

Isolation and characterization of a fungus *Aspergillus* sp. strain F-3 capable of degrading alkali lignin

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Abstract A fungus strain F-3 was selected from fungal strains isolated from forest soil in Dalian of China. It was identified as one *Aspergillus* sp. stain F-3 with its morphologic, cultural characteristics and high homology to the genus of rDNA sequence. The buds or thickened node-like structures are peculiar structures of hyphae of the strain. The fungus degraded 65% of alkali lignin (2,000 mg l⁻¹) after day 8 of incubation at 30°C at pH 7. The removal of colority was up to 100% at 8 days. The biodegradation of lignin by *Aspergillus* sp. F-3 favored initial pH 7.0. Excess acid or alkali conditions were not propitious to lignin decomposing. Addition of ammonium L-tartrate or glucose delayed or repressed biodegradation activities. During lignin degradation, manganese peroxidase (28.2 U l⁻¹) and laccase (3.5 U l⁻¹) activities were detected after day 7 of incubation. GC-MS analysis of biodegraded products showed strain F-3 could convert alkali lignin into small molecules or other utilizable

products. Strain F-3 may co-culture with white rot fungus and decompose alkali lignin effectively.

Keywords Alkali lignin · Biodegradation · *Aspergillus* sp. strain F-3 · GC-MS analysis

Introduction

Lignin, next to cellulose, is the second most abundant compound in plant biomass. And it is also known for resistance to microbial degradation because of its high molecular weight and presence of various biologically stable linkages. Microbial lignin degradation is drawing attention as an alternative to pulping or enzymatic hydrolysis of lignocellulosic materials. Delignification of wood by the alkaline pulping process produces millions of tons of kraft lignin annually of which little has been recovered and used. Studies on the biodegradation of lignin have significant implications on our understanding of the global carbon cycle and development of an environmentally friendly technique for selective removal of lignin in paper-making. Furthermore, the effective bioconversion of lignin is of importance to fully utilize it for new materials or energy. There was a breakthrough in the field of lignin biodegradation in 1983 when fungal ligninases and their hydrogen peroxide requirement were described by Tien and Kirk (1983). Since that time, biodegradation of lignin

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by white rot fungi and their lignolytic enzymes have been widely studied (Hatakka 2001; Pointing 2001; Baborová et al. 2006; Baldrian et al. 2005; Asgher et al. 2008). Most lignin research on bioconversion and biodegradation has been on wood-rot fungi, particularly white-rot fungi, and not on other lignolytic organisms. However, because of slow growth rates; easily counteracted by other lower microorganisms; damageable enzymes and production costs for them, etc., the direct use of single white rot fungi and their enzymes in industrial processes is still very difficult now. Molds derive energy not through photosynthesis but from the organic matter in which they live. Typically, molds secrete hydrolytic enzymes, mainly from the hyphal tips. These enzymes degrade complex biopolymers such as starch, cellulose and lignin into simpler substances which can be absorbed by the hyphae. In this way, molds play a major role in causing decomposition of organic material, enabling the recycling of nutrients throughout ecosystems. Members of the molds, as majority of microscopic forms, inhabit lignin-rich environments, and their relative lignin-degrading potential is worth of being studied. It was shown that some of them, such as *Aspergillus fumigatus*, *A. japonicus*, *A. niger*, and *A. terreus*, *Penicillium simplicissimum*, were capable of degrading both aromatic and carbohydrate components of water-soluble lignocarbhydrate complexes (LCC) or Kraft lignin (Milstein et al. 1984; Zeng et al. 2006). Although the function of mold species was noticed, few studies have so far described mold species occurring biodegradation of alkali lignin.

In this context, a fungus strain F-3 was isolated and characterized by its morphology and by rDNA sequencing. Its capacity to biodegrade alkali lignin was tested in liquid cultures and co-cultures with one white rot fungus—*Pleurotus ostreatus* strain G₅. The biodegraded residues of alkali lignin were analyzed with GC-MS.

Materials and methods

Chemicals and Media

All chemical reagents were of analytical or higher grade. Standard lignin: Sigma; alkali lignin was purchased from Wuhan East China chemicals co. Ltd

(Prepared from alkaline pulping wastewater, brown powder, mineral content $\leq 1.5\%$, pH 8–10, water content $\leq 5\%$, filtered through 80 mesh).

Medium: Isolation and storage medium: potato dextrose agar (PDA) medium. Screening medium (or selection medium): Crawford's liquid medium containing 2.0 g l^{-1} alkali lignin (Crawford et al. 1982). Biodegradation medium (or called as transparent medium) g per litre: Glucose 10, KH_2PO_4 2, MgSO_4 0.5, $(\text{NH}_4)_2\text{SO}_4$ 0.5, VB1 0.002, alkali lignin 2.0.

