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Effect of Biosurfactants on Laccase Production and Phenol Biodegradation in Solid-State Fermentation

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Abstract The effects of two biosurfactants, tea saponin (TS) and rhamnolipid (RL), on the production of laccase and the degradation of phenol by *P. simplicissimum* were investigated in solid-state fermentation consisting of rice straw, rice bran, and sawdust. Firstly, the effects of phenol on the fermentation process were studied in the absence of surfactants. Then, a phenol concentration of 3 mg/g in the fermentation was selected for detailed research with the addition of biosurfactants. The results showed that TS and RL at different concentrations had stimulative effects on the enzyme activity of laccase. The highest laccase activities during the fermentation were enhanced by 163.7%, 68.2%, and 23.3% by TS at concentrations of 0.02%, 0.06%, and 0.10%, respectively. As a result of the enhanced laccase activity, the efficiency of phenol degradation was also improved by both biosurfactants. RL caused a significant increase of fungal biomass in the early stage of the fermentation, while TS had an inhibitory effect in the whole process. These results indicated that RL could mitigate the negative effects of phenol on fungal growth and consequently improve laccase production and phenol degradation. TS was potentially applicable to phenol-polluted solid-state fermentation.

Keywords Rhamnolipid · Tea saponin · Laccase · Phenol · Solid-state fermentation · *P. simplicissimum*

Introduction

Phenol is an important raw material widely used in chemical industries, including coal conversion, petroleum refining, resins and plastics, wood preservation, metal coating, dyes,

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textiles, and pulp and paper [1]. It has been classified as a high-concern priority pollutant by the EPA because of its carcinogenicity, teratogenicity, and mutagenicity even at low concentrations [2]. Every year, large amounts of this compound are released into the environment by transport, by accidental discharge, by disposal of petroleum products, or through industrial effluents [3]. As a result, phenol-polluted industrial and agricultural solid wastes are widely produced, and their disposal should attract greater attention.

Much research has focused on the biological and physicochemical methods (e.g., solvent extraction, chemical oxidation, adsorption on activated supports, and biological remediation methods) to remove phenol from water and soil. However, there is still a lack of knowledge about its removal from industrial and agricultural solid wastes. In recent years, composting is considered a common bioremediation method for the disposal of these wastes. By doing this, removal of harmful organic pollutants could be achieved through a series of microbial metabolic processes. It has become a hot research concern as the toxic materials could be degraded into less toxic derivatives with lower costs, no secondary pollution, and the possibility of complete mineralization.

However, several difficulties are encountered in microbial biodegradation. First, the toxicity of these chemicals to microorganisms will reduce the efficiency of composting or solid-state fermentation processes. Second, the hydrophobic properties of phenolic compounds lead to mass transfer limitation during the biological degradation [4]. It has been found that some strains could be resistant to the toxicity within a certain concentration range, such as fungi (*P. simplicissimum* [5]), yeast (*Candida tropicalis* [6]), bacteria (*Pseudonomonas* sp. [7]), and so on. Some toxic materials are able to support the growth of some adapted microorganisms as sole carbon and energy source, and these microorganisms are served in the bioremediation of the phenol-polluted wastewater and soil. However, phenol, especially at high concentrations, is especially toxic or inhibiting to the growth of most types of micro-organisms, even to those species that have the metabolic capacity of using it as a growth substrate [8]. This would lead to the failure of the whole composting treatment.

Recently, the use of oxidoreductive enzymes, including peroxidases, laccases, and tyrosinases, to catalyze phenol in wastewaters has been investigated [9]. Laccases (1.10.3.2, *p*-diphenol:dioxygen oxidoreductases) are the most reliable enzyme for phenol removal. Large numbers of studies have been carried out on fungal laccases on this characteristic [9]. Since laccases are relatively nonspecific, they can induce the cross-coupling of pollutant phenols with naturally occurring phenols and then oxidize them to the corresponding anionic free radicals which are highly reactive [10]. Laccases can also detoxify highly reactive aromatic compounds by polymerizing them [11]. In addition, laccases, along with manganese peroxidase and lignin peroxidase, are a type of lignin-modifying enzymes [12, 13]. Zeng et al. [13] found that, when laccase activity was relatively low (e.g., 12.83–26.5 nkatals/g), lignocellulose degradation was improved during solid-state fermentation with rice straw, suggesting a polymerizing function of laccases in lignin degradation by *P. simplicissimum*.

It has been shown by previous reports that surfactants have a significant role in the decomposition of the substrate in submerged or solid substrate fermentation [14–17]. During composting, lignin in the raw material is transformed through a variety of biological and biochemical processes in which enzymes play an important role [14]. Surfactants could have stimulative effects on enzyme activities or production by microorganisms as their functions are to (1) improve the permeability of cell membrane to facilitate enzyme release [15, 16] and (2) enhance enzyme stability and prevent the denaturation of enzymes during hydrolysis by desorbing them from substrate [18]. In addition, surfactants could enhance the mobility of substrate to reduce mass transfer limitations in composting. Moreover, Liu et al. [19] found that surfactants can diminish the toxicity of phenol to the fungi *C. tropicalis*, increase cell

growth, and improve phenol removal in aqueous solution. In comparison with synthetic surfactants, biosurfactants have the advantages of biodegradability, low toxicity, and ecological compatibility, which have made it gain more and more attention.

