Original Article

Media optimization studies for enhanced biomass production of two lignolytic fungi

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Abstract

The present study evaluated the effect of 27 different Mineral Salt Medium-Lignin (MSM-L) formulations on the biomass production and sporulation of two laccase-producing *Aspergillus* species, *Aspergillus nomiae* and *Aspergillus niger*. Laccase activity was confirmed using the guaiacol plate assay, demonstrating the ligninolytic potential of both species. Furthermore, optimization of culture medium composition for enhanced growth and sporulation was carried out. The findings indicate that these two *Aspergillus* species may serve as promising candidates for laccase production and other biotechnological applications involving lignin degradation and bioremediation.

Keywords: *Aspergillus nomiae, Aspergillus niger,* fungal growth, laccase, lignin degradation, mineral salt medium.

Introduction

Lignolytic enzymes are extracellular enzymes produced by fungi for the degradation of lignin and other complex aromatic compounds present in plant biomass. Among these enzymes, laccase [benzenediol:oxygen oxidoreductase (E.C. 1.10.3.2)] is a multicopper-containing enzyme that catalyses the oxidation of a wide variety of aromatic compounds, accompanied by the concomitant reduction of molecular oxygen to water.[1] Laccase plays a significant role in lignin degradation and has gained considerable attention because of its applications in bioremediation, textile dye decolorization, paper and pulp industries, and wastewater treatment.[2-4] Fungal laccases are widely preferred for biotechnological applications over bacterial laccases due to their stability and sustained activity across a broad range of temperatures and pH.[3,5] Fungi are considered efficient producers of laccase, particularly filamentous fungi such as *Aspergillus* species.[6-8] The production of laccase is influenced by several nutritional factors, that include carbon and nitrogen sources, mineral composition, and incubation period. Optimization of these factors is important for improving enzyme production as well as fungal biomass formation.[3,9] Recent studies have focused on optimization of physiological and nutritional parameters to enhance laccase production in *Aspergillus* spp. under submerged fermentation conditions.[10] Mineral Salt Medium (MSM) is commonly used for fungal growth and enzyme production studies because it provides the essential nutrients required for fungal metabolism while allowing the modification of nutritional requirements for optimization studies. Different combinations of carbon and nitrogen sources in MSM can

significantly affect fungal growth and laccase production.[8,9] In the present study, qualitative screening of two characterized species of *Aspergillus* - *Aspergillus nomiae* and *Aspergillus niger* -was carried out using a guaiacol-containing medium, and their growth and biomass formation were subsequently evaluated under various MSM formulations.

Materials and Methods

Guaiacol Assay

Characterized strains of *A. nomiae* and *A. niger*, obtained from laboratory stock cultures maintained at the Department of Biotechnology, University of Kerala, were used in this study. Laccase activity was confirmed by a zone assay on SDA supplemented with 0.01% guaiacol, followed by incubation at room temperature for 7 days.

Media optimization with different MSM-L formulations.

Mineral salt medium supplemented with lignin (MSM-L) were prepared for media optimization studies. Lignin (1% w/v) was incorporated as the sole source of carbon. Three crucial components of the MSM were selected for optimization at three concentrations each: $NH_4H_2PO_4$ (0.5, 0.75 and 1 g/ L), $FeSO_4$ (0.01, 0.03 and 0.06 g/L), and $MgCl_2$ (0.5, 0.75 and 1 g/L), resulting in a total of 27 formulations.

The 3 × 3 × 3 optimization design used for the preparation of the various MSM formulations evaluated in this study is presented in Table 1. The same experimental design was applied to both *A. nomiae* and *A. niger* to facilitate comparison of their growth under identical culture conditions. For the preparation of each MSM formulation, autoclaved media components in the respective proportions outlined in Table 1 were dispensed into 50 mL sterile flasks containing double distilled water. The final volume of the medium was brought to 25 mL. The pH of the medium was subsequently adjusted to 6.5 using a 0.1 N HCl solution. The fungal strains were sourced from the mother culture. A 50 µL aliquot of the inoculum, taken from a vortexed, overnight MSM-L broth culture of each *Aspergillus* species, was transferred into

Table 1: 3 x 3 x 3 MSM-L formulations used in the study and their component concentrations

