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Optimization of Culture Conditions for Enhanced Laccase Expression by Marine *Acinetobacter* sp. M6

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Abstract

Laccases are versatile multicopper oxidases capable of oxidizing a broad range of phenolic and non-phenolic compounds, making them valuable for applications such as bioremediation, textile dye removal, pulp bleaching, and biosensor design. While fungal laccases are extensively studied, bacterial laccases—particularly those from marine environments—offer advantages in terms of stability under extreme conditions. In this study, we investigated laccase production by a marine *Acinetobacter* sp. M6 using three different growth media: Luria–Bertani (LB) broth, Nutrient Broth (NB), and Brain Heart Infusion (BHI). Enzyme activity was monitored at various incubation times using ABTS and guaiacol as substrates. LB medium supported the highest laccase output, with peak activity occurring between 72 and 84 hours. Supplementing LB with copper sulfate enhanced enzyme production, with 0.2 mM identified as the optimal concentration. ABTS was more sensitive than guaiacol for detecting enzyme activity. These findings indicate that LB medium supplemented with copper sulfate provides favorable conditions for efficient laccase production by marine *Acinetobacter* sp. M6, highlighting its potential for industrial and environmental applications.

Keywords

Laccase; Marine *Acinetobacter* sp.; Media optimization; ABTS assay; Copper induction

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INTRODUCTION

Laccases (EC 1.10.3.2) are copper-containing enzymes that catalyze the oxidation of a wide range of substrates, including phenols, aromatic amines, and lignin-derived compounds, while reducing molecular oxygen to water. Their broad substrate specificity makes them attractive for diverse applications such as dye decolorization, wastewater treatment, lignin degradation, and biosensor development.

Although fungal laccases have traditionally dominated research and commercial use, bacterial laccases are emerging as promising alternatives due to faster production, ease of genetic manipulation, and greater stability under extreme conditions such as high salinity, temperature, or pH. Marine bacteria, in particular, remain an underexplored source of robust enzymes capable of functioning efficiently in challenging industrial settings.

Within marine bacterial genera, *Acinetobacter* species are recognized for their metabolic versatility and ability to produce industrially relevant enzymes. Despite this, information on laccase production by marine *Acinetobacter* strains is limited. Since enzyme yields are strongly influenced by growth conditions and nutrient availability, optimizing the culture

environment is critical for maximizing laccase expression. Our study aims to evaluate the effects of different growth media and copper supplementation on laccase expression by marine *Acinetobacter* sp. M6 to identify the most favorable conditions for enzyme production.

MATERIALS AND METHODS

Microorganism

A marine isolate, *Acinetobacter* sp. M6, recovered from a coastal site, was used in all experiments.

Growth Media

The following media were tested for laccase production:

- Luria–Bertani (LB) broth (HiMedia)
- Nutrient Broth (NB) (HiMedia)
- Brain Heart Infusion (BHI) broth (HiMedia)

For copper induction studies, LB broth was supplemented with copper sulfate (Sigma) at concentrations ranging from 0 to 0.4 mM.

Culture Conditions

Cultures were incubated at 37 °C with shaking at 150 rpm. Samples were collected at 48, 72, 84, and 132 hours to evaluate time-dependent laccase production.

Laccase Activity Assays

ABTS Assay: Laccase activity was measured using 1 mM ABTS in 0.1 M glycine–HCl buffer (pH 3.0). Enzyme extract was incubated for 5 min at room temperature, and absorbance was measured at 420 nm. One unit (U) of enzyme activity was defined as the amount of enzyme oxidizing 1 µmol of ABTS per minute.

Guaiacol Assay: Oxidation of guaiacol was determined in a mixture containing 2 mM guaiacol in 10 mM sodium acetate buffer. Samples were incubated at 37 °C for 90 min, and absorbance was recorded at 450 nm. One unit of enzyme activity corresponded to the oxidation of 1 µmol of guaiacol per minute.

Statistical Analysis

All experiments were conducted in triplicate. Data are presented as mean ± standard deviation. Statistical significance was evaluated using one-way ANOVA.

RESULTS

Time-Dependent Laccase expression

Laccase activity increased gradually over time in LB medium, peaking between 72 and 84 hours. ABTS consistently detected higher activity than guaiacol, indicating greater sensitivity. Our findings suggest that maximal enzyme expressionmatches with the late exponential to early stationary phase of bacterial growth.

