

Full Length Research Paper

# Extracellular enzymes from brown-rot fungus *Laetiporus sulphureus* isolated from mangrove forests of coastal Tanzania

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A brown-rot fungus, *Laetiporus sulphureus* (Fr.) Murr., was isolated from Mweni, Oyster Bay and Mtoni Mangrove Forests in Dar es Salaam, Tanzania, and the biochemical properties of its extracellular enzymes were investigated. The crude culture filtrate was concentrated by ultrafiltration. Protein content and lignocellolytic enzyme activities were measured by photometric methods. The crude enzyme extract was purified by gel chromatography and characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The fungal filtrate had maximum manganese peroxidase (MnP) of 2.5 U/mL and lignin peroxidase (LiP) of 1 U/mL, but showed no laccase (Lac) activity. The enzyme extracts were able to oxidize rhemazol brilliant blue-R (RBB-R) dye and phenol, and could remove up to 90% color from raw textile effluent in immobilized culture. The purified peroxidases showed that the MnP from *S. sulphureus* has a molecular weight of 48 kDA. The study elucidated the extracellular enzymes profile of facultative marine *L. sulphureus* and provided basic information on their potential for biological wastewater treatment systems.

**Key words:** Brown-rot fungi, extracellular enzymes, biodegradation, electrophoresis.

## INTRODUCTION

In Tanzania, industrial discharges have led to serious coastal and marine environmental pollution. Untreated pollutants such as crude oil wastes, textile effluents, organochloride agrochemicals, pulp effluents and others have continued to be discharged to open water bodies. However, the country has a rich diversity of basidiomycetous fungi with potential applications in both *ex situ* and *in situ* biodegradation of such pollutants (Härkönen et al., 2003; Mtui et al., 2003; Masalu, 2004). The lingo-cellulosic enzymes from fungi can also be used in the introduction of environmentally sound biotechnologies for the polluting industries, and for treatment of wastewater or soils to remove persistent pollutants such as dichloro-diphenyltrichloroethane (DDT), polychlorinated biphenyls

(PCBs) and dioxins (Nakamura et al., 1997, 1999; Saparrat, 2000; Mtui and Nakamura, 2002; Coulibaly et al., 2003; Martinez et al., 2005). So far, in Tanzania most of research done has been focused on edible mushrooms, with more than 50 edible species being already identified (Härkönen et al., 2003; Mtui et al., 2003). However, research on the *L. sulphureus* inhabiting coastal areas including mangrove forests has not been carried out.

Fungi associated with decaying sea grass and mangrove plants contribute about 3% of the biomass per gram (dry basis) of detritus and nearly 77 - 100% nitrogen in decaying salt marsh grass ecosystems (Newel, 1993). The association and importance of terrestrial fungi with plant detritus in the coastal marine environment have so far received little attention. Worldwide, there is little published information on the lignin-degrading ability of the obligate and facultative marine fungi (Mtui and Nakamura, 2007). Moreover, the presences of MnPs,

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LiPs and Lacs in facultative and marine fungi have not been investigated except for few reports (Datta et al., 1991; Raghukumar et al., 1999). Currently, research on terrestrial fungi as a source of degradative enzymes has received a lot of interest. The abilities of terrestrial ligninolytic and non-ligninolytic fungi in the bioremediation of polycyclic aromatic hydrocarbon (PAHs), benzene-toluene-ethylbenzene-xylene (BTEX), chlorophenols, polychlorinated biphenyl, munitions waste and pesticides have been demonstrated (Gonzalo et al., 2005). However, the ability of marine fungi to degrade such toxins have not been investigated (Mtui and Nakamura, 2007).

Culture immobilization with polymeric matrices such as polyurethane foam (PUF) improves enzymatic production 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 et al., 1999).