Screening and characteristics of fungi

Soil samples were taken from the forest zone of Dalian, China, and used for isolation on plates of PDA medium with conventional dilution method (Barnett and Hunter 1972). The soil conditions are as follows: soil type—brown soil; pH 6–7; organic matter ≥ 75 ; N-total 2.5–4.3. Their ability to produce lignin modifying activities was then determined using screening medium. The isolates were inoculated to the screening medium and incubated at 30°C on a rotary shaker at 150 rpm. The decolourization of cultures was measured as biodegradation of alkali lignin. The most active isolate was further characterized. For macro-morphological observations, isolates were cultivated on plate and tubes of PDA medium at 28°C in the dark for 7 days. For microscopic observations, mounts were made in lactic acid from colonies or a drop of alcohol was added to remove air bubbles and excess conidia. Scanning Electron Microscopy (SEM) was performed using a JEM-1200EX electron microscope.

rDNA sequence analysis of strain F-3

Total DNA samples were used to amplify the ITS DNA (18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence gene) using TaKaRa Fungi Identification PCR Kit with TaKaRa Thermal Cycler Dice TP600 (TaKaRa BIO INC). The amplification products were gel purified using TaKaRa Agarose Gel DNA Purification Kit Ver. 2.0 (Code No. DV805A). DNA sequencing were performed using Seq Forward Primer (5'-CGCCAGGGTTTTCCAGTCACGAC-3') with an ABI PRISM™ 3730XL DNA Sequencer,

and the ABI Prism Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystem).

The partial sequence was subjected to BLAST analysis for homology analysis using the online option available at www.ncbi.nlm.nih.gov/BLAST (Altschul et al. 1997). The relative sequences from Blast results were aligned with ClustalX1.8 (Thompson et al. 1997) and constructed phylogenetic trees with MEGA4.2 (Tamura et al. 2007).

Biodegradation of alkali lignin by strain F-3

Biodegradation of alkali lignin: Dispense 100 ml of the transparent or screening medium with $2,000 \text{ mg l}^{-1}$ of alkali lignin into each 250 ml Erlenmeyer flask. The flasks were then plugged by cotton caps and the caps were again covered by non-absorbing paper with rubber band. The flasks were labeled accordingly and were sterilized by autoclaving at 110°C for 20 min. After autoclaving, the flasks were allowed to cool down at room temperature. 0.5 cm^2 mycelia of the selected strain from 7-day-old agar slants of PDA, served to inoculate Erlenmeyer flasks. The time course of lignin degradation was followed in shaking flasks at 30°C on a rotary shaker at 150 rpm. Disappearance of lignin was monitored by aseptically removing 5-ml samples for measurement of ultraviolet (UV) absorption spectra at 280 nm after centrifugation at 12,000 rpm for 10 min (Lundquist et al. 1977).

The colority of cultures was determined according to the CPPA standard method (1974).

All assays were performed at least in duplicate, with the corresponding controls. Both uninoculated media (called “blanks”) and inoculated autoclaved vials (called “controls”) were used.

Ligninolytic enzymes activity

Lignin peroxidase (LiP, EC1.11.1.14) activity was determined as described by Tien and Kirk (1984). The reaction mixture (in a total volume of 2 ml) in 0.125 mM sodium tartrate-HCl buffer (pH 4.5) contained 1.5 ml of extracellular culture fluid and 2.5 mM veratryl alcohol, 0.5 mM H_2O_2 . The reaction was monitored by measuring the change in A_{310} for 5 min. Manganese peroxidase (MnP, EC1.11.1.1) activity was determined as described by Glenn and Gold (1985). MnP reaction mixture (in a total volume of 2 ml) in 50 mM succinate-lactate buffer (pH 4.5)

contained 0.1 mM Manganese(II) sulfate, 0.1 mM H_2O_2 , 0.25 mM phenol red. The reaction was monitored by measuring the change in A_{610} for 5 min. And laccase (Lac, EC1.10.3.2) activity was determined as described by Niku-Paavola et al. (1990). The laccase reaction mixture (in a total volume of 2 ml) in 100 mM acetate buffer (pH 4.0) contained 1.5 ml of extracellular culture fluid and 0.5 mM ABTS as the substrate. The reaction was monitored by measuring the change in A_{420} for 5 min. One unit of enzymes activity was defined as the amount catalyzing 1 μmol of substrates oxidized per minute of reaction.

GC-MS analysis of degraded products

Sample preparation for the GC-MS analysis: 60 ml supernatant of centrifuged biodegraded liquids was adjusted pH to 1.0–2.0 with hydrochloric acid, and centrifuged at 12,000 rpm (17,000 g) for 10 min. The acidified supernatant was extracted three times with 20 ml ethyl acetate. The ethyl acetate phase extracts were dehydrated with sodium sulfate anhydrous and mixed then evaporated under vacuum in a rotary apparatus at 40°C . The residue was dissolved in 1.2 ml pyridine. 100 μl pyridine solvent was derivatized with 20 μl BSTFA (BSTFA + TMCS 99:1 Supelco, USA) in microreaction vials of 2 ml volume with mininert valves (Supelco Inc., Bellefonte, PA, USA) and kept at 50°C for 20 min. For the analysis an Agilent GC-MS system (6890N/5975B) with a quadrupole mass spectrometer and a capillary gas-chromatograph were used. The fused silica capillary column (30 m \times 0.25 mm i.d.) was coated with SE 30 (0.25 μm). Helium was used as carrier gas (0.7 bar) at a flow rate of 1.0 ml/min. The splitter/injector was kept at 300°C with a split ratio of 5:1. The column temperature was programmed as follows: 100°C (4 min) – $4^\circ\text{C}/\text{min}$ – 280°C (3 min). The ion source temperature was 250°C , the emission current 0.6–0.7 mA. The chromatograms were recorded by ion monitoring in the m/e range 50–500.

