Characterization of Lignocellulosic Enzymes from White-rot Fungus *Phlebia chrysocreas* Isolated from a Marine Habitat

[1]Godliving Mtui and [2]Yoshitoshi Nakamura
[1]Department of Molecular Biology and Biotechnology, University of Dar es Salaam,
P.O. Box 35179, Dar es Salaam, Tanzania
[2]Department of Life System, Institute of Technology and Science, The University of Tokushima,
2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

Abstract: Marine fungus *Phlebia chrysocreas* was isolated from decomposing mangrove leaves in Western Indian Ocean cost and cultured in the laboratory. Protein content and lignocellulosic enzyme activities were measured by photometric methods. Desalted and size-separated enzyme filtrates were resolved by sodium Docecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Isoelectric Focusing (IEF). The fungal filtrate had maximum Lignin Peroxidase (LiP), Manganese Peroxidase (MnP) and Laccase (Lac), activities of 45, 37 and 11 U mL^{-1}, respectively. *P. chrysocreas,* showed effective (92-100%) decolorization of synthetic azo dyes (Azure-B, Poly-B and Poly-R) in liquid cultures and completely decolorized textile wastewater in immobilized cultures. The LiP and MnP from *P. chrysocreas* purified by gel filtration chromatography revealed it to have molecular weights of 46 kDa and 47 kDa and isoelectric points of 4.0 and 3.8, respectively. Purified fractions had optimal reaction rates at temperature of 30°C for both LiP and MnP while optimal pH for LiP and MnP were 4.5 and 5.0, respectively. The study confirmed extracellular enzymes from *P. chrysocreas* to be potential degraders of organic pollutants and showed that facultative marine fungi that live under harsh seawater conditions are suitable for bioremediation of recalcitrant compounds.

Key words: Lignocellulosic enzymes, biodegradation, gel filtration chromatography, electrophoresis, isoelectric focusing

INTRODUCTION

Basidiomycetes fungi produce a wide range of extracellular cellulolytic and ligninolytic enzymes capable of degrading lignocellulosic wastes and other recalcitrant organopollutants into soluble substances. Three major classes of extracellular enzymes namely Manganese Peroxidase (MnPs), Lignin Peroxidase (LiPs) and Laccases (Lacs) are believed to be important in fungal degradation of lignin. MnPs and LiPs are heme proteins while Lacs are copper-containing proteins. Some wood degrading fungi contain all three classes of LMEs while the others contain only one or two of these enzymes. These enzymes are secondary metabolic products, differing in chemical compositions and are often species-specific (Härkönen *et al.,* 2003; Pointing *et al.,* 1998; Saparrat, 2000; Nakamura and Mtui, 2003). Due to their specificity and strong oxidative ability, they are capable of degrading a wide variety of recalcitrant pollutants including textile and pulp effluents, organohalide agrochemicals, petroleum by-products and other synthetic aromatic compounds that result to degraded environment and health hazards to human beings (Jager *et al.,* 1995; Kamei *et al.,* 2005).

Compared to terrestrial fungi, marine fungi have been the least studied. Only limited reports mostly on *Flavodon flavus* has so far been reported (Raghukumar *et al.,* 1994, 1996, 1999; Raghukumar and Paula, 2000; Raghukumar and Rivonkar, 2001; Raghukumar *et al.,* 2004a, b; Raghkumar, 2005). Although studies on *F. flavus* have shown that it produces all important lignocellulosic enzymes that could decolorize individual synthetic dyes (Raghukumar *et al.,* 1999), no attempts have been made to study the enzyme profiles and characteristics of other tropical marine lignocellulosic fungi. Therefore, the marine fungi of East African coast in the western Indian Ocean are an interesting subject of research.

Characterization of lignocellulosic enzymes is important in order to identify potential specific isoforms

Corresponding Author: Yoshitoshi Nakamura, Department of Life System, Institute of Technology and Science,
The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

that are suitable for remediation purposes. Chromatographical techniques have been shown to be conducive in separating individual proteins into isoenzymes in order to evaluate their properties The separated fractions are resolved by gel electrophoresis which is a powerful tool in the analytical separation of proteins such that proteins are separated according to their molecular sizes. On the other hand, in Isoelectric Focusing (IEF) is another biochemical technique in which protein molecules are separated as they migrate through a pH gradient under a strong electric field (Scopes, 1982; Deutscher, 1990). Although it was shown by Raghukumar *et al.* (1999) that concentrated filtrates of *F. flavus* (strain 312) from Arabian Sea cost has a wide range of isoelectric points (of pI 3-6) and relative molecular masses of 43-99 kDa (Raghukumar *et al.*, 1999) no studies have been done on other marine and facultative fungi to characterise their extracellular enzymes.

