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Research Article

DAIRY WASTE 'GHEE RESIDUE' INFLUENCE LACCASES PRODUCTION BY *PYCNOPORUS CINNABARINUS* AND ITS OPTIMIZATION

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ABSTRACT : The present study reveals that a potent laccases producer *Pycnoporus cinnabarinus* produces higher quantity of laccases in the Coll *et al.* Medium (M1 medium) and Potato dextrose broth medium (M4 medium) containing agricultural waste 'wheat bran' (1%w/v) after a great comparative study among the five laccases production media namely Coll *et al.* medium (M1 medium), Olga *et al*. medium (M2 medium), Slomczynski medium (M3 medium), Potato dextrose broth (PDB) medium (M4 medium) and nutrient salt medium (M5 medium). There was a tremendous increase in laccases production with alternative carbon source. There was about 265.2% increase in laccases production in M1 Medium with wheat bran (126.99±2.046 U/ml/min) which further increased by 305% (140.78±1.118 U/ml/min) after the addition of dairy waste 'Ghee residue'. In the presence of rice bran there was a reduction in laccase production, which however improved in the presence of Ghee residue by a good 63.8% (56.96±1.005 U/ml/min) compared to the M1 medium with glucose. Similarly in case of M4 medium, there was an increase in laccases production by 45.6% with wheat bran (89.46±1.861U/ml/min) and on addition of ghee residue it increased to about 95.6% (115.00±2.048U/ml/min). The beneficial effect of ghee residue was proved by comparative study on effect of other inducers namely copper sulfate, ammonium tartrate, ethanol and peptone. The maximum production was obtained in 9 days at pH 3.0 and temperature 35°C in potato dextrose broth medium (M4) with 1% w/v wheat bran and 2% w/v Ghee residue. The presence of phenolic compounds in the Ghee residue was found to be encouraging laccases production.

Key words: Laccases, *Pycnoporus cinnabarinus*, potato dextrose broth, wheat bran and Ghee residue.

INTRODUCTION

Laccases (*p*-benzenediol: oxygen oxidoreductase, E.C. 1.10.3.2) are multicopper enzymes widely distributed in bacteria, yeasts and plants and are mainly produced by white rot fungi, such as *Trametes versicolor, Pleurotus eryngii, Travetes villosa*, etc. (Morozova *et al.*, 2007). A wide range of phenolic and aromatic compounds are catalyzed by the oxidation reaction i.e. removal of electrons until molecular oxygen is reduced to water (Thurston, 1994 and Gianfreda *et al.*, 1999). Laccases act upon monophenol, diphenol, metoxiphenol, polyphenol, aniline, benzenotiole, aryldiamines substrates and many others. Kunamneni *et al.* (2008) stated that their range of action can extend to other substrates by the addition of small molecules, which act as mediators, to the reaction system. They can act upon a wide range of substrates; this has increased interest in laccases considerably over the last few decades, given the variety of biotechnological applications for these enzymes. Laccases are useful in the removal of phenolic compounds from wine, and in beer stabilization, paper pulp delignification and in the development of fuel cells and biosensors. In organic synthesis, they can transform functional groups or achieve the coupling of phenols and steroids and they are also used in bioremediation processes for industrial dyes and effluents (Ghindilis, 2000 ; Minussi *et al.*, 2002; Camarero *et al.*, 2007; Ponsoni *et al.*2007; Younes *et al.*, 2007; Kunamneni *et al.*, 2008). Unal and Kolankaya (2001) reported that in order to improve the color and quality of the Kraft pulp, chlorine based bleaching is adopted followed by discharging of waste waters containing chlorinated aromatics into water bodies**.** Water from such contaminated sources have cytotoxic and cytomutagenic effects on various living organisms ultimately harming human beings too.

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A similar process is also reported by Moorthi *et al.* (2007) in textile industries using harmful cytotoxic coloring dyes as the effluents are released in water bodies. Fungal laccases are environment friendly and help us reduce pollution and the toxicity of the currently used chemicals through their oxidation/reduction mode of action (Desai and Nityanand, 2011). As per the reports by Rodrı´guez and Angeles (2005), fungi are capable of using a wide variety of organic wastes as a source of carbon and nitrogen. So, an economically beneficial media can be formulated with easily available carbon and nitrogen sources. With an aim of producing such an ecofriendly enzyme in higher quantity, efforts were made to make it available industrially by using cheap and easily available agricultural and industrial wastes.

MATERIALS AND METHODS

Test Organism

The organism under study, *Pycnoporus cinnabarinus* was obtained from soil samples of forest area in Hosur, Tamil Nadu and maintained at Kakatiya University, Warangal, Andhra Pradesh under the supervision of Prof. M.A. Singara charya.

Maintenance of the Fungus

The fungal culture was maintained in pure form by inoculating it in malt extract agar (MEA) medium containing the following composition (g/L), 15g Malt extract powder, 1g Dipotassium hydrogen ortho phosphate, 1g Ammonium chloride, 15 ml Citric acid (1N), 20g Agar. The fungal cultures were inoculated in the plates containing MEA medium and incubated at 30^0C for 7 days.