*Laetiporus sulphureus* (Fr.) Murr. is a wood-inhabiting mushroom belonging to family Laetiporaceae and order Laetiporales. It is characterized by bright orange color, fleshy basidiocarps and tubular hymenopores (Banik et al., 1998; Fischer and Wagner, 1999; Zmitrovich et al., 2006). Terrestrial *L. sulphureus* has shown ability of decolorization of dyes (Yesilada, 1995; Shin et al., 1997), mineralization of 2, 4, 6-trinitrotoluene (Scheibner et al., 1997); degradation of benzo[a]pyrene (Wunch et al., 1997), inducing wood decay (Ferraz et al., 2001), and bioremediation of chromated copper arsenate-treated wood (Kartal et al., 2004). The strain has also been found to contain cytotoxic compounds (Yoshikawa et al., 2001; Tateno and Goldstein, 2003; Mancherno et al., 2004).

Purification and characterization of proteins is an important step in enzymology research. Electrophoresis - the movement of an electrically charged substance under the influence of an electric field - is a convenient method used in macromolecular separation. As they migrate through the gel, the movement of larger molecules is impeded relative of that of the smaller molecules. In polyacrylamide gel electrophoresis (PAGE), gels are made of free radical-induced polymerization of acrylamide and N,N'-methylenebisacrylamide in the buffer solution. The polymerization is induced by free radicals resulting from chemical decomposition of ammonium persulphate. N,N,N',N'-tetramethylethylenediamine (TEMED) is added to stabilize the reaction. The detergent sodium dodecyl sulphate (SDS) denatures protein. If the SDS complexes of different proteins are subjected to electrophoresis, they migrate and separate according to their peptide chain length, which is correlated to their molecular weights. The denaturation of proteins is achieved through boiling, presence of SDS, reduction of sulphide bonds with dithioerythritol (DTE) and the alkylation of the thiol groups with iodo acetyl amide (IAA) (Blobel and Dobberstein, 1975; Deutscher, 1990).

The present study involved collection and culturing of *L. sulphureus* from Tanzania's coastal mangrove ecosystem in order to elucidate the characteristics of its purified extra-

cellular enzymes. Furthermore, the potential of *L. sulphureus* extracellular enzymes in degrading recalcitrant organic compounds including textile effluent was investigated.

## MATERIALS AND METHODS

### Study site, sample collection and identification

The fungi were collected at Oyster Bay and Mtoni mangrove forests along the Indian Ocean coast in Dar es Salaam, Tanzania, and identified to be *Laetiporus sulphureus* (Fr.) Murr. based on morphological characteristics (Ainsworth, 1973; Ryvarden and Johansen, 1980; Arora, 1986; Buckzaki, 1992; Phillips, 1994; Bougher and Katrina, 1998; Harkönen et al., 2003; Zmitrovich et al., 2006). The fruiting bodies were brought to the laboratory for tissue culturing.

### Culture media and cultivation of mycelia

Solid media consisted of 5% (w/v) malt extract agar (MEA), 10 g/L glucose, 0.02 g/L malt extract and 20 mL of Kirk medium (Tien and Kirk, 1984). The liquid medium contained 2.7 g/L yeast extract, 10 g/L glucose and 25 mL Kirk medium. Solid medium cultivation was done in 10 mm - diameter petri dishes. Fresh 2 mm<sup>2</sup> tissues of fungal fruiting bodies were aseptically cultured in the petri dished and incubated at 30°C for 5 - 7 days for production of mycelial mats. Cultivation in liquid medium was done in 250 mL conical flasks 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 diameter mycelial mats from solid cultures and incubated in stationary condition at 30°C for up to 2 weeks.