In this study, we investigated the production and application of lignocellulosic enzymes from marine fungus *Phlebia chrysocreas* collected in the mangrove forests of coastal Tanzania. Furthermore, the active fractions of the purified enzymes were studied to determine their characteristics.

MATERIALS AND METHODS

Sampling site, fungi isolation and identification: The basidiomycetous fungus, *Phlebia chrysocreas* (TMIC31891), was isolated from decaying mangrove leaves collected at Mtoni Creek off the coast of Dar es Salaam, Tanzania. It was identified based on morphological and microscopic features (Suhara *et al.*, 2005; Zmitrovich *et al.*, 2006).

Culture media and cultivation of mycelia: Filter-sterilized full-strength seawater, 1:1 diluted seawater and distilled water were used in various experiments. Solid media consisted of 5% (w v^{-1}) Malt Extract Agar (MEA), 10 g L^{-1} glucose, 0.02 g L^{-1} malt extract and 20 mL of Kirk medium in 0.4 M phthalate buffer (pH 4.5) (Nakamura *et al.* 1997, 1999). The liquid medium contained 12.7 g L^{-1} malt extract, 10% carbon source (glucose, glycerol or bagasse) and 25 mL Kirk medium. Solid medium cultivation was done in 10 mm-diameter petri dishes. Leave discs (1 cm diameter) containing *P. chrysocreas* were cut and surface-sterilized with 0.5% sodium hypochlorite and then cultured in the petri dished at 30°C for 5-7 days for production of mycelial mats. Cultivation in liquid medium was done in 250 mL conical flasks (25 mL working volume) plugged with cotton wool and then covered with aluminium foil. The culture media was

sterilized by autoclaving at 121°C for 20 min. The flasks were then inoculated with 5 mm mycelial mats (from solid cultures) and incubated in stationary condition at 30°C for up to 2 weeks. *Phanerachaete chysosporium* (DSM 1556) maintained in agar slants was used as a positive control.

Decolorization of textile wastewater: Raw (dark-blue) wastewater from *Karibu* Textile Mill Ltd, Dar es Salaam, was sampled at various outlets and mixed thoroughly. The effluent was diluted (1:1) by using seawater followed by addition of 0.02% yeast extract. Fungal mycelia were then aseptically inoculated into the 250 mL flask (working volume 50 mL) and the reaction mixture was incubated at 30°C for 2 weeks in stationary condition. Supernatant of the culture was drawn at 2 day intervals and centrifuged at 5,000 rpm or 15 min. Control experiments were conducted using the same medium without inoculum. The color intensity (absorbance) was determined by using UV-Visible Thermo Stonic spectrophotometer, (UK) at 535 nm (Yang *et al.*, 2003).

Decolorization of synthetic dyes: The following reagent-grade synthetic dyes were used: Azure-B, Poly-B and Poly-R (Sigma). Stock solutions (1% w v^{-1}) were filter-sterilized (pore diameter 0.2 μm membrane) and the fungal mycelia were cultivated in stationary 250 mL Erlenmeyer flasks (working volume 50 mL). Decolorization of the supernatant was detected by UV spectrophotometer after centrifugation at 5000 rpm for 10 min. Absorbance was measured at λ_{max} of each dye (Raghkumar *et al.*, 1999). Color removal was reported as % decolorization = $(A_i-A_t)/A_i \times 100$. Where A_i is the initial absorbance before incubation and A_t is the absorbance after incubation time, t (Yang *et al.*, 2003).

Sample concentration and dialysis: Crude enzyme filtrates were successively filtered in 0.45 μm and 0.2 μm Acrodisc Syringe Filters (*Pall* Gelman Lab, USA). The 3 mL samples were concentrated 10-fold by ultrafiltration using 10 kDa cut-off Microsep centrifugal devise containing omega membrane (*Pall* Life Sciences, USA at 5000 rpm for 3 h at 4°C. The Micfrosep devise retained in the reservoir the concentrated proteins of molecular weight larger than 10 kDa, while lower molecular weight proteins and solvent passed through the membrane into the filtrate reservoir. The supernatant was collected and the total protein, total activity and specific activity were measured. The concentrated proteins were stored at 4°C for further analysis.