Cultivation in Liquid Media

Heinzkill *et al.* (1998) said Laccases production has been found to be highly dependent on the conditions for the fungus cultivation and according to Xavier et al. (2001) media supporting high biomass did not necessarily support high laccase yields. The test fungus was grown in following 5 different types of liquid media and all five media were prepared as per their composition (g/L) and the pH of was adjusted to 6.0 (Table.1).

Table.1 Laccases production Media

Media	**Composition gm/L**
Coll *et al.* medium M1	10g glucose,1.00g aspargine, 0.5g yeast extract, 0.5gK_2HPO_4, 1.00g$MgSO_4.7H_2O$, 0.001g $FeSO_4.7H_2O$,*
Olga *et al.* medium M2	3.0g peptone,10g glucose, 0.6g KH_2PO_4, 0.001g $ZnSO_4$, 0.4g K_2HPO_4, 0.0005g $FeSO_4$, 0.05g$MnSO_4$,0.5g $MgSO_4$*
Slomczynski medium M3	40.0g glucose,7.0g glycerol, 0.50g L-histidine,0.10g $CuSO_4$, 1.80g $NaNO_3$,1.80g NaCl,0.50g KCl0, 5.0g $CaCl_2.H_2O$,0.05g $FeSO_4.7H_2O$, 1.0g KH_2PO_4, 0.50g $MgSO_4$ *
Potato dextrose broth M4	200g potato extract, 20g dextrose,*
Nutrient salt mediumM5	10g glucose, 0.2g$(NH_4)_2PO_4$, 1.0g KH_2PO_4,0.5g $MgSO_4.7H_2O$, 0.5g KCl, 0.005g $FeSO_4.7H_2O$, 0.001g thiamine hydrochloride,*

* Addition of Ghee residue. The pH of all media was adjusted to 6.0 ± 0.2

Laccases Production

Five different laccases production media (M1, M2, M3, M4 and M5) were investigated to screen out the best production medium. In order to explore the efficiency of the dairy waste “Ghee residue”, all production media were prepared with minor modifications (Table.1). The modification was the addition of dairy waste “Ghee residue” (1%W/V) as an inducer to the medium. Later the media supporting better growth of fungi and enzyme production were prepared with altered carbon sources (rice Bran, wheat bran and saw dust) and inducer (Ghee residue) (Table. 2) and the production was compared to the production in original medium composition after 15 days of incubation at the temperature 28-30°C in an orbital shaker at 120rpm. For comparison a set of similar medium without addition of the industrial waste “ghee residue” was maintained in similar conditions.

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Table. 2 List of selected media used throughout the investigation

Media	Composition gm/L
Coll *et al*. medium M1	10g carbohydrates[a],1.00g aspargine, 0.5g yeast extract, 0.5gK_2HPO_4, 1.00g$MgSO_4.7H_2O$, 0.001g $FeSO_4.7H_2O$,*
Potato dextrose broth M4	10g carbohydrates[a] ,200g potato extract, 20g dextrose,*

[a]Either of the carbohydrate source may be added among glucose or wheat bran or saw dust or rice bran. * Addition of Ghee residue. The pH of all media was adjusted to 6.0 ± 0.2

Effect of Inducers

For comparison with the effect of dairy waste "ghee residue", other inducers like peptone, ethanol, ammonium tartrate and $CuSO_4$ were used and tested in the production medium. Each inducer was added in different concentrations ranging from 0.05% to 1.0% w/v in the selected medium for laccases production and incubated for 15 days at 28-30°C in an orbital shaking incubator (120rpm). The effects of various inducers were estimated based on the laccases production.

Extraction of Crude Enzyme from the Medium for assay

Crude enzyme was extracted by filtering the fungal mycelium using a pre-weighed filter paper after incubation and enzyme activity in the sample was spectrophotometrically determined by using Guaiacol as a substrate by monitoring the rate of product (dark brown color) formation. The mycelium obtained was dried in a hot air oven at 80^0C and the dry weight was recorded until a constant weight was obtained.

Optimization of Ghee residue concentration for laccases production

The laccases production medium (100 ml) was formulated with different concentrations of ghee residue. A set of Erlenmeyer flasks in triplicates containing 0.5 to 5.0% of Ghee residue was added in the production medium (adjusted to optimum pH) and incubated at optimum incubation temperature, shaking speed and time interval.

Extraction and Quantitative estimation of phenolic compounds from Ghee residue

0.5 gm of Ghee residue was dissolved in 5ml of hot concentrated H_2SO_4. By using methanol and dichloromethane the total phenolics present in Ghee residue was extracted and estimated by Folin -Ciocalteu method with gallic acid as a standard (Rasheeda and Gyana Prasuna, 2013).

Laccases production by submerged fermentation and optimization of physic- chemical parameters

In submerged fermentation (SmF) microorganisms were grown on a continuous liquid phase in selected production media. The optimum incubation period (with 3 days intervals), pH (3.0-9.0) and incubation temperature (20°C-60°C) with the best production medium was determined for laccases production. Simultaneously dry weight was also obtained.

Statistical Analysis

The results obtained were interpreted statistically using Minitab 16 software and Portable IBM SPSS Statistics v19.

