Phenanthrene degradation potential of lignolytic manglicolous filamentous fungus *Trichoderma* sp. CNSC-2

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ABSTRACT

Manglicolous filamentous fungi are ecologically important fungal communities involved in the degradation of organic compounds. Extracellular lignolytic enzymes released by mangrove fungi can degrade various xenobiotic compounds. In the present study, the manglicolous fungus *Trichoderma* sp. CNSC-2, isolated from the Indian Sundarban mangrove ecosystem, is explored for its ability to degrade polycyclic aromatic hydrocarbon (PAH) and determine the role of lignolytic enzyme in PAH degradation. Phenanthrene is a toxic, highly persistent, bioaccumulative three-ringed PAH with various adverse effects on surrounding. *Trichoderma* sp. CNSC-2 effectively reduces 50 mg l^{-1} phenanthrene concentration up to 64.05±0.75% within 10 days of incubation without further optimization. The degradation rate was further optimized under various pH, nutrient source, and Cu^{2+} concentrations. The involvement of the extracellular laccase enzyme was determined by ABTS assay. Laccase enzyme activity was found to be higher in the presence of phenanthrene on day 6 of incubation which coincided with the phenanthrene removal rate. The *Lac1* gene encoding for the laccase enzyme was identified. Further, GC-MS analysis revealed phenanthrene degradation metabolites, determining two possible metabolic pathways catalyzed by fungal laccase. The effect of enzyme inducers on laccase enzyme activity was observed by the addition of Cu^{2+} ion. Laccase activity was significantly higher in Phe+Cu^{2+} and Cu^{2+} induced culture over control ($P<0.0001$). Thus, the study suggests that *Trichoderma* sp. CNSC-2 can be a potential agent for bioremediation.

INTRODUCTION

Mangroves are thought to be the ecological hotspot for a various filamentous fungi, collectively called manglicolous fungi. These fungi demonstrate an unusual adaptation to stressful environmental parameters. The lignocellulosic enzymes derived from manglicolous fungi are known to participate in the biochemical transformation of complex organic compounds due to their low substrate specificity, thermostability, and improved catabolic properties[1,2]. Phenanthrene is highly persistent toxic PAHs, prolonged exposure of which causes several adverse effects[3]. Laccase enzyme from manglicolous fungus *Trichoderma* sp. CNSC-2, isolated from the Indian Sundarban Mangrove ecosystem plays a crucial role in phenanthrene degradation. The study showcases the effect of environmental parameters and enzyme activators on phenanthrene remediation to optimize the degradation process. Identification of degradation metabolites, catabolic genes, characterization of purified laccase by enzyme assays and zymogram, determined the involvement of the extracellular enzymatic system in phenanthrene degradation.

RESULTS

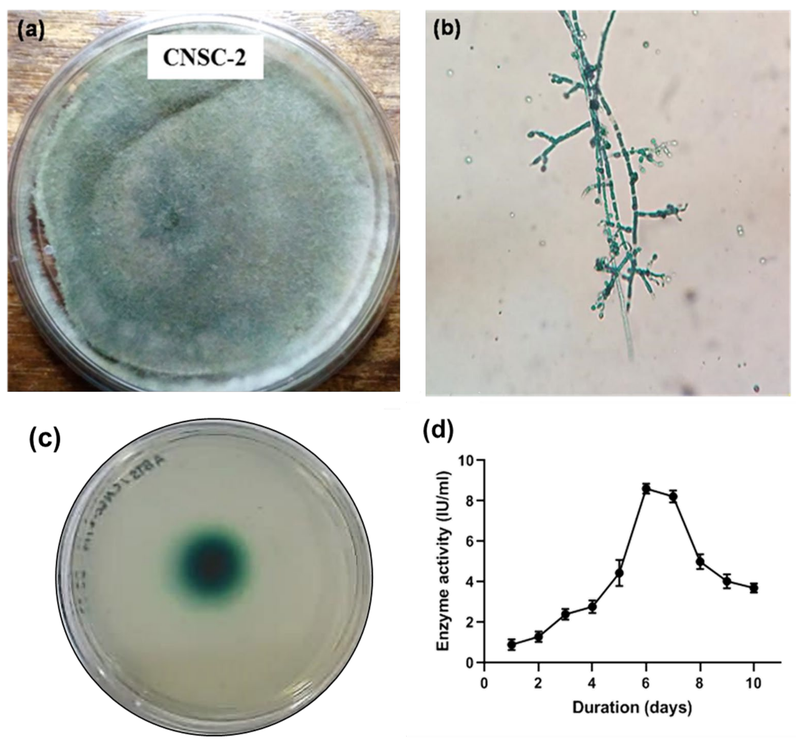

Fig. 1. Morphological characteristics of manglicolous fungus *Trichoderma* sp. CNSC-2. (a) Colony morphology, (b) Microscopic characteristics. Laccase activity (c) screening by ABTS plate assay, and (d) quantification at different time-intervals.

