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Banana Peel: A Potential Substrate for Laccase Production by *Aspergillus fumigatus* VkJ2.4.5 in Solid-State Fermentation

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Abstract In solid-state fermentation, among various solid supports evaluated, banana peel was found to be an ideal support and resulted into higher levels of laccase (6281.4± 63.60 Ul^{-1}) along with notable levels of manganese peroxidase production (1339.0± 131.23 Ul^{-1}) by *Aspergillus fumigatus* VkJ2.4.5. Maximum levels of laccase was achieved under derived conditions consisting of 80% of moisture level, 6 days of incubation period, 6% inoculum level, and an aeration level of 2.5 $lmin^{-1}$. A column-tray bioreactor was designed to scale up and economize the enzyme production in three successive cycles of fermentation using the same fungal biomass. Thermal and pH stability profiles revealed that enzyme was stable up to 50°C and at varying pH range from 5–9 for up to 2 h. The apparent molecular weight of laccase was found to be 34±1 kDa. MALDI-TOF/TOF analysis of the protein showed significant homology with maximum identity of 67% to other laccases reported in database.

Keywords Solid-state fermentation · *Aspergillus fumigatus* · Banana peel · Laccase · Manganese peroxidase · Bioreactor

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Introduction

Laccases (benzenediol: oxidoreductase) [EC 1.10.3.2] belong to the group of phenol oxidases [1] and are copper-containing enzymes. It can degrade lignin [2] by catalyzing the oxidation of large number of non-phenolic substrates in presence of suitable mediators, phenolic compounds, and aromatic amines. These utilize molecular oxygen as the electron acceptor and finally reduce it to water and CO_2 [3]. This is because of the enzyme's low degree of specificity to the reducing substrate [4]. Laccases have been reported from the different groups of plants, fungi, and bacteria [5]. Among these, white-rot fungi are considered as the major group of microbes that degrade lignin because they can produce extracellular laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) that are highly effective for lignin degradation [6, 7]. Some white rot fungi which are well known for this process are *Phanerochaete chrysosporium*, *Pleurotus* sp., *Trametes versicolor*, etc. [8, 9]. Laccase has also been reported from different genera of *Ascomycetes* such as *Aspergillus nidulans* [10], *Neurospora crassa* [11], *Podospora anserina* [12], *Myceliophthora thermophila* [13], and *Gaeumannomyces graminis* [14], etc.

It has the major application for pulp and paper industry as a natural bleaching agent for pulp delignification [15–17]. It has also been studied for effluent detoxification, textile dye bleaching [15], decolorization of kraft pulp waste [18], removal of chlorophenols and chlorolignins from bleach effluents [19], and in paper manufacturing to improve the pulp properties [20]. Along with laccase, MnP is also involved in lignin degradation during biobleaching [21–23]. It has also been reported by group of researchers that laccase and MnP act synergistically to degrade lignin [24, 25].

Production of laccases in cost-effective manner from microorganisms is a prerequisite for their use in industrial processes, which may be achieved by use of agro-industrial waste materials [26]. One such waste is banana peel which is available in bulk, and its disposal is a matter of concern for fruit-processing industries. Improper dumping of banana peels may result into ecological and environmental hazards. Banana is one of the highly consumed crops in the world and accounts 40% of the total world trade in fruits and fruit products. India is one of the largest producers of banana which is cultivated in 4.796×10^5 ha yielding 16.37×10^6 t of banana [27].

Use of banana peel as solid substrate in solid-state fermentation (SSF) may be a promising strategy for laccase production as it is an organic residual material rich in nutrients [28]. Solid-state fermentation process occurs in the absence of free flowing water, employing either a natural support or an inert support as a solid material. The process has lower energy requirement, produces lesser waste water, and is eco-friendly as it resolves the problem of solid waste disposal. A variety of solid substrates can be employed in SSF, which may have differences with respect to composition, size, mechanical resistance, porosity, and water-holding capacity. Bioreactors may be considered to scale up the production of enzyme. The SSF bioreactors should be designed with a material, which is economical, anticorrosive, and non-toxic to the process and organism. Other important aspect to be considered during the design of a bioreactor is the effective regulation of aeration, mixing, and heat removal. This could avoid problems related to an ineffective heat removal, evaporative loss of water from the substrate bed and thermal gradients, which affect the yield and quality of the desired product. Presently, SSF-based bioreactors are utilized at commercial scale leading to production of a range of value added products [29].

The present study evaluates laccase production from *Aspergillus fumigatus* VkJ2.4.5 employing banana peel as a solid substrate during solid-state fermentation. Various critical parameters for the solid-state fermentation have also been enumerated.

Materials and Methods

Microorganism

A. fumigatus VkJ2.4.5 developed in our laboratory from the wild-type *A. fumigatus* VkJ (ITCC 6035) (isolated from sugarcane industry waste site) after two successive stages of UV irradiation was used in this study. The strain was maintained on PDA slants, stored at 4°C and subcultured periodically.

Pre-treatment of Solid Substrate

Natural lignocellulosic materials (agro-residues) namely, poplar leaves, banana peel, wheat bran, rice bran, wheat straw, and bagasse were procured, washed, air dried, pulverized, and then utilized as the substrates for SSF. Banana peel (10 g) was first soaked for an hour in 30 ml of KOH (83 mM) to neutralize organic acids [30]. These were then washed thoroughly with double distilled water and dried. The treated banana peels were used as solid substrates.