RESULTS AND DISCUSSION

This paper summarizes the reports on the best production medium among five different selected media for the production of industrially most important laccases by submerged fermentation method. Among the five production media, potato dextrose broth medium (M4) was beneficial for maximum laccases production. In addition to the constituents of the media, the addition of industrial waste "Ghee residue" showed an increase in the production as well as in the fungal mass (Table.3).

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Table.3 Laccases activity by *P.cinnabarinus*

Media	*P.cinnabarinus*	
	U/ml/min	Dry wt.(mg/100ml)
M1	34.77±0.118	480±0.003
M2	0.490±0.095	330±0.005
M3	0.230±0.114	410±0.001
M4	61.45±0.101	840±0.008
M5	0.600±0.112	70±0.004
M1+G	70.06±0.152	690±0.006
M2+G	12.29±0.118	600±0.001
M3+G	9.10±0.128	1505±0.012
M4+G	77.54±0.110	2540±0.009
M5+G	38.35±0.084	975±0.014

Comparative study among five different laccases production media with and without Ghee residue abbreviated as M1= Coll *et al*. medium, M2=Olga *et al.* medium, M3=Slomczynski medium, M4=Potato dextrose medium, M5= Nutrient salt medium. M1+G=Coll *et al.* with Ghee residue, medium, M2+G =Olga *et al.* medium with Ghee residue, M3+G =Slomczynski medium with Ghee residue, M4+G =Potato dextrose medium with Ghee residue, M5+G = Nutrient salt medium with Ghee residue. All flasks incubated at 28-30°C for 15 days in an orbital shaker at 120rpm. Each value is an average of three replicates and ± indicates standard deviation among replicates**.** Though there was an increase in enzyme production in all five production medium in presence of ghee residue, more increase could be seen in Coll *et al.*, medium (M1) and potato dextrose broth medium (M4) (Table.1).

Best Carbon Source- Wheat Bran

There was about 240% and 265.2% increase in laccases production in M1 Medium with saw dust and wheat bran respectively. In the presence of rice bran there was a reduction obtained in laccase production, which however improved in the presence of Ghee residue by a good 63.8% (56.96±1.005 U/ml/min) compared to the M1 medium with glucose (34.77±0.118 U/ml/min). The M1 medium with wheat bran showed about 265% increase in laccases production (126.99±2.046 U/ml/min) which further increased by 305% (140.78±1.118 U/ml/min) after the addition of Ghee residue. But in case of saw dust it was showing a negative effect. In case of M4 medium, the effect of the agricultural and industrial wastes was quite different. Though there was an increase in laccases production by 76.4% in M4 medium with saw dust (108.43±1.514U/ml/min), further addition of ghee residue led to decrease in production. Where as with wheat bran there was about 45.6% increase in production (89.46±1.861U/ml/min) and on addition of ghee residue it increased to about 95.6% (115.00±2.048U/ml/min). In case of rice bran there was about 61% increase in production (98.72±0.435U/ml/min) which came down in the presence of ghee residue (Table.4). As the maximum laccases production was observed in M1 and M4 medium with wheat bran as a carbon source and more enhanced after the addition of Ghee residue (140.78±1.118 U/ml/min and 115.00±2.048 U/ml/min respectively) by *P.cinnabarinus*, this medium was considered for the further research work.

Best Inducer-Ghee Residue

A number of inorganic and organic inducers such as salts of copper, manganese, organic compounds, phenolics and natural products have been used for increasing synthesis of enzymes by microorganisms (Lee *et al.* 1999; Palmieri *et al.* 2000; Elishashvili *et al.* 2001; Laxmi and Khan 2010). These compounds have been found to have variable results depending on the organism, enzyme being studied, media or even the physical factors prevailing. Inhibitory effects of these on the production have been observed in a few cases (Lomascolo *et al*. 2003; Jose *et al*. 2005; Dekker *et al.* 2007). Lomascolo *et al.* (2003) revealed that ethanol is having encouraging effects on higher laccases production by *P.cinnabarinus* and phenolic compounds were tested for the encouraging effects on laccases production by *T.hirsuta* (Arora and Sandhu 1984).

Encouraged by the increase in laccases production obtained with the various carbon sources, the effect of inducers was also studied. Five different inducers namely copper sulphate, ethanol, ammonium tartrate, peptone and ghee residue were tested by inclusion in the production medium, at varying concentration. Gradual increase in laccases production with increasing concentration of inducer was observed. Among the five inducers employed, copper sulphate was the least encouraging inducer (Table. 3).

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Table.4 Laccases activity by *P.cinnabarinus* in M1 and M4 production media

100ml	U/ml/min	Dry wt. (mg/100ml)
M1	34.77±0.118	480±0.003
M1+SD	118.24±1.157	840±0.014
M1+SD+G	90.34±1.249	950±0.157
M1+WB	126.99±2.046	510±0.008
M1+WB+G	140.78±1.118	860±0.142
M1+RB	0.94±1.047	820±0.016
M1+RB+G	56.96±1.005	960±0.007
M4	61.45±0.101	840±0.008
M4+SD	108.43±1.514	1970±0.015
M4+SD+G	81.11±1.608	2790±0.007
M4+WB	89.46±1.861	3200±0.042
M4+WB+G	115.00±2.048	2720±0.063
M4+RB	98.72±0.435	2800±0.008
M4+RB+G	89.00±1.251	2940±0.010

M1 (Coll *et al*.) and M4 (PDB) medium with alternative carbon sources and with and without industrial waste Ghee residue, abbreviated as M1=Coll *et al.* medium, SD =Saw dust, G =Ghee residue, WB =Wheat bran, RB=Rice bran. M4=Potato dextrose broth, SD =Saw dust, G =Ghee residue, WB =Wheat bran, RB=Rice bran. Each value is an average of three replicates and ± indicates standard deviation among replicates.