Results and discussion

Isolation and identification of strain F-3

Bacteria can hardly grow well in most of forest soil because of a low pH value, and so the fungi are the

main decay producers. They will cause decay of lignin-containing dead leaves.

One strain F-3 was selected from the isolates of fungi from forest soil with selection medium. After day 8 of incubation, the fungus degraded 55% of alkali lignin, subsequently the discoloration rate slowed down gradually. The maximum biodegradation of alkali lignin was up to 65% (Fig. 1) at 12 days of incubation. Growth rate of F-3 on PDA medium is moderately rapid and texture of colonies is downy gradually to powdery; and colonies are 8–15 mm in diameter for 7 days (Fig. 2). Surface colony color ranges from white, pale pink, gray, to blue–green, while the reverse is uncolored. From the colony, it appears to be similar to *Penicillium* or *A. fumigatus* to some extent. Microscopic observations of appearance: mycelium composed of hyaline, branched, septate, smooth-walled hyphae with width of 3–12 μm . Conidiophores are smooth-walled and brittle, hyaline to pale brown, and with length ranging from 170–500 μm , width 5–12 μm ; conidial heads are loosely radiate, and with width 30–60 μm ; Conidiogenous cells biserial; vesicles spatulate to subclavate, 15–20 μm in diameter; metulaes 10–12 \times 4.5–5.5 μm ; phialides 8.5–10 \times 4.5–5.5 μm ; metulaes and phialides covering from 60% to almost the entire vesicle; conidia are globose, blue green, more or less rough-walled, and 3.5–4 μm in diameter; budes or thickened node-like structures 7–20 μm in diameter (Figs. 3, 4). The ITS region is a convenient universal marker for fungal species identification. The result of the partial ITS region DNA sequence of strain F-3 alignment based on BLAST analysis revealed that the

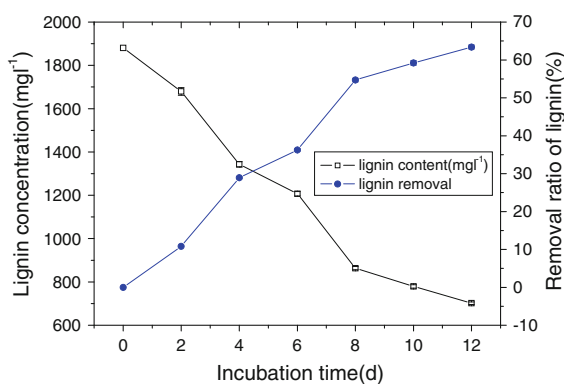


Fig. 1 The time course of biodegradation of alkali lignin by fungus strain F-3. *Open squares* lignin concentration (mg l^{-1}); *filled circles* lignin removal ratio (%)

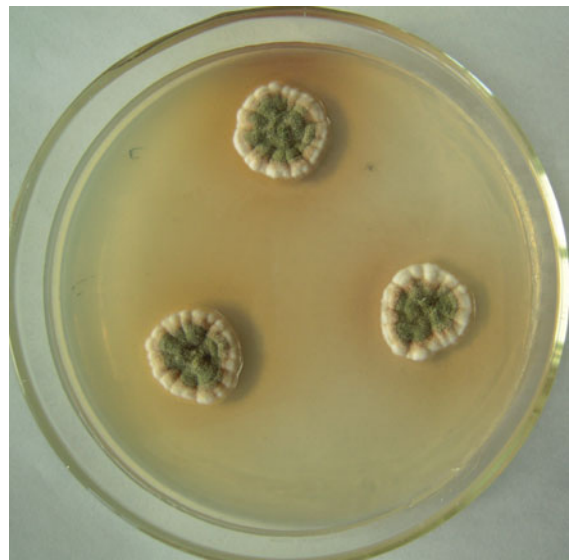


Fig. 2 The colonies of strain F-3 grown on PDA at 28°C for 7 days

isolate was identified as *Aspergillus* with high homology. There are many budes in hyphae of the strain F-3. This is a peculiar structure of hyphae compared with known species of *Aspergillus*. So we named it *Aspergillus* sp. strain F-3. The sequence was registered in the NCBI GenBank Data Library under the accession number GQ149340. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007). The evolutionary relations of the strain among the genus of *Aspergillus* were shown in the phylogenetic tree (Fig. 5).