Culture Conditions

The culture moistening medium used had the following composition (gl^{-1}): peptone, 3.0; glucose, 10.0; KH_2PO_4, 0.6; K_2HPO_4, 0.4; $ZnSO_4$, 0.001; $FeSO_4$, 0.005; $MnSO_4$ and $MgSO_4$, 0.05 [31]. The medium was autoclaved for 15 min (121°C) followed by inoculation with six discs(4 mm)/flask that were sliced out from the petri-plates containing freshly grown (72 h) *A. fumigatus* VkJ2.4.5. The fermentation was carried at 30°C in cotton-plugged Erlenmeyer flasks (250 ml) containing 6 g of chopped banana peel and required volume of culture medium to maintain the desired moisture level.

Derivation of Critical Parameters for Solid-State Fermentation

Critical parameters like incubation time, temperature, moisture level, aeration rate, and additional nutrients affecting the laccase production were derived by varying one parameter at one time and keeping others constant [32, 33].

Effect of Moisture Level

Effect of different moisture levels (20–100%) that was adjusted with media was evaluated. The fermentation was carried out for 8 days at 30°C. The derived moisture level for solid substrate attained by this step was used for subsequent experiments.

Effect of Incubation Time

The flasks were incubated for different time periods (2–10 days); other parameters were kept at their optimum conditions.

Effect of Inoculum Level

The effect of inoculum level on the laccase production was studied by varying the inoculum level (2–10%). The process was carried out at 30°C, keeping all other conditions up to their

optimum level. The optimum inoculum level achieved by this step was fixed for subsequent experiments.

Effect of Temperature

Fermentation was carried out at varying temperatures (20–40°C) to derive the suitable temperature for the process. The most suitable temperature attained by this step was used for further study.

Effect of Additional Nutrients

To analyze the influence of various additional nutrients; mustard oil cake, molasses, jaggery, yeast extract, and cheese whey were employed in media at 1% concentrations for laccase production.

Effect of Aeration Rate

The influence of aeration on laccase production by fungal strain was studied by supplementing sterile air into the fermentation medium. Air volumes of 1, 1.5, 2, 2.5, 3, and 3.5 $\mathrm{l\,min^{-1}}$ were supplied to the medium. The other derived parameters were kept at their optimal levels.

Scanning Electron Microscopy

The detailed morphological analysis of uninoculated and inoculated banana peel was carried out using scanning electron microscopy (SEM, LEO 435 VP, England). Samples were taken and subjected for fixation using a mixture (4:1) of glutaraldehyde (3%) and formaldehyde (2%) for 24 h. Following primary fixation, samples were washed thrice with double distilled water and then treated with the alcohol gradients (30%, 50%, 70%, 80%, 90%, and 100%) for dehydration. The retention time of samples in each alcohol series up to 70% alcohol gradient was 15 min, but later, the time period was increased to 30 min for alcohol concentrations ranging from 80% to 100%. After complete dehydration, samples were air dried and coated with gold-by-gold shadowing technique [34]. Electron photomicrographs were taken at desired magnifications.

Bioreactor Designing

Circular aluminum trays (4 nos.) with the dimension of 28×2 cm (diameter×height) were vertically arranged in the bioreactor. Fermentation was carried out in three upper trays. Tray at bottom contained sterile distilled water to maintain humidity in the chamber. Processing of banana peel was performed as described earlier. Appropriate amount (150 g) of solid substrate was mixed with fermentation medium to maintain the derived moisture level, autoclaved (121°C, 15 min, 15 psi), and layered evenly in the fermentation trays. Following inoculation, the microbial culture was allowed to grow over the support at 30°C. The inlet and outlet ports for air were provided in the bioreactor that had enabled the circulation of sterile air over the solid substrates. On completion of first fermentation cycle, content was

aseptically squeezed to release the accumulated enzyme followed by quenching the substrate with media, and the same set-up of fungal mat and the solid support was used for the successive batches of fermentation. The fermentation broth, thus recovered, was used for estimating the laccase and MnP activity. Finally, trays were subjected to UV sterilization to inactivate the spores generated and then sterilized further.

Characterization of Enzyme

Thermal and pH Stability

To determine the thermal stability of laccase, samples were pre-incubated for 2 h at temperatures ranging to 25–70°C. Assays were performed using 1.0 mM of 2, 2′-azino-bis-(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS) as substrate in 100 mM citrate buffer (pH 5.0). To estimate the pH optimum for the enzyme, laccase activity was measured with 1.0 mM of ABTS as substrate in 100 mM citrate buffer (pH 2.0–5.0), citrate-phosphate (pH 5.0–7.0), and Tris–HCl (pH 7.0–9.0) buffer.

Gel Electrophoresis and Zymogram Analysis

SDS-PAGE (12%) was performed as described [35]. Samples were treated with 1% SDS and β-mercaptoethanol and heat deactivated (100°C, 5 min). Proteins were visualized by staining with 0.25% Coomassie brilliant blue R-250. Non-denaturing gel electrophoresis was performed under the same conditions except that SDS and β-mercaptoethanol were not used, and samples were not boiled. Laccase activity in native gel electrophoresis was determined by soaking gel in 100 mM citrate-phosphate buffer (pH 5.0) containing 0.25% guaiacol [36]. The appearance of dark brown band indicated the presence of laccase. Molecular weight of the purified protein was determined using Bangalore Genei's (India) medium range (14.3–97.4 kDa) molecular weight protein markers.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Analysis

For matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF) analysis of the protein, culture broth was concentrated using Centricon C-10 (Amicon, USA) and then electrophoresed on native gel (12%). The half of the gel was stained with Coomassie brilliant blue R-250, and the other half was subjected to substrate staining (guaiacol) for zymogram analysis as described earlier. Protein bands in the CBB-stained gel with the one corresponding to the active band in zymogram were carefully excised and repeatedly washed with ammonium bicarbonate-acetonitrile buffer. Gel slice was dissolved in acetonitrile and vacuum dried followed by washing. Reduction was carried out with 1% dithiothreitol followed by 2% iodoacetamide modification and tryptic digestion for overnight and acidified by adding 5 μl of 10% tri-fluoroacetic acid. The digested product was mixed with α-cyano-4-hydroxycinnapic acid matrix in 1:1 ratio. The matrix was made by mixing 0.1% TFA to mixture of acetic acid and water (1:1). Spectra were recorded (Bruker Daltronix Autoflex TOF/TOF, Germany) in a positive mode, and the N_2 laser intensity was set at λ 337 nm. The peptide fragments obtained were analyzed with the Flex Analysis Software, and database homology search for protein identification was carried out manually using short sequence BLAST (Basic Local Alignment Search Tool) at NCBI (National Center for Biotechnology Information) [37].

Analytical Methods

Enzyme Assays

Laccase assay was performed using ABTS as substrate. Reaction in a total volume of 1 ml was carried out by taking 200 mM sodium phosphate/100 mM citric acid buffer at pH 5.0, enzyme extract, and 1.0 mM of ABTS. Reaction was monitored at 420 nm using UV–Vis spectrophotometer (Varian Cary 100 Bio, Australia) at 25°C [38].

MnP was estimated by 3-methyl-2-benzothiazoline hydrazone (MBTH)/3-(dimethylamino) benzoic acid (DMAB) assay where reaction mixture contained 0.07 mM MBTH, 0.99 mM DMAB, 0.3 mM $MnSO_4$, 0.05% H_2O_2, and 100 mM succinic-lactic acid buffer at pH 4.5. Reaction was measured at 590 nm at 25°C [39]. One unit enzyme was defined as the amount of enzyme required to convert 1 μmol of substrate to product in 1 min, under the assay conditions.

Estimation of Moisture Level

The moisture level of oven-dried (105°C, over night) banana peel with constant weight was estimated following KOH treatment, and the dry weight was noted. Moisture level of the solid medium was maintained by adding desired volume of fermentation medium to banana peel. After soaking, the sample was dried again as described above and % moisture level was calculated as follows [30], percent of moisture level (initial) of solid medium=(wt. of the wet banana peel−dry wt.)×100/dry wt.

Enzyme Extraction

Extraction of enzyme was carried out from the fermentation system with 100 ml distilled water. Each time the whole content was squeezed, extract was centrifuged (9,000×*g*, 10 min) to remove suspended particles and filtered to get rid of the fungal mycelia/spores, and clear supernatant was used as the enzyme source.

Reproducibility of Results

All experiments were performed in triplicates, and the reported experimental results represent the mean of three identical fermentation setups with ±S.E.

Results and Discussion

Solid-state fermentation process appears to be an economically viable system. The selection of a suitable strain, substrate, and various process parameters are crucial factors which affect the process [40]. The solid support used for the process is of vital consideration as it not only serves as an anchorage for the microbial cells but also provides nutrients and facilitate appropriate level of biomass generation for fermentation. Therefore, the chemical composition and the particle size of substrate are crucial for proper anchorage [41]. In present study, banana peel was selected as a novel solid support for microbial anchorage for higher levels of enzyme production in SSF. Uninoculated banana peel appeared porous and hence emerged as a better substrate for fungus to adhere and penetrate (Fig. 1a, b). Since banana peel is highly rich in carbohydrate, dry matter, total ash, ascorbic acid, potassium,

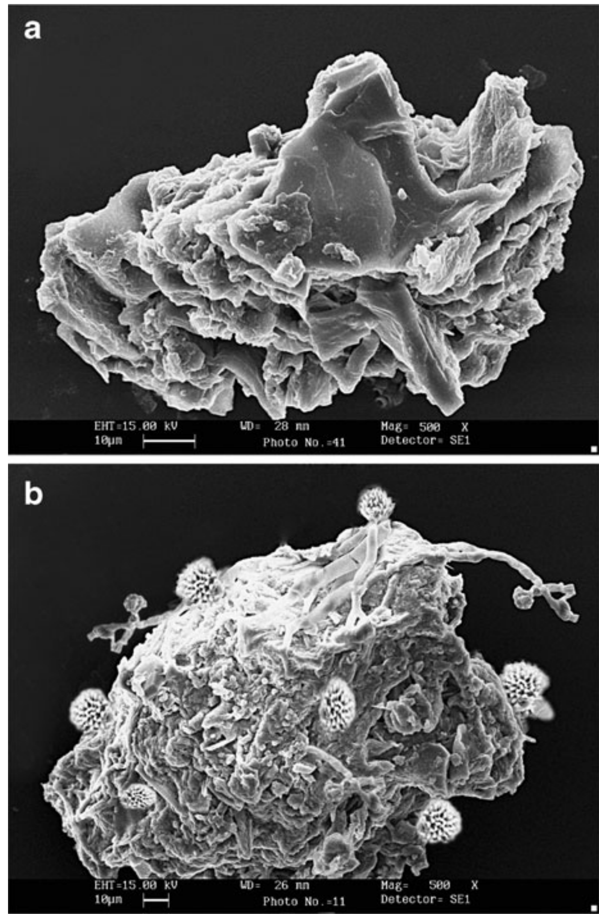

Fig. 1 Scanning electron micrographs of **a** uninoculated banana peel particle and **b** banana peel entrapped with *A. fumigatus* VkJ2.4.5 under solid-state fermentation