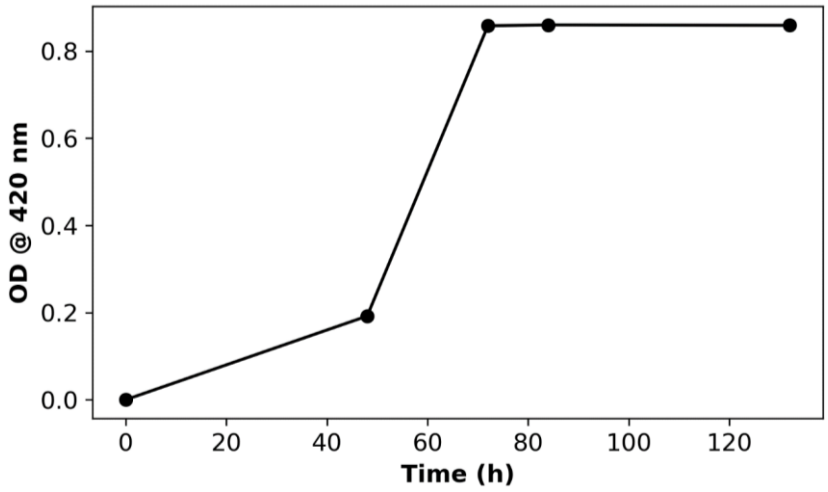

Figure 1: Time course of laccase expression by *Acinetobacter* sp. M6 in LB medium as determined by the ABTS assay. Enzyme activity increased notably between 48 and 72 h of incubation and showed little change at later time points.

Table 1. Laccase activity in LB medium (ABTS assay)

Sample	OD @ 420 nm
0 h	0.000
48 h	0.192
72 h	0.858
84 h	0.860
132 h	0.859

Effect of Copper Sulfate

Supplementing LB medium with copper sulfate enhanced laccase expression, with a maximum observed at 0.2 mM. Higher concentrations resulted in slight inhibition, likely due to metal toxicity. This highlights the dual role of copper as a cofactor and inducer of laccase expression.

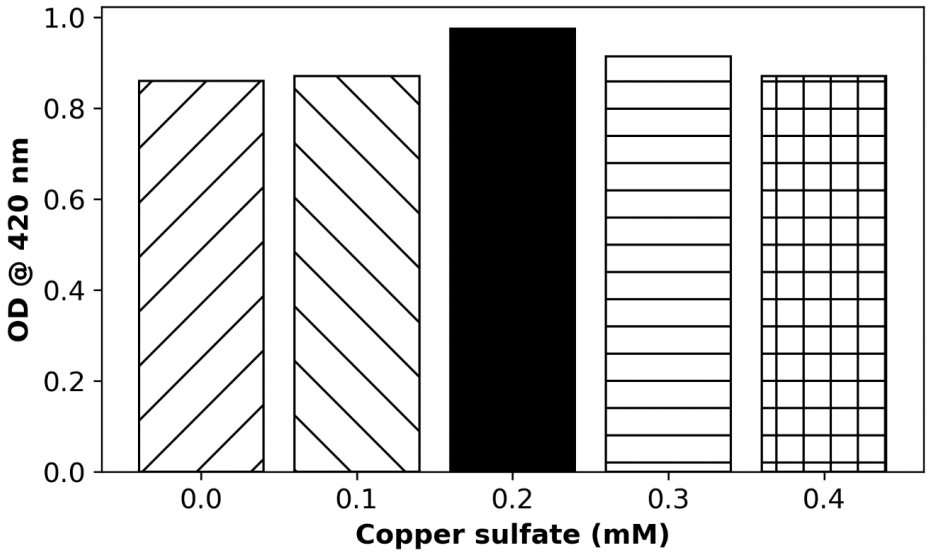

Figure 2: Influence of copper sulfate concentration on laccase activity of *Acinetobacter* sp. M6 measured using the ABTS assay. The highest enzyme activity was recorded at a copper sulfate concentration of 0.2 mM.