There are many budes or round and ovate thickened node-like structures in hyphae of strain F-3. It was recognized that the budes in the tip of hyphae were relative to the growth restarting (Horio and Oakley 2005). In general, node-like structures were less abundant in mycelia under natural conditions. These cell-like bulges are suggestive of intercalary spores. However, these are not reported to occur in the organism before, and no crosswalls (indicative of intercalary spore formation) are found. Perhaps the

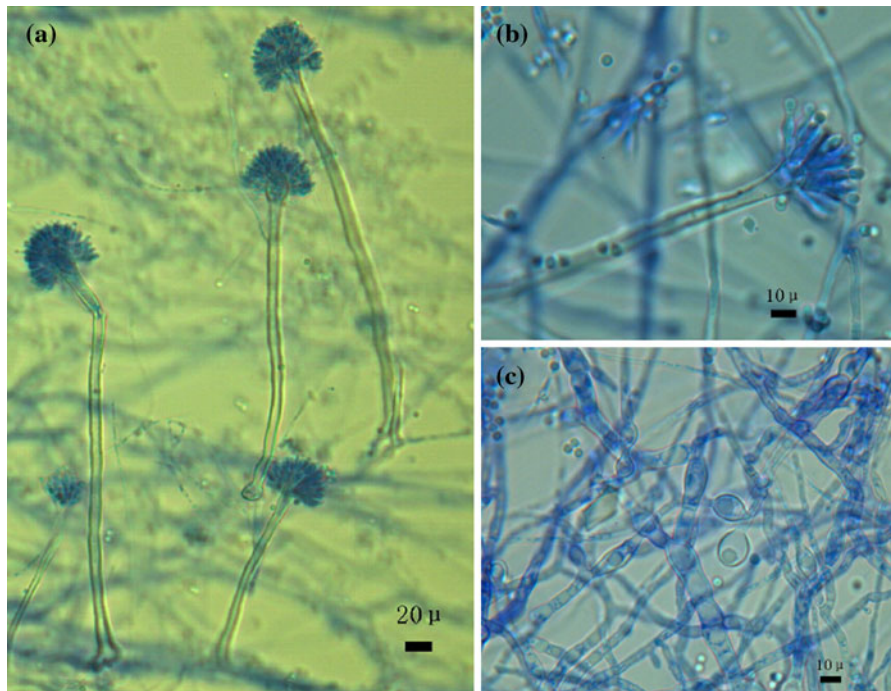


Fig. 3 The hyphae and conidia of strain F-3. **a** Conidiophore and conidial heads; **b** conidia; **c** hyphae and thickened node-like structures

nodes represent storage material in the hyphae and occur only when certain metabolic products are excessive, depending on the food source (Murphy et al. 1974). Luengo et al. (1986) found bulges were formed in the hyphae of a high penicillin-producing strain of *Penicillium chrysogenum* AS-P-78. When penicillin was accumulated in the broth, the size and number of bulges increase during the fermentation in parallel with penicillin accumulation. Several species of the genus *Aspergillus* form sexual spores within minute spherical shells (cleistothecia) with an envelope of thick-walled globose cells (hülle cells). It was found that lignin-decomposing enzyme—laccase localizes in hülle cells and cleistothecial primordia of *Aspergillus nidulans* (Hermann et al. 1983). So the presence of bulges or node-like structures in hyphae of strain F-3, may be relative to some products, such as lignin-decomposing enzymes.

Biodegradation of lignin and relative enzyme activities

As a result of isolation and screening, one of lignin degrading *Aspergillus* sp. strain F-3 was selected on the

basis of its color reduction efficiency in selection broth containing alkali lignin ($2,000 \text{ mg l}^{-1}$). The conditions for lignin degradation by fungi are varied for different species. The optimal pH was 4.5 and 6.0 for *Sporotrichum pulverulentum* (Kirk et al. 1978) and *P. ostreatus* B1 in bubble-column reactor (BCR) (Zhao et al. 2008) respectively. For *Aspergillus* sp. strain F-3, its biodegradation of lignin favored initial pH 7.0 (Fig. 6). Generally, the pH value of industrial wastewater which contains lignin such as cotton pulp black liquor is neutral to alkali. Strain F-3 could function under higher pH value than other fungi, which showed its prospective importance of using in industry.

The biodegradation of alkali lignin by fungi is always influenced by usable carbon sources. Studies have shown that degradation of alkali lignin by *Aspergillus* sp. strain F-3 was under C-limited condition, and depressed by glucose. The decolorization of alkali lignin could be delayed for 4 days by glucose at low concentration of 1 g l^{-1} whereas the removal rate was up to that of control at last. The biodegradation of lignin could be repressed with higher content of glucose (Fig. 7). Similar results are reported in other researches. *A. japonicus* OM-4

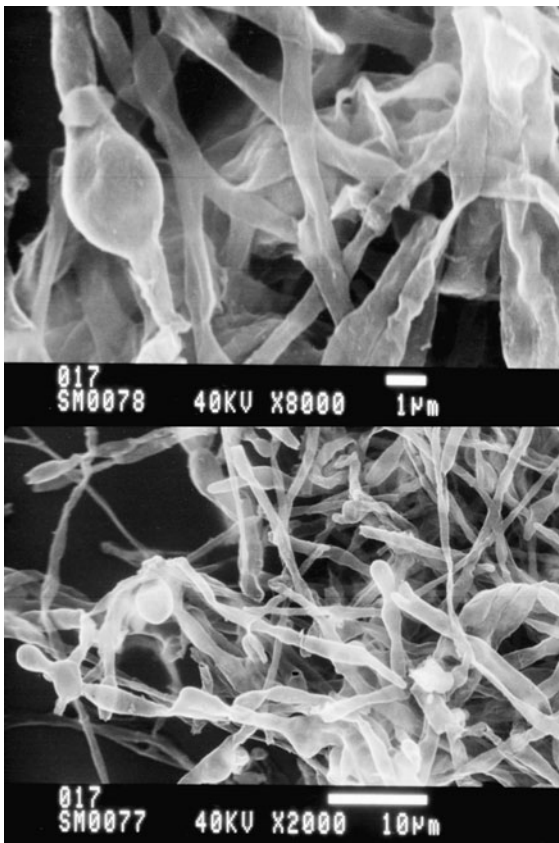


Fig. 4 Thickened node-like structures in hyphae of strain F-3 with scanning electron microscopy

degraded the lignin of the high molecular size soluble LCC (lignocarbohydrate complexes) and many phenolic compounds efficiently in the absence of