and other basic nutrients, could support fungal growth so the enhanced production of laccase may be attributed to nutritional components of the same [28]. The order of the substrate suitability for laccase and MnP production was: banana peel (5,792.0±40.95 Ul^{-1}; 1,334.66±167.32 Ul^{-1})>bagasse (4,561.65±69.11 Ul^{-1}; 1,133.67±57.18 Ul^{-1})>wheat bran (4,323.0±119.79 Ul^{-1}; 948.0±98.65 Ul^{-1})>poplar leaves (4,178.0±21.03 Ul^{-1}; 1,169.3±59.17 Ul^{-1})>wheat straw (3,843.6±76.52 Ul^{-1}; 1,071.3±149.19 Ul^{-1})>rice bran (3,454.66±83.47 Ul^{-1}; 1,133.6±57.53 Ul^{-1}). Laccase production using various agro-horticultural residual materials under SSF had been studied by several research groups [42–44]. Potential of nutrient rich banana peel for enzyme production had also been explored for laccase production, but most of the studies had involved the basidiomycete strains [45–47]. Present study for the first time reported simultaneous production of laccase and MnP employing banana peel as a solid substrate using an ascomycete strain, i.e., *A. fumigatus* VkJ2.4.5. Considerably higher laccase titers were produced by the strain VkJ2.4.5 in comparatively lesser time duration. *Trametes pubescens* produces 1,570 Ul^{-1} of laccase following 20 days of incubation [45]. Elisashvili et al. [46] had compared laccase production ability of several white-rot fungi using banana peel as a solid support and observed that *Funalia trogii* produces maximum laccase activity (988±74 Ul^{-1}) following 10–14 days

of fermentation. *Pleurotus florida* had also produced 5.4 Ug^{-1} of laccase utilizing banana peel as solid support following 10 days of incubation [47].

Effect of Moisture Level

A moisture level of 80% was found to be suitable for maximal levels of laccase and MnP (5,873.2±34.07 Ul^{-1}, 1,359.3±141.01 Ul^{-1}) production, respectively (Fig. 2a). The importance of moisture level in SSF and its influence on laccase production can be attributed to the interference of moisture in the physical properties of the solid particle. It is believed that porosity of substrate is reduced with increasing moisture level [48] and hence limiting the oxygen transfer [26]. The decrease in moisture leads to low availability of media nutrients to the fungus [33], resulting into low efficiency of the system.

Effect of Incubation Time

Laccase production at various time periods during the incubation period was monitored. Maximum levels of enzyme production were detected following 6 days of fermentation

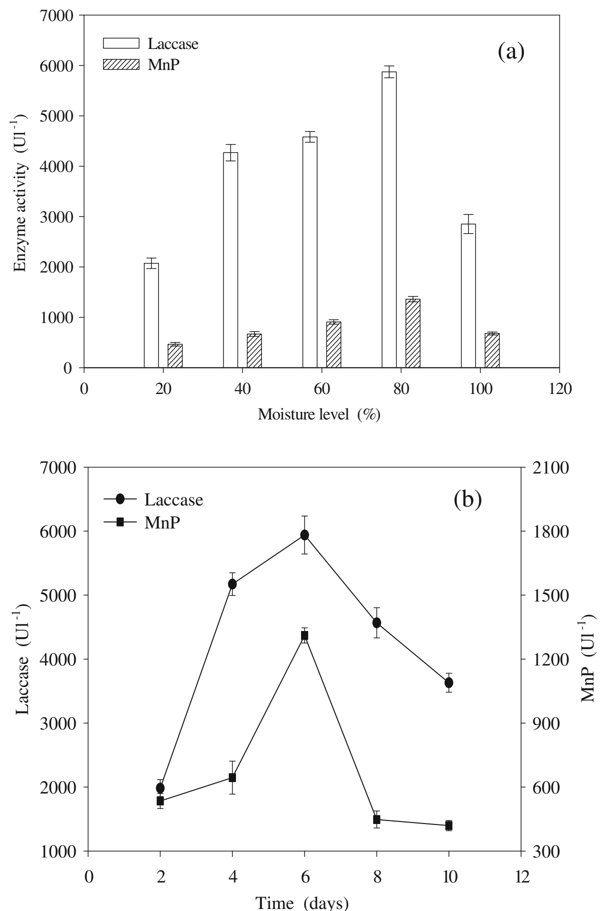

Fig. 2 Effect of **a** moisture level and **b** incubation time on laccase and MnP production by *A. fumigatus* VkJ2.4.5 using banana peel under solid-state fermentation

cycle (Fig. 2b). Initially (up to 48 h), enzyme production was lower and later increased exponentially (fourth to sixth days) and then declined. This change in enzyme production level may be attributed to acclimatization of fungi at the start of fermentation, followed by utilization of media and nutrients for increased enzyme production. Eventually, there was a drop in enzyme production level which may be due to nutrient depletion and product accumulation [49].

Effect of Inoculum Level

Level of inoculum for the process using varying concentrations of spores for laccase production was evaluated (Fig. 3a). Maximum production (5,945.0±69.50 Ul^{-1}) was observed when 6% inoculum was used in the process. However, levels of MnP production was slightly lower at this inoculum level. Lower level of laccase was detected when the inoculum level was higher or lower than the optimal inoculum level. A low inoculum level may lead to insufficient biomass generated causing reduced product formation, whereas a higher inoculum may lead to excessive biomass and drain out the substrate which is essential for product formation [50].