Table 2. Effect of copper sulfate on laccase activity

Copper sulfate (mM)	OD @ 420 nm
0.0	0.860
0.1	0.871
0.2	0.975
0.3	0.914
0.4	0.871

Comparison between media

LB medium showed the highest enzyme activity in both ABTS and Guaiacol assays, followed by BHI and NB. These results indicate that LB provides a more suitable nutrient media for laccase production by *Acinetobacter* sp. M6.

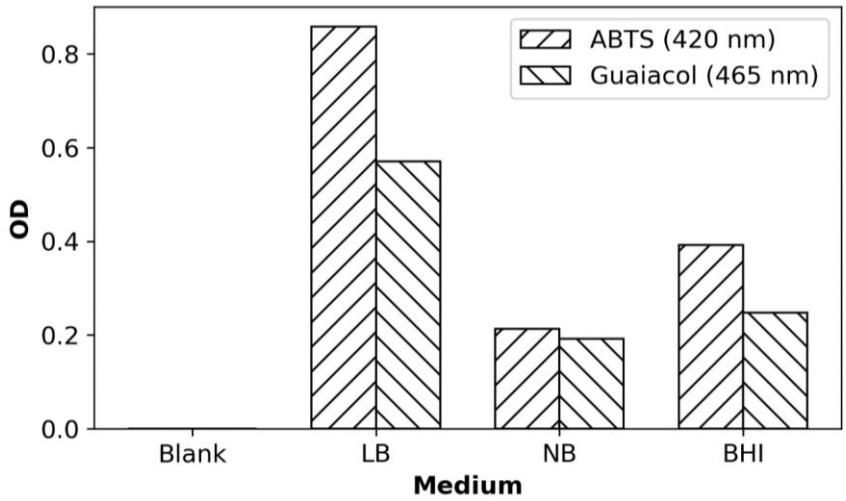

Figure 3. *Enzyme activity comparison across media.* LB medium supported the highest activity in both ABTS and Guaiacol assays, followed by BHI and NB.

Table 3. Laccase activity in different media

Sample	OD @ 420 nm (ABTS)	OD @ 465 nm (Guaiacol)
Blank	0.000	0.000
LB	0.858	0.571
NB	0.213	0.192
BHI	0.392	0.248

Plate Assay Confirmation

Guaiacol plate assays confirmed by spectrophotometric measurements. LB medium showed the most intense brown coloration, confirming higher extracellular laccase expression, while BHI and NB were less color and the control showed no activity.

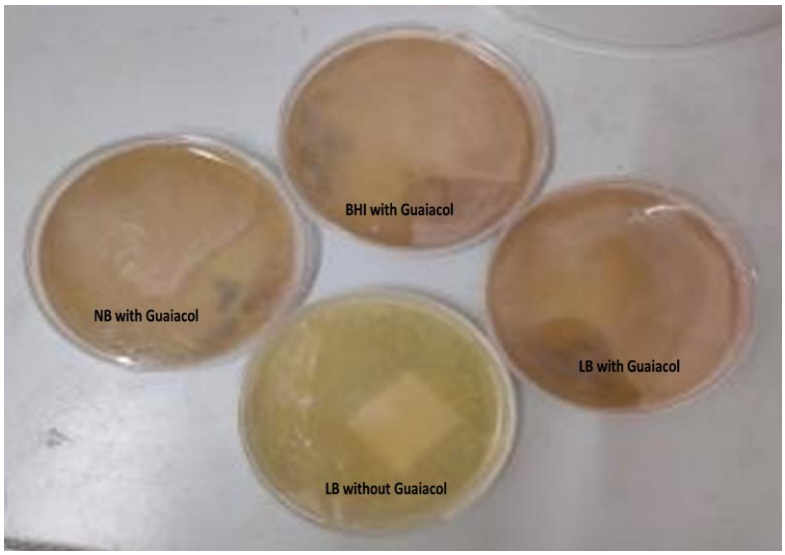

Figure 4. ***Representative guaiacol plate assay illustrating laccase activity of Acinetobacter sp. M6 cultured in four different media (LB, NB, BHI, and control). Distinct brown coloration indicates guaiacol oxidation. LB medium exhibited the most intense pigmentation, confirming higher extracellular laccase expression compared to BHI and NB. The plate assay visually validates spectrophotometric data, supporting LB as the optimal medium for enzyme expression.***

DISCUSSION

Our results showed that LB medium provides an optimal media for laccase expression in marine *Acinetobacter* sp. M6. The peak enzyme activity observed between 72 and 84 hours aligns with the late exponential or early stationary phase, suggesting a growth-phase-dependent regulation of laccase expression.

