTS was monitored spectrophotometrically by measuring the increase in absorbance at 420 nm after 10 min of incubation in an Evolution 202 UV vis spectrophotometer (Thermo Scientific, USA) [20]. One enzyme unit is defined as the amount of enzyme that oxidizes 1 µmol of ABTS per min. The enzyme unit was calculated using the formula [2]:

$$U/L = \frac{\Delta A \times Vt \times Df \times 10\text{^}6}{\varepsilon \times d \times Vs}$$

where, ΔA = change in absorbance; Vt = total volume measured; Df = dilution factor; 10^6 = correction factor; Vs = volume of enzyme; (ε) of ABTS= Extinction coefficient for the oxidation at 420 nm is 36000 M^{-1} cm^{-1}; d = path length of the optical cell is 1 cm.

Production and extraction of laccase

The macrofungal strains were grown in 500 ml Erlenmeyer flask, each containing 400 ml minimal medium and were supplemented with 0.001 g copper sulphate to produce high titres of laccase. The culture medium on their respective day of highest laccase production was filtered using glass wool in a way that no mycelium passes through it. The filtrate is referred to as the enzymatic extract.

Partial Purification of Laccase enzyme

For efficient precipitation of produced laccase, ammonium sulfate was added to the cell free enzymatic extract, to attain 75% saturation and the flask was kept in stirring condition at 4°C overnight. The saturated solution was centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was then resuspended in 100 mM sodium acetate buffer (pH 4.5) and dialyzed in a dialysis tubing (Sigma, USA) against 1 mM sodium acetate buffer (pH 4.5) to remove substances of low molecular weight including other ions that may interfere with the enzyme activity.

Determination of protein concentration

Protein concentration was quantified using the bicinchoninic acid (BCA) assay kit (Sigma, USA). The purple colour developed was recorded at 560 nm using Evolution 202 UV- vis spectrophotometer (Thermo Scientific, USA).