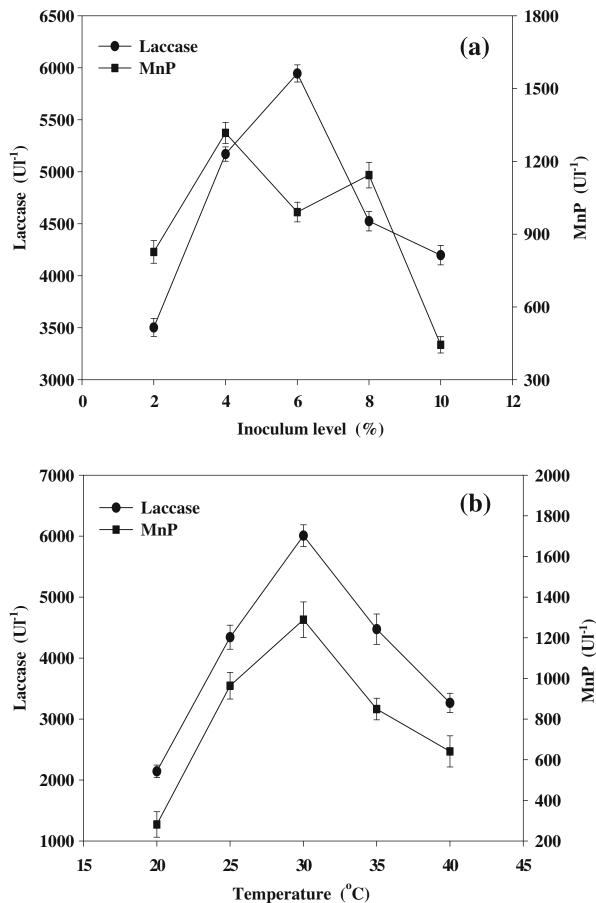

Fig. 3 Effect of **a** inoculum level and **b** temperature on laccase and MnP production by *A. fumigatus* VkJ2.4.5 using banana peel under solid-state fermentation

Effect of Temperature

The temperature also influences the levels of production. Maximum laccase (6,009.0± 73.33 Ul^{-1}) as well as MnP (1,289.0±102.06 Ul^{-1}) production was observed at 30°C (Fig. 3b). Maximal laccase production at this temperature may be beneficial as it can reduce the rate of evaporation during the incubation, whereas higher temperature may adversely affect the metabolic activity of microorganism [40].

Effect of Additional Nutrients

A range of additional nutrients were added to fermentation media at 1% concentration (Fig. 4a). Enhanced level of laccase and MnP (6,205.3±123.13 Ul^{-1}, 1,339.0±131.23 U l^{-1}) was observed when yeast extract (1%, *w*/*v*) was added into the fermentation media. The increase may be due to the vitamins, nitrogen, micronutrients, and additional growth-stimulating factors present in the yeast extract [51, 52].

Effect of Aeration Rate

Aeration rate is a significant entity influencing laccase production. An aeration rate of 2.5 $l min^{-1}$ led into maximum production of laccase (6,281.4±63.60 Ul^{-1}). Further

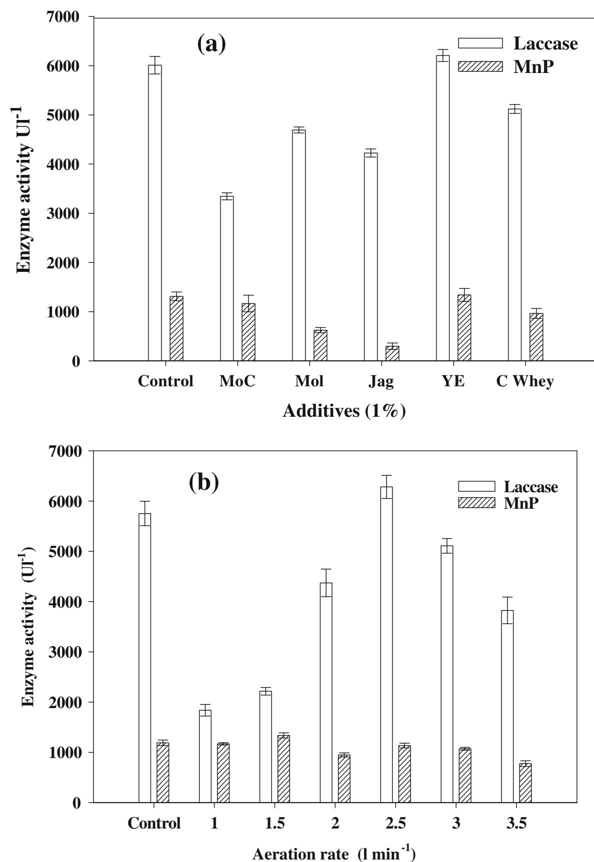

Fig. 4 Effect of **a** additives and **b** aeration rate on laccase and MnP production by *A. fumigatus* VkJ2.4.5 using banana peel under solid-state fermentation

increase in aeration rate was not supportive for laccase production (Fig. 4b). Heat generated during the process negatively affects the metabolism of fungi and hence product formation. Therefore, heating of solid matrix observed during process was regulated by aerating the fermentation process [26] to achieve higher levels of enzyme production.

Column-tray Bioreactor

A column-tray bioreactor was designed as shown in Fig. 5 for the production of enzyme. Enzymatic activities from the culture filtrate of the various fermentation cycles were detected. Significant levels of laccase and MnP (6,267.4±71.72 Ul^{-1}, 1,378.2±60.4 Ul^{-1}) were produced during I–III cycles of fermentation. Enzyme production in the IV and subsequent cycles decreased due to excessive biomass and sporulation of the fungal mycelia during the cycles (Fig. 6). The process is thus productive and cost effective as it utilizes the acclimatized fungal mat and biomass for successive batches of fermentation for enzyme production.