ABTS proved to be more sensitive than guaiacol, consistent with previous reports on bacterial laccases. Copper supplementation significantly increased enzyme production at 0.2 mM, confirming its role as both an essential cofactor and an inducer of gene expression. Higher copper levels slightly inhibited activity, likely due to metal toxicity affecting bacterial physiology.

Among the media tested, LB consistently outperformed BHI and NB, emphasizing that not all nutrient-rich media support optimal laccase expression. Visual confirmation through guaiacol plate assays reinforced the spectrophotometric data. These findings provide practical insights for optimizing culture conditions for bacterial laccases, which may be applied to industrial and environmental processes requiring stable, high-yield enzyme production.

CONCLUSION

Marine *Acinetobacter* sp. M6 efficiently produces laccase when grown in LB medium, with peak activity achieved between 72 and 84 hours. Supplementation with 0.2 mM copper sulfate further enhanced enzyme output. These optimized conditions offer a viable approach for scalable bacterial laccase production with potential applications in biotechnology and environmental remediation.

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REFERENCES

1. Baldrian P. Fungal laccases—occurrence and properties. *FEMS Microbiol Rev*. 2006;30(2):215-242.
2. Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G. Laccases: a never-ending story. *Cell Mol Life Sci*. 2010;67(3):369-385.
3. Ingle MR, Mishra RL. Production of laccase enzyme by *Trichodermaerinaceum*. *Indian J Appl Res*. 2016;6(10):26-28.
4. Jaeger KE, Kovacic F. *Acinetobacter* lipases: molecular biology, biochemical properties and biotechnological potential. *J IndMicrobiolBiotechnol*. 2002;31(9):391-400.
5. Kalra A, Chauhan R, Shavez M, Sachdeva S. Laccase production by *Trichodermaharzianum* and its role in lignin degradation. *Biotechnol*. 2013;3(1):37-44.
6. Kandasamy S, Sudhakar C, Prasath AR. Optimization of laccase production from *Bacillus subtilis* strain KSK02 utilizing bi substrates and their reactive red 120 dye degradation potential. *Biomass ConvBioref*. 2024; 15:22587-22601.
7. Kumar A, Chandra R. Determination of assay for laccase of *Bacillus subtilis* WPI with ABTS and guaiacol. *Indian J Microbiol*. 2012;52(4):558-564.
8. Li A, Zhu Y, Xu L, Zhu W, Tian X. Comparative study on the determination of assay for laccase of *Trametes* sp. *Int Scholars J Med Med Sci*. 2020;10(7):1-3.
9. Mishra SK, Srivastava SK. Production of extracellular laccase from *Bacillus subtilis* MTCC 1039 using different parameters. *BiosciBiotechnol Res Asia*. 2016;13(3):1235-1242.
10. Niku-Paavola ML, Raaska L, Itävaara M. Detection of white-rot fungi by a non-toxic stain. *Mycol Res*. 1988;92(2):215-220.
11. Pooalai R, Khongfak S, Leungtongkam U, Thummeepak R, Kunthalert D, Sitthisak S. Genomic analysis uncovers laccase coding genes and biosynthetic gene clusters encoding antimicrobial compounds in laccase producing *Acinetobacterbaumannii*. *Sci Rep*. 2022;12(1):10542.
12. Shraddha, Shekher R, Sehgal S, Kamthania M, Kumar A. Laccase: microbial sources, production, purification, and applications. *BioResources*. 2011;6(2):1446-1487.
13. Sidhu AK, Darade SB, Bhavsar PP, Gaikwad VB, Patil SN. Isolation, screening and optimization for laccase production by *Scytalidiumlignicola* under submerged fermentation. *Int J CurrMicrobiolAppl Sci*. 2017;6(4):2477-2491.
14. Strong PJ, Claus H. Laccase: a review of its past and future in bioremediation. *Crit Rev Environ Sci Technol*. 2011;41(4):373-434.