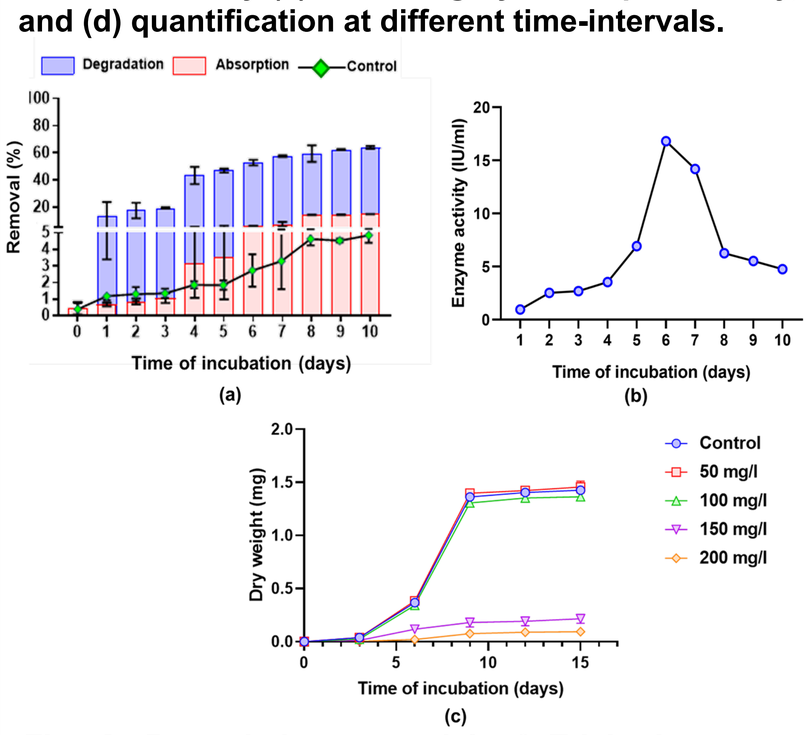

Fig. 2. Degradation potential of *Trichoderma* sp. CNSC-2 (a) Phenanthrene removal efficiency significantly enhanced with time, (b) Growth curve at different concentration of phenanthrene ($P < 0.0001$; Two-way ANOVA, Tukey's multiple comparison test), and (c) Laccase activity of culture induced in presence of 50 mg l^{-1} phenanthrene using ABTS.

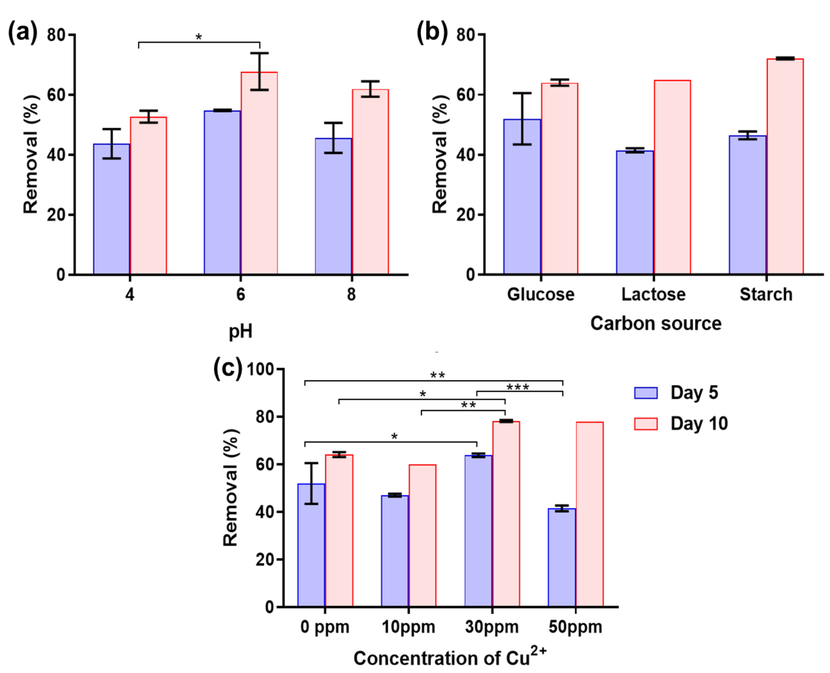

Fig. 3. Influence of various physicochemical parameters, (a) pH, (b) Carbon source, and (c) Cu^{2+} concentration on degradation of phenanthrene ($P<0.0001$; Two-way ANOVA, Dunnett's multiple comparison test).