Tea saponin (TS) and rhamnolipid (RL) are both typical biosurfactants. RL is one of the most extensively studied biosurfactants [14, 16, 17]. Liu et al. [17] reported that dirhamnolipid, at different concentrations, had certain stimulative effects on the enzyme activities of CMCase, xylanase, and lignin peroxidase in submerged fermentation. To our knowledge, however, there are few research activities on the effects of biosurfactants on laccase production by fungi in phenol-polluted solid-state fermentation. Therefore, it is valuable to add biosurfactants to such fermentation and determine whether they alleviate the toxicity of phenol to fungi and enhance enzyme production or activity to accelerate the decomposition of phenol and the other substrates.

In this study, TS and RL were used as the model biosurfactants, and their effects on simultaneous laccase production and phenol removal by *P. simplicissimum* in the solid-state fermentation were studied. The aim of this paper is to investigate the mechanism pertaining to the effects of biosurfactants on fungus growth and metabolism in the phenol-polluted solid-state fermentation process, especially at high concentrations of phenol. It may offer some useful information about the application of biosurfactants in compost or solid-state fermentation.

Materials and Methods

Microorganisms

The strain *P. simplicissimum* was isolated from the soil samples of Yuelu Mountain woodland in Changsha. *P. aeruginosa* was obtained from the China Center for Type Culture Collection (AB93066). The microorganisms were maintained on 3.9% potato dextrose agar (PDA) plates at 4 °C. For the biodegradation experiments, the fungus *P. simplicissimum* was inoculated on PDA plates at 30 °C for 7 days. After then, the spores were gently scraped with a sterile cotton swab and immersed into the sterile water until the concentration of the spore solution used for inoculation reached 10^7 mL^{-1}.

Biosurfactants

Biosurfactant RL was produced by *P. aeruginosa* and extracted from the fermentation medium as described by Zhong et al. [20].

TS isolated from tea seeds was purchased from Ningbo United Biotechnology Co. Ltd. (Zhejiang, China). It was a mixture of 76.1% triterpenoids saponins, and the molecular weight is 1,222.54. Its critical micelle concentration (CMC) was 540 mg/L, and the surface tension at CMC was 42.6 mN/m as measured by an automatic tension apparatus (JWY-2000).

Media and Inoculums

The content of solid media was an artificial mixture of kitchen waste and agricultural waste consisting of dried rice straw, rice bran, and sawdust in proportions of 60%, 30%, and 10%, respectively. All the materials were air-dried and shredded to a size of 1–2 mm in diameter. Every 70 g mixture in dry weight was put into 1,000-mL Erlenmeyer flasks. Firstly, phenol was added into the fermentation samples at three concentrations (i.e., 1, 3, and 5 mg/g) without biosurfactants. The sample without phenol was run as the control A1. Selected for detailed

research was 3 mg/g phenol, and then various concentrations of biosurfactants were added. The sample without biosurfactants was carried out as the control A2. The initial contents of TS were 0.02%, 0.06%, and 0.10%, while those of RL were 0.005%, 0.010%, and 0.015%, respectively. The samples were autoclaved at 115 °C for 30 min. These media were then allowed to cool and inoculated with 30 mL spores suspension containing 10^7 spores per milliliter from a 4-day-old sporulated culture grown on PDA slant at 37 °C. Then, these samples were kept in 37 °C incubator with 60% relative humidity. Each medium was stirred using a sterilized glass stick every 2 days. All results are mean values of duplicate experiments, and the standard deviations were consistently below 5%.

Enzyme Assays

Twenty milliliters of 0.2 M sodium acetate buffer (pH 5.0), along with 2.0 g of the sampled solid, was added into 50-mL flasks. The flasks were shaken at 200 rpm for 1 h on a rotary shaker at room temperature for the enzyme extraction. The mixtures were then centrifuged at 8,000 rpm for 10 min. The clear supernatant was then filtered through 0.8-μm filter membrane, and the crude enzyme solution was obtained. Laccase activity was determined with the method of Bourbonnais [21] using UV–visible spectrophotometer (Shimadzu Kyoto, Japan) with ABTS as the substrate. The enzyme activity of laccase was expressed as units of enzyme activity per gram dry weight of ferment substance (U/g DW). One unit of laccase activity (U) is defined as the amount of enzyme oxidizing 1 μmol of substrate per minute.