It is effective only in very minimum concentration and as the concentration increased it showed inhibitory effect on the fungal growth as well as to the productivity. The maximum increase in production (about 2.0 times more by *P.cinnabarinus*) was observed with increasing concentrations of Ghee residue in M4 medium. Ghee residue was dark brown in colour with pleasant odor and slightly bitter taste. Its texture is soft earlier, later became hard and granular on storage. It is soluble in hot concentrated H_2SO_4. Similarly the increasing concentrations of peptone led to approximately 1.9 times more enzyme production by *P.cinnabarinus* in M4 medium. The effect of increasing concentrations of Ghee residue (approximately 3.7 times higher) and peptone (approximately 3 times higher) by *P.cinnabarinus* respectively in M1 medium was also similar to that of M4 medium. Although a very good increase in laccases production in presence of peptone (116.2±0.016 U/ml/min) was observed in case of *P.cinnabarinus*, still more production was observed in presence of Ghee residue (120.9±0.006 U/ml/min) in M4 medium. Similarly increased production was obtained in M1+ WB+G (118.7±0.021 U/ml/min) when compared to M1+ WB+P (110.3±0.021 U/ml/min). Thus M4+WB+G and M1+ WB+G production medium was used in further investigations for *P.cinnabarinus* (Table.5).

Table 5. Effect of different inducers on enzyme production by *P.cinnabarinus*

%	100ml	$CuSO_4$	Ethanol	Ammonium tartrate	Peptone	Ghee residue
0.05%	M1	21.615±0.004	33.817±0.002	34.39±0.001	36.754±0.006	32.129±0.005
	M4	22.312±0. 012	43.671±0.006	30.06±0.005	61.97±0.008	60.433±0.003
0.1%	M1	11.121±0.002	41.31±0.003	13.36±0.008	42.78±0.008	34.98±0.008
	M4	11.704±0.008	55.66±0.012	8.002±0.006	78.80±0.002	68.663±0.003
0.25%	M1	1.101±0.003	85.83±0.003	7.42±0.013	96.99±0.006	83.41±0.013
	M4	1.901±0.006	83.11±0.015	2.349±0.015	81.86±0.005	97.80±0.023
0.5%	M1	Nil	116.4±0.009	0.440±0.012	106.6±0.015	115.2±0.012
	M4	Nil	113.9±0.016	1.468±0.009	103.7±0.013	116.7±0.009
1.0%	M1	Nil	116.8±0.005	0.257±0.003	110.3±0.021	118.7±0.021
	M4	Nil	119.9±0.004	1.054±0.002	116.2±0.016	120.9±0.006

Enzyme produced by *P.cinnabarinus* in M1 and M4 medium in presence of varied inducers at different concentrations at 28-30°C in an orbital shaking incubator for 15 days. Enzyme activity was calculated in U/ml/min. M1=Coll *et al.* medium, M4=Potato dextrose broth. Each value is an average of three replicates and ± indicates standard deviation among replicates**.**

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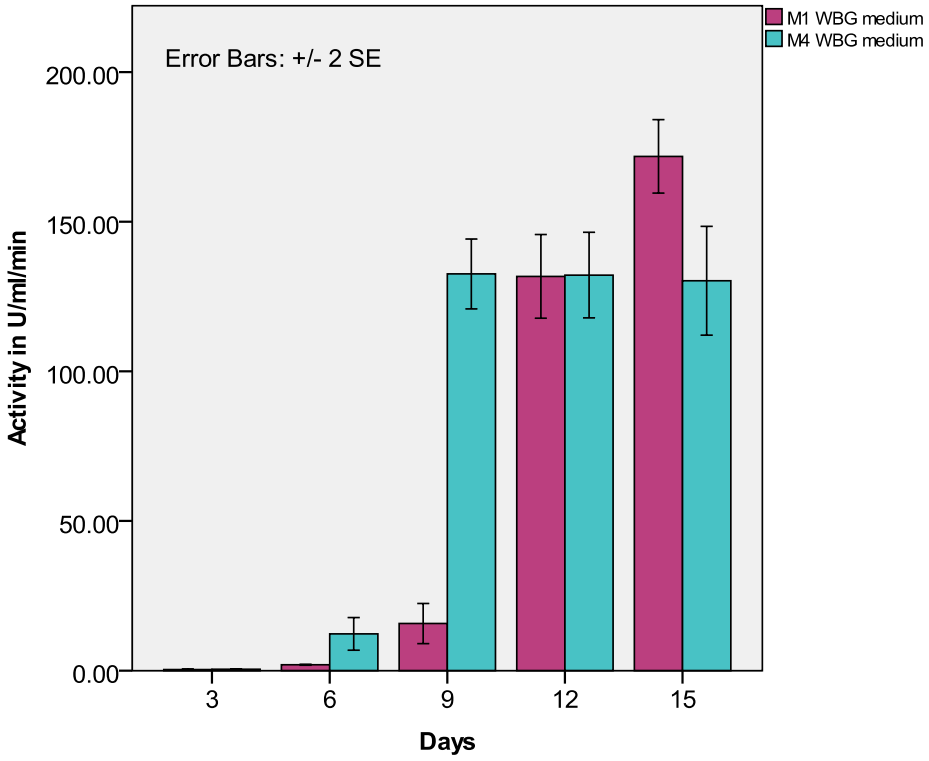
Optimum incubation period