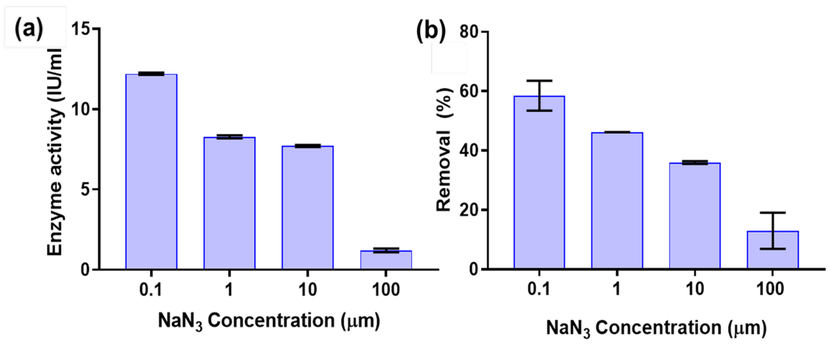

Fig. 4. Effect of NaN_3 concentrations on (a) Laccase enzyme activity and (b) phenanthrene removal efficiency.

Table 1. GC-MS analysis of the phenanthrene degradation metabolites by *Trichoderma* sp. CNSC-2.

Metabolite	Retention time (min)	m/z of major ion peaks (%)	Suggested structure
I	17.265	149 (M^+, 100), 154 (35.21), 70.10 (19.42), 150 (10.39), 57 (8.36)	Pthallic acid, isolbutyl 2-pentyl ester derivative
II	16.861	149 (M^+, 100), 156 (16.15), 150 (10.03), 113 (9.09), 55 (9)	1, 2 benzene dicarboxylic acid, butyl 2-methyl propyl ester derivative
III	23.814	73.10 (M^+, 100), 355 (82.04), 147 (78.59), 221 (73.36), 281 (56.51)	TMS derivative of benzoic acid
IV	17.421	154 (M^+, 100), 70.10 (46.58), 86 (15.67), 125 (13.43), 65.10 (10.07)	3, 5 dihydroxy benzoic acid

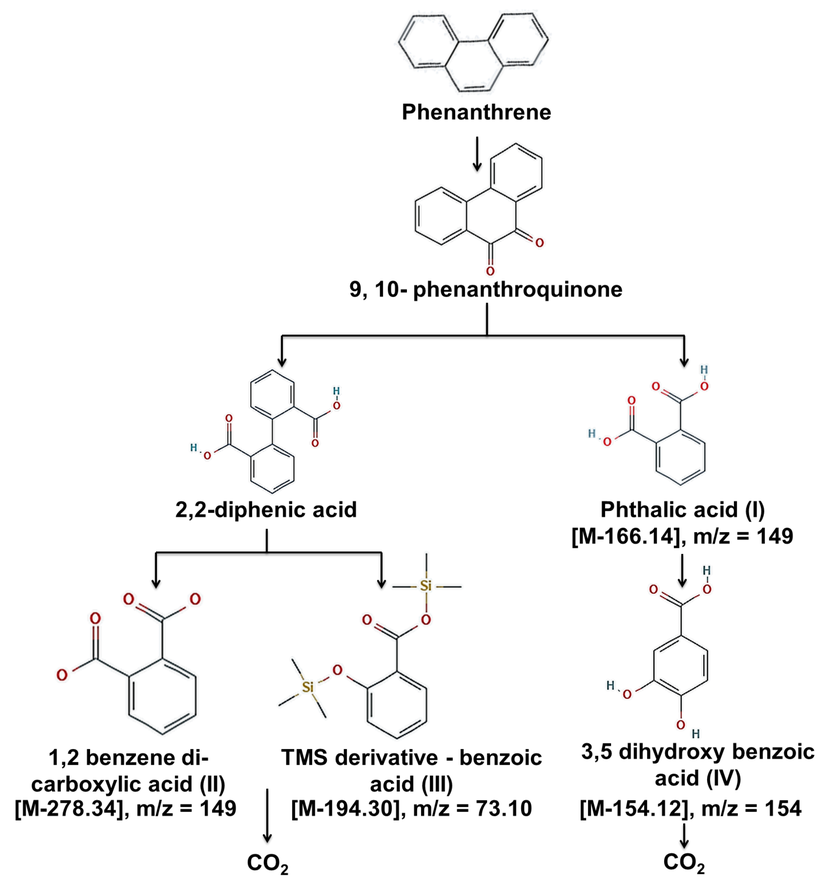

Fig. 5. Proposed degradation pathway for phenanthrene metabolism.