Phenol Analysis and Biomass Estimation

Ten milliliters of phosphate buffer solution (pH 2.0), along with 1.0 g of the sampled solid, was added to 50-mL flasks. Then, the flasks were shaken at 200 rpm for 1 h on a rotary shaker at room temperature for the extraction of enzyme. The mixtures were centrifuged at 8,000 rpm for 10 min. The clear supernatant was filtered through 0.45-μm organic microporous filter membrane, and then phenol solution was obtained. The phenol concentrations were analyzed by high-performance liquid chromatography (HPLC) using an HP1100 HPLC (Agilent Technologies, Santa Clara, CA, USA) system with a C18 column (150×4.6 mm, 5 μm; Agilent) and a column temperature at 35 °C. Acetonitrile/water (90:10, *v*/*v*) was used as the mobile phase at a flow rate of 1.0 mL/min. Absorbance was measured at a wavelength of 280 nm. Under such conditions, the retention time of phenol was 16.5 min, while data analysis was conducted using the Agilent GPC software.

The fungal ergosterol content was measured and taken as an equivalent value for biomass amount and cell growth. The ergosterol was extracted from the fungal biomass according to the method of Ruzicka et al. [22]. The content of ergosterol was determined by HPLC (Agilent 1100) analysis with a C18 column (150×4.6 mm, 5 μm; Agilent), a column temperature at 25°C, mobile phase of methanol and acetonitrile (98:2, *v*/*v*), flow rate at 1 mL/min, and UV detector at 282 nm. Under such conditions, the retention time of ergosterol was 8.1 min [14].

Results and Discussion

Effect of Biosurfactants on Laccase Activity

The effects of various concentrations of phenol on the enzyme activity of laccase produced by *P. simplicissimum* were shown in Fig. 1a. In the whole test process, 1 mg/g

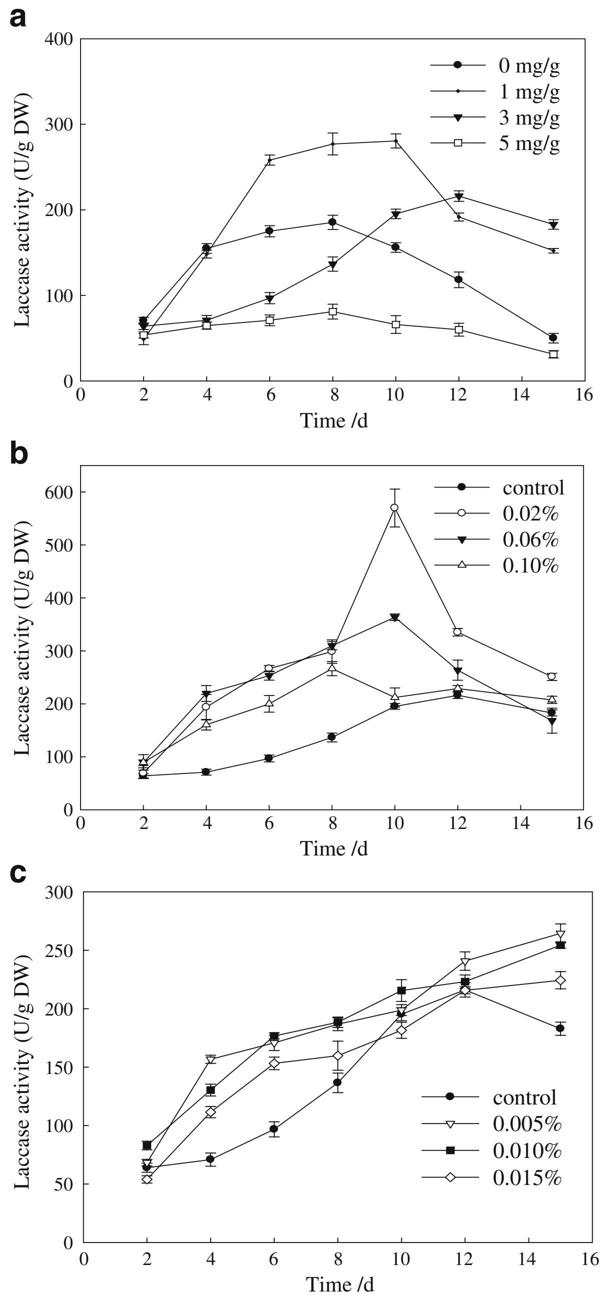

Fig. 1 Effects of phenol with or without biosurfactants on laccase activity as functions of their concentrations. **a** Treatment with phenol at different concentrations, **b** treatment with 3 mg/g phenol and TS at different concentrations, and **c** treatment with 3 mg/g phenol and RL at different concentrations. All of the results presented are mean values of triplicates and the standard deviations were consistently below 5%

phenol had a stimulatory effect on laccase activity. Laccase activity in the treatments with 1 mg/g phenol increased clearly to the highest level of 280.55 U/g DW at the 10th day; this was 51.4% higher than that of the control. Phenol at 3 mg/g restrained laccase activity during the first 9 days, while it stimulated laccase activity in the later stage, with the peak laccase activity of 216.09 U/g DW on the 12th day. Phenol at 5 mg/g totally suppressed

the laccase activity during the test duration, which confirmed the inhibitory effect of high concentrations of phenol on laccase production or activity. Selected for detailed research was 3 mg/g phenol.