Concentration of $NH_4H_2PO_4$ (g/L) (A)	Concentration of $FeSO_4$ (g/L) (B)	Concentration of $MgCl_2$ (g/L) (C)
0.05^1	0.01^1	0.5^1
		0.75^2
		1^3
	0.03^2	0.5^1
		0.75^2
		1^3
	0.06^3	0.5^1
		0.75^2
		1^3
0.75^2	0.01^1	0.5^1
		0.75^2
		1^3
	0.03^2	0.5^1
		0.75^2
		1^3
	$0.06l^3$	0.5^1
		0.75^2
		1^3
1^3	0.01^1	0.5^1
		0.75^2
		1^3
	0.03^2	0.5^1
		0.75^2
		1^3
	0.06^3	0.5^1
		0.75^2
		1^3

'A' *represents* $NH_4H_2PO_4$, 'B' *represents* $FeSO_4$, *and* 'C' *represents* $MgCl_2$, *while the numerical superscripts* 1, 2, *and* 3 *denote the concentration of each component in the medium. The 27 MSM formulations were labelled according to the concentrations of the individual components used in each formulation. For example,* $A^1B^3C^2$ *represents a medium in which the concentrations of* $NH_4H_2PO_4$, $FeSO_4$, *and* $MgCl_2$ *are 0.05g/L, 0.06 g/L, and 0.75 g/L respectively.*

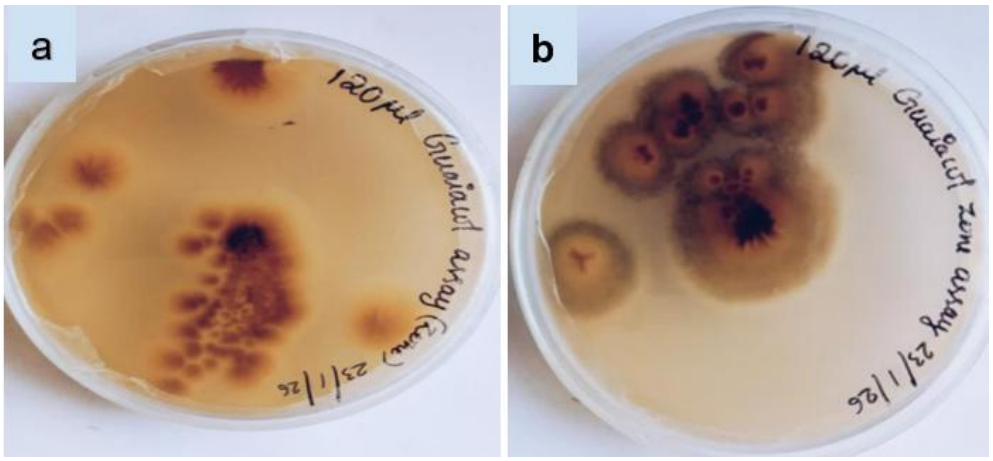

Figure 1: Guaiacol plate assay showing positive laccase activity. (a) *A. nomiae* (b) *A. niger*

Table 2: Qualitative scoring of biomass formation and sporulation in *A. nomiae* grown on various MSM formulations.

MSM formulations used for culture	Day of observation									
	DAY 3		DAY 6		DAY 10		DAY 17		DAY 20	
	g	*s*	*g*	*s*	*g*	*s*	*g*	*s*	*g*	*s*
$A^1B^1C^1$	+	0	++	S+	0	0	0	0	0	0
$A^1B^1C^2$	++	S+	++	S+	+	0	+	0	+	0
$A^1B^1C^3$	+	0	++	S+	0	0	0	0	+	0
$A^1B^2C^1$	+	0	++	S+	++	S+	++	S+	+	0
$A^1B^2C^2$	++	S+	++	S+	+	0	+	0	+	0
$A^1B^2C^3$	++	S+	++	S+	+	0	+	0	+	0
$A^1B^3C^1$	+	0	++	S+	+	0	+	0	+	0
$A^1B^3C^2$	+	0	++	S+	++	S+	++	S+	++	S+
$A^1B^3C^3$	+	0	++	S+	+	0	+	0	+	0

MSM=Mineral salt medium; G=Growth; s=Sporulation; 0, + and ++ indicate no, moderate and high growth or sporulation, respectively

Table 3: Qualitative scoring of biomass formation and sporulation in *A. niger* grown on various MSM formulations.