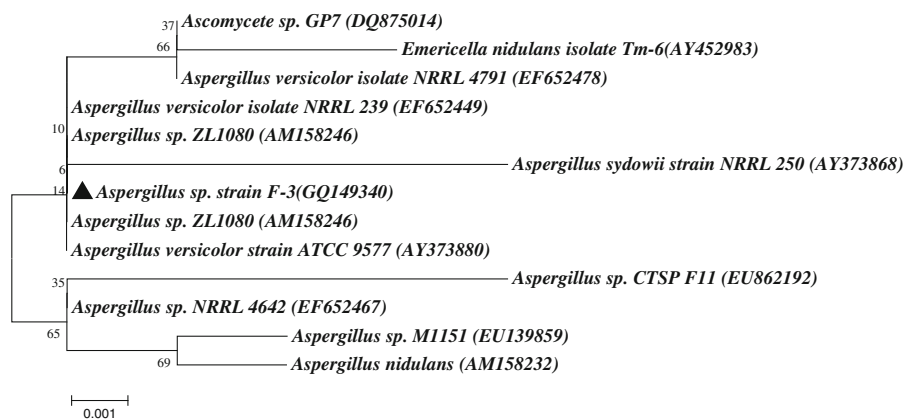


Fig. 5 Phylogenetic tree based on ITS region gene sequences showing relationships among strain F-3 and the most close type strain species of *Aspergillus*. Numbers at nodes indicate

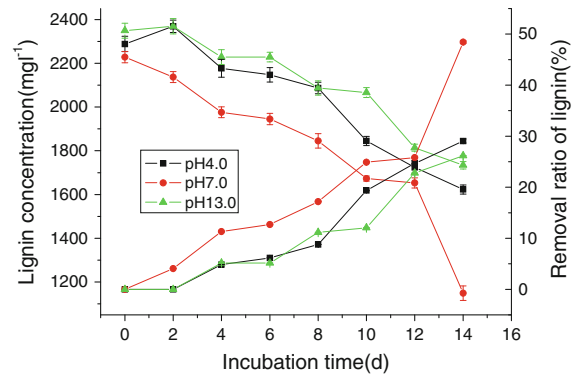


Fig. 6 Biodegradation of alkali lignin in selection medium at different initial pH (black filled squares—pH 4.0; red filled circles—pH 7.0; green filled triangles—pH 13.0)

additional carbon sources (Milstein et al. 1981). *Aspergillus wentii* could degrade aqueous fraction (AL-A) of alkali isolated straw lignin (AL) under C-limited condition (50% decrease in OD₂₈₁) (Janshekar et al. 1982). The activity of both endoglucanase and exoglucanase of *A. terreus* could be reduced by addition of carbon sources (Emtiazi et al. 2001). But there are different effects in other fungi. Both starch and phenolic compound(s) present in potato extract are capable of inducing laccase synthesis in *Pleurotus florida* (Nirmalendu et al. 1999).

Addition of ammonium L-tartrate at low concentration to selection medium could show relatively high degradation activity of lignin whereas its value was less than that of the control (Fig. 8). Alkali lignin was efficiently decomposed by strain F-3 in the

percentages of bootstrap support based on a neighbor-joining analysis of 1,000 resampled datasets. Bar, 0.001 substitutions per nucleotide position

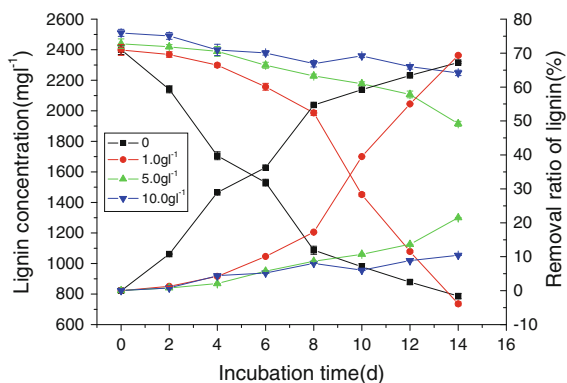


Fig. 7 Biodegradation of alkali lignin by strain F-3 under addition of glucose in selection medium (addition of glucose: black filled squares—0 g l⁻¹; red filled circles—1.0 g l⁻¹; green filled triangles—5.0 g l⁻¹; blue filled inverted triangles—10.0 g l⁻¹)