Desalting of proteins by gel chromatography: Enzyme desalting was done by using PD-10 column (void volume 3.5 mL) parked with Sephadex G-25 (dextran) gel

(Pharmacia Co., Sweden). The column was equilibrated with 10 mM Bistris [bis(2-hydroxyethyl)imino-tris (hydroxymethyl) methane] - HCl buffer (pH 6.5) and 3.5 mL of the buffer was used for elution. The eluent was collected and its absorbance at 280 and 405 nm was measured by UV-Visible spectrophotometer (Shimadzu Co. Ltd., Japan).

Enzyme purification by Gel Filtration Chromatography (GFC): The sepharose CL-4B column with void volume of 30 mL (Pharmacia, Sweden) was coupled to HPLC pump (LKB 2150, Sweden) with a flow rate of 0.5 mL min^{-1}. The column was equilibrated with Bistris-HCl buffer (pH 6.5). The enzyme eluent was separated by a fraction collector (Microcol TDC 80, Gilson, Sweden) set at 3 min intervals. The protein content of the eluted fractions were collected and monitored by a spectrophotometer (Shimadzu, Japan) at 280 nm for Laccases, while the heme protein (LiP and MnP) were monitored at 405 nm. Total proteins (mg L^{-1}) were determined by a Bradford (1976) method using protein dye and bovine serum albumin standard (Bio-Rad, USA).

SDS-PAGE and IEF analyses: Modified Laemmli (1970) method of SDS-PAGE analysis using 30% acrylamide:0.8% bis-acrylamide, 2 M Tris-HCl buffer (pH 8.8), 20% SDS, 0.02% $(NH_4)_2S_2O_8$ and 0.01 TEMED was used to resolve the purified proteins. Electrophoresis was carried out at 150 V and the gel staining was done using 0.05% (w v^{-1}) Coomassie Brilliant Blue (CBB) R 150 followed by de-staining at 10% methanol-acetic acid solution.

Isoelectric points (pIs) were determined by using IEF Unit, commercial ampoulines and protein markers (Pharmacia, Sweden) at pH range of 2.5-9.5. Elecrofocusing was carried out at 1,500 V and 50 mA for 1.5 h. The gels were stained by 0.01% (w v^{-1}) of CBB R 250 followed by destaining in 20% (v v^{-1}) ethanol-acetic acid, solution (Scopes, 1982; Deutscher, 1990).

Determination of enzymes activities: LiP activity was determined spectrophotometrically at 310 nm through the oxidation of veratryl alcohol to veratryl aldehyde (molar absorptivity, ϵ_{310}, = 9300 M^{-1}cm^{-1}). The reaction mixture contained 300 μl veratryl alcohol (8 mM), 600 μL sodium tartrate buffer (0.5 M, pH 4.5 at 27 ^{0}C), 60 μL mycelia liquid fraction and 1890 μL distilled water. The mixture was incubated for 2 min at 30°C and the reaction was initiated by addition of 150 μL H$_2$O$_2$ (5 mM). The absorbance was immediately measured in 1 min intervals after addition of H$_2$O$_2$. One Unit (U) of LiP activity was defined as activity

of an enzyme that catalyzes the conversion of 1 μ mole of veratryl alcohol per minute (Nakamura *et al.*, 1997, 1999).

Activity of MnP was measured following the method described by Wunch *et al.* (1997). In this method, guaicol was used as a substrate, then the increase in absorbance at 465 nm due to oxidation of guaicol was measured (ϵ_{465} = 12,100 M^{-1} cm^{-1}). The reaction mixture contained 300 μL sodium succinate buffer (0.5 M, pH 4.5 at 27°C), 300 μL guaicol (4 mM), 600 μL manganese sulphate (1 mM), 300 μL mycelial liquid fraction and 1200 μL distilled water. The mixture was incubated for 2 min at 30°C and the reaction was initiated by addition of 300 μL hydrogen peroxide (1 mM). The absorbance was measured immediately at 465 nm in one-minute intervals after addition of hydrogen peroxide one unit of MnP activity was defined as activity of an enzyme that catalyzes the conversion of 1 μmole of guiacol per minute.