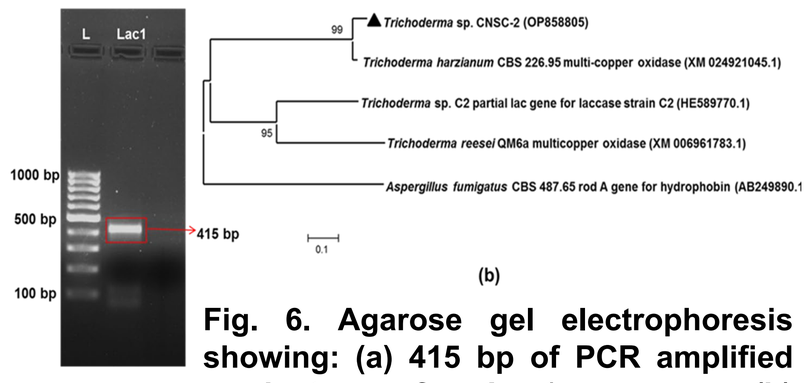

Fig. 6. Agarose gel electrophoresis showing: (a) 415 bp of PCR amplified products of *Lac1* gene, (b) Phylogenetic tree.

Table 2. Different culture conditions for laccase activity estimation.

Sl. No.	Sample type	Phenanthrene (mg/l)	Cu^{2+} (mg/l)
1	Control	-	-
2	Cu^{2+}	-	30
3	Phe+Cu^{2+}	50	30

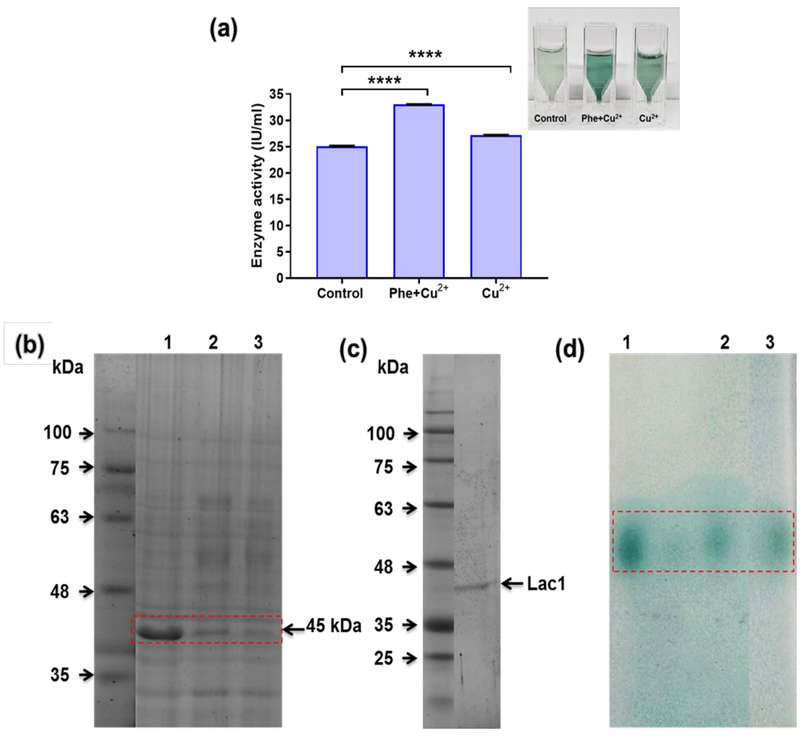

Fig. 7. Laccase enzyme of *Trichoderma* sp. CNSC-2 (a) Quantification of partially purified laccase activity, (b) SDS-PAGE analysis of extracellular laccase protein, (c) Single band represents the Lac1 protein at 45 kDa, and (d) Zymogram by ABTS in native PAGE, Lane 1: Phe+Cu^{2+}induced, Lane 2: Cu^{2+} induced and Lane 3: Control.

CONCLUSION

- Manglicolous fungus, *Trichoderma* sp. CNSC-2 was capable of degrading phenanthrene.
- The stimulation of the growth medium by Cu^{2+} ions significantly impacted the phenanthrene removal efficiency.
- Detection of four Phe degradation metabolites indicated the involvement of two different catabolic pathways mediated by extracellular laccase.
- The amendment of Phe+Cu^{2+} ions significantly increased the enzymatic activity of the purified extracellular laccase.
- The results suggested prominent role of extracellular laccase in the phenanthrene degradation.

REFERENCES

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