Assay of laccase activity

The partially purified laccase was diluted to a specific concentration and the

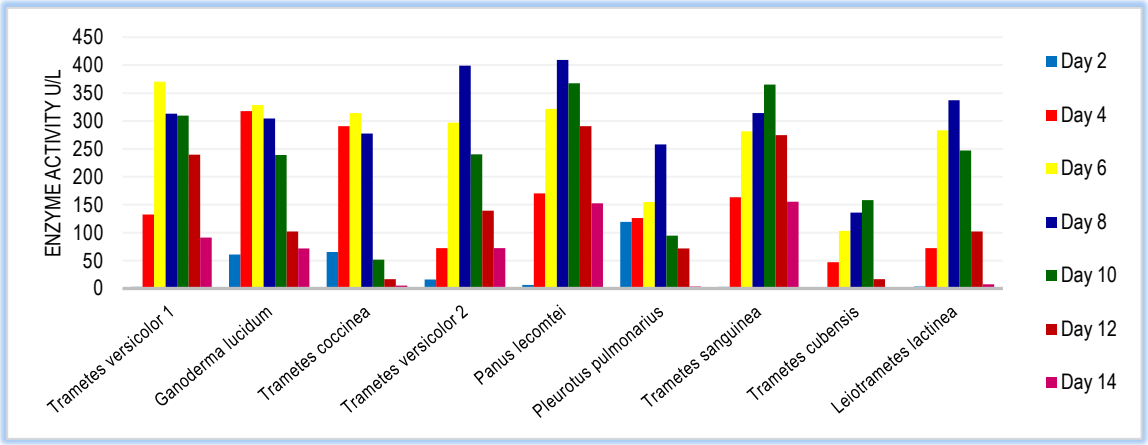

Fig-2 Activity in culture supernatant at different time intervals

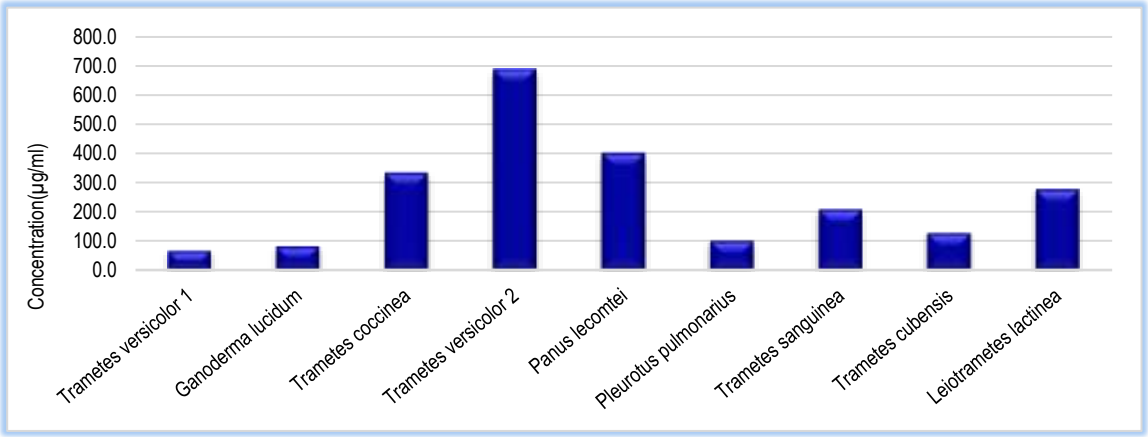

Fig-3 Determination of protein concentration in partially purified enzyme extract of different laccase producing macrofungal isolates