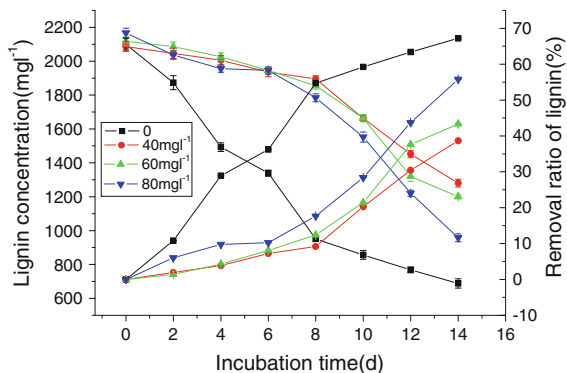


Fig. 8 Effect of addition of ammonium L-tartrate to selection medium on biodegradation of alkali lignin by strain F-3 (black filled squares—0 mg l⁻¹; red filled circles—40 mg l⁻¹; green filled triangles—60 mg l⁻¹; blue filled inverted triangles—80 mg l⁻¹)

biodegradation medium with addition of 1.0 g l⁻¹ yeast extract. In contrast, the ligninolytic activities of *P. ostreatus* strain G₅ was repressed (Fig. 9). It shows that at the early stage at 2–8 days, there was a significant biodegradation of lignin in culture, removal rate of lignin increased quickly up to its maximum 65% after day 8 of incubation. Ligninolytic enzymes of white-rot fungi are generally produced under N-limited media (Janshekar et al. 1982; Zhao et al. 2008). The fungi *A. wentii*, *Chaetomium cellulolyticum*, *Humicola fuscoatra* and *Sporotrichum thermophile* decreased the lignin absorbance only to a smaller extent (16–20%) under sufficient nitrogen supply. *P. ostreatus* was the

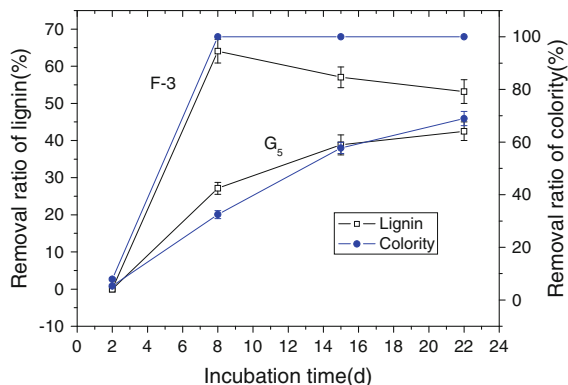


Fig. 9 Time course of biodegradation of alkali lignin by strain F-3 with *P. ostreatus* strain G₅ as reference in transparent medium with alkali lignin 2,000 mg l⁻¹, yeast extract 1 g l⁻¹ (open squares—removal of lignin; filled circles—removal of colority)

poorest strain in this respect (Janshekar et al. 1982). The correlation of biodegradation of lignin by *Aspergillus* sp. strain F-3 to N-sources is different from that of white-rot fungi to some extent. Compared with white-rot fungi—*P. ostreatus* strain G₅, *Aspergillus* sp. F-3 is not sensitive to nitrogen source in the beginning of alkali lignin degradation. As the control with white rot fungus—*P. ostreatus* strain G₅, the removal rates of lignin and colority by F-3 were higher than that of the former, about 65–70%.

Biodegradation of lignin by mixed cultivations of *S. pulverulentum*, *C. cellulolyticum*, *P. ostreatus*, *A. wentii* showed generally poorer results than those of pure cultures. This shows that the lignin degrading ability of different species may not be of a cumulative nature and hence can not be intensified by using a heterogenous culture of these species (Janshekar et al. 1981). As *Aspergillus* and white rot fungi are natural habitats in lignocellulosic materials, an attempt of co-cultures of *P. ostreatus* G₅ – *Aspergillus* sp. F-3 showed strain F-3 could co-grow with G₅ and decompose alkali lignin. In Fig. 10, slightly higher degrading rates appeared in co-cultures systems of white rot fungus-*Aspergillus* after day 7 of incubation. The removal of colority was higher than that of lignin. But on day 12, co-cultures systems did not have an advantage on removal of lignin. The immobilization of *A. niger* and *Phanerochaete chrysosporium* on polyurethane foam is considered. The different morphology of the obtained bioparticles seriously modify the productivity of citric acid and