Thermal and pH Stability

Laccase from *A. fumigatus* VkJ2.4.5 was found to be stable at higher temperature. Enzyme when incubated for 120 min at 60°C retained about 50% of its activity (Fig. 7a). The pH optimum of the enzyme was 5.0; enzyme at higher pH (i.e., up to pH 8.0) retained 50% of its activity (Fig. 7b). Thus, the laccase from *A. fumigatus* VkJ2.4.5 was stable at a wide range of temperature and pH and its stability profile was comparable with laccases from *Cyathus stercoreus* [53] and *Pycnoporus cinnabarinus* [54, 55]. The higher enzyme activity at pH 5 may partly be due to unfolding of molecule at acidic pH in the beginning which helps substrate to reach to active site [56]. In addition, it has been observed that high temperature resulted in the release of copper ions. The depletion of copper ions not only inactivates the enzyme but also uncouples the domains of copper-depleted protein leading to a decrease in enzyme activity [57].

Gel Electrophoresis and Zymogram Analysis

Culture supernatant from wild-type VkJ and mutant VkJ2.4.5 strains were analyzed on native polyacrylamide gels and subsequently subjected for zymogram analysis. The molecular weight of the enzyme thus detected was 34±1 kDa (Fig. 8a, b). Many of the fungal laccases mainly from *Phlebia radiata*, *Schizophyllum commune*, *N. crassa*, *Monocillium indicum* have been found to have molecular weight in the range of 62–100 kDa [5]. Two distinct laccase isozymes with the molecular weights of 77.6 and 52.5 kDa have been reported [58]. However, laccase of lower molecular weight (34–43 kDa) have been detected from *Ganoderma lucidum* KMK2 [59], *Tricholoma giganteum* [60], and *Pleurotus eryngii* (34 kDa) [61].

Determination of Internal Peptide Sequences

The internal amino acid sequencing of laccase was performed by MALDI-TOF/TOF analysis that provides significant insights into the nature of laccase and also the sequence homology search observations. Enzyme from *A. fumigatus* VkJ2.4.5 possesses

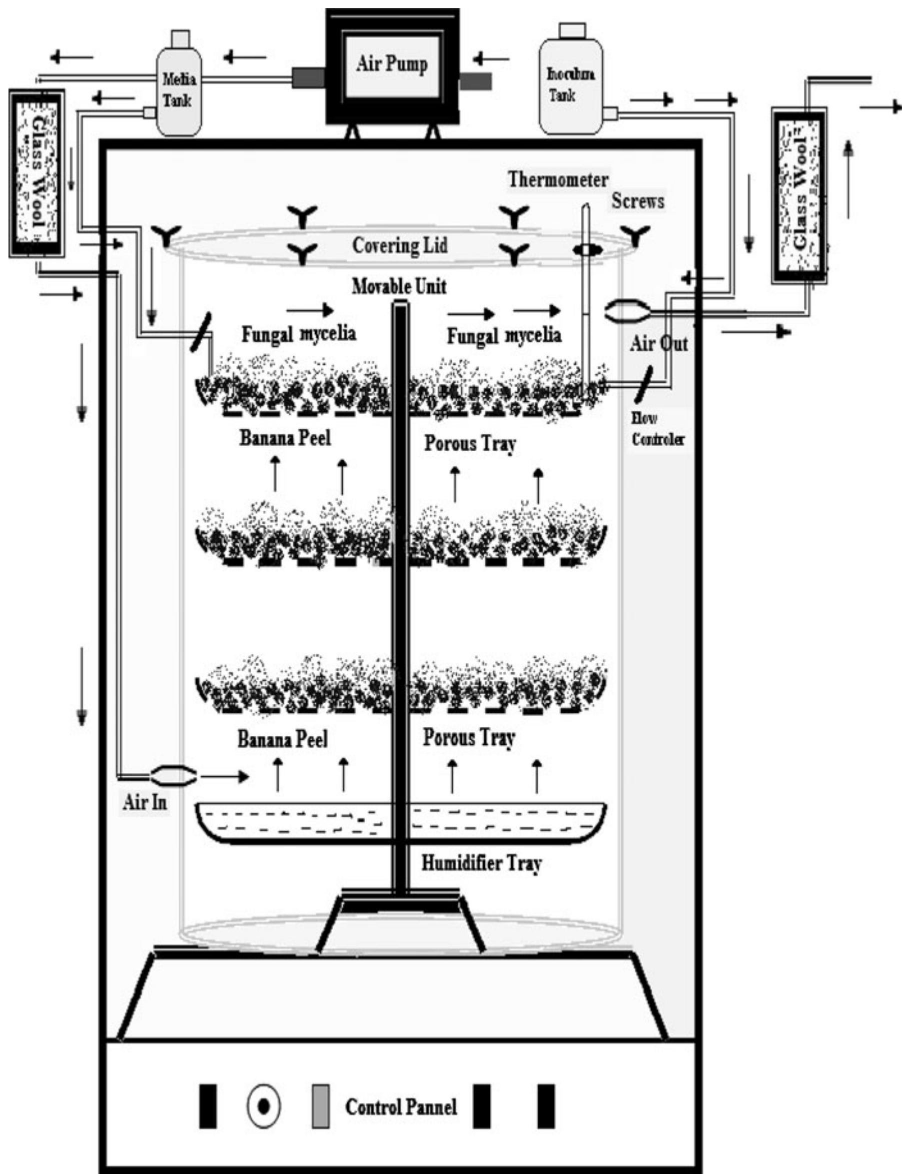

Fig. 5 Schematic representation of the column-tray bioreactor for batch production of laccase under solid-state fermentation

resemblance to those of reported laccase in ascomycetes, actinomycetes, plants, and basidiomycetes. All seven peptides generated during MALDI-TOF/TOF analysis of the *A. fumigatus* VKJ2.4.5 laccase were subjected to the homology search with other laccase from multi-copper oxidase family. The amino acid sequence of the generated peptides showed high levels of identity with laccase of ascomycetes mainly from *Fusarium oxysporum* strains (50%), *Phaeosphaeria* sp. (54%); actinomycetes like *Streptomyces cyaneus* (50%) and *Strongylocentrotus* sp. (50%) and plants like *Arabidopsis thaliana*