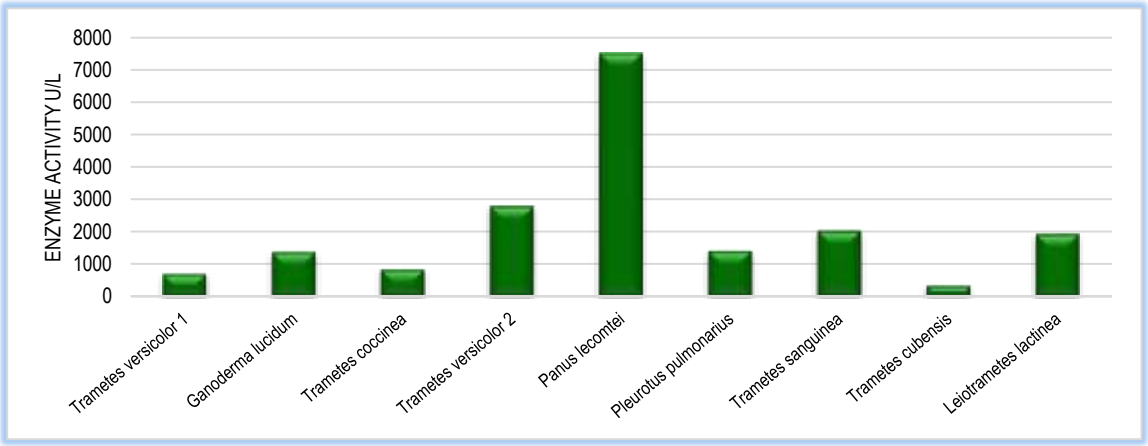

Fig-4 Laccase activity of the partially purified enzyme

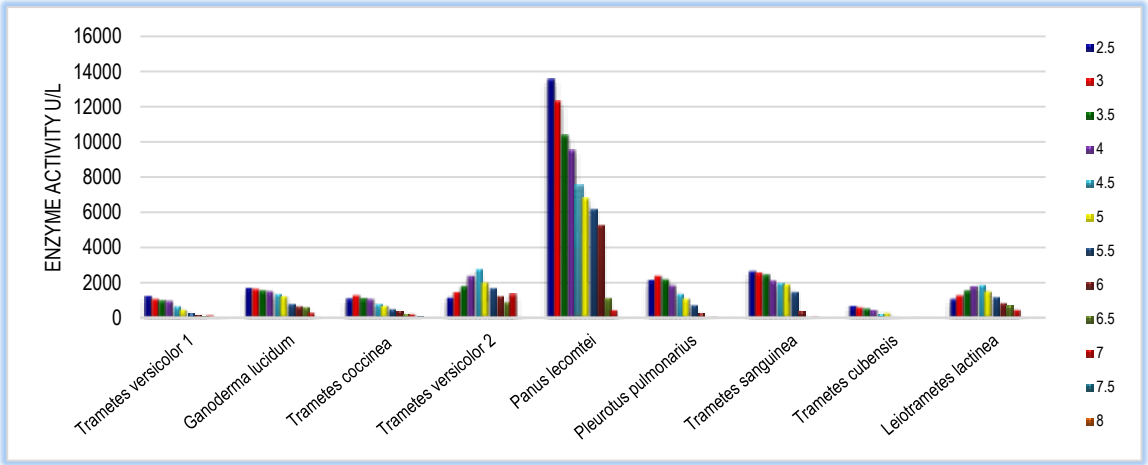

Fig-5 Effect of pH on laccase activity of different macrofungal isolates

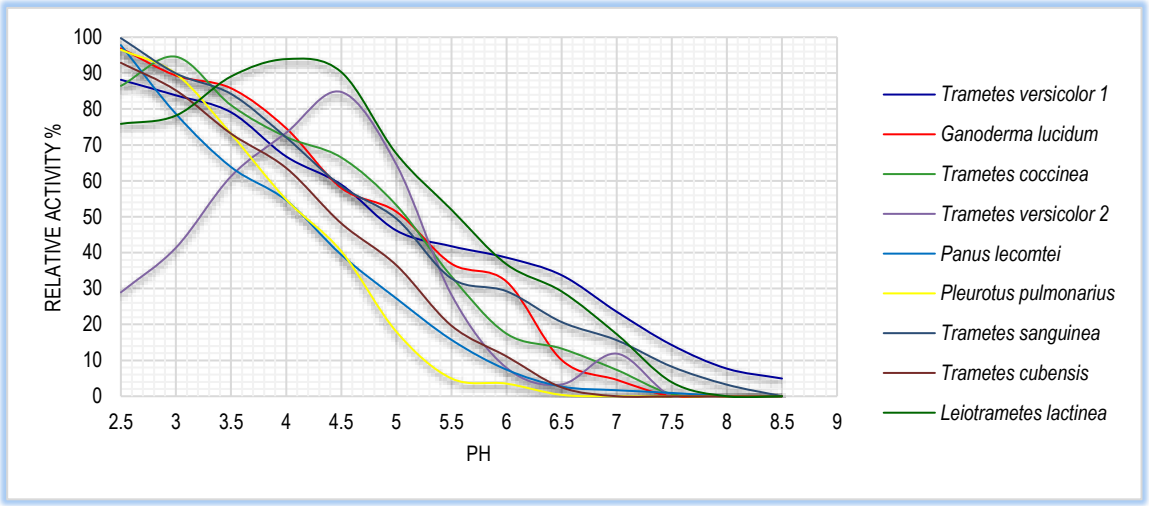

Fig-6 Determination of pH stability of different macrofungal laccases after 1 h of pre- incubation at 30°C

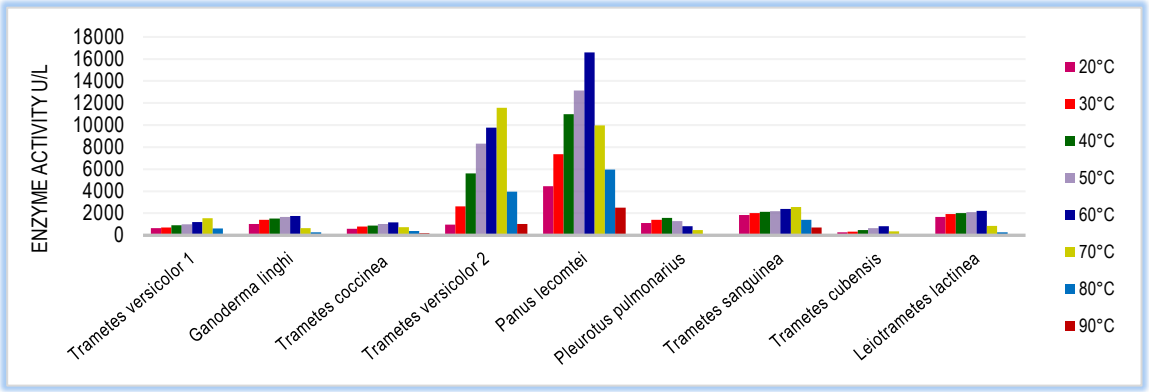

Fig-7 Effect of temperature on laccase activity of different macrofungal isolates