From the data of laccase activity in the sample treated with phenol at different concentrations, phenol had a certain inhibition on laccase activity, but within a certain concentration range it could stimulate *P. simplicissimum* to produce laccases. Some microorganisms possessed the ability to secrete enzymes used for phenol degradation. However, such ability of the strains was extremely weak. When these microorganisms gradually adapted to the phenol environment, they could be stimulated to produce the relevant enzymes by phenol as the inducer [23]. Ryan et al. [24] found that the use of aromatic inducer compounds, such as phenol and cresol, would promote the enzyme activity and production of laccase by *Trametes versicolor*. The effect on laccase production depended on bacteria species and the inducer characteristics and concentrations [23]. Production of laccase may improve the tolerance of the microorganisms to phenol and the degradation efficiency of phenol. However, phenol might play the roles in both inducing the production of laccase and suppressing the laccase activity, while the inhibition effects became stronger than the stimulative effects with increasing phenol concentration.

When the substrate was contaminated by 3 mg/g phenol, laccase activity was influenced by different concentrations of TS and RL (Fig. 1b, c). Laccase activity in the samples with TS or RL, in particular with TS, was significantly higher than that of the control in the early stage of the fermentation. Figure 1b showed that TS at 0.02% had the strongest stimulatory effect on laccase activity, where the peak laccase activity was 163.7% higher than that of the control. TS at 0.06% and 0.10% also increased the peak laccase activity by 68.2% and 23.3%, respectively. The stimulative effect of TS weakened when its concentration increased.

The effects of RL on laccase activity (Fig. 1c) were remarkably different from that of TS. Laccase activity in treatment with RL at different concentrations kept to a gradually upward trend during the whole process (15 days), while that with TS experienced a rise and a decline phase. During the whole process, laccase activity with RL was higher than that of the control. A lower concentration of RL was more effective to enhance laccase activity. Liu et al. [17] also reported that biosurfactant dirhamnolipid at lower concentration had more positive effect on CMCase activity. However, it was found in the experiment result that RL was less effective on laccase activity than TS.

In the presence of RL and TS, the laccase activity increased obviously after the same cultivation time. There was a process in phenol-contaminated solid-state fermentation involving interactions among substrate particles, phenol pollutants, water, microorganisms, and metabolic enzymes. When adding surfactants to such system, these interactions might have a change, which might overcome the problem of limited bioavailability of substrates in fermentation [25]. First, surfactants improved the permeability of the cell membrane to facilitate laccase release [15, 16] and improve phenol degradation. Similar phenomena about various surfactants, such as sodium dodecyl sulfate, Tween-80, diRL, and so on, in favor of other enzyme activities, including CMCase, xylanase, lignin peroxidase, and amylase, have also been reported previously [14, 16, 17]. Second, in enzyme hydrolysis of substrate, surfactants might promote enzyme stability and prevent the denaturation of enzymes by desorbing it from cellulose substrate [18]. The characteristics of the enzyme and the surfactant determine their interrelation, which can affect the stability or activity of enzyme in the hydrolysis of substrates [26].

Although an increase in the production or activity of laccase by *P. simplicissimum* was observed with the presence of either RL or TS, the effects of two surfactants were different. Several reports showed that the interaction between surfactant and enzyme could change the conformation and/or active site of the enzyme, which was dependent on the characteristics of the enzyme and the surfactant, such as the charge and size of head group, the length of the surfactant, and the hydrophobicity of its alkyl chain [26]. TS is a non-ionic biosurfactant, of which the partial structure is determined as 21-*O*-angeloyltheasapogenol F 3-*O*-[β-D-galactopyranosyl (1→2)] [β-D-xylopyranosyl (1→2)-α-L-arabinopyranosyl (1→3)]-β-D-glucopyranosiduronic acid [27]. RL is an anionic biosurfactant, of which the major component is the dirhamnolipid (diRL, 2-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxyalkanoyl-β-hydroxyalkanoate) [28]. Laccases are extracellular glycoproteins containing four atoms of copper, one of which coordinates a cysteine [29]. Laccase usually comprises 520–550 amino acids [29].

It was reported that the interactions involved both the electrostatic interactions between the surfactant head group and the charged amino acid residues of the enzyme and the hydrophobic interactions between the alkyl chains of the surfactant and the hydrophobic amino acid residues of the enzyme [26]. TS and RL both promoted the activity of laccase (Fig. 1b, c), but the structure of TS might be more complex with the charge of its head group and more hydrophobic alkyl chains at a different length. It might result in a better alteration of the enzyme's conformation and active site structure and hence affected more noticeably the catalytic properties of the enzyme. Moreover, non-ionic surfactant might play the roles both in activating and stabilizing enzyme activity, while anionic surfactant only does so in activating. Yang et al. [26] found that both anionic surfactant AOT and non-ionic surfactant Brij 52 showed the ability of activating tyrosinase, but tyrosinase was stabilized by Brij 52 while being destabilized by AOT. This might also happen in our case when the non-ionic surfactant TS was present. Hence, TS was a little more effective on laccase activity than RL.