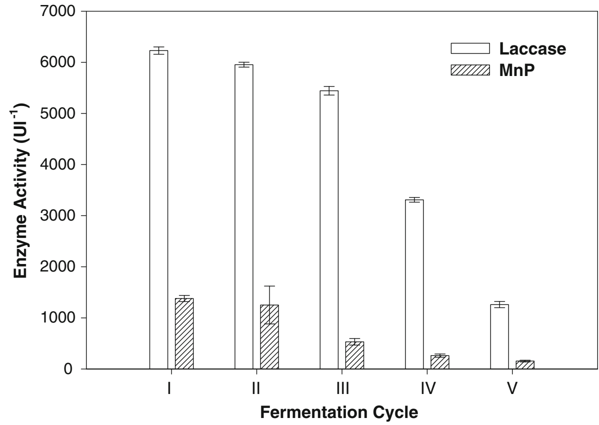

Fig. 6 Enzyme production during various cycles of fermentation in column-tray bioreactor

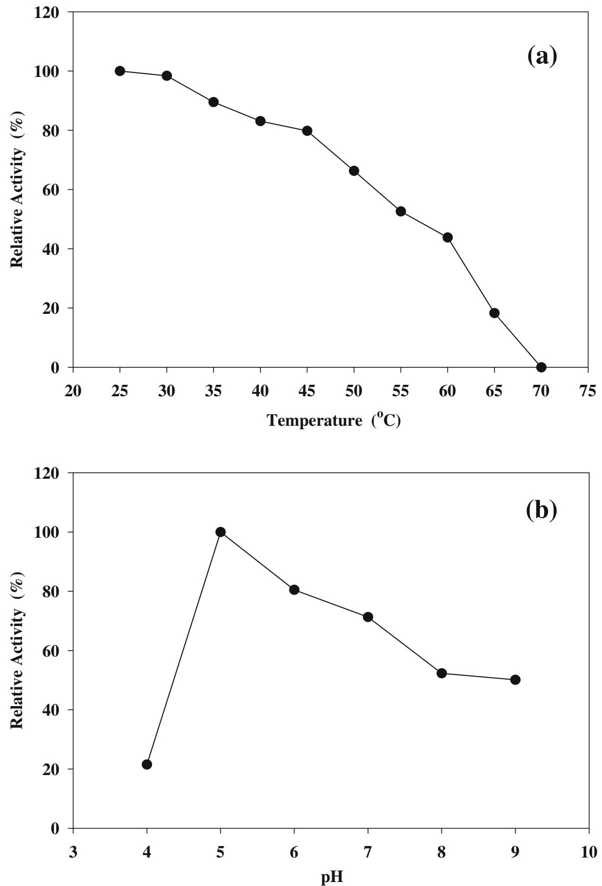

Fig. 7 Thermal (**a**) and pH stability (**b**) of laccase from *A. fumigatus* VkJ2.4.5

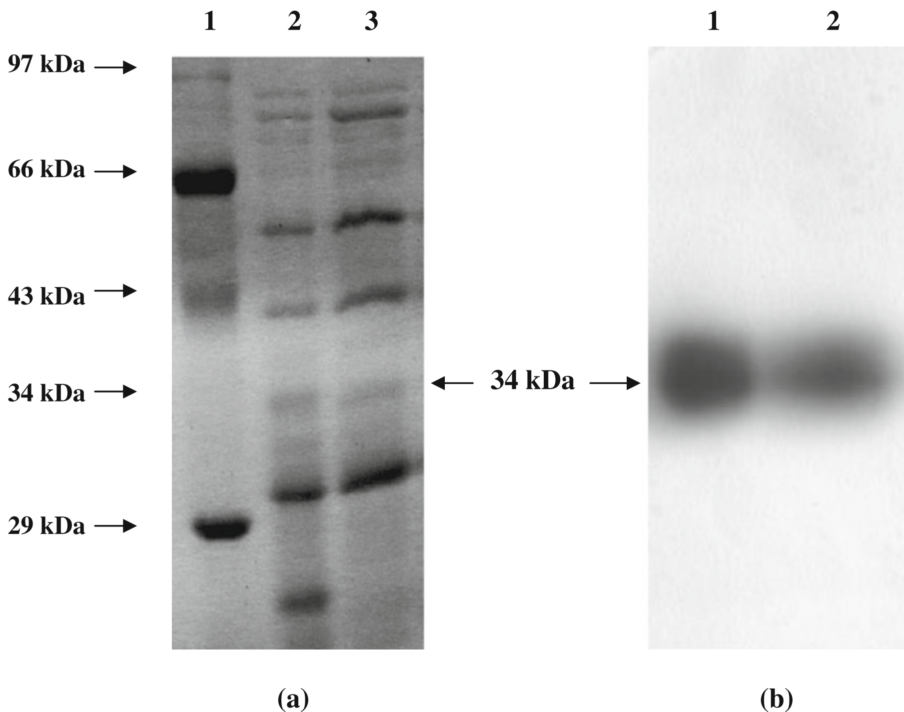

Fig. 8 SDS-PAGE (**a**) and zymogram (**b**) analysis of proteins produced by mutant *A. fumigatus* VkJ2.4.5 and wild-type *A. fumigatus* VkJ after 4 days of incubation **a**: *Lane 1*, marker protein; *Lane 2*, protein profile of mutant *A. fumigatus* VkJ2.4.5; *Lane 3*, protein profile of wild-type *A. fumigatus* VkJ, **b**: Zymogram analysis; *Lane 1*, laccase activity band of mutant *A. fumigatus* VkJ2.4.5; *Lane 2*, laccase activity band of wild-type *A. fumigatus* VkJ