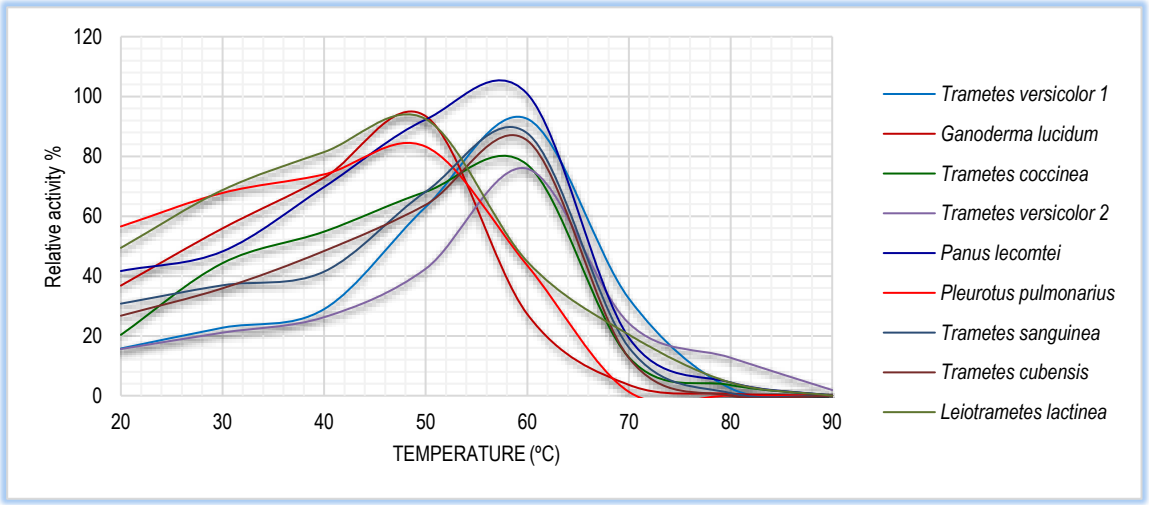

Fig-8 Temperature stability of different macrofungal laccases after 1 h of pre- incubation at pH 4.5

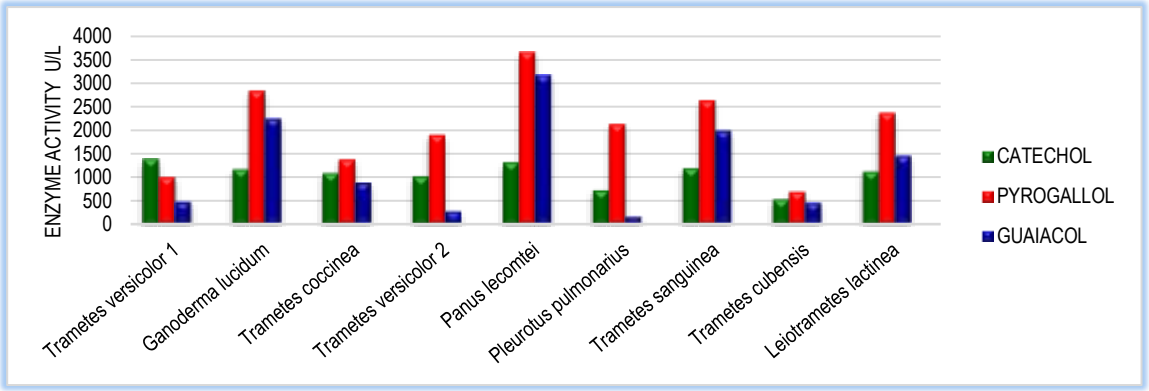

Fig-9 Substrate specificity of different macrofungal laccases using three aromatic compounds (catechol, pyrogallol, and guaiacol)

laccase activity was assessed spectrophotometrically using the same protocol described in described earlier.

Effect of pH on laccase activity and stability

The effect of pH on laccase activity was determined using pH 2.5, 3.0, 3.5 in sodium citrate buffer (0.1 M); at pH 4.0, 4.5, 5.0, 5.5 in sodium acetate buffer (0.1 M); at pH 6.0, 6.5 in phosphate buffer (0.1 M) and at pH 7.0, 7.5, 8.0 and 8.5 in Tris-HCL buffer (0.1 M). For the determination of pH stability, the enzyme solution was diluted in respective buffers and pre incubated for 1 h at 30°C. After 1 h of incubation, ABTS was added and the mixture was again incubated for another 10 min and the absorbance was recorded spectrophotometrically at 420 nm.

Effect of temperature on laccase activity and stability

The effect of temperature on laccase activity was determined by diluting the enzyme solution in 0.1 M sodium acetate buffer, pH 4.5 followed by incubation at different temperatures ranging from 20°C to 90°C for different periods. Then tubes was withdrawn after 10 min of incubation and cooled in ice water. For the determination of temperature stability, the enzyme solution was diluted in sodium acetate buffer, (0.1 M) pH 4.5 and pre incubated for 1 h at different temperatures ranging from 20°C to 90°C. After 1 h of incubation, ABTS was added. The mixture was then again incubated for 10 min for the reaction to occur and the absorbance was recorded spectrophotometrically at 420nm.

Substrate specificity of laccase

For determining substrate specificity of the partially purified laccase, 3 aromatic substrates were checked for their reactivity in place of ABTS. The substrates used were guaiacol (λmax = 470 nm), pyrogallol (λmax = 450 nm) and catechol (λmax= 450 nm). The enzyme assay was performed as described earlier and the test buffer used was 0.1 M sodium acetate buffer (pH 4.5). After incubating for 10 min at 30°C, the enzyme activity was recorded [6].