MSM formulations used for culture	Day of observation									
	DAY 3		DAY 6		DAY 10		DAY 17		DAY 20	
	g	*s*	*g*	*s*	*g*	*s*	*g*	*s*	*g*	*s*
$A^1B^1C^1$	+	0	+	0	+	0	++	S+	+	0
$A^1B^1C^2$	0	0	0	0	0	0	+	0	0	0
$A^1B^1C^3$	0	0	0	0	+	0	+	0	0	0
$A^1B^2C^1$	+	0	+	0	+	0	++	S+	+	0
$A^1B^2C^2$	+	0	+	0	+	0	++	S+	+	0
$A^1B^2C^3$	+	0	+	0	+	0	++	S+	+	0
$A^1B^3C^1$	0	0	0	0	0	0	+	0	+	0
$A^1B^3C^2$	0	0	+	0	0	0	+	0	+	0
$A^1B^3C^3$	+	0	+	0	+	0	++	S+	++	S+

MSM=Mineral salt medium; g=Growth; s=-Sporulation; 0, + and ++ indicate no, moderate and high growth or sporulation, respectively

MSM composition	Days of observation				
	3	6	10	17	20
$A^1B^1C^1$	1	2	0	0	0
$A^1B^1C^2$	2	2	1	1	1
$A^1B^1C^3$	1	2	0	0	1
$A^1B^2C^1$	1	2	2	2	1
$A^1B^2C^2$	2	2	1	1	1
$A^1B^2C^3$	2	2	1	1	1
$A^1B^3C^1$	1	2	1	1	1
$A^1B^3C^2$	1	2	2	2	2
$A^1B^3C^3$	1	2	1	1	1

(A) *Aspergillus nomiae*

MSM composition	Days of observation				
	3	6	10	17	20
$A^1B^1C^1$	1	1	1	2	1
$A^1B^1C^2$	0	0	0	1	0
$A^1B^1C^3$	0	0	1	1	0
$A^1B^2C^1$	1	1	1	2	1
$A^1B^2C^2$	1	1	1	2	1
$A^1B^2C^3$	1	1	1	2	1
$A^1B^3C^1$	0	0	0	1	1
$A^1B^3C^2$	0	1	0	1	1
$A^1B^3C^3$	1	1	1	2	2

(B) *Aspergillus niger*

MSM=Mineral salt medium; The intensity of colour is proportional to the extent of the observed response, increased colour intensity indicating a stronger response. For ease of interpretation, colour intensity is also represented numerically on a scale of 0 - 2, where 0 indicates no response and 2 represents the maximum response.

Figure 2: Heatmap analysis of fungal growth and sporulation in *A. nomiae* (A) and A. *niger* (B), from culture medium optimization studies

the sterile flasks. The cultures were then incubated at room temperature under controlled humidity without agitation (statically) to prevent mechanical damage to the developing mycelia. The preparation of the culture medium, inoculation, and incubation were carried out under aseptic conditions. Following incubation, visible biomass production and sporulation were monitored at specific intervals over a period of 20 days. Observations were recorded using a qualitative scoring system. Biomass formation was scored as follows: no growth was represented by '0', while increasing levels of biomass accumulation were denoted by '+' and '++', indicating moderate and high biomass production, respectively.

A similar scoring system was used to assess sporulation, where '0', '+', and '++' represented no, moderate, and high sporulation, respectively.

Heatmap analyses

Heatmap analyses of data derived from culture medium optimization studies were prepared using Microsoft Excel.

Results

Confirmation of laccase production using guaiacol assay

Qualitative screening for laccase activity using a guaiacol-containing medium confirmed laccase secretion in both *A. nomiae* and *A. niger* (Figure 1). The formation of a reddish-brown colouration around the fungal colonies confirmed the oxidation of guaiacol by laccase.[8]

Media optimization of nutrient conditions using different MSM-L formulations

Both *A. nomiae* and *A. niger* showed variations in biomass formation when grown in different MSM formulations. Some formulations of MSM showed enhanced biomass formation and sporulation after a definite period of incubation. Among the 27 MSM formulations evaluated, only nine supported enhanced fungal growth and were, therefore, selected for further comparative analysis using the growth-scoring system (Tables 2 and 3).