As the laccases production was found to be higher in both in M1 (M1+WB+G), M4 (M4+WB+G) by *P.cinnabarinus*, the optimization study for incubation period was carried out for the two mentioned media (M1 and M4). As a result maximum productivity was observed with in 9 days in M4 medium by both the test cultures where as it took 12-15 days in M1 medium. Minimum production was observed in 3 days in both M1 and M4 media by *P.cinnabarinus.* only 0.348U/ml/min and 0.464U/ml/min was obtained in M1 and M4 media respectively in 3 days of incubation but later it resulted in maximum yield in 15 days in M1 medium and with in 9 days in M4 medium (Graph.1). Although the laccases production is higher in M1 medium when compared to M4 by *P.cinnabarinus*, M4 medium was selected for further research due to its cheaper cost and the shorter time of incubation required for production. Other authors have also found different optimum incubation period of 12 to 20 for higher protein/ enzyme production based on the organism employed. The incubation period similarly was also found to be affected by the medium used, which supports our results (Abo-state *et al.* 2011). However Adebayo *et al.* (2012) and Patrick *et al.* (2010) found highest laccases production by mutant and wild strains of *P.pulmonarius* and *Trametes trogii* in 6-7 days and often isolated strains of fungi.



Graph.1 Optimization of incubation period

Laccases production liquid media, (M1) and M4 are composed of wheat bran and Ghee residue (1%W/V each). The vertical bars show the mean value of triplicates for enzyme activity measured in U/ml/min in every 3 days of time interval. M1=Coll *et al.* medium, WB=Wheat bran, G=Ghee residue.

The presence of Ghee residue is influencing the high production of laccases in both types of media by *P.cinnabarinus.* Ghee residue is beneficial in M4 (WBG) medium for the maximum production [Rasheeda *et al*, 2012].

Optimum pH

P.cinnabarinus was able to produce maximum quantity of laccases (108.32U/ml/min) at pH 3.0. As the pH of medium was increased beyond 3.0, there was a decrease in laccases production. The least or minimum quantity of laccases was observed in the medium with more basic pH i.e. 9.0 with 63.4% decrease in productivity (Graph.2).

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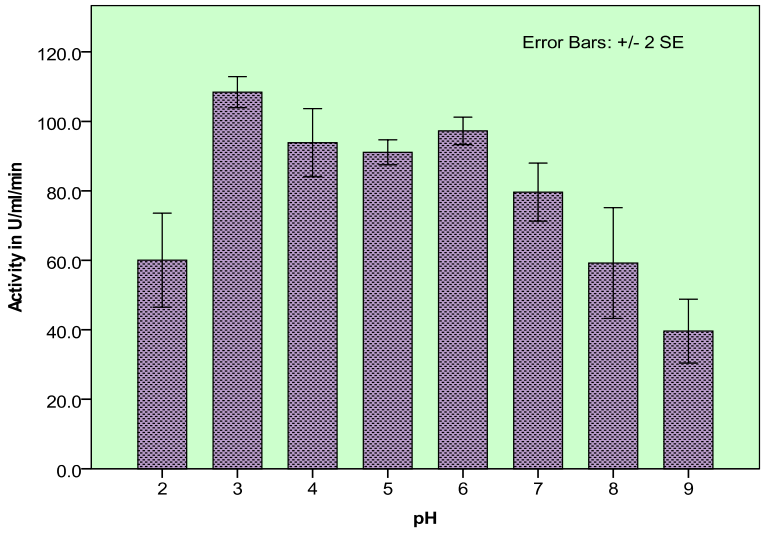
In line with our observations other authors have reported highest laccases at pH5.0 by fungal species like *Botryosphaeria* sp., *Schizophyllum commune*, *Rhizoctonia solani* (Thurston 1994; Vasconcelos *et al.* 2000; Pranjal, 2003; Adejoye *et al.* 2010; Elsayed *et al.*2012). Similarly Ascomycetes like *Alternaria arborescence* and *basidiomycete like Pycnoporus sanguineus* were reported to show maximum production of laccases in pH 4.2- 4.5 (Garcia *et al.* 2006; Christie and Shanmugam 2012).