Lac activity was measured by using the method described by Bourbounnais *et al.* (1995) based on the oxidation of 2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS). The rate of ABTS oxidation was determined spectrophotometrically at 420 nm (ϵ_{420}= 36,000 M^{-1} cm^{-1}). The reaction mixture contained 600 μL sodium acetate buffer (0.1M, pH 5.0 at 27°C), 300 μL ABTS (5 mM), 300 μL mycelial liquid fraction and 1400 μL distilled water. The mixture was incubated for 2 min at 30°C and the reaction was initiated by addition of 300 μL hydrogen peroxide. The absorbance was measured immediately in one-minute intervals after addition of hydrogen peroxide. One unit of laccase activity was defined as activity of an enzyme that catalyzes the conversion of 1 μmole of ABTS per minute.

RESULTS AND DISCUSSION

Production of extracellular enzymes by phlebia chrysocreas: Enzymes activities from *P. chrysocreas* grown in seawater containing Kirk's basal medium are shown in Fig. 1. The enzymes were produced during the idiophase when the fungi was carrying out secondary metabolism in carbon- and nitrogen-limited conditions. Enzymes production started from day 7 where LiP increased sharply and peaked at 45 U mL^{-1} in day 9. MnP increased steadily from day 8 and peaked at 37 U mL^{-1} in day 11. Lac was the least produced, the maximum of 10 U mL^{-1} being attained at day 12. The results were consistent with the work by Raghukumar *et al.* (1999) and Kondo *et al.* (2004) who demonstrated the ability of some costal marine fungi to produce major lignocellulolytic enzymes. The amounts of LiP and MnP and Lac are comparable to the amounts produced by the Tanzania's
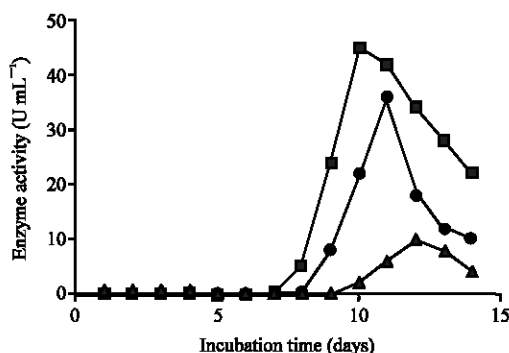
Fig. 1: Enzyme activities by *Phlebia chrysocreas* grown on stationary culture with Kirk's basal medium in seawater. Symbols: LiP (■); MnP (●), Laccase (▲)
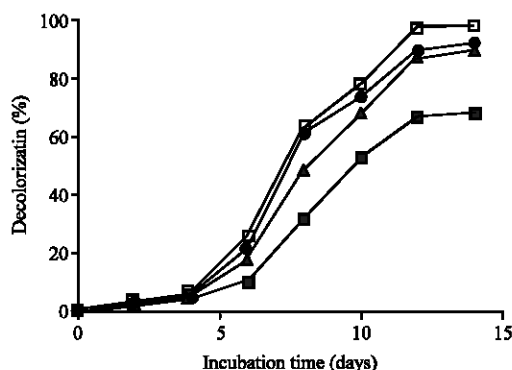

Fig. 2: Decolorization of 1% synthetic dyes and 1:1 diluted textile wastewater by cultures of *Phlebia chysocreas*. Azure-B (□), Poly-B (●), Poly-R (▲) and textile waste water(■)

terrestrial mushrooms (Mtui *et al.*, 2003; Mtui and Nakamura, 2004). The decrease in enzyme activities after their peaking is attributable to presence of protease in the medium (Nakamura *et al.*,1999; Mtui and Nakamura, 2002). The filtrate was therefore, harvested for purification after12 days of cultivation. The fact that all the major lignocellulolytic enzymes were present in the culture of *P. chrysocreas* is an indication that the stain is a strong degrader of organic substrates.

Decolorization of textile effluent and synthetic dyes: Decolorization of synthetic azo dyes and raw textile effluent by extracellular enzymes secreted by *P. chrysocreas* at different culture condition are shown in Fig. 2. For Azure-B, Poly-B and Poly-R, the color removal of 94-100% was achieved after 12 d of cultivation in seawater medium. As for the textile effluent, up to 64% decolorization was observed under the same culture

conditions. The decolorization of azo dyes contained in the synthetic dyes and raw wastewater shows that LiP, MnP and Lac produced by the fungus have broad substrate range. The findings are comparable to the report by Nilsson *et al.* (2006) who found that terrestrial white-rot fungus *Pleurotus flabellatus* could degrade real textile wastewater by 67%. Color removal by filamentous fungi has been attributed to be mainly due to biosorption to the mycelium (Yang *et al.*, 2003), but Gonzalo *et al.* (2005) and Raghukumar (2005) have shown that apart from adsorption, lignolytic enzymes are capable of carrying our catalytic and free-radical mediated breakdown of aromatic compounds including ring cleavage and thus they can be used for bioremediation of contaminated sites.