Effect of Biosurfactants on Phenol Degradation

As shown in Fig. 2a, phenol at different concentrations was degraded in the solid-state fermentation. Within 3 days, 1 mg/g of phenol was completely degraded by *P. simplicissimum*. In a previous report, *C. tropicalis* could also thoroughly degrade 2,000 mg/L of phenol in the mineral salt medium within 66 h [30]. The degradation efficiency was 39.5% and 18.9% for 3 and 5 mg/g of phenol on the 15th day, respectively. The result indicated that *P. simplicissimum* had high phenol degradation potential.

The effects of two biosurfactants on the degradation of 3 mg/g phenol were displayed in Fig. 2b, c. TS and RL at different concentrations both had stimulative effects on the degradation of phenol. Figure 2b showed that the degradation of phenol was obviously promoted by the addition of TS. The degradation efficiencies of phenol with TS at the concentrations of 0.02%, 0.06%, and 0.10% were 89.8%, 57.5%, and 42.7% over that of the control on the 15th day, respectively. As shown in Fig. 2c, the effects of RL on phenol degradation were not remarkable in the early stage. With the development of the process, the lowest concentration (0.005%) of RL played the strongest role in promoting the phenol degradation, which was 41.8% over that of the control on the 15th day. RL at 0.010% and 0.015% also increased the degradation efficiency by 30.4% and 22.6% after 15 days, respectively.

The tendency of phenol degradation in the treatments with two biosurfactants had some similar characteristics with the development of laccase activity. Laccase played a huge role

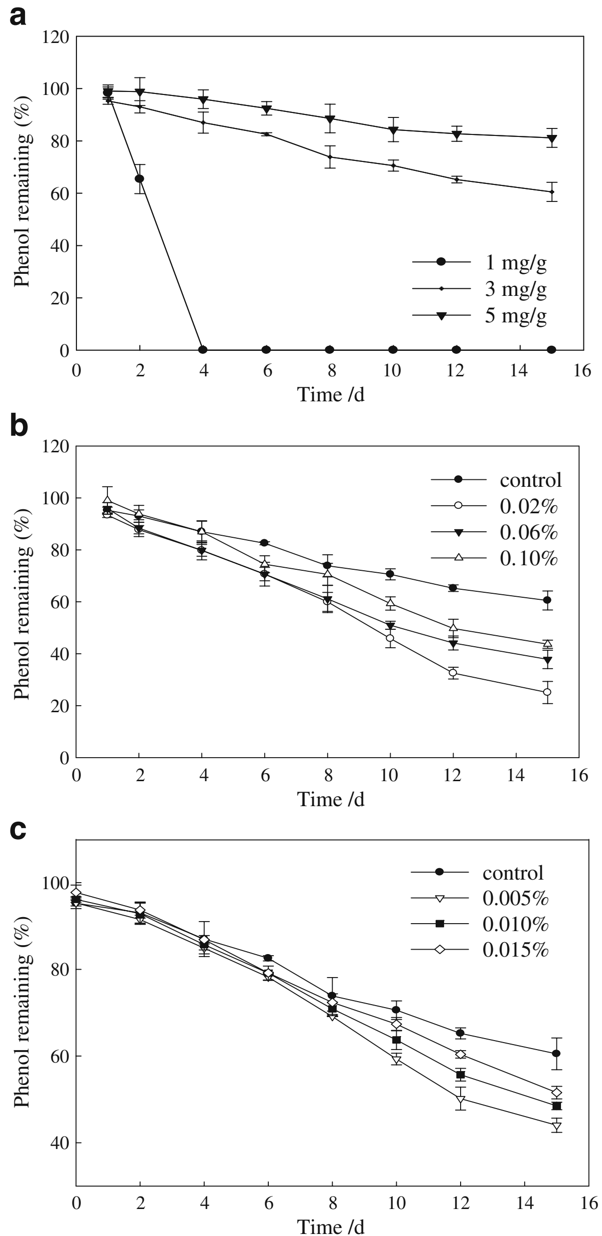

Fig. 2 Effects of biosurfactants on the degradation of phenol. **a** Treatment with phenol at different concentrations, **b** treatment with 3 mg/g phenol and TS at different concentrations, and **c** treatment with 3 mg/g phenol and RL at different concentrations

in phenol degradation. For phenolic substrates, oxidation by laccase resulted in the formation of an aryloxyradical, an active species that is converted to a quinone in the second stage of oxidation [31]. Besides, surfactants might affect the fluid transport of solid substrate, enzyme, and phenol in the hydrolysis process, which could increase their interfacial areas. This is because surfactants could alter the two-phase interaction between soluble enzymes and insoluble substrates in the fermentation system [18].