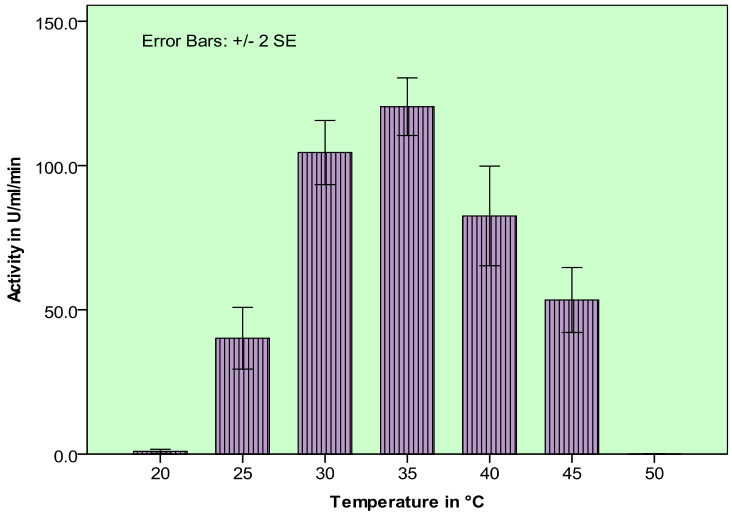


Graph.2 Optimization of pH

Laccases activity produced in the liquid medium (M4+WB+G) with varied pH by the test fungi. The vertical bars show the mean value of triplicates for the enzyme activity in U/ml/min after 9days of incubation period.

Optimum Temperature

Optimum temperature for incubating test fungus for laccases production in the M4 (M4+WB+G) production medium at the optimum pH 3.0 was examined. There was no or minimum productivity observed at the minimum (2-6°C) and maximum (50°C) temperatures. *P.cinnabarinus* was able to produce laccases with 0.961U/ml/min activity **(**Graph.3). Our results are supported by earlier reports showing highest laccases production by various fungi between 30-35°C (Adebayo *et al.* 2012; Christie and Shanmugam, 2012; Manimozhi *et al.* 2012). Though there are also reports on high laccases production even above 60°C by organisms such as *Lentinus strigellus*, *Pycnoporus sanguineus* and *Trametes modesta* (Shraddha *et al.* 2011) lower optimum temperature have also been reported such as 25°C for *Pycnoporus sanguineus and 30°C and Lentinus kauffmani* (Pointing *et al.* 2000; Johnsy and Kaviyarasan 2011).



Graph.3 Optimization of temperature

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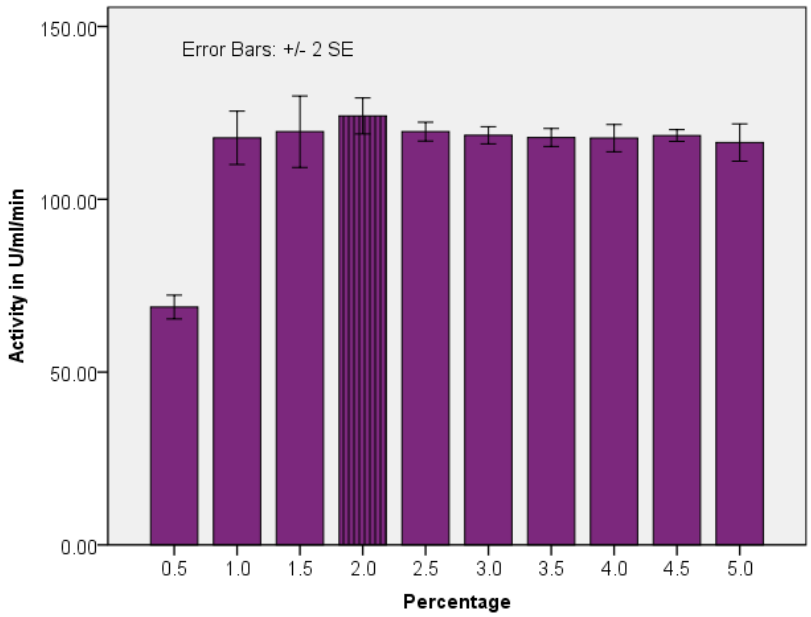
Laccases activity produced in the liquid medium (M4+WB+G) at varied temperatures by the test fungi. The vertical bars show the mean value of triplicates for the enzyme activity in U/ml/min after 9days of incubation period

Optimum concentration of Ghee residue

The concentration of Ghee residue was optimized in the production medium (M4+WB+G) by applying all the optimum conditions namely incubation time of 9 days, pH3.0 and temperature 35˚C. As a result *T.hirsuta* and *P.cinnabarinus* produced laccases with maximum activity when supplemented with 2% of ghee residue in the medium (Graph.4) Later as the concentration was increased, the production was neither increasing nor decreasing significantly.



Graph.4 Optimization of Ghee residue concentration

Laccases production by *P.cinnabarinus* (KR) in liquid medium (M4+WB+G) with varied concentrations (%) of Ghee residue. The vertical bars show the mean value of triplicates for enzyme activity in U/ml/min in 9 days.