Heatmap analyses of data

From the heatmap analysis of the quantitative scoring system, it was observed that both *A. nomiae* and *A. niger* exhibited distinct growth and sporulation patterns under different MSM formulations as may be seen in Figure 2.

Discussion

Aspergillus species can inhabit a wide variety of ecological environments, with soil serving as their primary habitat.[11] In soil ecosystems, they play a crucial role as saprophytic decomposers of organic plant materials. However, certain species are also recognized as opportunistic pathogens. For instance, *Aspergillus fumigatus* is widely reported to cause severe allergic reactions and life-threatening systemic infections in humans, collectively known as aspergillosis.[12] Additionally, some members of this genus act as phytopathogens that target economically important crops.[11] Despite their pathogenic potential, many *Aspergillus* species are beneficially utilized in industrial biotechnology for the large-scale production of enzymes, vitamins, organic acids, and other bioactive secondary metabolites.[13]

The present study shows that MSM composition significantly influences fungal growth in *Aspergillus* spp. which is consistent with previous reports on the effects of nutritional factors on fungal growth. [3,8] Enhanced growth of fungal isolates in lignin-containing MSM suggests their ability to utilize lignin through lignolytic enzymes such as laccase. This observation indicates that *Aspergillus* spp. can be utilised for lignin degradation.[2]

The quantitative scoring of data from media optimization studies revealed that among the 27 MSM formulations tested, $A^1B^3C^2$ and $A^1B^3C^3$ consistently supported enhanced growth and sporulation in A. *nomiae* and *A. niger* respectively, throughout the period of observation. Therefore, $A^1B^3C^2$ and $A^1B^3C^3$ combinations were selected as the optimal media for culturing *A. nomiae* and *A. niger* respectively. *A. nomiae* reached maximum growth on day 6, while in *A. niger* this was on day 17, indicating differences in nutrient utilization and growth response. Heatmap analysis is a powerful data visualization tool widely used in fungal studies to represent complex datasets using colour gradients, thereby enabling rapid identification of patterns, clusters, and correlations.[14] In the present study, the heatmap analysis of growth and sporulation responses of *Aspergillus* spp. maintained on different MSM formulations corroborated the results obtained through qualitative scoring. The approach proved useful in identifying the optimal composition of medium components for enhanced fungal growth and sporulation.

It is observed in the present study that both fungal species exhibited enhanced growth at a comparatively lower $NH_4H_2PO_4$ concentration of 0.5g/L. Fungi are well known for their ability to degrade lignin. Lignin contains very little or no readily available nitrogen and hence, is not considered a significant source of nitrogen; but it may serve as a carbon-rich substrate, for fungal growth.[15,16] Fungi are well known for their ability to degrade lignin. During lignin degradation, fungi secrete extracellular ligninolytic enzymes such as laccases, lignin peroxidases, and manganese peroxidases, which break down the complex lignin polymer into smaller aromatic compounds that can be further metabolized. The production of these enzymes is often stimulated under conditions of nitrogen limitation, indicating that low nitrogen availability can enhance lignin degradation and ligninolytic activity.[2] The present study shows that both species of *Aspergillus* can survive and grow well in a culture medium with relatively low nitrogen content. This observation further supports the existence of a nitrogen-limitation response in *Aspergillus* spp. in which reduced ammonium availability in the culture medium appears to have caused increased secretion of laccase for lignin degradation. Thus, under conditions of low nitrogen availability, lignin may contribute indirectly to fungal growth by providing carbon and energy through its degradation products.[15,16] In the present study, MSM supplemented with 1% lignin was used for culturing the fungal isolates based on standardization trials. Thus, with lignin as the sole carbon source, both fungal species may have relied on ligninolytic enzymes for substrate

utilization, for growth and sporulation.[8] It is further observed that different culture medium formulations significantly influence ligninolytic enzyme production and fungal growth.[9,17,18]

The successful growth of fungi in MSM indicates their ability to utilize complex carbon sources under nutrient-limited conditions. MSM provides essential inorganic nutrients while minimizing the availability of readily metabolizable carbon, thereby promoting the utilization of alternative substrates such as lignin and lignocellulosic compounds. Several studies have reported that fungi, particularly white-rot and lignolytic species, exhibit substantial growth and enzymatic activity in MSM supplemented with lignin or related aromatic compound.[10,19]