Zymogram analysis

Zymogram of the partially purified laccase was determined by polyacrylamide gel electrophoresis (NATIVE- PAGE) in a vertical electrophoresis apparatus (Mini-PROTEAN® Electrophoresis System - Bio-Rad, USA). Native PAGE gel was loaded with 1 µg non denatured protein samples and subjected to electrophoresis at 50 V for 3 h. The gel was then incubated in 100 ml of sodium acetate buffer (0.1 M, pH 4.5) containing 0.01% guaiacol for 30 min to visualize laccase bands on the gel.

Result and Discussion

Preliminary Plate Screening

Of the thirty macrofungal strains screened for laccase enzyme activity, only nine strains [Table-1] were found to be laccase positive showing significant brick red colored zone [Fig-1] around and under the growing mycelia due to the oxidation of guaiacol. The zone of oxidation was different for different species basically depending upon the growth stage and growth rate of the mycelia indicating that each macrofungus have different ability of secreting laccase.

SN	Strain Name	Accession No.
1	*Trametes versicolor* (1)	MK370665
2	*Ganoderma lucidum*	MK370666
3	*Trametes coccinea*	MK168589
4	*Trametes versicolor* (2)	MK370667
5	*Panus lecomtei*	MK168585
6	*Pleurotus pulmonarius*	MK370668
7	*Trametes sanguinea*	MK168587
8	*Trametes cubensis*	MK168588
9	*Leiotrametes lactinea*	MK168586

Quantitative estimation of Laccase activity

The positive strains were further studied in broth cultures for quantitative determination of laccases. Detectable amount of laccase production was observed with all the nine macrofungal strains starting from 2nd day with a gradual decrease in the enzyme production on the 12th day for all the cases [Fig-2]. Among the nine strains, three strains namely *Trametes versicolor* (1), Ganoderma lucidum and *Trametes coccinea* were able to produce high titres of enzyme on the 6th day of inoculation. Four other strains *Trametes versicolor* (2), *Pleurotus pulmonarius, Leiotrametes lactinea, Panus lecomtei* were able to produce highest amount of laccase on the 8th day. But in case of *Trametes cubensis* and *Trametes sanguinea*, it was seen that the enzyme production increased gradually and reached its peak on the 10th day. The difference in maximal activity of laccase in different species is due to the different growing patterns of the macrofungal species as some of them had a fast growth rate than others. Based on this property, the degradation of the supplemented lignin by macrofungal species could also vary resulting in differences in the production of the enzyme. Moreover, due to structural variation of laccase in different isolates, the enzyme activity may vary. The decline in production of the enzyme is proposed to be due to the rise in fungal biomass production, competition for available nutrients, degradation of lignin and proteolysis. In concordance, Tinoco *et al* (2011) reported that lignin synergistically induced the production of laccase in *Pleurotus ostreatus* [29]. Several authors reported that laccase enzyme activity in macrofungi depends upon factors such as nutrient source and growth conditions (pH, temperature) [3], [4].

Determination of protein concentration

The protein concentration of *T. versicolor* (2) was found to be highest which was 688.7 µg/ml followed by *P. lecomtei* with an average protein concentration of 400.5 µg/ml and *T. coccinea* with 332 µg/ml which were quite high in comparison to the other macrofungal strains *viz. T. sanguinea, T. cubensis, P. pulmonarius* while the least was found in case of *G. lucidum* (79.3 µg/ml), *T. versicolor* (1) (63.1 µg/ml) [Fig-3].

Characterization of partially purified Laccase

The partial purification procedure yielded a recovery of total laccase activity that was five to ten times higher than the crude enzyme extract for most of the isolates [Fig 4]. The production of laccase increased apparently when lignin was present in the broth culture and the supplementation of copper, even at low concentrations, resulted in a marked increase in laccase activity. Copper atoms which serve as cofactors in the catalytic site of laccase are reported to be one of the most effective and commonly used inducer of laccase in fungi [8]. Moreover, Stoilova *et al* (2010) reported that in the presence of KCl, NaCl, $CaCl_2$, $MnSO_4$ and $MgSO_4$, laccase from *T. versicolor* demonstrated high laccase activity [27]. Galhaup and Haltrich (2001) also reported that laccase activity was enhanced in the white-rot fungus *T. pubescens* in presence of copper [11]. A remarkably high amount of laccase activity was recorded by *P. lecomtei* (7511 U/L at pH 4.5) as shown in [Fig-4] when the medium was supplemented with 1 mM of copper sulfate. With exception to *T. cubensis*, all the other eight macrofungal strains recorded laccase enzyme activity of >500 U/L.