Effect of Biosurfactants on Fungal Biomass

As shown in Fig. 3a, the fungal biomass was decreased with increasing the initial phenol concentration. The results indicated that phenol exerted marked toxicity to the fungal cells

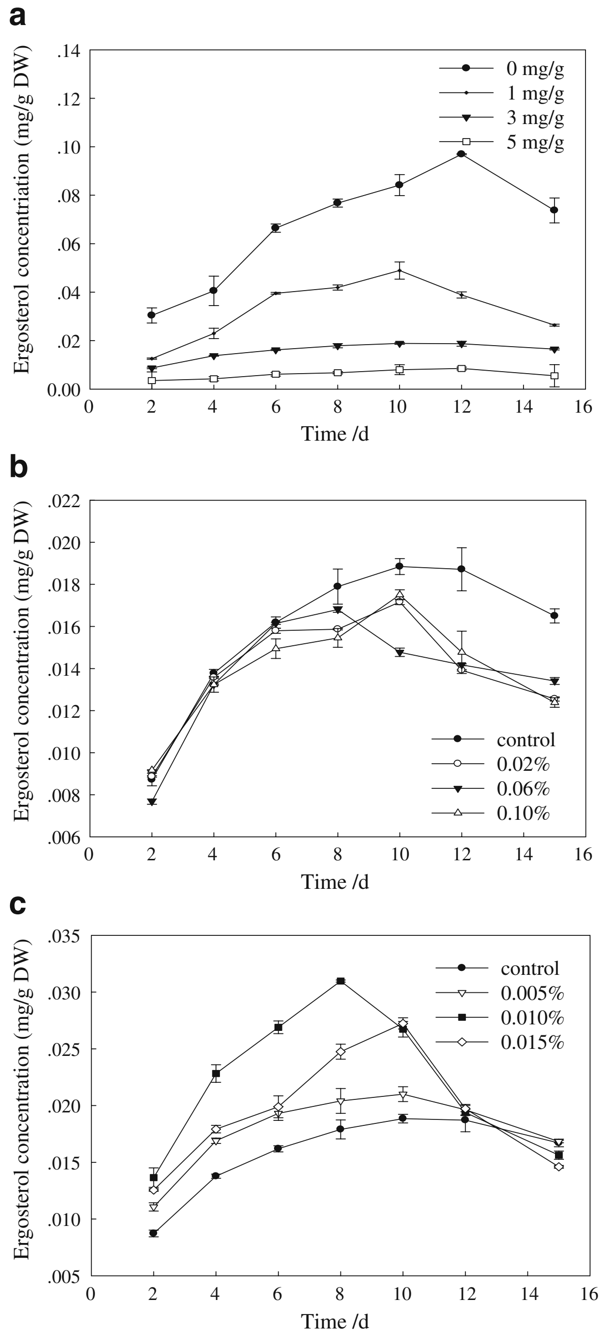

Fig. 3 Effects of phenol and biosurfactants on the fungal biomass estimated by ergosterol. **a** Treatment with phenol at different concentrations, **b** treatment with 3 mg/g phenol and TS at different concentrations, and **c** treatment with 3 mg/g phenol and RL at different concentrations

since phenol can change membrane functioning and then influence protein-to-lipid ratios in the membrane [32]. Liu et al. [19] reported that phenol could cause cellular lysis in several strains of *C. tropicalis* and affect their growth. A similar phenomenon might occur to *P. simplicissimum*. However, some cells were still able to survive in 5 mg/g phenol. The result showed that some *P. simplicissimum* cells had a strong tolerance to phenol.

RL and TS at different concentrations had opposite effects on fungal biomass (Fig. 3b, c). Figure 3b showed that biosurfactant TS had inhibition effects on fungal biomass, especially in the later stage. While in the presence of RL, the cell growth rate was remarkably improved during the early stage. For example, the ergosterol of fungal cells was 0.031 mg/g DW on the 10th day in the presence of 0.010% RL, which was 73.1% higher than that of the control. Meanwhile, it was 0.027 mg/g DW on the 8th day with 0.015% RL, which was 44.7% higher than that of the control. Then, the fungal biomass declined quickly, which was even lower than that of the control in the later stage. The results indicated that the biodegradable biosurfactant RL could diminish the toxicity of phenol to fungal cells and consequently improve the cell growth in the early stage of the fermentation.

It is found in the experiment result that RL had a little stimulatory effect on fungal growth while TS had a suppression effect in the phenol-pollution solid-state fermentation—that is because surfactants may interact directly with microorganisms, since the steric or conformational compatibility of surfactants with cell membrane lipids [33]. It might be effectively absorbed to these membranes and form a protective layer to alleviate the toxicity of phenol on fungi. In many previous studies, RL did not exhibit any inhibitory effect on microorganism growth [14, 19]. Note that Liu et al. [19] found that biosurfactant monoRL might diminish the toxicity of phenol to fungi (*C. tropicalis*) and then increase the cell growth. The adsorption of the relatively hydrophobic phenol by the surfactant micelles could decrease the amount of free phenol that might suppress laccase activation [34]. However, saponins occur widely in plant species and exhibit a range of biological properties, both beneficial and harmful. Most previous studies indicated that TS had a good inhibition activity on bacteria, fungi, and yeast [35]. However, saponins from *Sesbania pachycarpa*, in contrast, enhanced the growth of *Ruminococcus* sp. [36]. The different response of microbes to saponins may be due to the types of saponins present [36]. In addition, TS had an inhibitory effect on the growth of *P. simplicissimum* (Fig. 3b) but had a stimulative effect on laccase activity (Fig. 1b). These results suggested that laccase activity is not necessarily related to fungal biomass, which was consistent with the phenomena that when 0.5 g ammonium nitrate was supplemented in the solid-state fermentation with rice straw the fungal biomass declined acutely following the maximum on day 18 while laccase activity demonstrated a gradually rising trend [13].