Table 1 shows the decolorization of 1:1 diluted textile waste and mycelial biomass of *P. chrysocreas* after 12 days of cultivation in different media and growth conditions. Complete delolorization and highest biomass production were achieved when glucose was used as a carbon source in Polyurethane Foam (PUF)-immobilized mycelia. However, glycerol and baggase were also favorable carbon sources resulting over 90% decolorization. When half strength seawater was used instead of distilled water, there was no significant difference in decolorization rates and biomass production. Seawater cultures performed better (95% compared to 64% decolorization) in immobilized culture than free liquid cultures. The results are consistent with the reports by Shin *et al.* (1997) and Yang *et al.* (2003) who observed significant delolorization by terrestrial fungi and Raghukumar *et al.* (1999, 2004) who reported the same trends for facultative marine fungi. This study shows that bagasse from sugar cane waste can be used as a cheap substrate for fungi cultivation and fungal bioremediation. In addition, for efficient degradation, immobilization with PUF improves enzymatic reactions and biomass growth because free enzymes may be subjected to thermal instability, susceptibility to protease attack and problems of reusing them after completion of the reaction (Nakamura *et al.*, 1999; Raghukumar, 2005; De Souza *et al.*, 2006). It is apparent that decolorization of wastes alone may not always solve the pollution problem completely (De Souza *et al.*, 2006). Therefore, it is necessary to carry out studies on mechanisms of enzyme degradability and detoxification. Future research should be focused towards bioprospecting of native or bioengineered strains that overproduce lignin-degrading enzymes for complete degradation of recalcitrant pollutants.

Purification of lignin peroxidase from Phlebia chrysocreas: The concentrated enzyme filtrates of

Table 1: Decolorization of 1:1 diluted textile wastewater and mycelial dry weight at various cultivation conditions after 12 days of stationary culture (50 mL medium volume) cultivation. Each value is an average of triplicate samples

Medium composition		Liquid culture		PUF-immobilized culture	
Solvent in Kirk's basal medium	Carbon source (10 % w/v)	Decolorization (%)	Mycelial dry weight (mg)	Decolorization (%)	Mycelial dry weight (mg)
Distilled water	Glucose	84.3	1132.2	100	1820.9
	Glycerol	82.2	962.6	94.4	1332.6
	Bagasse	79.7	512.2	90.9	1119.1
Half-strength seawater	Glucose	80.8	884.8	97.3	1439.3
	Glycerol	74.9	618.5	92.2	1137.3
	Bagasse	70.1	502.9	90.0	989.4
Seawater	Glucose	64.4	773.1	95.0	1051.7
	Glycerol	62.1	522.7	86.6	892.1
	Bagasse	60.3	442.0	85.2	773.4

Table 2: Purification of lignin peroxidase from the culture filtrate of *Phlebia chrysocreas*. (Fraction 1 = LiP: Fraction 2 = MnP)

Procedure		Total activity (U)	Total protein (mg)	Specific activity (U mg^{-1})	Yield (%)	Purification (fold)
Culture filtrate (0.2 μm membrane filter)		4533.5	185.5	24.4	100	1
Microsep 10 kDa membrane		1812.2	71.4	25.4	40.0	1.1
Sephadex G-25 size separation chromatography		994.5	27.2	36.6	21.9	1.5
Sepharose CL-4B gel filtration chromatography-	Fraction 1	817.4	0.9	908.3	18.0	37.2
	Fraction 2	571.1	0.7	818.9	12.6	33.6

P. chrysocreas, pretreated by desalting gel chromatography, were subjected to gel filtration chromatography that resulted to two dominant fractions containing LiP and MnP. As shown by the SDS-PAGE analysis in Fig. 3, the fractions resulted to sharp bands between 46 kDa and 47 kDa. Spectral analysis at 280 (overall proteins) and 405 nm (heme proteins) showed that the eluted fractions were heme proteins. The results are comparable to studies by Raghukumar (1999) which showed that relative molecular masses of marine *Flavodon flavus* (strain 312) isoforms ranged from 41.5 to 99 kDa, signifying the versatility of facultative marine fungi in degrading a variety of lignocellulosic substrates and xenobiotics.