### Plate-tests for RBB-R decoloration and guaiacol oxidation

Solid 2% (w/v) MEA plates containing 0.005 g/L rhemazol brilliant blue-R (RBB- R) (Sigma) were inoculated with 5 mm diameter agar plugs from a 7 day old mycelia of *L. sulphureus* previously grown on 2% (w/v) MEA. Plates were incubated at 30°C in the dark until they were totally colonized. Peroxidases and oxidases production were revealed by formation of a halo in the medium resulting from RBB-R decolorization. Similarly, 7 mm agar plug from 7-day old mycelium grown in 2 % (w/v) MEA was used to inoculate triplicate plates with 0.2 sugarcane bagasse powder, 0.01 guaiacol (Sigma, UK) and 1.6 g agar per 100 mL solution. The plates were incubated for 7 days before being evaluated on the basis of the formation of a reddish coloured zone in the culture medium resulting from guaiacol oxidation (Okino et al., 2000).

### Decolorization of raw textile wastewater

Raw effluent wastewater from *Karibu* Textile Mill Ltd., Dar es Salaam, was sampled at various outlets and mixed thoroughly. This dark blue effluent was diluted (1:1) by using distilled water, followed by filter -sterilization and addition of 0.02% yeast extract. Fungal mycelia were then aseptically inoculated into 250 mL flask and the reaction mixture was incubated at 30°C for 2 weeks under aerobic, stationary incubation conditions. For immobilization cultures, 1 cm cubes of PUF were added into the flasks such that they wholly covered the base of the flask and remained half immersed in the media. Samples were drawn at 2 day intervals and the color intensity (absorbance) was determined by using UV-Visible *Thermo Stonic* spectrophotometer (UK) at the  $\lambda_{max}$  of the dye (535 nm).

### Sample pretreatment

Crude enzyme filtrates were successively filtered in 0.45 and 0.2  $\mu\text{m}$  *Acrodisc* syringe filters (*Pall* Gelman Lab, USA). Three millilitres triplicate samples were concentrated 10-fold by ultrafiltration using *Microsep* devise containing Omega membrane (*Pall* Life Sciences, USA) and centrifuged at 5000 rpm for 3 h at 4°C. The *Microsep* 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 concentrated proteins were stored at 4°C for further analysis.

### Spectrophotometric assay

The spectrometric assay at 280 nm wavelength was used to estimate the presence of proteins in the filtrates by using UV-visible spectrophotometer (*Shimadzu* Co. Ltd., Japan). The absorbance of the samples was also determined at  $A_{260}$  (nucleic acids absorb maximally at this wavelength). The protein concentrations were calculated according to the following empirical equation which takes into account the correction of the interference by nucleic acids (Deutscher, 1990):

$$\text{Protein concentration (mg/ml)} = 1.55 (A_{280} - 0.76 A_{260})$$

### Enzyme assays

Liquid culture medium containing 1% (w/v) glucose, 0.02% ammonium oxalate, 0.02% yeast extract and 0.5 M sodium acetate buffer (pH 4.5) in 30 mL of Kirk's basic medium (Tien and Kirk, 1984; Nakamura et al., 1997, 1999) was used. Lignin peroxidase (LiP) activity was determined spectrophotometrically at 310 nm through the oxidation of veratryl alcohol to veratryl aldehyde (molar absorptivity,  $\epsilon_{310} = 9300 \text{ M}^{-1} \text{ cm}^{-1}$ ). The reaction mixture contained 300  $\mu\text{L}$  veratryl alcohol (8 mM), 600  $\mu\text{L}$  sodium tartrate buffer (0.5 M, pH 4.5 at 27°C), 60  $\mu\text{L}$  culture supernatant, and 1890  $\mu\text{L}$  distilled water. The mixture was incubated for 2 min at 30°C and the reaction was initiated by addition of 150  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (5 mM). The absorbance was immediately measured in one-minute intervals after addition of  $\text{H}_2\text{O}_2$ . One unit (U) of LiP activity was defined as activity of an enzyme that catalyzes the conversion of 1  $\mu\text{mole}$  of veratryl alcohol per minute.