Effect of pH on laccase activity and stability

The efficacy of laccase in many industrial applications will depend on its proclivity to inactivate over time under the conditions of its particular reaction environmen*T.* Since the tertiary structure of the enzyme and its functional attributes mainly depend on pH and temperature, the enzyme can be denatured at extremes of pH and temperature [1]; [24]. The shift of an enzyme to a moderate pH or temperature condition can sometimes result in the refolding of the tertiary structure of the enzyme with a corresponding return of catalytic activity. The effect of pH on laccase activity was examined with pH ranging from 2.5 - 8.5 at a fixed temperature of 30°C. The optimum pH for the laccase produced by *T. sanguinea, G. lucidum, T. versicolor* (1), *P. lecomtei, T. cubensis* was pH 2.5 while *T. versicolor* (2), *L. lactinea* had a pH optima at 4.5 followed by *T. coccinea and P. pulmonarius* at pH 3.0 using ABTS as substrate [Fig-5]. For determination of pH stability, the enzyme was incubated for 1 h at different pH ranging from 2.5 to 8.5 at a fixed temperature of 30°C. The optimum pH for all the nine strains were acidic pH ranging from 2.5 -4.5. A severe decline in the enzyme activity was noticed in the alkaline pH. Early reports suggesting that fungal laccase typically exhibit pH optima in acidic pH range and the enzyme activity at higher pH decreased due to

the binding of a hydroxyl ion to the T2/T3 coppers of laccase interrupting the internal electron transfer from T1 to T2/T3 centres [8],[22]. The pH stability profile showed that the laccase produced by *T. sanguinea, G. lucidum, T. versicolor* (1), *P. lecomtei, T. cubensis* were able to retain more than 90% of their activity at pH of 2.5 while *T. versicolor* (2), *L. lactinea* were stable at 4.5 and *P. coccineus, P. pulmonarius* at pH of 3.0 retaining 80% of their activity respectively after 1 h of pre- incubation at 30ºC [Fig-6]. But, a severe decline in the activity was observed when the enzyme samples were incubated at alkali pH (6-8.5) suggesting that the enzymes were more stable at an acidic pH. Previous reports also suggested that the optimal range for the laccase isoforms secreted by *T. pubescens* is between pH 3.0 and 4.5 (Viswanath *et al* 2014), though some exceptions have also been reported [30,1].

Effect of temperature on laccase activity and stability

Effect of temperature on laccase activity of the nine strains was studied at temperature 20ºC - 90ºC. It was seen that the activity of enzyme progressively elevated as the ambient temperature is raised from 20ºC to 70ºC [Fig-7]. Enzyme produced by macrofungal strains are susceptible to thermal denaturation, at high temperatures (70ºC - 90ºC) and all enzyme activity vanished after 1 h of incubation. Laccases from different macrofungal strains have been reported to show highest activities having an optimum temperature ranging from 40ºC -70ºC. [14,20,27]. Most enzymes are also inactivated at elevated or even moderate conditions of temperature and some can even be inactivated at temperatures at or below 10ºC [22]. Thermal inactivation is believed to be mainly caused by denaturation of the tertiary structure through protein unfolding or disruption of the enzyme's active site [5, 17, 21-27]. In concordance with the earlier reports, it was noticed that the laccase produced by *T. versicolor* (1), *T. versicolor* (2) and *T. sanguinea*, *L. lactinea* were able to retain over 90% of its activity at 60 ºC after 1 h of incubation while all the other strains had a thermal stability at 50ºC - 60ºC [Fig 8].

Substrate specificity of laccase

Laccase catalyze the one electron oxidation of a wide variety of organic and inorganic substrates including mono, di and polyphenols [16]. Thurston (1994) stated that catechol is a good substrate for laccase but guaiacol is often better [28]. In this study, three ortho substituted aromatic substrates were selected to study the substrate specificity of the partially purified laccases. It was observed eight strains *viz. L. lactinea, G. linghi, P. pulmonarius, T. cubensis, P. lecomtei, T. coccinea, T. versicolor* (2), *T. sanguinea* were capable of oxidising guaiacol and pyrogallol to varying degrees. But *T. versicolor* (1) had more affinity towards catechol over pyrogallol and guaiacol [Fig-9]. However, it has been noticed that laccase produced by *P.* lecomtei have more affinity towards the pyrogallol and guaiacol like substrates. Laccases can be grouped according to their preference for ortho-, meta- or para- substituted phenols though several reports reveal that ortho- substituted phenols such as guaiacol and catechol are better substrates than meta or para substituted phenols [12].