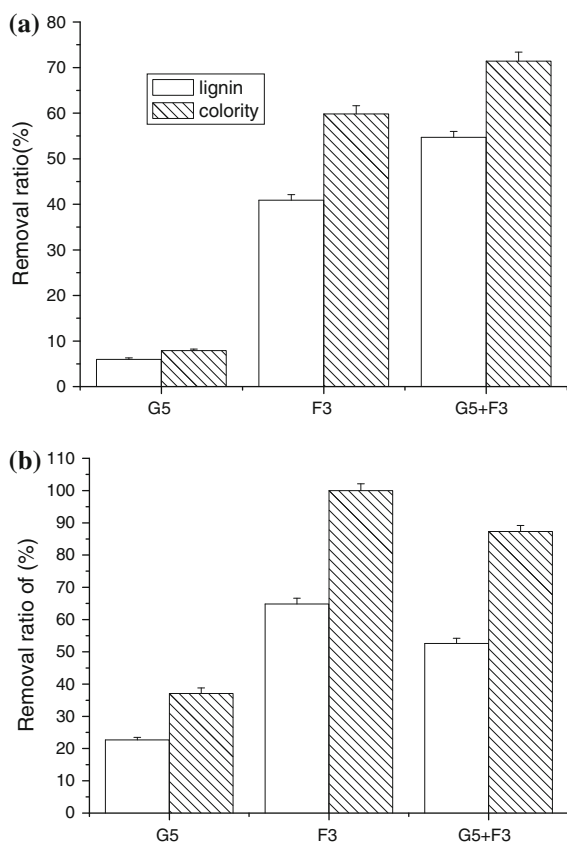


Fig. 10 The removal of alkali lignin and colority in transparent medium with $2,000 \text{ mg l}^{-1}$ of alkali lignin by *Aspergillus* F-3 and *P. ostreatus* G₅ under pure or co-culture conditions (**a** duration of 7 days; **b** duration of 12 days)

extracellular peroxidases (lignin peroxidase, LiP, and manganese peroxidase, MnP) by *A. niger* and *P. chrysosporium*, respectively. The best results are obtained in both cases when fungi developed inside the cube foam (Sanromán et al. 1996). Submerged pure cultures which are either suspended or immobilized on synthetic support material have been used for most of studies. Although immobilized cultures have been shown to be very effective in dye decolorization (Palma et al. 1999; Zhang et al. 1999), the aging process of the mycelium may limit the length of the process (Palma et al. 1999). The ability of *T. versicolor* to produce enzymes during growth was exploited in the investigation of long-term decolorization activity of *T. versicolor* in sequencing batch reactors (Borchert and Libra 2001). By shearing suspended pellets of *T. versicolor* in each cycle, high and stable degrees of

decolorization of reactive textile dyes were repeatedly found without decrease in activity over time in pure culture. However, decolorization efficiency decreased because bacterial contamination occurred easily. Judy et al. (2003) investigated four strategies for their potential in optimizing the use of white rot fungi in nonsterile culture for treating wastewater containing textile dyes. He considered that the two strategies of tolerance of low pH and production of extracellular enzymes of white rot fungi did not show potential. The third strategy, the limitation of nitrogen, had potential as limited nitrogen source could suppress bacterial growth in some extent. And the fourth strategy, which was considered as the most potential in further development, was the addition of cost-effective fungi inocula in processing system. From our results, strain F-3 could co-exist with white rot fungus and degrade lignin efficiently. In any case, this is of importance to put them in industrial use because community of microorganisms is surely better than one species or strain in decomposition of organic pollutants based on micro-ecology principle.

For some other microorganisms, they are capable of degrading lignin and may contain ligninolytic enzymes. It was shown (Emtiazi et al. 1999) that *A. terreus* had peroxidase and laccase activities when grown on wheat straw and removed COD (chemical oxygen demand) of textile dyes effluent. The enzyme activities of *Aspergillus* sp. strain F-3 were measured by us. There were activities of manganese peroxidase (MnP), and laccase (Table 1) in biodegradation culturing solution. LiP activity was not detected in the strain. The ligninolytic enzymes of strain F-3 are only partial supports for its strong biodegradation ability of alkali lignin as the enzymes activities were not detected in high level. There may be other enzymes connected with the degrading process (Emtiazi et al. 2001). The mechanism of strain F-3 for decomposing lignin still needs further study.

GC-GS analysis of products decomposed

GC-MS is used in the present study because it has been proved to be a suitable technique for analyzing low molecular weight compounds released from lignin due to degradation (Kirk et al. 1978; Janshekar et al. 1982). Small molecular products could be realized through

Table 1 Ligninolytic enzymes activities of *Aspergillus* sp. strain F-3

Strain	Laccase activity		MnP activity	
	(U l ⁻¹)	(U g ⁻¹ dry biomass)	(U l ⁻¹)	(U g ⁻¹ dry biomass)
F-3	3.5	1.0	28.2	8.2

In transparent medium with alkali lignin 2.0 g l⁻¹, yeast extracts 1.0 g l⁻¹, shaking incubation (150 rpm) at 30°C for 7 days

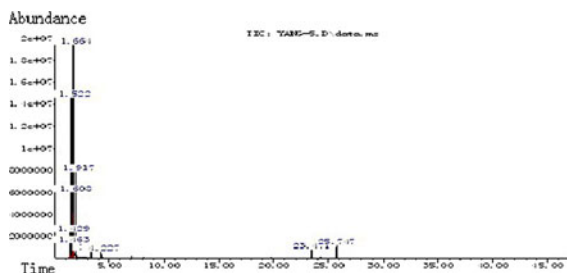


Fig. 11 Total ion chromatogram of the ethyl acetate-soluble portion from alkali lignin after biodegradation by *Aspergillus* sp. F-3 in shaking culture

GC-MS analysis of products degraded by *Aspergillus* sp. strain F-3. The results showed that alkali lignin could be effectively degraded by F-3 and be converted into broken phenol (C₃–C₈) and other compounds (Figs. 11, 12). Jouni’s study showed the α -hydroxyl groups in the propane chain of both phenolic and nonphenolic tetramers were first oxidized symmetrically in two successive steps to give monoketones and diketones (Jouni et al. 1987), then these ketone metabolites were decomposed through C _{α} –C _{β} cleavage. The metabolic route may resemble Jouni’s results according to products degraded by F-3. But some products, such as 1,4-anthracenediol, were intricate now. They may be metabolites of F-3 or resultant products from alkali lignin. Butyl cyclohexyl phthalate may be transformed from abietic acid, which is the most abundant of several closely-related organic acids that constitute most of rosin, the solid portion of the oleoresin of coniferous trees (Table 2). The pathway and mechanism of alkali lignin decomposed by strain F-3 still need further studies. However F-3 is possessed of potential value in bioconversion and biodegradation of lignin and relative compounds.