Activity of manganese peroxidase (MnP) was measured following the method described by Wunch et al. (1997) whereby guaicol was used as a substrate. The increase in absorbance at 465 nm due to oxidation of guaicol ( $\epsilon_{465} = 12,100 \text{ M}^{-1} \text{ cm}^{-1}$ ) was measured. The reaction mixture contained 300  $\mu\text{L}$  sodium succinate buffer (0.5 M, pH 4.5 at 27°C), 300  $\mu\text{L}$  guaicol (4 mM), 600  $\mu\text{L}$  manganese sulphate (1 mM), 300  $\mu\text{L}$  culture supernatant and 1200  $\mu\text{L}$  distilled water. The mixture was incubated for 2 min at 30°C and the reaction was initiated by addition of 300  $\mu\text{L}$  hydrogen peroxide (1 mM). The absorbance was measured in 1 min intervals after addition of hydrogen peroxide. One unit of MnP activity was defined as activity of an enzyme that catalyzes the conversion of 1  $\mu\text{mole}$  of guaicol per minute.

Laccase activity was measured by using the method described by Bourmnais et al. (1995) based on the oxidation of the substrate 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS). The rate of ABTS oxidation was determined spectrophotometrically at 420 nm. The reaction mixture contained 600  $\mu\text{L}$  sodium acetate buffer (0.1 M, pH 5.0 at 27°C), 300  $\mu\text{L}$  ABTS (5 mM), 300  $\mu\text{L}$  mycelial liquid fraction and 1400  $\mu\text{L}$  distilled water. The mixture was then incubated for 2 min at 30°C and the reaction was initiated by addition of 300  $\mu\text{L}$  hydrogen peroxide. The absorbance was measured immediately in one-minute intervals after addition of hy-

drogen peroxide. One Unit of laccase activity was defined as activity of an enzyme that catalyzes the conversion of 1  $\mu\text{mole}$  of ABTS ( $\epsilon_{410} = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$ ) per minute.

### Purification of extracellular proteins by gel chromatography

Preliminary desalting and protein-buffer exchange was done by using PD-10 column (void volume 3.5 ml) parked with Sephadex G-25 gel (*Pharmacia* Co., Sweden). The column was equilibrated with 10 mM [bis (2-hydroxyethyl)imino-tris (hydroxymethyl) methane] (Bistris) – HCl buffer (pH 6.5). 3.5 mL Bistris-HCl buffer was added and the eluent was collected in test tubes. The desalted samples were subjected to CL-6B Sepharose column (void volume 30 ml) parked with agarose gel (*Pharmacia*, Co. Sweden) and coupled to HPLC pump (LKB 2150, Sweden) at a flow rate of 0.5 ml/min. The column was equilibrated with Bistris-HCl buffer (pH 6.5). 2 mL enzyme concentrate was injected and the eluent was pumped through a variable wavelength monitor with a detector (LKB 2151, Sweden) set at 280 nm. The fraction collector (*Microcol* TDC 80, Gilson, Sweden) set at 3 min was coupled to the system. Total proteins (mg/L) were determined by a Bradford method (Bradford, 1976) using protein dye and bovine serum albumin standard (*Bio-Rad*, USA).

### Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

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%  $(\text{NH}_4)_2\text{S}_2\text{O}_8$  and 0.01 TEMED (*Midget* LKB Broma gel casting unit, USA) 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) coomassie brilliant blue (CBB) R 150 followed by de-staining at 10% methanol-acetic acid solution (Scopes, 1982).