Concentration of phenolics in Ghee residue

Concentration of total phenolics in 0.5gm of ghee residue which was condensed to 1ml followed by a series of treatment for the separation of Phenolics was calculated in mg of Gallic acid. It was estimated as 2.52 mg of total phenolics present in 1gm ghee residue. Thus it was calculated as total phenolics of approximately 5mg/gm of Gallic acid were supplied to the test fungus in the production medium (2% W/V Ghee residue) for achieving higher laccases production (Rasheeda *et al.,* 2013). Since laccases oxidize phenolic compounds (Pcs), they may also induce its production. Bravo (1998), stated that phenolic compounds are a diverse group of chemicals (over 8000 currently known), produced as secondary metabolites by most plants, as natural deterrents to grazing animals. Pcs get incorporated into milk and milk products. Connella and Foxa (2001) reported that Pcs are found in considerable amounts in ruminant milk (mg/Kg) and it was also found to be present in Gee residue.

CONCLUSION

Ghee residue, by virtue of its chemical composition, nutritional quality, physical characteristics, bulk of production and long shelf life permitting its collection and centralized handling has great potential and is more amenable to exploit its utilization. Ghee residue can be utilized in a number of products like chocolate burfi, samosa filling, chapatis etc. However, most dairy plants in India have not been utilizing ghee residue profitably except for fat extraction. Most of the ghee residue goes to waste (*Butter and Ghee Booklet No. 286*].

In order to utilize whole ghee residue commercially it can be used as an important component in the production medium for laccases production to put it in the market.

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REFERENCES

Abo-State M.A.M., Khatab O., Abo-EL Nasar A. and Mahmoud B. (2011). Factors Affecting Laccase Production by Pleurotus ostreatus and Pleurotus sajor-caju. World Applied Sciences Journal, 14 (11):1607-1619.

Arora D.S. and Sandhu D.K. (1984). Laccase production and wood degradation by Trametes hirsuta. Folia Microbiol., 29, 310-315.

Telma A. G., Maria^ ngela F.S. and Cirano J.U*. (2006). Properties of laccases produced by Pycnoporus sanguineus induced by 2, 5-xylidine. Biotechnology Letters, 28: 633–636.

Lee,Y., Jung K.H., Lee C.H and Park Y. H. (1999). Enhanced production of laccase in *T.versicolor* by addition of ethanol. Biotechnol.Lett., 21, 965-968.

Pointing S.B., Jones E.B.G., Vrijmoed L.L.P. (2000). Optimization of laccase production by *Pycnoporus sanguineus* in submerged liquid culture. Mycologia, 92, 139-144.

Shraddha, Ravi Shekher, Simran Sehgal, Mohit Kamthania, and Ajay Kumar. (2011). Laccase:Microbial Sources, Production, Purification, and Potential Biotechnological Applications. Enzyme ResearchVolume Article ID 217861, 11 pages doi:10.4061/2011/217861.

Adebayo E. A., Oloke J. K., Achana Y., Bora T. C. (2012). Improvement of Laccase Production in *Pluerotus pulmonarius*-LAU 09 by Mutation. Journal of Microbiology Research, 2(1): 11-17.

Adejoye O.D. and Fasidi I. O. (2010). Effect of Cultural Conditions on Biomass and Laccase Production in Submerged Medium by Schizophyllum Commune (Fries), a Nigerian Edible Mushroom, EJEAFCHE, 9 (3):600-609.

Airong Li, Yue Zhu, Liang Xu, Wenqing Zhu and Xingjun Tian. (2008). Comparative study on the determination of assay for laccase of Trametes sp. AJB, 2 (8):181-183.

Arzu (Taspinar) unal, Nazif kolankaya. (2001). Dechlorination of Bleached Kraft Pulp by Laccase Enzyme Produced from Some White-Rot Fungi, Turk J Biol. 25 (1):67-72.

Bravo L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Lead Review Article; NUTR REV., 56 (11):317-33.

Camarero S., Ibarra D., Martínez A., Romero J., Gutiérrez A. and Del Río J. (2007). Paper pulp delignification using laccase and natural mediators, Enzyme Microb. Technol. 401: 1264-1271.

Arul Diana C. S. and Shanmugam S. (2012). Analysis of fungal cultures isolated from anamalai hills for laccase enzyme production effect on dye decolorization, antimicrobial activity, *ijpaes*, 2 (3):143-148.

Connella J.E. O and Foxa P. F. (2001). Significance and application of phenolic compounds in the production and quality of milk and dairy products: a review, INT DAIRY J., 11, 103- 120.

Robert Dekker F.H., Aneli Barbosa M., Ellen Giese C., Saulo Godoy D.S. and Luiz Covizzi G. (2007). Influence of nutrients on enhancing laccase production by Botryosphaeria rhodina MAMB-05. International Microbiology, 10, 177-185.

Desai S. S. and Nityanand C. (2011). Microbial laccases and their applications: A review. Asian J. Biotechnol. 3 (2): 98-124.

Elishashvili V., Parfar H., Kachlishvili E., Chichua D., Bakradze M. and Kokhreidze N. (2001). Ligninolytic activity of basidiomycetes grown under submerged and solid state fermentation on plant raw material (saw dust of grapevine cuttings). Advances in Food Science, 23, 117-123.

Elsayed M. A., Elshafei M., Hassan M. M., Haroun B. M., Othman A. M. (2012). "Optimization of Laccase Production from *Penicillium martensii* NRC 345", Advances in Life Sciences, 2(1):31-37.