Isoelectric Focusing (IEF) analysis of two main fractions separated by gel filtration chromatography demonstrated distinct bands at pI 3.8 and pI 4.0 (Fig. 4). The low pI values indicate that the number of acidic groups in the structures of lignocellulosic enzymes from *P. chrysocreas* exceeds the number of basic groups. The IEF values obtained from this study are comparable to the values of pI 3 to pI 6.0 reported by Ranghkumar (1999) for *F. flavus* strain (312). It is worth noting that laccase fraction could not be clearly resolved in SDS-PAGE or IEF because the fraction collected had very low protein content. Optimized enzyme production will be the focus of the future research.

Table 2 summarizes the purification procedures of the main lignocellulosic enzymes from *P. chrysocreas*. The purification of LiP and MnP fractions by gel filtration chromatography gave a final specific activities of 908.3 and 818.9 U mg^{-1} protein with 8.3 purification-fold

Table 3: Substrate specificities for purified LiP (Fraction 1) and MnP (Fraction 2) from *Phlebia chrysocreas*

Substrate	Maximum absorbance (nm)	Activity (U mg^{-1} protein)	
		Fraction 1	Fraction 2
Veratryl alcohol	310	908.8	1.3
Guaiacol	465	66.9	819.4
ABTS	420	327.3	330.9
Catechol	398	82.9	127.2
2,4-dimethoxyphenol	468	234.7	398.1
o-phenylenediamine	440	322.6	432.4
p-phenylenediamine	459	546.1	531.3

and a recovery rate of 37.2 and 33.6%, for fractions 1 and 2, respectively. The purification process, judged by the final specific activities, was fairly successful. It was therefore confirmed that *P. chrysocreas* possess heme proteins that are characteristic to LiP and MnP. The results are comparable to reports by Coll *et al.* (1993), Shin *et al.* (1997) and Nakamura *et al.* (1999) who demonstrated successful purification of lignocelluosic enzymes by fungal filtrates from terrestrial basidiomycetes fungi.

Substrate specificities for the purified enzymes are shown in Table 3. The specific activities varied from 1.3 to 819 U mg^{-1} protein depending on the substrate used. The fact that Fraction 1 readily oxidized veratryl alcohol at 310 nm confirms it to be purified LiP. Similarly, guaiacol was mostly oxidized at 465 nm by Fraction 2 suggesting that it is a MnP. The study elucidated *P. chrysocreas* to posses degrading enzymes which are non-specific to substrates and, therefore, are capable of oxidizing a variety of aromatic environmental pollutants.

The effects of pH on relative activities of purified LiP and MnP were examined at pH values ranging from 3.0 to
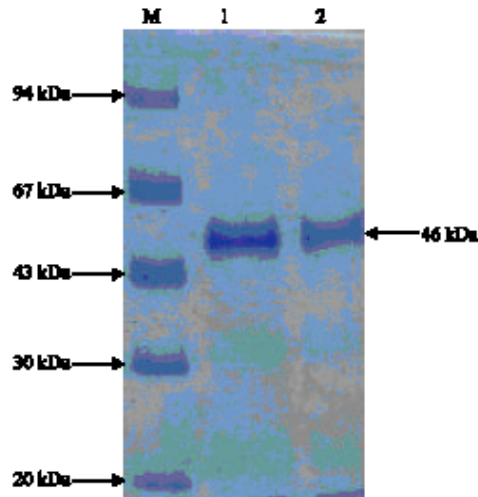
Fig. 3: SDS-PAGE analysis of LiP (Lane 1) and MnP (Lane 2) from *Phlebia chrysocreas* purified by gel filtration chromatography. M: Marker proteins: Phosphorylase-b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa)
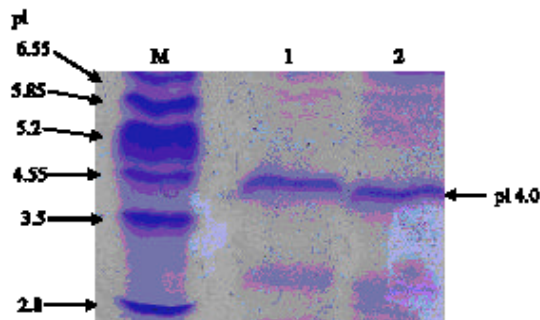