Zymogram Analysis

Native PAGE analysis indicated the presence of laccase enzyme in all the samples with different migration rates on the gel. The different banding patterns revealed in zymogram suggested the presence of different laccase isozymes. Two laccase isoenzymes were revealed in the Native PAGE for *T. versicolor* (1), G. *lucidum*, *P. pulmonarius, T. versicolor* (2) whereas *P. lecomtei, T. coccinea, T. sanguinea, L. lactinea, T. cubensis* produced a single laccase band [Fig-10].

Earlier, different laccase isoenzymes were reported from *T. multicolour*, *P. ostreatus* and many more in Cu^{2+} ions containing cultures [26]. It can be hypothesised that the isoenzymes produced by different strains could be due to the expression of different laccase genes during oxidative stress, gene duplication or allelic variability of the same gene in the same locus or several other factors such as nutrient levels, culture conditions, and developmental stage as well as by the addition of different inducers to cultural media [12]. Due to the presence of different genes encoding laccases and multimeric forms, variations in the molecular size of laccase is observed frequently. Low molecular weight laccases (43 kDa – 45 kDa) has also been reported earlier from different macrofungal strains such as *T. hirsuta* (MTCC 11397) [8].

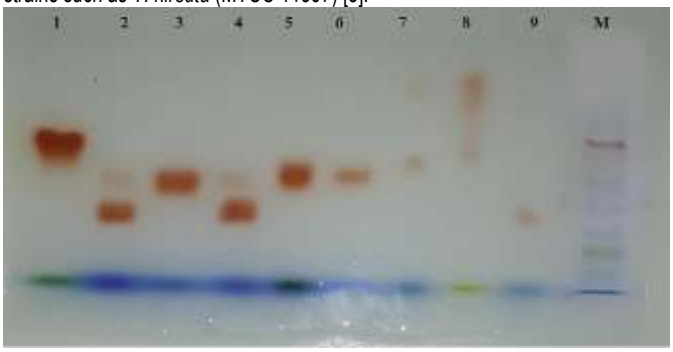

Fig-10 Zymogram analysis determining laccase enzyme (Lane 1: *P. lecomtei*; Lane 2: *T. versicolor*; Lane 3: *T. sanguinea*; Lane 4: *G. lucidum*; Lane 5: *L. lactinea*; Lane 6: *T. coccinea*; Lane 7: *T. versicolor*; Lane 8: *P. pulmonarius*; Lane 9: *T. cubensis* ; Lane 10: Protein marker)

However, high molecular weight laccases are also abundant Dwivedi *et al* (2011) demonstrated the structural and functional relationship of laccase between bacteria, fungi and plants concluding that though all the three laccases exhibited a common 3-D architecture structurally, there exhibited certain significant differences in conformity at catalytic site along with their functional diversity and evolutionary relationship[9].

Conclusion

Macrofungi are cosmopolitan in nature and occur seasonally in various habitats all over the North Eastern region [23]. Very few researches have been conducted on the potential laccase producers of macrofungal species in the NE India, making it mandatory for undertaking the documentation and conservation of this resource, considering the wide range of application of the enzyme in industrial sector. From this study, it could be conferred that there is inter specific as well as intra specific diversity in structural and functional characteristics of macrofungal laccases. As per our knowledge, it is the first report on *P. lecomtei* producing laccase that can tolerate high temperature and extreme pH with high efficiency. Further studies on genetic variability and regulation mechanisms of these macrofungal laccases may open up new possibilities for application of these enzymes in the industrial, environmental and agricultural sectors.

Application of research: Laccases are versatile oxidoreductases, and their versatility lies in the high oxidation potential that makes them perfect candidate for biotechnological applications. With plethora of applications in industrial sectors, including paper pulp bleaching, detoxification, baking, manufacturing gluten-free breads, beverages (wine, juice and beer) stabilization, bioremediation, manufacturing of biosensors and biofuels, laccases certainly have important role to play in green chemistry

Research Category: Agricultural Biotechnology

Acknowledgement / Funding: Authors are thankful to acknowledge the Science and Engineering Research Board (SERB), Department of Science and Technology, Govt. of India for providing fund for conducting the research work. Authors are grateful to the Department of Agricultural Biotechnology and DBT-AAU Centre, Assam Agricultural University for rendering the laboratory space and instrumentation facilities for carrying out the study.