Ghindilis A. (2000). Direct electron transfer catalyzed by enzymes: application for sensor development, Biochem. Soc. Trans. 28: 84-89.

Gianfreda L., Xu F. and Bollag J.M. **(**1999). Laccases: A useful group of oxidoreductive enzymes, Biorem. J. 3, 1-26.

Heinzkill M., Bech L., Halkier T., Schneider P.,**(**1998). Characterization of Laccases and Peroxidases from Wood-Rotting Fungi (Family Coprinaceae) AEM, 64 **(5)**:1601-1606.

Johnsy G. and Kaviyarasan V. (2011). Effect of Nutritional and Environmental Conditions on Production of Extracellular Laccase under Submerged Culture Conditions in *Lentinus Kauffmanii.* Int J Curr Pharm Res., 3(4):105-109.

José Renato P. Cavallazzi, Catarina M. Kasuya, Marcos A. Soares. (2005). Screening of inducers for laccase production by *Lentinula edodes* in liquid medium. Brazilian Journal of Microbiology, 36, 383-387.

Kunamneni A., Camarero S., García-Burgos C., Plou, F. J., Ballesteros A. and Alcalde M. (2008). Engineering and applications of fungal laccasess for organic synthesis. Microb. Cell Factories. http:/www.microbialcellfactories.com/content/7/I/32.

Sampoorna Laxmi M.V. and Md.Mazharuddin Khan. (2010). Effect of Natural Phenolic and Lignin rich Inducers on the Production of Laccases by Streptomyces griseus MTCC 4734. International Journal of Engineering Science and Technology, 2(6) 2130-2132.

Lomascolo A., Record E., Herpoël-Gimbert I., Delattre M., Robert J.L., Georis J., Dauvrin T., Sigoillot J.C., Asther M. (2003). Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer. J. Appl Microbiol., 94(4):618-624.

Manimozhi M., Kaviyarasan V. (2012). Screening the Effect of Nutritional Parameters on Biomass and Laccase Production in Submerged Medium by Litter Decomposing Basidiomycete *Agaricus Heterocystis.* Int J Pharm Pharm Sci, 4 (3): 592-599.

Minussi R., Pastore G. M. and Duran N. (2002). Potential applications of laccase in the food industry, *Trends Food Sci. Technol*. 13: 205-216.

Morozova O. V., Shumakovich G. P., Gorbacheva M. A., Shleev S. V. and Yaropolov A. I. (2007). "Blue" Laccases. *Biokhimiya* 72 (10):1396-1412.

Palmieri, G., Giardina, P., Bianco, A., Capasso, A., and Sannia, G. (2000). A novel white laccase from P.ostreasus. J.Biol.Chem., 272(3):1301-31307.

Patrick *et al.* (2010). Optimized production of lignin peroxidase, manganese peroxidase and laccase in submerged cultures of *trametes trogii* using various growth media compositions. Tanzania journal of science, volume 36.

Ponsoni C. et al. (2007). Laccase catalyzed dimerization of hydroxystillenes. *Adv. Synt. Catal.* 349: 1497-1506.

Pranjal, B. (2003). *Production of laccase by the phytopathogenic fungus Rhizoctonia solani.* PhD thesis, Murdoch University. Division of Science and Engineering, School of Biological Sciences and Biotechnology, Murdoch University, Perth, Western Australia.

Rasheeda K.et al. (2012). Exploitation of industrial waste ghee residue in laccases production by *Pycnoporus cinnabarinus* strain sybc-l14, IJPAES, 4 (2012) 2 1-10.

Rasheeda K. *et al.* (2013). Evaluation of Total Phenolic content in Ghee residue: Contribution to Higher Laccase Production. Microbiology Journal, Volume 3, Number 1, 12-20.

Sathiya moorthi P., Periyar selvam S., Sasikalaveni A., Murugesan K., and Kalaichelvan P. T. Decolorization of textile dyes and their effluents using white rot fungi, Afr J Biotechnol ,6 (2007) 4, 424-429.

Susana Rodrı´guez C. and Angeles S. (2005). Coconut flesh: a novel raw material for laccase production by Trametes hirsuta under solid-state conditions. Application to Lissamine Green B decolorization, J Food SCI 71 (2):208-213.

Thurston C. (1994). The structure and function of fungal laccases, Microbiology, 140, 19–26**.**

Butter and Ghee Booklet No. 286, Dairy Management and Milk Products: DMMPS -16.

Vasconcelos, A.F.D., Barbosa, A.M., Dekker, R.F.H., Scarmínio, I.S., Rezende, M.I. (2000). Optimization of laccase production by *Botryosphaeria* sp. in the presence of veratryl alcohol by the response-surface method. Process Biochem., 35, 1131-1138.

Xavier A. M. R. B., Evtuguin D. V., Ferreira R. M. P. and Amado F. L. (2001). Laccase production for lignin oxidative activity. Proceedings of the 8th International Conference on Biotechnology in the Pulp and Paper Industry, 4-8 June, Helsinki, Finland.

Younes B. S., Mechichi T., Sayadi S. (2007). Purification and characterization of the laccase secreted by the white rot fungus *Perenniporia tephropora* and its role in the decolourization of synhthetic dyes. J. Appl. Microbiol. 1033-1042.