Fig. 4: Isoelectric focusing of LiP (Lane 1) and MnP (Lane 2) purified by gel filtration chromatography. Marker proteins: Pepsinogen pI 2.8, amyloglucosidase (pI 3.5), soybean trypsin inhibitor (pI 4.55), B-lactoglobulin A (pI 5.2, bovine carbonic anhydrase B (pI 5.85), human carbonic anhydrase B (pI 6.55)

8.0. The bell-shaped curve obtained in Fig. 5 shows the optimum pH for purified LiP and MnP fractions to be 5.0 and 4.5, respectively. Enzyme reaction rates increased steadily from pH 3 to pH 4, peaking at pH 4-5 and then decreased rapidly, before ceasing at pH 9. It is noteworthy that at pH 7-8, there was still some limited enzyme activities. This suggests that enzymes from *P. chrysocreas* could carry out *in situ* degradation of recalcitrant substrates in marine environments which
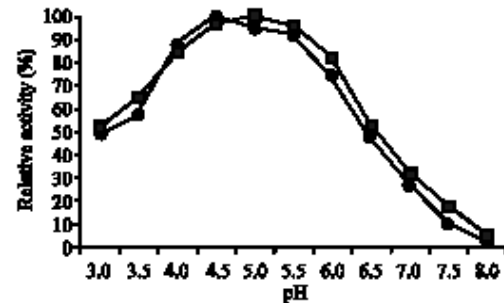

Fig. 5: pH dependence on relative activities of purified Fraction 1 (LiP, ■) and Fraction 2, (MnP, ●) from *Phlebia chrysocreas* at 30°C under standard assay conditions with 0.1 M citrate buffer (pH 3-6) and 0.1 M phosphate buffer (pH 6-8)
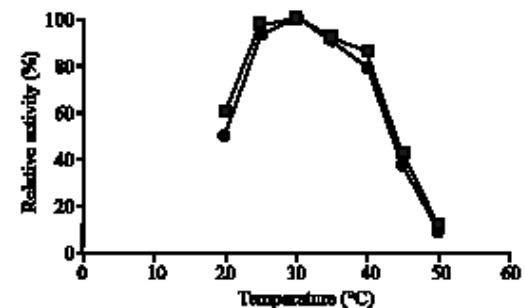

Fig. 6: Temperature dependence on relative activities of purified Fraction 1 (LiP, ■) and Fraction 2, (MnP, ●) from *Phlebia chrysocreas* using 20mM sodium acetate buffer (pH 4.5) under standard assay conditions

have alkaline pH. The acidic pH optima for purified lignolytic enzymes from *Pleurotus ostreatus* and *Bjerkandera adusta* were also demonstrated by Shin *et al.* (1997) and Nakamura *et al.* (1999).

The effect of temperature on relative activities of purified LiP and MnP fractions are shown in Fig. 6. Optimum temperature for both LiP and MnP isoforms were 30°C, The reaction rates stayed high at a temperature range of 25-40°C, below and above which a sharp decline was observed. Comparative optimal temperature values of 27-30°C have been reported by Nakamura *et al.* (1999) for terrestrial fungi. The findings suggest that the extracellular enzymes from *P. chrysocreas* are suited for the tropical climates and therefore conducive for bioremediation of environmental pollutants.

CONCLUSION

Our results have shown that marine fungus *Phlebia chrysocreas* (TMIC31891 isolated from marine

environment had maximum lignin peroxidase, manganese peroxidase and laccase activities of 45, 37 and 11 U mL^{-1}, respectively. *P. chrysocreas*, showed effective (92-100%) decolorization of synthetic (Azure-B, Poly-B and Poly-R) azo dyes in liquid cultures and completely decolorized textile wastewater in immobilized cultures. The purified enzymes revealed *P. chrysocreas* to have relative molecular weights of 46 and 47 kDa and isoelectric points of 4.0 and 3.8 corresponding to LiP and MnP, respectively. Purified fractions for LiP and MnP had an optimal temperature of 30°C and optimal pH of 4.5 and 5.0, respectively. The study confirmed *P. chrysocreas* to be one of the potential degraders of marine pollutants and showed that facultative marine fungi that live under harsh seawater conditions have important roles in bioremediation of xenobiotics. Future research should focus on optimized enzymes production and toxicity studies of the decolorized organic pollutants.

ACKNOWLEDGEMENT

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