## RESULTS AND DISCUSSION

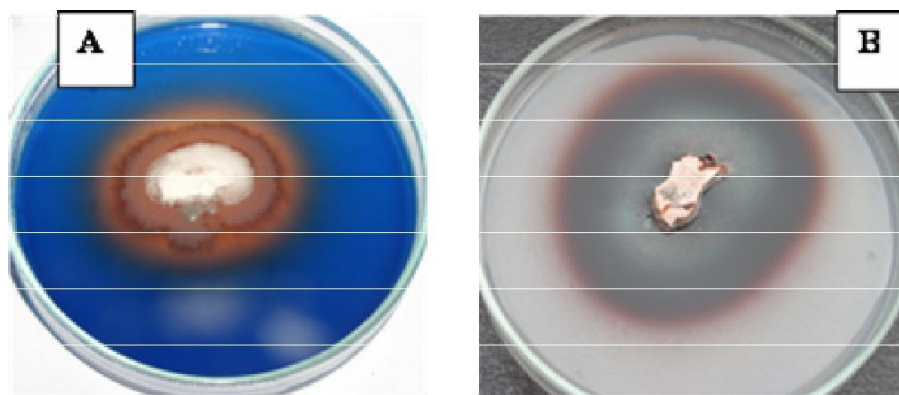
*L. sulphureus* inhabiting the mangrove forests and bordering areas were found growing on the bases of dry or decomposing mangrove shrubs at the periphery of the sea shoreline (Figure 1). They have fleshy yellowish-orange basidiocarps measuring 5 - 10 cm and tubular hymenopores resembling those of polyporales. The fact that some *L. sulphureus* was also found in the neighboring non-mangrove tree communities suggests that the strain is a facultative marine fungus.

### Rhemazol brilliant blue-R (RBB-R) decolorization and guaicol oxidation

Figure 2 shows the degradation potential of enzymes from *L. sulphureus*. A halo was formed around the as it grew, completely decolorizing the blue-colored substrate after 2 weeks of incubation. The ability of *L. sulphureus* enzymes to degrade the aromatic model compounds is an indication that they are the main decomposers of cellulose, hemicellulose and lignin contained in the mangroves trees. This implies that the enzymes can also be used in detoxification of aromatic pollutants such as agrochemicals and industrial effluents.



**Figure 1.** *L. sulphureus* growing on a base of mangrove shrub (left) and decomposing mangrove log in the seashore (right). Note the characteristic morphological features such as the yellowish-orange basidiocarps, tubular hymenophores and firm attachment to the substrates.

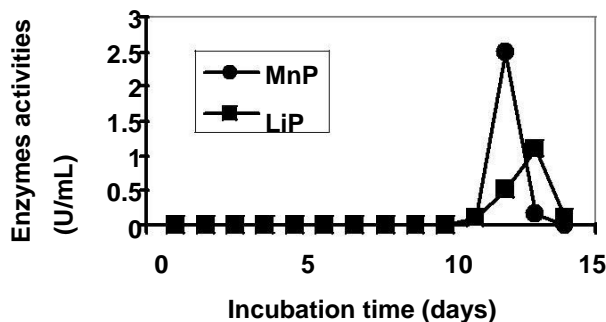


**Figure 2.** MEA agar plates showing RBB-R decolorization (A) and guaiacol oxidation (B) by mycelial cultures of *L. sulphureus* after 7 days of incubation. The plates were completely oxidized after 14 days.

The degrading ability of the basidiomycetous fungi is thought to be due to the action of their extracellular oxidative enzymes (Wunch, et al., 1997; George et al., 2000). The involvement of LiP and MnP suggests that hydrogen peroxide is responsible for the initial breakdown of aromatic and non aromatic molecules and oxygen may play a role in the initial degradation of the cellulose and lignin (Enoki et al., 1989). This observation reveals the potential of *L. sulphureus* to degrade recalcitrant organic pollutants. It serves as a model system for bioremediation of marine ecosystems polluted by recalcitrant hazardous chemicals. The results are consistent with the findings by Okino et al. (2000) and Masalu (2004) who demonstrated that both wood-inhabiting and non-wood inhabiting basidiomycetous fungi are capable of degrading RBB-R dye and other xenobiotic compounds. The decolorization ability is the result of synergetic action of multiple enzymes including peroxidases and oxidases (George et al., 2000).