Conclusion

In this study, *P. simplicissimum* completely degraded 1 mg/g phenol in the control within 3 days, suggesting its potential in the degradation of phenol at high concentrations. Biosurfactants TS and RL had a simulative effect on the activity of laccase in the solid-state fermentation with 3 mg/g phenol. The highest laccase activities during the fermentation were enhanced by 163.7%, 68.2%, and 23.3% by TS at concentrations of 0.02%, 0.06%, and 0.10%, respectively. As a result of the enhanced laccase activity, the efficiency of phenol degradation was also improved by both biosurfactants. Biosurfactants at lower concentrations were more effective for the enhancement of laccase activity and phenol

degradation. These results indicated the potential application of the biosurfactants in the bioremediation of phenols.

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References

1. Caza, N., Bewtra, J. K., Biswas, N., & Taylor, K. E. (1999). Removal of phenolic compounds from synthetic wastewater using soybean peroxidase. *Water Research, 33*(13), 3012–3018.
2. Autenrieth, R. L., Bonner, J. S., Akgerman, A., Okaygun, M., & McCreary, E. M. (1991). Biodegradation of phenolic wastes. *Journal of Hazardous Materials, 28*(1–2), 29–53.
3. Zhao, G. H., Zhou, L. C., Li, Y. F., Liu, X. X., Ren, X. J., & Liu, X. L. (2009). Enhancement of phenol degradation using immobilized microorganisms and organic modified montmorillonite in a two-phase partitioning bioreactor. *Journal of Hazardous Materials, 169*(1–3), 402–410.
4. Banerjee, I., Modak, J. M., Bandopadhyay, K., Das, D., & Maiti, B. R. (2001). Mathematical model for evaluation of mass transfer limitations in phenol biodegradation by immobilized *Pseudomonas putida*. *Journal of Biotechnology, 87*, 211–223.
5. Fraaije, M. W., Pikkemaat, M., & Van Berkel, W. J. H. (1997). Enigmatic gratuitous induction of the covalent flavoprotein vanillyl-alcohol oxidase in *Penicillium simplicissimum*. *Applied and Environmental Microbiology, 63*(2), 435–439.
6. Rocha, L. L., Aguiar Cordeiro, R., De Cavalcante, A. E., Nascimento, R. M., Do, R. F., Silveira Martins, S. C., et al. (2007). Isolation and characterization of phenol-degrading yeasts from an oil refinery wastewater in Brazil. *Mycopathologia, 164*, 183–188.
7. Mollaei, M., Abdollahpour, S., Atashgahi, S., Abbasi, H., Masoomi, I., Rad, F., et al. (2010). Enhanced phenol degradation by *Pseudomonas* sp. SA01: gaining insight into the novel single and hybrid immobilizations. *Journal of Hazardous Materials, 175*(1–3), 284–292.
8. Annachhatre, A. P., & Gheewala, S. H. (1996). Biodegradation of chlorinated phenolic compounds. *Biotechnology Advances, 14*, 35–56.
9. Niladevi, K. N., & Prema, P. (2008). Immobilization of laccase from *Streptomyces psammoticus* and its application in phenol removal using packed bed reactor. *World Journal of Microbiology & Biotechnology, 24*, 1215–1222.
10. Bollag, J. M., Shuttleworth, K. L., & Anderson, D. H. (1988). Laccase-mediated detoxification of phenolic compounds. *Applied and Environmental Microbiology, 54*, 3086–3091.
11. Thurston, C. F. (1994). The structure and function of fungal laccases. *Microbiology, 140*, 19–26.
12. Petri, W., & Andreas, K. (2008). Laccase application in the forest products industry: a review. *Enzyme and Microbial Technology, 42*, 293–307.
13. Zeng, G. M., Yu, H. Y., Huang, H. L., Huang, D. L., Chen, Y. N., Huang, G. H., et al. (2006). Laccase activities of a soil fungus *Penicillium simplicissimum* in relation to lignin degradation. *World Journal of Microbiology and Biotechnology, 22*, 317–324.
14. Zeng, G. M., Shi, J. G., Yuan, X. Z., Liu, J., Zhang, Z. B., Huang, G. H., et al. (2006). Effects of Tween 80 and rhamnolipid on the extracellular enzymes of *Penicillium simplicissimum* isolated from compost. *Enzyme and Microbial Technology, 39*, 1451–1456.
15. Ahuja, S. K., Ferreira, G. M., & Moreira, A. R. (2004). Production of an endoglucanase by the shipworm bacterium *Teredinobacter turnirae*. *Journal of Industrial Microbiology & Biotechnology, 31*, 41–47.
16. Reddy, R. M., Reddy, P. G., & Seenayya, G. (1999). Enhanced production of thermostable-amylase and pullulanase in the presence of surfactants by *Clostridium thermosulfurogenes* SV2. *Process Biochemistry, 34*, 87–92.
17. Liu, X. L., Zeng, G. M., Tan, L., Zhong, H., Wang, R. Y., Fu, H. Y., et al. (2008). Effects of dirhamnolipid and SDS on enzyme production from *Phanerochaete chrysosporium* in submerged fermentation. *Process Biochemistry, 43*, 1300–1303.
18. Helle, S. S., Duff, S. J. B., & Cooper, D. G. (1993). Effect of surfactants on cellulose hydrolysis. *Biotechnology and Bioengineering, 42*(5), 611–617.
19. Liu, Z. F., Zeng, G. M., Wang, J., Zhong, H., Ding, Y., & Yuan, X. Z. (2010). Effects of monorhamnolipid and Tween 80 on the degradation of phenol by *Candida tropicalis*. *Process Biochemistry, 45*(5), 805–809.