(67%) and *Rhus vernicifera* (50%). Further, low identity level was observed with laccase from basidiomycetes like *Pleurotus* sp. (35%), *Trametes* sp. (33%), and *Pycnoporus sanguineus* (27%) (Table 1).

The notable performance of fungi producing higher levels of laccase on banana peel may be attributed to rich nutrient content of banana peel. The agro residual materials like banana peel therefore can be attributed to be of potential significance due to their usage for the production of commodities of commercial significance. Laccase and MnP have a wide range of applications viz. in biobleaching, bioremediation, and dye decolorization. It has also been observed that the synergistic action of laccase and MnP may degrade lignin effectively [25]. The laccase and MnP-less mutants from *T. versicolor* were found to be largely incapable for degrading lignin as compared to strain possessing laccase and MnP activity [24]. Laccase and MnP from *Rigidoporus lignosus* were also studied for lignin degradation. When isolated, neither laccase nor MnP alone was able to solubilize the radioactive lignin. In contrast, higher lignin solubilization was observed when both enzymes were added to reaction medium at the same time. Thus, laccase and MnP appear to act synergistically for degrading the lignin [62]. Simultaneous and large scale production of laccase and MnP in the column-tray bioreactor provides a promising approach for enhanced biodegradation of lignin. Thus, banana peel appears to be an economical substrate for production of industrially compatible laccase and MnP by *A. fumigatus* VkJ2.4.5, which can be further utilized for biobleaching applications.

Table 1 Sequence homology for internal peptides of *Aspergillus fumigatus* VKJ2.4.5 laccase by NCBI BLAST short sequence search

Accession no.	Organism		Sequence		Identity (%)
Peptide 1	*Aspergillus fumigatus*	39	-MMMIER-	44	-
ABS19941	*Fusarium oxysporum*	582	GMMMVIQD	589	50
NP195724	*Arabidopsis thaliana*	433	KMMFPERK	440	67
BAB63411	*Rhus vernicifera*	410	LMIEYGEA	417	50
Peptide 2	*Aspergillus fumigatus*	45	-VRGEEEER-	52	-
ABP49580	*Streptomyces cyaneus*	103	LVRDDEEDAL	112	50
NP199621	*Arabidopsis thaliana*	166	DVRVEVEEFV	175	63
XP00120228	*Strongylocentrotus* sp	142	IVREPEEDNP	148	50
Peptide 3	*Aspergillus fumigatus*	15	-KGGEEEGEAK-	24	-
ABS19943	*Fusarium oxysporum*	239	DGIYTEEAEAEM	250	40
AAN17283	*Phaeosphaeria* sp	77	HVSGEGVGEYAK	85	40
XP001177603	*Strongylocentrotus* sp	436	NRMQNEEGEAIH	447	50
Peptide 4	*Aspergillus fumigatus*	16	-GGEEEGEAKGR-	26	-
ABS19941	*Fusarium oxysporum*	264	GLGNEEALLPGTF	276	36
ABK58288	*Streptomyces* sp	249	PAGEHPAELDGRL	261	45
AAN17283	*Phaeosphaeria* sp	75	VSGEGVGEAKMNV	87	54
XP001177603	*Strongylocentrotus* sp	437	RMQNEEGEAIHWH	449	45
Peptide 5	*Aspergillus fumigatus*	1	-MPLALDSDGGESAR-	14	-
ABS19942	*Fusarium oxysporum*	484	VEDPLALQASLKLPQNH	498	29
ABP49580	*Streptomyces cyaneus*	214	PGIVHQIGSDGGLLPRP	228	29
AAN17292	*Phaeosphaeria halima*	216	QDMPKALEVGFDDDLGS	232	29
Peptide 6	*Aspergillus fumigatus*	27	-QMTREWLSTLGR-	38	-
ABS19938	*Fusarium oxysporum*	430	YNSPTLLLSKLGNH	443	33
ABK58288	*Streptomyces* sp	74	VIVLDDWLDGIGRT	87	33
XP789245	*Strongylocentrotus* sp	215	VIMMSDWTDTLSMQ	228	33
Peptide 7	*Aspergillus fumigatus*	1	-MPLALDSDGGESARK-	15	-
ABS19942	*Fusarium oxysporum*	483	VEDPLALQASLKLPQNH	499	27
ABP49580	*Streptomyces cyaneus*	212	PGIVHQIGSDGGLLPRP	228	27
AAN17292	*Phaeosphaeria halima*	215	QDMPKALEVGFDDDLGS	231	27

Conclusions

Present study therefore emphasizes that in view of escalating cost of substrates for microbial cultivation especially in developing countries, it has become imperative to explore organic materials available freely or with low cost as a promising source for microbial cultivation. Banana peel appears to be an excellent alternative, and its utilization by the microbes could be implied to effective waste management and simultaneously for the production of industrially viable products.

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