### Production of lignin peroxidase and manganese peroxidase

Figure 3 shows the time courses of lignocellulosic enzymes activity by *L. sulphureus*. The enzymes were produced during idiophase when the fungus was carrying out secondary metabolism in nitrogen-deficient condition. Generally, the enzymes production started after 10 days of incubation. MnP increased sharply from day 11 and peaked at 2.5 U/mL in day 12. LiP increased steadily from day 12 and peaked at 1.0 U/ mL in day 13. It is noteworthy that laccase assay was also carried out but no activity was observed. The results are consistent with the work by Raghukumar et al. (1999) and Kondo et al. (2004) who demonstrated the ability of some coastal marine fungi such as *Flavodon flavus* and *Phlebia* sp. to produce major lignocellulolytic enzymes. The amounts of LiP and MnP are comparable to the amounts produced by the Tanzania's terrestrial mushrooms including *Oudemansia*



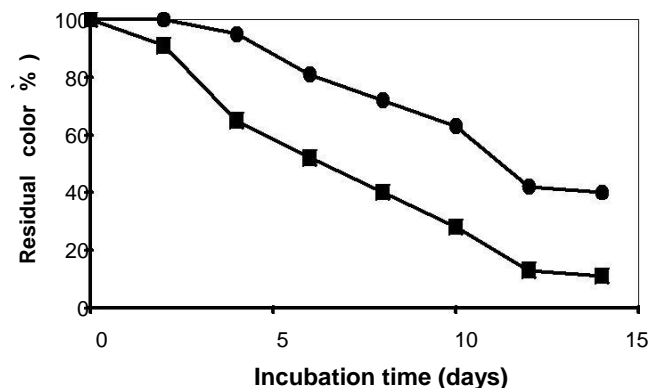
**Figure 3.** Time courses of lignocellulolytic enzymes production by *L. sulphureus* grown in half-strength sea water containing Kirk medium (pH 4.5, Temperature 30°C).

*siella* sp., *Pleurotus flabellatus* and *Bjerkandera adusta* (Mtui et al., 2003; Mtui and Nakamura, 2004).

The decrease in MnP and LiP activities after 12 and 13 days of incubation, respectively, could be attributed to the presence of protease in the reaction mixture (Nakamura et al., 1999; Mtui and Nakamura, 2002). The filtrate was therefore harvested for purification after 12 days of cultivation. The presence of both LiP and MnP suggests that *L. sulphureus* is one of the brown-rot fungi responsible for cellulose and lignin degradation through production of H<sub>2</sub>O<sub>2</sub> via oxygen radical in the presence of molecular oxygen (Enoki et al., 1989).

### Decolorization of textile effluent

Figure 4 shows the effect of the crude enzymes from *Laetiporus sulphureus* in decolorization of raw wastewater collected from Karibu Textile Mill in Dar es Salaam, Tanzania. It was found that *L. sulphureus* could decolorize the raw azo dyes by 60% in liquid culture and 90% in polyurethane foam (PUF) immobilized culture after 14 days of incubation. Immobilized culture performed better (33% more efficient) than liquid culture due to enhanced mycelial growth as a result of PUF support. The upper portions of the mycelial mats were not submerged in water, a condition that enhanced enzyme productivity due to sufficient aeration (Nakamura et al., 1997, 1999). This improvement of decolorization rate due to culture immobilization provides an important advantage in terms of process efficiency and cost effectiveness. The decolorization of azo dyes contained in the raw wastewater in the absence of a redox mediator shows that the fungus produces enzymes that have broad substrate range. The findings are comparable to the work by Yesilada (1995), Scheibner et al. (1997), Wunch et al. 1997, Ferraz et al. (2001) and Kartal et al. (2004) who reported the degradative abilities of terrestrial strains of *L. sulphureus* on recalcitrant organic substrates. Color removal by filamentous fungi has been attributed to be mainly due to biosorption to the mycelium (Yang et al., 2003). However,