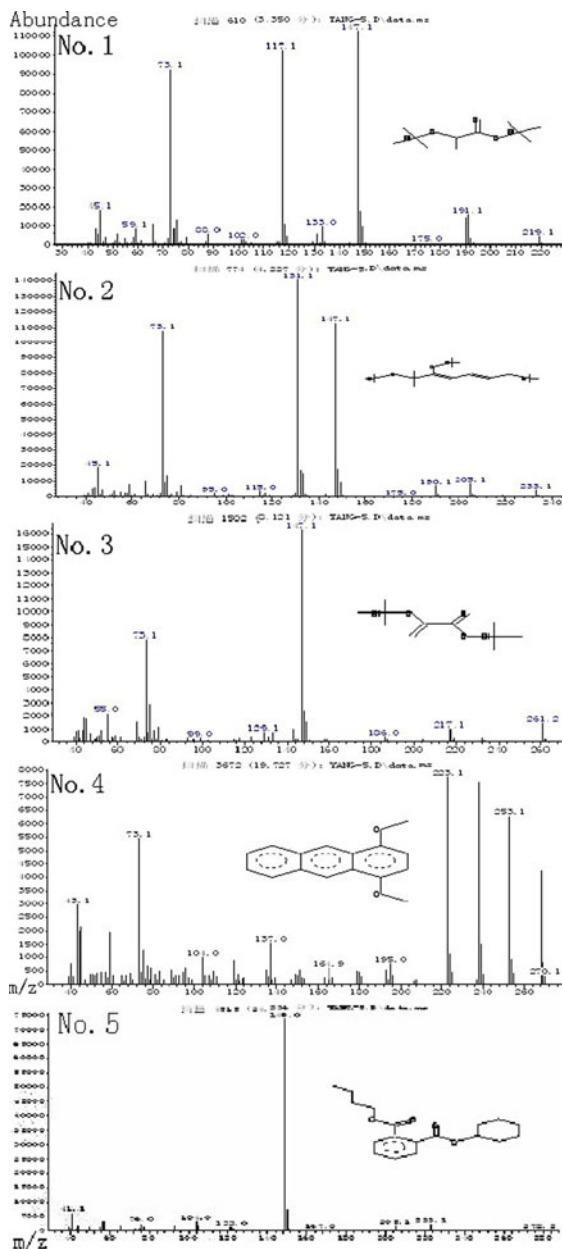


Fig. 12 Mass spectral data of alkali lignin-derived products in the ethyl acetate-soluble portion

Conclusions

The newly isolated *Aspergillus* sp. strain F-3 has peculiar node-like structures in its hyphae. This morphological character is different from present known species or strains of *Aspergillus*. A hypha extends only at its tip and the tapered region involved in this process is called the extension zone during its

Table 2 Lignin degradation products of *Aspergillus* sp. strain F-3 analyzed by GC-MS

Number	Retention time (min)	Molecular formula	Compounds name
1	3.350	C ₃ H ₆ O ₃	2-Hydroxypropanoic acid
2	4.227	C ₈ H ₁₄ O ₃	(2 <i>E</i> ,4 <i>Z</i>)-6-methylhepta-2,4-diene-1,5,6-triol
3	8.121	C ₃ H ₄ O ₃	2-Hydroxypropenoic acid
4	19.727	C ₁₄ H ₁₀ O ₂	1,4-Anthracenediol
5	24.236	C ₁₈ H ₂₄ O ₄	Butyl cyclohexyl phthalate

growth. The bulges in the tip of hyphae were relative to the growth restarting. These cell-like bulges are different from suggestive intercalary spores, as no crosswalls were found. So the presence of bulges or node-like structures in hyphae of strain F-3, may be relative to the accumulation of some products, which may participate in alkali lignin-decomposing process.

Aspergillus sp. strain F-3 is possessed of ligninolytic enzymes activities and is capable of degrading alkali lignin alone. GC-MS analysis of products decomposed by strain F-3 showed alkali lignin can be degraded to small molecules or converted into utilizable products.

The strain can effectively degrade alkali lignin under co-culture system of *Pleurotus*—*Aspergillus*. It implied their potential values in industrial use. Molds grow rapidly and with high antibacterial ability in natural environment. Many different mold species vary enormously in their tolerance to temperature and humidity extremes. Certain molds can survive in harsh conditions such as the snow-covered soils of Antarctica, refrigeration, highly acidic solvents, and even petroleum products such as jet fuel. The system of co-exist of *Pleurotus*—*Aspergillus* may keep degradation activity under non-sterilized conditions with its relative strong antimicrobial contamination.

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