Conclusion

The present study demonstrated that both *A. nomiae* and *A. niger* exhibited enhanced biomass production in culture media containing comparatively a lower concentration of $NH_4H_2PO_4$ (0.5 g/L), with optimum growth observed on the 6^{th} and 17^{th} days of incubation, respectively. Since lignin served as the sole carbon source, the increased biomass under these conditions suggests efficient lignin utilization, which is probably associated with enhanced ligninolytic enzyme activity, including laccase production. The MSM-L formulations designated as $A^1B^3C^2$ for *A. nomiae* and $A^1B^3C^3$ for *A. niger* yielded the best results in supporting fungal growth and sporulation. These formulations may therefore be considered potentially suitable media for laccase production by *Aspergillus* species.

References

1. Gianfreda L, Xu F, Bollag JC. Laccases: a useful group of oxidoreductive enzymes. Bioremediation Journal 1999; 3: 1–26.
2. Baldrian P. Fungal laccases – occurrence and properties. FEMS Microbiol Rev 2006; 30(2): 215–42.
3. Madhavi V, Lele SS. Laccase: properties and applications. BioResources 2009; 4(4): 1694–717.
4. Couto SR, Herrera, TJL. Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 2006; 24(5):500–13.
5. Mate DM, Alcalde M. Laccase engineering: from rational design to directed evolution. Biotechnol Adv 2015; 33(1): 25-40
6. Bagewadi ZK, Mulla SI, Ninnekar HZ. Purification and characterization of endo β-1,4-d-glucanase, xylanase and laccase from Aspergillus niger. 3 Biotech 2017; 7: 302
7. Shekher SR, Sehgal S, Kamthania M, Kumar A. Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme Res 2011; 2011:217861.
8. Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G. Fungal laccases and their applications in bioremediation. Enzyme Res 2014;2014:163242.
9. Papagianni, M. Fungal morphology and metabolite production in submerged mycelial processes. Biotechnology Advances 2004; 22(3): 189–259.
10. Zaidi S, Mirza SA. Optimization of laccase production parameters by Aspergillus niger from different ecological sites of Lahore. Punjab Univ J Zool 2024;39(2):141-9.
11. Niji QN, Babalola OO, Mwanza M. Soil Aspergillus Species, Pathogenicity and Control Perspectives. J Fungi 2023; 9(7): 766.
12. Frías-De-León GM, García-Salazar E, Acosta-Altamirano G. Virulence attributes in Aspergillus fumigatus. In: Razzaghi-Abyaneh M, Rai M, Shams-Ghahfarokhi M, Rodrigues-Morales AJ, editors. Aspergillus and Aspergillosis- Advances in Genomics, Drug Development, Diagnosis and Treatment. London (UK):Intech Open Limited;2023. Available from: https://www.intechopen.com/chapters/87187 [Last accessed on 2026 May 15].
13. Hou X, Liu L, Xu D, Lai D, Zhou L. Involvement of Lae A and velvet proteins in regulating the production of mycotoxins and other fungal secondary metabolites. J Fungi (Basel) 2024; 10(8): 561.
14. Feng W, Wang J, Jin Q, Guo Z, Cai W. Analysis of Soil Fungal Community Characteristics of Morchella sextelata Under Different Rotations and Intercropping Patterns and Influencing Factors. Agriculture 2025;15(8): 823.
15. Kumar V, Prasher IB. Ligninolytic enzymes production by endophytic fungus Diaporthe phaseolorum under the influence of different carbon and nitrogen sources. Studies in Fungi 2021; 6(1): 531–42.
16. Dashtban M, Schraft H, Syed TA, Qin W. Fungal biodegradation and enzymatic modification of lignin. Int J Biochem Mol Biol 2010; 1(1): 36–50.
17. Cairns T C, Nai C, Meyer V. How a fungus shapes biotechnology: 100 years of Aspergillus niger research. Fungal Biol Biotechnol 2018; 5: 13.
18. Upadhyay P, Shrivastava R, Agrawal PK. Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech 2016; 6: 15.
19. Elisashvili V, Kachlishvili E, Penninckx M. Effect of growth substrate, method of fermentation, and nitrogen source on lignocellulose-degrading enzymes production by white-rot basidiomycetes. J Ind Microbiol Biotechnol 2008; 35(11): 1531–8.

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