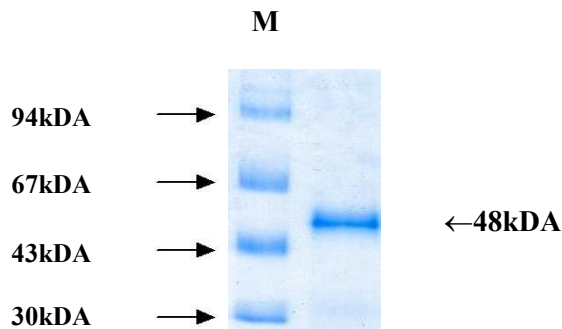


**Figure 4.** Decolorization of half-strength textile wastewater inoculated by *L. sulphureus* followed by incubation at 30°C. The color intensity was measured at 475 nm. Symbols: Liquid culture (○) PUF-immobilized culture (◻).

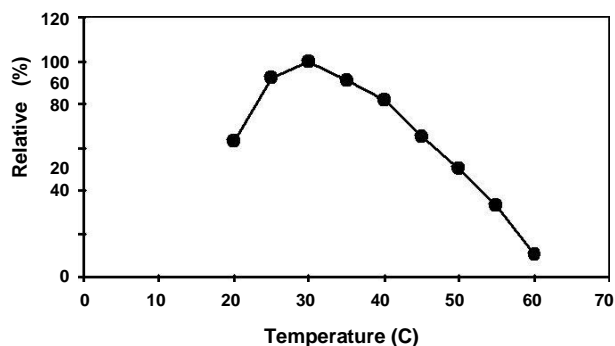
Gonzalo et al. (2005) has shown that LiP and MnP are capable of carrying out catalytic and free-radical mediated breakdown of aromatic compounds including ring cleavage. The fact that degradative enzymes from *L. sulphureus* could not degrade all the organic compounds in the wastewater shows the complexity of systems associated with the degradation of aromatic molecules. A combined action of various lignolytic enzymes is therefore needed for complete degradation of recalcitrant organic compounds.

### Purification of peroxidases from *L. sulphureus*

The *L. sulphureus* filtrates, concentrated by ultrafiltration had overall protein content of 5.2 mg/ml. The concentrated and desalted sample, subjected to gel filtration chromatography, gave dominant peaks containing peroxidases. The main peak containing MnP was pooled and resolved in SDS-PAGE. The protein concentration of this fraction was 1 mg/ml. As shown in Figure 5, a broad distinct single band was resolved after staining with CBB. When compared to the standard marker proteins, the band was found to correspond to molecular weight of 48 kDA. The results are comparable to studies by Karhunen et al. (1990), Bonarme and Jeffries (1990), Matsubara et al. (1996) and Heinfling et al. (1998) who observed the molecular weights of MnP from lignolytic fungus *Phlebia radiata*, IZU 154, *Phanerochaete chrysosporium* and *Bjerkandera adusta* to be 49 kDA, 43 kDA, 42 kDA and 45 kDA, respectively. The active fraction of purified LiP could not be resolved in SDS-PAGE, most likely because the amount produced was too little (0.1 mg/ml protein). Optimized production of LiP from *L. sulphureus* by varying different culture parameters will be the focus of future studies. In order to resolve the specific isoforms of MnP, more advanced purification techniques including ion exchange chromatography may be needed.



**Figure 5.** SDS-PAGE of enzyme filtrate from *Laetiporus sulphureus* purified by gel filtration chromatography. Separated MnP appeared at 48 kDa. (Lane M: Standard Molecular Marker proteins: Phosphorylase b (94 kDa), Bovine serum albumin (67 kDa), Ovalbumin (43 kDa) and Carbonic anhydrase (30 kDa).