***Principle Investigator or Chairperson of research: Dr Robin Chandra Boro**
University: Assam Agricultural University, Jorhat, 785013
Research project name or number: Wild mushroom from NE India: Evaluation of their nutritional status and medicinal properties (EEQ/2016/000631)

Author Contributions: RCB conceived the idea; TB, DJH and RCB designed the study; MK, TG, SD performed DNA isolation and identification; TB, SD performed the laccase activity assay; TB, AP, DJH performed the partial purification of laccase, Native –PAGE analysis; MB, RCB supervised the study; TB prepared the manuscript and figures.

Author statement: All authors read, reviewed, agreed and approved the final manuscript. All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Microbial Biotechnology Lab, Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat

Cultivar / Variety name: Nil

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Baldrian *P.* (2006) *FEMS Microbiology Reviews* 30, 215–242.
[2] Baltierra-Trejo E., Márquez-Benavides L. & Sánchez-Yáñez J. M. (2015) 119,126–131.
[3] Boominathan K. & Adinarayana Reddy C. (1996) 62, 3739-3744.
[4] Buswell J. A., Cai Y. & Chang S. (1995) *FEMS Microbiology Letters* 128,81–87.
[5] D'Amico S., Marx J. C., Gerday C. & Feller G. (2003) 278,7891–7896.
[6] Devasia S. & Nair A. J. (2016) *International Journal of Current Microbiology & Applied Science* 5,127–137.
[7] Dhakar K. & Pandey A. (2013) *Enzyme Research*, 2013.
[8] Dhakar K., Pandey A. (2014) *Advances in Natural Sciences*. 8,14–20.
[9] Dwivedi U. N., Singh *P.*, Pandey V. *P.* & Kumar A. (2011) *Journal of Molecular Catalysis B Enzyme*. 68,117–128.
[10] Fang Z., Liu X., Chen L., Shen Y., Zhang X., Fang W. and Xiao Y. (2015) *Biotechnology for Biofuels*, 8(1), 1-12.
[11] Galhaup C. & Haltrich D. (2001) *Applied Microbiology & Biotechnology* 56,225–232.
[12] Giardina *P.* (2010) *Cellular and Molecular Life Sciences* 67,369–385.
[13] Kadri T. (2017) *A review. Journal of Environmental Science* 51,52–74.
[14] Kiiskinen L.-L., Ratto M. & Kruus K. (2004) *Journal of Applied Microbiology*, 97,640–646.
[15] Kunamneni A., Ballesteros A., Plou F. J. & Alcalde M. (2007) *Communicating current research and educational topics and trends in applied microbiology*,233–245.
[16] Madhavi V. & Lele S. S. (2009) *Bioresources* 4,1694–1717.
[17] Mäkelä M. R., Hildén K. S., Hakala T. K., Hatakka A. & Lundell T. K.(2006) *Current Genetics* 50, 323–333.
[18] Manole A., Herea D., Chiriac H. & Melnig V. (2008) *Cuza University Science Annals. Lasi* 4,17–24.
[19] Marques De Souza C. G. & Peralta R. M. (2003) *Journal of Basic Microbiology*. 43,278–286.
[20] More S.S., PS, R. and Malini S.(2011) *Enzyme Research*.
[21] Murugesan K., Arulmani M., Nam I.H., Kim Y.M., Chang Y.S. and Kalaichelvan P. T. (2006) *Applied Microbiology and Biotechnology*. 72(5),939-946
[22] Palmer T. & 1944-. (E. Horwood,1991).
[23] Parveen A., Khataniar L., Goswami G., Jyoti Hazarika D., Das *P.*, Gautom T. Chandra Boro, R. (2017) *International Journal of Current Microbiology and Applied Science*, 6(12),275-297.
[24] Perna V., Agger J. W., Holck J. & Meyer A. S. (2018) *Scientific Reports*. 8,1–9.
[25] Quaratino D., Federici F., Petruccioli M., Fenice M. & D'Annibale A. (2006) *Antonie Van Leeuwenhoek* 91,57–69.
[26] Shrestha *P.*, Joshi B., Joshi J., Malla R. & Sreerama L.(2016) *Biomed Research International* 2016,1–10.
[27] Stoilova I., Krastanov A. & Stanchev V. (2010) *Advances in Bioscience and Biotechnology*. 01,208–215.
[28] Thurston C. F. (1994) *The structure and function of fungal laccases. Microbiology* 140,19–26.
[29] Tinoco R., Acevedo A., Galindo E. & Serrano-Carreón L. (2011) *Journal of Industrial Microbiology and Biotechnology*. 38, 531–540.
[30] Viswanath B., Rajesh B., Janardhan A., Kumar A. *P.* & Narasimha G. (2014) *Enzyme Research*. 2014.
[31] Xu F. (1997) *Journal of Biological. Chemistry* 272,924–928.