20. Zhong, H., Zeng, G. M., Liu, J. X., Xu, X. M., Yuan, X. Z., Fu, H. Y., et al. (2008). Adsorption of monorhamnolipid and dirhamnolipid on two *Pseudomonas aeruginosa* strains and the effect on cell surface hydrophobicity. *Applied Microbiology and Biotechnology, 79*, 671–677.
21. Bourbonnais, R., Paice, M. G., Freiermuth, B., Bodie, E., & Borneman, S. (1997). Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. *Applied and Environmental Microbiology, 63*(12), 4627–4632.
22. Ruzicka, S., Norman, M. D. P., & Harris, J. A. (1995). Rapid ultrasonication method to determine ergosterol concentration in soil. *Soil Biology and Biochemistry, 27*, 1215–1217.
23. Jiang, Y., Wen, J. P., Li, H. M., Yang, S. L., & Hu, Z. D. (2005). The biodegradation of phenol at high initial concentration by the yeast *Candida tropicalis*. *Biochemical Engineering Journal, 24*(3), 243–247.
24. Ryan, D., Leukes, W., & Burton, S. (2007). Improving the bioremediation of phenolic wastewaters by *Trametes versicolor*. *Bioresource Technology, 98*, 579–587.
25. Volkering, F., Breure, A. M., & Rulkens, W. H. (1998). Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation, 8*, 401–417.
26. Yang, Z., Deng, J., & Chen, L. F. (2007). Effect of ionic and non-ionic surfactants on the activity and stability of mushroom tyrosinase. *Journal of Molecular Catalysis B, Enzymatic, 47*, 79–85.
27. Morikawa, N., Li, N., Nagatomo, A., Matsuda, H., Li, X., & Yoshikawa, M. (2006). Triterpene saponins with gastroprotective effects from tea seed (the seeds of *Camellia sinensis*). *Journal of Natural Products, 69*, 185–190.
28. Noordman, W. H., Brusseau, M. L., & Janssen, D. B. (2000). Adsorption of a multicomponent rhamnolipid surfactant to soil. *Environmental Science & Technology, 34*, 832–838.
29. Mougin, C., Jolivalt, C., Briozzo, P., & Madzak, C. (2003). Fungal laccases: from structure–activity studies to environmental applications. *Environmental Chemistry, 1*, 145–148.
30. Minussi, R. C., Pastore, G. M., & Durán, N. (2007). Laccase induction in fungi and laccase/N–OH mediator systems applied in paper mill effluent. *Bioresource Technology, 98*(1), 158–164.
31. Majeau, J. A., Brar, S. K., & Tyagi, R. D. (2010). Laccases for removal of recalcitrant and emerging pollutants. *Bioresource Technology, 101*(7), 2331–2350.
32. Keweloh, H., Weyrauch, G., & Rehm, H. J. (1990). Phenol-induced membrane changes in free and immobilized *Escherichia coli*. *Applied Microbiology and Biotechnology, 33*, 66–71.
33. Rouse, J. D., Sabatini, D. A., Suflita, J. M., & Harwell, J. H. (1994). Influence of surfactants on microbial degradation of organic compounds. *Critical Reviews in Environmental Science and Technology, 24*(4), 325–370.
34. Zeng, G. M., Xu, K., Huang, J. H., Li, X., Fang, Y. Y., & Qu, Y. H. (1998). Micellar enhanced ultrafiltration of phenol in synthetic wastewater using polysulfone spiral membrane. *Journal of Membrane Science, 310*, 149–160.
35. Li, Y., Du, Y. M., & Zou, C. (2009). Effects of pH on antioxidant and antimicrobial properties of tea saponins. *European Food Research and Technology, 228*, 1023–1028.
36. Wina, E., Muetzel, S., Hoffmann, E., Makkar, H. P. S., & Becker, K. (2005). Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure in vitro. *Animal Feed Science and Technology, 121*, 159–174.