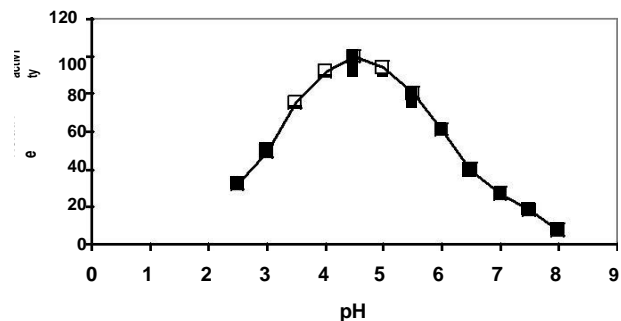


**Figure 6.** Temperature dependence on relative activity of purified manganese peroxidase from *L. sulphureus* using 20 mM sodium acetate buffer (pH 4.5) under standard assay conditions.

### Characteristics of purified manganese peroxidase

Substrate specificities for the purified MnP are shown in Table 1. The specific activities varied from 12.3 to 1,119.4 U/mg protein depending on the substrate used. The fact that the purified enzyme fraction readily oxidized guaiacol at 465 nm confirmed it to be MnP. The study revealed that *L. sulphureus* possesses degrading enzymes which are non-specific to substrates and therefore capable of oxidizing a variety of aromatic environmental pollutants.

The effect of temperature on relative activities of purified MnP fractions is shown in Figure 6 where the optimum temperature was found to be 30°C. The reaction rates remained relatively high within 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 also been reported by Nakamura et al. (1999) for terrestrial fungi. These findings suggest that the extracellular enzymes from *L. sulphureus* are suited



**Figure 7.** pH dependence on relative activities of purified manganese peroxidase from *L. sulphureus* at 30°C under standard assay conditions with 0.1 M citrate buffer (pH 2 - 6) and 0.1 M phosphate buffer (pH 6 - 8).

for the tropical climates and therefore conducive for bioremediation of environmental pollutants at ambient temperatures.

The effects of pH on relative activities of purified MnP were examined at pH values ranging from 2.5.0 to 9.0. The bell-shaped curve obtained in Figure 7 shows the optimum pH for purified MnP fraction to be 4.5. Enzyme reaction rates increased steadily from pH 3 to pH 4, peaking at pH 4.5 and then decreased sharply with increased pH, before ceasing at pH 9. It is noteworthy that at pH 8, there was still some limited enzyme activity, suggesting that enzymes from *L. sulphureus* could carry out *in situ* degradation of recalcitrant substrates in marine environments which are normally of alkaline pH range (7.5 - 8.0). The findings are comparable to the results by Shin et al. (1997) and Nakamura et al. (1999) who demonstrated the abilities of *Pleurotus ostreatus* and *Bjerkandera adusta* to maximally degrade substrates at acidic pH values.

### Conclusion

This study has shown that extracellular enzymes from *L. sulphureus*, a brown-rot basidiomycetous fungus collected off the coast of Dar es Salaam, Tanzania, had appreciable activities of MnP (2.5 U/mL) and LiP (1 U/mL), but showed no laccase activity. The enzyme extracts were able to oxidize RBB-R dye and phenol. Furthermore, 90% decolorization of textile effluent was achieved after 14 days of incubation. SDS-PAGE showed that purified MnP from *L. sulphureus* has a molecular weight of 48 kDa, and the purified MnP fraction had maximum activities at pH 4.5 and the temperature of 30°C. Optimizing production of LiP from *L. sulphureus* by varying different culture parameters will be the focus of future studies. Our work provides basic information on extracellular lignocellulosic enzymes from facultative marine *L. sulphureus* and elucidates their potential for bioremediation of polluted coastal and marine ecosystems.

**Table 1.** Substrate specificities for purified manganese peroxidase from *L. sulphureus*.

Substrate	Maximum absorbance (nm)	Activity (U/mg protein)
Veratryl alcohol	310	12.3
Guaiacol	465	1119.4
ABTS	420	340.3
Catechol	398	227.4
2,4-dimethoxyphenol	468	193.9
o-phenylenediamine	440	342.7
p-phenylenediamine	459	321.4

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